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 University of Diyala

College of Veterinary Medicine

**The Effect of Different Types of Anticoagulants on Some Physiological and Chemical Properties of Bovine Blood Samples**

A seminar submitted to the council of veterinary medicine at university of Diyala in partial fulfillment of requirement for the degree of Bachelors in surgery and veterinary medicine

**By**

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**{ قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لا يَعْلَمُونَ }**

**صدق الله العظيم**

**الزمر‏:الاية ‏9**

**الاهـــــــــــداء**

**الى من كان سندي وقوتي وملاذي بعد الله ( ابي رحمه الله) .**

**الى من تعجز كلماتي وتنحني هامتي لعظيم عاطائها شمس حياتي التي لا تغيب وسبيلي الى الجنة (امي الغالية).**

**الى من شدو ازري وشاطروني امري ووقفوا بجانبي ( اخواتي واخي).**

**الى رفقاء دربي ونعمة الله علي ... اصدقائي وصديقاتي**

 **الى من مهدوا لنا طريق العلم والمعرفة ... جميع اساتذتنا الافاضل**

**الى من اخلصت النية في التعليم فنورت عقولاً وهذبت نفوساً وربت اجيالاً الى السراج الذي يحرق نفسه لينير الطريق لغيره... دكتورتي الفاضلة منى .**

**Abstract**

The aim of this study was to estimate the some changes in bovine blood samples physiological component ( hemoglobin, packed cell volume and plasma platelets count)and plasma physical properties ( clarity and volume)and chemical properties (pH and calcium concentration) when blood samples test tubes contain four types of anticoagulants (EDTA ethyline diamine tetracetic acid, sodium oxalate, sodium citrate and heparin).

Blood samples were obtained from five cows (3 male and2 female ) 2-7 years old at College of Veterinary Medicine, university of Diyala. The samples were collected in four test tubes containing EDTA, sodium citrate, sodium oxalate and heparin. Hemoglobin and packed cell volume were estimated immediately then the blood samples were centrifuged for 15 min at 3000rpm to obtain plasma for evaluating clarity, volume, pH, PPC and Ca ion concentration. The results revealed that of hemoglobin and PCV was significantly higher in heparin group than oxalate and citrate groups with non significant differences with EDTA group, while the plasma obtained from different types of anticoagulants appeared with high degree of clarity, beside the volume of plasma was non significant increase in oxalate and citrate group as compared to other groups, also the different anticoagulants revealed no effect on pH,on the other hand the PPC and calcium concentration was significantly increased in heparin group as compared to other groups. in conclusion the anticoagulant heparin was more reliable anticoagulant for bovine blood samples.

 Chapter one Introduction

 Anticoagulants are additives that inhibit the clotting of blood thereby ensuring that the concentration of the substance to be measured is changed as little as possible before the analytical process (Guder, 2001). Such substances occur naturally in leeches and blood sucking insects. anticoagulants can be used an injection like heparin and orally as coumarin; some anticoagulants are used in medical equipments such as test tubes, blood transfusion bags like EDTA and citrate and renal dialysis equipment (Narayanan,1993 ). Anticoagulants are closely related to anti platelets drugs and thrombolytic drugs by manipulating the various pathways of blood coagulation cascade that built upon the initial platelets thrombus (Di Nisio, et al, 2005).

 The anticoagulants which are used in this study are: EDTA: ethylene diamine tetra acetic acid strongly and irreversibly chelates calcium ion to prevent blood from clotting (Banfi, et al, 2007 ). Oxalate has mechanism similar to that of citrate used in florid oxalate tubes used to determine glucose and lactate . Citrate is in liquid form it is bind to calcium ion but not as strongly as EDTA (NCCLS,2000). Heparin is a biological substance usually made from pig intestine, it work by activating anti thrombin III which blocks thrombin from clotting (Edens , et al, 1993 ). The effect of various type of anticoagulant on plasma biochemistry have been studied in man and various animals but limited information exist for bovine blood component and plasma ;Therefore this project was design to determine the effect of these anticoagulant on some equine blood samples component and plasma physical and chemical properties through study the following parameters: 1.packed cell volume PCV. 2.Hemoglobin Hb. 3.clarity. 4.volume. 5.pH 6.plasma platelets count. 7.calcium concentration.

1

Chapter two Review of literatures

 Anticoagulants : are additives that inhibit blood from clotting, Venous, arterial or capillary blood samples in which the concentration and properties of cellular and extracellular constituents remain relatively unaltered when compared with in vivo state (Harr, et al, 2005). Platelets and coagulation factors are activated when blood vessels are punctured, and their activation continues in sample containers that do not contain anticoagulant (Li et al 2004).

Serum or plasma: Historically, serum was preferred assay material for determination extracellular concentration of constituents in blood ; today plasma is preferred for many but not all laboratory investigation because the constituents in plasma are better reflecting the pathological situation than serum through some changes of constituents can be avoided by using anticoagulants (Agnese et al, 1983; Guder et al,1998).

Advantage of using plasma

1-time saving: plasma samples can be centrifuged directly after sample collecting un like serum in which coagulation is completed after 30 minutes.

2-higher yield: 15-20% more in volume of plasma than of serum can be isolated from the same volume of blood.

3-prevention of coagulation –induced interference :the coagulation process change the concentration of numerous constituents of extracellular fluid beyond their maximum allowable limit the changes are induced by the following mechanism:

1. increase in concentration of platelets component in serum as compare to

2

1. plasma(e.g potassium, phosphate, magnesium, aspirate amino transferase, lactate dehydrogenase, serotonin, neurase, specific enolase, zinc) relase of amide-NH3from fibrinogen induced by action of clotting factor XIII.
2. Decrease in concentration of constituents in serum as a result of cellular metabolism and coagulation process(glucose, total protein, platelets)
3. Activation of cell lysis of erythrocytes and leukocyte in non coagulate blood (cell free hemoglobin, cytokines).

Certain constituents should only be measured in plasma (e.g. neuron-specific enolase, serotonin, ammonia) to obtain clinically relevant results.

Disadvantage of plasma over serum: The addition of anticoagulants may interfere with certain analytical methods or change the concentration of constituents to be measured:

a. contamination with cat ions:NH4, Lithium, Na, K.

b. assay interference caused by metals complexing with EDTA and citrate(e.g. inhibiton of alkaline phosphates activity by zinc binding, inhibition of metallo-proteinase, inhibition of metal depended cell activation in function tests, binding of calcium (ionized) to heparin (Wallace, 2000).

c.interference by fibrinogen in heterogenous immunoassayes (Banks,2000)

d.inhibition of metabolic of catalytic reaction by heparin: eg.Taq polymerease in poly merase chain reaction (Numata et al,1998).

e.interferance in the distribution of ions between intracellular and extracellular

 spaces (e.g Cl, NH3) by EDTA, citrate (Guder,1998).

3

f.serum electrophoresis is can be perfomed only after pre- treatment to induce coagulation in plasma(Bank,2000)

Laboratory use: Laboratory instrument, blood transfusion bags, medical and surgical equipment will get clogged up become non operational if blood is allowed to clot. In addition, test tube used for laboratory blood clotting. Apart from heparin most of these chemicals work by bind calcium ion, preventing the coagulation protein from using them.

-ethylene diamine tetra acetic acid EDTA: is a polyprotic acid containing four carboxylic acid groups and two amine groups with one –pair electrons that chelate calcium and several other metal ions irreversibly to prevent blood from clotting.

-citrate is in liquid form in the tube and is used for coagulation tests, as well as in the blood transfusion bags. It bind calcium but not strongly as EDTA. Correct proportion of this anticoagulants to blood is crucial because of dilution and it can be reversed with the addition of calcium it can be in the form of sodium citrate or acid citrate-dextrose.

-oxalate has a mechanism similar to that of citrate it is used in fluoride oxalate tubes used to determine glucose and lactate levels.

-heparin : a hetrogenous anionic poly saccharide used clinically as anticoagulant, its activity is primarily derived from its binding to serine proteas inhibitory antithrombin III, a potent inhibitor of thrombin (factor IIa) and factor Xa. Heparin is a complex natural product and it is in vitro synthesis is not yet possible due to the difficulty of organizing the many bio synthetic enzyme required for its synthesis. The principle natural sources for heparin include procine intestine and bovine lung produced by mast cell and basophiles (Goosen et al,1991).

4

Chapter three Materials and methods

**Table(1): Equipments and instruments used in this study with their suppliers and sources.**

|  |  |  |
| --- | --- | --- |
| SOURCES | SUPPLIERS | INSTRUMENT |
| Germany | Denver instrument | Sensitive balance |
| Germany | Hettich | Centrifuge |
| Germany | Termarks | Incubator |
| India | Labtech | Micropipette |
| USA | Spectro PC | Spectrophotometer |

|  |  |  |
| --- | --- | --- |
| Germany | Mission | Hb –hemoglobin testing system |
| Germany | Olympus | Microscope |
| Germany | WITEG | Neubaner slide |

**Table( 2): Chemical substances used in this study with their suppliers and sources.**

|  |  |  |
| --- | --- | --- |
| Chemicals | Suppliers | Sources |
| EDTA | HIMEDIA | India |
| Sodium oxalate | Analar | England |
| Sodium citrate | Analar | England |
| Heparin | Leo pharmaceutica | Denmark |
| Calcium kit | Cromatest | Barcalona |
| pH paper | universalindKator 0-14 pH | Germany |

5

Preparation of anticoagulants:( NCCLAS,2000)

1.EDTA ethylene diamine tetra acitic acid: 0.1 mL 10% dissolved EDTA solution for 5 ml of blood.

2.sodium oxalate:0.5ml of 3.8%solution of sodium oxalate for 5ml of blood.

3.sodium citrate: 0.5 ml of 3.8% solution of sodium citrate for 5 ml of blood.

4.heparin:100 unites for 5ml.

Blood samples:

Twenty mL of blood samples were obtained from five cow(3male and 2 female), 2-7 years old at College of Veterinary Medicine/University of Diyala. The blood samples were collected from jugular vein by disposable syringe gage 16, in four tubes containing the following anticoagulants: EDTA, sodium oxalate, sodium citrate and heparin.

Parameters assist in this study:

These blood samples were analyzed within 3hr of collection in physiology laboratory for assisting the following parameters:

1.physiological: including hemoglobin and packed cell volume measured by kit of Hb testing system.

Then the blood samples test tubes were centrifuged at 3000rpm for 15 minutes to obtain plasma for assisting the following plasma chemical parameters:

2.degree of plasma clarity.

3.volume of plasma: drawling the plasma by mechanical graduated pipette from each sample. 6

4.pH of plasma: by putting few drops of plasma samples on the pH paper then compared with pH paper colors standard after 3minutes..

5.plasma platelets count : hemocytometer slide, pipette of RBC red blood cell and Ress-Echar solution which composed from:(sodium citrate 3.8gm,formaldedhyde 40% 0.2ml,brilliant cresyl blue 0.05gm, distalled water 100ml

6.plasma calcium concentration: O- cresolphthalein complex methods:

Procedure :

|  |  |  |  |
| --- | --- | --- | --- |
|  | Blank  | Standard  | Sample  |
| Mixture R1+R2  | 2000 micro litter  | 2000 micro litter  | 2000 micro litter  |
| Distilled water  | 50 micro litter  | ------- | ------ |
| Standard /R4 | ------- | 50 micro litter |  |
| Sample  |  |  | 50 micro litter  |

Calculation:

Sample

-----------x conc. Of standard calcium in mg/dL (10mg/dL)

 Standard

Statistical analysis: all data was performed on the basis of one way analysis (ANOVA) ,at p<0.05 significantly level and specific group differences were determined using least significant differences (LSD) test (Steel and Torri,1988 ).

7

Chapter four Results and Discussion

1.hemoglobin Hb concentration and packed cell volume PCV: the results of present study showed that the anticoagulant heparin group revealed a significant elevation p≤0.05 as compared to oxalate and citrate with non significant differences p≥0.05 with EDTA anticoagulant group , this results may be attributed to that heparin protect the red blood cell membrane shape and osmolarity this finding come in line with (Majeed and Salah,2007).

2.clarity: all blood samples with different type of anticoagulant produce clear plasma, the high degree of clarity come from no any hemolysis were detected in any sample, this result agree with(Ceron et al,2004).

3.volume:in table (3) appear that non significant increase in plasma volume in oxalate and citrate anticoagulant groups as compared to EDTA and heparin anticoagulant groups this is may be due to diluting effect (Kamal and Mohri,2015).

4. concentration of H+ion pH the data of table (3) illustrated that heparin anticoagulant group produced non significant p≥0.05 increase in H ion concentration as compared with other remain groups. In veterinary diagnostics the blood pH is an important indicator of homeostasis. which mean that hydrogen ion concentration kept within limits with any type of used anticoagulants(Sobiech et al,2005).

5.plasma platelets count ppc: according to table (3) the heparin anticoagulant group revealed a significant increase p≤0.05 in ppc as compared to remain groups, this mean that heparin does not produce structural and functional damage to platelets ,this finding come in line with (Kasten et al 2008) and disagree with (Puzzini et al,2016).

8

6.calcium ion concentration Ca++ :the data pertaining in table (3) that heparin group produce a significant increase p≤0.05 in calcium ion concentration as compared to remain groups. Ethylene Diamine Tetra Acetic acid (EDTA) exert their actions as anticoagulants byinhibiting thrombin in blood and chelating the

calcium ions ( Pramina et al ,2013).

Table (3):The effect of different type of anticoagulants on Haemoglobin , packed cell volume and plasma clarity, volume, pH, plasma platelets count and calcium concentration of bovine blood samples

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  Groups Parameters | EDTA | Sodium oxalate | Sodium citrate | Heparin  | LSD value |
| Hb gm/100ml | 20.7 AB±1.13 | 19.1 B±0.76 | 18.5 B±1.11 | 22.3 A±1.12 | 3.139 |
| PCV%  | 32.6 AB±1.69 | 27.4 B±1.03 | 26.6 B±1.86 | 34.0 A±2.05 | 5.10 |
| Clarity | +++ | +++ | +++ | +++ |  |
| VolumemL | 2.2 a±0.22 | 2.7 a ±0.29 | 2.6 a ±0.34 | 2.4 a±0.15 | 0.796 |
| pH | 8.8 a±0.20 | 8.6 a±0.24 | 8.8 a±0.20 | 8.4 a±0.24 | 0.670 |
| PPCCell/ | 40.2×103 B±3.97 | 40.0×103 B±2.70 | 40.1×103 B±3.68 | 42.5×103 A±2.62 | 2.15 |
| Camg/100mL | 9.2 B±0.22 | 11.9 B±0.95 | 10.9 B±1.32 | 16.8 A±2.68 | 4.86 |

The data expressed as mean ± standard error SE, significant level p<0.05, capital letters mean significant differences, small letters mean non significant differences p≥0.05.

9

Chapter five Conclusions and Recommendations

Conclusions:

1.heparin produce the relible anticoagulant for bovine blood sample for hematology estimation.

 2.the elevation in PPC and Calcium ion concentration in heparin indicate the

 efficient of its anticoagulant effect.

Recommendations:

1. study the other physiological properties of bovine blood samples like blood film, erythrocyte sedimentation rate, differential white blood cell.
2. study the bovine biochemical composition of plasma like total protein , glucose, cholesterol….
3. Comparative with serum.
4. Study the changes in physiological and chemical properties of bovine blood sample during the second and third day.

10

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11

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12

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13

الخلاصة

 الغرض من اجراء هذه الدراسة هو تحديد بعض التغيرات في مكونات الدم الفسلجية مثل(الهيموكلوبين وحجم كريات الدم المرصوص وعدد الصفيحات الدمية في البلازما) وبعض الصفات الفيزياوية لبلازما الدم مثل( درجة النقاوة وحجم البلازما) وبعض الصفات الكيمياوية مثل(درجة الحموضة وتركيز ايون الكالسيوم) لعينات دم الابقلر في انابيب اختبار حاوية على اربعة انواع من موانع تخثر الدم مثل الاثيلين دايمين تترا اسيتك اسد والاوكسالات والستريت والهيبارين.

 جمعت عينات الدم من خمسة ابقار (3 ذكور و2 اناث) تراوحت اعمارهم من 2-7 سنوات في كلية الطب البيطري جامعة ديالى ووضعت في اربعة انابيب اختبار حاوية على موانع التخثر المذكورة اعلاه. تم فحص الهيموكلوبين وحجم كريات الدم المرصوص مباشرة بعد جمع العينات في الانابيب ثم وضعت انابيب الاختبار في جهاز الطرد المركزي مدة 15 دقيقة وبسرعة 3000 دورة /دقيقة للحصول على البلازما لإجراء الفحوصات الفيزياوية والكيميائية المذكورة اعلاه

 اظهرت النتائج بان الهيموكلوبين وحجم كريات الدم المرصوص ارتفعت معنويا في مجموعة الهيبارين مقارنة مع مجموعة الاوكسلات والستريت وغير معنويا مع مجموعة الاثلين دايمين تترا اسيتك اسد ، كانت درجة النقاوة عالية في جميع نماذج البلازما الى جانب حجم البلازما الذي ارتفاعه غير معنوي في مجموعة الاوكسلات والستريت عن بقية المجاميع ، من جانب اخر كان الارتفاع تركيز الصفيحات في البلازما وتركيز ايون الكالسيوم معنويا في مجموعة الهيبارين عن بقية المجاميع، يستنتج من هذه الدراسة بأن مانع التخثر الهيبارين اكثر ملائمة لدم الابقار

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ديالى

كلية الطب البيطري

**تأثير انواع مختلفة من موانع التخثر على بعض الصفات الفسلجية والكيميائية لنماذج دم الابقار**

بحث مقدم إلى مجلس كلية الطب البيطري جامعة ديالى كأحد متطلبات نيل شهادة البكالوريوس في الطب والجراحة البيطرية

من قبل الطالبة

زكية خالد خليل

بإشراف

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