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Study the Effect ofsome Plant Leaves onHyperlipidemic Rats

Hamdia A. Helal , Magda K. El-Shaer , Abeer N. Abdel Rahman AL-Zahraa S. Abd-Alla

Abstract:

This study was carried out to evaluate the anti-hypercholesterolemicrats effects of spearmint(*Menthe spicataL.) ,sage(Salvia officinalis L.*) and okra (*Abelmoschusesculentus)* leaves.Thirty two male mature albino rats weighing 150±10g per each, were used in this study and divided into threegroups, the first group was kept as a control (-ve) group (n=4), while the groups were fed on hypercholesterolemicdiet (n=28) for 3 weeks to induce hypercholesterolemicrats.The tested plant leaves powder (PLP) were given to the rats at the leaves of 3 and 6% from the basel diet for 28 days. At the end of the experiment, serum total cholesretol(TC), triglycerides(TG), high density lipoprotein (HDL-c), AST, ALT, ALP, urea, creatinin, uric acid were determined Low density lipoprotein(LDL-c),Verylow density lipoprotein (VLDL-c) , and Atherogenic index (A.I) were calculated.The results indicated that tested plants leaves significantly (P≤0.05) decreased serum TC, TG, LDL.c, VLDL.c and increased HDL.cAlso, the tested plants leaves improved liver and kidney functionsand prevent some of the adverse histopathological changes in heart. The obtained findingshypothesis that tested plant leavescontaining several compounds those are able to improve the adverse effects hypercholesterolemicdiet.So,Theobtainedresults recommended such plants by a moderate amount in our diets, are more benefits.

Keywords:T.C, T.G,HDL-c, LDL-c, VLDL-c, liver and kidney functionsofhypercholesterolemic diet, plant leaves, histopathologicalexamination.

Introduction

Hyperlipidemia is a condition characterized by an elevation of any or all lipid profile and/or lipoproteins in the blood. Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk**(Amit*et al.*, 2011)**, dyslipidemia (abnormal amount of lipids in the blood) can also describe elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL).Hyperlipidemia is the major precursor of lipid related ailment such as atherosclerosis, coronary artery disease and also involved in sudden death syndrome. The main cause of hyperlipidemia includes changes in lifestyle habits in which risk factor is mainly poor diet i.e. with a fat intake greater than 40 percent of total calories, saturated fat intake greater than 10 percent of total calories; and cholesterol intake greater than 300 milligrams per day or treatable medical conditions **(Durrington, 1995 and Amit*et al.*, 2011)**.

Okra(*Abelmoschusesculentus L*.) okra bast, a multicellular fiber was analyzed and the estimated average chemical compositions of okra bast fiber (OBF) (Abelmoschusesculentus variety) are 67.5 % a-cellulose, 15.4 % hemicelluloses, 7.1 % lignin, 3.4 % pectic matter, 3.9 % fatty and waxy matter and 2.7 % aqueous extract. It is clear that the main constituents of OBF are a-cellulose, hemicelluloses and lignin and the rest are very minor in proportion, so render a little influence to the structure of OBF **(Sathish and Eswar , 2013).**

The leaves of this plant gives nutritional benefits like protein, niacin, riboflavin, phosphorus, zinc, copper, potassium, vitamins A, B, C and K, thiamine, magnesium, folate, calcium and manganese. The superior fibre found in okra helps to stabilize the blood sugar by curbing the rate at which sugar is absorbed from the intestinal tract **(Chopra *et al.*, 1996).**

Common sage *(Salvia officinalis)*sage leaves is generally characterized by thujones, with -thujone usually predominating (18–43%) over -thujone (3–8.5%), camphor (4.5–24.5%), 1,8-cineole (5.5–13%), -humulene (0–12%), -pinene (1–6.5%), camphene (1.5–7%), and bornyl acetate (2.5% maximum) **(Bruneton, 1999).**

Common sage, since ancient times, has been an ingredient in perfumes, a flavoring in a variety of food preparations, and a medicinal plant used in folk medicine for the treatment of a variety of ailments , where many studies mentioned that sage have many of biological activities, such as antioxidant, antibacterial,

hyperglycemic and anti-inflammatory activities **(Alarcon-Aguilar *et al.*, 2002 and Lima, 2006).**

*S. officinalis*leaves showed inhibitory effect against the pancreatic lipase activity and eventually was effective in reducing body weight and obesity **(Ninomiya *et al.*, 2004).**

Abdel Moneim*et al.*(2011)found that spearmintcontainedmoisture (76.01 ± 0.033)%, fiber (2.1 ± 0.03)%, ash (3.48 ± 0.001)%, protein (1.75 ± 01.)%, fat (3.20 ± 0.003)%, and carbohydrates (14.46 ± 0.15)%. On the other hand, the acid value, peroxide value, iodine value, free fatty acids, refractive index at 27Cº and density at room temperature were 0.0614, 1.0, 0.564, 0.0305, 1.4572 and 0.8395, respectively.

Plant leaves from the genus Mentha are used for antimicrobial, antiviral and insecticidal activity The plants are aromatic, stimulant and carminative. The infusion of leaves affords a remedy for rheumatism and indigestion **(Gupta,1991 and Franzio*set al.*, 1997) .**

Spearmint is used in Iran as flavouring agent in food products such as cheese and doogh besides, spearmint is added in Indian and Italian cuisine, either in fresh or dried form, to fish and shellfish plates before or after cooking **(Kizil *et al.*, 2010).**

so,This study aimed to evaluated the effect of the tested leaves on the biochemical parameters of hyperlipidemicrats .

Materials and methods

Materials:

Plants

Common sage , spearmint and okra leaves were washed properly with water to remove the mud or dust if any initially it was dried in a sun for an hour than was shade dried completely. The dried leaves were then powdered by means of wood grinder and then stored in airtight containers The leaves of plant were collected and dried, and then the dried plant samples were ground well into a fine powder and stored in darkish bags for later use.

Rats

Thirty two adult male albino rats weighing 150±10 g were obtained from medical Insects Research Institute ,Doki,Cairo. The rats were maintained under controlled humidity temperature (25± 2°C) and light (12h light/ 12h dark). The Rats were placed in standard ventilated cages and maintained under standard laboratory conditions were obtained from the animal house of the faculty of Home

Economics, Menoufia University with free access to food and water and studies were carried out in strict guidelines for the care of laboratory animal.

Diet

Basal diet contained 10 % fat ,14% casein (protein) , 10 % sucrose, 4% salt mixture, 1% vitamin mixture , 5% fiber , 0.3% Dl-methionine , 0.2% choline and corn starch up to 100g were obtained from Gomhoria Co. Dokki Egypt.

Methods:

Preparation of material:

leaves of Common sage , spearmint and okra were washedproperly with water to remove the mud or dust if any initially it was dried in a sun for an hour then was shade dried completely. The dried leaves were powdered by means of wood grinder and was sieved through sieve no. 40 and stored in airtight containers **(Kannur and Hukkeri, 2006).**

The induction of experimental hyperlipidemia:

Rats were fed on high fat diet to inducehyperlipidemia . The diet contained the following composition 10 % fat ,14% casein (protein), 10 % sucrose, 4% salt mixture, 1% vitamin mixture , 5% fiber , 0.3% Dl-methionine , 0.2% choline and corn starch up to 100g **(Negm, 2002).**

Experimental design:

The experimental was done in the Faculty of Home Economics, Menoufia University, Shebin El-kom . Rats were maintained under controlled humidity temperature (25± 2°C) and light (12h light/12hdark).

Rats were divided into three main groups as following:

-**The frist main group:**(n=4) was fed on the basal diet as negative control group .

-**The second main group**:(n=28) hyperlipidemic rats in this group rats were fed on high fat diet by 10 % for three weeks , then divided into 7 subgroups as following :

G2:Hyperlipidemic rats +basal diet (positive control) .
G3:Hyperlipidemic rats + basal diet containing 3% common sage .
G4:Hyperlipidemic rats + basal diet containing 6% common sage .
G5:Hyperlipidemic rats + basal diet containing 3% spearmint .
G6:Hyperlipidemic rats + basal diet containing 6% spearmint .
G7:Hyperlipidemic rats + basal diet containing 3% okra .
G8:Hyperlipidemic rats + basal diet containing 6% okra .

Blood sampling and organs:

Blood samples were collected after 12 hours fasting at the end of the experiment using the abdominal aorta in which the rats were scarified under ether anetheized. Blood samples were received in to clean dry centerfuge tubes and left to clot at room temperature, then centerfuged for 10 minutes at 3000 rpm to separate the serum. Serum was carefully aspirate, transferred in to clean cuvet tubes, and stored frozen at-20^{o}C for analysis. All serum samples were analyzed for determination the following parameters:Lipid profile,cholesterol, tri-glycerides(T.G), high density lipoprotein(HDL-c), low density lipoprotein(LDL-c),very low density lipo protein(VLDL-c),urea, creatinin, uric acid, alkaline phsphatase (ALP),aspartate amino transferase (AST) and glucose .At the same time, the organ (heart) removed, washed in salin solution, dried by filter paper, weighted, and stored frozen in formalin solution 10% for histopathological testing according to method mentioned by **(Drury and Wallington, 1980).**

Analytical methods:

Enzymatic calorimetric determination of triglycerides was carried out according to **(Fassati and prencipe 1982) .**

The principle use of total cholesterol determination according to (**Allen, 1974).**HDL.c could be determined by the same method used for total cholesterol, according to **Lopez (1977) .**

The calculation of VLDL.c (very low density lipoproteins) and LDL.c were carried out according to **Lee and Nieman(1996)** as follows:

VLDL (mg/dl) = Triglycerdes/5.

LDL (mg/dl) = (Total cholesterol – HDL) – VLDL .

Urea was determinated according to the enzymatic method of **(Patton andCrouch 1977)**.

Creatinine was determinated according to kinetic method of **(Henry, 1974).**

The intensity of this red color formed is proportional to the uric acid concentration in the sample **(Schultz, 1984).**

Enzymatic calorimetric determination of alkaline phosphates (ALP) was carried out according to **(Belfield and Goldberg, 1971).**

Aspartate transaminase (AST)and alanine phosphatase (ALT)activities were measured according to method described by (**Yound, 1975)**and**(Tietz, 1976)** .

Histopathological investigation:

Small specimens from heart were collected from all experimental groups, fixed in 15% neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80, and 90%), cleared in xylene and embedded in paraffin. Sections of (4 - 6) µm thickness were prepared and stained with Hematoxylin and Eosin according to **(Bancroft *et al.*, 1996).**

Statistical Analysis :

All statistical analysis was done using statistical package for social sciences (SPSS) version, 17.0 windows and differences in means were compared using the students' t-test and one-way analysis of variance (ANOVA) Error of probability or $P \leq 0.05$ was considered significant **(Ben-Chioma*et al.*,2014) .**

Results and Discussion

Effect of some plant leaves on total cholesterol of hyperlipidemic rats:

Data presented in table (1) showed that effect of some plant leavesokra,sage and spearmint on total cholesterol and triglyceridesthe mean value of all treated groups were significantly lower ($p \leq 0.05$) when compared with positive control.Theseresults are in agreement with **Camacho, (1996) and Jorge *et al.*, (1998)** The results indicated that triglycerides and total cholesterol were also lower by feeding on sage and okra .

Table(1):Effect of some plant leaves on fasting total cholesterol and triglycerides (mg/dl) of hyperlipidemic rats

parameter / Groups	T.C (mg/dl) Mean ±SD	T.G (mg/dl) Mean ±SD
G1:Control (-ve)	69^{g}±1	79.3^{g}±0.2
G2: Control (+ve)	96^{a}± 0.5	143^{a}± 1
G3:Okra (3%)	89^{b}± 0.02	134.5^{b}± 0.25
G4: Okra (6%)	86.3^{c}± 0.2	104.5^{c}± 0.18
G5:commonsage(3%)	86^{c}± 0.1	100.6^{d} 0.1
G6:commonsage(6%)	85^{d} ± 0.2	90.3^{e}± 0.29
G7:Spearmint(3%)	83.3^{e}± 0.3	90.3^{e}± 0.21
G8:Spearmint(6%)	82.01^{f}± 0.2	86^{f}± 0.5

Effect of some plant leaves on fasting serum HDL ,LDLandVLDL (mg/dl) of hyperlipidemic rats:

Data presented in table (2) showed that effect of some plant leavesOkra,sage and Spearmint on HDL ,LDL andVLDLthe mean value

of all treated groups were significantly lower ($p \leq 0.05$)withLDL and VLDLwhen compared with positive control. but in caseHDLthe mean value of all treated groups were significantly higher($p \leq 0.05$) when compared with positive control.These results are in agreement with **Camacho, (1996) and Jorge *et al.*, (1998)** The results indicated that VLDL.c andLDL.c were also lower but in caseHDL.cwere significantly higher($p \leq 0.05$).

Table(2):Effect of some plant leaves on fasting serum HDL.c ,LDL.candVLDL.c (mg/dl) of hyperlipidemic rats

parameter / Groups	HDL.c (mg/dl) Mean ±SD	LDL.c (mg/dl) Mean ±SD	VLDL.c(mg/dl) Mean ±SD
G1:Control (-ve)	$23.46^{a} \pm 0.2$	$29.64^{f} \pm 0.2$	$15.86^{f} \pm 0.02$
G2: Control (+ve)	$19.7^{e} \pm 0.2$	$47.82^{a} \pm 0.2$	$28.6^{a} \pm 0.11$
G3:Okra (3%)	$20.03^{d} \pm 0.3$	$42.10^{e} \pm 0.11$	$26.9^{b} \pm 0.11$
G4: Okra (6%)	$20.31^{d} \pm 0.2$	$45.1^{b} \pm 0.1$	$20.9^{c} \pm 0.12$
G5:Common sage(3%)	$21.3^{c} \pm 0.3$	$44.56^{c} \pm 0.11$	$20.1^{c} \pm 0.03$
G6:Common sage(6%)	$21.71^{c} \pm 0.2$	$45.24^{b} \pm 0.04$	$18.1^{d} \pm 0.05$
G7:Spearmint(3%)	$22.02^{b} \pm 0.2$	$43.24^{d} \pm 0.04$	$18.06^{d} \pm 0.02$
G8:Spearmint(6%)	$22.02^{b} \pm 0.2$	$42.80^{e} \pm 0.2$	$17.19^{e} \pm 0.19$

Effect of some plant leaves on liver enzymatic(AST), (ALT) and (ALP) U/L of hyperlipidemic rats:

Data presented in table (3) showed that effect of some plant leavesOkra,sage and Spearmint on AST ,ALT andALPthe mean value of all treated groups were significantly lower ($p \leq 0.05$)withAST ,ALTandALPwhen compared with positive control. These results are in agreement with **Draper and Hadley, (1990)** The results indicated that AST ,ALT andALP were also lower by feeding on some plant leavesOkra,sage and Spearmint.

Table(3): Effect of some plant leaves on liver enzymatic (AST), (ALT) and (ALP) U/L of hyperlipidemic rats

parameter / Groups	AST (U/L) Mean ±SD	ALT (U/L) Mean ±SD	ALP (U/L) Mean ±SD
G1:Control (-ve)	$82.7^{g} \pm 0.14$	$38.74^{g} \pm 0.2$	$374^{g} \pm 1$
G2: Control (+ve)	$110.7^{a} \pm 0.3$	$68.7^{a} \pm 0.3$	$613.6^{a} \pm 1.5$
G3:Okra (3%)	$106.28^{b} \pm 0.3$	$56.3^{b} \pm 0.3$	$584.7^{b} \pm 2.5$
G4: Okra (6%)	$103^{c} \pm 0.55$	$50.8^{c} \pm 0.18$	$507.1^{c} \pm 3$
G5:Commonsage(3%)	$102.8^{d} \pm 0.22$	$46.3^{d} \pm 0.3$	$501.1^{c} \pm 3$
G6:Commonsage(6%)	$102^{d} \pm 0.23$	$42.8^{e} \pm 0.2$	$470.5^{d} \pm 2.8$
G7:Spearment(3%)	$96.31^{e} \pm 0.2$	$42.3^{e} \pm 0.2$	$451.2^{e} \pm 2.04$
G8:Spearment(6%)	$90.73^{f} \pm 0.1$	$40.31^{f} \pm 0.11$	$418.03^{f} \pm 2.2$

Effect of some plant leaves on kidney functionsurea,creatinine and uric acid of hyperlipidemic rats:

Data presented in table (4) showed that effect of some plant leavesOkra,sage and Spearmint on urea, creatinine and uric acidthe mean value of all treated groups were significantly lower ($p \leq 0.05$)withurea, creatinine and uric acidwhen compared with positive control. These results are in agreement with **Cheeseman and Slater, (1993)**The results indicated that urea, creatinine and uric acidwere also lower by feeding on some plant leaves Okra, sage and Spearmint.

Table(4):Effect of some plant leaves on kidney functions urea, creatinine and uric acid of hyperlipidemic rats

parameter / Groups	urea (mg/dl) Mean ±SD	creatinine(mg/dl) Mean ±SD	uric acid (mg/dl) Mean ±SD
G1:Control (-ve)	13.2^{g}±0.11	0.8^{f}±0.02	1.5^{g}±0.03
G2: Control (+ve)	35.49^{a}± 0.17	1.4^{a}± 0.1	5^{a} ± 0.23
G3:Okra (3%)	31.03^{b}± 0.17	1.3^{b}± 0.03	4.5^{b} ± 0.02
G4: Okra (6%)	30.4^{c}± 0.19	1.1^{c}± 0.1	2.5^{c}± 0.03
G5:Commonsage(3%)	29.4^{d}± 0.2	1^{c}± 0.05	2.4^{d}± 0.01
G6:Commonsage(6%)	29.1^{d} ±0.2	0.97^{d}± 0.02	2.1^{e} ± 0.11
G7:Spearmint(3%)	28.5^{e}± 0.5	0.80^{e}± 0.03	2.1^{e}± 0.01
G8:Spearmint(6%)	24^{f} ± 0.23	0.8^{f}± 0.01	1.8^{f}± 0.05

Histopathological examination of heart :

Microscopical examination of heart of rat from group 8 (negative control) revealed the normal histological structure of cardiac myocytes (photo 16 and 17).group1(positive control)revealedcongestion of myocardial blood vessels (photo1) and intermuscularoedema (photo2). Meanwhile, sections from groups 2 and 3 (Okra 3% and Okra 6%) revealed no histopathological changes (photo 3, 4, 5 and 6). However, sections from group 4 (sage3%) revealed no histopathological changes exceptintermuscularoedema (photo 7 and 8). Some examined sections from group 5 (sage 6%) showedfocal necrosis of cardiac myocytes associated with inflammatory cells infiltration (photo 9), whereas, other sections from this group as well as sections from group 6 (Spearment3%) revealednohistopathological changes (photo 10, 11 and 12). On the other hand, heart of rats from group 7 (Spearment 6%) showed congestion of myocardial blood vessels (photo 13). focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (photo 14) and intermuscularoedema (photo 15).

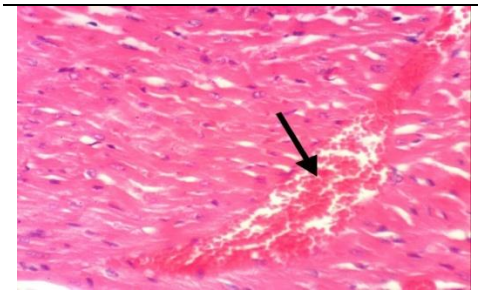

photo (1): Heart of rat from group 1 showing congestion of myocardial blood vessels (H & E X 400).

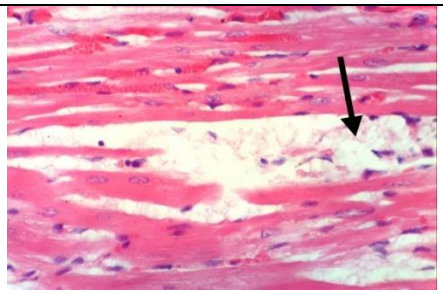

photo (2): Heart of rat from group 1 showing intermuscularoedema (H & E X 400).

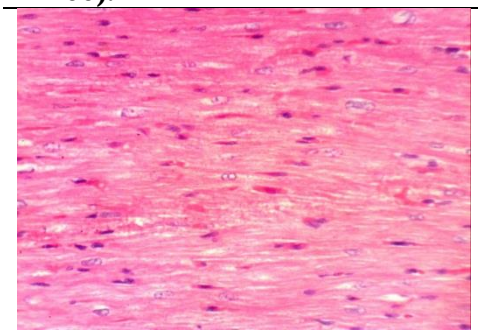

photo (3): Heart of rat from group 2 showing no histopathological changes(H & E X 400).

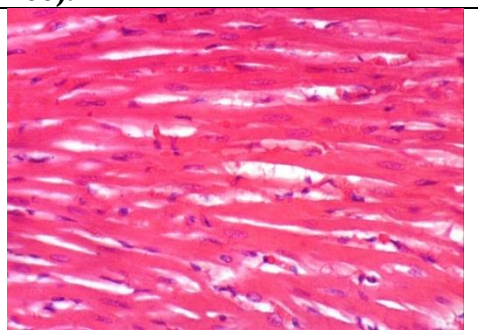

photo (4): Heart of rat from group 3 showing no histopathological changes(H & E X 400).

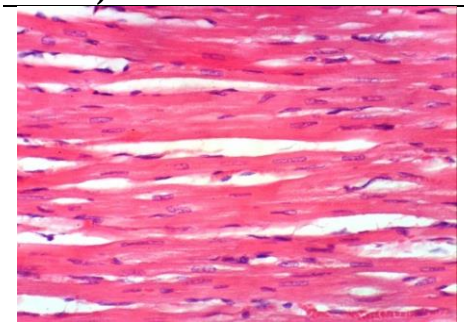

photo (5): Heart of rat from group 3 showing no histopathological changes(H & E X 400)

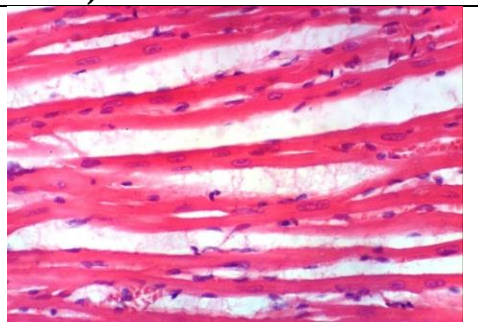

photo (6):Heart of rat from group 3 showing no histopathological changes(H & E X 400).

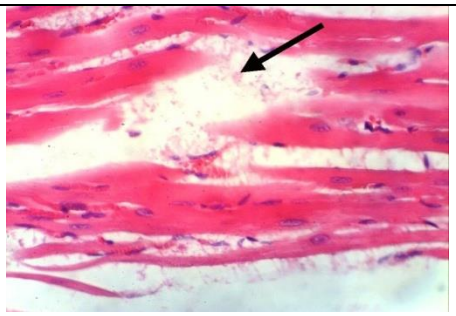

photo (7): Heart of rat from group 4 showing intermuscularoedema (H & E X 400).

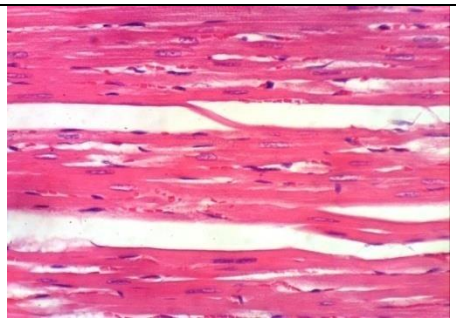

photo (8): Heart of rat from group 4 showing no histopathological changes(H & E X 400).

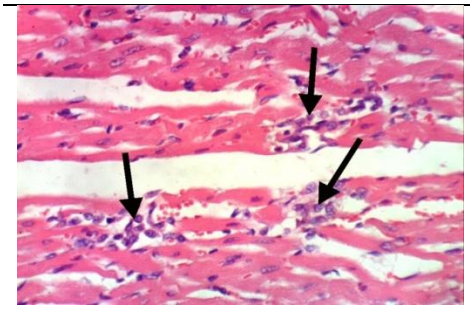

photo (9): Heart of rat from group 5 showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (H & E X 400).

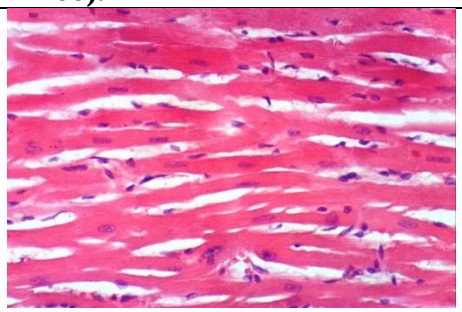

photo (10): Heart of rat from group 6 showing no histopathological changes (H & E X 400).

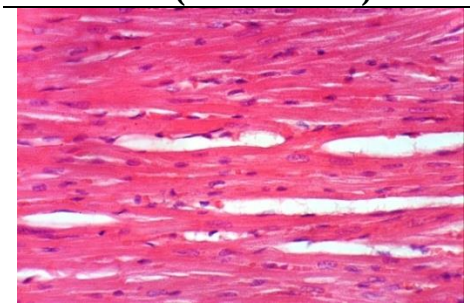

photo (11): Heart of rat from group 6 showing no histopathological changes (H & E X 400).

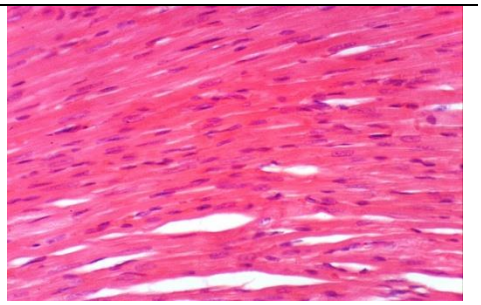

photo (12): Heart of rat from group 6 showing no histopathological changes (H & E X 400).

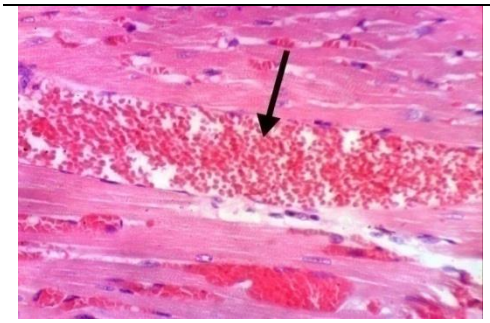

photo (13): Heart of rat from group 7 showing congestion of myocardial blood vessels (H & E X 400).

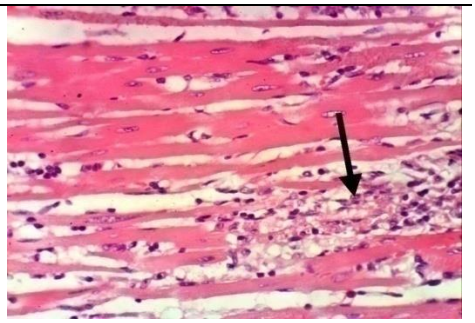

photo (14): Heart of rat from group 7 showing focal necrosis of cardiac myocytes associated with inflammatory cells infiltration (H & E X 400).

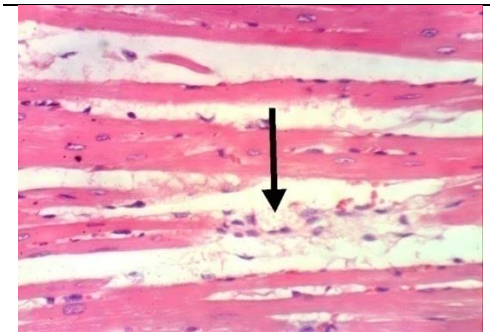

photo (15): Heart of rat from group 7 showing intermuscularoedema (H & E X 400).

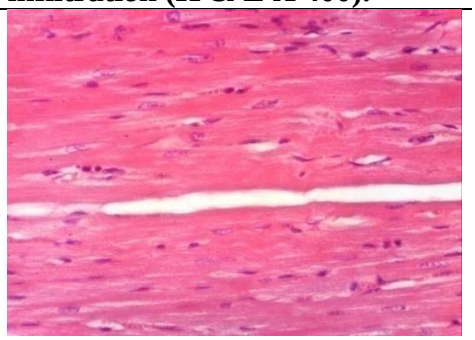

photo (16): Heart of rat from group 8 showing the normal histological structure of cardiac myocytes (H & E X 400).

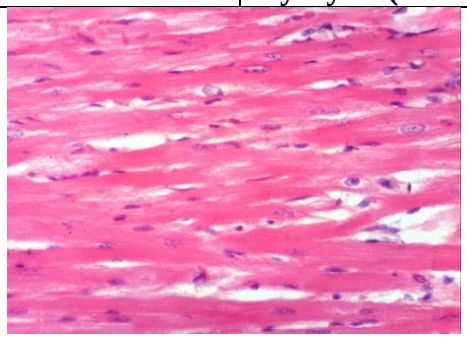

photo (17): Heart of rat from group 8 showing the normal histological structure of cardiac myocytes (H & E X 400).

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دراسة تأثير أوراق بعض النباتات على الفئران المصابة بإرتفاع دهون الدم

حمدية أحمد هلال ، ماجدة كامل الشاعر ، عبير نزية عبد الرحمن
الزهراء سعد عبد اللة

الملخص العربى:

أجريت هذه الدراسه لمعرفة التأثير المضاد لارتفاع الكوليستيرول لمسحوق أوراق كل من الباميا والمريميه والنعناع على الفئران المصابة بارتفاع الكوليسترول . تم استخدام ٣٢ فأر من ذكور فئران الألبينووالتى تزن ١٥٠ ±١٠ جم تم تقسيمها الى مجموعتين , أحدهما استخدمت كمجموعه ضابطه سالبه (-), بينما المجموعهالتانيه المختبرة ٢٨ فأر قد تم تغذيتهم على الوجبة المرتفعة الكوليسترول لمدة ثلاثة أسابيع (٢١) يوملاحداث ارتفاع فى الكوليسترول للفئران . ثم تم اضافة مساحيق الاوراقالنباتيه محل الدراسهالىالوجبهالأساسيه للفئران بنسبة ٣% , ٦% وذلك لمدة ٢٨ يوم . وفى نهاية التجربه تم عمل التحاليل التاليه: تقدير الكوليستيرول الكلى, الجليسريداتالثلاثيه , الليبوبروتينات مرتفعة الكثافه , اليبوبروتينات منخفضة الكثافه , الليبوبروتيناتالمنخفضه جدا فالكثافه. كما تم أيضا تقدير كل من وظائف الكبد والكلى وعمل فحص هيستوباثولوجى للقلب. وقد أوضحت النتائج المتحصل عليها وجود انخفاض معنوى ($p\leq0.05$) فى مستويات دهون الدم ووظائف الكبد والكلى , بينما لوحظ وجود ارتفاع معنوى($p\leq0.05$) فى مستوى الليبوبروتينات المرتفعة الكثافه, كما أكدت نتائج الفحص الهيستوباثولوجى للقلب ما تم التحصل عليه من التحاليل البيوكيميائيه. ويرجع هذا التحسن الى احتواء الأوراقالنباتيه محل الدراسه على العديد من المكونات الحيويهالفعالهالتى تحسن من التاثير السيئ للوجبهعاليه الدهون , ولذلك نوصى بالاهتمام باستخدام هذه النباتات بكميات معتدلهفى وجباتنا اليوميه .

الكلمات المفتاحية : الكوليسترول الكلى, الجلسريدات الثلاثيه, الليبوبروتينات مرتفعة الكثافه, الليبوبروتينات منخفضة الكثافه,اوراق النباتات , وظائف الكبد والكلى, الفئران المصابه بارتفاع الكوليستيرول و الفحوصات الهيستوباثولوجيه.