Ministry of higher education and scientific research

Diyala university /collage of veterinary medicine

The effect of the alcoholic extract of *Celery* on low density lipoprotein (LDL) and histology of aorta in albino male rabbits

A Report
Submitted to the Council of the College of Veterinary Medicine – University of Diyala in Partial Fulfillment of the Requirements for the Degree of Bachelors Science in Veterinary Medicine

By

Alaa Ismail Nasser

Supervised

By

A.L. Anas A. Humadi

2014 **1435**

بسم الله الرحمن الرحيم

وَإِذَا أَنْعَمْنَا عَلَى الْإِنْسَانِ أَعْرَضَ وَنَأَى بِجَانِبِهِ
وَإِذَا مَسَّهُ الشَّرُّ كَانَ يَئُوساً ۞ قُلْ كُلٌّ يَعْمَلُ عَلَى شَاكِلَتِهِ
فَرَبُّكُمْ أَعْلَمُ بِمَنْ هُوَ أَهْدَى سَبِيلاً ۞ وَيَسْأَلُونَكَ
عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ
الْعِلْمِ إِلَّا قَلِيلاً ۞

صدق الله العظيم

(الاسراء: 83 – 85)

DEDICATION

I would like to present this Modest Effort to my father and mother

Whose support is amazing and never ending

My brothers and sister:

For motivating me.

I am blessed to have you as family and supporting in everything

Thank you

ACKNOWLEDGEMENT

It's from absolute oneness of God from no god but Allah alone. I thank God for his support and influence on my life and work. Not only did he provide the project of my interest , but also he encouraged me through the assistance of the expertise of many knowledgeable and caring people. I have been blessed with the presence of many people who have assisted me with this research.

I would like to express my deep appreciation and sincere thanks to my supervisors Assist Lecture Dr. Anas A. Humadi . for helping and guiding me during the course of the work. I would also like to thank the deanery Assist Professor Dr. Sami A.A Al Mamoory of the Veterinary Medicine Collage .

I want to thank my parents, brothers and sisters for their support and patience who did not spare the least effort to bring this work to light ,through years of study.

Finally , I want to thank all my friends Ahmed Kamel Awaad and Ahmed Talib Jassam those help me to complete my research.

Alaa

2014

Summary

The aim of this study was to investigate the effect of alcoholic extract of *Celery* on low density lipoprotein (LDL) , cholesterol and pathological changes in aorta of white male rabbits .The study was carried out for 60 days on 20 mature white male rabbits , divided into two groups (control and treated) . The rabbits of treated group were fed on ordinary rabbit diet containing 2% cholesterol for 60 days .At day 30 of experiment the animals were given daily oral dose of 1 gm/kg body weight of alcoholic extract of Celery by stomach tube .The results showed a significant increase $P< 0.05$ in LDL – cholesterol at days 15 and 30 and a significant decrease at days 45 and 60 of the experiment .The histopathological changes of aorta at day 30 of the experiment showed hyperplasia of endothelial cells and ulcer in tunica intima , granulation tissue in tunica intima and media at day 45 with mononuclear cells infiltration in tunica media at day 60 of the experiment .

Introduction

hypercholesterolemia is the term used to describe high level of cholesterol in blood , its characterized by elevated level of LDL – cholesterol , normal or low level of HDL-cholesterol and normal or elevated level of triglycerides (1 and 2) . Lipids mainly cholesterol , cholesterol esters and triglycerides enter the tunica intima probably from blood across a damaged endothelium . Much of the lipid is phagocytosed by foam cells probably macrophages and myointimal cells but some eventually become free and more lipid accumulates when bloated foam cells undergo cell death (3) . Atherosclerosis is a chronic disease of large and medium size arteries with hardening and loss of elasticity of arterial walls which lead to narrowing of arterial lumen , the lesion starts initially as fatty streaks development into fatty plaques in smooth muscle cells migrate to the tunica intima and proliferate as foam cells arise from monocytes derived macrophages in the tunica intima and then necrosis with deposition of extracellular cell lipid and cholesterol crystals (4 and 5) .The plant Celery is an important salad plant ,containing of the bulbous roots , green leaves and the stem . The leaves sprout directly from the roots , these are compound with long stalk which is big and succulent (6) .

materials and methods

1-Experimental animals :

Twenty white male rabbits were obtained from local market at age 5-6 months , they were reread in clean cages at room temperature about 20-25 C and fed on pellets , green silages and watered freely with tap water . Animals were left for four weeks for adaptation before beginning of the experiment .

2- Experimental procedure :

1-Control group : 10 rabbits were fed on ordinary rabbits diet for 60 days .

2-Treated group : 10 rabbits were fed on ordinary diet containing 2% cholesterol for 60 days . Thirty days after begin of the experiment , the animals were given daily oral dose of 1 gm/kg body weight of alcoholic extract Celery by stomach tube for 30 days .

3-Cholesterol diet :

Cholesterol powder was dissolved by diethylether solution (99%) the solution was spilled upon the pellets and let to dry and then given to the rabbits (7) .

4-Biochemical tests :

Blood samples were withdrawn from marginal ear vein immediately transferred to a tube containing heparin (1000 U) and centrifuged (3000 U) for 10min. , Serum was separated immediately and kept at -20C until assayed.

The biochemical tests were carried out at days (0,15,30,45 and 60) of experiment .

Lipid profile tests performed in this study , including:

Low density lipoprotein (LDL - cholesterol) by using kit (linear com. Spain) , the procedure :

1-Attend the reagents and samples in room temperature .

2-The samples , blank and calibrator as represented in the following table :

Tube	Blank	Sample	Calibrator
R1	300 ul	300 ul	300 ul
Sample		4 ul	
CAL			4 ul

3-Mixed and incubator for 5 min. at 37C.

4-Added :

R2	100 ul	100 ul	100 ul

5-Mixed and incubate further 5 min. at 37 C.

6-After that reading the absorbance of the sample and calibrator at 600 nm against the reagent blank .

Calculation : LDL – cholesterol (mg/dl) = absorbance of sample/ absorbance of calibrator x concentration of calibrator

5- Histopathological examination :

Specimens from aorta were taken from the animals at day (30,45 and 60) of experiment they were fixed in 10% formaline for 72 hours , samples were washed , dehydrated by ascending grades of ethanol alcohol concentration 70% , 90% and 100% , then cleared by xylol

and embedded in paraffin wax with melting of 58C in electric oven for 15 min. . Paraffin blocks were sectioned as thin cross and longitudinal sections serially (5 um thickness) by manual rotary microtome , floated in water bath at 45C and fixed on a slide by mayers albumin (8) . Sections were stained with Hematoxylin and Eosin (H and E) .

6- Preparation of the crude ethanolic extract of *Celery :*

Preparation of the crude extract of celery leaves was done according to (9) : the plant material was washed , dried in open air and crushed into powder . Fifty grams of the powdered plant were transferred into decanter of one liter . Volume of 250 ml of ethanolic alcohol 70% were added to decanter . The decanter was placed upon magnetic stirrer and let to be mixed well at room temperature . Insoluble matter was filtered 72 hours later by medical gauze and then by filter paper . steps were repeated in medical gauze and filter papers to get the sweating . Sweating was centrifuged (3000 r.p.m) for 10 min. collected in sterile petri dish and let to dry in oven under temperature of 37 C .

7- Statistical analysis :

Statistical analysis of data of the present study was performed on the basis of two way analysis of variance (ANOVA) using significant level of $P< 0.05$ (10) .

Results and discussion

1-Biochemical observations :

Values of LDL – cholesterol showed significant increase P< 0.05 at days 15 and 30 (176.16± 8.28 and 274.26± 8.50) respectively with significant decrease at day 45 and 60 (197.05± 5.27 and 131.26± 4.38) respectively compared with day zero and control group.

Day	Control	Treated
0	89.7± 5.31	84.26± 4.74
15	93.28± 3.17	176.16± 8.28
30	91.98± 4.75	274.26± 8.50
45	85.8± 4.63	197.05± 5.27
60	91.78± 2.83	131.26± 4.38

2- Histopathological changes :

Day 30 of experiment : hyperplasia and metaplasia of endothelial cells with ulcer appeared in tunica intima with fatty droplets in both tunica intima and media (fig. 1) . Day 45 of experiment : hyperplasia and metaplasia of endothelial cells , mild vaculation in tunica intima and media with granulation tissue present in tunica media (fig.2) . Aorta at day 60 of experiment : showed mild degeneration in tunica intima and media with vaculation , mononuclear cells mostly lymphocytes and macrophages infiltration in tunica media and adventitia (fig.3) .

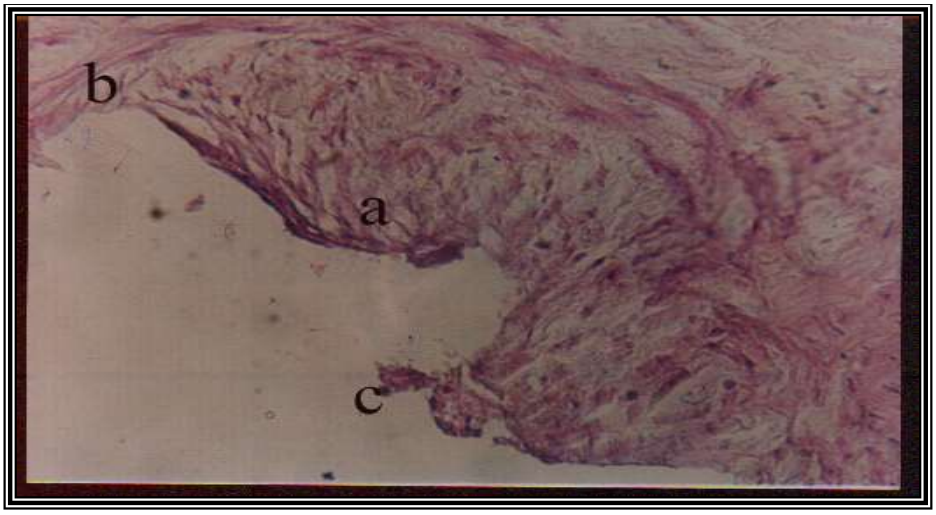

Fig. 1 : Microscopic section at day 30 in aorta of rabbit no treated with alcoholic extract of Celery for days 30 : a) Hyperplasia of endothelial cells b) ulcer of tunica intima c) slaughing and desqumation of endothelial cells . X40 : H & E stain

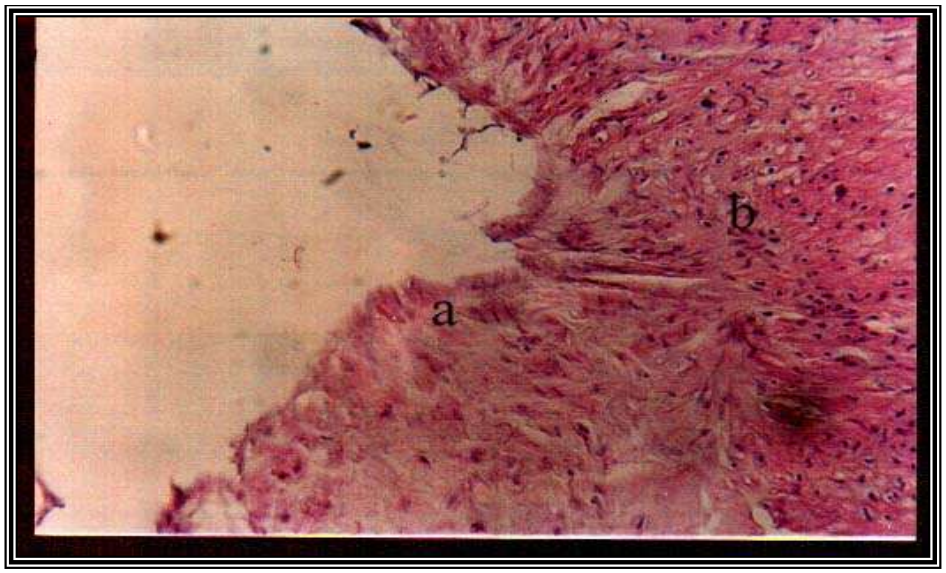

Fig. 2 : Microscopic section at day 45 in aorta of rabbit treated with alcoholic extract of Celery for days 45 : a) Hyperplasia of endothelial cells b) granulation tissue in tunica intima and media . X 40 : H & E

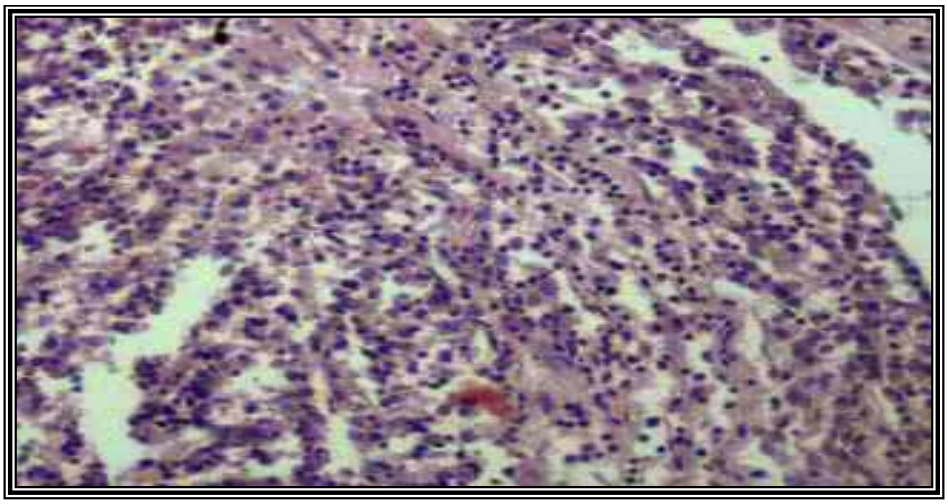

Fig. 3 : Microscopic section at day 60 in aorta of rabbit treated with alcoholic extract of Celery for days 60 : Mononuclear cells infiltration in tunica media . X 40 : H & E stain .

Rabbits treated with alcoholic extract of Celery showed decrease in concentration of total cholesterol in serum , the extract of celery contains flavonoides and lipase enzyme which cause lysis of lipid and convert the triglycerides and fatty acids . It could be considered as an important material in dissolving cholesterol lead to inhibition of feed back regulation (11) Sterols play an important role in the metabolism of lipids through inhibition of absorption of cholesterol , increased secretion of bile salts and also inhibition of synthesis of triglyceride in the liver leading to decrease level of LDL in blood and decrease of cholesterol in the liver . This could be considered as on antagonist to cholesterol of blood which helps in preventing atherosclerotic lesion (12) .Burtis and bucar ,2000 (13) found that the extract of celery contains saponine which ia an antioxidant having cell protective properties by preventing oxidation of lipid especially LDL . Other authors stated that the presence of ellagic acid in high concentration in alcoholic extract of Celery lead to

decreased activity of LDL and prevented information enter the body weight (14) .Epidemiologic studies suggested that low levels of antioxidants are associated with a high risk of cardiovascular diseases and their increased intake appears to be protective (15) .The presence of foam cells and fatty droplets in the endothelium of tunica inyima in aorta at day 30 has been considered as one of the most important defense mechanisms in atherosclerosis due to migration of macrophage from tunica adventitia to intima and then engulfed the fatty droplets to form foam cells (16 and 17).Feeding rabbits with cholesterol rich diet has been shown to increase lipid peroxidation and expose the animals to increased oxidative stress (18) .Celery extract contains active ingredients which may inhibit the level of cholesterol , such as tannin in high concentration which lead to stimulation of interlukin -1 in macrophages leading to hyperplasia , infiltration of macrophages , Tannin plays role in healing tissue and form protective layer over the exposed tissue (19 and 20). These findings appeared in day 45 and 60 in aorta .Rimm et.al. (1996) pointed out the relationship between intake of flavonoides and risk of coronary heart diseases leading to prevent development of atherosclerosis in arteries due to the strong role of flavonoid antioxidant mechanism and also preventing the permeability of lipids to endothelium .The present study indicated that hypercholesterolemia lead to elevation of LDL cholesterol in contrast to the uptake of native (unoxidized) LDL by the LDL (apolipoproteins B and E) receptor on macrophages . The uptake of oxidized LDL by the scavenger – receptor pathway is not subject to negative – feedback regulation and thus results in massive uptake of cholesterol by macrophages (22)

References

(1) Anderson , R. A. ; Jacqueline A. and Shumake R. L. (2001) . Hypercholesterolemia . Circ. Res. , 103 : 79-95 .

(2) Zubay , G. (2001) . Metabolism of cholesterol . Biochemistry , 3rd ed. Wm. C. brown publishers Melbourny , England : 635-657 .

(3) Stevens , A. ; Lowe , J.S. and Young , B. (2002) . Wheaters Basic Histopathology . 3rd ed. Churchill Livingstone . Tokyo : 86 – 95 .

(4) Schwartz , C.J. ; Valente , A.J. ; Sprague , E.A. and Kelley , J.L.(1991) . The pathogenesis of atherosclerosis : an overview Clin. Cardiol ., 14 suppl 111- 116 .

(5) Frei, B. (1999) . On the role of vitamin C and other antioxidants in atherogenesis and vascular dysfunction P.S.E.B.M., 222 : 196-204 .

(6) Dobelis , I.N.(1986) . Magic and medicine of plants , Pleasantville , NY: Readers Digest Assoc.

(7) Wilens , S.L. and Stumpf , H.H. (1955) . Nodular and fatty glomerular lesion in rabbits on cortisone . Am. J. Path., 34 : 275 – 287 .

(8) Luna , L.G.(1968) . Manual of Histological Staining Methods . 3rd ed. Mc Graw . Hill book company , New York , Toronto . London ; 69 .

(9) Haberland , M.E. ; Olch, C.L. and Fogelma , A.M. (1984) . Role of lysines in mediating interaction of medified low density lipoproteins with the scavenger receptor of

human monocyte macrophages J. Boil .Chem., 259 : 11305-11311 .

(10) Chap , TLE.(2003) . Introductory Biostatistics . John Wiley and Sons Pub. Inc. New Jersey .

(11) Roberts , K.; Murray , D.G. ; Peter , A.M. and Victor, W.R. (2000) . Lipid Transport and Storage . In. : Harpers biochemistry . 25th ed. Printed in USA : 268- 285

(12) Pilar , O. ; Francis , J. ; Sanchez-Muniz , S.R. and Crmen , C. (2001) . Eicosanoid protection , thrombogenic ratio and serum and LDL peroxidase in normo and hypercholesterolemia post menopausal women consuming two olic acid rich diets.British J.Nutr.,85:41-47.

(13) Burtis , M. and Bucar , F.(2000) . Antioxidant activity of Negilla Sativa essential oil . Pathotherapy Res., 14(5)323 – 328 .

(14) Mukhtar , H.; Das, B.J. and Del, T.(2006) . Protection against 3 – methylchoanthrene induced skin tumorigenesis by ellagic acid . Biochem. Biophys. Res. Commun., 119 : 751 – 757 .

(15) Jialal , J. and Devaraj , S.(2003) . Antioxidants and atherosclerosis : Don't throw out the baby with the bath water . Circulation , 107 : 926 – 928 .

(16) Kumer , V. ; Cotran , R.S. and Robbins , S.L.(2003) . Robbins Basic Pathology . 7th ed. Saunders , Philadelphia : 328 – 338 .

(17) Leborgne , L. ; Pakala , R.; Dilcher , C.; Seabron , D.; Fermin , O. and Waksman , T.R.(2005) . Effect of Antioxidants on atherosclerotic plaque formation in ballon

– denuded and irradiated Hypercholesterolemic Rabbits ., J. Cardiovasc. Pharmacol., 46 : 540 – 547 .

(18) Mahfouz , M.M. ; Kawano , H. and Kummerow , F.A.(2000) . Cholesterol rich diets have different effects on lipid peroxidation , Cholesterol oxides and antioxidant enzymes in rats and rabbits , J.Nut. Biochem. , 11: 293-302 .

(19) Hayashi , S. ; Funatogawa , K. ; Yoshida , T. and Hatano , T.(2004) . Antibacterial activity of hydrolysable tannins derived from medicinal plants against helicobacter pylori . Microbiol . Immunol ., 48: 251-261 .

(20) Murayama , T. ; Kishi , N. ; Furukawa , T. and Miyamoto , K. (2005) . Agrimoniin an antitumor tannin of Agrimonia pilosa . Anticancer Res., 12 : 1471 – 1474 .

(21) Rimm , E.B. ; Katan , M.B. and Acerio , A(1996) . Relation between intake of flavonoides and risk of coronary heart disease in male health professional Ann. Inter. Med., 125 : 384 – 389 .

(22) Yia – Herttuala , S. ; Palinski , W. and Rosen , m. (1989) . Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man . J.Clin. Invest., 84 : 1086 – 1095 .

الخلاصة

هدف هذه الدراسة هو معرفة تأثير المستخلص الكحولي لنبات الكرفس على البروتينات الدهنية واطئة الكثافة والتغيرات المرضية النسجية التي تحدث في الشريان الابهر لذكور الأرانب المهقاء . كانت فترة التجربة ستون يوما" استخدمت فيها عشرين من ذكور الأرانب المهقاء ، قسمت الأرانب لمجموعتين كل مجموعة مكونة من عشرة أرانب (مجموعة السيطرة ومجموعة المعالجة) غذيت الأرانب في مجموعة المعالجة على غذاء الأرانب الاعتيادي مع إضافة 2% من الكولسترول للعلف لمدة ستين يوما" وأعطي المستخلص الكحولي لنبات الكرفس يوميا" منذ اليوم 30 ولغاية نهاية التجربة بجرعة 1 غم لكل كيلو غرام من وزن الحيوان بواسطة اللي المعدي .لوحظ ارتفاع معنوي $P< 0.05$ في قيم البروتين الدهني واطئ الكثافة في اليوم 15 و 30 وانخفاض معنوي $P< 0.05$ في الأيام 45 و 60 من التجربة .أوضحت التغيرات المرضية النسجية في شريان الابهر في اليوم 30 من التجربة حدوث فرط تنسج ووجود تقرحات في الغلالة الداخلية ، أما في اليوم 45 من التجربة فقد شوهد وجود نسيج حبيبي في الغلالة الداخلية والوسطية ، أما في اليوم 60 من التجربة فقد شوهد ارتشاح للخلايا وحيدة النواة في الغلالة الوسطية .

وزارة التعليم العالي والبحث العلمي

جامعة ديالى/ كلية الطب البيطري

دراسة تأثير المستخلص الكحولي لنبات الكرفس على البروتينات الدهنية واطئة الكثافة والتغيرات النسيجية في الابهر لذكور الارانب المهقاء

مشروع بحث

مقدم إلى مجلس كلية الطب البيطري جامعة ديالى كجزء من متطلبات نيل شهادة البكالوريوس في علوم الطب البيطري

من قبل الطالب

علاء أسماعيل ناصر

المشرف

م.م. انس عبد المجيد حمادي

1435 **2014**