Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Baghdad
College of Veterinary Medicine

TREATMENT OF INDUCED HYPOZINCEMIA IN LOCAL BREED GOATS WITH NANOPARTICLES AND CONVENTIONAL ZINC OXIDE

A Dissertation

Submitted to the Council of the College of Veterinary Medicine-University of Baghdad In Partial Fulfillment of Requirements for the Degree of Doctor of Philosophy in Veterinary internal and Preventive medicine

By
RAAD MAHMOOD HUSSEIN
Supervised by
Assist. Prof. Dr. Alaa Kamil Mahmood

2020 A.D 1441A.H

بِسمِ الله الرحْمنِ الرحم

فَتعالى اللهُ المَلِكُ الحَق وَلا تَعْجَلْ بِالقُرآن

مِنْ قَبْلِ أَنْ يُقْضَى إِلَيكَ وَحْيُهُ

وقُلْ رَب زِدْنِي عِلْما

صدق الله العظيم

سورة ط (الآية - 114)

Supervisor Declaration

I certify that this dissertation (Treatment of induced hypozincemia in local breed goats with nanoparticles and conventional zinc oxide) has been prepared under my supervision at the College of Veterinary Medicine/ University of Baghdad in partial fulfillment of the requirements for the degree of Doctor of philosophy in Veterinary Medicine/ Internal and Preventive Veterinary Medicine.

Assist. Prof.
Dr. Alaa Kamil Mahmood
College of Veterinary Medicine
Baghdad University
(Supervisor)
Date: / /

In view of the available recommendations, I forward this dissertation for debate by the examining committee.

Prof. Dr. Ahmed H. Fathullah AL-Bayati

Vice Dean for Postgraduate Studies and Scientific Affairs
College of Veterinary MedicineBaghdad University

Date: / /

Examination Committee Certification

We the members of the examining committee, certify that this dissertation entitled (Treatment of induced hypozincemia in local breed goats with nanoparticles and conventional zinc oxide) presented by Raad Mahmood Hussein is sufficient in fulfilling the scope and quality for the purpose of awarding for the degree of doctor of philosophy in Veterinary Medicine/ Veterinary internal and Preventive Medicine.

Signature
Prof. Dr. Afaf Abdulrahman Yousif
College of Vet. Medicine / University of Baghdad
(Chairman)

Signature
Prof. Dr. Majid Shayal Rhaymah
College of Medicine / University of Misan
(Member)

Signature
Prof. Dr. Salah Mahmood Ashour Al-Kubaysi
College of Vet. Medicine / University of Fallujah
(Member)

Signature
Assist. Prof. Dr. Oday Kareem Luaibi
College of Vet. Medicine / University of Baghdad
(Member)

Signature
Assist. Prof. Dr. Inam Badr Falih
College of Vet. Medicine / University of Baghdad
(Member)

Signature
Assist. Prof. Dr. Alaa Kamil Mahmood
College of Vet. Medicine / University of Baghdad
(Member)

Approved by the council of the college of veterinary medicine- University of Baghdad

Signature
Prof. Dr. Afaf Abdulrahman Yousif
Head Department of Internal and Preventive Medicine

Signature
Prof. Dr. Hameed Ali Khadim
Dean of the college

Date of examination 22 / 7 / 2020

Declaration

I hereby declare that the dissertation is my original work except for quotations and citations which have been dully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at the University of Baghdad or other institutions.

Name: Raad Mahmood Hussein

Date: / / 2020

Dedication to:

-My mother and father. Those who taught me and overwhelmed me with their love and prayers, was the greatest motivation of my life.

- My wife. She was my best help in my life.

- My brothers and sisters. Those who supported me and who carved their love in my heart.

- My sons and daughters. The future of my life.

- Everyone, I love them and love my friends.

Raad

Acknowledgement

First of all I would like to thank Allah the merciful God for helping me in completing this study.

The first person I would like to thank is my supervisor, Assist. prof .Dr. Alaa kamil mahmood for his supervision, guidance, constructive advice and giving most of his time and knowledge, advice and guidance along the period of my study.

My thanks to Prof. Dr. Hameed Ali Khadim, Dean of the College of Veterinary Medicine and Prof. Dr. Ahmed H. Fathullah AL-Bayati, the vice Dean of post –Graduate Studies and Scientific Research for their encouragement.

My thanks to the head of department of Internal and Preventive Medicine Prof. Dr. Afaf Abdulrahman Yousif, and all staff member for their assistance and generosity.

Special thanks and appreciations Prof. Dr. Nazar Jabbar Al-Khafaji Department of Medicine, College of Veterinary Medicine, University of Diayla, for his supporting and continuous encouragement along the period of my study.

My thanks to the Deanship of the College of Veterinary Medicine Diyala University for their help in completing the study.

My thanks go to all the teachers in the Department of Internal Medicine, Faculty of Veterinary Medicine, University of Diyala, for their help and generosity.

I thank the members of the Biochemistry Department, Faculty of Veterinary Medicine. Diyala University of Diyala, for their help in the completion of serological tests.

Special thanks to the teachers staff who contributed in helping me to accomplish this study. I mention them, Dr. Ramzi Al-Agele, Dr. Bassem Mansour, Dr. Ali Ibrahim, Dr. Anas A. Humdi and my colleague Dr. Ahmed Hanash, University of Diyala.

I would like to thank Dr. Issa Daham from the Technological University for facilitating the completion of the tests for the zinc oxide nanoparticles. My thanks to everyone who helped me and did not mention the name, to them all my respect.

Raad

Abstract

The current study was conducted to assess the efficiency of nanoparticles and conventional zinc oxide in treatment of experimentally induced zinc deficiency in goats, in depending on the health status and growth performance of animals, clinical signs, path histological hematological analysis, some biochemical changes, general immune response, additionally to histopathological changes and the healing of wounds which experimentally induced. Twenty five apparently healthy, Iraqi local breed goats, of 5-6 months old with 15.52 ±1.05 kg. BW, were used, during the period of December 2018 to June 2019 in the field of the College of Veterinary Medicine / University of Diyala / Iraq. The study divided to three main parts. The first part include identification and characterization of zinc oxide Nanoparticles. Second part, zinc deficiency were induced experimentally in the local breed goats, Third part included treatment with zinc oxide nanoparticles and conventional zinc oxide. In the second part zinc deficiency was induced in all goats during the period, December 2018 for 10 weeks. The goats were fed concentrated feed with a high concentration of calcium which was added at 400-500 g / head / day. In the third part of study, the animals were divided into 5 groups, each group included 5 animals. Those in Group (I) left without treatment as control group, while those in group (II , III) were treated orally by zinc oxide nanoparticles in dose rate of 25 and 75mg/kg respectively. Meanwhile, animals in groups (IV , V) were treated orally by zinc oxide 25 and 75mg/kg respectively, once weekly for 10 weeks.

The main dependent clinical parameters in second part were examination of (body weight, temperature, pulse rates and respiratory rates) in addition to examination of mucous membranes and monitoring animal behaviors. Blood samples were collected at zero, 2^{nd} ,4^{th} ,6^{th} and 8^{th} weeks of study, for counting and estimation of erythrocytes count (RBC), hemoglobin concentration (Hb), packed cell volumes(PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular hemoglobin (MCH), Total and differential leucocytes count (TLC, DLC), in addition estimation of serum level of zinc. In the third part dependent parameters as in second parts, with addition to clotting time (CT), Bleeding time (BT). Blood samples were collected at zero, 3^{rd} , 6^{th} and 9^{th} weeks, which submitted to the same examinations in second part above. In addition to that, platelet count and biochemistry parameters included serum level of zinc, total protein, albumin and globulin.

Immune response includes phagocytic index assay and interleukin 1β. Total serum anti oxidant and comet assay also estimated. The third part of study also included, inducing cutaneous wound, two circular areas, on dorsum of each animal used in the study. The healing steps of wounds were followed through macroscopic examination and measurement of wound diameters weekly, as well as histological examination of the skin and skin samples were collected at day 3rd , 7th, 14th and 28 post operative day (POD).

The results of the first part showed the physical specifications of the nanoparticles of zinc oxide. In the scan electron microscope SEM, the images raveled the shape, size of the collected molecules with diameters ranging from 1-50 nm. X ray diffraction (XRD) pattern showed that all the diffraction peaks indexed of pure ZnO Nanoparticles. UV-Vis absorbance spectroscopy analysis revealed the absorption peak was observed at 389.3 nm. Particle size (PS) showed the mean diameter 41.2 nm. In the second part, clinical signs were appearance retardation in growth and losing hair of head and back with hyperkeratosis of the skin. Heart rates significantly increased ($P < 0.05$) in 6th week, and respiratory rates significantly decreased ($P < 0.05$) in 3rd and 6th weeks. Total red blood cells counts were significantly increased ($P < 0.05$) in the 8th week in comparison with 0-week. Hb, PCV, MCV and MCH concentration significantly increased. Serum zinc was significant depressed, starting from the 2nd week till the end of study of 2nd part, so the lowest level (7.61µmol/L) in the 8th week compared with 0-week (11.34 µmol/L). Significance decrease in Lymphocytes, while Eosinophil significantly increased at 8week compared with 2nd ,6th week, respectively.

In the third part, clinical signs results showed marked improvement, and the animals seemed close to the natural appearance except in one animal in 3rd group. Body weight increased significantly in the groups II, III and V, respectively, compared to I group. The respiratory rate increased significantly in the group I compared to the groups II,III.IV and V. RBC, Hb, PCV and MCV values increased significantly in groups II and III. MCHC increased significantly in the IV and V groups compared to the other groups. The amount of MCH significantly increased in the III group compared to the other groups in the 9th week. Results of total leukocyte showed a significant increase in group V compared to other groups in the 9th week. The percentage of neutrophils increased significantly in the I group compared to other groups. The percentage of lymphocytes increased significantly in the IV groups compared to other groups in the 9th week. Monocytes percentage significant increased in groups I and II in comparison with III, IV and V

groups at 6th week. Eosinophil percentage significant increased in group III and V in comparison with I, II and IV at 9th week. Clotting time significant increased and highest value was in the 9th week in all groups. Between groups significantly increased in groups I and IV in comparison with groups II, III and V at 6th week only while in the 9th week significant increased in groupI in comparison with other groups. Bleeding time significant increased in groups I in comparison with II, IV and V groups at 9th week. Platelet count result decreased significantly in group I in comparison with other groups at 9th week. Zinc serum value increased significantly in groups II and III in comparison with groups I, IV and V at 9th week. Serum total protein and globulin significant increased in groups II and III in comparison with groups I, IV and V at 9th week. Serum albumin significantly decreased in II group at 9th weeks in in comparison with 0-week.

The results of comet assay uncovered lowest amount of DNA damage in treatment groups in comparison with control group. The results of phagocyte index significant increased in groups III and IV in comparison with other groups. Interleukin 1 Beta (IL-1B) value significantly increased at 9th week in comparison with 0-week in all groups, but it was lower in the treatment groups compared to group I. Total antioxidant values significant increased in group II in comparison with other groups. The macroscopic and histological examination results of wound healing showed that the Nano groups in particular 75 mg / kg better than other groups, and evidence for this is shown in complete epithelium restoration with normal entire tissue formation at 28 post operative days. The study concluded that the nanoparticles of zinc oxide have better advantage compared to ordinary zinc oxide, and there were noticeable changes in blood and biochemical values, and wound healing in addition to improving the health of animals. Which gives an indication of promising medical applications for the use of zinc oxide nanoparticles in the veterinary field to improve animal flocks.

LIST OF CONTENTS

List of tables

List of Figures

List of Abbreviations

Abbrev	Full name
AAS	Atomic absorption spectrophotometry
ADF	Acid detergent fiber
BCS	Body scoring
BT	Bleeding time
BW	Body weight
Ca	Calcium
CP	Crude protein
CT	Coagulation time
Cu	Copper
Cu- Zn SOD	Copper-Zn superoxide dismutase
DLC	Differential leucocytes count
DM	Dry matter
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
EDTA	Ethylene demine tetra acetic acid
EGF	Epidermal growth factor
ELISA	Enzyme-Linked Immunosorbent Assay
Fe	Iron
Fl	Fimtoliters
FWHM	full width at half maximum
GFs	Growth factors
GIT	Gastrointestinal tract
GIT	Gastrointestinal tract
H α E	Hematoxylin and Eosin
Hb	Hemoglobin
HRP	Horseradish Peroxidase
IL -1β	Interleukin-1β

MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
Mg	Magnesium
MMPs	Matrix metalloproteinase
NADPH- oxidase	Nicotinamide adenine dinucleotide phosphate -oxidase
NBT	Nitro blue Tetrazolium
NDF	Neutral detergent fiber
NMs	Nano materials
NP	Nanoparticle
NPs	Nanoparticles
OD	Optical density
P	phosphor
PCV	Packed cell volume
PDGF	Platelet-derived growth factor
Pg	Pico gram
PKC	Protein kinase C
PMN	Polymorph nuclear leukocytes
POD	Post-operative days
PRF	Platelet-rich fibrin
PRP	Platelet-rich plasma
PS	Particle size
RBCs	Red blood cells
RBCs	Red blood cells count
ROP	Reactive oxygen production
ROS	reactive oxygen species
Se	Selenium
SEM	Scanning electron microscope
TGF-ß1	Transforming growth factor-ß1
TLC	Total leucocytes count
TNF	Tumor necrosis factor

TNF-α	Tumor necrosis factor α
UV–Vis	Ultraviolet–visible spectroscopy
XRD	X-ray diffractions
ZIP	zinc transporter protein
Zn	Zinc
ZnO	Zinc oxide

Chapter one
Introduction

1. Introduction:

Goats are considered as perfect animals to keep because of their great ability to living under severe conditions, and due to their ability to produce high-quality meat, hair and milk(Silanikove, 2010) and human used the goats for domestication in all civilizations (Nomura et al., 2013). This domestications was register in Iraq and beside Iran since 10,000 calibrated calendar. The goats and sheep were raised in pastures in the area around Syria and Turkey extended to the Levant through southeastern, to the high Zagros mountain (Zeder and Hesse, 2000).

For essential cell metabolism all trace elements have vigorous role to keep animal healthy. Any lack in these trace elements leading to disrupt in animal health and their productions (Underwood and Suttle, 1999). Therefore, optimal concentration for these trace element should be supplied as mineral supplementations to avoid any lack (Suttle, 2010). Lack of some trace elements data in goat bring to the surface using some of these data from dairy cattle for ideal concentrations in goats (NRC, 2007). Any evaluations of trace elements in animals need deep information in how are they deeply deposit in the body (Bellof and Pallauf, 2007). Though, there is need to do clinical trails in order to investigate the trace elements necessities after slaughtering as newly published studies (Ji et al., 2014; Zhang et al., 2015).

Trace elements amount in forage depend on some factors such as type of the soil, way of fertilization, forage type, age of the plant, pasture botanical composition, climate conditions and season (Ramírez-Perez et al., 2000). In young ruminants, macro and micro element deficiencies contribute to metabolic disorders and clinical changes in individuals and in the herd. The mineral content of feed should be regularly monitored to improve

animal health and promote healthy growth and development of animals (Justyna and Katarzyna, 2014).

From ancient times, Zinc is discovered which is transitional metallic element and is widely distributed in the air, water, and food appliances. The medicinal properties of zinc in the form of calamine were recognized about 3,000 years ago in the Ebers Papyrus and in olden Ayurvedic manuscripts in Indian medicine Prasad, (1995), but Raulin, (1869) observation that the mold Aspergillus niger not grow on a zinc-deficient medium since importance of zinc in biological systems (Prasad, 1995; Jones and Williams, 2004). In all live cells, zinc is present in tiny concentrations as new reports have found, as cofactors as a key enzyme systems after injury repair systems, protein synthesis and cell replication (Lansdown et al., 2007).

Nanotechnology is a contemporary field of science which has a dominant role in the day life aspects. It deals with production, manipulation and use of material ranging Nanometers (Kavitha et al., 2013). Nanoparticle (NP) having a size of 1-100 nm in one dimension used significantly about medicinal chemistry, atomic physics, and certain known fields (Jaison et al., 2018). Nano-medicine is a new branch of nanotechnology that use Nanoparticles for therapeutic targets such as drug delivery, especially into Central Nervous System, imaging, diagnostic and treatment of tissue damages (Kreuter, 2005; Chandra et al., 2011; Cho and Borgens, 2012).

History and development of Nano materials (NMs) indicate the Ancient Egyptians, used NMs from 4000 years ago, they synthesize 5 nm diameter NPs for hair dye based on a synthetic chemical process (Walter et al., 2006). Though, the Egyptians were prepared and used “Egyptian blue” as first synthetic stain by a mixture Nanometers-sized glass and quartz about 3rd century BC (Johnson-McDaniel et al., 2013).

Introduction

Zinc oxide nanoparticles (ZnO NPs) when compared to other metal oxide nanoparticles, is less toxic and widely used in medicine such as antibacterial, drug delivery, wound healing, anti-inflammation, anticancer, and bio imaging (Xiong, 2013; Zhang and Xiong, 2015; Mishra et al., 2017). It promotes growth, modulates the immune system and reproduction of the animals. All doses of specifications showed various effects on animal performances. Without indirectly contamination of the environment, ZnO NPs can be replace conventional Zn sources in small doses and at better results. Though, there is a need to optimize the dose and duration of ZnO NP supplementation for livestock, depending on its biological effects. The actual bioavailability of ZnO NP in livestock remains to be worked on (Partha et al., 2016).

1.2 Aims of the study

1. Induction zinc deficiency in goats experimentally.
2. Study the efficiency of Zinc Oxide Nano-particles and conventional zinc oxide, in goats suffer from experimental zinc deficiency, by dependence on:-

- Clinical signs, growth performance and health status of experimental goats.
- Hematological, some biochemical changes and general immune response.
- Histopathological changes and healing of cutaneous wounds induced experimentally.

Chapter two
Review of literature

Chapter two. Review of literature

2.1. Zinc

Zinc (Zn) is unique vital element that requirement to body growth and main physiological processes. It is essential to enzyme activity for about (250 to 300) enzymes and takes part in numerous metabolic and enzymatic roles in the body of animals (Miao et al., 2013). Zinc is a necessary element requisite in ruminants, so primary studies confirmed that Zn deficiencies led to problems in growth, reproduction and immune response of ruminants (Underwood and Suttle, 1999). Mineral lack or imbalance observed in approximately everywhere in the world causes important losses in both production and economy (Constable et al., 2017). It is reported that the losses caused by trace elements are as important, as the losses duo to infectious and parasitic diseases (Smith, 2002).

2.1.1. Zinc functions in the body

Zinc is a component of several metaloenzymes and transcription factors (O'Dell, 2000), which it shows a significant role in the metabolism of vital nutrients in ruminants (Jia et al., 2008). This metal is the second most profuse trace element in the body, and it is not stored in the body organs (Zalewski et al., 2005).

The two major sources of Zn, include ZnO and ZnSO4. H2O used by the animal feed industry (Wedekind and Baker, 1990). The wide range of uses of ZnO, is attributed to their unique physical characteristics, as well as catalytic, magnetic, antimicrobial and ultraviolet light absorption properties (Fan and Lu, 2005; Kumari and Li, 2010). So, that the important functions include:

2.1.1.1. Zinc functions in growth

Zinc is important for regular appetite, but the mechanism of zinc effect on appetite unclear. Zinc-regulated genes are involved in signal transduction, responses to oxidative stress or growth and energy employment (Cousins et al., 2003). Moreover, to its role in nucleic acid and protein synthesis, carbohydrate metabolism, oxygen transport and activate transcription of growth factors (Berg and Shi, 1996).

2.1.1.2. Functions of zinc on immune systems

Zinc is essential in regulation of the immune response(Haase and Rink, 2009), many researchers have previously revealed a solid impact of zinc deficiency on cell-mediated immunity, including several T-cell defects (Wellinghausen and Rink, 1998; Kahmann et al., 2006; Honscheid et al., 2009; Haase and Rink, 2009). In contrast, the amount and reactivated of myeloid cells rise during zinc deficiency (Fraker and King, 2004). It has been exposed that zinc deficit induces pro-inflammatory cytokine synthesis and reactive oxygen production(ROP) in myeloid cells, but in the sum of studies reported that these mechanisms are not completely understood (Cousins et al., 2003; Bao et al 2003; Prasad et al., 2010; Dubben et al., 2010).

Zinc-dependent proteins production various central roles within cells, for instance, apoptosis, metabolic processing, extracellular matrix (ECM) regulation and antioxidant defense (Cronin et al., 2003; Tomlinson et al., 2008; Prasad, 2009; Cho et al., 2016). It contributes in both the inflammatory and immune systems, in addition to metabolism of vitamin A(Kinal et al., 2005). Also, Wessels et al., (2013) investigated the effect of zinc deficiency on the production of the pro-inflammatory cytokines, tumor necrosis factor (TNF) and interleukin-1β (IL -1β) in pro-myeloid cells. But Spears, (2003) found the low Zn deficiency does not harm cell-mediated or humeral immune responses in ruminants.

2.1.1.3. Functions of zinc on skin

Kruczynska, (2004) indicated that zinc is participate in rapidly-dividing cells, counting those of the epidermis, Zinc-deficient diets or high levels of dietary components that prevent absorption and utilization can cause zinc-responsive dermatosis (Reuter et al., 1987). Miller at al., (1964) documented loss of hair and hyper keratinized skin in male growing goats experimentally fed a little zinc diet, also skin lesions have been reported by Neathery et al., (1973) in mature goats experimentally nourished with a zinc-deficient diet.

Krametter-Frotscher et al., (2005) those concluded that zinc- responsive dermatosis in the goats was detected history cutaneous signs, histopathological changes, serum zinc level, and the response to zinc treatment. So, cutaneous lesions re-appeared in the face of normal dietary amount of zinc and increased interfering minerals, this disease is associated with hereditary damage of zinc absorption from the gastrointestinal tract. This information is the detailed case report of secondary zinc deficiency because of a hereditary intestinal tract mal-absorption in goats.

2.1.1.4. Other function of zinc

Zinc widely be existent in whole body tissues, including the brain, muscle, bone, and skin, in addition to its plays crucial roles in proteins and nucleic acid synthesis, hematopoiesis, and neurogenesis (Smijs and Pavel, 2011; Sahoo et al., 2007). The study results by Lenka et al., (2016) indicated that a feed enriched with various forms of Zn had a significant influence on the quantity of individual protein fractions (albumins, α1, α2, β1, β2 a γ-globulins) of blood serum.

O'Dell, (1992) and Vallee and Falchuk, (1993) documented that zinc has been identified in more than 300 different enzymes, of which alcohol dehydrogenase, alkaline phosphatase, angiotensin-converting enzyme, matrix metalloproteinase (MMPs), and

superoxide dismutase. In addition to its role in cytoprotective proteins (Zhu et al., 2004), regulators of adult hematopoietic stem cells (Hock and Orkin, 2006), closely involved in intracellular signaling and neurotransmission (Beyersmann and Haase, 2001; Siemes et al., 2004).

2.1.2. Zinc distribution and kinetics in the body

The non-plasma Zn is mostly bound to carbonic anhydrase in the erythrocytes (erythrocyte membrane) Coni et al., (1996), and low blood Zn level are associated with increased erythrocytes hemolysis due to per-oxidative damage (Bettger et al., 1978). Zinc absorption in the intestine of ruminants happens strictly in the small intestine, only about 2% of the total Zn absorption happens in large intestine (Hampton et al., 1976). To maintain Zn homeostasis, when additional dietary Zn 238-638 ppm in fed (Miller et al., 1967).

Kincaid and Cronrath, (1979) explained that Zinc is chiefly transported bound to albumin and transferrin after uptake in the portal blood. In consequence, little blood albumin concentrations impede blood zinc transport and uptake from intestinal cells into the blood stream, subsequently zinc absorption from the intestinal lumen. Through the first days after a single oral dose, the liver is the highest Zn-retaining organ in goats, after that Zn released from liver, then bound with albumin and amino acids (Cousins, 1985).

2.1.3. Bio-availability, absorption and metabolism of Zinc

A number of studies have indicated genetic predisposition that results in reduced absorption of zinc in sheep and goats. Therefore, when these animals are identified, they may need to treat zinc for life (Mullowney and Baldwin, 1984; Smith and Sherman, 1994). Bozym et al., (2010) reported that metabolically active zinc is found inside cells at Pico to Nano molar concentrations and in the submicromolar to the micro molar

concentrations in extracellular. The zinc transporter protein (ZTP) is responsible for it uptake from the extracellular milieu or intracellular vesicles (Zhang et al., 2016).

In the study of Spears, (2003) found the most important pathway of Zn absorptions is facilitated by transport proteins situated in the intestinal brush border membrane. Homeostatic regulator of absorption of these proteins and molecules of the subsequent transport link from the cell lumen toward the bloodstream. The absorption of minerals is influenced by certain factors like interactions with other elements in the intestinal lumen, insoluble compounds, intestinal pH, phosphates, oxalates and other ingredients of the diet. Feng et al., (2009) demonstrated the absorption of Zn in the body is very smaller amount and differs according to age and gastrointestinal tract sites of the animal.

The digestibility of dry matter (DM), organic matter, and crude protein (CP) increased when the diets were supplemented with 1 g/day organic zinc (Salama et al., 2003). In contrast, Jia et al., (2008) indicated that DM, CP, acid detergent fiber (ADF), and neutral detergent fiber (NDF) digestibility were not affected by dietary zinc in goat.

2.1.4. Excretion

Excretion of Zn happens through the feces from bile secretion, pancreatic juice, directly via the intestinal wall and by the urinary excretion that considered to be minor (Underwood and Suttle, 1999). In goats (46 ppm Zn dietary) and 12% of an intravenous dose of zinc excreted in the feces after 28 days, whereas 0.5% of dose was excreted through the urine, while in the zinc-deficient goats (6 ppm Zn), these values were excreted 9% and 0.3%, respectively (Miller et al., 1966). In other study on growing goats and calves by Miller, (1967) reported that zinc excretion into the feces in goats higher than in their bovine.

2.1.5. Effects of mineral deficiency on the health of young ruminants

Suttle, (2000) reported that macronutrients and micronutrients play four key roles in the body: structural, physiological, catalytic and regulatory, so the catalytic role of minerals is probably the greatest important function.

In ruminants, Justyna and Katarzyna, (2014) found that insufficiency amount of mineral cause inhibition of metabolic pathways that required for common body function. Severe microelement deficiency are manifested by signs corresponding to the role of the deficient element in the body. Thus help in right diagnosis, but in a minor deficiency, the signs are non-specific and difficult to diagnose due to low intensity. Subclinical deficiencies are observed more frequently than severe ones. Also, low-quality feed and impaired or increased demand for minerals during intensive growth, pregnancy and lactation can led to mineral deficiencies.

2.1.6. Hematological and biochemical profiles of goats

Hematological, biochemical, and mineral profiles are significant to be determined because they provide information about animals health status (Madan et al., 2016). Hematological and biochemical variables of blood are commonly used to monitor and assess the health, nutritional and physiological status of ruminants (Gupta et al., 2007; Al-Eissa et al., 2012). Blood manufacture of animal might be influenced by several factors such as nutrition, management and stress in addition to anti-nutritional element, and that might affect blood values. The hematological and geochemical indices are a guide and image of the effects of dietary treatment on the animals in terms of the type, and amount of nutrition consumed, and were available for the animals to protecting its geochemical and metabolically necessities (Tambuwal et al., 2002 ; Ewuola et al., 2004).

Therefore, it is necessary to know the most important physiological characteristics of blood and biochemistry in goats before explaining the disease of zinc deficiency in goats.

2.1.6.1. Physiology of blood in goats

Blood is one of the vital tissues of animals and expresses the efficiency of production and functional, and it is a accurate pointer of the physiological condition of the animal, so the consists of blood in two parts, the first is liquid part (plasma), and second is cellular part which involves of three types of cells are white blood cells, red blood cells and blood platelet (Coles, 1986; Harvey, 2008).

Roubies et al., (2006) and Yokus et al., (2006) revealed that knowing the animal's vital blood and chemical values help the veterinarian enhance clinical diagnosis, assess the severity of the condition, use appropriate therapies, and assess the economic benefits. The results of these laboratory values are interpreted by comparing them with the natural values of clinically healthy animals, which is found as clinical evidence in calculating scales.

2.1.6.1.1. Erythrocyte

Coles, (1986) defines the term Erythron is used to describe the mass of red blood cells circulating in the blood. For its manufacture, a sufficient amount of globin, as well as certain other elements such as iron, copper, cobalt and some vitamins, should be provided. If all these factors are available in sufficient quantities, with which can manufacture hemoglobin molecules of natural erythrocytes.

The red blood cells mature in an orderly manner in bone marrow after the stimulation of the rubriblast with the hormone Erythropoietin, its with no nuclei and no organelles, and ther no ability to synthesize proteins. The full complement of functional proteins must be present by the time the reticulocyte matures (Olver et al., 2010; Lodish et al., 2010). Typical RBCs shape for several animals is disc or biconcave disc (discoid) resulting in a high surface area to volume ration. Central pallor can be detected in these species on investigation of a peripheral blood smear (Barger, 2010).

Red blood cells for goats are usually 2.5-3.9 μm in diameter and lifespan of 125 days. The cell has a disc shape with a large amount of hemoglobin, and this facilitates and increases the efficiency of the gas exchange process in a steady way, with increasing hemoglobin molecules. (Dellmann and Eurell, 1998; Barger, 2010; Nezar and Mohamed, 2014).

Hemoglobin is intracellular protein responsible for transporting oxygen in blood. It occupies approximately 33% of the volume of red blood cell also Golgi apparatus, centrioles and mitochondria diminish. Sixty percent of red blood cell volume consists of water and 40% composed of solids nearly 90% of solid material is conjugated protein composed globin and pigment hem (Shaikat et al., 2013).

Douglas et al., (2010) noticed the common technique for evaluating the functional status of red blood cells depends on the total number of blood units in a certain volume of blood and found the ratio of the size of blood corpuscles, and measurement of blood hemoglobin concentration can be calculated through these three parameters to calculate the size of red blood cells, calculate the amount of hemoglobin mobile and determines its efficiency.

Furthermore, Darmolo et al., (2005) confirmed that there is a significant difference in the blood and chemical values between the breeds of the goats. This is due to the overall metabolic nature of the goats, so it is necessary to draw a suitable basic map of the natural physiological values of the different breeds of the goats, assist to evaluate management applications, nutrition, and health status more accurately and more realistic.

Also, Douglas et al., (2010) demonstrated cpraine red blood cells (RBCs) are some of the smallest of mammalian RBCs and do not aggregate or deform as readily as RBCs of other species. They are conical to a triangular when staining a blood smear and have a pale, anisocytosis region. Holman and Dew, (1965) found during the first month of the

life of young goats and a difference in the form of red blood cells, Poikilocytosis in young goats especially sick and between the age of 1-3 months.

2.1.6.1.2. Leukocytes

Douglas et al., (2010) showed that differential cell counts of white blood cell (WBC) are useful for monitoring inflammatory states, hematopoiesis, oncology, and immunology in goats. Ovine and caprine WBC differential cell counts change with age. Neutrophils dominate the profile during the first 2 weeks of postnatal life. By 3 weeks of age, lymphocytes are dominant cells with a neutrophil: lymphocyte ratio of 0.6 in kids. In ewes and does, parturition is accompanied by minor changes in the RBC count and WBC differential cell counts, such as neutrophilia and lymphopenia.

Neutrophils from sheep and goats are contain primary, secondary, and tertiary granules. When compared to other species, tertiary granules are numerous, large and dense (Bertram, 1985; Harvey, 2001).

The nuclei of most ruminant eosinophils are the band or bilobed and surrounded by numerous, intensely red-stainedd, small, round, refractile, and cytoplasmic granules against the background of a sparse basophilic cytoplasm (Harvey, 2001).

Caprine basophils are rarely seen in peripheral blood. When observed they contain numerous, small, electron-densese, intensely basophilic - staining cytoplasmic granules, that may completely mask the nucleus (Anosa, 1993). Basophils occur infrequently in peripheral blood and they are not reliably quantified. Marked basophilia is uncommon and may be associated with eosinophilia (Douglas et al., (2010).

Caprine lymphocytes are small to medium in size and not readily confused with monocytes. They have a sparse blue to gray cytoplasm that frequently contains variably sized and shaped magenta granules of unknown significance (Smith and Sherman, 1994). Kids begin life with a greater proportion of granulocytes than lymphocytes. Within 3

months of age, lymphocytes represent 70 – 80% of the total WBC population. Within a few years, the lymphocytes begin a slow relative drop in numbers (Mbassa and Poulsen, 1991; Douglas et al., 2010).

Caprine monocytes are round to convoluted - shaped WBCs with a diameter of 13 – 19 µm. The nucleus is large, indented to bilobed, and contains a diffuse chromatin pattern. The cytoplasm is gray and contains small, indistinct, and magenta to eosinophilic granules when stained with Wright's stain. Cytoplasmic vacuoles are common and more irregular in shape than those seen in some large lymphocytes (Anosa, 1993; Harvey, 2001).

2.1.6.1.3. Platelets

Wright's stained blood platelets are distributed singly and in aggregates with azurophilic granules of variable size and shape. Cytoplasmic projections are rare. Normal survival time in the blood is reported to be about 10 days. Giant platelet forms and pseudopodia are associated with recent proliferation from the parent megakaryocytes (Meyers, 1985).

2.1.6.2. Physiology of proteins in goats

Proteins are an important components of many proteins, enzymes, and hormones in the body, including albumin and globulin. The protein is essential for the transport of food, oxygen and iron (MoraG, 2002; Douglas et al., 2010).

Opara et al., (2010) showed that the animal and health status of goats, especially total protein and albumin are influenced by nutrition and physiological status. Abdalla et al., (2009) noted that the difference in season affects on albumin levels in the body, as the level of albumin in the dry summer is lower than the other seasons with the increase of globulin in the dry summer.

Juma et al., (2009) observed a significant increase in total protein concentrations and albumin during the final stage of pregnancy, and decreased during the week after birth in the black goats in northern Iraq.

Variation in albumin levels indicates liver diseases, malnutrition, skin lesions such as dermatitis and burns or dehydration (Burtis and Ashwood, 1999). The concentration of blood proteins alteration in animals exposed to external or internal challenges, such as inflammation and surgical trauma (Murata et al., 2004).

2.1.7. Zinc deficiency in goats

2.1.7.1. Etiology of Zinc deficiency in goats

Similarly to other microelement deficiencies, Zn deficiency can be both primary, when milk and feed do not supply animals with adequate quantities of the element, and secondary, when feed has sufficient levels of zinc, but its availability from feed is reduced by Zn opponents (copper, magnesium, calcium, phosphates, and divalent iron compounds) and amino acid deficiency (Constable et al., 2017).

Hambidge et al., (1989) reported that reduced zinc concentration in plasma response to inflammation, stress, and trauma. On the other hand, catabolism in tissue throughout starvation can release Zn into the blood stream, causing a temporary increase in circulating Zn levels.

Singer et al., (2000) found in the affected ram was being fed with a diet of alfalfa legume high in calcium, which can block zinc absorption. Decrease zinc absorption occurs when that increased calcium and phosphorus consumption. Genetic predisposition may has a depressed zinc absorption that found in certain breeds of goats. Goats with this genetic feature may necessitate lifelong zinc supplementation in the presence of high calcium intake (Linklater and Smith, 1993).

2.1.7.2. Risk factors of zinc deficiency

A primary zinc deficiency due to low dietary zinc in ruminants is rare but it occurs (Lamand, 1984). Several factors influence the obtainability of zinc from soils, including nitrogen and phosphorus concentration, soil pH rises above 6.5, legumes and aging of the plant. While the important factors reason in secondary zinc deficiency including excessive dietary sulfur and amplified calcium-phosphorus intake led to decrease zinc absorption (Lamand, 1984; Linklater and Smith, 1993; Anderson et al., 2002).

2.1.7.3. Pathogenesis

The effects of zinc deficiency are widespread and influence on numerous body organ, due to its ubiquitous and complex nature, in addition to the hereditary and dietary problems, and can manifest clinically by gastrointestinal tract mal-absorption syndromes (Linklater and Smith, (1993), growth retardation (Hagmeyer et al., 2014; Boycott et al., 2015), immune dysfunction (Gammoh and Rink, 2017), and impaired wound healing (Zorrilla et al., 2006), so the main pathogenesis effects in goats include:

2.1.7.3.1. Growth

Zinc deficiency results in a reduced feed consumption in all animals, and is probably cause declined in growth rate in growing animals and loss of body weight in mature animals (Underwood and Suttle, 1999). Zinc also responsible in keeping hoof tissues through incitement growth of epidermal cells, production of keratin, improved wound healing and improved cellular integrity (Ginn et al., 2007).

In study of Wenbin et al., (2009) established that the Zn supplementation to a diet containing 22.3 mg Zn/kg DM diet increased growth gain in Cashmere goats, on the other hand, Silva et al., (2013) showed that the increased feed intake by elevation trace element content of Canindé goats and body weight (BW) changing from 15 to 25Kg.

Araujo et al., (2010) designated that Zn expressed based on the metabolic weight, a linear rise was detected in response to the increased dose of supplementation. Wherefore, increased mineral consumption and weight gain led to a better body growth of the animals (Mahgoub and Lu, 1998; Fernandes et al., 2007; Araújo et al., 2010; Fernandes et al., 2012).

2.1.7.3.2. Immunity

Zinc is a component of thymulin (a hormone produced by thymic cells that regulate cell-mediated immunity), and also is a component of many metalloenzymes such as Copper-Zn superoxide dismutase (Cu- Zn SOD) (NRC, 2007). Also, Zn influences the immune system (Shankar and Prasad, 1998). This trace element has a profound effect on host defense mechanisms relative to infectious disease. The animals were deficient in Zn reveal atrophy of the thymus gland, depressed cell-mediated immunity (Xingen Lei, 2011), and modulating both innate and adaptive immune responses (Wessels et al., 2013).

Alonso et al., (2004) reported that decrease the adverse effect of heavy metals exposed and improve the blood lymphocyte population, cell-mediated immune system and phagocytosis ability by supplementation Zn in the diet of animals . The lack of zinc, in malnutrition or following the disease affect on immune cell functions, producing a higher incidence of infections, increased production of reactive oxygen species (ROS) and pro inflammatory cytokines (Pisoschi and Pop, 2015). Gammoh and Rink, (2017) observed zinc changes immune function from myeloid-derived cells and inflammatory signaling to lymphocyte differentiation, antibody production and regulate immune homeostasis.

2.1.7.3.3. Reproductive

Hostetler et al., (2003) and Robinson et al., (2006) reported that the Zn has a controlling ability in maintaining the integrity of the epithelial cells of the reproductive tissues, embryonic implantation, uterine involution and tissue repair after parturition (Apgar, 1985). Zinc deficiency in male could affect on spermatogenic process and sex organs development, and in females it could affect in estrus, gestation and lactation phase (Smith and Akinbamijo 2000).

2.1.7.3.4. Hematology

The life of all living organisms depends on the blood and its usefulness to assess the health status, and diagnose different types of diseases in animals (Tambuwal et al., 2002). Red blood cells (RBCs) deliver the body organs with oxygen transport, carbon dioxide transport, and buffering of hydrogen ions (Harvey, (2010). Mammalian RBCs are constantly replaced through erythropoiesis, a deficit in the total number of RBCs is named anemia. Zinc is considered as a main factor for erythropoiesis in addition to iron, folate, and vitamin B12 (Hayden et al., 2012).

There are lesions of the arteriolar walls of the dermis relationship with a deficiency of zinc (Constable et al., 2017). Ibrahim et al., (2016) concluded that induced Zinc deficiency has a adverse influence on the common health condition as well as on some hematological and immunological parameters in sheep. On other hand, Elamin et al., 2013) believed that results of dietary zinc supplementation with 33mg zinc/kg had no effect on performance of blood indices and blood biochemistry of Nubian goat kids.

2.1.7.4. Clinical findings

Naturally, happening cases of zinc-responsive dermatosis in goats are seldom designated. Some studies confirmed the presence of zinc deficiency in goats caused by

food deficiency, this is indicated by the of numerous studies by Schulze and Üstdal, (1975), in Turkish Angora goats Nelson et al., (1984) and in pygmy goats (Reuter et al., 1987). Also, experimental zinc deficiency in goats has also been described by study of Miller et al., (1964) and Neathery et al., (1973).

The disease has been detected in mature goats, and characterized by poor growth and infertility and significant decrease in concentration of zinc in serum, but without additional clinical signs, its can happen in sheep grazing pastures containing less than 10 mg/kg Zinc (Underwood, 1999).

In the study of Krametter-Froetscher et al., (2005) reported zinc deficiency in two cases of dairy goats and not associated with a zinc-deficient diet, they are described by hyperkeratosis skin, hair loss and pruritus, especially prominent on the back, legs, udder, face, and ears. Biochemical examination reveled to low concentrations of serum zinc in both goats, skin lesions completely resolved after long oral zinc supplementation. This study diagnosed that zinc deficiency in these goats was due to hereditary malabsorption of dietary zinc.

Study in Iraq by Khaleel, (2013) found that the local goat affected naturally with hypozincemia in Al-Najaf province, and the main signs include alopecia, the skin is rough, thickened, wrinkled, cracked and dandruff. So, pale mucous membranes, loss of appetite, decreased growth ratio, swelling of joints and pica.

In other studies on goats showed alopecia, rough hair coats, weight loss, abnormal hoof growth, gingivitis, conjunctivitis, scaling and crusting of the skin of the face and neck (Neathery et al., 1973; Schulze and Üstdal, 1975; Singer et al., 2000; Mcgavin and Zachary, 2007). Bowing of the hind legs, stiffness and swelling of joints are signs of the impaired skeletal functions, and anorexia. Later on, the bones, hoof, and horn may be

weak and infectious pododermatitis (foot rot in sheep and goats) may happen more frequently (Skerman, 2000).

2.1.7.5. Pathology

Al-Saad et al., (2010) observed that histopathology of the skin biopsies revealed parakeratosis, sometimes hyperkeratosis, moderate acanthuses, intracellular edema, pseudoepitheliomatous hyperplasia, and heavy infiltration of mononuclear cells as well as lymphocytes in the dermis.

2.1.7.6. Treatment

In animals are used a sufficient amount of microelement, containing mostly inorganic zinc oxide and zinc sulfate or organic zinc in the chelated form. Chelated resulting from the reaction of cation Zn supplied by soluble salts with amino acids through the formation of covalent coordination bonds (Mandal et al., 2007).

Constable et al., (2017) reported that the orally treatment of zinc by a dose of 250 mg zinc sulfate daily, for 4 weeks lead to a clinical cure of zinc deficiency in goats. Garg et al., (2008) explained that used organic zinc in ruminant diets has increased. Average daily gain was better in Angora goats supplemented with ZnMet compared to a ZnO group (Puchala et al., 1999). While some researchers have investigated the effect of ZnO on the growth rate when used as a food supplement in livestock (Kincaid et al., 1997; Puchala et al., 1999 and Phiri et al., 2009), and blood mineral levels of Markhoz goat kids (Khaleel, 3013), but studies on ZnO are limited.

In the zinc deficiency case , can be administer larger doses of zinc than recommended. This must progress weight gains and feed conversion ratio as well as ensure higher concentrations of zinc in the animal (NRC, 2007). In sheep were observed Zn toxicity led

to histopathological changes in the pancreas, liver, kidney, rumen, abomasum, small intestine and adrenal gland (Allen et al., 1983).

2.2. Nanotechnology

The term "Nano" is derived from a Greek word meaning “dwarf” or very small. Nanomaterials include metals, ceramics and polymeric materials or composite materials. Their defining characteristic "is a very small feature size in the range of 1–100 nanometers". One nanometer spans 3–5 atoms lined up in a row (Richa, 2012).

Nanotechnology has become the head of research and led to revolution in livestock zone. With the introduction of this developing field, wide varieties of Nanoparticles are being manufactured and used for a broad range of applications (Zhao and Castranova, 2011). Nanotechnology, introduced in half a century ago, its a term surrounding the science, engineering, and applications of submicron materials contains the harnessing of the unique physical, chemical, and biological properties of Nano scale materials in essentially novel and beneficial ways. The complexities of nanotechnology, still in the initial step of development, and the broad possibility of these potential applications, have become progressively important (Sargent and John, 2014).

Ding et al., (2018) demonstrated that Veterinary Medicine also entered into a new phase of Nanotechnology, it has great influence on the way that we practice veterinary medicine and increase the protection of domestic animals, production, and income to the farmers through use of Nanomaterial’s.

2.2.1. Principles of Nanotechnology

Scenihr, (2010) demonstrated that materials, structures, devices and systems of controlled shape, size and morphology at the nanometer scale have been developed purposely for medical and food requests. Nano-particles can be divided into three groups

as a function of their size; big particles if their diameter is greater than 500 nm, medium size ranged from 100 nm to 500 nm, and ultrafine particles if their diameter is less than 100 nm. Two essential methods are used in nanotechnology, the first "bottom-up" method, materials and devices are built from molecular components which accumulate themselves chemically by principles of molecular recognition (Kralj and Makovec, 2015). The second "top-down" method, Nano-objects are constructed from larger entities without atomic-level control (Rodgers, 2006).

2.2.2. Pathways of nanoparticle absorption

O' Hagan, (1996) found that Nanoparticles can cross the gastrointestinal tract (GIT) in numerous ways. Smaller particle diameter is sooner the diffusion through GIT mucus to spread in the cells of the intestinal inside layer, followed by across through the GIT barrier to blood stream. Transport occurs by passive diffusion through the mucosal cells. Smaller particle size improved absorption, and reaches deeper into the tissues, in addition to lesser particles that are capable of being taken up by the villus epithelium of intestine, and directly pass in the bloodstream, then mainly hunted by the liver and spleen (Hillery et al., 1994). The gold nanoparticles smallest (10 nm) spreading in body after intravenous administration, and showing widespread tissue distribution. Also, the absorption, distribution, breakdown, and excretion of mineral particles in the body is dependent on physicochemical characteristics (De Jong et al., 2008).

2.2.3. Zinc Oxide Nanoparticles (ZnO NPs)

Zinc oxide nanoparticles is a metal oxide semiconductor, the focus on zinc oxide nanoparticles has amplified in recent years due to its wide application such as biomedical systems (Anbuvannan et al., 2015; Prasad and Jha, 2009). Zinc oxide nanoparticles have great benefits, because the low-cost, and safe production can be setting up easily

(Jayaseelan, et al., 2012). ZnO NPs show great semiconducting properties, and high excite binding energy like high catalytic activity, optic, UV filtering properties, anti-inflammatory and wound healing (Pulit-prociak et al., 2016; Mirzaei and Darroudi, 2017), tissue repair, food preservative and as feed additive (Raguvaran et al., 2015).

In the review of (Raguvaran et al., 2015) explained that numerous uses to ZnO NPs in veterinary sciences, and compared with ordinary ZnO powder, ZnO nanoparticles have a large specific surface area and small size effect, and show extensive application potential in microbial and fungal inhibition (Li et al., 2012; Cha et al., 2015).

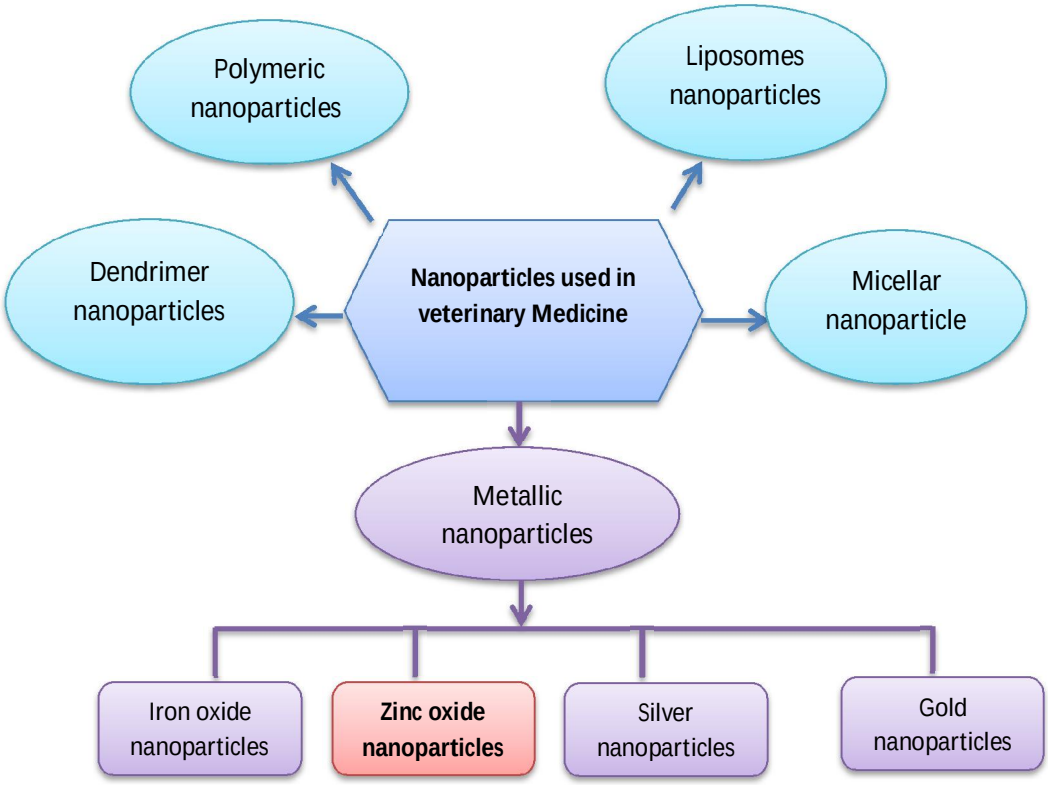
2.2.3.1. Synthesis of Zinc oxide nanoparticles

The biological action of Nanoparticles depends on factors as well as surface chemistry, size distribution, particle morphology, and particle reactivity in solution. The approaches for stable ZnO NPs preparation synthesis have been commonly developed in recent years, which mainly contain the chemical precipitation method, sol-gel method, solid-state pyrolytic method, solution-free mechano-chemical method, and biosynthesis method. Thus, the progress of Nanoparticles with controlled structures that are uniform in size, morphology, and functionality is critical for several biomedical applications. The ZnO NPs occurring in a very rich variety of sizes and shapes will provide a wide range of properties (Jinhuan et al., 2018).

2.2.3.2. Kinds of Nano material used in veterinary medicine

The Nano materials have been used for disease diagnosis, treatment, drug delivery, animal breeding, reproduction and animal nutrition, the different Nanomaterial that have been used in veterinary medicine including liposomes nanoparticles, micellar nanoparticles, polymeric nanoparticles, dendrimer nanoparticles, metallic nanoparticles, and carbon nanoparticles (Figure 2-1) (Ding et al., 2018).

Zinc oxide nanoparticles as one of the greatest significant metal oxide nanoparticles, are commonly employed in numerous fields due to their particular physical and chemical properties (Smijs and Pavel, 2011). So, progressively used ZnO in care products, such as cosmetics and sunscreen, since the strong UV absorption properties (Newman et al., 2009).



Figure(2-1): Shows the different Nanomaterial that have been used in veterinary medicine (Ding et al., 2018).

2.2.3.3. Characterization and applications of Zinc oxide nanoparticles

Zinc oxide has been used as antimicrobial in animal feed, wound healing and different skin disorders (Raguvaran et al., 2015). On other hand, study by Manuja et al., (2012) demonstrated that numerous applications of ZnO NPs in veterinary sciences include antibacterial, antineoplastic, wound healing and angiogenic (Figure 2-2). Also, it used for treat various illnesses in animals like meningitis, tumors, and diseases caused by intracellular pathogens. To treatment these diseases, the drug has to penetrate inside cells

and able to cross the blood-brain barrier. This is unlikely with existing macromolecular therapeutic substances, but the nanoparticles can destroy intracellular pathogens and brain tumors due to their small size. Zinc oxide has a high chemical stability, strong photosensitivity, and low-toxicity property (Ficociello et al., 2016). On other hand, Rasmussen et al., (2010) and Ma et al (2015) reported that ZnO NPs, as a new kind of the low-cost and low-toxicity Nanomaterial.

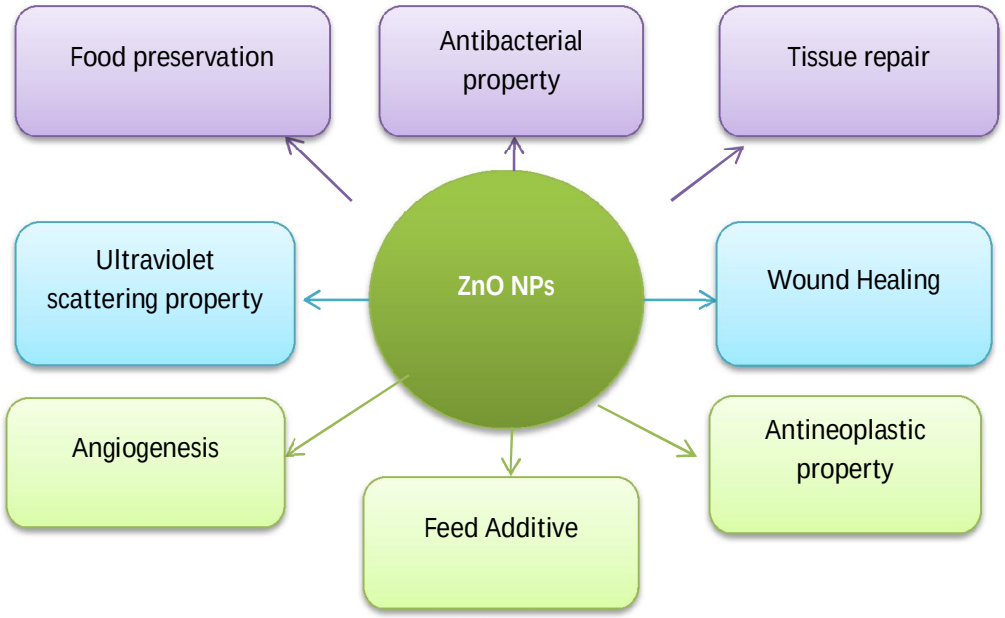


Figure (2-2): Showed the potential applications of ZnO NPs in veterinary sciences (Raguvaran et al., 2015).

2.2.3.4. Effect of the ZnO NPs supplementation on biological systems

Partha et al., (2016) and Kanti et al., (2018) suggested that supplementation of Nano minerals improved the growth, digestive efficiency, immunity, antioxidant status, milk production and other performance of a different groups of animals. Bioavailability of minerals from its inorganic sources is quite low so these minerals are added 20-30 fold higher than the normal requirement of animals, which can lead to excess excretion of these minerals in the feces resulting in environmental pollution, and it may affect the

balance of other minerals. Interaction among two minerals or more minerals can decrease bioavailability.

Nano minerals increase bioavailability due to the increase in the surface area, higher surface activity, great catalytic efficiency, and solider adsorbing ability. Nano minerals are used for enhancing the bioavailability of minerals in livestock which helps progress the growth, production, health status of animals, and immunomodulatory. Thus, Zn NP can be used at minor doses in livestock feed to provide better results than the conventional Zn sources and indirectly avoids environmental contamination(Partha et al., 2016).

2.2.3.5. Toxicity of Zinc oxide nanoparticles

The possible risk of great concentrations of Zn NP is still unidentified, and their toxicological data are somewhat uncommon (Argmann et al., 2005). The transition from micro particles to Nanoparticles (< 100 nm in diameter) includes an increment of the surface area, among other changes in properties. A greater surface area of the Nanoparticles lets higher interactions with other organic and inorganic molecules. Many properties of the metals in the Nano scale are not until now determined (Francisco et al., 2008). Limited knowledge of the toxic effects of these substances on ruminants highlights that need for immediate research to identify their possible adverse effects when used as a nutritional supplement in livestock (Mohamed et al., 2018).

2.3. The wound and effect of Zinc on wound healing

Wounds are a one of main cause of physical disabilities, so healing is a vital mechanism to maintain a normal body structure and function (Wang et al., 2017). Repair of impaired skin is essential for the surgical manipulations. Wound healing remains a challenging clinical problem, require effectual management to restrict morbidity and

mortality. Availability of appropriate trace elements like zinc, which work as enzyme cofactors and structural components in wound healing (Majumder and Kamath, 2005; Olaifa and Fadason, 2016).

Mihai et al., (2019) reported that wound healing by using develop technique for succeeds rapid recovery. The classic method to wound management is embodied by topical treatments, such as antibacterial, to avoid infection and support a correct wound-healing process. Submicroscopic particles of metals like silver, gold and zinc nanoparticles are increasingly being used in skin management, due to their beneficial effect on hastening wound healing, as well as treating and inhibiting bacterial infections.

2.3.1. Skin Anatomy and Physiology

Alonso et al., (1996) and Bassert, (2002) showed that the skin is known as the integumentary system, its larger and widespread organ systems in the body forming the external covering of the body and constituting 20 % of its total mass. The skin is a complex biological material acting as the interface between the body and the environment. It keeps subcutaneous structures and forms a first barrier between the delicate internal mechanisms of the body and the harsh elements of the outside world.

The skin is promote in the maintenance and regulation of body temperature and excretes water by sweating, salts and organic wastes. It is also considered as a vital sensual organ, that takes information from the environment via touching and force conveying this input to regions of the central nervous system. It is responsible for protection, external sensory awareness, immunological defense, wound healing, perception and excretion. It is also synthesis of vitamin D and the storage of nutrients (Mobini, 2013 and Razvia et al., 2015). Besides its biological importance, it has an economic value as a raw material in the industry (Ozfiliz et al., 2002). Loss of a large

portion of the skin integrity arising due to injury or illness generally leads to disabilities or death (Robson et al., 2001).

Skin is made up of outside ectodermal epithelial cells layer known as epidermis and under dermis made up of mesodermal connective tissue (Dyce et al., 1996). The epidermis, generally in mammalian, is consist of stratified squamous epithelium which are regenerated continuously starting with proliferation, migration, differentiation, and cornification from basement membrane toward the surface of the skin, which contains many of cells like keratinized cells and melanocytes (Al-Barwari et al., 1988).

Saleemm et al., (2016) reported that the outermost level, the epidermis, an epithelial layer of ectodermal origin. The thickness of these layers is varying according to different species of animals as well as different layers of the body in the same animal depending on the body position. The classification of the thickness of the epidermis of the skin is generally divided into two parts; the thick epidermis and thin epidermis. The thin epidermis is covered the largest part of the body while thick epidermis found in the palm, sole, and tail of animals.

The skin second layer is called dermis (middle layer) which is thicker than the epidermis, and gives the skin its flexibility and strength since composed of fibrous and elastic tissue (made mostly of collagen and elastin) (Saleemm et al., 2016), and classified as dense irregular connective tissue extends to the hypodermis (Abdul Raheem and Al-Hety, 1997; Al-Barwari et al., 1988).

James et al., (2006) stated that the third layer lies in the subcutaneous tissue or panniculus, which contains small lobes of fat cells known as lipocytes. These lobules are separated from the fat cells or adipocytes by the fibrous barrier consisting of blood vessels and large collagen. The thickness of the panniculus varies depending on the location of the skin.

2.3.2. Wounds

A wound is defined as damage or disruption to the normal anatomical structure and function" (Robson et al., 2001). This may variety from a simple disruption in the epithelial integrity of the skin or it can be deeper, include subcutaneous tissue with damage to further structures such as vessels, muscles, nerves, tendons, parenchymal organs and also bones (Alonso et al., 1996).

Lazurus et al., (1994) and Demetriou and Stein, (2011) indicated that the wounds can get up from pathological processes that begin externally or internally. They can have an accidental or planned etiology or result by a disease process.

2.3.2.1. Classification of wounds

Wounds can classified in many ways depend upon whether they are open or closed, period since the injury, original etiology, degree of contamination and skin disruption. Also, it can be clinically categorized as acute and chronic, With regard to the recovery time frame, due to the time is an important factor in injury repair (Bischoff et al., 1999; Robson et al., 2001; Mickelson et al., 2016).

2.3.2.2. Wound healing

Wound healing is a physiological response to skin damage that is necessary for all tissue systems (Nussbaum et al., 2018). It is a lively and very controlled process including cellular, humoral and molecular mechanisms (Reinke and Sorg, 2012). Wound healing arises at the moment of injury as the wound is a disruption to the anatomic structure and the functional continuity of alive tissues (Robson et al., 2001).

Pei-Hui et al., (2017) reported that wound is an intricate process, which can be divided into a sequence of phases, including the first phase is coagulating fibrin clot formation (hemostasis, occurs within seconds to 1 h), the second phase is inflammatory

response (within minutes to days), third phase of wound healing including cell proliferation, re-epithelialization, granulation and angiogenesis (extend from 18–24 h after wounding and lasts for days to weeks) and in the fourth-phase, matrix remodeling and scar formation (start at 5–7 days after injury and persist months to years). The other overlapping phases include phases dynamic coordination of soluble mediators (reactive oxygen species (ROS), chemokines, cytokines and growth factors), extracellular matrix (ECM) turnover and cellular cross-talk amongst platelets, infiltrating immune cells, resident keratinocytes, endothelial cells, fibroblasts, epithelial cells, and stem cells.

Skin wound healing depends upon the availability of suitable trace elements serving as enzyme cofactors and structural components in tissue healing (Lansdown et al., 1999). Zinc has a important role in several process of wound repair. Hemostasis occurs after wounded tissue through coagulation and clot formation, and quickly followed by immune cells infiltration and inflammation response as a means to the wound of damaged tissue and microbes invasion, thus preventing infection and allowing granulation, Fibroblast, epithelial cells, keratinocytes, and endothelial cells, will proliferate and migrate into injuries to deposit ECM and re-populate the injury sites. Lastly, deposition of matrix and clearance regulates the development of scar formation and ECM remodeling (Figure 2-3) (Pei-Hui et al., 2017).

2.3.2.3. The Phases of wound healing

2.3.2.3.1. Hemostasis and platelets phase

The early phase assists in the protection of the vascular system to keep the functionality of the organs. Serious consequences of injury to the blood vessels, and clot formed, becomes necessary to stop bleeding and provides a medium for the cells involved in subsequent steps of hemostasis and inflammation. The main mechanism of hemostasis is accomplished by activation and grouping platelet cells. Platelets are able to respond to

vascular injury by grouping at the site of damage to form an early clot subsequent by fibrin deposition to further strengthen the plug, with different pro-inflammatory cytokines and growth factors are released by the clot and wound tissue (Didar et al., 2017).

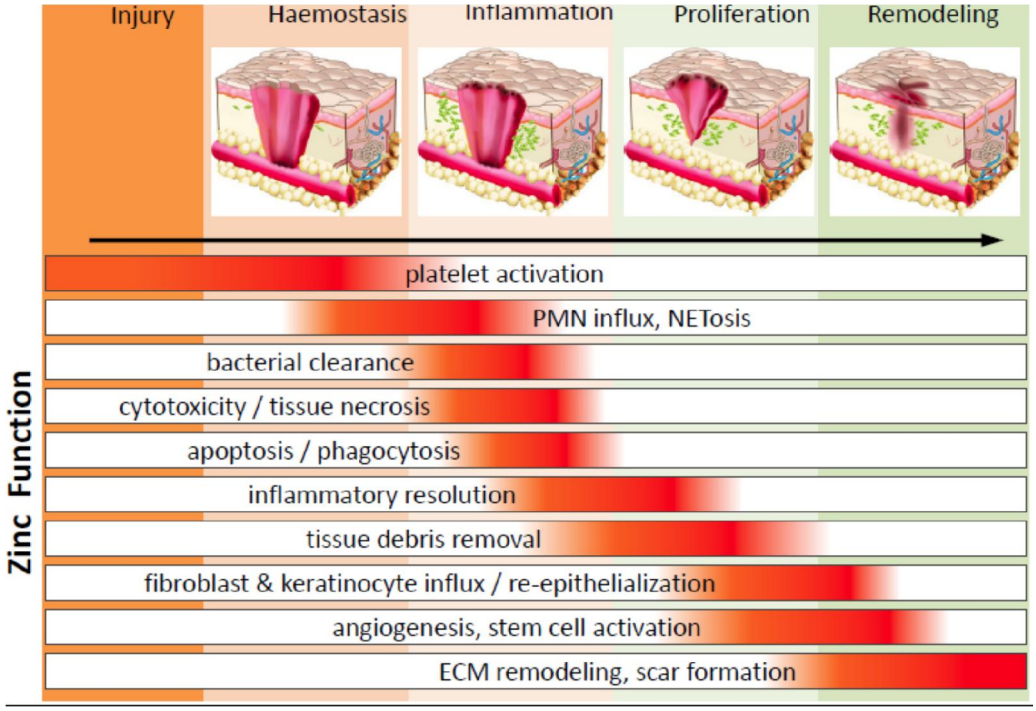


Figure (2-3): Zinc functions throughout the wound healing stages (Pei-Hui et al., 2017).

Platelets are a natural source of numerous growth factors and cytokines that regulate blood coagulation, tissue repair, and bone mineralization. Degranulation of platelets led to release of transforming growth factor-ß1 (TGF-ß1), platelet-derived growth factor (PDGF), fibrinogen, epidermal growth factor (EGF), histamine, and hydrolytic enzymes. Addition to the angiogenic cascade assists in wound healing (Harrison and Cramer, 1996; Kliche and Waltenberg, 2001; Nikolidakis and Jansen, 2008).

In study of Falah and Serwa, (2018) conclusion the use of platelet-rich plasma (PRP) gel and platelet-rich fibrin (PRF) matrix, as improved therapy for open chronic or non-healing wounds, accelerates epithelialization and scar formation in goats, the study

demonstrated the beneficial effect of both PRP and PRF as a biological wound healing enhancer.

When the injury occurs, fluid of blood and lymphatic vessels rapidly enters the wound site and clears out antigens and microbes. After this initial rush, the hemorrhage must be restricted. This is completed through three mechanisms: vasoconstriction, aggregation of platelets and activation of the coagulation cascade. Vasoconstriction restrictions the amount of blood but only takes a limited minutes enough time to deposit the necessary elements for clot formation in the wound bed. Longer-term hemostasis results from the development of a fibrin clot (Reinke and Sorg, 2012). Zinc is play the important role in improving platelet activity and aggregation (Heyns et al., 1985; Marx et al., 1991), and zinc is mediated through Protein kinase C (PKC) mediated tyrosine phosphorylation of platelet proteins (Taylor and Pugh, 2016; Watson et al., 2016). Exogenous zinc treatment induced zinc access into the platelet cytosol. The intriguing role of zinc on pathophysiological thrombus formation in tissue injury is still generally unknown (Morrell et al., 2014).

2.3.2.3.2. Inflammation and immune defense phase

This phase is an elegant process including organization between a variety of cell types. This phase begins during and after hemostasis. The necessary of this phase is to purify the wound and recruit fibroblasts, and it can be subdivided in two-phase, the early phase of inflammation involves neutrophils employment, and late phase involves the entry of monocytes to the wound and their transformation into macrophages (Reinke and Sorg, 2012).

2.3.2.3.2.1. Early Phase of inflammation (the action of Neutrophils)

Neutrophils that enter the wound site through adjacent blood vessels with increased endothelial permeability. This occurs within a maximum of 48 hours post injury (Werner and Grose, 2003; Reinke and Sorg, 2012). It granular polymorph nuclear leukocytes (PMN), which often act as one of the first responders to tissue injury and bacterial infection. The neutrophils function can destroy microbes through phagocytosis, secrete proteases that are essential for debridement and immune stimulation. These cells have a role in increasing the response via releasing mediators such as tumor necrosis factor α (TNF-α) and Interleukin-1 beta (IL-1β) (Reinke and Sorg, 2012).

Zinc supplementation was reduced plasma oxidative stress markers, inflammatory cytokines, chemokines, and reduced secretory cell adhesion molecules, which represent important biomarkers of cell damage-associated inflammation in endothelium and platelets (Vruwink et al., 1991; Bao et al., 2010; Prasad, 2014).

2.3.2.3.2.2. The late phase of inflammation (involves the entry of monocytes)

Monocytes are pro-inflammatory innate immune cells, it were respond to chemokine's and migrate to wounded tissue, where they stick to endothelial cells, infiltrate tissue and differentiate into macrophages, and clearing pathogens and damaged tissue. Zinc has conflicting roles in monocyte and endothelial adhesion. It has been shown that zinc-deficiency, as well as zinc oxide treatment, result in elevated monocyte adhesion (Lee et al., 2012; Suzuki et al., 2014). Zinc modulation of monocyte differentiation into pro-inflammatory (M1) macrophages or immune-regulatory of wound healing (M2) macrophages (Dierichs et al., 2017).

Pei-Hui et al., (2017) found M1 macrophages are important for early inflammation, microbial and debris clearance, while M2 macrophages are involved in immune suppression and later tissue remodeling and repair. Zinc deficiency and supplementation

promote M1 phenotypes and also inhibiting M2 differentiation. Maintaining an appropriate equilibrium between M1/M2 macrophage populations is complex and important during wound healing. Explaining the effect of zinc on macrophage phenotypes and functions will assistance in the advancement of wound healing treatments.

Mature B-cells and plasma cells are producing antibodies that detect injured tissue. These antibodies serve as signals by which macrophages recognize and phagocytize damaged cells. Zinc deficiency results in lowered populations of both precursor and mature B-cells and can reduce antibody production (Fraker and King, 2004; Iwata et al., 2009).

2.3.2.3.3. Inflammatory resolution and tissue proliferation phase

The importance of this phase is to resolve inflammation. Proliferation of both epithelial and dermal elements results in re-epithelialization of the wound and laying down of the primary extracellular matrix. The process where epithelial cells proliferate and repopulate damaged tissue for wound closure (Fraker and King, 2004 ; King et al., 2005).

The study of Reinke and Sorg, (2012) described that re-epithelialization occurs when the migration of keratinocytes, which oscillate from the wound edge, through the clot, and across the wound. Keratinocytes are attached via a matrix mat to the basal lamina. So, before migration occurs, it must be separated. To tolerate thrombosis, advanced keratinocytes must regulate protein synthesis to achieve fibrinolysis. Once keratinocytes from the wound edges meet each other in the center of the wound.

2.3.2.3.3.1. Angiogenesis (Neovascularization)

Hinsbergh et al., (2001) explained the term angiogenesis is the formation of new blood vessels from existing vessels to provides the oxygenation and nutrients necessary

for the growth of new tissue in the wound". It happens secondary to endothelial progenitor cells, a derived of hematopoietic stem cells(Wu et al., 2007).

During the angiogenesis stage, after a few days of wounding, wound edge capillaries shoot into the wound and branch out to form a micro vascular network (Shaw and Martin, 2009). For process to initiate, the basement membrane of intact endothelial cells must be tainted to release them for movement into the extracellular matrix. At this time, the endothelial cells proliferate, and during hemostasis the fibrin matrix formed and acts as a support for attacking micro vascular endothelial cells. Subsequent endothelial cells form a lumen, a basement membrane is developed and the vessels are stabilized (Hinsbergh et al., 2001).

2.3.2.3.3.2. Granulation tissue creation and fibroblast proliferation

Iocono et al., (1998) described that fibroblasts begin to migrate to the wound in two days of injury, at seven to fourteen days post-injury they proliferate become highest density. The function of fibroblast is replacement of the original fibrin matrix with collagen-rich granulation tissue and other function, include production and release of components of the extracellular matrix (glycosaminoglycan and proteoglycans). Fibroblasts are drawn from fit dermis, bone marrow progenitor cells, circulating fibroblasts and multi potent cells in the dermis (Shaw and Martin, 2009). Granulation tissue replaced during the remodeling phase and it is described by dense vascularization, fibroblast and myofibroblast populations and macrophages (Iocono et al., 1998).

Pei-Hui et al., (2017) showed that the collagenase and plasminogen stimulants break down fibrin clots in conjunction with zinc-based matrix metalloproteinase (MMPs), which digests the skin basal membranes and ECM, allowing for cell proliferation, migration and angiogenesis. Also, the apoptosis and necrotic cells stimulate the

multiplication of epidermal cells and migration of the granule tissue to repair the epidermis.

Neovascularization or angiogenesis process initial simultaneous with re-epithelialization, endothelial cells migrate and proliferate into wound places to formation new blood vessels, thus supplying vital oxygen and nutrients for the cells development in the wound. Vivo results showed that zinc has been effective for angiogenesis (Li and Chang, 2013).

2.3.2.3.4. Wound resolution and remodeling

Resolution and remodeling are the last stage, in this phase the tissue remodeling and differentiation leading to recovery of the skin (Diegelmann and Evans, 2004). Rebuilding of the dermis happens by reform of the matrix collagen (Braiman-Wiksman et al., 2007), and fibroblasts development into myofibroblasts, causing wound contraction and closure (Gabbiani, 2003). The wounds resolution and remodeling of the matrix extend from twenty one days after infection and can last up to a year. It is evident by the programmed cell death of the granulation tissue cells (fibroblasts and macrophages) (Reinke and Sorg, 2012). Extracellular matrix is composed of a complex mixture of insoluble molecules (collagens, laminins, fibronectin, integrin, and heparin sulfate proteoglycans), and provide a solid supportive matrix scaffold for cells. The extracellular matrix network also acts as a tank of a great several of cytokines, growth factors, active and latent cellular migration, adhesive epithelium, and wound contraction (Tracy et al., 2016).

Broughton et al., (2006) reported that in this stage new collagen tissues were found in an arranged group to increase the wound's ductile strength. Type III collagen is replaced by type I collagen, yielding strength can be up to 80% of that of the original skin (Broughton et al., 2006; Reinke and Sorg, 2012).

The essential protein for epidermal wound repair are the Extracellular matrix (ECM) remodeling matrix metalloproteinase (MMPs), which secreted by different cells types such as inflammatory cells, keratinocytes, endothelial cells, and fibroblasts. Matrix metalloproteinase (MMPs) are zinc-dependent endopeptidases, which act to modulate growth factor activation, degradation and composition of ECM processing of junction adhesion molecules between cell with other cell (Xue et al., 2006; McCarty et al., 2012; Martins et al., 2013; Caley et al., 2015).

Zinc ions are required for optimal MMPs function in vitro (Tezvergil-Mutluay et al., 2010), but, the molecular mechanism by how zinc controls MMPs function in vivo is still not fully understood because of the complexity of their regulation in wound healing (Pei-Hui et al., 2017).

2.3.2.4. Clinical perspectives on the effect of zinc on wound healing

Lansdown et al., (2007) and Kogan et al., (2017) stated that zinc plays impotent role in wound healing and can be seen from two angles first, the effect of zinc deficiency and the second, the effect of zinc supplementation (topical or systemic) on wound repair. There is relationship between zinc deficiency and delayed wound healing. So, the treatment for zinc deficiency improves wound healing compared to those with zinc deficiency.

A link was found between zinc deficiency and delayed wound healing (Kogan et al., 2017), and due to the abundance and quality of the skin, it can be seen that moderate zinc deficiency leads to rough skin slow wound healing, so it is important to re-evaluate the potential benefits that zinc treatment offers in wound management. Despite this, there are many clinical studies that have proven the benefits of using oral or topical zinc treatment in wound management, but differences in the method of treatment and the composition of the zinc preparations used have obscured the true effectiveness of zinc, but evidence is

now available to show that zinc is not only useful in wound healing but provides an effective level anti-infection procedures (Lansdown et al., 2007).

Olaifa and Fadason, (2016) suggested that the level of copper and zinc prevalence in the skin and hair of goats and both male and female could be a factor affecting animal wound healing. Zinc deficiency resulting from genetic or nutritional causes can lead to pathological changes and delayed wound healing. It was found that oral zinc supplementation may be useful in treating leg ulcers with zinc deficiency (Lansdown et al., 2007). Also, prolonged supply of zinc to wounds led to enhances its healing ability. Additionally, ZnO increases collagen degradation in necrotic wounds (Agren et al., 1991).

Studies of Xiong, (2013) and Oyarzun-Ampuero et al., (2015) described advances in drug delivery using the ZnO-NPs technology, which received much attention for wound healing due to their effective cell penetration, immunomodulation and antimicrobial ability, and this augurs well for future promising research on the use of nanoparticle technology in various biological branches.

Chapter three
Materials and Methods

3. Chapter three: Materials and Methods

3.1. Materials:

3.1.1. Instruments and equipments:

Table (3-1) Instruments and equipments used:

No.	Equipment	Origin
1	Microscope	Genex lab U.S.A
2	Micropipette	Slamed Germany
3	Refrigerator	Beko Turkish
4	Freezer	regal Turkish
5	Disposable syringe (different size)	Meheco China
6	Slides	Meheco China
7	Distillatory	Rowa GmbH Germany
8	Incubator	Binder GmbH Germany
9	Hotplate stirrer	Lab Inco China
10	Sensitive balance	KERN and Sohn GmbH Germany
11	Test tubes for blood collection with gel and clot activator	APCO Jordan
12	Test tubes with anti coagulant (EDTA) for blood collection	APCO Jordan
13	Test tubes with anti coagulant (heparin) for blood collection	APCO Jordan
14	Capillary tub	Vitrex medical Denmark
15	Spectrophotometer	Apel co., ltd. japan
16	Hemocytometer	Germany
17	Hb Hemoglobin Test Strips	Mission^{r} system-Germany
18	Centrifuge	Germany
19	Disc balance (0 -175kg)	China
20	Stethoscope	China
21	Eppendorf tub	China
22	Beakers 25,50, 100 ml	HBG England

No.	Equipment	Origin
23	Conical flask 250,500, 1000 ml	HBG England
24	Cylinders 50, 100, 250 ml	HBG England
25	Metal and plastic racks	Meheco China
26	Micro-titer plate	China
27	Surgical set	England
28	Screw capped tubes	Exceller England
29	Electrical clipper	Moser Germany
30	Electrical grinder	Panasonic Japan
31	ECG	Japan
32	Micropipette	Labtech Korea
33	Scan electron microscope	Shemadzu/ Japan
34	UV-Visible spectrophotometer	Shemadzu/ Japan
35	X'pert Pro diffractometer	Shemadzu/ Japan

3.1.2. Chemicals and stains

Table (3-2) The chemicals and stains

No.	Chemicals	Origin
1	Ethanol (70%)	APCO Jordan
2	Methanol (70%)	APCO Jordan
3	Formalin 40%	BDH England
4	Potassium hydroxide	BDH England
5	Giemsa stain	Syrbio Syria
6	Nitroblue tetrazolium stain	Alpha chemika India
7	Eosin	Sigma(USA)
8	Haematoxylin	Foukal AG. Switzerland
9	Phosphate buffer saline	APCO Jordan
10	Lidocaine hydrochloride, (2%)	Media, Syria
11	Xylazine hydrochloride	Xyla –MD. Germany
12	R.B.C. Diluting Fluid (Hayem's)	APCO Jordan
13	W.B.C. Diluting Fluid	APCO Jordan

No.	Chemicals	Origin
14	Limestone (Calcium)	Local market
15	Calcium di-phosphat	DCP – 18 India
16	Nano Zinc oxide	Sky Spring Nanomaterials, inc. Houston. USA
17	Zinc oxide	Local market (Skyspring Nanomaterials, Inc. Houston, USA)

Table (3-3) ELISA and Chemicals Kits used in the study and their manufacturer

No.	Kits	Origin
1	IL-1B	Bioassay Technology Laboratory. Shanghai. China
3	Zinc	LTA s.r.l.-s.u.-Via Milano, Italia
4	Total serum protein	Spectrum, Hannover, Germany.
5	Albumin	Spinreact Spain
6	Total anti oxidant	Bioassay Technology Laboratory. Shanghai. China

3.1.3. Animals

Twenty five apparently healthy Iraqi local breed goats, of both sexes, 5-6 months old and 15.52 ±1.05 kg body weight, were used in this study, carried from December 2018 to June 2019 in farm of Veterinary Medicine College, University of Diyala, Diyala Governorate, Iraq.

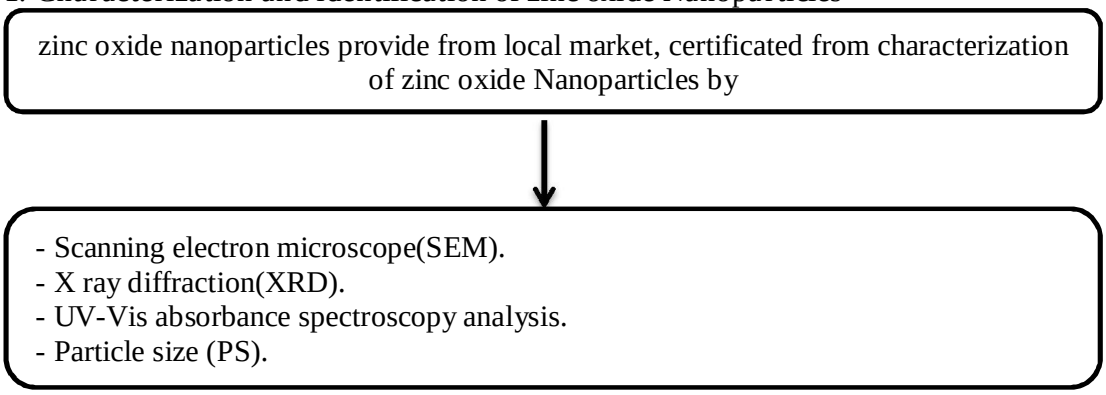
3.1.3.1. Clinical examination of animals

All animals were subjected to internal parasite examination with biochemical tests of the liver and kidney function before the study.The goats were treated as prophylactic measures by used Ivermectin and Oxyclozanid at recommended doses (0.2 mg/Kg B.W.), (15 mg/Kg B.W.) respectively, bi doses two weeks intervals. During two weeks of adaptation the clinical examination of mucous membranes, appetite, behaviors, body condition, were carried according to Constable et al., (2017).

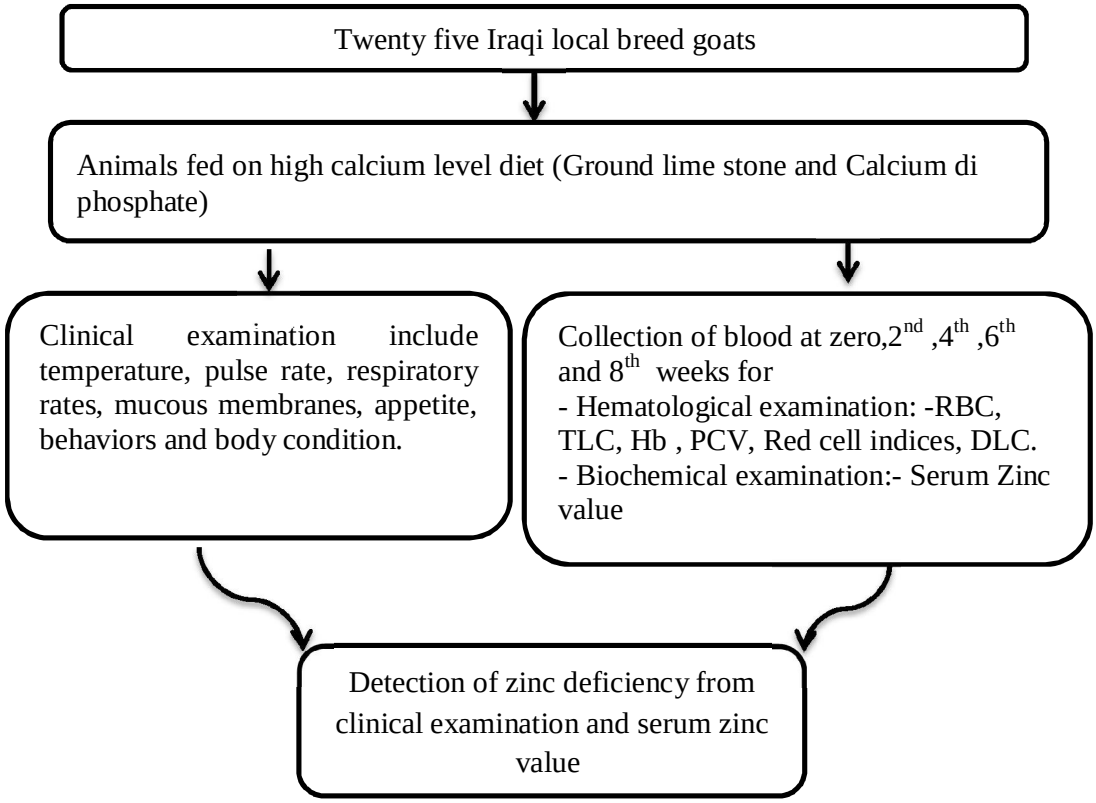
3.2. Methods:

3.2.1. Experimmantal design:- The current study was designed into three main parts; identification and characterization of Zinc Oxide Nanoparticles, induced zinc deficiency in experimental goats and study the treatment of goats have deficiency by Zinc Oxide Nano particles and conventional Zinc Oxide (Diagrams 3 – 1).

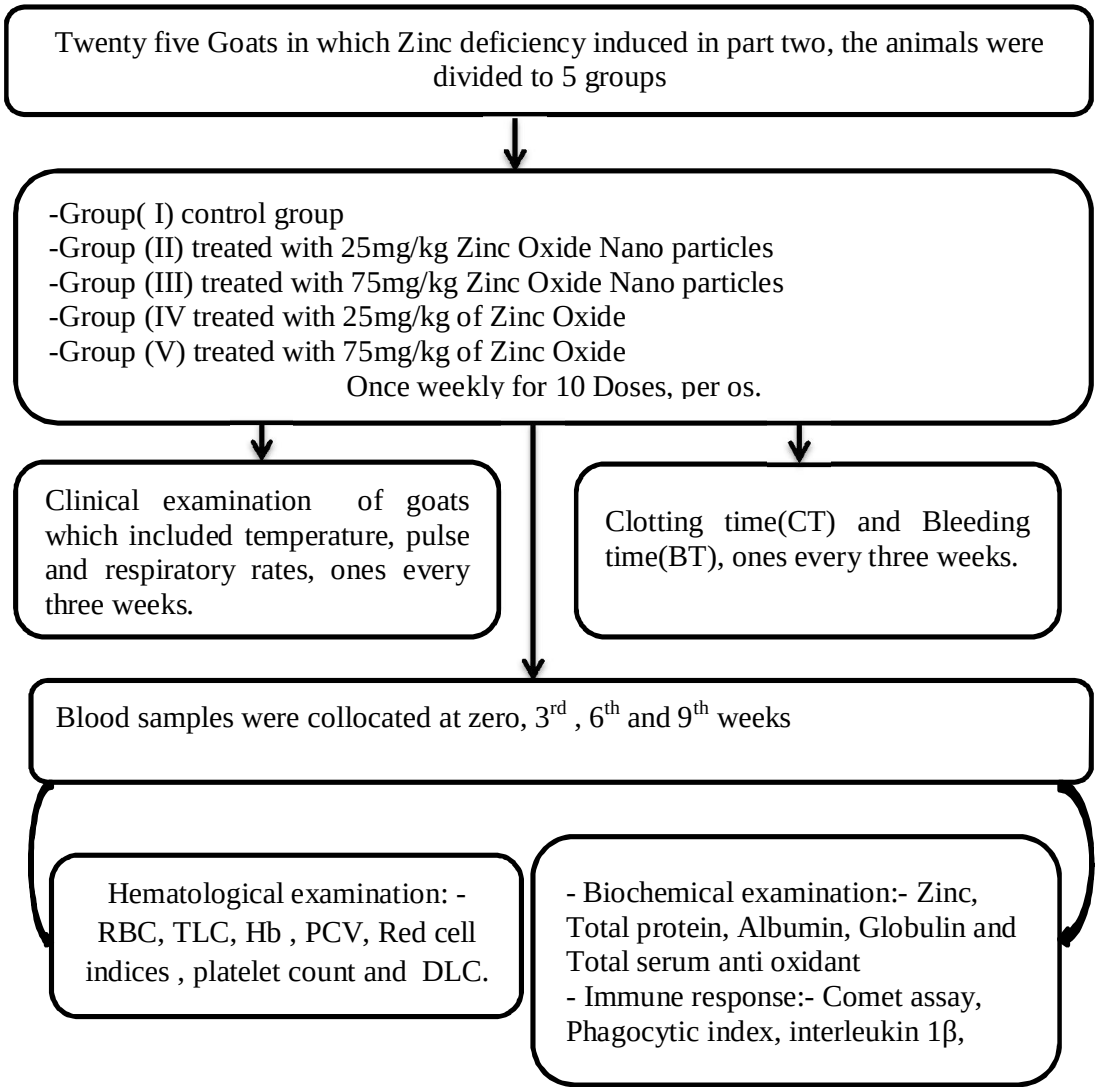
1. Characterization and identification of zinc oxide Nanoparticles



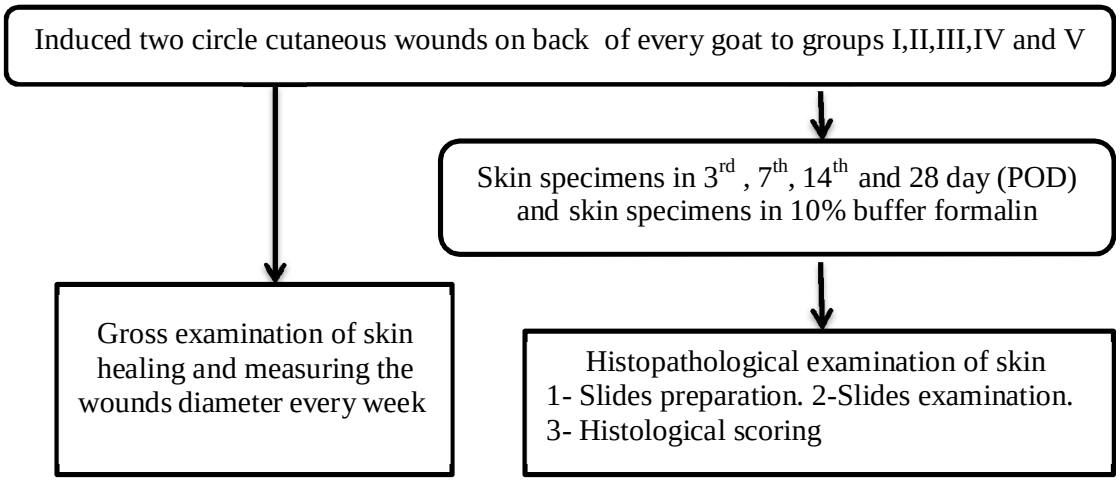
2- Induced zinc deficiency in the animals of study



3. A. Study treatment by Zinc Oxide Nano particles and conventional Zinc oxide



3. B. Study of induced skin wounds and assessment it's healing



3.2.2. First part (Characterization and identification of Zinc oxide Nanoparticles)

Zinc Nanoparticles were purchased from the local Iraqi market and were tested with the following techniques: -

3.2.2.1. Scanning electron microscope (SEM).

Morphological specifications and size of ZnO nanoparticles were defined according to (Shamhari et al., 2018) by using SEM (Shemadzu/ Japan, Nano LAB-MOST, SEM MAG:50.0 KX, SEM HV: 20.0 KV) in the Ministry of Science and Technology.

3.2.2.2. Ultraviolet–visible spectroscopy (UV–Vis) analysis.

Absorption coefficient and the gap energy were calculated for optical properties as described by (Dhuha et al., 2018). The absorption spectrum was measured by using UV-Visible spectroscopy (Schimadzu 1601 spectrophotometer) (Measure mode = ABS, High value = 3.0000ABS, Scan speed = 1000 nm/min, Accessory = Single cell), in the University of Technology, Nanotechnology.

3.2.2.3. X-ray diffractions (XRD)

The average particle size and phase detection of particles were evaluated by X-ray diffractions (XRD) pattern using X'pert Pro diffract meter (Shemadzu/ Japan) that was used to affirm the crystal phases and size, in the University of Technology, Nanotechnology. It was carried out using X-ray diffractometer with Cu- Kα crystal radiation (λ = 1.541 A°) scanning at a speed of (10 º deg/min) for (2theta) range of (20 º-80 º). The diffraction peaks were identified by comparison with (00-036-1451) card/ Variable Slit Intensity. The full widths at half maximum (FWHM) in the XRD was used to determine the crystallite size by Scherer's equation. The strain values η, and the values of dislocation densities δ, were calculated by (Khors Zak, et al 2012 and Khitam et al., 2018) (Figure 3-1).

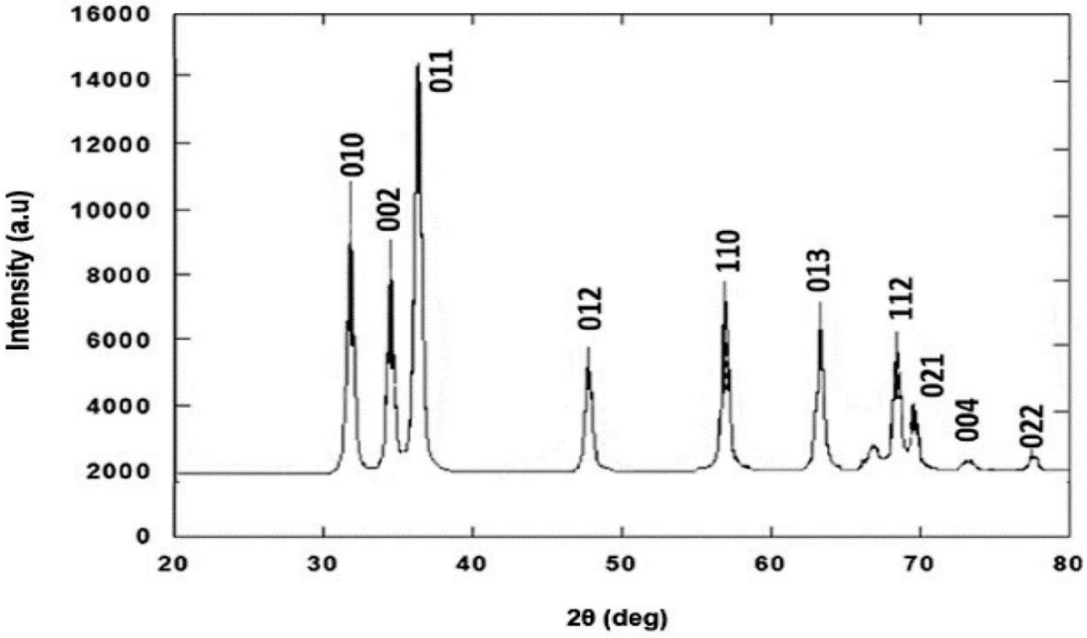


Figure (3-1): XRD pattern of ZnO Nanoparticles as standr carve (Shamhari *et al.,* 2018).

3.2.2.4. Particle size (PS)

Particle size (PS) was determined according to the method as described by Shamhari et al., (2018) by used Brookhaven instruments Corp. (90Plus Particle Sizing Software Ve 5.34), laser in the University of Technology, Nanotechnology. Diluted suspensions are prepared, in the range of 0.0001 to 1.0% v / v, using appropriate moisturizing and / or dispersing agents, if necessary. Small ultrasound is sometimes helpful in breaking up loose clumps. Small sample volumes are used as 50 µl. Sample is refundable. U-shaped, disposable, polystyrene cells are used to suspend aqueous and ethanol. It takes a few minutes for the sample and the cell to balance with the effectively controlled temperature environment inside Nano Brook (ISO, 2009).

3.2.3. Method of completion of the current study on goats

The study on experiment goats was divided into two main steps. The first step of the study included the inducation of zinc deficiency experimentallyin goats, whereas, the

second step included treatment with nanoparticles zinc oxide and conventional zinc oxide. These two steps represent the second and third parts respectively.

3.2.3.1. Second part (experimental induction of zinc deficiency in goats)

1. Preparation of goats

All the animals were used in this part were exposed to experimental induction of zinc deficiency during December 2018 and continued for 10 weeks. The dependent parameter were clinical examination including pulse, respiration rates, body temperature and body weight which were carried according to Constable et al., (2017), as well as monitoring for any abnormal changes in behavior, appetite, characters of feces, urine, and hair and skin changes.

In the second part the animals fed on basal high calcium and composition of basal diet of the experimental ration. Experiment animals were fed on green fodder and barley straw. Also, it were fed on the blended feed mixture with a daily ration of 400-500 g / head / day. The straw and water libitum until the end of the study at the tenth week. In the third part of the study, animals fed on barley cereals 500 g / head / day. In addition to straw, green fodder and water until the end of the study.

2. Induction of Zn deficiency

Induction of Zn deficiency was carried out by increase the calcium and phosphorus level in the ration, addition of ground limestone and Calcium Di-Phosphate with diet, modification to the method adopted by (Ibrahim et al,. 2016). Grind limestone to powder by using electric mill. Composition of the experimental ration. Mineral content was determined in the Ministry of Science and Technology, according to Association of Official Analytical Chemists methods (AOAC, 1990). The trace elements in experimental ration were analysis by using 10 g of pouwdered seed taken and mixed with

0.5 ml of concentrated nitric acid and 2 ml of 60% prechloric acid in a conical flask, the mixture was kept for 24hrs covered with watch glass. The trace elements were determined by (Shimadzu AA-670, Flame Atomic Absorption Spectrophotometer) at optimum separation condition. Other mineral contents was determined by using the flame system of the atomic absorption spectrophotometry (AAS), (Shimadzu 760. Japan, Koyota). The powder were ashes at 550˚ C overnight and the ash was dissolved in concentrated nitric acid and filtered, diluted to 50 ml with deionized water and the absorbance of the samples was red directly on the AAS.

Table -3.4: composition of basal diet

Element	%
Ground yellow corn	35.5
Barley beans	32
Wheat beans	28
Ground lime stone	2
Calcium di phosphate	2
Plain salt	0.5
Supplemental vitamin B12	2.3 µg/kg

Modification to the method adopted by (Ibrahim et al,. 2016)

3. Blood sampling

In two parts of study on experimental goats the blood samples were collected from jugular vein according to (Pugh, 2002), two sets of blood samples were obtained from each goat every two week till the end of the experiment (10 weeks), the first set of samples were collected (2.5 ml blood) in labeled test tube containing ethylene demine tetra acetic acid (EDTA) as an anticoagulant. The second set of blood samples (5ml) were allowed to flow freely and gently over the inner surface of a clean and dry tube. The samples were allowed to clot in slanting position at room temperature for overnight in

refrigerated, then the samples were centrifuged at 3000 rpm for 10 minutes, the clear sera were aspirated carefully by automatic pipette and transferred into clear dry labeled Eppendorf tubes and stored at -20◦C till examination.

4. Hematological parameters

A. Total leucocytes count (TLC) and Differential leucocytes count (DLC)

Total leucocytes count (TLC), were estimated by the haemocytometer method according to Coles, (1986), as well as Brockus and Andreasen, (2003). By using a special pipette and a glass slide with chambers (Haemocytometere chamber) and solution (Turk's solution). To count the celles in the optical microscope under a magnifying glass lens (40 X) and the TLC in four large side squares. Then, calculated multiplied by factor (50), according the following equilibrium of corpuscles counting (cell/mm^3) was applied: No. of corpuscle /mm^3 = no. cells counted ×50. Blood film were made and stained by Giemsa stain according to the method mentioned by Coles (1986), to differential leucocytes count (DLC).

B. Red blood cells (RBCs) count

Red Blood Cell (RBCs) counts were estimated by the haemocyatometer method according to Coles, (1986), Brockus and Andreasen, (2003). By used a special pipette for red blood cells and a special glass slide with chambers known as Haemocytometere slide for counting blood cells and using the solution of Haym’s solution. The count of coruscles is by the optical microscope under a magnifying glass lens (40 X) and the erythrocytes are in (5) secondary squares, then the following equilibrium of corpuscles counting (cell/mm^3) was applied: No. of corpuscle /mm^3 = no. cells counted ×10000.

C. Determination of Hemoglobin (Hb) concentration and Packed cell volume (PCV)

Values of the Hemoglobin (Hb) concentration and Packed cell volume (PCV) were estimated by the Hb Hemoglobin Test Strips Method (Mission system-Germany).

D. Mean corpuscular volume (MCV)

Values of MCV were calculated according to the method described by Tietz, (1999) as in the equation: MCV= PCV (%) x 10 / RBC (M/L) = Fimtoliters (Fl)

E. Mean corpuscular hemoglobin concentration (MCHC)

MCHC were calculated according to the method described by Tietz, (1999) as in the equation: MCHC= Hb(g/dl) x 100/ PCV(%) = g/dl

F. Mean corpuscular hemoglobin (MCH)

MCH were calculated according to the method described by Tietz, (1999) as in the equation: MCH= Hb (g/dl) x 10/ RBC (million) = Pico gram (Pg)

5. Zinc analysis

The level of serum Zinc was determined by the atomic absorption spectrophotometer as described by Fuwa et al,. (1964), using the commercial kit for colorimetric determination of zinc in serum (LTA s.r.i 20060, Bussero (Milan) Italy.

Procedure

Reagents	Blank	standard	sample
Work reagent	1ml	1ml	1ml
Distilled water	50µl	--	--
Standard	--	50µl	--
Sample	--	--	50µl
Mix and read the absorbance against blank at 578 nm			

Zn µmol/l =(A(sample)/A(standard)*30.6

3.2.3.2. Third part (Treatment part)

3.2.3.2.1. Treatment by zinc oxide nano particles and conventional zinc oxide.

In this part after induction of Zinc deficiency in goats (second part), the animals were randomly divided in to 5 equel groups, 5 animals in each group. Control group (group I) leaved without any treatment, group (II) treated orally by 25mg/kg Zinc Oxide Nano

particles, group (III) treated orally by 75mg/kg with Zinc oxide Nano particles. group (IV) treated orally by 25mg/kg of Zinc Oxide, group (V) treated orally by 75mg/kg of Zinc Oxide, once weekly for 10 doses. Equal volumes of water and glycerol were mixed, then zinc oxide Nanoparticles and zinc oxide were added to this mixture (as a stabilizer for ZnO NPs at room temperature) modification to the method adopted by (Wang et al., 2018), from the mixture prepared a suitable concentration for each treatment group, the control group was given a mixture of water and glycerol only. After that, the dose for each animal was calculated according to the dose, weight and group, after that orally treated by disposable syringe one dose/week (Figur 3-2).

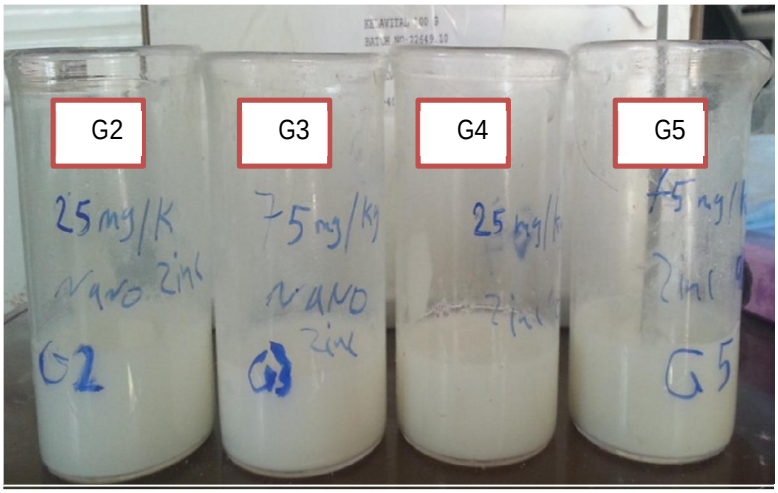


Figure (3-2): Show stability of ZnO NPs and ZnO in mixture water and glycerol.

1. Clinical examination of animals

In this part, the dependent parameter were clinical examination included pulse, respiration rate, body temperature, body weight and changes response on skin and hair were carried according to Constable et al., (2017), also body scoring system (BCS) method has used, BCS estimate of the muscle and fat development of an animal in dorsal and lumbar regions to predict fat stores in goats, Goats are given a BCS of 1 (very thin) to 5 (very fat), based on the level of muscling and fat deposition around the loin region according to Detweiler et al., (2008).

2. Hematological parameters

The hematological examination every three weeks till to the end of the experiment. In this part, the hematological parameters depended on the same methods in the second part, and in addition to made Clotting time (CT), Bleeding time (BT) and platelet count.

A. Clotting time (Capillary Tube Method)

Clotting time were calculated throw skin punctured, the first drop of blood was wiped away, and capillary tubes was filled with blood. The capillary tube was holed between the thumb and index finger of both hands, gently break off small pieces every 30 second until a strand of fibrin is seen. The interval between the appearance of blood and the appearance of a fibrin strand is the coagulation time according to (Coles, 1986; Webb et al., 2004).

B. Bleeding time

A small and clean deep puncture was made in dry skin with a No. 11 Bard-Parker bland. Carefully and without touch the skin, blood accumulate was removed with filter paper every 30 second. When blood no longer appears from the puncture site, the endpoint had been reached the interval between the appearance of blood and the blood stop, so the measure of bleeding time is according to (Coles, 1986; Webb et al., 2004).

C. Platelet count

Platelet count from stained blood film, which that prepared for hematologic examination, average in 10 high-power field on a blood film microscopically and multiplying by 15,000 gives a platelet count in per microliter as the below equation:

Platelets per microliter = average of platelets in 10 fields × 15000 (Webb et al., 2004).

3. Biochemical study

A. Total protein (Biuret Reagent)

Method: Colorimetric method (Biuret reagent).

Methods for detection total serum protein are according to Doumas and Biggs, (1976), by using the commercial kit for colorimetric determination of protein in serum. In alkaline medium, the copper reacts with the peptide bonds of proteins to form the specific pink to purple biuret complex. Sodium potassium tartrate stops copper hydroxide precipitation, and potassium iodide prevents the auto reduction of copper. The color intensity was directly proportional to the protein concentration. It was determined by measuring the increase in the absorbance spectrophotometer at 546nm.

Reagents (R)

Sodium hydroxide	750 mmol/L
Copper sulfate	12.0 mmol/L
Sodium potassium tartarate	40.9 mmol/L
Potassium iodide	19.8 mmol/L

Procedure

Reagents	blank	standard	sample
Reagents R(mL)	1ml	1ml	1ml
Standard (µL)	--	20	--
Sample (µL)	--	--	20
Mix, Incubate for 10 minutes at room temp. Measure absorbance of specimen (A specimen) and standard (A standard) against reagent blank within 30 minutes. against the blank on wavelength 546 nm.			
Calculation: $\frac{(\quad)}{(\quad)}$ = / in the sample			

B. Serum albumin

The level of serum albumin was determined by the absorption spectrophotometer as described by Fuwa et al., (1964), using the commercial kit for colorimetric determination of albumin in serum (spin react Spain).

Reagents

R	Bromcresol green PH 4.2 0.12 mmol/L
Albumin cal	Albumin aqueous primary standard 5g/dL

Procedure

Reagents	blank	standard	sample
R(mL)	1ml	1ml	1ml
Standard (µL)	--	5	--
Sample (µL)	--	--	5
Mixed and incubated for 10 min. at room temperature(15-25˚c) and read the absorbance(A) of samples and standard, against the blank on wavelength 630 nm			
Calculation: $\frac{(\ )}{(\ )}$ (.) = / lbumin in the sample			

C. Serum globulin

Globulin value was determined by subtracting the albumin value from the total protein value (Coles, 1986).

Calculation: Total protein value - Albumin value = Globulin value

4. Immunological and Antioxidant study

A. Comet Assay

Comet assay in order to determine Deoxyribonucleic acid (DNA) damage. OxiSelect comet assay kit was used to perform the test according to (Olive et al., 2001; De Boeck et al,. 2000). Comet Assay is a single cell gel electrophoresis assay for evaluation of cellular DNA damage. First, individual cells are mixed with molten agarose before application to the OxiSelect™ 96-Well Comet Slide. These fixed cells are then exposed to a lysis buffer and alkaline solution, which denatures the DNA. Lastly, the samples are electrophoresed in a horizontal chamber to separate intact DNA from damaged fragments. Following electrophoresis, the samples are dried, stained with a DNA dye, and pictured by fluorescence microscopy. Under these conditions, the damaged DNA (containing

cleavage and strand breaks) will migrate further than intact DNA and produce a “comet tail” shape.

B. Phagocytic index of neutrophil and macrophage

Phagocytic index was made by using Nitro blue Tetrazolium (NBT) test according to Freeman and King, (1972).

Method

Nitro blue tetrazolium (NBT) was made up as a 0.2% solution in saline, gentle heating to dissolve fully (stock solution). the working NBT solution was made by mixing equal volumes of stock 0.2% solution and the phosphate-buffered saline. This solution made freshly for each batch of tests,

Blood samples were collected from jugular vein, blood was obtained (3 mL) in glass tubes, contain heparin as anticoagulant. By pipette 0.1 ml of blood was putted into a well of the plastic tray, and 0.1 ml of working NBT solution was added into each well containing blood and mix the contents, so the tray was covered with another tray to ensure humidity, and was incubated at 37º C for 15 minutes, and the same period at room temperature, was transferred the blood-NBT mixture by using Pasteur pipette, was made careful coverslip smears and allow them to dry in air, and fixed smears in methanol for three minutes, then stained with Giemsa stain. Examined under the 40x objective and the number of neutrophils containing the formazan deposit as a percentage, formazan deposit occurs in two forms, a single large black deposit in the cytoplasm of the affected cell, this represents the phagocytic vacuole (phagsome), the second forms multiple black speckles, randomly distributed in cytoplasm.

C. Measuring Interleukin1 Beta.

Goat interleukin 1 Beta was measuring by using Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Bioassay Technology Laboratory ELISA Kit. China) (Appendix 3-1).

D. Measuring total antioxidant

Principle: Measuring total antioxidant capacity assay

The principle of total antioxidant capacity assay was perrformed according to Apak, et al,. (5008). The sample or standard acts to reduce Cu^{++} to Cu^{+} is combined action of the antioxidants. This reduced form of copper will selectively appearance a 2:1 complex with the chromogenic reagent. This complex is stable and has an absorption maximum at 450nm. A known concentration of Trolox is used to generate are ference curve to compare those reading obtained by the samples. Data can be expressed as Mm copper reducing equivalents or in Mm Trolox equivalents.

Antioxidants + Cu^{+2} $\longrightarrow$ Cu^{+}

Cu^{+} + 2,9-dimethyl-1,10-phenanthroline $\longrightarrow$ complex at 450nm

Reagents

1- R1 (Dilution Buffer): Ammonium acetate (NH_4Ac) buffer PH=7.0 was prepared by dissolving 19.27 g of NH_4Ac in water and completed the volume to 250 ml.

2- R2 (Copper Solution): Copper (II) chloride solution at a concentration of 10^{-2} M was prepared from $CuCl_2.2H_2O$ weighing 0.4262 g, dissolving in H_2O and diluting to 250 mi with water.

3- Trolox Standard 2 mM (0.05 g Trolox/100 ml) (mwt 250.279 g/mol)

4- Stop solution: Neocuproine (Nc) (2,9-dimethyl-1,10-pheenanthroline) solution at a concentration of $7.5 * 10^{-3}$ M was prepared by dissolving 0,039 g Nc in 96% Et OH, the volume was completed to 25 ml with ethanol.

Assay procedure

Allow dilution buffer, copper solution and stop solution to equilibrate to room temperature for 30 minutes prior to running the assay. Dilute both sample and standards 1:40 in the provided dilution buffer.

A-Added 200µl of diluted samples or standards in each well.

B -Added 50µl of the Cu solution and incubate 3 minutes at room temperature.

C-Stop solution (50µl) added to each well.

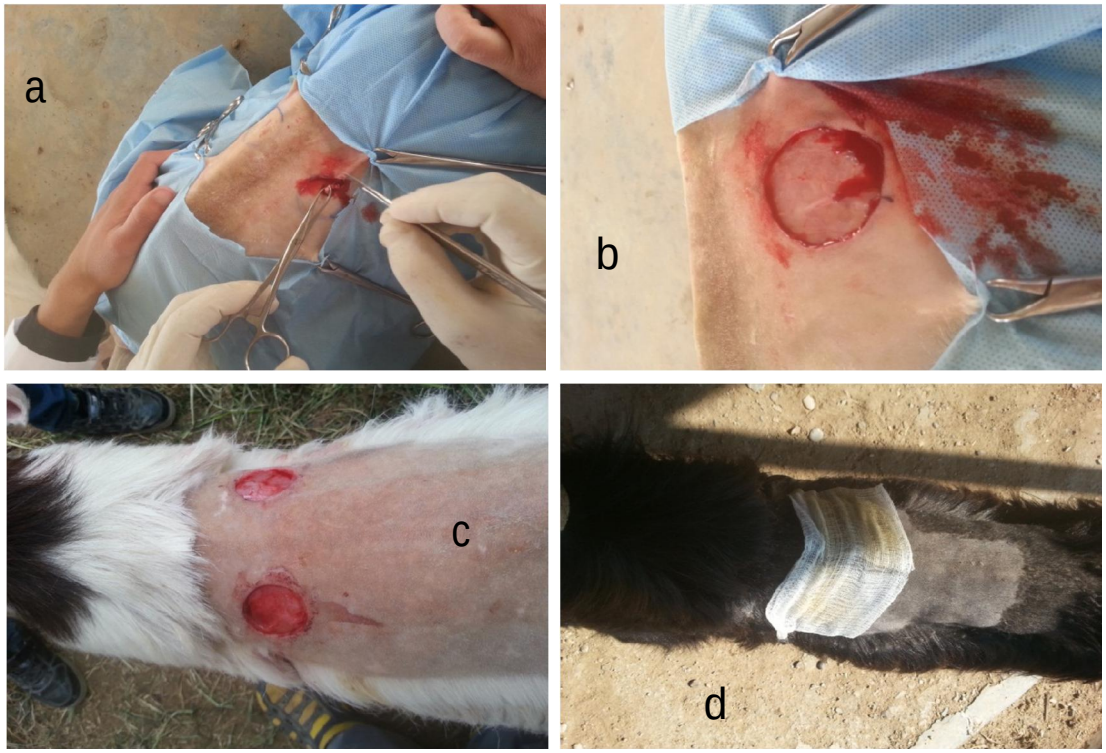
D- The plate was read a second time at 450nm.

3.2.4. Wounds healing study

Cutaneous wounds was induced in (25) animals, (5) animals of each control and treated group for evaluation the cutaneous wound healing.

3.2.4.1. Surgical procedure

Food was withdrawn for 24 hours. and water restricted 12 hours. before surgery. Under light sedation, by using intramuscular injection of xylazine hydrochloride in a dose of 0.2 mg / kg B.W. and local anesthesia by using inverted L - shape technique at wound borders with lidocaine hydrochloride 2%, in a dose 1 ml for 1cm of tissues. A sharp sterilized scalpel was used and bleeding reduced by the use of pressure gauze and shortening of surgery duration. The full thickness of the skin within the incision was then carefully stripped away by sharp dissection from its underlying muscle. All excisions were made using scalpel blade and forceps with particular care taken that wound edges were sharply defined. Skin and subcutaneous tissues were removed to make two circular full-thickness skin wounds (3.5-4.0 cm) on the dorsal sides of the back of each animal (one wound on each side), 10 cm apart after preparation of the area in routine surgical manner. The site of operation was covered with gauze dressing for all wounds (Figures, 3- 3(a, b, c, d)) according to (Olaifa and Fadason, 2016).



Figures (3- 3(a, b, c, d)): show method of surgical procedure

3.2.4.2. Gross examination of wounds

The grossly examination of skin wounds was done for all animals, to assessment normal grossly wounds healing and detect any abnormal grossly changes or pus formation by wound contamination.

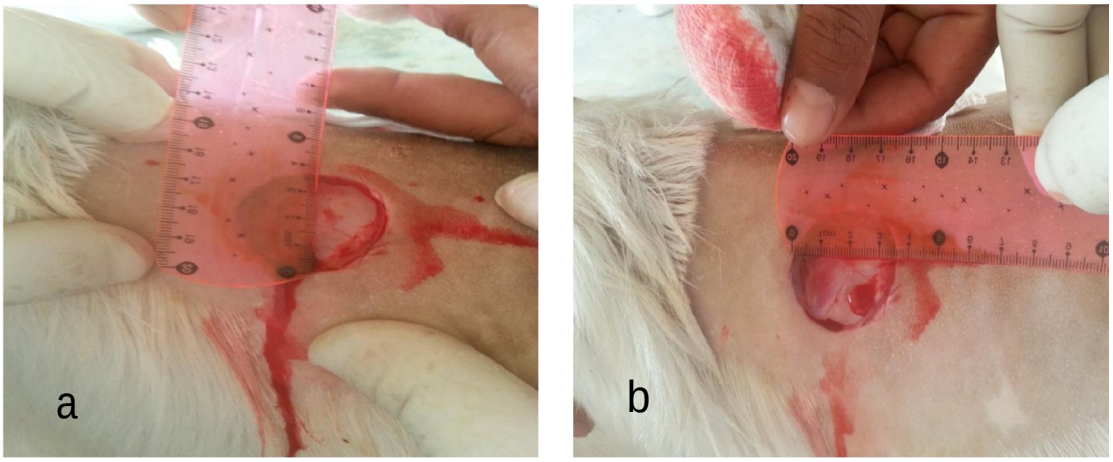
3.2.4.3. Histopathological examination

Biopsies taken from edges and center of wounds which was collected in the 3rd, 7th ,14th and 28th post-operative days (POD), tissues specimens kept and fixed in 10% buffer formalin solution directly, after 72hrs. Fixation the tissues were washed with tap water and then processing was routinely done with a set of upgrading alcoholic concentration from 70% to absolute 100% for 2hrs. in each concentration to dehydrate tissues from water (dehydration), after that clearance was done by xylol, the next step included infiltrated with liquid paraffin wax at 58˚c on two stages, and blocks of specimens were made with paraffin wax, then sectioned by rotary microtome at 5µm for all tissues. Then,

these sections were hang up slides and staining by Hematoxylin and Eosin (H α E) stain to detect the prominent changes in wound healing process of wounded area under light microscope (Anderson and Gordon, 1999). Assessment of histological condition wound healing is based on histological scoring of the case of wound healing method according to (Gupta and Kumar, 2015).

3.2.4.4. Wound dimensions measure

Under local anesthetic effect by using 10% lidocaine hydrochloride spray, the wound contraction and healing was assessed by weekly measurements of wound size, the length of the mid-horizontal and mid-vertical sides of the wound with the aid of a ruler graded in millimeters positioned at the borders of wound in clock method according to Van Rijswijk, (2013); Olaifa et al., (2016) (Figures, 3- 4(a, b)), and the means of all wounds were taken in each week of the experiment, for wound contraction evaluation according to (João De Masi et al., 2016).



Figures (3- 4): showed method of wound dimensions measure,(a) mid-vertical, (b) mid-horizontal

3.2.5. Statistical Analysis

All data denoted as means ± SE. One way analysis of variance (One-way ANOVA) by using SPSS, followed by using different letter to represent the significant differences

among means of groups at the level of statistical significant ($P < 0.05$) (Joda, 2008). One-way ANOVA with Dunnett's post test performed using GraphPadPrism version 6.04 for Windows, GraphPadSoftware,La Jolla California USA,www.graphpad.com.

Chapter four
Results

4. Chapter four: Results

4. 1. Results of Zinc Oxide Nanoparticles analysis

4.1.1 Scanning electron microscopic analysis (SEM):

The result of scanning electronic microscopy (SEM) was showed a specific morphology and size of ZnO. Particles clearly execute the spherical structural formation, some small particles which observed to be bit agglomerated (Figure 4 – 1(a)). In following image it can be clearly observed that the particles possess nearly spongy like and oval like structural nanoparticles, and the diameters of ZnO nanoparticles varying between 1-50 nm (Figure 4 -1(b)).

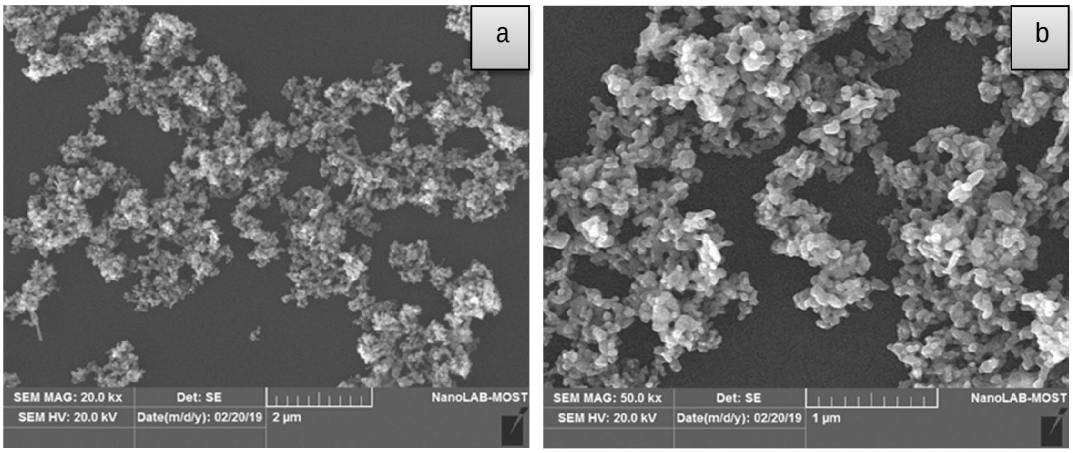


Figure (4 - 1): SEM image of Zinc Oxide Nanoparticles, a. show small particles which observed to be bit agglomerated. b. observed oval like structured particles,with diameters between 1-50nm.

4.1.2. X- Ray Diffraction (XRD)

XRD pattern of ZnO NPs is shown in (Figure 4 – 2), based on the XRD pattern, ZnO NPs has high purity of quartzite crystalline structure as the diffraction peak is seen to be intense and narrower. This result was also being compared with the given standard XRD pattern of ZnO (JCPDS 36-1451) for confirmation purpose. The pattern shows that all the diffraction peaks indexed to the hexagonal phase of pure ZnO Nanoparticles, ZnO Nanoparticles diffraction peaks appearing to scattering angles (2θ) = 31.88°, 34.52°, 36.39°,

47.64°, 56.70°, 62.95, 66.48. 68.03, 69.16 and 77.05. Strongest 3 peaks in 36.39, 31.88 and 34.52 respectively.

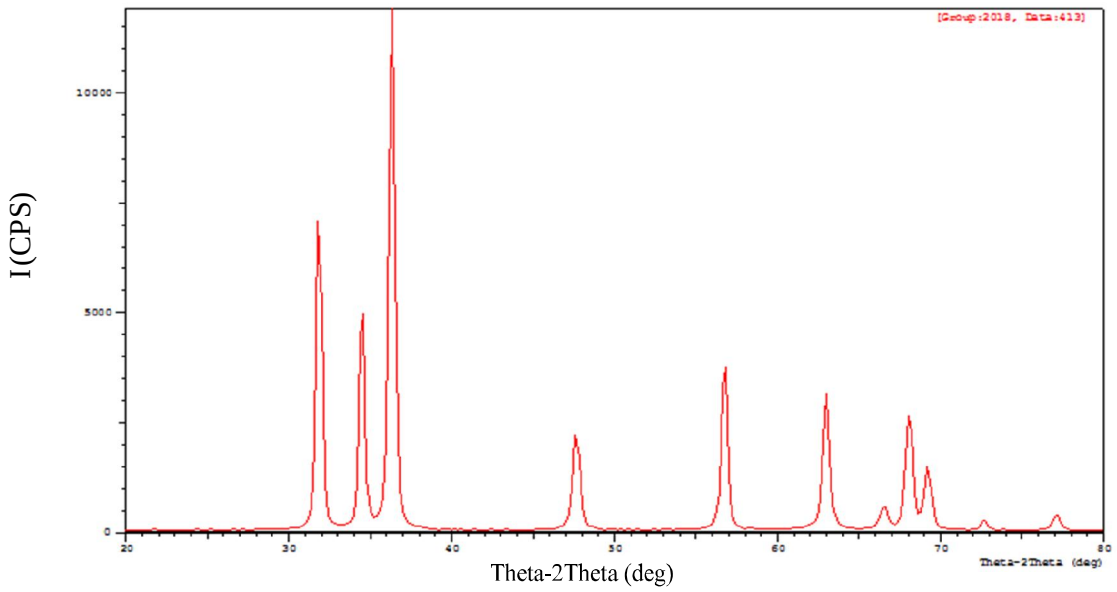


Figure (4 – 2): XRD patterns of ZnO Nanoparticles, pure ZnO Nanoparticles, ZnO Nanoparticles diffraction peaks appearing to scattering angles (2θ) = 31.88°, 34.52°, 36.39°, 47.64°, 56.70°, 62.95, 66.48. 68.03, 69.16 and 77.05. Strongest 3 peaks in 36.39, 31.88 and 34.52 respectively.

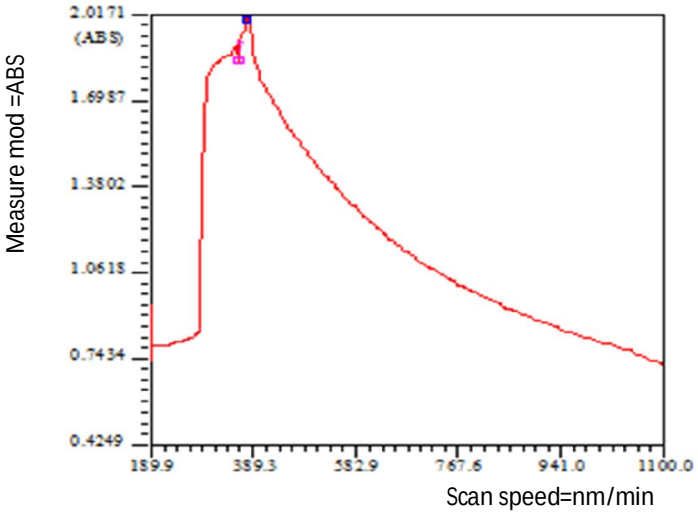
4.1.3. UV-Vis Absorption Spectrum

UV-Vis spectroscopy was confirm the formation of ZnO NPs. The absorption spectrum of ZnO NPs was shown in (Figure 4 – 3). The UV-Vis measurement was performed after the ZnO NPs was dispersed in ethanol. The absorption peak was observed at 389.3 nm, which attribute to the intrinsic band-gap of ZnO absorption.

4.1.4. Particle size

Particle size also performed to further confirm the size of Zinc Oxide Nanoparticles was shown in (figure 4 - 4), and the mean diameter 41.2 nm.

Graph



Parameter settings

Function = SPECTRUM
Start wavelength = 190.0 nm
Stop wavelength = 1100.0 nm
Measure mode = ABS
Low value = 0.0000 ABS
High value = 3.0000 ABS
Scan speed = 1000 (nm/min)
Accessory = Single cell

Figure (4 – 3): UV-Vis Absorption Spectrum show the absorption peak at 389.3 nm.

Brookhaven Instruments Corp.
90Plus Particle Sizing Software Ver. 5.34

Date: Nov 29, 2018
Time: 23:48:28
Batch: 0

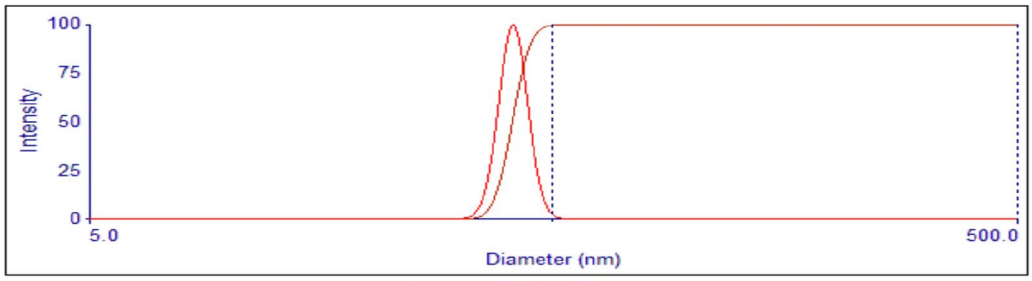
Sample ID 87-0
Operator ID 0
Notes laser

Measurement Parameters:

Temperature	= 25.0 deg. C	Runs Completed	= 1
Liquid	= Ethanol	Run Duration	= 00:00:30
Viscosity	= 1.084 cP	Total Elapsed Time	= 00:00:30
Ref.Index Fluid	= 1.357	Average Count Rate	= 541.2 kcps
Angle	= 90.00	Ref.Index Real	= 1.590
Wavelength	= 660.0 nm	Ref.Index Imag	= 0.000
Baseline	= Auto (Slope Analysis)	Dust Filter Setting	= 30.00

Measurement Results:

Effective Diameter: **41.1 nm**
Polydispersity: **0.005**
Baseline Index: **0.0/100.00%**



Lognormal Distribution

Figure (4 – 4): the apparent particle size of Zinc Oxide Nanoparticles, average diameter 41.2 nm.

4. 2. Experimental induction of zinc deficiency

The result of analysis of the feed sample for the elements calcium, magnesium, potassium, zinc, copper and phosphorus appeared on the following concentrations: 1349.6, 972.3, 58.12, 1.83, 1.52 and 3.2 mg / 100g respectively (Table 4-1).

Table 4-1: analysis of the experimental ration

Element	mg/100g
Calcium Ca	1349.6
Magnesium Mg	972.3
Potassium K	58.12
Zinc Zn	1.83
Copper Cu	1.52
Phosphorus P	3.2

First clinical signs in the present study manifested 30 days post starting the study in some animals, included loss of hairs around the eyes and near of nostrils as well as, rough hairs. In the next days, the clinical signs became clear in most experimental animals. The main clinical signs in current study were retardation in growth of hairs, especially on legs, head, rough hair coat, and losing hairs on head, limbs and scrotum. Rough skin, dandruff, alopecia and crusting. Swollen joints, poor growth, fissuring of the hooves, deformity with overgrowth of hooves. Loss of appetite, pruritus and emaciation (Figures 4-5,6,7,8,9).

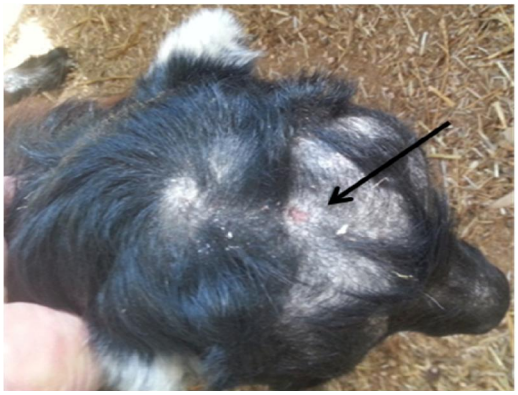


Figure (4-5): Goat showed rough, loss hair on head region (10th week of study)

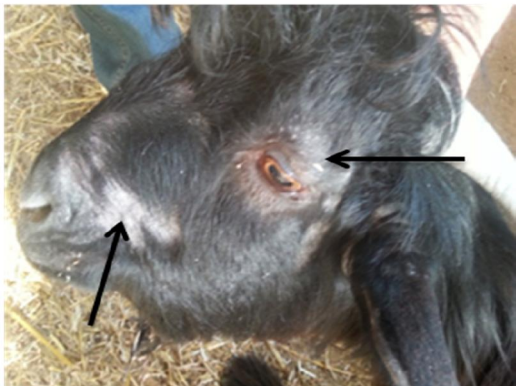


Figure (4-6): Goat showed rough, loss hair on face. (10th week of study)

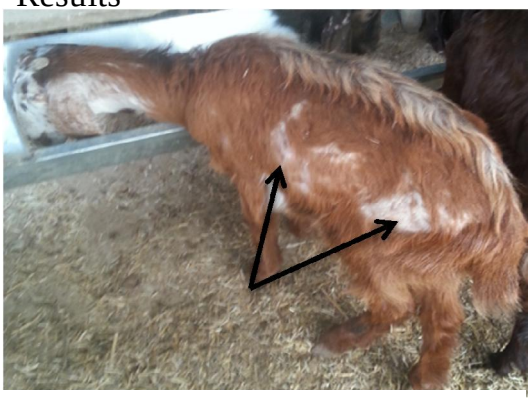


Figure (4-7): Goat with rough hair, hair loss and emaciation (10th week of study)

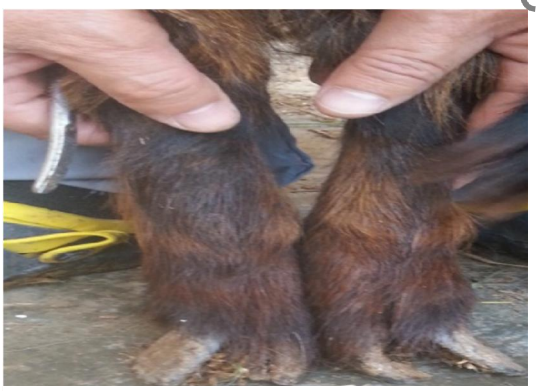


Figure (4-8): fissures and over growth of the hooves (10th week of study)

Figure (4-9): Goat with rough skin, hair loss and emaciation (10th week of study)

The results revealed that heart rates were significantly decreased in 2nd and 4th week in comparison with 0, 6th and 8th week, but significantly increased in 6th week in comparison with other weeks, the highest heart rate (129.55±5.52 beat/min) in 6th week, so the lowest rates were in 2nd week (115.9±3.829 beat/min).

There were non-significant changes in body temperature during the study, and the lowest was in 2nd week (38.40±0.09 ^{o}C) in comparison with the highest (38.62±0.12 ^{o}C) in 8th week.

The respiratory rates were significantly decreased in 2nd and 6th weeks in comparison with 0 week of the study, the lowest rates was in 2nd week (26.0±0.79). body weight was no significantly changes between 6th and 8th weeks and the highest weight was in 8th week (18.02±1.56 Kg) in comparison with 0 week (15.53±1.05 Kg) (Table -4- 2).

Table (4 -2) Clinical parameters and weight in goats.

Parameters / Time	Weeks				
	0	2	4	6	8
Temperature ^{o}C	38.58±0.13	38.40±0.09	38.55±0.48	38.54±0.15	38.62±0.12
Heart rate beat/min	123.7±2.80	115.9±3.82 9a	116.65±4.65 a	129.55±5.52 abc	123.9±5.33
Respiratory rate/min	32.6±1.95	26.0±0.79a	31.4±1.0	26.2±1.28a	30.40±1.68
Weight/ Kg	15.52±1.05	17±1.19a	17.05±1.46a	18±1.49abc	18.02±1.56 abc

Values are Mean ± SE. a. Means significance in comparison with 0 week b. In comparison with 2nd week, c. In comparison with 4th week, significance at $P < 0.05$.

The results of Hb, PCV, MCV and MCH at 0 week significantly decreased in comparison with average of normal blood value in goats less than one year (microcytic normochromic anemia). The results of red blood corpascles count were significantly decreased in 2nd week incomparison with 0 week, then rised in 4th, 6th and 8th weeks, the highest counts of erythrocyte was in 6th week (14.06±0.80 ×10^{6}/µl) compared with lowest in the 2nd week (9.5±0.54 ×10^{6}/µl).

Hemoglobin concentration were significantly increased concentetion in the 2nd, 4th and 6th weeks in comparison with 0 week, the highest concentetion was in 6th week (6.45±0.22 g/ dl), while the lowest concentetion (5.19±0.06 g/ dl) in 0 week.

PCV percentage increased in the 2nd ,4th ,6th and 8th weeks in comparison with 0-week the highest percentage was in 6th week (19.0±0.63%) in comparison with the lowest percentage in 0 week (15.55±0.20%).

MCV were significantly increased in the 2nd,4th,6th, and 8th weeks in comparison with the 0 week, and the highest was in the 2nd week (20.77±1.22 Fl) in comparison with (13.42±1.33 Fl) in 0 week.

MCHC did not show significant changes, so the lowest level was in 2nd week (33.51±0.42 g/dl) in comparison with the highest level (34.23±0.23 g/dl) in 0 week.

MCH significantly increased in the 2nd,4th ,6th, and 8th weeks in comparison with the 0 week the highest was in 2nd week (6.88±0.46 Pg) in comparison with lowest (4.33± 0.38 Pg) in 0 week. Serum zinc level was significantly depressed, starting from the 2nd week till the end of study, so it was the lowest level (7.61±0.28 µmol/L) compared within 0 week (11.34±0.70 µmol/L) (Table -4- 3).

Table (4 - 3) Hematological parameters and serum Zn in experimentally induction zinc deficiency in goats.

Parameters / **Time**	Normal value	Weeks				
		0	2	4	6	8
RBC $\times 10^6/\mu l$	12.92± 0.70	11.0±0.53	9.5±0.54 a	11.52±0.57**a** b	14.06±0.80**a** b	12.54±0.55**a** b
Hb g/ dl	9.94±0.50	5.19±0.06A	6.3±0.26 **a**	6.07±0.22 **a**	6.45±0.22 a	5.96±0.19
PCV %	29.45±1.26	15.55±0.20 A	18.7±1.03 **a**	17.95±0.64 a	19.0±0.63 a	17.75±0.55 a
MCV Fl	23.39±1.10	13.42±1.33 A	20.77±1.22 **a**	16.20±2.23 a	15.14±1.71 a	14.64±0.74 a
MCHC g/ dl	33.63±0.42	34.23±0.23	33.51±0.42	33.83±0.35	33.91±0.25	33.59±.0.38
MCH Pg	7.63±0.36	4.33±0.38A	6.88±0.46 **a**	5.44±0.27 a	5.13±0.58 **a**	4.90±0.24 **a**
Serum Zn µmol/L		11.34±0.70	9.17±0.43 **a**	8.94±0.49 a	7.62±0.17 abc	7.61±0.28 abc

Values are Mean ± SE. A. Means significance in comparison with normal value according to (Hussain and Salman 2012) a. Means significance in comparison with 0 week b. In comparison with 2nd week, c. In comparison with 4th week, significance at $P < 0.05$.

The results of the total leucocytes count were non significantly decreased in 2nd week (5678.1±366.13×10^3/µl) in comparison with (7079 ±953.56×10^3/µl) in 0 week but increased to its highest level (7480.66±246.7×10^3/µl) all non-significantly changes. Neutrophil percentage significant increased in 4th week(53.75±3.06%) in comparison with 0 weeks (39.70±2.28%).

Lymphocytes significant decreased in the 4th week in comparisons with 0 ,2nd ,6th and 8th weeks, the highest level was in 0 week (54.95±2.71%) and other no significant differences. Eosinophil significantly decreased in 2nd (1.35±0.28%) and 6th week (1.80±0.38%) in comparisons with 0 week (2.55±0.41%), 4th week (2.7±0.57%) and 8th weeks (2.3±0.59%). Monocytes and Basophiles none significant differences (Table 4- 4).

Table (4 - 4): Total leucocytes count and differential leucocytes after experimentally induction of zinc deficiency in goats.

Parameter / Time	Weeks				
	0	2	4	6	8
TLC X103/µl	7079±953.56	5678.1±366.13	7480.66±246.7	6824.45±323.45	5824.5±559.32
N %	39.70±2.28	41.60±2.81	53.75±3.06 a	42.20±2.26	42.5±1.55
L %	54.95±2.71	54.60±2.99	38.95±2.99 ab	51.75±2.59 c	52.65±3.81 c
M%	3.75±0.66	2.25±0.43	3.50±0.51	2.95±0.44	2.85±0.36
E %	2.55±0.41	1.35±0.28 a	2.7±0.57 b	1.80±0.38 ac	2.3±0.59 bd
B %	0.45±0.19	0.50±0.14	0.65±0.17	1.05±0.22	0.75±0.17

Values are Mean ± SE. a. Means significance in comparison with 0 week b. In comparison with 2nd week, c. In comparison with 4th week, d. In comparison with 6th week, significance at $P < 0.05$.

4. 3. Results of treatment with ZnO NPs and conventional ZnO.

4. 3. 1. Clinical study of treatment and control groups.

After stopped first ration which used in the second part of current study by a new ration the clinical signs showed changes in the appetite of animal and increased food consumption, skin changes developed after the 7th day from started the treatment characterize by hair growth and changes in color, decreased crust and restricted the lesion of hyperkeratosis, in the 4th week after treatment, new hair growth was observed in the affected areas, and in the 6th week after treatment abnormal lesions on the skin disappeared and the skin and hair

became normal in appearances except in one animal their was crust and rough skin, their was no large different between treated groups and control group in the clinical signs.

Body weight significantly increased in all groups including, control group (group I), Zinc Oxide Nano particle 25mg/kg group (group II), Zinc Oxide Nano Particle 75mg/kg group (group III), Zinc Oxide 25mg/kg group (group IV) and Zinc Oxide 75mg/kg group (group V) during the study in comparison with the starting point (zero day), the best increased were in groups II, III and V, respectively in comparison with group I and group IV. Between groups significant increased were in groups II and III in 3rd week in comparison with groups I, IV and V, and in group II in 6th week in comparison with other groups, and in groups II and III in 9th week in comparison with groups I, IV and V. The body scoring showed the highest values were in group II and III (2.25±0.25) and lowest values were in group I (1.625±0.37), in lumbar scoring, in sternal scoring the highest values in groups II and III (2.625±0.23, 2.375±0.23), respectively, and lowest values in group I (Table 4 – 5).

Table 4 – 5: body scoring parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III)) and Zinc Oxide (25mg/k (IV) and75mg/k (V)) of goats.

Parameter	Groups		
		Lumbar	Sternal
Body Scoring	G I	1.625±0.37	1.5±0.20
	G II	2.25±0.25	2.62±0.23
	G III	2.25±0.25	2.37±0.23
	G IV	1.75±0.25	1.75±0.43
	G V	1.875±0.42	2.0±0.35

Body temperature showed no significant changes. Heart rates were significantly decreased in 6th week in comparison with 0- week in group II and non significant changes in other groups, between groups non significant changes. Respiratory rats were increased significantly in group I in week 9th in comparison with 0-week, in group II significant

increased in 3rd week after that decreased in 6th and 9th weeks, in group III significant increased in 3rd week and 6th week in comparison with 0-week, but in 9th week decreased, in group IV increased significantly in all weeks in comparison with 0-week, and significant increased in 3rd week in comparison with 6th and 9th week. Among groups, significant increased in group IV in 3rd week in comparison with other weeks, in 6rd week the significant lowest rate in group II in comparison with the other weeks, in 9th week the significant highest rate in groups I, V and IV, repactivally in comparison with groups II and III (Table 4-6) (figure 4-10,11).

Table 4-6: Show body weight and clinical parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III)) and Zinc Oxide (25mg/k (IV) and75mg/k (V)) of goats.

Parameter / Groups		Weeks			
		0	3	6	9
Weight/ Kg	G I	17.12±2.13aA	18.87±2.38aA	22.37±2.55bA	23.62±2.30bA
	G II	17.75±1.31aA	20.12±1.08bB	24.62±1.06bcB	26.25±0.85bcB
	G III	17.37±0.85aA	20±0.97bB	23.87±1.32bcA	26.37±1.21bcdB
	G IV	16.27±0.16aA	18.62±0.55bA	21.87±1.28bcA	24.25±1.31bcdA
	G V	17.37±1.79aA	19.37±2.52aA	23.75±3.11bA	26±2.73bA
Temperature ^{o}C	G I	39.02± 0.18	39.17± 0.22	39± 0.12	39.22± 0.17
	G II	38.2±0.40	39.25±0.20	38.27±0.56	38.95±0.17
	G III	38.97±0.08	39.6±0.21	39.2±0.07	39.15±0.10
	G IV	38.25±0.08	39.37±0.14	39.15±0.21	39±0.14
	G V	38.62±0.14	39.06±0.24	38.95±0.09	39.25±0.18
Heart rate beat/min	G I	122±7.77aA	117±8.69aA	121±7.7aA	121±6.19aA
	G II	122.25±2.09bA	122.5±7.18aA	118.25±1.18aA	119.5±2.62aA
	G III	119±12.04aA	116.5±6.44aA	118±6.21aA	118±3.46aA
	G IV	120±6.87aA	121±3.41aA	121.25±9.49aA	118±10.51aA
	G V	121.5±5.96aA	120±13.95aA	122±5.03aA	119±6.60aA
Respiratory Rate/min	G I	30.5±0.95aA	33.5±2.75aA	33±2.38aBC	36±1.63bBC
	G II	30±3.46aA	32.5±1.70bA	27±3.41aA	29±1aA
	G III	28±3.26aA	32.75±2.13bA	32±1.63bB	31.5±0.95aA
	G IV	28±4.89aA	43±1.91bcdB	35.5±1.70bcBC	32±3.26aB
	G V	31±3.41aA	32.5±1.70aA	35.5±0.5aBC	34±2.5aB

Values are M ± SEM: a, b, c, d significant difference at a level of $P < 0.05$ in comparison with in the same group. A, B, C, D significant in comparison between groups, significance at $P < 0.05$.

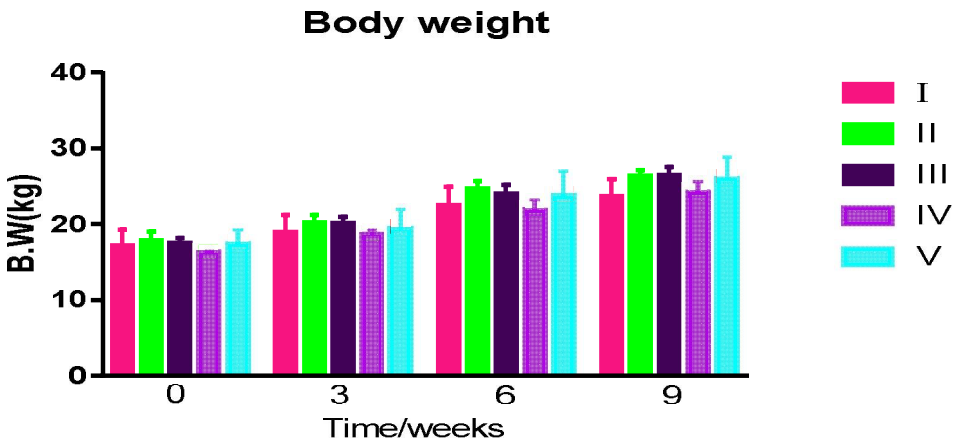


Figure (4 -10): Show body weight parameters in diffrrent groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

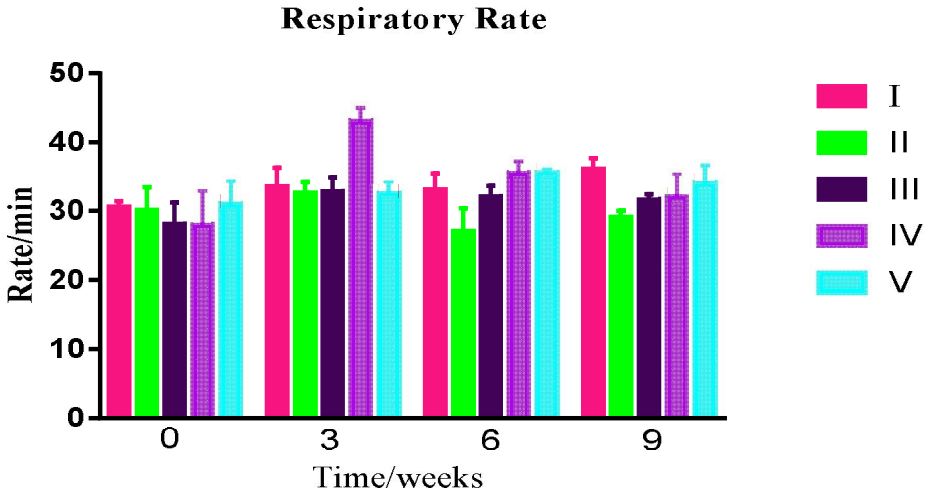


Figure (4 -11): Show respiratory rate in diffrrent groups control (I), Zinc Oxide Nanoparticle 25mg/k (II) and 75mg/k (III) and Zinc Oxide 25mg/k (IV) and75mg/k (V) of goats.

4. 3. 2. Results of hematological analysis of treated and control groups.

The results of Red Blood Cells count refer to significant increase in 9th week in comparison with the previous weeks in group I, while in group II and III significant increased in 6th and 9th weeks in comparison with 0-week and 3rd week, while it increased significantly in 9th week in comparison with 3rd and 6th weeks in group V, between groups significant increase in groups II and III at 6th in comparison with groups I, IV and V, and in 9th week significant increased in group II in comparison with groups I, III, IV and V (Figure 4-12).

Hemoglobin concentration (Hb) increased significantly in all groups in 9th week in comparison with 0-week, between groups hemoglobin concentration significantly increased in group III in comparison with other groups at 9th week. The result of Packet Cells Volume (PCV) percentage showed significant increase in all groups in 9th week in comparison with 0-week, between groups the significant increased were in groups I, II, III and IV in comparison with V group at 3rd week, while in 9th week significant increased in groups II and III in comparison with groups I, IV and V(Figure 4-13,14).

MCV increased significantly from 3rd week to 9th week in comparison with 0-week in groups I, II, III, IV and V. Between groups MCV significant increased in groups I and III in comparison with groups II, IV and V at 3rd week, at 6th week the lowest mean in group II in comparison with other groups, and highest significantly mean at 9th week in group II in comparison with other groups.

MCHC decreased significantly from 3rd to 9th weeks in comparison with 0-week in groups I, II, III and V, but in group IV decreased significantly in 3rd and 6th weeks in comparison with 0-week and 9th week. Significant increased between groups at 9th week in groups IV and V in comparison with other groups.

MCH increased significantly in 3rd , 6th and 9th weeks in comparison with 0-week in groups I, II, III and V, and in group IV significant increased in 6th and 9th weeks in comparison with 0-week. Between groups significant increased in group V in comparison with I, II, III and IV groups at 6th week, and significantly increased in group III in comparison with I, II, IV and V groups at 9th week (Table 4-7).

Table 4-7: RBC, Hb, PCV, MCV, MCHC and MCH parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III)) and Zinc Oxide (25mg/k (IV) and75mg/k (V)) of goats.

Parameter / Groups		Weeks			
		0	3	6	9
RBC $\times 10^6/\mu l$	G I	12.03±0.63aA	11.53±0.40aA	12.05±0.51aA	12.93±0.55b A
	G II	11.0±0.91aA	12.03±0.41aA	14.33±0.96bB	14.70±0.94b B
	G III	11.64±1.08aA	11.06±0.80aA	13.89±0.89bB	14.13±1.79bA
	G IV	12.69±0.52aA	12.98±0.84aA	12.35±1.15aA	13.25±2.10aA
	G V	13.06±1.57aA	11.62±0.59aA	11.0±1.24aA	13.7±1.58bA
Hb g/dL	G I	5.67±0.11aA	7.67±0.11bA	9.33±0.19bcA	10.03±0.58bcdA
	G II	5.32±0.08aA	7.7±0.13bA	9.74±0.16bcA	10.67±0.30bcdA
	G III	5.85±0.37aA	7.47±0.39bA	10.08±0.72bcA	12.18±0.48bcdB
	G IV	6.97±0.53aA	7.72±0.32bA	9.54±0.56bcA	10.23±0.67bcA
	G V	5.95±0.41aA	7.22±0.52bA	9.3±0.30bcA	10.23±0.56bcdA
PCV %	G I	16.5±0.28aA	25.5±0.28bB	30±0.57bcA	32.5±1.84bcdA
	G II	16.5±0.86aA	25.25±0.47bB	31.25±0.47bcA	34.25±0.85bcdB
	G III	17.25±1.10aA	25.5±0.64bB	32.25±2.17bcA	38.75±1.37bcdBC
	G IV	17.75±0.75aA	25.75±0.85bB	31±1.73bcA	31.25±2.25bcA
	G V	17.5±1.32aA	23.25±1.37bA	30.5±1.19bcA	32±1.77bcA
MCV Fl	G I	13.81±0.68aA	22.16±0.54bB	24.29±0.88bcB	24.59±0.64bcA
	G II	15.50±2.18aA	21.04±0.74bA	22.43±1.81bA	24.60±1.59bcA
	G III	15.24±1.67aA	23.34±1.43bB	24.94±0.39bB	28.78±3.66bcB
	G IV	15.67±0.56a	20.07±1.35bA	25.43±1.33bcBC	25.52±4.23bcA
	G V	13.94±1.80aA	20.05±0.92bA	25.60±1.20bcBC	24.84±2.25bcA
MCHC g/dL	G I	34.39±0.28bA	30.10±0.42aA	31.09±0.03aA	30.86±0.23aA
	G II	32.48±1.37bA	30.50±0.46aA	31.19±0.03aA	31.15±0.14aA
	G III	33.91±0.28bA	30.74±0.50aA	31.22±0.13aA	31.42±0.27aA
	G IV	33.13±1.23bA	30.23±0.33aA	30.76±0.38aA	32.78±0.34bB
	G V	34.36±3.17bA	30.58±2.48aA	30.51±0.33aA	32.08±1.57aB
MCH Pg	G I	4.75±0.27aA	6.67±0.18bA	7.79±0.40bcA	7.74±0.27bcA
	G II	4.94±0.44aA	6.41±0.18bA	6.92±0.60bA	7.36±0.58bA
	G III	5.17±0.58aA	6.80±0.29bA	7.32±0.59bA	9.03±1.11bcB
	G IV	5.56±0.60aA	6.0±0.33aA	7.83±0.47bA	8.3±1.39bA
	G V	4.75±0.62aA	6.22±0.34bA	8.66±0.67bcB	7.71±0.85bA

Values are M ± SEM: a, b, c, d significant difference at a level of $P < 0.05$ in comparison with in the same group. A, B, C, D significant in comparison between groups, significance at $P < 0.05$.

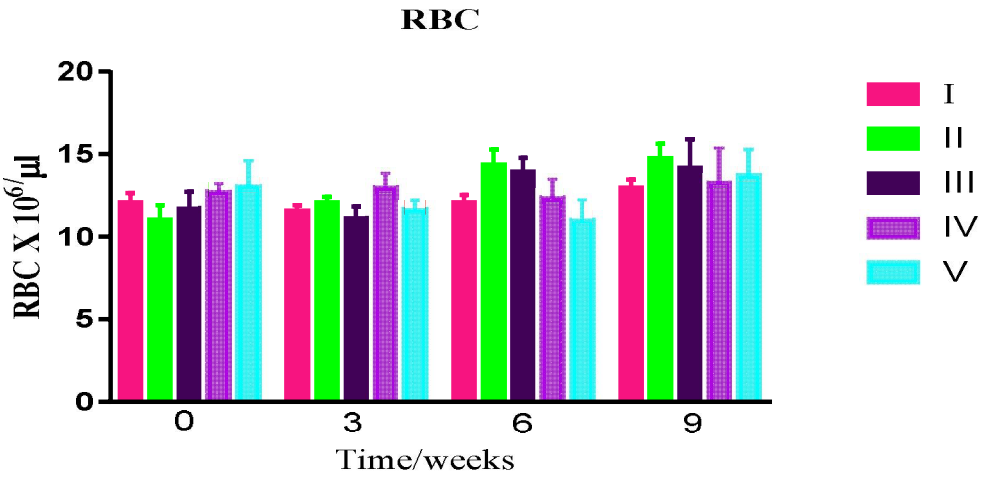


Figure (4-12): Show RBC parameter in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

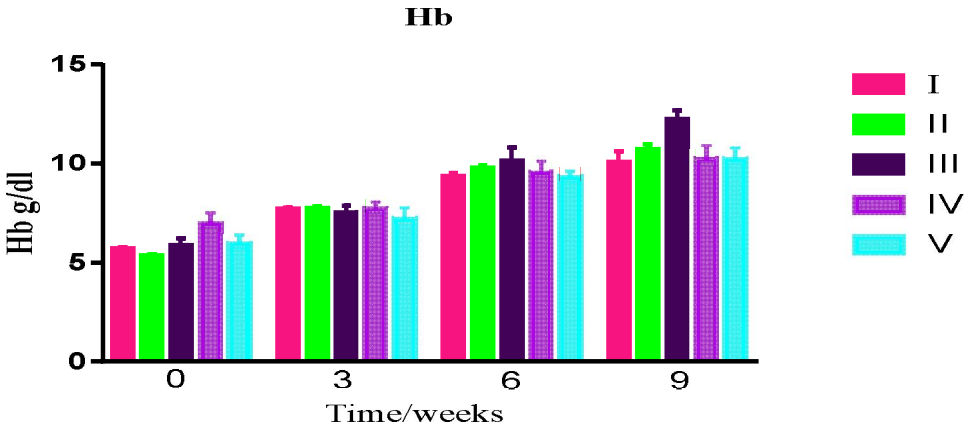


Figure (4-13): Show Hb parameter in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

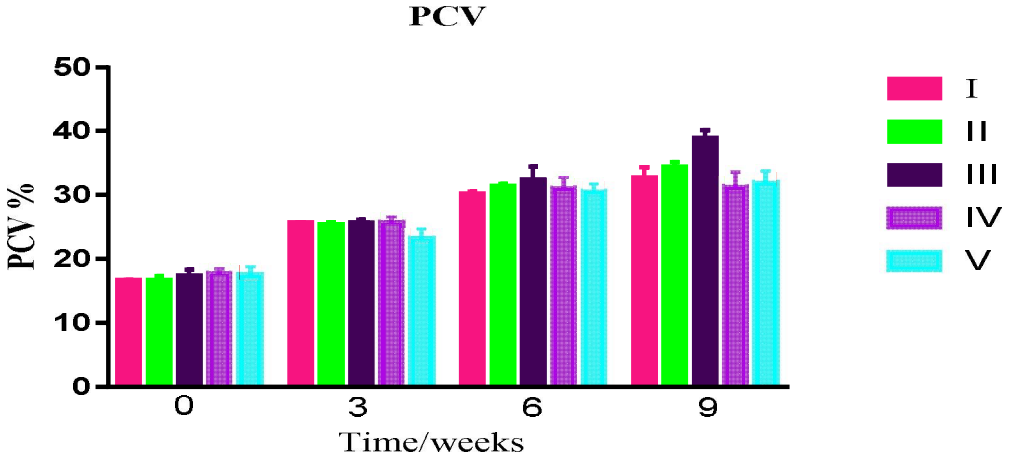


Figure (4-14): Show PCV parameter in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

The results of the total leucocytes count were significant increased in 3rd and 9th weeks in comparison with 0-week in groups I, and significant increased in 9th week in comparison with other weeks in groups II, while in group IV significant increased in 6th and 9th weeks in comparison with 0-week and 3rd weeks, all so significant increased in 3rd and 9th weeks in comparison with 0-week and 6th week in group V. Between groups, significant increased in group III and V in comparison with other groups at 3rd week, but significant increased in group V in comparison with I, II, III and IV groups at 9th week (Figure 4-15).

Neutrophils percentage increased significantly in 3rd and 9th weeks in comparison with 0-week and 6th weeks in group I, in group II significant increased at the 3rd week in comparison with other weeks, decreased significantly in 9th week in comparison with 0-week, 3rd and 6th weeks in group III, while decreased significantly in 9th week in comparison with 3rd week in group IV, and increased significantly in 3rd week in comparison with 0-week after that decreased significantly in 6th and 9th weeks in comparison with 3rd week. Among groups, significantly decreased in group III and IV in comparison with I, II, V groups, in 9th week significant increased in group I in comparison with groups II, III, IV and V and the lowest value in groups III and IV(Figure 4-16).

Lymphocyte percentage decreased significantly in 3rd ,6th and 9th weeks in comparison with 0-week in group I, in group II decreased significantly in 3rd week in comparison with 0-week, 6th and 9th weeks, while in groups III decreased significantly in 3rd and 6th weeks in comparison with 0-week and after that significant increased in 9th week in comparison with 3rd and 6th weeks. In group IV significant decreased in 6th week in comparison with 0-week and 3rd week, but significantly increased in 9th week in comparison with the previous weeks. Significantly decreased in 3rd , 6th and 9th weeks in comparison with 0-week in group V. Among groups, at 3rd week significant increased in groups III and IV in comparison with groups I, II and V, but in 6th week significant increased in groups II and V in comparison

with other groups while in 9th week significant increased in groups IV in comparison with groups I, II, III and V(Figure 4-17).

Monocytes percentage increased significantly in 3rd week in comparison with other weeks in group II. In group III significant decreased in 3rd , 6th and 9th weeks in comparison with 0-week, while in group IV significant decreased in 6th and 9th weeks in comparison with 0-week and 3rd week. But in group V decreased significantly in 6th week in comparison with other weeks. Between groups decreased significantly in group V in comparison with other groups in 3rd week. In 6th week significant increased in groups I and II in comparison with III, IV and V groups (Figure 4-18).

Eosinophil percentage decreased significantly in 3rd week in comparison with 0-week, 6th and 9th weeks, in group III significant increased in 6th and 9th weeks in comparison with 0-week and 3rd week, and increased in 6th week in comparison with other weeks in group IV, and increased significantly in 3rd and 9th weeks in comparison with 0-week and 6th week in group V. Between groups significant decreased in group I with other groups and highest value in group V, but at 6th week significant decreased in group II in comparison with other groups and decreased in groups I and V in comparison with groups III and IV, while in 9th week significant increased in group III and V in comparison with I, II and IV. Basophiles percentage showed non significant changes within and between groups (figures 4 –19) (Table 4-8).

Table 4-8: WBC, N%, L%, M%, E% and B% parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III)) and Zinc Oxide (25mg/k (IV) and75mg/k (V)) of goats.

Parameter	Groups	Weeks			
		0	3	6	9
TLC $X10^3/\mu l$	G I	5449±615aA	6471±254bA	5415.5±371aA	6083±382bA
	G II	5910±707aA	5139±282aA	5393±474aA	6971±315bA
	G III	5771±296aA	6010±487aB	5782±326aA	6110±192aA
	G IV	5198±432aA	4821±322aA	6376±757bA	6924±399bA
	G V	5793±384aA	7130±428bBC	5743±357aA	8215±344bcB
N %	G I	42.5±2.5aA	48±2.85bB	43.25±1.70aA	49.5±4.55bBC
	G II	42.75±1.93aA	50.08±2.04bB	45±2.04aA	43.25±2.32aB
	G III	42.5±2.32bA	42±1.77bA	44±3.82bA	35±3.53aA
	G IV	40.75±4.80aA	45.5±3.27bA	43.5±8.10aA	35±5aA
	G V	41±4.41aA	51.25±7.02bB	46±5.40aA	45.25±5.25aB
L %	G I	50.5±1.93bA	43±3.39aA	47.25±4.58aA	44±5.58aA
	G II	49.75±1.31bA	41.25±1.88aA	48.25±1.70bB	49±4.32bA
	G III	52.75±2.17bA	47.5±5.23aB	41.5±3.20aA	54.75±8.75bA
	G IV	52.5±4.52bcA	50.25±2.86bB	43.75±4.62aA	58±5.80bcB
	G V	51.75±2.78bA	43±5.11aA	49.25±4.02aB	48.5±4.57aA
M%	G I	3.75±0.85aA	5±2.38aB	3.5±0.86aB	3.25±0.85aA
	G II	3±0.70aA	4.75±0.75bB	3.25±0.75aB	3.5±0.64aA
	G III	3.25±0.62bA	4±1.82aB	2.25±0.47aA	2.25±1.03aA
	G IV	3.75±0.75bA	3.75±0.25bB	2.25±0.75aA	2.75±1.54aA
	G V	3.25±0.47bA	2.75±0.25bA	1.75±0.25aA	3.5±0.28bA
E %	G I	3±1.91bA	0.75±0.47aA	4.5±2.90bB	2±0.91bA
	G II	3.25±1.31aA	2.5±1.32aB	2.75±0.75aA	2.75±1.18aA
	G III	2.25±1.93aA	3.75±1.43aB	8.75±5.49bBC	6±3.43bB
	G IV	2.5±0.5aA	2.25±0.25aB	7.25±1.60bBC	2.25±0.25aA
	G V	2.75±0.47aA	5±1.68bBC	4±1.41aB	4±0.70bB
B %	G I	0.75±0.47	0.5±0.28	1±0.40	1±0.40
	G II	0.5±0.28	1±0.47	0.5±0.5	0.5±0.28
	G III	1±0.70	1±0.70	1.25±0.25	1±0
	G IV	0.75±0.25	1±0.40	0.75±0.25	0.5±0.28
	G V	0.75±0.25	0.5±0.5	1±0.57	0.75±0.47

Values are M ± SEM: a, b, c, d significant difference at a level of $P < 0.05$ in comparison with in the same group. A, B, C, D significant in comparison between groups, significance at $P < 0.05$.

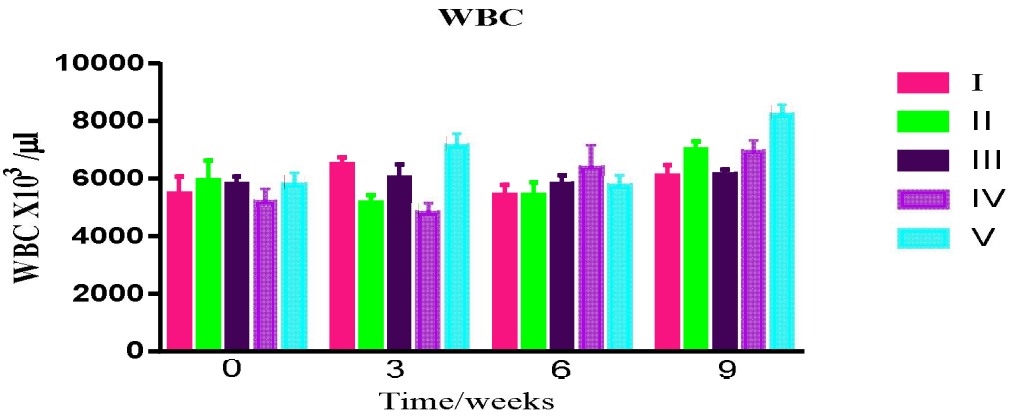


Figure (4-15): Show WBC in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

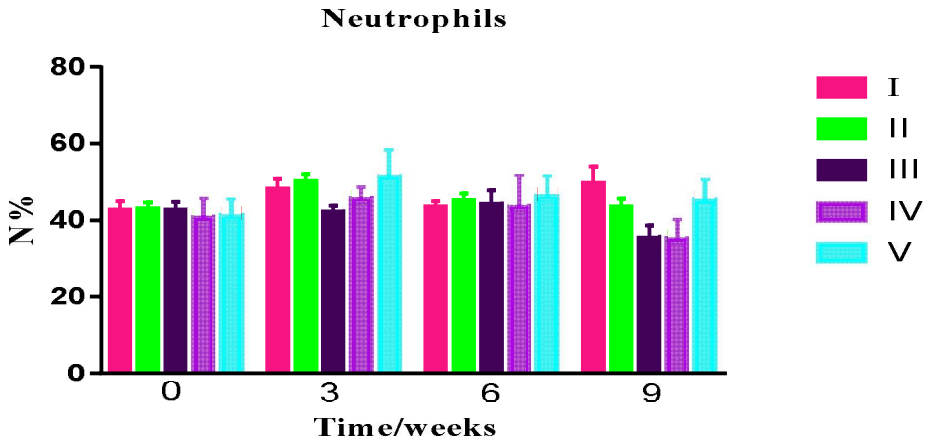


Figure (4-16): Show N% in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

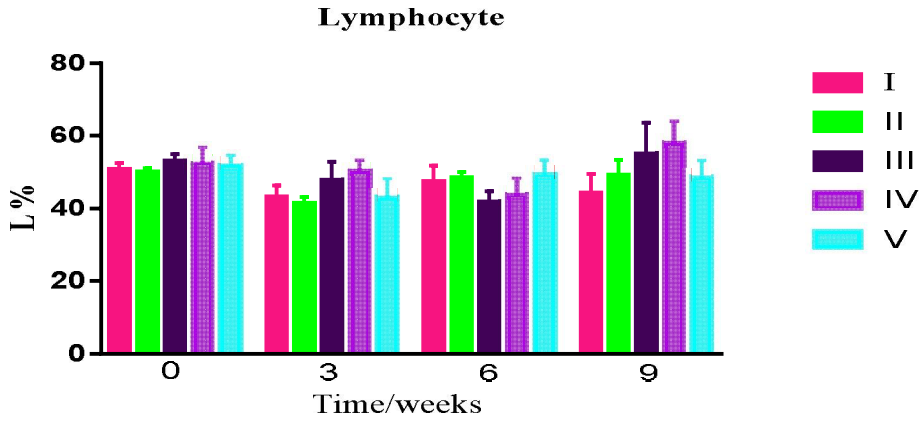


Figure (4-17): Show Lymphocyt % in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

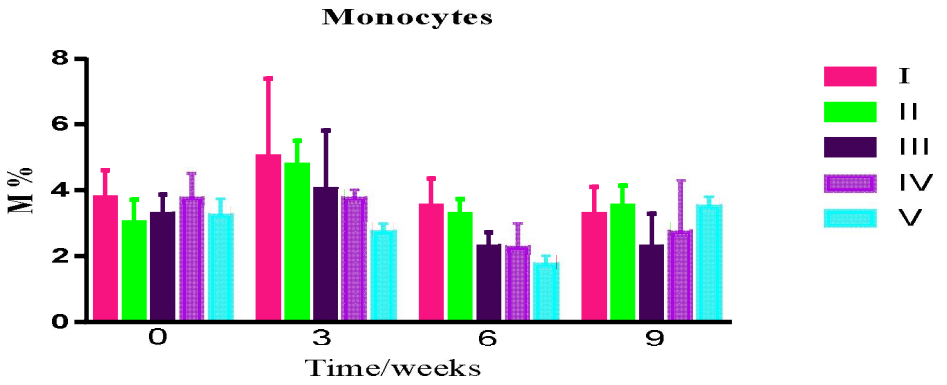


Figure (4-18): Show Monocytes % in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

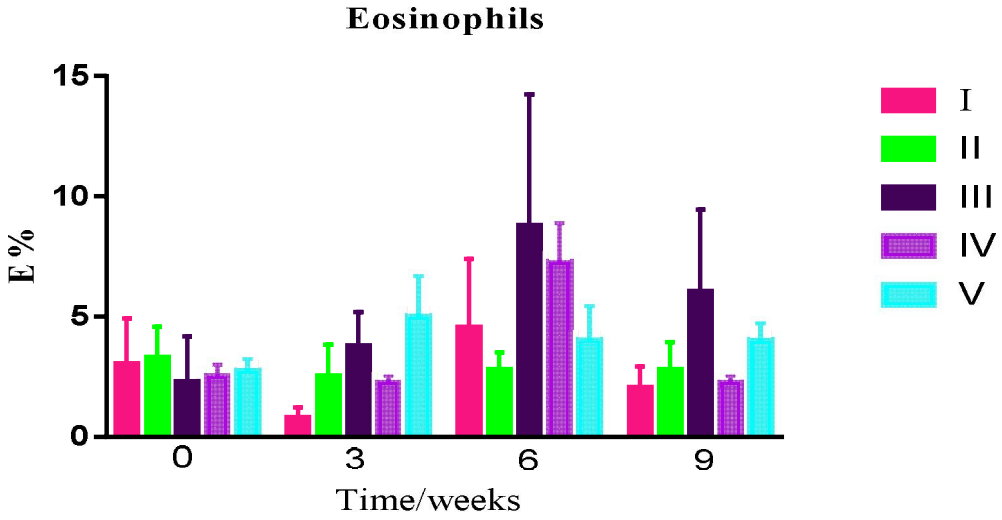


Figure (4-19): Show Eosinophils % in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

Clotting time within groups showed significant increased and highest value was in the 9th week in all groups. Between groups significantly increased in groups I and IV in comparison with groups II, III and V at 6th week only while in the 9th week significant increased in groupI in comparison with other groups (Figure 4-20). Bleeding time was significant increased in 6th and 9th weeks in comparison with 0-week and 3rd weeks in group I, while in group II significant decreased in 3rd , 6th and 9th weeks in comparison with 0-week, but the results of groups III, IV showed non significant changes, while in group V increased significantly in 3rd week in comparison with other weeks. Between groups significant

increased in groups III and V in comparison with groups I, II and IV at 3rd week, and significantly increased in groups I, III and IV in comparison with groups II and V at 6th week, after that in 9th week significant increased in groups I in comparison with other groups (Figure 4-21). Platelet count significantly decreased in 9th week in comparison with 0-week, 3rd and 6th weeks in group I. In group III significant increased in 3rd and 6th weeks in comparison with 0-week and 9th weeks, while in groups IV and V significant decreased in 9th week. Between groups plat let count result showed significant decreased in group V in 3rd week, while in 6th week significant decreased in groups I, IV and V in comparison with groups II, III, and in 9th week significant decreased in group I in comparison with other groups (figures 4-22) (Table 4-9).

Table 4-9: Show Clotting time, Bleeding time and Plat let parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III)) and Zinc Oxide (25mg/k (IV) and75mg/k (V)) of goats.

Parameter / Groups		Weeks			
		0	3	6	9
Clotting time CT /sec	G I	152.5±18.87aA	173.75±13.44aA	192.5±16bA	200±13.98bA
	G II	153±17.55Aa	169.5±15.39aA	167.5±6.29aA	187.5±22.86b A
	G III	151.25±6.25aA	157.5±18.08aA	170±9.12b A	195±5bcA
	G IV	152.5±11.08aA	182.5±19.73bA	200±14.14b B	198.75±17.83bA
	G V	155±6.45aA	155±18.48aA	175±22.17aA	176.25±14.86bA
Bleeding time BT/sec	G I	46.25±3.14a A	50±2.04aA	53.75±2.39bB	53.75±3.75bB
	G II	52±4.78bA	44.5±3.57aA	43.75±2.39aA	47.25±2.62aA
	G III	53.75±3.75aA	53.75±3.14aB	49.25±1.49aB	51.75±3.14aA
	G IV	47.5±4.78aA	52.5±4.78aA	58.75±5.90aBC	51.25±5.15aA
	G V	48.75±5.54aA	57.5±4.33bB	46.25±3.75aA	53.75±5.54aA
Plat let per uL	G I	1249.87±206.14bcA	1021±167.14bB	884.25±126.84bA	273±49.62aA
	G II	1445.25±409.22aA	1151±44.65aB	1194.75±65.88aB	1217.37±39.88aBC
	G III	1097.87±121.75aA	1251±24.86bB	1234.87±122.05aB	989.87±128.62aB
	G IV	1393.5±322.60bA	1098.75±166.66bB	1024.12±165.12bA	641.62±260.56aB
	G V	1490.62±311.81bA	886.87±88.31aA	969±247.64aA	903.75±52.72aB

Values are M ± SEM: a, b, c, d significant difference at a level of $P < 0.05$ in comparison with in the same group. A, B, C, D significant in comparison between groups, significance at $P < 0.05$.

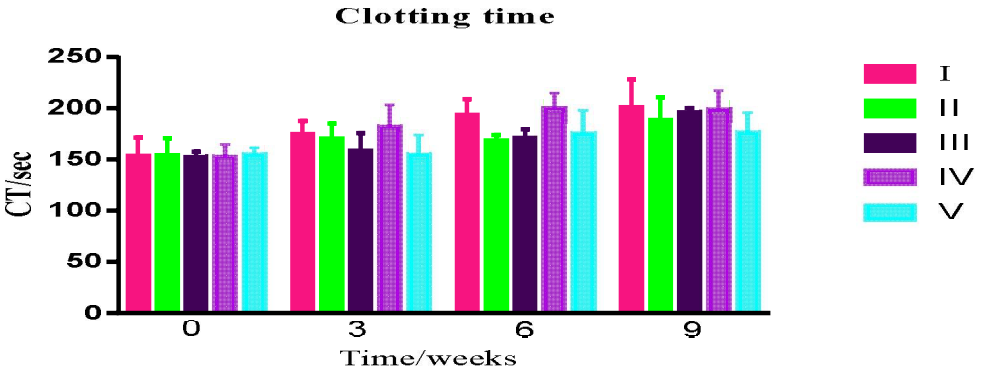


Figure (4- 20): Show clotting time parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

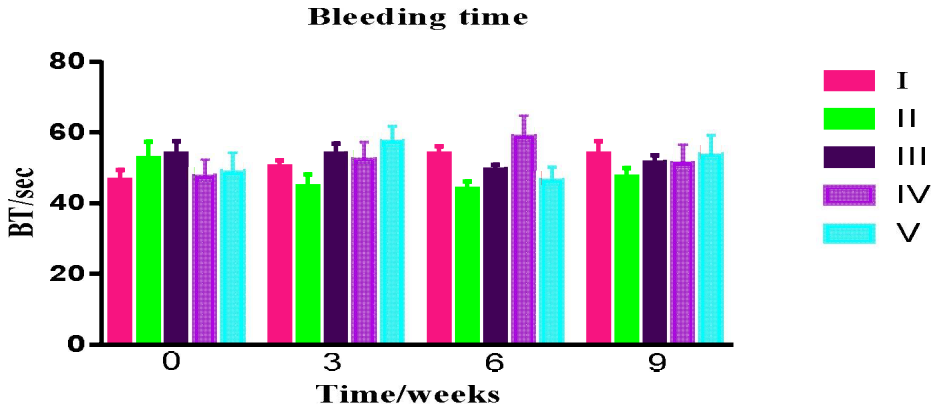


Figure (4- 21): Show bleeding time parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

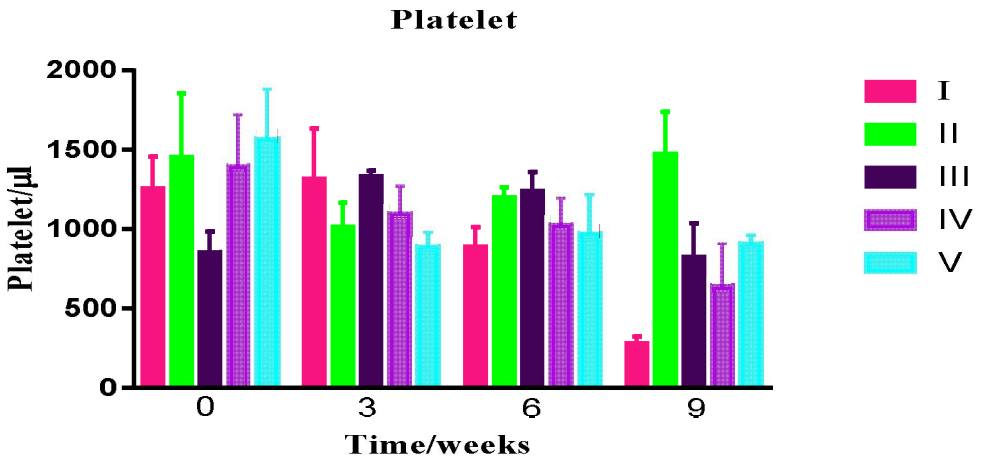


Figure (4- 22): Show platelet parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

4. 3. 3. Results of biochemistry, immunity and antioxidants

Zinc serum level increased significantly with highest value in the 9th week in comparison with the 0-week within groups II, III, IV and V, but in group I showed non significant changes. Among groups, group III and V showed increased significantly in comparison with other groups at 6th week, while in 9th week increased significantly in groups III and V in comparison with groups II and IV, and increased significantly in groups II and III in comparison with groups I, IV and V(Figure 4-23).

Total protein significantly decreased in 3rd ,6th and 9th weeks in comparison with 0-week in group I and IV while in group II, III and V significantly decreased in 3rd and 6th week in comparison with 0-week after that increased significantly in 9th week in comparison with 3rd and 6th weeks. Among groups showed decreased significantly in group V in comparison with other groups at 3rd and 6th weeks, while in 9th week significant increased in group II and III in comparison with groups I, IV and V(Figure 4-24). Serum albumin significantly decreased in II group at 9th weeks in in comparison with 0-week(Figure 4-25).

Globulin significantly decreased in 3rd and 6th weeks in comparison with 0-week and 9th week in groups I, II and III, but in groups IV and V decreased significantly in 6th week in comparison with other weeks. Between groups showed increased significant in groups II and III in comparison with groups I, IV and V figure (4-26) (Table 4-10).

Table 4-10: Show serum Zinc, Total protein, Albumin and Globulin values in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III)) and Zinc Oxide (25mg/k (IV) and75mg/k (V)) of goats.

Parameter	Groups	Weeks			
		0	3	6	9
Zinc µmol/L	G I	6.61±0.51aA	6.72±0.99aA	7.39±1.49aA	10.01±3.84aA
	G II	7.18±0.71aA	7.28±1.14aA	8.04±0.65aA	15.82±1.41bB
	G III	7.81±0.29aA	8.33±1.94aA	12.64±1.18bB	16.86±2.38bcB
	G IV	7.10±0.49aA	7.29±0.67aA	9.21±0.92bA	10.9±1.53bA
	G V	7.08±0.60aA	7.20±1.54aA	10.89±1.73bB	13.67±2.13bcA
Total protein g/dL	G I	5.84±0.53bA	5.17±0.24aB	5.28±0.45aB	5.18±0.09aA
	G II	6.60±0.15bcA	5.17±0.22aB	5.45±0.20aB	5.77±0.30bB
	G III	6.53±0.24bcA	5.04±0.27aB	5.28±0.31aB	5.92±0.28bB
	G IV	6.84±0.21bA	5.76±0.74aB	5.04±0.26aB	5.19±0.66aA
	G V	6.30±0.67bcA	4.62±0.15aA	4.59±0.12aA	5.37±0.29bA
Albumin g/dL	G I	3.64±0.22aA	4.13±0.09bA	4.23±0.15bA	3.64±0.06aA
	G II	4.17±0.26bA	4.14±0.15aA	4.03±0.33aA	3.67±0.10aA
	G III	3.94±0.20aA	3.88±0.12aA	3.94±0.05aA	3.64±0.15aA
	G IV	3.82±0.19aA	3.93±0.15aA	4.15±0.15aA	3.3±0.12aA
	G V	3.93±0.10aA	3.43±0.16aA	4.12±0.07aA	3.69±0.23aA
Globulin g/dL	G I	2.20±0.27bcA	1.04±0.19aA	1.05±0.38aA	1.53±0.05bA
	G II	2.43±0.28bA	1.03±0.15aA	1.42±0.42aA	2.09±0.23bB
	G III	2.59±0.05bA	1.15±0.15aA	1.34±0.36aA	2.27±0.26bB
	G IV	3.01±0.19bcA	1.83±0.83bA	0.89±0.14aA	1.89±0.58bA
	G V	2.37±0.61bcA	1.18±0.13bA	0.47±0.15aA	1.68±0.40bA

Values are M ± SEM: a, b, c, d significant difference at a level of $P < 0.05$ in comparison with in the same group. A, B, C, D significant in comparison between groups, significance at $P < 0.05$.

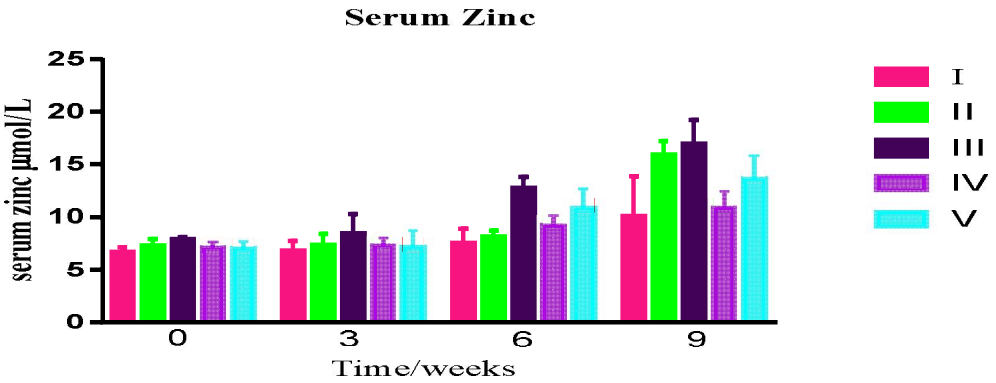


Figure (4-23): Shown serum Zinc values in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

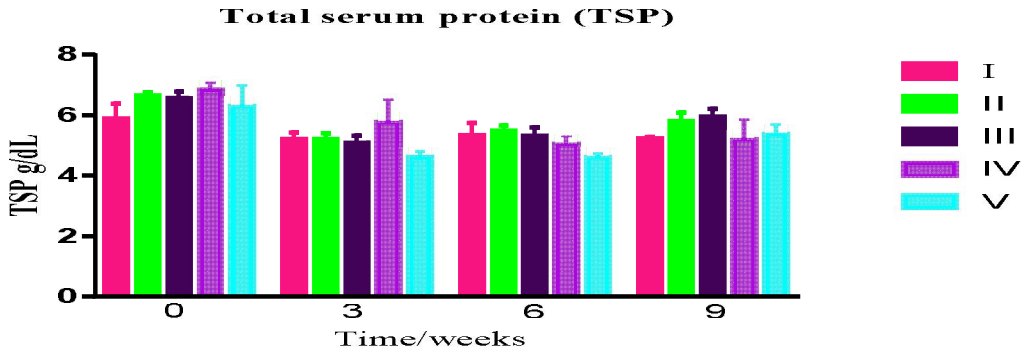


Figure (4-24): Show Total serum protein values in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

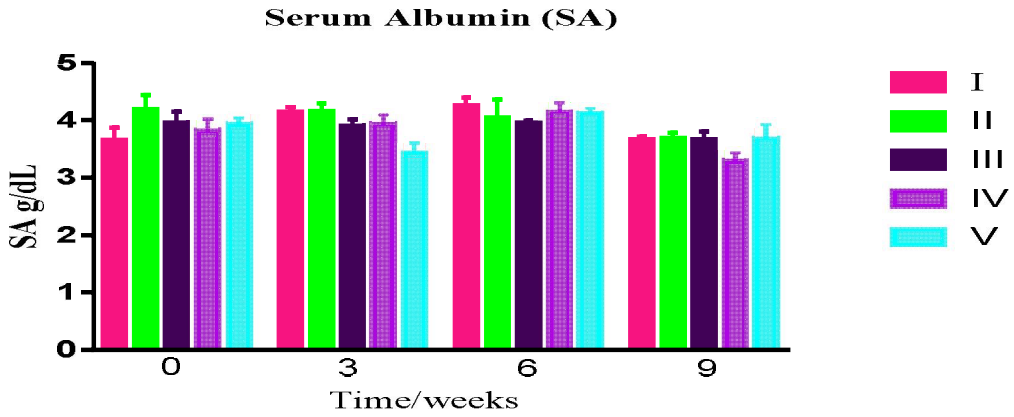


Figure (4-25): Show serum Albumin values in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

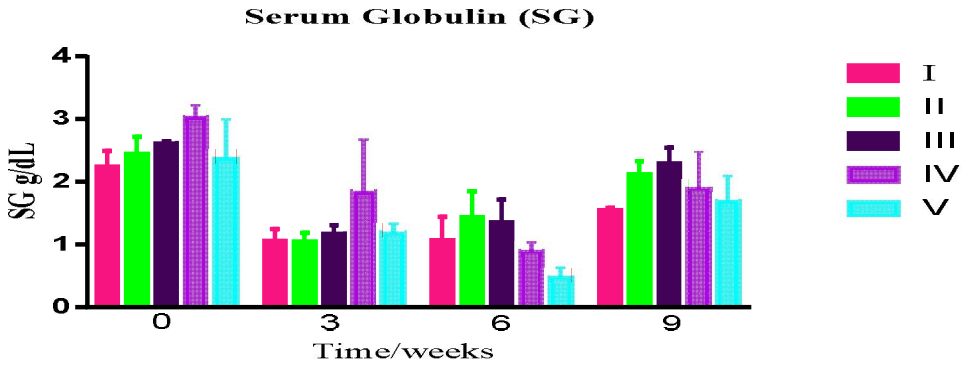


Figure (4-26): Show serum Globulin values in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

The results of comet assay showed lowest amount of DNA damage in treated groups in comparison with control (table 4 – 11) (Figure 4-27(a, b, c, d, e)).

Table 4 – 11: Show Comet assay% parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats at 9th week post treatment.

Parameter	Groups		
		No damage %	Damage %
Comet assay %	G I	87.42	12.58
	G II	88.11	11.89
	G III	88.89	11.11
	G IV	88.77	11.23
	G V	89.0	11.0

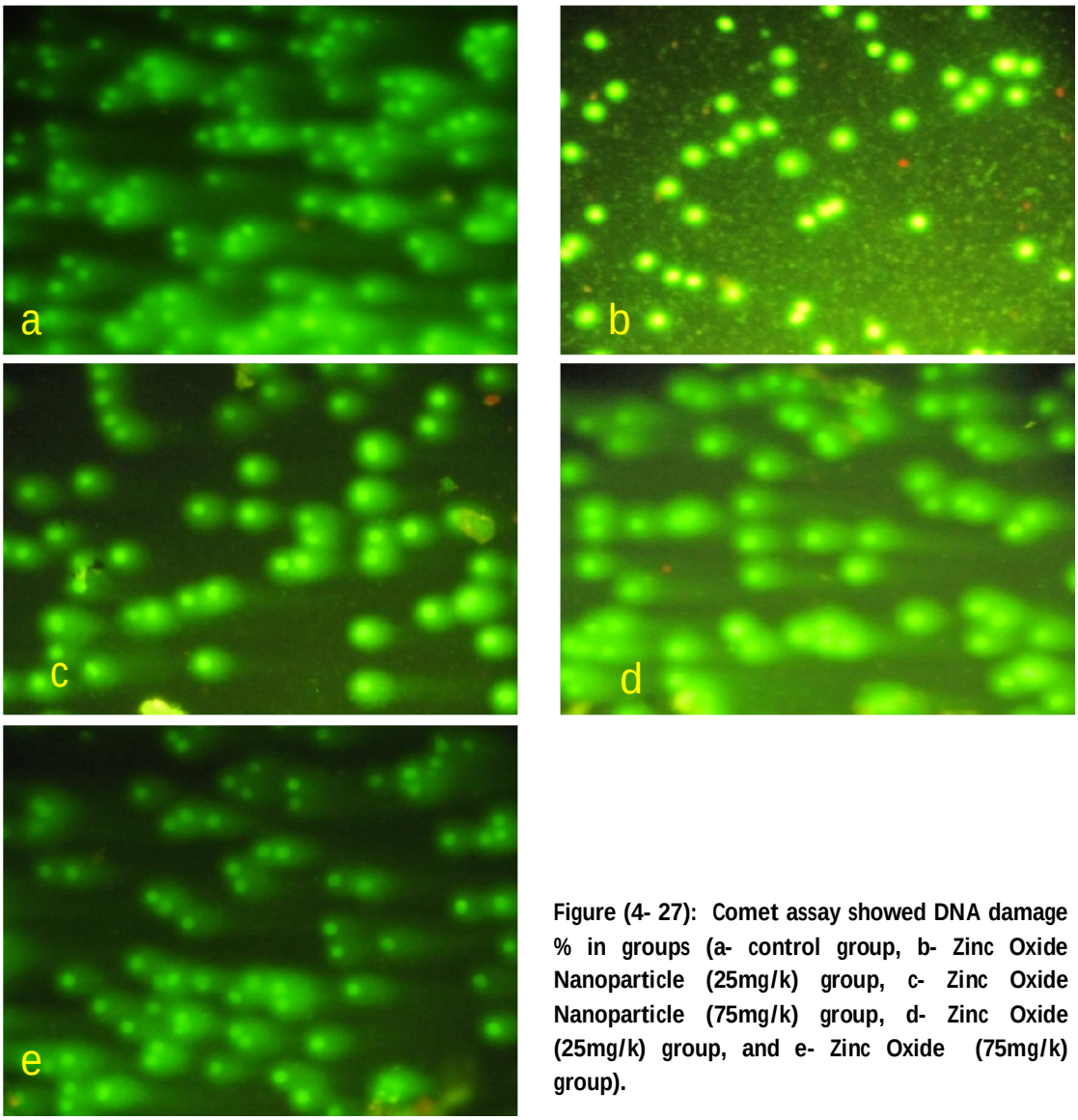


Figure (4- 27): Comet assay showed DNA damage % in groups (a- control group, b- Zinc Oxide Nanoparticle (25mg/k) group, c- Zinc Oxide Nanoparticle (75mg/k) group, d- Zinc Oxide (25mg/k) group, and e- Zinc Oxide (75mg/k) group).

The results of phagocyte index (neutrophils containing the formazan deposit) showed the formazan deposit as one or more large black, different in shape and size deposit in the cytoplasm of the cell (Figure 4-28(a, b, c)). Phagocyte index within groups showed increased

significantly in 9th week in treated and control groups in comparison with 0-week. but groups showed significant increased in groups III and IV in comparison with groups I, II, and V at 9th week, and highest value was in group III (Figure 4-29). Interleukin 1 Beta (IL-1B) value within groups showed significantly increased at the weeks progressed up and down to the 9th week in comparison with 0-week, but between groups the results showed that treatment groups (II, III, IV and V) lowest values in comparison with control group (I) (figures 4-30)(Table 4-12).

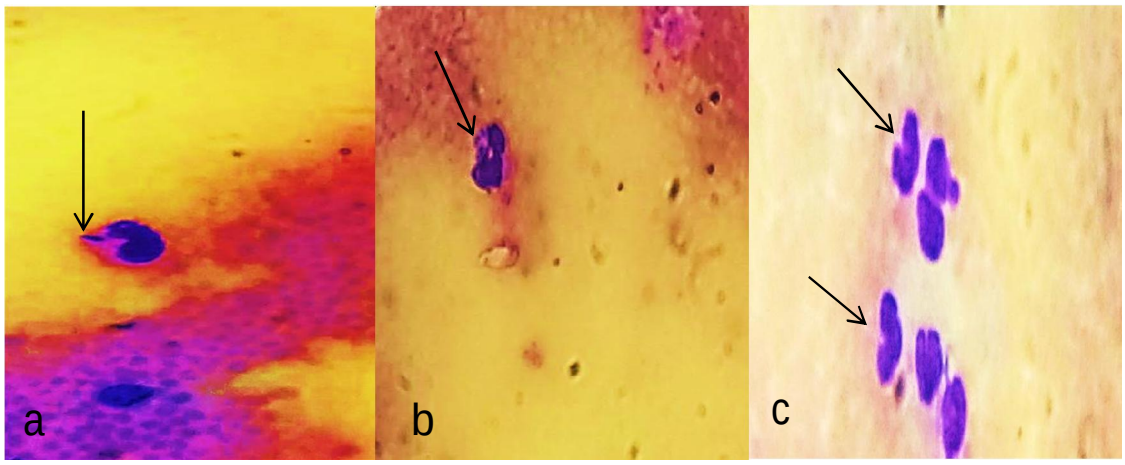


Figure (4- 28): Show (a, b) positive Phagocyte and (c) negative phagocyte of neutrophils

Table 4-12: Show Phagocyte index and IL-1B parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III)) and Zinc Oxide (25mg/k (IV) and75mg/k (V)) of goats.

Parameter / Groups		Weeks			
		0	3	6	9
Phagocyte index %	G I	14.08±1.51bA	13.69±0.89bA	13.43±2.16aA	16.78±1.39bA
	G II	14.6±1.22aA	14.33±3.17aA	15.01±0.57aA	19.85±3.88bA
	G III	14.46±0.98aA	14.46±1.87aA	21.60±3.13bB	22.39±1.59bBC
	G IV	14.83±0.57aA	16.25±0.97bB	17.76±2.87bB	19.91±1.31bcB
	G V	13.65±2.07aA	13.44±1.73aA	26.24±8.27bcBC	16.50±2.06bA
IL-1B ng/L	G I	64.25±11.75aA	157.13±51.50bA	113.70±27.31bB	321.30±45.38bcB
	G II	58.94±5.40aA	159.38±17.92bcB	137.58±6.06bBC	133.57±35.04bA
	G III	63.61±12.25aA	157.97±23.52bB	157.61±7.53bBCD	130.16±30.69bA
	G IV	59.60±6.83aA	161.78±38.96bcB	108.02±39.33bB	130.41±41.57bA
	G V	61.51±17.05aA	110.11±26.11bA	85.38±38.33aA	123.08±28.97bA

Values are M ± SEM: a, b, c, d significant difference at a level of $P < 0.05$ in comparison with in the same group. A, B, C, D significant in comparison between groups, significance at $P < 0.05$.

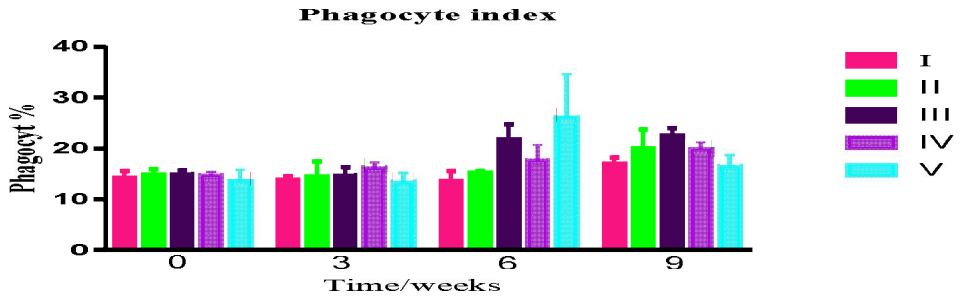


Figure (4- 29): Show Phagocyte index parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

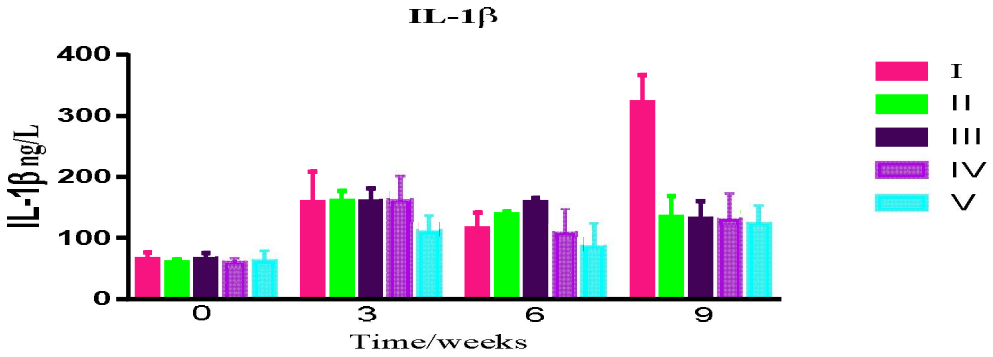


Figure (4- 30): Show IL-1B parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

Total antioxidant values within groups increased significantly 9th week in all groups except group V which showed non significantly increased in comparison with 0-week. Between groups, showed significant increased in group II in comparison with groups I, III, IV and V at 9th week (Table 4 – 13).

Table 4 – 13: Show Total antioxidant parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

Parameter	Groups	Time	
		0- week	9- weeks
Total antioxidant µL	G I	1434.79±52.55aA	1582.02±27.13bA
	G II	1430.64±29.54aA	1625.28±14.73bB
	G III	1401.31±16.29aA	1607.20±22.65bA
	G IV	1421.88±91.79aA	1605.26±36.86bA
	G V	1480.64±105.81aA	1555.54±45.65aA

Values are M ± SEM: a, b, c, d significant difference at a level of P < 0.05 in comparison with in the same group. A, B, C significant in comparison between groups, significance at P < 0.05.

4. 3. 4. The results of wounds healing

4. 3. 4.1. Macroscopic results

The results of circular wounds gradually narrowing in sigificatly level in its diameters, with the narrowest diameters were in 5th week in comparison with 0-week within each group. While between groups, all treated groups showed narrowest diameters in comparison with the control values (group I) (Table 4 – 14). Data of macroscopic appearance follow-up in the period of induced wounds showed, inflammatory signs in wounded area with systemic reaction, characterized by anorexia, depression and lethargy in the first three days post operation, when disappeared gradually within first 3 days to become within normal values. At 3rd to 7th days the wound healing showed normal response to injury (inflammatory phase), local inflammatory reaction persisted and were graded from slight to moderate inflammatory swelling with bloody clots formation, inflammatory exudate without signs of infection and the wound itself did not ooze in all groups of animals during this period (Figure 4- 31).

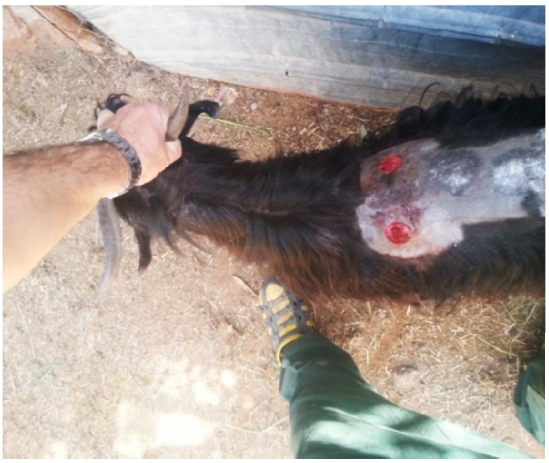


Figure (4- 31): Show wounded area moderat swelly with bloody clot formation in the 3rd day post operation.

Table 4 – 14: Wounds dimension parameters in groups control (I), Zinc Oxide Nanoparticle (25mg/k (II) and 75mg/k (III) and Zinc Oxide (25mg/k (IV) and75mg/k (V) of goats.

Parameter	Groups	Time				
		1st week	2nd weeks	3rd weeks	4th weeks	5th weeks
Wounds dimension mm	G I	32.75±1.05bcdA	29.75±1.49bcdB	20.87±1.80bcB	14.25±0.25bB	9.37±1.43aBC
	G II	32.87±1.68bcdA	29.62±1.24bcdB	20.62±1.46bcB	9.75±0.52bA	6.5±0.45aB
	G III	32.62±1.37bcdA	28.37±2.61bcdB	18±1.77bcA	9.62±2.89bA	3±1.41aA
	G IV	31.25±1.63bcdA	28.12±0.89bcdB	18.87±0.82bcA	10.87±0.80bA	5.5±1.67aB
	G V	30.75±2.41bcdA	25.12±1.06bcdA	19.12±2.33bcA	13.12±1.35bB	7.37±1.46aB

Values are M ± SEM: a, b, c, d significant difference at a level of $P < 0.05$ in comparison with in the same group. A, B, C, D significant in comparison between groups, significance at $P < 0.05$.

At 15th day post operation, diameters of wound were decreased, wounds were remodeled , made up new blood vessels, and had eventually scar formation, and in 30th day complete closed to the circle of wounds and infused of its edges and significant decreased in diameters of scar tissue, and the treated with Zinc oxide Nano particles group III revealed the best wound healing (Figure 4- 32, 33, 34, 35, 36).

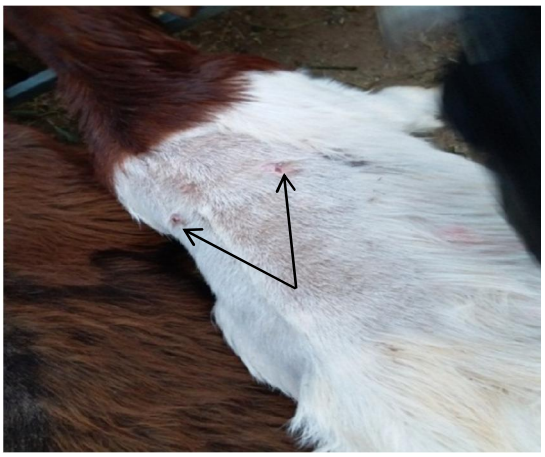


Figure (4- 32): Show multiple scar formation in th healed site of group I at 30 day post operation.

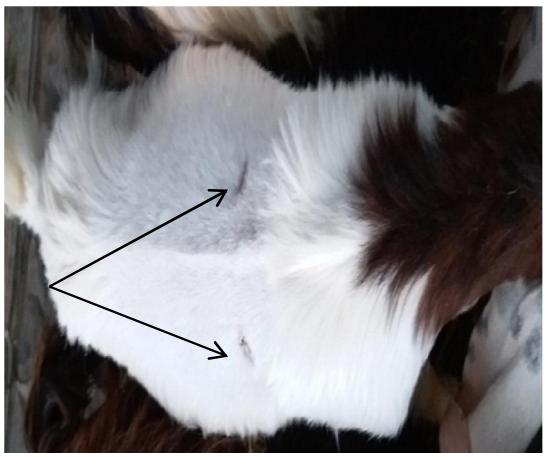


Figure (4- 33): Show complet closure in healed site of group II at 30 day post operation.

Figure (4- 34): Best wound healing of group III at 30 day post operation.

Figure (4- 35): Limited scar area in group IV at 30 day post operation.

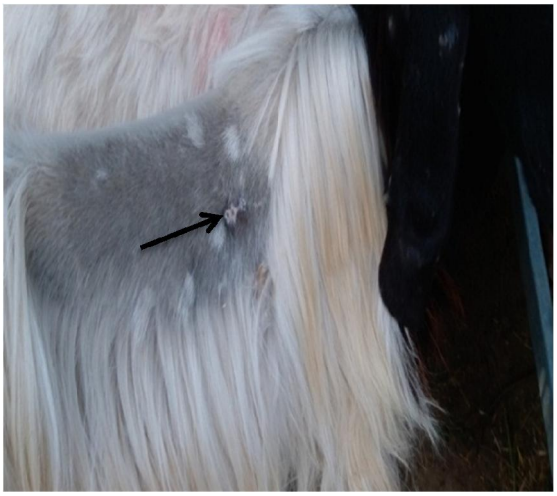


Figure (4- 36): Small scar tissue in group V at 30 day post operation.

At day 40th, the results showed disappear of wounds and as the normal skin and hair in group III, but the other groups little markets appear in wounds location. The current study also reported that wounds of group III were some how similar to normal skin, with less hypertrophic scarring and nearly normal hair on the skin surface. Although, the clinical sings showed that similar in wounds healing of treated and control groups but, treated with Zinc oxide Nano particles group III revealed the best wound healing (Table 4 – 15) (Figure 4- 37, 38, 39, 40,41).

Table 4 – 15: Macroscopic score of wounds healing at 45 POD in control and treated groups of goats

Time	No	Groups				
		I	II	III	IV	V
45 POD	1	Scar 3mm	Healed nearly	Better healing ++ve	Healed nearly	Scar 3mm
	2	Scar 5mm	Scar 3mm	Better healing ++ve	Scar 4mm	Healed nearly
	3	Scar 3mm	Scar 3mm	Healed nearly	Scar 5mm	Healed nearly
	4	Scar 3mm	Healed nearly	Healed nearly	Scar 3mm	Scar 3mm
	5	Scar 4mm	Healed nearly	Healed nearly	Scar 3mm	Scar 3mm

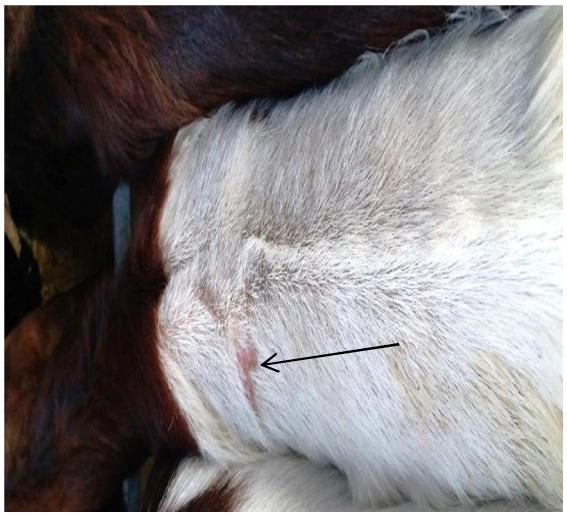


Figure (4- 37): Moderat wound clusur in group I at 40 day post operation.

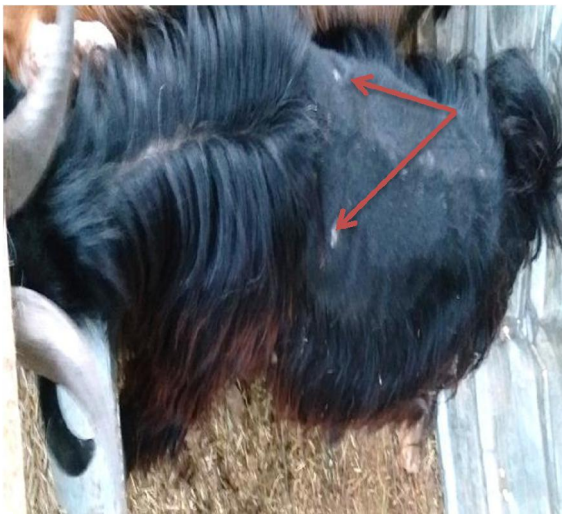


Figure (4- 38): Limited scar tissue with partial hair replacement in group II at 40 day post operation.

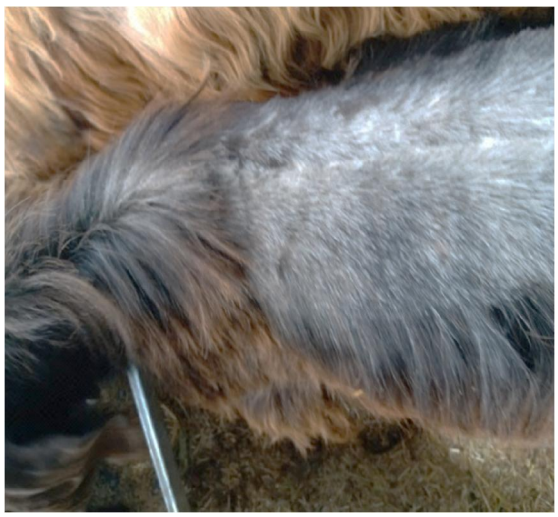


Figure (4- 39): Normal skin appearance with hair replacement in group III at 40 day post operation.

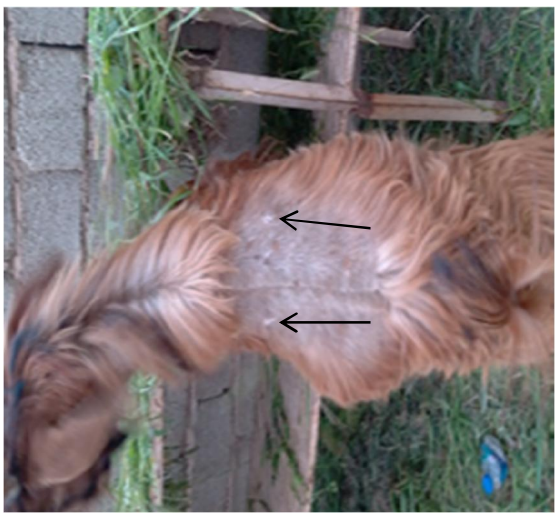


Figure (4-40): Reminant of scar tissue appearance in group IV at 40 day post operation.

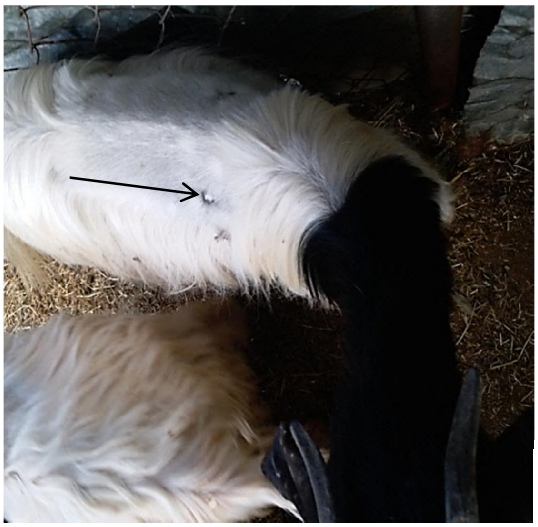


Figure (4- 41): Limited scar with moderate hair growth in group V at 40 day post operation.

4. 3. 4.2. Histopathological results

In the current study, the histological examination was performed via comparison between non treated (control group) and treated wound healing at different times and different concentration of substances. At day 3, indicated presence of inflamation cells, accumulation of exudates, regeneration of epidermis, and proliferation of fibrous connective tissue were observed to understand the normal healing process, so the infiltration of reactive cells including neutrophils, macrophages, and lymphocytes were present in control and treated groups (Table 4 – 16). The progress of wound healing in the control section showed large area of dermal tissue uncovered by epidermal tissue. This area was observed covering with the necrotic debris and accumulation of fibrin, and it was also invaded with immature blood capillaries and occupied by granulation tissues (Figure 4-42). In contrast, at the same time wound healing treated by 25mg/kg Zinc Oxide Nano particles revealed some detectable progression. This progression was observed via development of new matrix deposition along with aggregation of many proliferating fibroblasts and less amount of necrotic debris. The same results were indicated in wound healing treated by 75mg/kg Zinc Oxide Nano particles, but with much more progression in the formation and organization of proliferating fibroblast, it was also showed that there were moderate amounts in the numbers of inflammatory cells

(figure 4-43, 44). Additionally, treated wound with 25mg/kg Zinc Oxide showed slightly to moderate changes in the progression of wound healing comparison with previous treatments (figure 4-45,46).

Table 4 – 16: Histopathological score of wounds healing at 3 POD in control and treated groups of goats

Time	Group	parameters								
		Agt	Ii	Re	Ic	Fbf	Nv	Cd	Nt	Sca
3 day	I	2	1	0	marked	0	mild	mild	+	0
	II	2	2	0	moderate	mild	moderate	mild	+	0
	III	2	2	0	moderate	mild	moderate	mild	+	0
	IV	2	2	0	mild	mild	moderate	mild	+	0
	V	2	1	0	marked	mild	moderate	mild	+	0

Agt: Amount of granulation tissue. Ii: Inflammatory infiltrate. Re: re-epithelization. Ic: Inflammatory cells. Fbf: fibroblasts formation. Nv: Neo vacuolization. Cd: collagen deposition. Nt: Necrotic tissue. Sca: Skin cell assessment. Amount of granulation tissue (profound-1, moderate-2, scanty-3, absent-4). Inflammatory infiltrate (plenty 1, moderate 2, a few 3). Re-epithelialization: migration of keratinocytes-1, bridging of cells-2, keratinization-3. Inflammatory cells: absence/presence (mild/moderate/marked). Fibroblasts: absence/presence (mild/moderate/marked). New vessels: absence/presence (mild/moderate/marked). Collagen: absence/presence (mild/moderate/marked).

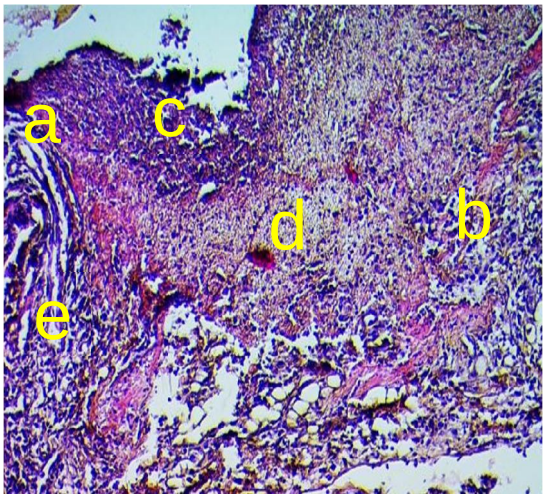


Figure (4-42): Photomicrograph shows the progression of normal wound healing (control (GI)) at day 3, (a) represents the thin fibrin, while (b) indicated the infiltration of inflammatory cells, (c) ulcer formation, (d) represent granulation tissue, and(e) vascular dermal tissue (H &E: 10 X).

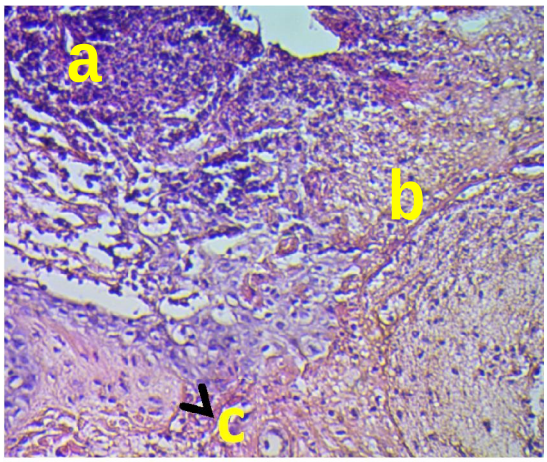


Figure (4-43): Photomicrograph illustrates the process of wound healing (GII) at day 3, (a) necrotic tissue, (b) represents granulation tissue,(c) blood vessels. (H &E: 10 X).

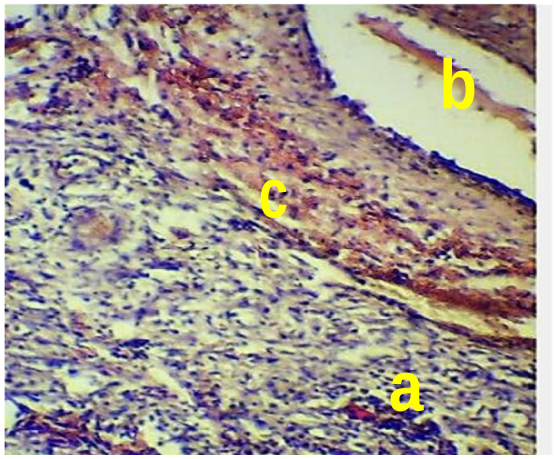


Figure (4-44): Photomicrograph illustrates the process of wound healing (GIII) at day 3, (a) indicated the infiltration of inflammatory cells, (b) epidermis (c) represents granulation tissue. (H &E: 40 X).

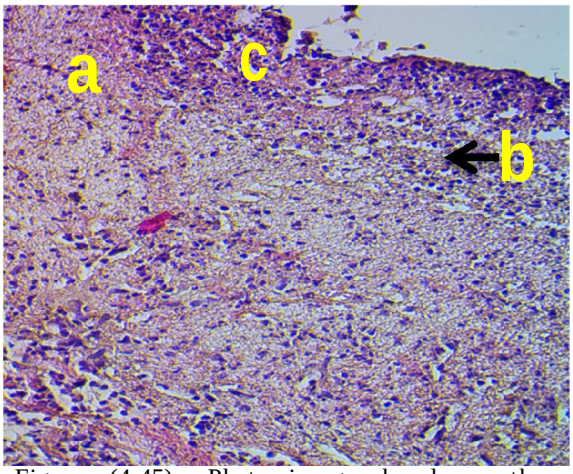


Figure (4-45): Photomicrograph shows the progression of wound healing (GIV) at day 3, (a) represents the thin fibrin, while (b) indicated the infiltration of inflammatory cells, (c) indicated necrotic tissues. (H &E: 40 X).

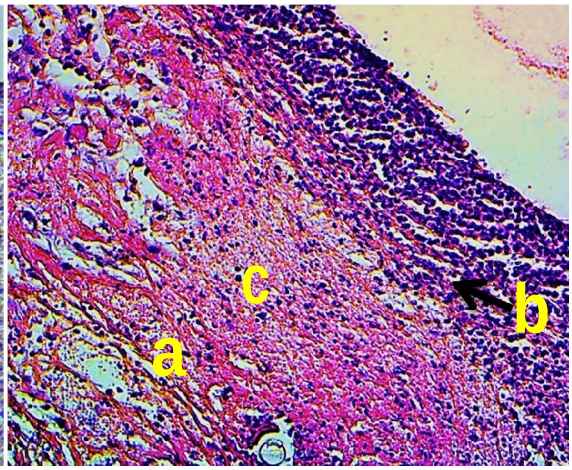


Figure (4-46): Photomicrograph shows the progression of normal wound healing (GV) at day 3, (a) immature granulation tissue, while (b) indicated the infiltration of inflammatory cells, (c) represents granulation tissue. (H &E: 40 X).

At day 7, indicated that the progression of wound healing in the control section was observed still covering with the necrotic debris and accumulation of fibrin, and granulation tissues, so fibroblast increased and inflammatory cells decreased in comparison with control groups at day 3 (figure 4-47). While, at the same time wound healing treated by 25mg/kg Zinc Oxide Nano particles revealed some visible progression. This progression was observed via development and increasing in the amounts of matrix deposition along with aggregation of many proliferating mature fibroblasts, blood capillary and less amount of inflammatory

cells. The same results were showed in wound healing treated by 75mg/kg Zinc Oxide Nano particles, but with much more progression in the formation and organization of mature fibroblast, and it was also showed that there were moderate amounts in the numbers of inflammatory cells (figure 4-48, 49). While treatment with 25mg/kg Zinc Oxide showed slightly to moderate changes in the progression of wound healing in comparison with previous treatments. However, the progress of wound healing in group treated by 75mg/kg Zinc Oxide revealed that there was a clear progression in the cellularity of dermal tissue and an abundant of mature fibroblast. This stage also indicated a clear diminished in the numbers of inflammatory cells and observed an increasing in the amounts of matrix deposition a long with new mature blood capillaries (figure 4-50,51) (Table 4 – 17).

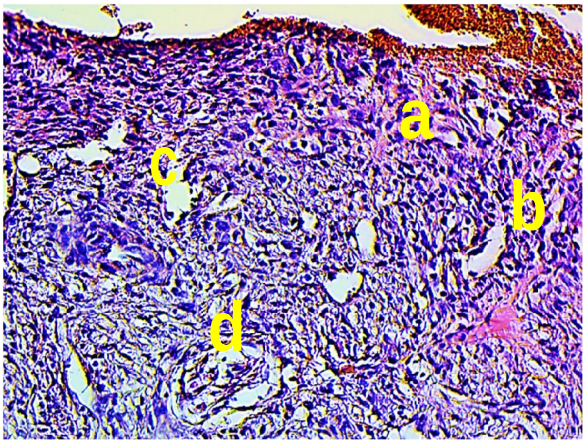


Figure (4-47): Photomicrograph shows the progression of normal wound healing (control (GI)) at day 7, (a) represents the thin fibrin, while (b) indicated the infiltration of inflammatory cells, (c) indicated fibroblast, whereas (d) immature granulation tissue. (H &E: 40 X).

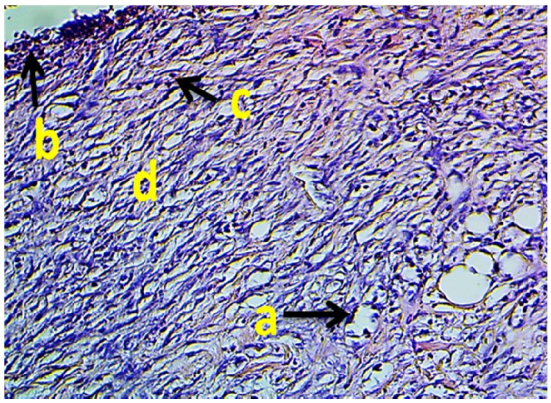


Figure (4-48): Photomicrograph shows the progression of wound healing (GII) at day 7, (a) represents blood vessels, while (b) indicated mild infiltration of inflammatory cells, (c) indicated fibroblast, (d) mmature granulation tissue (H &E: 40 X).

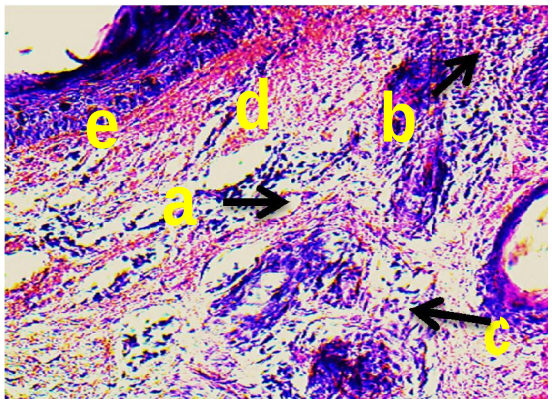


Figure (4-49): Photomicrograph shows the progression of wound healing (GIII) at day 7, (a) represents blood vessels, while (b) indicated mild infiltration of inflammatory cells, (c) indicated fibroblast, (d) represents granulation tissue, (e) mild re-epithelization (H &E: 10 X).

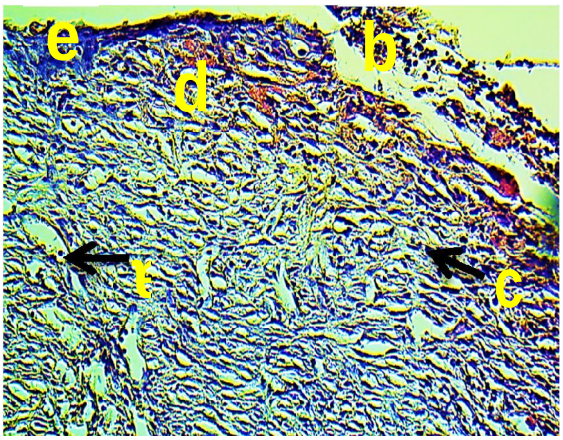


Figure (4-50): Photomicrograph shows the progression of wound healing (GIV) at day 7, (a) represents blood vessels, while (b) indicated mild infiltration of inflammatory cells, (c) indicated fibroblast, (d) represents granulation tissue, (e) mild epidermal epithelization (H &E: 40 X).

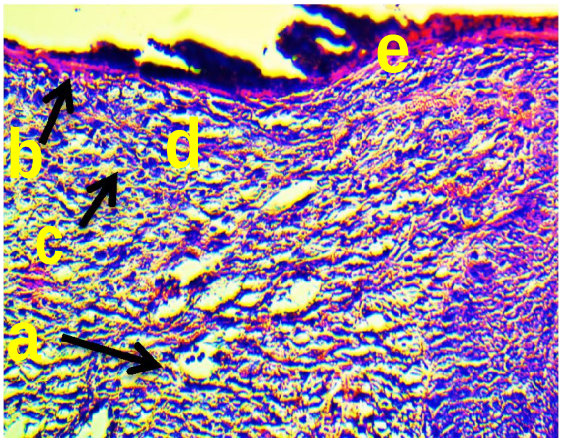


Figure (4-51): Photomicrograph shows the progression of wound healing (GV) at day 7, (a) represents blood vessels, while (b) indicated mild infiltration of inflammatory cells, (c) indicated fibroblast, (d) represents granulation tissue, (e) re-epithelization (H &E: 10 X).

Table 4 – 17: Histopathological score of wounds healing at 7 POD in control and treated groups of goats

Time	Group	parameters								
		Agt	Ii	Re	Ic	Fbf	Nv	Cd	Nt	Sca
7 day	I	2	2	1	moderate	moderate	moderate	moderate	+	Epidermal migration and proliferating cell
	II	1	3	1	mild	moderate	marked	moderate	–	Epidermal migration and proliferating cell
	III	1	3	2	mild	marked	marked	marked	–	Epidermal migration and proliferating cell
	IV	2	3	2	mild	marked	marked	moderate	+	Epidermal migration and proliferating cell
	V	2	2	3	moderate	marked	marked	marked	–	Epidermal migration and proliferating cell

Agt: Amount of granulation tissue. Ii: Inflammatory infiltrate. Re: re-epithelization. Ic: Inflammatory cells. Fbf: fibroblasts formation. Nv: Neo vacuolization. Cd: collagen deposition. Nt: Necrotic tissue. Sca: Skin cell assessment. Amount of granulation tissue (profound-1, moderate-2, scanty-3, absent-4). Inflammatory infiltrate (plenty 1, moderate 2, a few 3). Re-epithelialization: migration of keratinocytes-1, bridging of cells-2, keratinization-3. Inflammatory cells: absence/presence (mild/moderate/marked). Fibroblasts: absence/presence

(mild/moderate/marked). New vessels: absence/presence (mild/moderate/marked). Collagen: absence/presence (mild/moderate/marked).

At day 14, the progress in wound healing showed the clear generation of thin epidermal tissue. This layer of re-epithelization of epidermal tissue covering the wound with scar tissue formation, addition to that the collagen and fibroblast in dermal tissue were appeared more organization and replacement of the initial fibrin matrix with collagen rich granulation tissue (Table 4 – 18), decreased inflammatory cells in control group (figure 4-52). While, in wounds healing treated by 25mg/kg Zinc Oxide Nano particles at the same time revealed more detectable progression. This progression was observed via development of mild thin epidermal re-epithelization completely covering the wound, collagen and fibroblast more organization along with aggregation of many mature fibroblasts, blood capillary and less amount of inflammatory cells. The same results were indicated in wound healing treated by 75mg/kg zinc oxide nano particles, but it was observed an obvious formation and organization of mature fibroblast, thin walled capillary and formation of a new hair follicles (figure 4-53, 54). Additional treatment with 25mg/kg zinc oxide showed degeneration changes in the development of wound healing contrast with previous treatments. However, the progress of wound healing in group treated by 75mg/kg zinc oxide revealed that there were advance development in the re-epithlization and formation of new skin layer but still there was this layer of hyperplastic area (figure 4-55,56).

Table 4 – 18: Histopathological score of wounds healing at 14 POD in control and treated groups of goats

Time	Group	parameters								
		Agt	Ii	Re	Ic	Fbf	Nv	Cd	Nt	Sca
14 day	I	1	1	2	marked	marked	moderate	mild	_	Epidermal migration and hyperplasia
	II	1	3	2	absence	marked	marked	mild	_	Epidermal migration and hyperplasia
	III	0	3	3	absence	marked	marked	moderate	_	Granulation tissue formation and Epidermal hyperplasia Collagen fiber deposition
	IV	2	2	2	mild	marked	marked	moderate	+	Epidermal migration and hyperplasia
	V	2	2	2	mild	marked	marked	marked	_	Granulation tissue formation and Epidermal hyperplasia Collagen fiber deposition

Agt: Amount of granulation tissue. Ii: Inflammatory infiltrate. Re: re-epithelization. Ic: Inflammatory cells. Fbf: fibroblasts formation. Nv: Neo vacuolization. Cd: collagen deposition. Nt: Necrotic tissue. Sca: Skin cell assessment. Amount of granulation tissue (profound-1, moderate-2, scanty-3, absent-4). Inflammatory infiltrate (plenty 1, moderate 2, a few 3). Re-epithelialization: migration of keratinocytes-1, bridging of cells-2, keratinization-3. Inflammatory cells: absence/presence (mild/moderate/marked). Fibroblasts: absence/presence (mild/moderate/marked). New vessels: absence/presence (mild/moderate/marked). Collagen: absence/presence (mild/moderate/marked).

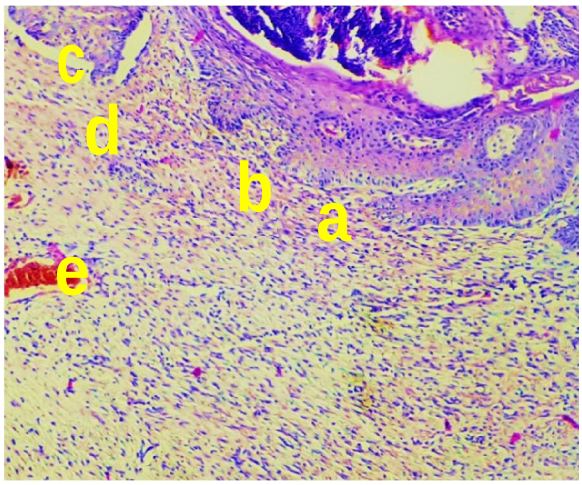


Figure (4-52): Photomicrograph shows the progression of normal wound healing (control(GI) at day 14, (a) thin epidermal tissue, while (b) indicated granulation tissue, whereas (c) scar tissue, (d) infiltration of inflammatory cells, (e) hemorrhage. (H &E: 10 X).

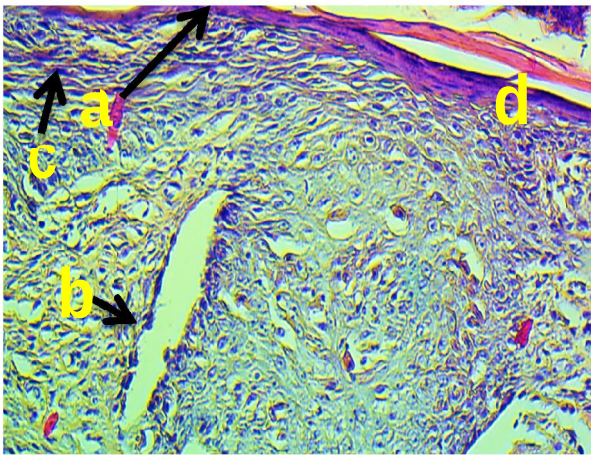


Figure (4-53): Photomicrograph shows the progression of wound healing (GII) at day 14, (a) thin epidermal tissue, while (b) represents blood vessels, (c) indicated fibroblast, (d) mild re-epithelization (H &E: 40 X).

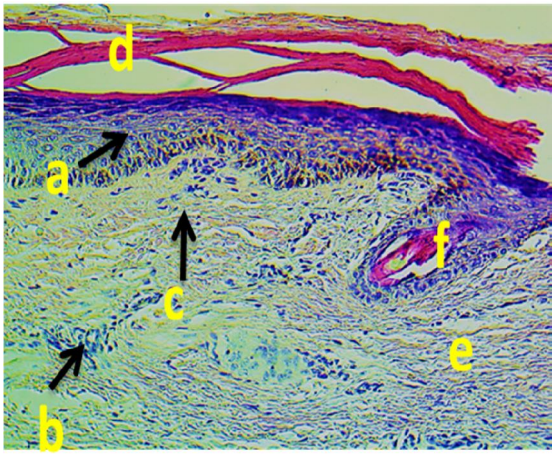


Figure (4-54): Photomicrograph shows the progression of wound healing (GIII) at day 14, (a) epidermal tissue, while (b) represents blood vessels, (c) indicated fibroblast, whereas (d) keratin, (e) organization of collagen fibers, (f) generation of new hair follicle. (H &E: 10 X).

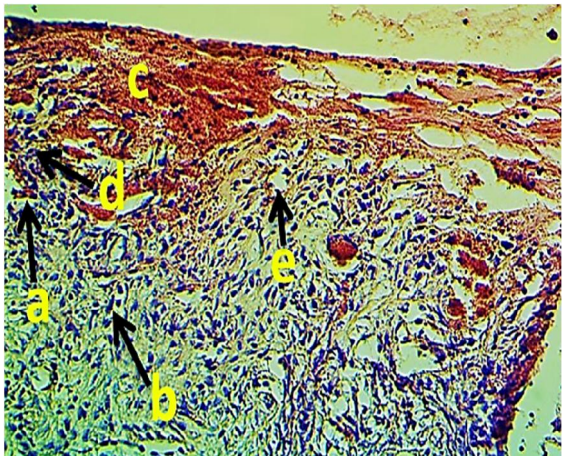


Figure (4-55): Photomicrograph shows the progression of wound healing (GIV) at day 14, (a) thin epidermal tissue, while (b) indicated fibroblast, whereas (c) necrotic debris, (d) inflammatory cells,(e) blood vessels. (H &E: 40 X).

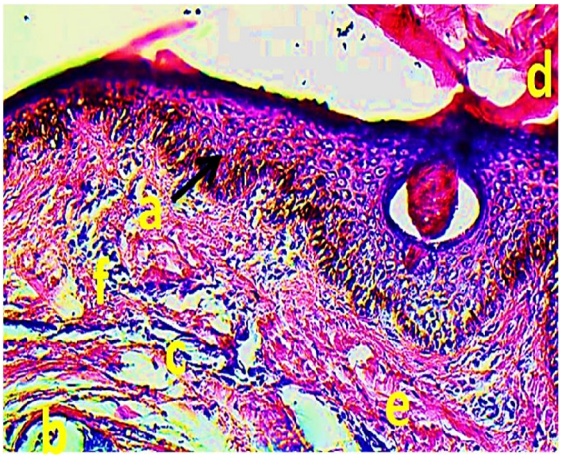


Figure (4-56): Photomicrograph shows the progression of wound healing (GV) at day 14, (a) epidermal tissue, while (b) represents blood vessels, (c) indicated fibroblast, whereas (d) keratin, (e) organization of collagen fibers, (f) mild inflammatory cells. (H &E: 40 X).

At day 28, the control group section showed a clear and a complete re-epithelization but still there was a remaining some localization of inflammatory cells. The dermis showed remodeling in the connective tissue, but it was also showed delay in the organization of interconnecting collagen fibers, hair follicular growth and sebaceous glands (figure 4-57). Compared between the treatment groups of 25mg/kg zinc oxide nano particles and 75mg/kg zinc oxide nano particles revealed there were a complete re-epithelizatio and with final

epidermal layers. In this staged, the healing showed complete clearance from the inflammatory cells and new generation of skin, this was appeared obviously in wound healing treated with 75mg/kg than 25mg/kg (figure 4-58, 59). In the group treated with 25mg/kg zinc oxide indicated presence keratin with mild dermatitis, in the group treated 75mg/kg zinc oxide found evidence of the complete re-epithelizatione and proliferation of dermal collagen fibers (figure 4- 60,61)(Table 4 – 19).

Table 4 – 19: Histopathological score of wounds healing at 28 POD in control and treated groups of goats

Time	Group	parameters								
		Agt	Ii	Re	Ic	Fbf	Nv	Cd	Nt	Sca
28 day	I	3	1	3	marked	marked	moderate	moderate	+	Granulation tissue and matrix formation Inflammation dermal closure
	II	4	3	3	absence	marked	marked	marked	_	Late stage of matrix remodeling
	III	4	3	3	absence	marked	marked	marked	_	Late stage of matrix remodeling
	IV	3	2	3	mild	marked	moderate	marked	-	Inflammation dermal closure
	V	3	2	3	mild	marked	marked	marked	_	Late stage of matrix remodeling

Agt: Amount of granulation tissue. Ii: Inflammatory infiltrate. Re: re-epithelization. Ic: Inflammatory cells. Fbf: fibroblasts formation. Nv: Neo vacuolization. Cd: collagen deposition. Nt: Necrotic tissue. Sca: Skin cell assessment. Amount of granulation tissue (profound-1, moderate-2, scanty-3, absent-4). Inflammatory infiltrate (plenty 1, moderate 2, a few 3). Re-epithelialization: migration of keratinocytes-1, bridging of cells-2, keratinization-3. Inflammatory cells: absence/presence (mild/moderate/marked). Fibroblasts: absence/presence (mild/moderate/marked). New vessels: absence/presence (mild/moderate/marked). Collagen: absence/presence (mild/moderate/marked).

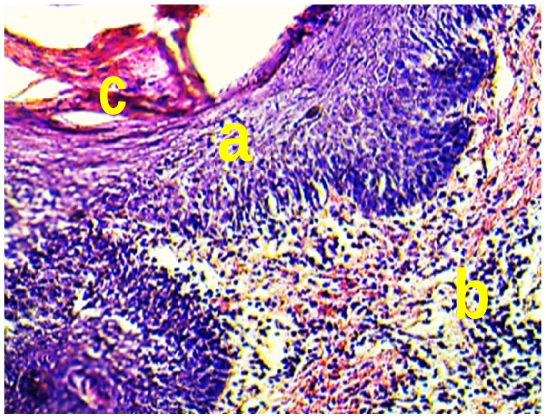


Figure (4-57): Photomicrograph shows the progression of normal wound healing (control GI) at day 28, (a) epidermal tissue with presence of dermatitis, while (b) indicated inflammatory cells, and (c) scar tissue. (H &E: 40 X).

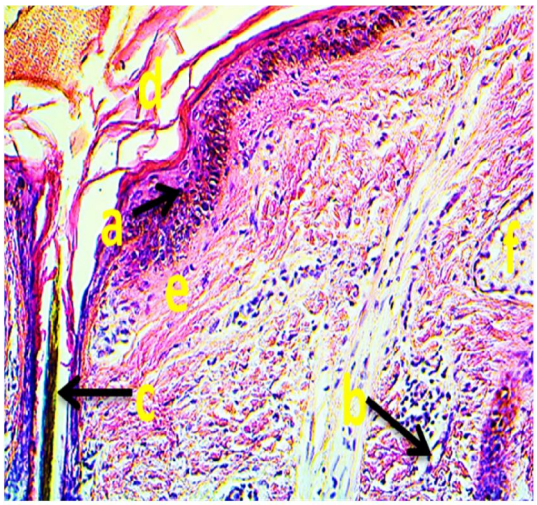


Figure (4-58): Photomicrograph shows the progression of wound healing (GII) at day 28, (a) epidermal tissue, while (b) represents blood vessels, (c) hair follicular, whereas (d) keratin, (e) organization of collagen fibers,(f) sebaceous gland. (H &E: 40 X).

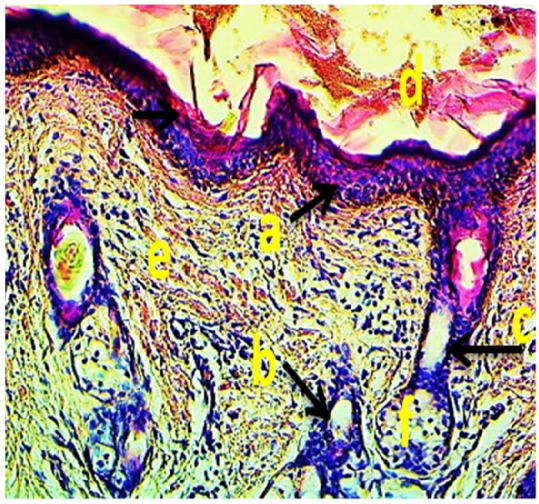


Figure (4-59): Photomicrograph shows the progression of wound healing (GIII) at day 28, (a) indicates of epidermal tissue, while (b) represents blood vessels, (c) hair follicular, (d) kiratin, (e) organization of collagen fibers,(f) sebaceous gland. (H &E: 40 X).

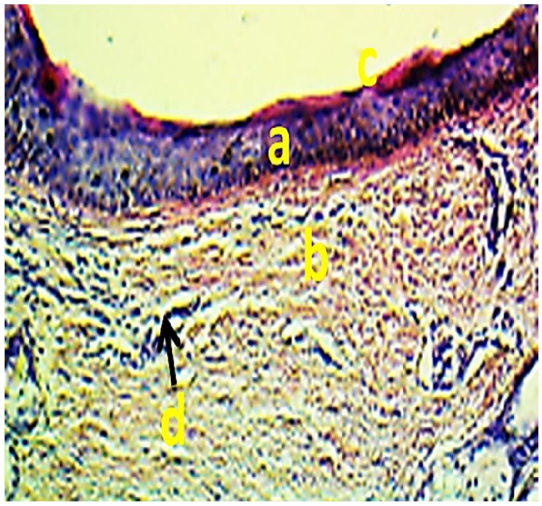


Figure (4-60): Photomicrograph shows the progression of wound healing (GIV) at day 28, (a) epidermal tissue, (b) organization of collagen fibers,(c) kiratin, while (d) indicated presence of mild dermatitis. (H &E: 40 X).

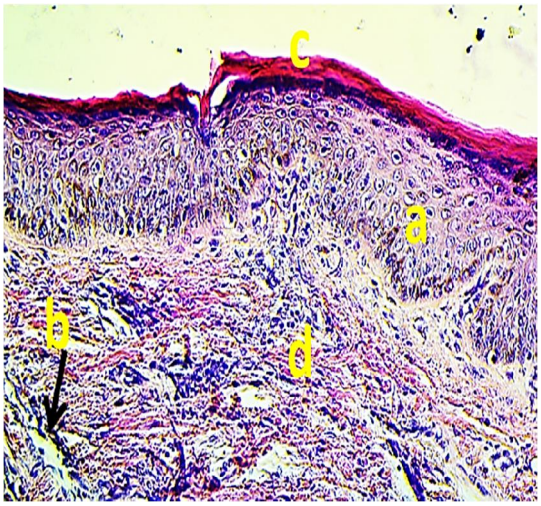


Figure (4-61): Photomicrograph shows the progression of wound healing (GV) at day 28, (a) epidermal tissue, while (b) represents blood vessels, (c) fibrin, (d) proliferation of dermal collagen fibers. (H &E: 40 X).

Chapter five
Discussion

5. Chapter five: Discussion

5. 1. Study of ZnO NPs analysis

In the current study, the results of analysis ZnO NPs by using scanning electron microscopy (SEM), X-Ray Diffraction (XRD), UV-Vis absorption Spectroscopy and particle size (PS) measurement similar to results of several researches like Khors Zak et al., (2012), Khitam et al., (2018), Dhuha et al., (2018), and Shamhari et al., (2018), also in this study similar of images of SEM with images of production manufacturer. This result was also the same pattern in compared with the given standard XRD pattern of ZnO (JCPDS 36-1451) for confirmation purpose. The SEM images it can be showed the particles with size less than 100nm were formed, also it provided clear idea about the particle separation, and shape of ZnO NPs are separated smoothly and not highly affected by agglomeration, these results are consistent with the findings of (Ananthu and Renjanadevi, 2016). In the current study, the XRD of the ZnO shows broad peaks at values of 31.88°, 34.52°, 36.39°, 47.64°, 56.70°, 62.95, 66.48. 68.03, 69.16 and 77.05, which are typical for the ZnO structure. Based on the XRD pattern, ZnO NPs has high purity of quartzite crystalline structure as the diffraction peak is seen to be intense and narrower. The peak shown is broad which indicates that the particles are smaller, which were also described in previous literature (Bai et al., 2015).

The average particle size has been determined from full width at half maximum (FWHM) of the diffraction peaks using Scherer's equation (Ananthu and Renjanadevi, 2016). This study stated ZnO Nano powder particles with crystallite size of 41.2nm, similar methodology was reported by Salah, et al., (2011) and used such ZnO in antimicrobial activity. Also, laser ablation technique utilizes a laser beam to remove

particle from solid or a liquid surface. Spherical ZnO with average diameter of 35nm was reported by Ismail et al., (2011), they used pulsed laser ablation in double distilled water. The absorption peak was observed at 375 nm, which attribute to the intrinsic band-gap of ZnO absorption. Similar result of absorption band that represent ZnO NPs was also obtained from previous research in which the range of absorption and were from 355 to 380 nm. (Shamhari et al., 2018).

5. 2. Experimental induction of zinc deficiency study

The current study, showed that the increasing of calcium lead to decrease in zinc which led to zinc deficiency induction, this fact insured by Arrayet et al., (2002) who report that the most important factors that predispose to zinc deficiency are increasing calcium and phosphorus (decrease zinc absorption), a diet rich in legume (high calcium) or high phosphorus grain supplement with no added minerals leding to interfering with zinc absorption in intestine. The Legumes may contain a smaller amount of zinc than grasses grown on soil.

Zinc-deficient diets or high levels of dietary components that bind zinc and prevent absorption and utilization can cause zinc-responsive dermatitis and a few field cases of zinc-responsive dermatitis caused by nutritional imbalances have been documented in small ruminants (Nelson et al., 1984; Reuter et al., 1987). These findings are somewhat similar to the current study, in addition to the clinical signs that look like to those of Miller et al., (1964), they were found hair roughness, hair loss and excessive keratin skin in male goats fed a low-zinc diet. Similar skin lesions to current study have been described by Neathery et al., (1973) in mature goats experimentally fed with a zinc-deficient diet, in addition to horn changes, abnormal hooves and cracked on nostrils, skin lesions previously described in goats suffering from zinc deficiency caused by a deliberate or accidental zinc-deficient diet were similar in many respects to those seen in

study cases by (Krametter-Froetscher et al., 2005). Because zinc availability in ruminants may be influenced by many factors and cause a secondary deficiency of zinc. These consist of the consumption of young grass, nourishing on late-cut hay, and highly dietary sulphur can also affect the digestibility of zinc (Constable et al., 2017). Sheep and goats have a small, zinc storage unit, for that reason the clinical signs and laboratory abnormalities associated with zinc deficiency occur quickly after removal of zinc from diets and return to normal after supplementation because sheep and goats are able to absorb zinc very efficiently at low intake Suttle, and Jones, (2007). These findings are consistent with the findings of the current study that the defect in the diet (increased calcium) led to the emergence of signs of zinc deficiency clearly.

Anorexia with no significant increase in animal weights in the period from 6^{th} to 8^{th} week was observed in this study. Droke et al., (1993) were explain that consumption foods decreased due to loss appetite in Zn deficient sheep, which represented by significant decrease in the rumen movement. There is a relationship between animal appetite and concentration of some amino acids, so that changes in appetite are associated with changes in the concentration of amino acid-derived (neuro transmitters) in the brain, thus some trace elements deficiency such as Zn may decrease the appetite because it hypothesized that the sense of taste is mediated through the salivary zinc- dependent as a result low salivary zinc concentration leads to a decline of taste (Kennedy et al., 1998), and diminution appetite (Failla, 2003). On the other hand, reduced appetite has been also reported in buffalo calves affected with Zn deficiency (Al-Saad et al., 2006), with reduced appetite and rumen movement induced reduction in the body weight in goats (Van Wouwe, 1989). Thus, there was no significant increase in body weight over the current study period.

Discussion

Alopecia was the frequent sign in goats with Zn deficiency in this study. This result was seen in other animals, in cattle (Constable et al., 2017; Sharma and Joshi, 2005). Buffalo calves (Failla, 2003) sheep (Ibrahim et al., 2016). The skin alopecia and abnormality belong to the zinc of an wide-ranging of metallo-enzymes and acts as a cofactor for RNA and DNA polymerases (Gooneratne et al., 1989), therefore, zinc necessity for the active proliferation and differentiation of epidermal keratinocytes (Ogawa et al., 2016), that may be explained the skin changes that found in this study. In the current study, the increased in heart rates. Result was agreed with Al-Saad et al., (2006) and Khaleel, (2013), that may because zinc deficiency is responsible for an increased oxidative stress which leads to cellular destruction (EFSA, 2014), and the role of zinc as an antioxidant (Prasad, 2009), so the zinc deficiency lead to accumulation free radicals and damage heart muscles and lead to increase heart rate. The respiratory rate was decreased, these results were different with (Al-Saad et al., 2006), and this may be due to the fact that animals suffering from zinc deficiency naturally take the chronic phase, and in this study the breeding system is different (the animals are reserved in the barn), this reduces stress on the animal, which lead to respiratory signs are not clearly visible.

The drop in blood values at the beginning of the experiment may be due to poor management, lack of nutrition and the effect of climate on natural pastures, and this is consistent with what was indicated by Egbe-Nwiyi et al., (2000) and Ebiegberi, (2009). RBC, Hb, and PCV values were observed differences in the current study than those previously reported by (Aggarwal et al., 2015; Kimber and Pai, 2000; Olayemi, et al., 2009; Piccione et al., 2010; Rice and Hall, 2007). Age, breed, and environments have been reported to influence on the hematological values of goat in the arid zone (Egbe-Nwiyi et al., 2000; Zumbo et al., 2011; Zamfirescu, 2009). The disease could also

influence the hematological parameters in goats (Sulaiman et al., 2010). Because rare research that available about information on zinc requirements for native in Iraqi local breed of goats using the factorial approaches, for that reason, it is challenging to compare these results with another value generated with similar animals to the ones used in this research. So, new studies on the animals nutrient should be undertaken, containing different breeds and different environments because of the influence of breed and environment on the requirements of the animals. Thus, these differences must be taken into account when a diet is formulated (Marcos Jácome et al., 2017).

In the induced zinc deficiency, serum zinc concentration was significantly decreased from the 2nd week. Plasma zinc concentration can fall with response to other factors unconnected to Zn status or dietary Zn consumption, including infection, inflammation, stress, and trauma. On the other hand, tissue catabolism during starvation can release Zn into the circulation, causing a transient increase in circulating Zn levels (Hambidge et al., 1989). Singer et al., (2000) found in the ram was being fed with a diet of alfalfa legume high in calcium which can block zinc uptake, so it can be concluded that the main factor, which could prevent zinc absorption, was due to high dietary calcium. Decrease zinc absorption happens for the reason that increased calcium and phosphorus consumption, and some breeds of goats were found in genetic predisposition may have a depressed zinc absorption, so may require lifelong zinc supplementation in the appearance of high calcium (and other minerals) intake (Linklater and Smith, 1993).

A significant decrease in a lymphocyte in Zn deficient goats may indicates that Zn deficiency had an effect on cell- mediated immunity (Bires et al., 1992; Droke and Spears, 1993). A significant decrease in lymphocytes in Zn deficient goats in the current study may be attributed to that zinc deficiency decreases the activity of serum thymine (thymes hormone), which is required for maturation of T-helper cells, for avoid cell-

mediated immune dysfunction (Prasad, 2007). The immune system function is impaired even in cases of moderate Zn deficiency (Shahraz and Ghaziani, (2005). In this study, pruritus in goats that occurs because imbalance of calcium, magnesium, and phosphorus in the body (Lugon, 2005). Eosinophils are multifunctional leukocytes implicated in the pathogenesis of numerous inflammatory processes such as non-specific tissue injury (Pruritus) or response to a variety of stimuli, eosinophils are recruited from the circulation into the tissue where they modulate immune responses through multiple mechanisms. Previous studies have suggested that localized eosinophil-nerve interactions at sites of inflammation significantly alter tissue innervation. Thus, eosinophil -nerve interactions provide a potential mechanistic link between eosinophil-mediated events and neurosensory responses (Dagmar et al., 2010). The eosinophil cells may be decreased because of migration of eosinophil from circulatory blood vessels to the skin tissue, response to stimuli of tissue injury (Pruritus) (Lugon, 2005).

5. 3. Study of treatment by ZnO NPs and conventional ZnO.

5.3.1. Clinical signs

The results clinical signs agree with (Constable et al., 2017), which reported that dose responses quickly after zinc administration in zinc deficiency animals. Also, Krametter-Froetscher et al., (2005) concluded that zinc-responsive dermatitis was diagnosed in two goats on the basis of history, cutaneous signs, histopathological features, and serum zinc concentration, in addition to managing response to zinc therapy. They, also were explained that, although zinc analysis of the feed can provide tentative support to a diagnosis of a zinc-responsive dermatitis, its value is limited by sampling technique where by the diet tested, must be representative of the patient’s intake within the herd, and the amount quantified must be assumed to be assimilated by the animal. The results of the current study, showed that body weight increased significantly in groups

treated by ZnO NP in comparison with control group, this result agree with Kanti et al., (2018), which reported that Nano minerals are used for enhancing the bioavailability of minerals in livestock, which are helpful in improving growth, production and health status of animals. Also they conclusion that minerals as nanoparticles decrease intestinal mineral antagonism, thereby reducing excretion and environmental pollution. In addition to Nano minerals are having a great potential as mineral feed supplements in animals even at very lower doses than the conventional sources by increasing their bioavailability in biological system due to the increase in the surface area, surface activity and catalytic efficiency of Nano minerals.

On the other hand, dietary zinc supplementation with (33mg zinc/kg) had no effect on the performance, blood indices and blood biochemistry of Nubian kids (Elamin et al., 2013). Similar observations were reported by Pal et al., (2010) and Mandal et al., (2007), throughout supplementation with organic source of Cu and Zn did not differ in final body weight, body weight gain, average daily gain, and feed efficiency. These findings are inconsistent with the results of the current study because the animals were treated after undergoing experimental zinc deficiency, and this explains the rapid response after zinc therapy and this is supported by Constable et al., (2017). While, the control group was left fed on the diet only to obtain zinc so, the changes are limited and slow compared to the treated groups, on the other hand, in the most previous research used conventional ZnO whereas in this study used ZnO NP, we conclude that the response to zinc treatment depends mainly on the zinc level of the animal before treatment and the zinc nanoparticles produce a faster response.

In addition that, some study suggested that may be due to sufficient amount of zinc in the basic ration (Elamin et al., 2013). Also, similar conclusion in the study by Aditia, et al., (2014) they found the control diet which contained 73.306 ppm of Zn suggested that

Zn concentration in the diet was adequate, which could have reduced potential for responses in body weight change, average daily gain, feed intake, and feed efficiency. Puchala et al. (1999) stated that the effect of dietary Zn supplementation, regardless of form, depends on the animal's nutrient status. Also, they reported that the amount of Zn in plasma of Angora goats was 0.72 mg/l. But, Elamin et al., (2013) found plasma zinc concentration varied within the range of 0.59 to 1.23 mg/l, which shows that zinc concentration in the basal diet was adequate for normal blood Zn concentration, leading to normal growth of goats.

On the other hand, Al-Arabiy, et al. (2013) results indicated that goat kids fed the control diet containing 22.12mg Zn/kg DM had a growth similar to that of the Zn supple-minted kids. In addition that they suggest that this level of Zn in the basal diet was adequate for normal growth of Marko goat kids and supplementation of 20 and 40 ppm zinc from both ZnO and nano ZnO sources had no effect body weight. Therefore, this study relied on the introduction of deficiency of zinc so that the changes resulting from the treatment effect can be clearly seen between the treatment groups and the control group. Also, between the treatment groups with Nanoparticles of zinc oxide and the groups of ordinary zinc oxide treatment on the other. It is concluded from all these studies that the status of animals in the present study is different from what it was in those studies, this study was designed on the basis of the development of zinc deficiency for animals before being treated, and the result was a clear change in body weight and health status.

No significant difference was detected in body temperature and heart rate between groups. Respiratory rate was significantly increased in control and ZnO groups in comparison with ZnO NP groups, this is consistent with the results of the study reported

that the respiratory rate affected by zinc value in the body, and indicated role of ZnO NP as antioxidant (Prasad, 2009).

The results of body scoring are similar to those described by many researchers such as Detwiler et al., (2008); Morgan-Davies et al., (2008) and Phythian et al., (2011), they noted that the state of the body is the outcome of on-farm husbandry and management, and therefore body scoring can be a key tool in the on-farm assessment and management of welfare. The body scoring of goat is a method to evaluate the nutritional status or the amount of body fat deposition. It can also play an important role in goat marketing (Koyuncu and Öziş Altınçekiç, 2013). The current study, this importance to examine the body scoring, and may be a good indicator in assessing the body weight and health status of the animal.

5. 3. 2. Hematological study of treated and control group

The results showed that the blood parameters(RBC, PCV, Hb) significant increased in all groups after the exclusion of a diet containing a large amount of calcium, and compensation for a balanced diet. That refers to the role of zinc on synthesis and maturation blood cells, these facts were explained by Chen et al., (2017), about stimulation of RBC formation by zinc signal appears to be common among different animals. Such findings were cited by Yen-Hua et al., (2018), when they found that combination of zinc with serum transferrin stimulated erythropoiesis in the rat blood. Also, study indicate that zinc supplementation influences hemoglobin production, and similar results have been found in the effect of low zinc supplementation on hemoglobin levels in some pathological conditions that cause decreased of hemoglobin in the body (Fukushima et al., 2009). On other hand, zinc supplementation has helped improved PCV

levels, RBC proportions, and testosterone levels. For this reason, increased RBCs may be caused by androgen metabolism (Haboubi et al., 1988; Olorunnisomo et al., 2012).

These studies results disagree with other studies such as Elamin et al., (2013), they found the zinc Supplementation were not significantly affected on the value of PCV, RBCs and WBCs in goat Kids, and low estimates for RBCs were reported by Ukanwoko et al., (2013). Also Perme et al., (2013) found similar results, supplementation of 40 ppm of Zn in the form of Zn-methionine did not affect blood hemoglobin or PCV values in goats. Variations responses in the present study in comparison with other studies may be due to composition of rations, zinc level, source of zinc supplied, bioavailability of zinc, animal species or breed and duration of the experimental period (Elamin et al., 2013), Also, the animals in the present study were subjected to experimental induced zinc deficiency as mentioned earlier, and different doses with different administration methods were used, resulting in differences in the results of the current study with other studies. In current study, the results agree with study of Perme et al., (2013), they reported that were no abnormal findings at hematological parameters (RBCs count, PCV value and Hb concentration) in all experimental groups which indicate that absence of harmful effect of ZnO and ZnO NPs on erythrogram.

The current study, refer to increased in WBC in treated and control groups except group III, this result disagree with study of Elamin et al., (2013) which reported that value of WBCs were not significantly affected by feedingd on the basal ration supplement with zinc as zinc sulfate 33mg/kg, this differences with current study may be because normal inflammatory response to induced wounds. Neutrophils cells are granular polymorph nuclear leukocytes (PMN), which often act as one of the first responders to tissue injury and bacterial infection (Reinke and Sorg, 2012), group III treated by (75mg ZnO NP) showed non significant increased because the role of ZnO NP as anti-inflammatory

(Xiong, 2013; Oyarzun-Ampuero et al., 2015). The Lymphocyte count results agree with Partha et al., (2016) and Kanti et al., (2018) suggested that supplementation of Nano minerals improved the growth, digestive efficiency, immunity, antioxidant status, and milk production of animals. Gammoh and Rink, (2017) observed Zinc alters immune responses in a multitude of ways ranging from myeloid-derived cells and inflammatory signaling to lymphocyte differentiation and antibodies production. The ability of zinc to regulate immune homeostasis, by stimulation of lymphocytes when using phyto-mitogens, and this stimulation is widely used to measure immune competence (Weigel et al., 1992).

The results showed a lower platelet count in the control group compared to treatment groups. In a study on zinc effects showed that hyperzincemia affects increased coagulation, and hypozincemia led to poor platelet aggregation and increased bleeding time. Considering the importance of zinc as an essential element, its participation in regulation of the equilibrium between pro- and anti-thrombotic factors (Tubek et al., 2008). There are some similarities in the results of this study with what mentioned by Tubek et al., (2008), but the lack of studies on goats in this area prevented a deeper explanation on the impact of nanoparticles of zinc oxide on the bleeding time and clotting time. On other hand, proved ZnO nanoparticles (diameter 70 nm) at a concentration of 1 mg/mL prolonged clotting time and provoked a weak clot, this procoagulant effect decreased at lower concentrations reaching the detection limit at 10 ng/mL. Nanoparticles in high concentrations reproduce the surface charge effects on blood coagulation previously observed with large particles or solid metal oxides. However, nanoparticles with different surface charges equally well stimulate coagulation at lower concentrations (Steuer et al., 2014). This stimulation may be an effect which is not directly related to the

surface charge. The differences between these results and the results of the present study are due to differences in blood zinc levels as well as the impact of Nano scale differences.

5. 3. 3. Biochemistry, immunity and antioxidants study

The results of serum zinc, showed significant increased in all groups at 9th week in comparison with 0-week and significant increased in ZnO nanoparticle groups(II, III) in comparison with control and conventional ZnO groups, this results incompatible with different studies. In many studies were refer to that effect of zinc added in the diet of Angora goats (Puchala et al., 1999; Eryavuz et al., 2002), and dairy goats (Salama et al., 2003), also had no effect on performance, blood indices and blood biochemistry of Nubian goat kids by dietary zinc supplementation (33mg zinc/kg) (Elamin et al., 2013), and serum minerals level were not affected in kids supplemented 20-40 ppm nano of ZnO (Zaboli et al., 2013).

In contrast, the some studies reported that serum level of zinc was increased by oral administration of Nano zinc particles in lambs fed 20 mg/kg zinc NPs daily for 25 days Najafzadeh et al., (2013), but this elevation was not significant. On the other hand, Jia et al., (2008), they found the concentration of Zn in plasma was significantly higher in supplemented groups as compared with the control by supplemented inorganic Zn in the diet of Cashmere goats. Also Phiri et al., (2009) reported an increased plasma Zn concentration in goats supplemented with Zn in the form of zinc oxide. Differences in the present study in comparison with other studies may be due to composition of rations, zinc level, source of zinc supplied, bioavailability of zinc, animal species or breed and duration of the experimental period, and that's what was supported by Elamin et al., (2013). However, zinc oxide treated nanoparticles were higher in their values than other groups, and this is consistent with (Shim, et al., 2014), and those who reached in their studies that ZnO NPs are dissolved in the blood, a high concentration of ionic zinc could

be generated. Later, an in vitro test was conducted to differentiate the effects between ionic zinc and NPs, and role of zinc in nucleic acid, proteins, albumin and globulin synthesis (O'Dell, 2000).

The results of total protein, albumin and globulin agree with study Lenka et al., (2016), they were reported that various forms of Zn had a significant influence on the quantity of individual protein fractions. On the other hand, the globulin value was compatible with Spears, (2003), they reported that ruminants marginal Zn deficiency does not impair cell-mediated or humeral immune responses, and observed increased in the immunoglobulin level in blood serum by supplementing organic Zn (Kinal et al., 2005). In current study showed significant decreased in the 3rd and 6th weeks in comparison with 0-day, and after that increased significantly in 9th week, this changes it may be because by surgical truma, Murata et al., (2004) found that blood proteins that change in concentration in animals subjected to external or internal challenges, such as inflammation and surgical trauma. In the current study, a response of similar magnitude has been previously described in calves (Conner et al., 1988). Albumin concentration declines following an inflammatory condition Fe´lix et al., (2008), biochemical changes in blood in Awassi lambs following elective castration (AL-Zghoul et al., 2008).

The results of phagocyte index in this study refer to significant increased in all groups in comparison with 0-week, and increased in group III in comparison with other groups these results agreement with study which showed that ZnO NPs induced significant increase in phagocytic percentage and phagocytic index when were used compared to control negative group Mobarez et al., (2018), and induce elevation of immunoglobulin (IgG1) and (IgGE) (Roy et al., 2014). Regarding to neutrophils, that are the primary line of defense in innate immune response, the differ between studies in results of innate immunity may be due to that the difference in the criteria of investigated

ZnO NPs (Mobarez et al., 2018). In addition to the tendency ZnO NPs to incorporate with highly proliferated cells such as lymphocytes (Christa et al., 2015).

In current study Interleukin 1β (IL-1β) result showed significant decreased in treated groups by ZnO NPs and conventional ZnO comparison with control group. Mobarez et al., (2018) explored the production of the pro inflammatory cytokines tumor necrosis factor (TNF)α and interleukin1β (IL-1β) in pro myeloid cells influence by zinc deficiency, focusing on the role of epigenetic and redox-mediated mechanisms as possible explanations for zinc-deficiency-induced changes. Other studies recommend a strong influence of zinc in the regulation of pro inflammatory cytokine expression in mononuclear cells (Haase and Rink, 2009; Bao et al., 2003). Similarly, increased IL-1β cytokine synthesis through zinc deficiency induced by dietary means, as a consequence of leishmaniasis or cancer (Black, 2001; Clark et al., 2003; Bao et al., 2003). These studies discus the results of current study about role zinc in decrease IL-1B, but the increasing within group in comparison with 0-week that is normally because inflammatory response to inducing wound in experimental animals. Transient hypozincemia is a physiological effect due to hepatic zinc uptake during systemic inflammation induced by pro inflammatory cytokines (Liuzzi et al., 2005; Cousins et al., 2006). Prolonged decreased of serum zinc causes increased IL-1β and TNFα production, initialing a vicious circle and inducing permanent zinc uptake by the liver. Subsequently, this would explain the steadily increased elevated level of pro inflammatory cytokines as well as the development of chronic inflammation. It is also responsible for tissue destruction, fetal defects and immune dysfunctions reported during zinc deprivation (Wellinghausen and Rink, 1998; Fraker and King, 2004; Prasad et al., 2007; Mobarez et al., 2018). On the other hand, the immune modulating (activating) effect of ZnO NPs mainly on Th1 (T-cytotoxic) lymphocytes through innate and cell – mediated immunity (Hanley et al.,

2009). The ability of ZnO NPs to initiate Th1 differentiation is mediated by its ability to induce TNF-α (Lappin and Campbell, 2000), also, clarify the immune – suppressive effect of ZnO NPs on Th2 lymphocytes Wessels et al., (2013). Kim et al., (2014) reported the immunosuppressive effect of ZnO NPs as a sequence of innate immunity (natural killer cells) and consistently Th2 cytokines in ZnO NPs -fed mice.

During vitamin D3 (VD3) induced differentiation of pro myeloid HL-60 cells, intracellular free zinc levels decrease, whereas IL-1β and TNFα expression increases and the IL-1β promoter is remodeled into an open conformation (Dubben et al., 2010; Wessels et al., 2010). zinc deficiency might be able to support VD3 induced differentiation by the activation of some monocytes genes. Wessels et al.,(2013) conclusion that zinc deficiency leads to chromatin remodeling, facilitating IL-1β and TNFα mRNA transcription after appropriate stimulation. These findings provide a link between zinc deficiency and the induction of IL-1β and TNFα production via epigenetic as well as oxidant-mediated signaling pathways.

Total antioxidant results significant increased in all groups in comparison with 0-week, but between groups, total antioxidant value significantly low in group I in comparison with other groups, these results similar to a number of studies describing the role of zinc as an antioxidant (Mackenzie et al., 2006; Prasad, 2009), provided that alternative mechanisms for the ruling of gene expression by zinc. Signal transduction, is the majority of zinc-regulated genes are involved in responses to oxidative stress or in growth and energy utilization (Cousins et al., 2003), that is explain the ability of ZnO NPs to induce pro inflammatory cytokine expression is stable with the recognized relationship between oxidative stress and inflammation (Federico et al., 2007). On the other hand, ZnO supplemented rabbits group showed marked increase in activities of hepatic and renal catalase in compare with control group may be due to indirect

antioxidant effect of Zn which reduces formation of free radicals and acts as inhibitor of NADPH oxidase and an integral metal of Cu, Zn-SOD. Also, it induces metallothioneina protein with antioxidant properties and increases the protein sulfhydryl groups stability (Powell, 2000; Prasad, 2009).

The results of comet assay showed little lowest amount of DNA damage in treatment groups in comparison with control, this results disagree with many studies, so that the Elham et al., (2018) regarding to the effect of ZnO NPs on the bone marrow, and they were detect significant genotoxic effect by using comet assay, also, Kumar et al., (2015); Sliwinska et al., (2015) ; Simón-Vázquez et al., (2016) attributed this marked effect to the accessibility of ZnO NPs to the nuclei, and hence interaction with enzymes involved in detection of DNA breaks. Although, the current study not showed significant role of ZnO and ZnO NPs protective damage to DNA but the same time not causes higher oxidative DNA damage, this differences with previous study may be because highly resistant goats to oxidative stress.

5. 3. 4. Wounds healing

In this study macroscopic findings showed that wounds healing in goats have high intends to repair and healing process with limited inflammatory reaction at the site of wounds in all groups . In the other way, the groups treated by ZnO NPs showed more faster healing and better response than other groups, these results are agree with the results of studies by Lansdown et al., (2007) and Kogan et al., (2017) which were explained that the zinc plays important role in wound healing and treated zinc deficiency results in improving wound healing compared to those with zinc deficiency. Also, the results of current study, which documented important dose amount of ZnO NP in wound healing, are similar to results of study by Al – Zubaedi, (2017), which showed that better healing is achieved in a concentration 20% followed by that of 10% by using silver

nanoparticles in cutaneous wounds in rabbits. Additionly this study consistent with the study that indicated significant results of wound contraction rate, epithelialization and histopathology of the healed tissues of rats that confirmed the promising wound healing property of ZnO NP (Ekta et al., 2018).

Moreover, at day 3 histological results in this study indicated presence of reactive cells (neutrophils, macrophages, and lymphocytes), accumulation of exudates, regeneration of epidermis, and proliferation of fibrous connective tissue were observed to understand the normal healing process, these results revealed to normal response in early stage of wounds healing, so it may be because released molecules of chemo-attractants for neutrophils, which enter the wound site and increased endothelial permeability, the same as also has been reported by (Werner and Grose, 2003; Reinke and Sorg, 2012). But the same time the treatment of groups with 25mg/kg ZnO NPs and 75mg/kg ZnO NPs respectively, revealed to progression was observed via development of new matrix deposition along with aggregation of many proliferating fibroblasts and less amount of necrotic debris, These results agree with Padmavathy and Vijayaraghavan, (2008), which were revealed to that ZnO has both antibacterial and anti-inflammatory properties, and accelerates the healing of both acute and chronic wounds. Also, this experiment indicated that decreased inflammatory reaction in treating groups in comparison with control group, this may be correlated to the ability of zinc in modulating both innate and adaptive immune response, additional to alters immune responses in a multitude of ways ranging from myeloid-derived cells and inflammatory signaling to lymphocyte differentiation and antibody production (Pei-Hui et al., 2017). Similarly, a recent trail reported that zinc could participate in modulation of monocyte differentiation into pro-inflammatory (M1) or immune-regulatory/wound healing (M2) macrophages (Dierichs et al., 2017). Another explanation could be that the connected to the decreases of inflammatory reaction,

addition to ZnO NPs may be has an affect on bacteria when used in different concentrations and it showed the ability of high concentration as compared to low amount (Khitam et al., 2018). More studies are needed in order to confirm this findings in different concentration.

The regulated and promotion phases of wound healing depends on the several factors (Guo and Dipietro, 2010; Dhivya et al., 2015). One these factors is the availability of appropriate trace elements serving as enzyme cofactors and structural components in tissue repair (Lansdown et al., 1999). Furthermore, Zinc has a significant function in regulated and promotion, and also on many cells over the entire process of wound repair (Pei-Hui et al., 2017), so these results agree with results of study by Bao et al., (2003), and his coworkers, which can explain the role of zinc supplementation in reduced plasma levels of oxidative stress markers, decreased ex vivo production of inflammatory cytokines, chemokine's and reduced secretary cell adhesion molecules which represent important biomarkers of cell damage-associated inflammation in endothelium and platelets (Bao et al., 2010 ; Prasad, 2014).The main observation of histological imaging at day 7^{th} including many proliferating mature fibroblasts, newly blood capillary with much more progression in treated groups, these changes in cells proliferation are similar to findings which were reveled to that the moment when granulation tissue begins to cover wound surface, marks the transition to the proliferative phase and represented by activation of fibroblasts which produce collagen and other extracellular matrices, such as neoangiogesis (Negut et al., 2018), so the results of current study are parallel with studies which reported that ZnO NPs play a significant role in angiogenesis (Manuja et al., 2012; Li and Chang, 2013).

Development and highly maturation of fibroblast in groups which treated by ZnO NP, may be because of increase fibroblasts drawn from healthy dermis, bone marrow

progenitor cells, circulating fibroblasts and multi potent cells in the dermis (Shaw and Martin, 2009). Granulation tissue is only temporary and will be replaced during the remodeling phase. It is characterized by dense dermal vascularization, fibroblast populations and macrophages (Iocono et al., 1998), these results synergy with the current study in the role of zinc in formation of granulation tissue (Maywald et al., 2017).

In the current study on day 14, histological findings showed a clear generation of thin epidermal layer. This layer of re-epithelium of skin tissue covering the wound with the formation of scar tissue. These fact were more advanced in groups treated with ZnO NPs , especially in the 75 treated group, where serious hair follicle development was observed, and changes were observed in groups treated with ZnO but to a lesser extent. Observations of this study are consistent with the studies that indicated the importance of ZnO NPs in promoting keratinocytes migration, thus improving Re-epithelialization (Vijayakumar et al., 2019) and (Ekta et al., 2018).

Looking at the histological results on day 28 of the present study, it is clear that the ZnO NPs are very close in shape to normal skin and the dermis and sub-dermal layers appear to cover the wound, and in this stage the healing showed complete clearance from the inflammatory cells and new generation of epidermal layer , so these results analogous with Aksoy et al., (2010) which they reveled that ZnO NP accelerate the healing of both chronic and acute wounds, because of its epithelialization and bacteriostatic properties. On the other hand, these results are very similar with the results mentioned by Naraginti et al., (2016) which they indicated that appearance of fibroblasts, complete re-epithelialization, neovascularization, and fewer inflammatory cells were observed in the tissue obtained from the ZnO NP treated group, which formed the basis for the increase of collagenation at the wound site.

Discussion

Our study reported that among the parameters used for the analysis of healing wounds including cell proliferation, re-epithelialization, collagen deposition, granulation, angiogenesis, and scar formation (Pei-Hui et al., 2017). Several observation of Xiong, (2013) and Oyarzun-Ampuero et al., (2015) described the advances in drug delivery with ZnO technology has received considerable attention for the treatment of wounds due to their effective cell penetration, immunomodulation and antimicrobial capacity. This fact could be has a main role in the process of regeneration and wound healing in the current trail. To sum up, wound healing is influence by many factors as in this study the concentration, particle size and the time for giving the zinc oxide. In the near future may be more evidence is a viable to confirm this statement.

Conclusions and Recommendations

6. Conclusions and Recommendations

6. 1. Conclusions

1- The study showed the advantage with treatment by zinc oxide Nano particles groups size 41.1 compared with the conventional zinc oxide groups. which gives an indication of promising medical applications of the use of Nano zinc oxide in veterinary field to improve animal herds.

2- It was concluded from the current study that the imbalance of the diet has a direct effect on zinc absorption, even if the amount of zinc in the diet is within the recommended amounts.

3-The severity of clinical signs on the goats are associated with the degree of deficiency and the period of exposure.

4- There are noticeable changes in blood and biochemical parameters with even body growth, in addition to clear difference when comparing all the parameters in the treatment phase from the criteria in the stage of induction of deficiency and the results of zinc oxide Nano particles show best result in comparison with other groups.

5- Although clinical signs and histopathological changes showed that wound healing was similar in the treatment groups and the control group, but groups treated with nanoparticles of zinc oxide, especially the treatment group at a dose of 75 mg / kg revealed the best wound healing..

6. 2. Recommendations

1- Limited knowledge of the toxic effects of ZnO NPs on ruminants highlights, this needs for immediate researches to identify their possible adverse effects when used as a nutritional supplement in livestock, and study the effect of prolonged exposure on animal and environment.

2- Conduct extensive studies on the effect of zinc oxide nanoparticles on natural micro flora in ruminants and on the local immunity of the digestive system, and effect of nano scale size of zinc oxide nanoparticles on the biological activity in the body.

3- Extensive studies on the using of zinc oxide nanoparticles as an antibacterial drug.

4- Study the effect of mixing zinc oxide nanoparticles with nanoparticles of other essential elements and determining the optimal ratios in diets of different animals, and determine the method that gives the best results and easy methods.

References

A.O.A.C. (1990). Official Methods of Analysis. Association of Official Analytical Chemists, The Association: Arlington, VA, Vol. II, 15th ed. Sec.985.29.

Abdalla, MA.; Mariam, Y. and Yahin, Y. (2009). Seasonal Variation in Erythrocytic and Leukocytic Indices and Serum protein of female Nubian Goats. Middle East J Sci Res; 4 (3): 168-74.

Abdul Raheem, MH. and Al-Hety, MS. (1997). Histological and morphological study of the skin of the black goat. Iraq, J. Vet. Sci; 10: 59-71.

Aditia, M.; Sunarso, CC. ; Sevilla, C. and Angeles, AA. (2014). Growth Performance and MineralStatus on Goats (Caprahircuslinn.)Supplemented with ZincProteinateandSeleniumYeast. Int. j. sci. eng.; 7(2): 124-9.

Aggarwal, M.; Chua, TK.; Pinard, MA.; Szebenyi, DM. and McKenna, R. (2015). Carbon Dioxide "Trapped" in a beta- Carbonic Anhydrase. Biochemistry; 5(4): 6631-8.

Agren, MS.; Chvapil, M. and Franzen, L. (1991). Enhancement of re-epithelialization with topical zinc oxide in porcine partial-thickness wounds. J. Surg. Res.; 50: 101–5.

Aksoy, B.; Atakan, N.; Aksoy, HM. et al., (2010). "Effectiveness of topical zinc oxide application on hypertrophic scar development in rabbits," Burns.; 36 (7):1027–35.

Al –Zubaedi, RM. (2017). Induced cutaneous wound healing in rabbits under the cover of silver nanoparticles. RJPBCS. ;8(4) : 620.

Al-Arabi, KZ. ; Bahari, AA. And Abbasalipourkabir, R. (2013). Role of dietary nano-zinc oxide on growth performance and blood levels of mineral: A study on in Iranian Angora (Markhoz) goat kids. JPHS;2(1): 19-26.

Al-Barwari, SE.; Al-Alousi, RS. and Nassir, JK. (1988). A valuable method for the preparation of pure epidermal sheets from vertebrate skin & its advantages in histology & cell kinetics. Iraq. J. Vet. Sci.; 29: 253- 65.

Al-Eissa, MS.; Alkahtani, S.; Al-Farraj, SA.; Alarifi, SA.; Al-Dahmash, B. and Al-Yahya, H. (2012). Seasonal variation effects on the composition of blood in Nubian ibex (Capra nubiana) in Saudi Arabia. Afr. J. Biotechnol.; 11(5):1283-6.

Allen, JG.; Masters, HG.; Pee,t RL.; Mullins, KR.; Lewis, RD. and Skirrow, SZ. (1983). Zinc toxicity in ruminants. J Comp Pathol.; 93:363–77.

Alonso, JE.; Lee, J. and Burgess, AR. (1996). The management of complex Orthopaedic injuries. Surg. Clin. North. Am.; 76: 879 – 903.

Alonso, ML.; Montaña, FP.; Miranda, M.; Castillo, C.; Hernández, J. et al. (2004). Interactions between toxic (As, Cd, Hg and Pb) and nutritional essential (Ca,Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Zn) elements in the tissues of cattle from NW Spain. Biometals; 17: 389-97.

Al-Saad, KM.; Al-Sadi, HI. and Abdul-Majeed MO. (2010). Clinical, hematological, biochemical and pathological studies on zinc deficiency (Hypozincemia) in Sheep. Vet. Res.; 3 (2): 14-20.

Al-Saad, KM.; Al-Sadi, HI. and Abdul-Majeed, MO., (2006). Clinical and

pathological studies on naturally occurring zinc deficiency (hypozincemia) in buffalo calves. Proceedings of the 4th Scientific Conference of College of Veterinary Medicine, (SCCVM'06), University of Mosul, Mosul, Iraq:97-107.

AL-Zghoul, MB.; AL-Rukibat, RK.; Talafha, AQ.; Ababneh, O. and Bani- Ismail, Z. A. (2008). Cellular and some biochemical changes in blood and peritoneal fluid constituents in Awassi lambs following elective castration. Am J Anim Vet Sci; 3(1): 23-7.

Ananthu, C. and Renjanadevi, B. (2016). Preparation of Zinc Oxide Nanoparticles and its Characterization Using Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD). Procedia Technology; 24: 761-6.

Anbuvannan, M.; Ramesh, M.; Viruthagiri, G.; Shanmugam, N. and Kannadasan, N. (2015). Synthesis, characterization and photocatalytic activity of ZnO nanoparticles prepared by biological method, Spectrochim. Acta A Mol. Biomol. Spectrosc. ;143 :304–8.

Andarson, DE.; Ring, DM. and Pugn, DG. (2002). Diseases of integumentary System.In: sheep and goat medicine, Pugh, D.G (Ed.). 1st Edn, W.B. Saunders Co., Philadelphia, :214-5.

Anderson, G. and Gordon, KC. (1999). Tissue processing, microtome and paraffin sections. In: Bancroft, J. D. & Stevens, A.; Theory and practice of histopathological techniques, 4th ed. Edinburgh, Churchill Livingstone: 47-68.

Anosa, VO. (1993). Ultrastructure of developing and mature Caprine leukocytes . Anat Histol Embryol; 22 :328 – 41.

Apgar, J. (1985). Zinc and reproduction. Annual Reviews of Nutrition; 5:43-68.

Apak, R.; Güçlü, K.; Özyürek, M. et al (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. Microchim Acta.;160: 413–9.

Araújo, MJ.; Medeiros, AN.; Teixeira, IA.; Costa, RG.; Marques, CA.; Resende, KT. and Mello, GM. (2010). Mineral requirements for growth of Moxotó goats grazing in the semi-arid region of Brazil. Small Ruminant Res 93:1-9.

Argmann, CA.; Chambon, P. and Auwerx, J. (2005). Mouse phenogenomics: the fast track to systems metabolism. Cell Metab.; 2:349–60.

Arrayet, JL.; Oberbauer, AM.; Famula, TR.; Garnett, I.; Oltjen, JW.; Imhoof, J.; Kehrli, Jr, ME. and Graham, TW. (2002). Growth of Holstein,s calves from birth to 90 days: the influence of dietary zinc and BLAD status. J Anim Sci; 80:545-52.

Bai, X.; Li, L.; Liu, H.; Tan, L.; Liu, T. and Meng, X. (2015)."Solvothermal synthesis of ZnO nanoparticles and anti-infection application in vivo," ACS App Mat Int; 7 (2): 1308–17.

Bao, B.; Prasad, AS.; Beck, FW.; Fitzgerald, JT.; Snell, D.; Bao, GW.; Singh, T. and Cardozo, LJ. (. 2010). Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: A potential implication of zinc as an atheroprotective agent. Am. J. Clin. Nutr; 91: 1634–41.

Bao, B.; Prasad, AS.; Beck, FW. and Godmere, M. (2003). Zinc modulates mRNA levels of cytokines. Am J Physiol Endocrinol Metab.; 285: E1095–102.

References

Barger, AM. (2010). Erythrocyte morphology. In: Weiss, D. J. & Wordrop, K. J. ed. Schalm's Veterinary Hematology. 6th Ed. Wiley-Blackwell Publishing Ltd, Ames. U.S.A, Iowa. :144-51.

Bassert, JM. (2002). The Integument and Related Structures. In Colville, T, and Bassert, J., M. Clinical Anatomy and Physiology for Veterinary Technicians. M. Mosby, USA.: 122-141.

Bellof, G. and Pallauf, J. (2007). "Deposition of Copper, Iron, Manganese and Zinc in the Empty Body of Growing Lambs of the Breed German Merino Land sheep." Ani Con;1(6): 827–34.

Berg, JM. and Shi, Y. (1996). The galvanization of biology: a growing appreciation for the roles of zinc. Science; 271:1081–5.

Bertram, TA. (1985). Neutrophilic leukocyte structure and function in domestic animals . Adv Vet Sci Comp Med; 30 : 91 – 129.

Bettger, WJ.; Fish, TJ. And O'Dell, BL. (1978). Effects of Cu and Zn status of rats on erythrocyte stability and superoxide dismutase activity. Proceedings of the Society for Experimental Biology and Medicine; 158: 279-82.

Beyersmann, D. and Haase, H. (2001). Functions of zinc in signaling, proliferation and differentiation of mammalian cells. Biometals; 14: 331–41.

Bires, J.; Bartko, P.; Juhasova, Z. and Kovarova, E., (1992). Biologic quality of colostrum and calves from dairy cows injected with zinc during pregnancy. Vet Med (Praha); 37: 641-50.

Bischoff, M.; Kinzl, L. and Schmelz, A. (1999). The complicated wound. Unfallchirurg; in German; 102:797– 804.

Black, RE. (2001). Micronutrients in pregnancy. Br J Nutr.; 85(2):S193–7.

Boycott, KM.; Beaulieu, CL.; Kernohan, KD.; Gebril, OH.; Mhanni, A.; Chudley, AE.; Redl, D.; Qin,W.; Hampson, S.; Kury, S. et al. (2015). Autosomal-Recessive Intellectual Disability with Cerebellar Atrophy Syndrome Caused by Mutation of the Manganese and Zinc Transporter Gene SLC39A8. Am. J. Hum. Genet.; 97: 886–93.

Bozym, RA.; Chimienti, F.; Giblin, LJ.; Gross, GW.; Korichneva, I.; Li, Y.; Libert, S.; Maret,W.; Parviz, M.; Frederickson, CJ. et al. (2010). Free zinc ions outside a narrow concentration range are toxic to a variety of cells in vitro. Exp. Biol. Med.; 235: 741–50.

Braiman-Wiksman, L., Solomonik, I., Spira, R., Tennenbaum, T. (2007). Novel insights into wound healing sequence of events. Toxicol Pathol. ;35:767-79.

Brockus, CW. and Andreasen, CB. (2003). Erythrocytes. Pages 3–45 in K.S. Latimer, E.A. Mahaffey, and K.W. Prasse, eds. Duncan and Prasse's veterinary laboratory medicine clinical pathology. Iowa State University Press, Ames, IA.

Broughton, G.; Janis, JE. and Attinger, CE. (2006). Wound healing: an overview. Plast. reconstr. surg.; 117(7): 1–32.

Burtis, CA. and Ashwood ER. (1999). Book Review Tietz Textbook of Clinical Chemistry. editors 3rd edition. Saunders Clin Chem Lab Med. by Walter de Gruyter Berlin; 37(11/12):1136.

Caley, MP.; Martins, VL. and O'Toole, EA. (2015). Metalloproteinases and Wound Healing. Adv. Wound Care; 4: 225–34.

Cha, SH.; Hong, J.; Mcguffie, M.; Yeom, B.; Vanepps, JS. and Kotov, NA. (2015). Shape-Dependent Biomimetic Inhibition of Enzyme by Nanoparticles and Their Antibacterial Activity. ACS Nano; 9: 9097–105.

Chandra, S.; Barick, KC. and Bahadur, D. (2011). Oxide and hybrid nanostructures for therapeutic applications. Adv. Drug Deliv. Rev.; 63(14-15): 1267-81.

Chappell, RL.; Anastassov, I.; Lugo, P. and Ripps, H. (2008). Zinc-mediated feedback at the synaptic terminals of vertebrate photoreceptors. Exp. Eye Res.; 87: 394–7.

Chen, YH.; Shiu, JR.; Ho, CL. and Jeng, SS. (2017). Zinc as a Signal to Stimulate Red Blood Cell Formation in Fish.Int. J. Mol. Sci. :18.

Cho, JG.; Park, S.; Lim, CH.; Kim, HS.; Song, SY.; Roh, TY.; Sung, JH.; Suh, W.; Ham, SJ.; Lim, KH. et al. (2016). ZNF224, Kruppel like zinc finger protein, induces cell growth and apoptosis-resistance by down-regulation of p21 and p53 via miR-663a. Oncotarget, ;7:31177–90.

Cho, Y. and Borgens, RB. (2012). Polymer and nano-technology applications for repair and reconstruction of the central nervous system. Exp. Neurol.; 233(1):126–44.

Christa, YW.; Molina, MR.; Louzada, A.; Murdaugh, KM.; Donaghey, TC. and Brain, JD. (2015). Effects of zinc oxide nanoparticles on Kupffer cell phagosomal motility, bacterial clearance, and liver function. INT J Nanomed, ;(10):4173–84.

Clark, AR.; Dean, JL. and Saklatvala, J. (2003). Post-transcriptional regulation of gene expression by mitogen-activated protein kinase p38. FEBS Lett; 546: 37–44.

Coles, EH. (1986). Veterinary Clinical Pathology. 4th Edition, W.B. Saunders Company, Philadelphia, :17-19.

Coni, E.; Bocca, A.; Coppolelli, P.; Caroli, S.; Cavallucci, C. and Marinucci MT. (1996). Minor and trace element content in sheep and goat milk and dairy products. Food Chemistry, ;57:253-60.

Conner, JG.; Eckersall, PD.;Wiseman, A. et al. (1988). Bovine acute phase response following turpentine injection. Res Vet Sci. ;44:82–8.

Constable, PD.; Hinchcliff, KW.; Done, SH. and GR.NBERG, W. (2017). Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats, 11th edition, Volumes 1 and 2. Elsevier Ltd. Printed in China.

Cousins, RJ. (1985). Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiological Reviews, ;65:238-309.

Cousins, RJ.; Blanchard, RK.; Moore, JB.; Cui, L.; Green, CL.; Liuzzi, JP. et al (2003). Regulation of zinc metabolism and genomic outcomes. J Nutr. ; 133:1521S–6S.

Cousins, RJ.; Liuzzi, JP. and Lichten, LA. (2006). Mammalian zinc transport, trafficking, and signals. J Biol Chem. ;281:24085–9.

References

Cronin, L. and Walton, PH. (2003). Synthesis and structure of [Zn (OMe) (L)] [Zn(OH)(L)]_2(BPh4), L=cis, cis-1,3,5-tris[(E,E) -3-(2) furyl) acrylideneamino] cyclohexane: Structural models of carbonic anhydrase and liver alcohol dehydrogenase. Chem. Commun. (Camb. Engl.), ;13:1572–3.

Dagmar, S.; Andrew W. and Marc E. (2010). Organ-specific eosinophilic disorders of the skin, lung and gastrointestinal tract. J Allergy Clin Immunol. ;126(1):3–13.

Daramolo, JO.; Adeloye, AA.; Fatoba, T. and Soladoye, AO. (2005). Hematological and biochemical parameters of West African Dwarf goats. Livestock Res. Rural Dev. ;17(8):21-7.

De Boeck, M.; Touil, N.; De Visscher, G.; Vande, PA. and Kirsch-Volders, M. (2000). Validation and implementation of an internal standard in comet assay analysis. Mutat Res. ;469(2):181-97.

De Jong, WH.; Hagens, WI.; Krystek, P.; Burger, MC.; Sips, AJ. and Geertsma, RE. (2008). Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. Biomaterials, ;29(12):1912-9.

Dellmann, H. D. and Eurell, J. (1998). Textbook of veterinary histology. 5th Ed., Lippincott Williams and Wilkins, Philadelphia, ;10:235.

Demetriou, J. and Stein, S. (2011). Causes and management of complications in wound healing. In Practice. ;33: 392–400.

Detweiler, GT.; Gipson, RC.; Merkel, AG. and Sahlu, T. (2008). Body Condition Scores in Goats. Pages 127-133 in Proc. 23rd Ann. Goat Field Day, Langston University, Langston, OK.

Dhivya, S.; Padma, VV. and Santhini, E. (2015). Wound dressings - A review. Biomedicine (Taipei), ;5(4):22.

Dhuha, KS.; Raghad, D. and Ahmed, NA. (2018). Biosynthesis of zinc oxide nanoparticles by hot aqueous extract of Allium sativum plants. J. Pharm. Sci. & Res. ;10(6): 1590-6.

Didar, U.; Sekhon, S. and Gupta, AS. (2017). Platelets and Platelet-Inspired Biomaterials Technologies in Wound Healing Applications. ACS Biomater. Sci. Eng. ;4 (4) :1176–92

Diegelmann, RF. and Evans, MC. (2004). Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci. ;9 :283-9.

Dierichs, L.; Kloubert, V. and Rink, L. (2017). Cellular zinc homeostasis modulates polarization of THP-1-derived macrophages. Eur. J. Nutr. ;57(6):2161-9.

Ding-Ping, B.; Xin, YL.; Yi, H. and Xi-Feng, Z. (2018). Theranostics Aspects of Various Nanoparticles in Veterinary Medicine. Int. J. Mol. Sci. ;19:3299.

Douglas, JW.; Jane Wardrop, K. and Schalm, OW. (2010). Schalm's Veterinary Hematology, 6th ed.USA. Wiley – Blackwell.

Doumas, BT. and Biggs, HG. (1976). standard Methods of clinical chemistry. Academic Press, N.y. ;7:175 .

Droke, EA. and Spears, JW., (1993). In vitro and in vivo immunological measurements in growing lambs fed diets deficient, marginal or adequate in zinc. J. Nutrition- Immunology, ;2(1) :71-90.

References

Droke, EA.; Spears, JW.; Armstrong, JD.; Kegley, EB. and Simpson, RB. (1993). Dietary zinc affects serum concentrations of insulin and insulin-like growth factor I in growing lambs. J nutrition, ;123:13-19.

Dubben, S.; Honscheid, A.; Winkler, K.; Rink, L. and Haase, H. (2010). Cellular zinc homeostasis is a regulator in monocyte differentiation of HL-60 cells by 1 alpha,25-dihydroxyvitamin D3. J Leukoc Biol. ;87:833–44.

Dubben, S.; Honscheid, A.; Winkler, K.; Rink, L. and Haase, H. (2010). Cellular zinc homeostasis is a regulator in Monocyte differentiation of HL-60 cells by 1 alpha, 25-dihydroxyvitamin D3. J Leukoc Biol. ;87:833–44.

Dyce, KM.; Sack, WO. and Wensing, CJG. (1996). Text Book of Veterinary Anatomy. 2nd Edn, WB Saunders. :347.

Ebiegberi, M.N. (2009). Hematological characteristics and performance of West African Dwarf Goats fed crude oil contaminated forage. Afr. J Biotech. Vol. 8(4) :699-702.

EFSA, Feedap Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed) (2014). Scientific Opinion on the potential reduction of the currently authorised maximum zinc content in complete feed. EFSA J. ;12(5): 3668−745.

Egbe-Nwiyi, TN.; Nwaosu, SC. and Salami, HA. (2000). Hematological values of apparently healthy sheep and goats as influenced by age and sex in the arid zone of Nigeria. Afr. J. Biomed. Res. ;3:109-15.

Ekta, Y.; Deepika, S.; Pankajkumar, Y. and Amita, V. (2018). Ameliorative effect of biofabricated ZnO nanoparticles of Trianthema portulacastrum Linn. on dermal wounds via removal of oxidative stress and inflammation. RSC Adv. ; 8 :21621–35.

Elamin, KM.; Dafalla ,NA.; Abdel Atti, KA. and Tameem Eldar, AA. (2013). Effects of Zinc Supplementation on Growth Performance and some Blood Parameters of Goat Kids in Sudan. IJPABRS;1(1):1-8.

Elham, A.; Hanan, A.; Naglaa, M.; Abdel-Fatah, A.; Essam, M.; Maha, Z. and Hesham, S. (2018). Evaluation the Effectiveness of Green Zinc Oxide Nanoparticles on The Anti-Inflammatory Effect of Dexamethasone and Its Side Effects in Rats. SVU-Int J Vet. Sci;1(1): 25-54.

Eryavuz, A.; Durgan, Z. and Keskun, E. (2002). Effects of ration supplemented with zinc on some rumen and blood parameters, mohair production and quality in faunated and defaunated Angora goats. Turk J Vet Anim Sci, ;26:753-60.

Ewuola, EO.; Falayan, OA.; Cibore, FA.; Adebunmi, AI.; Akanji, RA.; Ogunlade, JT. and Adeneye, JA. (2004). Physiological response of growing west African Dwarf goats fed groundnut shell-based diets as concentrate supplements. BOWEN J. Agric. ;1 (1): 61-9.

Failla, ML. (2003). Trace elements and host defense: recent advances and continuing challenges. J nutrition, ;133:1443s-7s.

Falah, HK. and Serwa, I.S. (2018). Clinical and histopathological evaluation of using platelet-rich plasma and platelet-rich fibrin matrix in treatment of induced chronic open wounds in Bucks. Asian J Pharm Clin Res. ;11(5) :337-41.

Fan, Z. and Lu, JG. (2005). Zinc oxide Nanostructures: synthesis and properties. J Nanosci Nanotechnol. ;5:1561–73.

Fe´lix, HD.; Fernando, T.; Silvia, M.; Asta, T.; Laura, SV. and Jose,´ J. (2008). Acute phase protein responses in goats. J VET DIAGN INVEST. vetd-20-05-06.3d. ;20(5):45-49.

Federico, A.; Morgillo, F.; Tuccillo, C.; Ciardiello, F. and Loguercio, C. (2007). Chronic inflammation and oxidative stress in human carcinogenesis. International Journal of Cancer, ;121(11): 2381-6.

Feng, M.; Wang, ZS.; Zhou, AG. and Ai, DW. (2009). The effects of different sizes of nanometer zinc oxide on the proliferation and cell integrity of mice duodenum-epithelial cells in primary culture. Pak J Nutr. ;8:1164–6.

Fernandes, MH.; Resende, KT.; Tedeschi, LO.; Fernandes, JS.; Silva, HM.; Carstens, GE.; Teixeira, IA. and Akinaga, L. (2007). Energy and protein requirements for maintenance and growth of Boer crossbred kids. J. Anim. Sci. ;85:1014-23.

Fernandes, MH.; Resende, KT.;Tedeschi, LO.; Teixeira, IA. and Fernandes Jr, JS. (2012). Macromineral requirements for the maintenance and growth of Boer crossbred kids. Journal of Animal Science, ;90:4458-66.

Ficociello, G.; Zanni, E.; Cialfi, S.; Aurizi, C.; Biolcati, G.; Palleschi, C.; Talora, C. and Uccelletti, D. (2016). Glutathione S-transferase _-subunit as a phenotypic suppressor of pmr1D strain, the Kluyveromyces lactis model for Hailey-Hailey disease. Biochim. Biophys. Acta. ;1863 :2650–7.

Fraker, PJ. and King, LE. (2004). Reprogramming of the immune system during zinc deficiency. Ann. Rev. Nutr. ;24 :277–98.

Francisco, HS.; Facundo, R. and Diana, CP. (2008). Fidel MG, Alberto EM, Amaury DJPG, Humberto TP, Gabriel MC. The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide and gold. NANOMED-NANOTECHNOL, ;4:237-40.

Freeman, R. and King B. (1972). Technique for the performance of the nitro-blue tetrazolium (NBT) test. Technical methods, :912-4.

Fukushima, T.; Horike, H.; Fujiki, S.; Kitada, S.; Sasaki, T. and Kashihara, N. (2009). Zinc deficiency anemia and effects of zinc therapy in maintenance hemodialysis patients. Ther. Apher. Dial. ;13 :213–9.

Fuwa, K.; Pablo, P.; Robert, M. and Vallee, BL. (1964). Determination of Zinc in Biological Materials by Atomic Absorption Spectrometry. Anal. Chem. ;36 :2407–11.

Gabbiani, G. (2003). The myofibroblast in wound healing and fibrocontractive diseases. J Pathol. ;200 :500-3.

Gammoh, NZ. and Rink, L. (2017). Zinc in Infection and Inflammation. Nutrients, ;9 :624.

Garg, A K.; Vishal, M. and Dass, R S. (2008). Effect of organic zinc supplementation on growth, nutrient utilization and mineral profile in lambs. Anim. Feed Sci. Technol. ;144:82-96.

Ginn, PE.; Mansell JE. and Rakich, KL. (2007). Skin and Appendages. In: Jubb,

Kenndey and Pathology of Domestic Animals, Maxie, M.G. (ED). Elsevier, Edinburgh, London, :620-32.

Gooneratne, S.; Buckley, W. and Christensen, D. (1989). Review of copper deficiency and metabolism in ruminants. Can. J. of Ani. Sci. ;69 :819-45.

Guo, S. and Dipietro, LA. (2010). Factors affecting wound healing. J OF Den. R. :89(3):219-29.

Gupta, A and Kumar, P. (2015). Assessment of the histological state of the healing wound. Plast Aesthet Res. ;2:239-42.

Gupta, AR.; Putra, RC.; Saini, M. and Sawrup, D. (2007). Haematology and serum biochemistry of Chital (Axis axis) and barking deer (Muntiacus muntjak) reared in semi-captivity. Vet. Res. Commun. ;31:801-8.

Haase, H. and Rink, L. (2009). Functional significance of zinc-related signaling pathways in immune cells. Annu Rev Nutr. ;29:133–52.

Haboubi, NY.; Baker, MA.; Gyde, OH.; Small, NA. and Haboubi, N. (1988). Zinc supplementation and erythropoiesis in the elderly. J. Clin. Pathol. ;41 :706.

Hagmeyer, S.; Haderspeck, JC. and Grabrucker, AM. (2014). Behavioral impairments in animal models for zinc deficiency. Front. Behav. Neurosci. ;8: 443.

Hambidge, KM.; Goodall, MJ.; Stall, C. and Pritts, J., (1989). Post-prandial and daily changes in plasma zinc. J Trace Elem Electrolytes Health Dis MEDLINE; 3(1):55-7.

Hampton, DL.; Miller, WJ.; Blackmon, DM.; Gentry, RP.; Neathery, MW.; Lassiter, JW.; Kincaid, RL. and Stake, PE. (1976). Zinc absorption from the small intestine in young calves. J. Dairy Sci;59: 712-5.

Hanley, C.; Thurber, A.; Hanna, C.; Punnoose, A.; Zhang, J. and Wingett, DG. (2009). The Influences of Cell Type and ZnO Nanoparticle Size on Immune Cell Cytotoxicity and Cytokine Induction. Nanoscale Research Letters, ;4(12):1409-20.

Harrison, P. and Cramer, EM. (1996). Platelet -granules. Blood Reviews, ;7:52-62.

Harvey, JW. (2010). Erythrocyte biochemistry. In: Weiss, D. J. & Wordrop, K. J. editors. Schalm's Veterinary Hematology. 6th Ed. Wiley-Blackwell Publishing Ltd, Ames. U.S.A, Iowa. :131-5.

Harvey, JW. (2008). The erythrocyte: physiology, metabolism, and biochemical disorders. In: Kaneko, J.J., Harvey, J.W. and Bruss, M.L. editors. Clinical Biochemistry of Domestic Animals. 6th edition. San Diego: Acedemic Press. U.S.A. 173-92.

Harvey, JW. (2001). Atlas of Veterinary Hematology: Blood and Bone Marrow of Domestic Animals . Philadelphia : Saunders, ;87-91.

Hayden, SJ.; Albert, TJ.; Watkins, TR. and Swenson, ER. (2012). Anemia in critical illness: Insights into etiology, consequences, and management. Am. J. Respir. Crit. Care Med. ;185 :1049–57.

Heyns Adu, P.; Eldor, A.; Yarom, R. and Marx, G. (1985). Zinc-induced platelet aggregation is mediated by the fibrinogen receptor and is not accompanied by

release or by thromboxane synthesis. Blood, ;66 :213–9.

Hillery, A.; Jani, P. and Florence, A. (1994). Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60 nm polystyrene particles. J DRUG TARGET;2(2):151-6.

Hinsbergh, VW.; Collen, A. and Koolwijk, P. (2001). Role of fibrin matrix in angiogenesis. Annals of the New York Academy of Sciences, ;936: 426–37.

Hock, H. and Orkin, SH. (2006). Zinc-finger transcription factor Gfi-1: versatile regulator of lymphocytes, neutrophils and hematopoietic stem cells. Curr Opin Hematol;13:1–6.

Holman, H. and Dew, S. (1965). The blood picture of the goat. Changes in Hb concentration and physical measurements occurring with age. Res. Vet. Sci. ;6:245.

Honscheid, A.; Rink, L. and Haase, H. (2009). T-lymphocytes: a target for stimulatory and inhibitory effects of zinc ions. Endocr Metab Immune Disord Drug Targets;9:132–44.

Hostetler, CE.; Kincaid, RL. and Mirando, MA. (2003). The role of essential trace elements in embryonic and fetal development in livestock. The Veterinary Journal, ;166:125-39.

Hussain, RM. And Salman, KO. (2012). Morphological classification of anemia in Local goats native. Al – Anbar Journal of Veterinary Sciences,;5(2):7-12.

Ibrahim, SO.; Helal, MA.; Abd El Rraof, YM. and Elattar, HM. (2016). Experimental study on zinc deficiency in sheep. BVMJ;31(1):110-8.

Iocono, J.; Ehrlich, H.; Gottrup, F. and Leaper, D. (1998). The biology of healing. In: Leaper DJ, Harding KG (eds). Wounds: Biology and Management. Oxford, New York: Oxford University Press. :10–22.

Ismail, RA. ; Ali, AK. ; Ismail, MM. and Hassoon, KI. (2011). “Preparation and characterization of colloidal ZnO nanoparticles using nanosecond laser ablation in water,” Appl. Nanosci;1(1) :45-9.

ISO, (2009). “Particle Size Analysis—Laser Diffraction Methods,” International Standards Organization ISO. 13320.

Iwata, Y.; Yoshizaki, A.; Komura, K.; Shimizu, K.; Ogawa, F.; Hara, T.; Muroi, E.; Bae, S.; Takenaka, M.; Yukami, T. et al. (2009). CD19, a response regulator of B lymphocytes, regulates wound healing through hyaluronan-induced TLR4 signaling. Am. J. Pathol. ;175 :649–60.

Jaison, J.; Ahmed, B.; Yen, S.; Alain, D. and Michael, K. (2018). Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol. ;9 :1050–74.

James, WD.; Berger, TG. and Elston, DM. (2006). Andrews’ diseases of the skin: Clinical dermatology. Philadelphia: Elsevier Saunders, 10th ed. : 203-13.

Jayaseelan, C.; Rahuman, AA.; Kirthi, AV.; Marimuthu, S.; Santhoshkumar, T.; Bagavan, A. et al., (2012). Novel microbial route to synthesize ZnO nanoparticles using Aeromonas hydrophila and their activity against pathogenic bacteria and fungi, Spectrochim. Acta A Mol. Biomol. Spectrosc. ;90 :78–84.

Ji, SK.; Xu, GS.; Diao, QY.; Jiang, CG.; Deng, KD.; Tua, Y. and Zhang, NF. (2014). Net zinc requirements of Dorper × thin-tailed Han crossbred lambs. Livestock Science, ;167:178-85.

Jia, WB.; Jia, ZH.; Zhang, W.; Wang, RL.; Zhang, SW. and Zhu XP. (2008). Effects of dietary zinc on performance, nutrient digestibility and plasma zinc status in Cashmere goats. Small Ruminant. Research, ;80:68-72.

Jinhuan, J.; Jiang, P. and Jiye, C. (2018). The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications. BIOINORG CHEM APPL;3:1-18.

João De Masia, EC.; Camposa, A.; João De Masi, FD.; Soatti Rattia, MA.; Shin Ikea, I. and João De Masi, RD. (2016). The influence of growth factors on skin wound healing in rats. Braz. J. Otorhinolaryngol. ;82(5):512-21.

Joda, M. (2008). Statistical analysis spss. Joda, M. (2008) The Progressive Statistical Analyses by Using SPSS. 1st Edition, Churchill Livingstone, Edinburgh.

Johnson-McDaniel, D.; Barrett, CA.; Sharafi, A. and Salguero, TT. (2013). Nanoscience of an Ancient Pigment. J. Am. Ceram. Soc. ;135 :1677–9.

Jones, PW. and Williams, DR. (2004). The use and role of zinc and its compounds in wound healing. Met Ions Biol Syst. ;41:139–83.

Juma, FT.; Maroff, NN. and Mahmood, KT. (2009). Effect of some hormones on reproductive performance and some serum biochemical changes in synchronized black goats. Iraqi J. Vet. Sci.;23(2) :57-61.

Justyna, R. and Katarzyna, Ż. (2014). Effects of mineral deficiency on the health of young ruminants. J. Elem.;19(3):915-28.

Kahmann, L.; Uciechowski, P.; Warmuth, S.; Malavolta, M.; Mocchegiani, E. and Rink, L. (2006). Effect of improved zinc status on T helper cell activation and TH1/TH2 ratio in healthy elderly individuals. Biogerontology, ;7:429–35.

Kanti, R.; Sadhana, O.; Alok, M.; VK, M.; Chandrakanta, R. and Sandeep, K. (2018). Impact of supplementation of mineral nano particles on growth performance and health status of animals: A review. Stud Zool. Entomol. J., ;6 (3): 1690-4.

Kavitha, KS.; Syed, B.; Rakshith, D.; Kavitha, HU.; Yashwantha Rao, HC.; Harini, BP. and Satish, S. (2013). Plants as green source towards synthesis of nanoparticles. Int. Res. J. Biol. Sci. ;2(6): 66- 76.

Kennedy, KJ.; Rains, TM. and Shay, NF. (1998). Zinc Deficiency Changes Preferred Macronutrient Intake in Subpopulations of Sprague-Dawley Outbred Rats and Reduces Hepatic Pyruvate Kinase Gene Expression. J Nutr. Jan. ; 128(1): 43-9.

Khaleel, ZK. (2013). Clinical and biochemical studies on zinc deficiency (hypozincemia) of goat and sheep. Kufa j. vet. Sci.;4(1) :69-74.

Khitam, SS.; Alhtheal, ED. and Azhar, JB. (2018). Effect of Zinc Oxide nanoparticals preparation from Zinc Sulphate (ZnSo4) against gram negative or gram positive microorganisms in vitro. Iraqi .J.Vet.Med;42(1): 18-22.

Khors Zak, A.; Majid, WH.; Ebrahimizadeh Abrishami, M.; Yousefi, R.; Parvizi, R. (2012). Synthesis, magnetic properties and X-ray analysis of Zn0.97X0.03O nanoparticles (X = Mn, Ni, and Co) using Scherrer and size-strain plot

methods. Solid State Sci. ;14 :488–94.

Kim, CS.; Nguyen, HD.; Ignacio, RM.; Kim, JH.; Cho, HC.; Maeng, EH.; Kim, YR.; Kim, MK.; Park, BK and Kim, SK. (2014). Immunotoxicity of zinc oxide nanoparticles with different size and electrostatic charge. International Journal of Nanomedicine, ;9(2): 195-205.

Kimber, MS. and Pai, EF. (2000). The active site architecture of Pisum sativum beta-carbonic anhydrase is a mirror image of that of alphacarbonic anhydrases. The EMBO journal. ;19 :1407-18.

Kinal, S.; Korniewicz, A.; Jamroz, D.; Zieminski, R. and Slupczynska M. (2005). Dietary effects of zinc, copper and manganese chelates and sulphates on dairy cows. J Food Agric Environ. ;3:68–172.

Kincaid, RL.; Chew, BP. and Cronrath, JD. (1997). Zinc oxide and amino acids as sources of dietary zinc for calves: Effects on uptake and immunity. J. Dairy Sci.;80:1381-8.

Kincaid, RL. and Cronrath, JD. (1979). Effects of dietary zinc upon tissue zinc and percent unsaturated plasma-zinc binding capacity. . J. Dairy Sci.;62: 572-6.

King, LE.; Frentzel, JW.; Mann, JJ. and Fraker, PJ. (2005). Chronic zinc deficiency in mice disrupted T cell lymphopoiesis and erythropoiesis while B cell lymphopoiesis and myelopoiesis were maintained. J. Am. Coll. Nutr. ;24 :494–502.

Kliche, S. and Waltenberg, J. (2001). VEGF receptor signaling and endothelial function. IUBMB Life, ;52 :61-6.

Kogan, S.; Sood, A. and Garnick, MS. (2017). Zinc and Wound Healing: A Review of Zinc Physiology and Clinical Applications. Wounds Compend. Clin. Res. Pract. ;29 :102–6.

Koyuncu, M. and Öziş, Altınçekiç, S. (2013). Importance of body condition score in dairy goats. MJAS;3(2) :167–73.

Kralj, S. and Makovec, D. (2015). "Magnetic Assembly of Superparamagnetic Iron Oxide Nanoparticle Clusters into Nanochains and Nanobundles". ACS Nano. ;9 (10): 9700–7.

Krametter-Froetscher, R.; Hauser S. and Baumgartner W. (2005). Zinc-responsive dermatosis in goats suggestive of hereditary malabsorption: two field cases. Vet. Dermatol. ;16 :269–275.

Kreuter, J. (2005). “Application of Nanoparticles for the Delivery of Drugs to the Brain” .International Congress Series. ;1277: 85–94.

Kruczynska, H. (2004). Excess is also unhealthy (in Polish). Hoduj z Glowa. ;3:12-5.

Kumar, A.; Najafzadeh, M.; Jacob, BK.; Dhawan, A. and Anderson, D. (2015). Zinc oxide nanoparticles affect the expression of p53, Ras p21 and JNKs: an ex vivo/in vitro exposure study in respiratory disease patients. Mutagenesis, ;30(2): 237-45.

Kumari, L. and Li, WZ. (2010). Synthesis, structure and optical properties of zinc oxide hexagonal microprisms. Cryst Res Technol.;45:311–5.

Lamand, M. (1984). Zinc deficiency in ruminants. Irish Vet J. ;38:40-7.

Lansdown, AB.; Sampson, B. and Rowe, A. (1999). Sequential changes in trace metal, metallothionein and calmodulin concentrations in healing skin wounds. J Anat.;195(3):375–86.

Lansdown, BG.; Ursula Mirastschijski, MD.; Nicky Stubbs, RN.; Elizabeth Scanlon, RN. and Magnus, SA. (2007). "Zinc in Wound Healing: Theoretical, Experimental, and Clinical Aspects." Wound Repair Regen;15(1): 2–16.

Lappin, MB. and Campbell, JD. (2000). The Th1-Th2 classification of cellular immune responses: concepts, current thinking and applications in haematological malignancy. Blood Reviews, ;14(4): 228-39.

Lazurus, GS.; Cooper, DM. and Knighton, DR. (1994). Definitions and guidelines for assessment of wounds and evaluation of healing. Arch. Dermatol, ;130: 489 – 93.

Lee, S.; Eskin, SG.; Shah, AK.; Schildmeyer, LA. and McIntire, LV. (2012). Effect of zinc and nitric oxide on monocyte adhesion to endothelial cells under shear stress. Ann. Biomed. Eng. ;40 :697–06.

Lenka, K.; Leoš, P.; Alena, P. and Jaroslav, F. (2016). Blood serum protein in periparturient goats supplemented with various forms of zinc. ACTA VET. BRNO. ;85: 387-94.

Li, H. and Chang, J. (2013). Bioactive silicate materials stimulate angiogenesis in fibroblast and endothelial cell co-culture system through paracrine effect. Acta Biomater. ;9 :6981–91.

Li, Y.; Zhang, W.; Niu, J. and Chen, Y. (2012). Mechanism of Photogenerated Reactive Oxygen Species and Correlation with the Antibacterial Properties of Engineered Metal-Oxide Nanoparticles. ACS Nano. ;6:5164–73.

Linklater, KA. and Smith, MC. (1993). Color atlas of diseases and disorders of the sheep and goat. London : Wolfe Pub., an imprint of Mosby Year Book Europe Ltd., English.

Liuzzi, JP.; Lichten, LA.; Rivera, S.; Blanchard, RK.; Aydemir, TB.; Knutson, MD. et al., (2005). Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. Proc Natl Acad Sci U S A. ;102: 6843–8.

Lodish, H.; Flygare, J. and Chou, S. (2010). From stem cell to erythroblast: Regulation of red cell production at multiple levels by multiple hormones. NIH Public Access. ;62(7):492–6.

Lugon, J. (2005). Uremic pruritus: A review. Hemodial Int. ;9:180–8.

Ma, YY.; Ding, H. and Xiong, HM. (2015). "Folic acid functionalized ZnO quantum dots for targeted cancer cell imaging," Nanotechnology, ;26(30) :305702.

Mackenzie, GG.; Zago, MP.; Erlejman, AG.; Aimo, L.; Keen, CL. and Oteiza, PI. (2006). alpha-Lipoic acid and N-acetyl cysteine prevent zinc deficiency-induced activation of NFkappaB and AP-1 transcription factors in human neuroblastoma IMR-32 cells. Free Radic Res. ;40:75–84.

Madan, J.; Sindhu, S.; Gupta, M. and Kumar, S. (2016). Hematobiochemical profile and mineral status in growing beetal goat kids. J. Cell Tissue Res. ;16: 5517-22.

Mahgoub, O. and Lu, C. D. (1998). Growth, body and carcass tissue distribution in goats of large and small sizes. Small Ruminant Res;27:267-78.

Majumder, M. and Kamaath, JV. (2005). Herbal concept of wound healing. Journal of Pharmaceutical Research, ;4(1): 1-7.

Mandal, GP.; Dass, RS.; Isore, DP.; Garg, AK. and Ram, GC. (2007). Effect of zinc supplementation from two sources on growth, nutrient utilization and immune response in male crossbred cattle (Bos indicus × Bos taurus) bulls. Anim Feed Sci Technol. ;138: 1–12.

Manuja, A.; Kumar, B. and Singh, RK. (2012). Nanotechnology developments: opportunities for animal health and production. Nanotechnology Development, ;2: 17-25.

Marcos Jácome, A.; Ariosvaldo Nunes, M. ; Izabelle, AM. ; Roberto, GC. ; Carlo, AT. ; Kléber, TR. and Gabriel, MP. (2017). Trace mineral requirements for growth of Moxotó goats grazing in the semiarid region of Brazil. Braz. J. An. Sci.;46(3):231-9.

Martins, VL.; Caley, M. and O'Toole, EA. (2013). Matrix metalloproteinases and epidermal wound repair. Cell Tissue Res. ;351 :255–68.

Marx, G.; Krugliak, J. and Shaklai, M. (1991). Nutritional zinc increases platelet reactivity. Am. J. Hematol. ;38 :161–5.

Maywald, M.; Meurer, SK.; Weiskirchen, R. and Rink, L. (2017). Zinc supplementation augments TGF-beta1-dependent regulatory T cell induction. Mol. Nutr. Food Res. ;61(3).

Mbassa, GK. and Poulsen, JS. (1991). Influence of pregnancy, lactation and environment on hematological profiles in Danish Landrace dairy goats Caprahircus) of different parity. Comp Biochem Physiol, ;100 :403 – 12.

McCarty, SM.; Cochrane, CA.; Clegg, PD. and Percival, SL. (2012). The role of endogenous and exogenous enzymes in chronic wounds: A focus on the implications of aberrant levels of both host and bacterial proteases in wound healing. Wound Repair Regen, ;20 :125–36.

Mcgavin, MD. and Zachary, JF. (2007). Pathological basis of veterinary disease. 4th Edn. Mosby Publishing, St, Louis, MO., USA., :622-30.

Meyers, KM. (1985). Pathobiology of animal platelets. Adv Vet Sci Comp Med. ;30 : 131 – 65.

Miao, X.; Sun, W.; Fu, Y.; Miao, L. and Cai, L. (2013). Zinc homeostasis in the metabolic syndrome and diabetes. Front. Med. ;7 :31-52.

Mickelson, MA.; Mans, C. and Colopy, SA. (2016). Principles of Wound Management and Wound Healing in the Exotic Pets. Vet. Clin. North. Am. Exot. Anim. Pract. ;19(1): 33–53.

Mihai, MM.; Dima, MB.; Dima, B. and Holban, AM. (2019). Nanomaterials for Wound Healing and Infection Control. Materials, ;12 :2176.

Miller, WJ.; Pitts, WJ. and Clifton, CM. (1964). Experimentally produced zinc deficiency in the goat. Journal of Dairy Science, ;47: 556–8.

Miller, WJ.; Blackmon, DM.; Gentry, RP.; Pitts, WJ. and Powell, GW. (1967).

Absorption, excretion, and retention of orally administered zinc-65 in various tissues of zinc-deficient and normal goats and calves. Journal of Nutrition. ;92: 71-8.

Miller, WJ.; Blackmon, DM.; Powell, GW.; Gentry, RP. and Hiers, JM. (1966). Effects of zinc deficiency perse and of dietary zinc level on urinary and endogenous fecal excretion of 65Zn from a single intravenous dose by ruminants. J. Nutrition. ;90: 335-41.

Mirzaei, HH. and Darroudi, M. (2017). Zinc oxide nanoparticles: biological synthesis and biomedical applications, Ceram. Int. ;43:907–14.

Mishra, PK.; Mishra, H.; Ekielski, A.; Talegaonkar, S. and Vaidya, B. (2017). "Zinc oxide nanoparticles: a promising nanomaterial for biomedical applications," Drug Discov. Today;22(12) :1825–34.

Mobarez, A.; Hanan, A.; Naglaa, M.; Abdel-Fatah, A.; Essam, M.; Maha, Z. and Hesham S. (2018). Evaluation the Effectiveness of Green Zinc Oxide Nanoparticles on The Anti-Inflammatory Effect of Dexamethasone and Its Side Effects in Rats. SVU-Int J Vet Sci.;1(1) :25-54.

Mobini, A. (2013). A quantitative evaluation of different regions of skin in adult Iranian native sheep. Vet. Medicina. ;58(5) :260–3.

Mohamed, I.; Kenneth, W. and Cristina, M. (2018). Nanotechnology considerations for poultry and livestock production systems – a review Ann. Anim. Sci. ;18(2) :319–34.

MoraG, GK. (2002). Vet. Lab. Med. (Clinical Biochemistry and Hematology). 2nd. ed. Blackwell Sci. :74-130.

Morgan-Davies, C.; Waterhouse, A.; Pollock, M. L. and Milner, JM. (2008). Body Condition Score as An Indicator of Ewe Survival Under Extensive Conditions. Anim. Welf. ;17 :71–7.

Morrell, CN.; Aggrey, AA.; Chapman, LM. and Modjeski, KL. (2014). Emerging roles for platelets as immune and inflammatory cells. Blood, ;123 :2759–67.

Mullowney, P. and Baldwin, EW. (1984). Skin disease of goats. Veterinary Clinics of North America: Large Animal Practice, ;6 :143–54.

Murata, H.; Shimada, N. and Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. Vet J. ;168:24-40.

Najafzadeh, H.; Ghoreishi, SM.; Mohammadian, B.; Rahimi, E.; Afzalzadeh, MR.; Kazemivarnamkhasti, M. and Ganjealidarani, H. (2013). Serum biochemical and histopathological changes in liver and kidney in lambs after zinc oxide nanoparticles administration. Vet World. ;6(8) :534-7.

Naraginti, S.; Kumari, PL.; Das, RK.; Sivakumar, A.; Patil, SH. and Andhalkar, VV. (2016). Amelioration of excision wounds by topical application of green synthesized, formulated silver and gold nanoparticles in albino Wistar rats.Mater. Sci. Eng C. ;62 :293–300.

Neathery, MW.; Miller, WJ.; Blackmon, DM. et al.(1973). Effects of long term zinc deficiency on feed utilization, reproductive, characteristics, and hair growth in sexually mature male goat. J. Dairy Sci.;56 :98–105.

Negut, I.; Grumezescu, V. and Grumezescu, AM. (2018). Treatment Strategies for

Infected Wounds. Molecules, ;23(9) :E2392.

Nelson, DR.; Wolff, WA.; Blodgett, DJ. et al (1984). Zinc deficiency in sheep and goats: three field cases. JAVMA-J AM VET MED A;184 :1480–5.

Newman, MD.; Stotland, M. and Ellis, JI. (2009).“The safety of Nano sized particles in titanium dioxide- and zinc oxide based sunscreens,” Journal of the American Academy of Dermatology, ;61(4) :685–92.

Nezar, A. and Mohamed, M. (2014). Preliminary study of the influence of red blood cells morphometry on the species determinism of domestic animals. Veterinary World, ;7(4):219-23.

Nikolidakis, D. and Jansen, JA. (2008). The biology of platelet-rich plasma and its application in oral surgery: literature review. Tissue Engineering Part B Reviews, 14(3) :249–58.

Nomura, K.; Yonezawa, T.; Mano, S.; Kawakami, S.; Shedlock, AM. et al. (2013). Domestication Process of the Goat Revealed by an Analysis of the Nearly Complete Mitochondrial Protein-Encoding Genes. PLOS ONE. ;8(8): e67775.

NRC - National Research Council (2007). Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. 1st rev.ed. National Academy Press, Washington, DC.

Nussbaum, SR.; Carter, MJ.; Fife, CE.; Da Vanzo, J.; Haught, R.; Nusgart, M. and Cartwright, D. (2018). An economic evaluation of the impact, cost, and medicare policy implications of chronic nonhealing wounds. Value Health, Doi.10:1016/j. jval. ;21(1) :27-32.

O' Hagan, DT. (1996). The intestinal uptake of particles and the implications for drug and antigen delivery. J. Anat.;189:477-82.

O’Dell, BL. (1992). Zinc plays both structural and catalytic roles in metalloproteins. Nutr Rev. ;50: 48–50.

O’Dell, BL. (2000). Role of zinc in plasma membrane function. J Nutr. ;130: 1432S–6S.

Ogawa, Y.; Kawamura, T. and Shimada, S. (2016). Zinc and skin biology. Archives of biochemistry and biophysic, :1-7.

Olaifa, AK. and Fadason ST. (2016). Studies on Zinc and Copper Ion in Relation to Wound Healing in Male and Female West African Dwarf Goats. Niger. J. Physiol. Sci. ;31 :171-6.

Olaifa, AK.; Samuel, OO. and Banjoko, OM. (2016). Effects of Topical 17β Estradiol on Wound Healing and Hemato-Biochemical Changes In West African Dwarf Goats. International Journal of Livestock Research, ;6 (8) :15-26.

Olayemi, FO.; Oboye, OO.; Azeez, IO.; Oyagbemi, AA. and Soetan, KO. (2009). Influence of management systems and sex on hematology of West African dwarf goat. Afr. J. Agric. Res. ;4(11) :1199-202.

Olive, L.; Durand, E.; Banath, P. and Johnsto, J. (2001). Analysis of DNA damage in individual cells. Methods in cell biology, ;64part B :235-49.

Olever, CS.; Andrews, GA.; Smith, JE. And Kaneko, JJ. (2010). Erythrocyte structure and function. In: Weiss, DJ. and Wordrop, KJ. Editor. Schalms Veterinary Hematology.6th Edition. Wily-Blakwell Publishing Ltd, Ames. USA,Iowa.

P123-30.

Olorunnisomo, OA. ; Ewuola, EO. and Lawal, TT. (2012). Intake and Blood Metabolites in red Sokoto Goats fed Elephant Grass and Cassava Peel Silage . J. Anim. Prod. Adv.;2(9) :420-8.

Opara, MN.; Udevi, N. and Okoli, IC. (2010). Hematological parameters and Blood Chemistry of Apparently Healthy West African Dwarf (Wad) Goats in Owerri, South Eastern Nigeria.New York Science Journal, ;(8) :68-72.

Oyarzun-Ampuero, F.; Vidal, A.; Concha, M.; Morales, J.; Orellana, S. and Moreno-Villoslada, I. (2015). Nanoparticles for the Treatment of Wounds. Curr. Pharm. Des. ;21 :4329–41.

Ozfiliz, N.; Balikcier, M.; Erdost, H. and Zik, B. (2002). Histological and morphometric features of the skin of native and hybrid (f2) sheep. Turk. J. Vet. Animal Sci. :429–38.

Padmavathy, N. and Vijayaraghavan, R. (2008). Enhanced bioactivity of ZnO nanoparticles - an antimicrobial study. Sci Technol Adv Mater, ;9:1-7.

Pal, DT.; Gowda, NK. ; Prasad, CS. ; Amarnath, R.; Bharadwaj, U.; Sureshbabu, G. and Sampath, KT. (2010). Effect of copper- and zinc-methionine supplementation on bioavailability, mineral status and tissue concentrations of copper and Zn in ewes. J. of Trace Elements in Medicine and Biology. ;24:89–94.

Partha, S.; Somu, BN.; Duraisamy, R.; George, D. and Sellappan, S. (2016). Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. Animal Nutrition, ;2:134-41.

Pei-Hui, L.; Matthew, S.; Haichang, L.; Peter, HU.; Steven, M.; Steinberg, and Jianjie M. (2017). Zinc in Wound Healing Modulation. Nutrients, ;10(16) :1-20.

Perme, B.; Dass, RS.; Garg, AK. and Sethy, K. (2013). Effect of inorganic and organic copper and zinc supplementation on haematological and blood biochemical profile of male kids. Indian J. Anim. Prod. Manag. ;29(3-4):30-3.

Phiri, EC.; Viva, M.; Chibunda, RT. and Mellau, LS. (2009). Effect of zinc supplementation on plasma mineral concentration in grazing goats in sub-humid climate of Tanzania. TVJ;26(2):92-6.

Phythian, CJ.; Michalopoulou, E.; Jones, PH.; Winter, AC.; Clarkson, MJ.; Stubbings, LA.; Grove-White, D.; Cripps, PJ. and Duncan, JS. (2011). Validating Indicators of Sheep Welfare Through a Consensus of Expert Opinion. Anim. ;5: 943–52.

Piccione, G.; Casella, S.; Lutri, L.; Vazzana, I.; Ferrantelli, V. and Caola, G. (2010). Reference values for some haematological, haematochemical and electrophoretic parameters in the Girgentana goat. Turk. J. Vet. Anim. Sci. ;34(2):197-204.

Pisoschi, AM. and Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. Eur. J. Med. Chem. ;97:55–74.

Powell, SR. (2000). The antioxidant properties of zinc. J. Nutr;130:1447s–54s.

Prasad, AS. (1995). Zinc an overview. Nutrition. ;11: 93–9.

References

Prasad, AS. (2007). Zinc: mechanisms of host defense. J. Nutr;137: 1345-9.

Prasad, AS. (2009). Zinc role in immunity, oxidative stress and chronic inflammation. Curr Opin Clin Nutr Metab Care. ;12:646–52.

Prasad, AS. (2014). Zinc An antioxidant and anti-inflammatory agent: Role of zinc in degenerative disorders of aging. J. Trace Elem. Med. Biol. ;28: 364–71.

Prasad, AS.; Bao, B.; Beck, FW. and Sarkar, FH. (2010). Zinc-suppressed inflammatory cytokines by induction of A20-mediated inhibition of nuclear factor-kappaB. Nutrition, ;27(7-8):816-23.

Prasad, AS.; Beck, FW.; Bao, B.; Fitzgerald, JT.; Snell, DC.; Steinberg, JD. et al. (2007). Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. Am J Clin Nutr. ;85:837–44.

Prasad, K.; Jha, AK. (2009). ZnO nanoparticles: synthesis and adsorption study, Nat. Sci. ;1 :129–35.

Puchala, R.; Sahlu, T. and Davis, JJ. (1999). Effects of zinc methionine on performance of Angora goats. Small Ruminant Res;33:1-8.

Pugh, DG. (2002). Atext Book of Sheep and Goat medicine. 1st edition. W. B. Saundes,USA. :7211-16.

Pulit-prociak, J.; Chwastowski, J; Kucharski, A. and Banach, M. (2016). Applied surface science functionalization of textiles with silver and zinc oxide nanoparticles, Appl. Surf. Sci. ;385 :543–53.

Raguvaran, R.; Anju, M. and Balvinder, KM. (2015). Zinc Oxide Nanoparticles: Opportunities and Challenges in Veterinary Sciences. Immunome Res.;11(2):1-8.

Ramirez-Perez, AH.; Buntinx, SE. and Rosiles, R. (2000). “Effect of Breed and Age on the Voluntary Intake and the Micro mineral Status of Non-Pregnant Sheep.” Small Ruminant Research, ;37(1):231–42.

Rasmussen, JW.; Martinez, E.; Louka, P. and Wingett, DG. (2010). “Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications,” Expert Opin Drug Deliv.;7(9):1063–77.

Raulin J. (1869). Chemical studies on vegetation. Ann Sci Nat. ;11:93–9.

Razvia, R.; Suria, S.; Sarmaa, K. and Sharma, R. (2015). Histomorphological and histochemical studies on the different layers of skin of Bakerwali goat. J. Appl. Anim. Res. ;(43)2:208–13.

Reinke, JM. and Sorg, H. (2012). Wound repair and regeneration. Europ. Surg. Res. ;49(1):35–43.

Reuter, R.; Bowden, M.; Besier, B. et al. (1987). Zinc responsive alopecia and Hyperkeratosis in Angora goats. Aust Vet J;64:351–2.

Rice, CG. and Hall, B. (2007). Hematologic and biochemical reference intervals for mountain goats (Oreamnos americanus): effects of capture conditions. Northwest Sci. ;81(3):206-14.

Richa, S. (2012). Synthesis and Characterization Techniques of Nanomaterial’s. Int. J. Green Nanotechnol. Biomed.;4:1:17-27.

References

Robinson, JJ. ; Ashworth, CJ.; Rooke, JA. ; Mitchell, LM. and McEvoy, TG. (2006). Nutrition and fertility in ruminant livestock. Animal Feed Science and Technology, ;126:259-76.

Robson, MC.; Steed, DL. and Franz, MG. (2001). Wound healing: biologic features and approaches to maximize healing trajectories. Curr. Probl. Surg. ;38:72 – 140.

Rodgers, P. (2006). "Nanoelectronics: Single file". Nature Nanotechnology. doi:10.1038/nnano.2006.5.

Roubies, N.; Panousis, N.; Fytianou, A.; Katsoulos, PD.; Giadinis, N. and Karatzias, H. (2006). Effects of age and reproductive stage on certain serum biochemical parameters of Chios sheep under Greek rearing conditions. J. Vet. Med. A Physiol. Pathol. Clin. Med. ;53:277-81.

Roy, R.; Kumar, S.; Verma, AK.; Sharma, A.; Chaudhari, BP.; Tripathi, A. and Dwivedi, DM. (2014). Zinc nanoparticles provide an adjuvant effect to or albumin via Th2 response in Balb /c mice. Int Immunol;26(3): 159-72.

Sahoo, S. ; Maiti, M.; Ganguly, A.; George, JJ. and Bhowmick, AK. (2007). “Effect of zinc oxide nanoparticles as cure activator on the properties of natural rubber and nitrile rubber,” J. Appl. Polym. Sci.;105(4):2407–15.

Salah, N.; Habib, SS. ; Khan, ZH. et al., (2011). “High-energy ball milling technique for ZnO nanoparticles as antibacterial material,” Int J Nanomed;6:863–9.

Salama, AA.; Caja, G.; Albanell, E.; Such, X. and Caslas, R. (2003). Effects of dietary supplements of zinc-methionine on milk production, udder health and zinc metabolism in dairy goats. J Dairy Res. ;70: 9–17.

Saleemm, MA.; Habeeb, RS. and Abdullah, M. (2016). Comparative histological study of the skin layers of black goats in north Iraq. AL-Qadisiyah. J. Vet. Med. Sci. ;15(2):25-30.

Sargent, J. and John, F. (2014). ”The National Nanotechnology Initiative: overview, reauthorization, and appropriations issues.” library of congress washington dc congressional research service, december16.

Scenihr, (2010). (Scientific Committee on Emerging and Newly Identified Health Risks) .Scientific Basis for the Definition of the Term “nanomaterial”, adopted 8 December.

Schulze, A. and Üstdal, KM. (1975). Mögliche Ursachen der Alopezie türkischer Angora-Ziegen. Berl Munch Tierarztl;88: 66-73.

Shahraz, S. and Ghaziani, T. (2005). A comprehensive textbook of drug information. 3rd ed. Tehran: Teimourzadeh, :744-5.

Shaikat, AH.; Hassan, MM.; Khan, SH.; Islam, MN.; Hoque, MA.; Bari, MS. and Hossain, ME. (2013). Haematobiochemical profiles of indigenous goats (Capra hircus) at Chittagong, Bangladesh. Vet. World, ;6(10):789-93.

Shamhari, NM.; Boon, SW.; Suk, FC. and Kuan, YK. (2018). Synthesis and Characterization of Zinc Oxide Nanoparticles with Small Particle Size Distribution. Acta Chim. Slov. ;65 :578–85.

Shankar, AH. and Prasad, AS. (1998). Zinc and immune function: the biological basis of altered resistance to infection. AM J Clin Nutr, ;68:447S-63S.

Sharma, MC. and Joshi, C. (2005). Therapeutic efficacy of zinc sulphate used in clustered model treatment in alleviating zinc deficiency in cattle and its effect on hormones, vitamins and production parameters. Veterinary research communications, ;29 :609-28.

Shaw, TJ. and Martin, P. (2009). Wound repair at a glance. J. Cell Sci. ;122(18): 3209–13.

Shim, KH.; Hulme, J.; Maeng, EH.; Kim, M. and An, SS. (2014). Analysis of zinc oxide nanoparticles binding proteins in rat blood and brain homogenate. International Journal of Nanomedicine. ;9(Suppl 2) :217–24.

Siemes, C.; Quast, T.; Klein, E.; Bieber, T.; Hooper, NM. and Herzog, V. (2004). Normalized proliferation of normal and psoriatic keratinocytes by suppression of SAPPalpha-release. J Invest Dermatol, ;123: 556–63.

Silanikove, N. (2010). The physiological basis of adaptation in goats to harsh environments. Small Rumin. Res., ;35: 181-93.

Silva, CM.; Medeiros, AN.; Costa, RG.; Sales, EP.; Silva, AM. and Lima Jr, VL. (2013). Micromineral nutritional requirements for weight gain in Canindé goats under grazing in the Brazilian semiarid. Acta Sci. Anim. Sci.;35:173-9.

Simón-Vázquez, R.; Lozano-Fernández, T.; Dávila-Grana, A. and González-Fernández, A. (2016). Metal oxide nanoparticles interact with immune cells and activate different cellular responses. Int J Nanomed;14(11): 4657-68.

Singer, LJ.; Herron, A. and Altman, N. (2000). Zinc responsive dermatopathy in goats. Contemp. Top. Lab. Anim. Sci. ;39:32-5.

Skerman, TM. (2000). Foot scald and foot rot in goats, Sheep and Beef Cattle Society of the New Zealand Veterinary Association. Proceedings of the Society's 17th Seminar; Waikato University, :65-9.

Sliwinska, A.; Kwiatkowski, D.; Czarny, P.; Milczarek, J.; Toma, M.; Korycinska, A.; Szemraj, J. and Sliwinski, T. (2015). Genotoxicity and cytotoxicity of ZnO and Al2O3 nanoparticles. Toxicol. Mech. Methods;25(3):176-83.

Smijs, TG. and Pavel, S. (2011). “Titanium dioxide and zinc oxide Nanoparticles in sunscreens: focus on their safety and effectiveness,”Nanotechnology, Science and Applications, ;4:95–112.

Smith, MC. and Sherman, DM. (1994). Zinc deficiency. In: Smith MC, Sherman DM eds. Goat Medicine, 1st edn. Philadelphia: Lea & Febiger, :34.

Smith, BP. (2002). Large animal internal medicine. Mosby, Inc. Third Ed., USA.

Smith, MC. and Sherman, DM. (1994). Blood, lymph, and immune systems. In: Goat Medicine. Malvern PA : Lea & Febiger :193 – 229.

Smith, OB. and Akinbamijo, OO. (2000). Micronutrients and reproduction in farm animals. Anim. Reprod. Sci. :60-1,:549-60.

Spears, JW. (2003). Trace mineral bioavailability in ruminants. J Nutr. ;133:1506S–9S.

Sri Sindhura, K.; Selvam, PP.; Prasad, T. and Hussain, OM. (2014). Synthesis, characterization and evaluation of effect of phytogenic zinc nanoparticles on soil exo-enzymes. Appl Nanosci. ;4:819-27.

Steuer, H.; Krastev, R. and Lembert, N. (2014). Metallic oxide nanoparticles stimulate

blood coagulation independent of their surface charge. J Biomed Mater Res B Appl Biomater. ;102(5):897-902.

Sulaiman, EG.; Arslan, SH.; Al-Obaidi, QT. and Daham, E. (2010). Clinical, hematological and biochemical studies of babesiosis in native goats in Mosul. Iraqi J. Vet. Sci. ;24(1):31-5.

Suttle, NF. (2010). Mineral Nutrition of Livestock, 4th Edition. Wallingford, Oxfordshire, UK : CABI.

Suttle, NF. (2000). Minerals in Livestock Production. Underwood Memorial Lecture. Asian-Aus.J. Anim. Sci. ;13(Suppl):1-9.

Suttle, NF. and Jones, G. (2007). Micronutrient imbalance. In: disease of sheep, Aitken, I.D. (Ed). 4th Edu., Blackwell Publishing, Oxford, UK. :377-92.

Suzuki, Y.; Tada-Oikawa, S.; Ichihara, G.; Yabata, M.; Izuoka, K.; Suzuki, M.; Sakai, K. and Ichihara, S. (2014). Zinc Oxide Nanoparticles induce migration and adhesion of monocytes to endothelial cells and accelerate foam cell formation. Toxicol. Appl. Pharmacol. ;278:16–25.

Tambuwal, FM.; Agale, BM. and Bangana, A. (2002). Haematological and Biochemicalvalues of Apparently Healthy Red Sokoto goats. Proceedings of 27th Annual conference Nigerian society of Animal production (NSAP) March 17-21, 2002 FOTA, Akure.

Taylor, KA. and Pugh, N. (2016). The contribution of zinc to platelet behaviour during haemostasis and thrombosis. Metallomics Integr. Biomet. Sci. ;8 :144–55.

Tezvergil-Mutluay, A.; Agee, KA.; Hoshika, T.; Carrilho, M.; Breschi, L.; Tjaderhane, L.; Nishitani, Y.; Carvalho, RM.; Looney, S.; Tay, FR. et al. (2010). The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices. Dent. Mater. ;26:1059–67.

Tietz, NW. (1999). Text Book of Clinical Chemistry. 3rd Edn., W.B. Saunders, USA., pp: 809-861.

Tomlinson, ML.; Garcia-Morales, C.; Abu-Elmagd, M. and Wheeler, GN. (2008). Three matrix metalloproteinases are required in Vivo for macrophage migration during embryonic development. Mechan. Dev. ;125:1059–70.

Tracy, LE.; Minasian, RA. and Caterson, EJ. (2016). Extracellular Matrix and Dermal Fibroblast Function in the Healing Wound. Adv. Wound Care. ;5: 119–36.

Tubek, S.; Grzanka, P. and Tubek, I. (2008). Role of zinc in hemostasis: a review. Biol Trace Elem Res. ;121(1):1-8.

Ukanwoko, AI.; Ironkwe, MO. and Nmecha, C. (2013). Growth Performance and Hematological Characteristics of West African Dwarf Goats Fed Oil Palm Leaf Meal – Cassava Peel Based Diets. J. Anim. Prod. Adv.;3(1): 1-5.

Underwood, EJ. and NF. Suttle. (1999). The mineral nutrition of livestock, 3rd ed. CABI Publishing, CAB International, Wallingford, Oxon, UK.

Valle, BL. and Falchuk, KH. (1993). The biochemical basis of zinc physiology. Physiol Rev; 73: 79–118.

Van Rijswijk, L. (2013). Wound Wise: Measuring Wounds to Improve Outcomes. Am. J. Nurs (AJN);113 (8):60 – 1.

References

Van Wouwe, JP. (1989). Clinical and laboratory diagnosis of acrodermatitis enteropathica. Eur. J. Pediatr.;149: 2-8.

Vijayakumar, V.; Samal, SK.; Mohanty, S. and Nayak, SK. (2019). Recent advancements in biopolymer and metal nanoparticle-based materials in diabetic wound healing management. Int. J. Biol. Macromol. ;122:137–48.

Vruwink, KG.; Fletcher, MP.; Keen, CL.; Golub, MS.; Hendrickx, AG. and Gershwin, ME. (1991). Moderate zinc deficiency in rhesus monkeys. An intrinsic defect of neutrophil chemotaxis corrected by zinc repletion. J. Immunol. ;146: 244–9.

Walter, P.; Welcomme, E.; Hallégot, P.; Zaluzec, NJ.; Deeb, C.; Castaing, J.; Veyssière, P.; Bréniaux, R.; Lévêque, JL. and Tsoucaris, G. (2006). Early use of PbS nanotechnology for an ancient hair dyeing formula. Nano Lett. ;6(10):2215-9.

Wang, L.; Qin, W.; Zhou, Y.; Chen, B.; Zhao, X.; Zhao, H.; Mi, E.; Mi, E.; Wang, Q. and Ning, J. (2017). Transforming growth factor β plays an important role in enhancing wound healing by topical application of Povidone-iodine. Scientific Reports, ;7 (1): 991.

Wang, Z.; Hongwei, L.; Fanghua, T.; Jinxia, M. and Xiaofan, Z. (2018). A Facile Approach for the Preparation of Nano-size Zinc Oxide in Water/Glycerol with Extremely Concentrated Zinc Sources. Nanoscale Res. Lett.;13:202.

Watson, BR.; White, NA.; Taylor, KA.; Howes, JM.; Malcor, JD.; Bihan, D.; Sage, SO.; Farndale, RW. and Pugh, N. (2016). Zinc is a transmembrane agonist that induces platelet activation in a tyrosine phosphorylation- dependent manner. Metallomics Integr. Biomet. Sci. ;8: 91–100.

Webb, DI.; Parker, L. and Webb, K. (2004). Platelet count assessment from peripheral blood smear (PBS). Alaska Med. ;46(4):92-5.

Wedekind, KJ. and Baker, DH. (1990). Zinc bioavailability in feed-grade sources of zinc. J. Anim. Sci.;68:684-9.

Weigel, KA.; Kehrli, ME.; Freeman, AE.; Thurston, JR.; Stear, MJ. et al., (1992). Association of Class I bovine lymphocyte antigen complex alleles with in vitro blood neutrophil functions, lymphocyte blastogenesis, serum complement and conglutinin levels in dairy cattle. Vet Immunol Immunopathol. ;27: 321-35.

Wellinghausen, N. and Rink, L. (1998). The significance of zinc for leukocyte biology. J Leukoc Biol 1998;64:571–7.

Wenbin, J.; Xiaoping Z.; Wei Z.; Jianbo C.; Cuihua G. and Zhihai J. (2009). Effects of Source of Supplemental Zinc on Performance, Nutrient Digestibility and Plasma Mineral Profile in Cashmere Goats. Asian-Aust. J. Anim. Sci. ;22(12):1648–53.

Werner, S. and Grose, R. (2003). Regulation of wound healing by growth factors and cytokines. Physiol. Rev. ;83:835-70.

Wessels, I.; Fleischer, D.; Rink, L.; Uciechowski, P. (2010). Changes in chromatin structure and methylation of the human interleukin-1beta gene during monopoiesis. Immunology. ;130:410–7.

Wessels, I.; Haase, H.; Engelhardt, G.; Rink, L. and Uciechowski, P. (2013). Zinc deficiency induces production of the pro-inflammatory cytokines IL-1β and TNFα in pro-myeloid cells via epigenetic and redox-dependent mechanisms. J Nutr Biochem;24: 289–97.

Wu, Y.; Wang, J.; Scott, PG. and Tredget, EE. (2007). Bone marrow-derived stem cells in wound healing: a review. Wound Repair Regen.;15(1) :S18-26.

Xingen, Lei, (2011). 14th International Symposium on Trace Elements in Man and Animals. Exploring Trace Elements: Science, Innovation, & Application. September 19-24, 2011, Meeting in Se-Enriched Enshi, China.

Xiong, HM. (2013). ZnO nanoparticles applied to bio imaging and drug delivery. Adv. Mater. ;25: 5329–35.

Xue, M.; Le, NT. and Jackson, CJ. (2006). Targeting matrix metalloproteases to improve cutaneous wound healing. Expert Opin. Ther. Targets, ;10: 143–55.

Yen-Hua, C.; Hui-Lin, F. and Sen-Shyong, J. (2018). Zinc Supplementation Stimulates Red Blood Cell Formation in Rats. Int. J. Mol. Sci. ;19:2824.

Yokus, B.; Cakir, DU.; Kanay, Z.; Gulten, T. and Uysal, E. (2006). Effects of seasonal and physiological variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. J. Vet. Med. A Physiol. Pathol. Clin. Med. ; 53: 271-6.

Zaboli, K.; Aliarabi, H.; Bahari, AA. and Abbasalipourkabir, R. (2013). Role of dietary nano zinc oxide on growth performance and blood levels of mineral: A studyon in Iranian Angora (Markhoz) goat kids. Int. j. pharm. res. health sci. ;2(1):19-26.

Zalewski, PD.; Ai, QT.; Dion, G.; Lata, J.; Chiara, M. and Richard, ER. (2005). Zinc metabolism in airway epithelium and airway inflammation: basic mechanisms and clinical targets: A review. Pharmacol. Ther.;105:127- 49.

Zamfirescu, S.; Topoleanu, I. and Nadolu, D. (2009). Observations concerning hematological profile in goat. Lucrari Seria Stiintifice. ;52:86-91.

Zeder, M. and Hesse, B. (2000). The initial domestication of goats (Capra hircus) in the Zagros Mountains 10,000 years ago. Sciences, ;287: 2254–7.

Zhang, T.; Sui, D. and Hu, J. (2016). Structural insights of ZIP4 extracellular domain critical for optimal zinc transport. Nat Commun. ;7:11979.

Zhang, ZY. and Xiong, HM. (2015). “Photoluminescent ZnO nanoparticles and their biological applications,” Materials. ;8(6):3101–27.

Zhao, J. and Castranova, V. (2011). Toxicology of Nanomaterial’s used in Nano medicine. J Toxicol Environ Health B Crit Rev. ;14: 593-32.

Zhu, CH.; Ying, DJ.; Mi, JH.; Zhang, W.; Dong, SW.; Sun, JS. and Zhang, JP. (2004). The zinc finger protein A20 protects endothelial cells from burns serum injury. Burns. ;30:127–33.

Zorrilla, P.; Gomez, LA.; Salido, JA.; Silva, A. and Lopez-Alonso, A. (2006). Low serum zinc level as a predictive factor of delayed wound healing in total hip replacement. Wound Repair Regen. ;14:119–22.

Zumbo, A.; Sciano, S.; Messina, V.; Casella, S.; Rita di Rosa, A. and Piccione, G.

(2011). Hematological profile of Messinese goat kids and their dams during the first month post-partum. Anim. Sci. Pap. Rep. ;29(3):223-30.

Appendix 3-1: Measuring Interleukin1 Beta

Assay principle

The kit is an Enzyme-Linked Immunosorbent Assay (ELISA).The plate has been pre-coated with Goat IL-1 Beta antibody. IL-1Beta present in the sample was added and binds to antibodies coated on the wells, and then biotinylated Goat IL-1Beta antibody is added and binds to IL-1 Beta in the sample, then Streptavidin-HRP is added and binds to the Biotinylated IL-1 Beta antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution was then added and color develops in proportion to amount of Goat IL-1 Beta, then reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

Reagent preparation

A- All reagents were brought at room temperature before used.

B- Standard reconstitute the 120µl of the standard (320ng/L)with 120µl of standard diluent to generate a 160ng/L standard stock solution. Allowed the standard to 15 mints with gentle agitation prior to making dilution. prepared duplicate standard points by serially diluting the standard stock solution (160ng/L) 1:2 with standard diluent to produce 80ng/L, 40ng/L, 20ng/L and 10ng/L solution.

C- Wash buffer dilute 20ml of wash buffer concentrate 25x into deionized or distilled water to yield 500 ml of 1x wash buffer.

Assay procedure

A- All reagents were bring at room temperature before used.

B- The strips inserted in the frames for use.

C- Added 50µl standard to standard well.

D- Added 40µl sample to sample wells and then added 10µl anti-IL-1 Beta antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells, mixed well, covered the plate with a sealer and incubated 60 minutes at 37˚c.

E- The plate was washed 5 times with wash buffer.

F- Substrate solution A and B added to each well and incubate for 10 minutes at 37˚c in the dark.

G- Stop solution added to each well, the blue color changed into yellow.

H- Optical density (OD value) of each well determined immediately using a micro plate reader set to 450 nm within 10 minuets after added the stop solution.

الخلاصة

هدفت الدراسة الحالية لتقييم الكفاءة العلاجية لجسيمات أوكسيد الزنك النانوية وأوكسيد الزنك التقليدي ، في علاج نقص الزنك المستحدث في المعز تجريبيا، بالاعتماد على العلامات السريرية وأداء النمو والحالة الصحية للحيوانات وفحوصات الدم، بعض التغيرات الكيموحيوية والاستجابة المناعية العامة فضلا عن التغيرات المرضية والتئام الجروح الجلدية المستحدثة تجريبياً. استخدم في هذه الدراسة خمسة وعشرون معز محلي عراقي سليمة ظاهريا ،تراوحت اعمارها من 5-6 أشهر وتراوحت اوزانها 15.52 ± 1.05 كجم , للفترة من كانون الاول 2018 ولغاية حزيران 2019 في حقل كلية الطب البيطري/ جامعة ديالى، العراق. قسمت الدراسة إلى ثلاثة اجزاء رئيسية. شمل الجزء الأول دراسة توصيف وتحديد الجسيمات النانوية لأوكسيد الزنك. الجزء الثاني استحدث نقص الزنك التجريبي في المعز المحلي، والجزء الثالث العلاج بجسيمات أوكسيد الزنك النانوية وأوكسيد الزنك التقليدي. في الجزء الثاني تم استحداث نقص الزنك في جميع المعز للفترة من كانون الاول 2018 واستمر لمدة 10 أسابيع. تم اعطاء معز التجربة عليقة تحتوي على تركيز عالي من الكالسيوم بعد إضافته الى خليط الاعلاف المركزة وبمقدار 400-500غرام / رأس / يوم. في الجزء الثالث قسمت الحيوانات إلى خمسة مجموعات (5) حيوانات لكل مجموعة. مجموعة (I) اعتبرت مجموعة سيطرة تركت بدون علاج ،بينما في المجموعة (II و III) عولجتا بالجسيمات النانوية لأوكسيد الزنك بتجريعهما بجرعة 25 مجم / كجم و 75 مجم / كجم على التوالي. ،بينما في المجموعة (IV و V) عولجتا بأوكسيد الزنك بتجريعهما بجرعة 25 مجم / كجم و 75 مجم / كجم على التوالي. مرة واحدة أسبوعيا ب 10 جرعات.

تضمنت الفحوصات السريرية في الجزء الثاني (درجة الحرارة, معدل النبض ,معدلات التنفس) ,الأغشية المخاطية وسلوك الحيوان. جمعت عينات الدم عند الاسابيع 0 , 2 , 4 , 6, 8 لأجل تقيم وحساب عدد الكريات الحمر (RBC)، تركيز خضاب الدم (Hb)، حجم الخلايا المرصوصة (PCV) ، معدل حجم الكرية (MCV)، معدل تركيز الخضاب في الكرية (MCHC) ومعدل الخضاب الكروي (MCH) , العدد الكلي والتفريقي للخلايا البيض (TLCوDLC) , بالإضافة إلى مستوى الزنك في الدم. في الجزء الثالث اعتمدت الفحوصات السريرية في الجزء الثاني بالإضافة إلى زمن التخثر (CT) وزمن النزيف (BT), وجمعت عينات الدم في الاسابيع 0, 3, 6, 9, وشملت الفحوصات نفسها في الجزء الثاني بالإضافة الى عد الصفائح الدموية، الفحوصات الكيموحيوية (الزنك ، البروتين الكلي ، الألبومين والغلوبيولين) في مصل. الاستجابة المناعية شملت اختبار مؤشر البلعمة والنترلوكين 1 ب. تم قياس مستوى مضادات الأكسدة الكلي في مصل الدم و اختبار المذنبات ايضا. الجزء الثالث من الدراسة تم استحداث جروح جلدية, جرحين دائريي الشكل على الظهر لكل من حيوانات التجربة. بعد ذلك تمت متابعة مراحل شفاء والتئام الجروح من خلال الفحص العياني وقياس اقطار الجروح كل أسبوع , وكذلك الفحص النسيجي للجلد وجمعت العينات الجلدية في الايام 3 ، 7 ، 14 و 28 بعد العملية الجراحية.

أظهرت نتائج الجزء الاول لتوصيف الجسيمات النانوية لأوكسيد الزنك, في المسح الصوري للمجهر الإلكتروني (SEM) اظهرت الشكل وحجم الجزيئات المجتمعة بأقطار تتراوح ما بين 1-50 نانومتر. اظهر فحص نمط حيود الأشعة السينية (XRD) أن جميع قمم الحيود المفهرسة لجسيمات اوكسيد الزنك النانوية النقية. أظهر نتائج التحليل الطور الطيفي للأشعة فوق البنفسجية أن ذروة الامتصاص عند 389.3 نانومتر. وأظهر متوسط القطر لحجم الجسيمات (PS) 41.2 نانومتر. في الجزء الثاني ، اظهرت العلامات السريرية تأخر النمو وفقدان شعر الرأس والظهر مع تقرن الجلد. وارتفعت معدلات ضربات القلب

معنويا $P < 0.05$ في الأسبوع السادس ، كما انخفضت معدلات التنفس معنويا $P < 0.05$ في الأسبوعين الثالث والسادس. سجل العدد الكلي لخلايا الدم الحمراء زيادة معنوية$P < 0.05$ في الاسبوع الثامن مقارنة مع الاسبوع 0. زاد معنويا تركيز Hb, PCV , MCV و MCH. انخفض مستوى الزنك معنويا بدءًا من الأسبوع الثاني وحتى نهاية الدراسة التجريبية ، وأدنى مستوى(7.61 ملي مول / لتر) في الاسبوع الثامن مقارنةً بالأسبوع 0 (11.34 ملي مول / لتر). الخلايا اللمفاوية انخفاض معنويا ، ازدادت الحمضات معنويا في الاسبوع الثامن مقارنة بالأسبوع الثاني والسادس على التوالي.

في الجزء من الثالث, اظهرت العلامات السريرية تحسن ملحوظ وكانت الحيوانات قريبة من المظهر الطبيعي إلا في حيوان واحد. اما وزن الحيوان ازداد معنويا في المجموعات II ,III وV على التوالي مقارنة بالمجموعة I. اما معدل التنفس ازداد معنويا معدل التنفس في مجموعة السيطرة مقارنتا مع المجموعات المعالجة. وسجلت قيم العد الكلي لكريات الدم الحمر (RBCc) ، خضاب الدم(Hb) ، حجم الخلايا المرصوصة (PCV) و معدل حجم الكرية(MCV) ارتفاعا معنويا في المجاميع التي عولجت بجسيمات اوكسيد الزنك النانوية. اما معدل تركيز خضاب الكرية (MCHC) ارتفعت معنويا في المجموعتين IV وV مقارنة بالمجاميع الأخرى. وكذلك معدل خضاب الكرية (MCH) ارتفعت معنويا في المجموعة III مقارنة بالمجاميع الاخرى عند الأسبوع التاسع. وأظهرت نتائج العد الكلي لخلايا الدم البيض(WBCc) زيادة معنوية في المجموعة V بالمقارنة مع المجاميع الاخرى عند الأسبوع التاسع. ارتفعت نسبة العدلات المئوية معنويا في المجموعة I مقارنة بالمجاميع الاخرى, وازدادت النسبة للخلايا اللمفاوية معنويا في المجموعة IV مقارنة بالمجاميع الاخرى عند الأسبوع التاسع. كما ارتفعت النسبة للخلايا الوحيدة معنويا في المجموعتين I وII بالمقارنة مع المجموعات III , IV وV عند الأسبوع السادس. اما نسبة الحمضات ارتفعت معنويا في المجموعة III وV مقارنة مع المجاميع I , II وIV عند الأسبوع التاسع. ارتفع زمن تخثر الدم معنويا في كل المجموعات وكانت أعلى قيمة في الأسبوع التاسع.اما بين المجموعات زاد بشكل ملحوظ في المجموعات I و IV بالمقارنة مع المجموعات II , III وV في الأسبوع السادس فقط بينما في الأسبوع التاسع ارتفعت معنويا في المجموعة I مقارنة مع المجموعات الأخرى. وقت النزف ارتفع معنويا في المجموعة I مقارنةً بالمجاميع II , IV وV عند الأسبوع التاسع. وقد انخفض عدد الصفيحات معنويا في المجموعة I مقارنة بالمجاميع الأخرى عند الأسبوع التاسع. وزادت قيمة الزنك في المصل معنويا في المجموعتين II و III بالمقارنة مع المجموعات I , IV وV عند الأسبوع التاسع. اما مقدار البروتين الكلي والغلوبيولين في المصل ارتفع معنويا في المجموعتين II و III مقارنة بالمجاميع I , IV وV عند الأسبوع التاسع. وانخفض معنويا مستوى الألبومين في المصل في المجموعة II في الأسبوع التاسع مقارنة مع الاسبوع 0.

وأظهرت نتائج فحص المذنب أدنى كمية من الحمض النووي المتحطم في مجاميع العلاج بالمقارنة مع مجموعة السيطرة. وزادت نتائج مؤشر البلعمة معنويا في المجموعتين III وIV مقارنة بالمجموعات الاخرى. وارتفعت قيمة (Interleukin 1 Beta (IL-معنويا عند الأسبوع التاسع مقارنةً بالأسبوع 0، ولكن كانت أدنى في المجاميع المعالجة مقارنة بالمجموعة I. اما مستوى مضادات الاكسدة الكلي ارتفع معنويا في المجموعة II بالمقارنة مع المجموعات الأخرى. وأظهرت نتائج الفحص العياني والنسيجي لشفاء الجروح أن المجاميع المعالجة بالجسيمات النانوية و بشكل خاص 75 ملغ / كغم أفضل من المجموعات الاخرى والدليل على ذلك تبين في إعادة الظهارة الكاملة مع تكوين طبيعي كامل لأنسجة البشرة عند اليوم 28 بعد العملية الجراحية. واستنتجت الدراسة ان لجسيمات أوكسيد الزنك النانوية افضلية بالمقارنة بأوكسيد الزنك العادي, وكان هنالك

تغييرات ملحوظة في قيم الدم والقيم الكيموحيوية التئام الجروح بالإضافة الى تحسن الحالة الصحية للحيوانات. والذي يعطي مؤشرا على التطبيقات الطبية الواعدة لاستخدام الجسيمات النانوية لأوكسيد الزنك في المجال البيطري لتحسين قطعان الحيوانات.

جمهورية العراق
وزارة التعليم والبحث العلمي
جامعة بغداد
كلية الطب البيطري

علاج نقص الزنك المستحدث في المعز المحلي بالجسيمات النانوية والاعتيادية لأوكسيد الزنك

اطروحة مقدمة الى
مجلس كلية الطب البيطري في جامعة بغداد وهي جزء من متطلبات نيل درجة دكتوراه فلسفة في الطب البيطري/ الطب الباطني والوقائي البيطري

تقدم بها الطالب

رعد محمود حسين

بإشراف

ا.م .د علاء كامل محمود

1441 هجري

2020 ميلادي