Hypolipidemic Effect of Apigenin Extracted From Parsley (*Petroselinum sativum* L.) Leaves In Cadmium Chloride Treated Rats (Part II)

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Abstract

Cadmium has been reported to have cumulative effects on mortality ,cardiovascular , renal and neurologic .flavonoids (apigenin) are naturally occurring phytochemicals possessed divers pharmacological effects hypolipidemic and antioxidant activity .this study was carried out to investigate the protective effect of the flavonoids extracted from (*Petroselinum sativum*) parsley leaves on some parameters related to cardiac risk in adult male rats exposed to 50 ppb cadmium chloride in drinking water . Crude flavonoids were extracted from parsley leaves . Further purification of flavonoids was performed by gel permeation column chromatography (TLC) . Thirty adult Albino male rats randomly and equally divided into three group (10 rats for each) and were treated daily for 60 days as follows : The rats were kept on ordinary tab water as control (group c) , received drinking water containing 50 ppb of cadmium chloride (group T1) and simultaneously given orally 150mg/kg B.W. of flavonoids (apigenin) extracted from parsley in addition to 50ppb of cadmium chloride in drinking water (group T2). Fasting blood sample were collected by cardiac puncture technique at 0,30 and 60 days of experiment for measuring the flowing parameters : serum lipid profile including: a. triacylglycerol (TAG) .b. serum total cholesterol (TC) .c. serum high density lipoprotein (HDL-C). d. serum low density lipoprotein (LDL-C).e. serum very low density lipoprotein (VLDL-C) concentration .Besides section from heart, aorta were taken at the end of experiment for histopathological study .The results showed that the yield of crude flavonoids from parsley leaves were approximately 2.68% of dray leaves .Purification of crude flavonoids on Sephadex LH-20 clarified three peaks activity and the proportions of the purified fraction P1, P2 and P3 were 14.06, 82.43 and 3.5% respectively. Thin layer chromatography confirmed that p2 was pure apigenin . the result revealed that animal exposed to 50 ppb of cdcl2 water for 60 days caused cardiac damage manifested by a significant elevation in serum TC,TAG,HDL-C,VLDL-C and LDL-C concentrations, with a significant depression in serum HDL-C concentration . Gavage of flavonoids and Cd concurrently caused a significant correction of the previous studies parameters . Histological section of the heart and aorta of cd treated (T1) group revealed an occurrence of early signs of atherosclerotic lesions . Such histological change could not be observed in heart and aorta of animal in group

T2 after oral intubation of flavonoids (apigenin). It could be concluded that apigenin is effective in prevention the deleterious effect of cadmium chloride on major rick factors of cardiovascular system.

Key Words: Cadmium chloride, apigenin, lipid profile, heart, parsley.

التاثير الخافض للدهون للابجنين المستخلص من اوراق نبات المعدنوس في الجرذان المعاملة بكلوريد الكادميوم

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المستخلص

اجريت هذه الدراسه لمعرفه الدور الوقائي القلبي للفلافونيدات المستخلصه من اوراق البقدونس في ذكور الجرذان البالغه المعرضه لكلوريد الكاميوم 50 جزء بالبليون في ماء الشرب . تم استخلاص الفلافينونات الخام من اوراق البقدونس ، ث تم تنقيه (الابجنين) على هلام عمود التحلل الوني على السيفادكس LH–20 ومن ثم تشخيصه باستخدام كروموتورفيا الطبقه الرقيقه . وقد بلغت كميه المتسخلص الخام من اللفلافينونات 2.68 غم لكل 100 غم من الاوراق الجافه ، واوضحت نتائج التنقيه على عمود التحلل اللوني وجود ثلاث قيم بتركيز 14.06,82.43,3,51% وقد اكدت نتائج الكوموتوكرافيا الطبقه الرقيقه ان P2 هو الابجنين الذي تمت تنقيته وقد بلغت تركيز الابجنين النقي 47% من الاوراق الجافه للبقدونس . تم تقسيم 30 من ذكور الجرذان البالغه عشوائيا الى ثلاثه مجاميع (عشر حيوانت/مجموعه) وعوملت كلتالي لمده 60 يوما : اعطيت المجموعه الاولى الناء العادي واعدت مجموعه سيطره (Group C))، في حين اعطيت حيوانات المجموعه الثانيه ماء شرب محتوي على 50 جزء بالبليون من كلوريد الكاميوم(Group T1) ، اما حيوانت المجموعه الثالثه فقد اعطيت ماء الشرب المحتوي على 50 جزء بالبليون من كلوريد الكاميوم بالاضافه الى تجريع الفموي للفلافونيدات المستخلصه من اوراق البقدنوس بتركيز 150 ملغم/كغم من وزن الجسم (Group T2) ، تم جمع عينات الدم الوخز القلبي في الفترات 0 و30 و60 يوما من التجربه لغرض قياس تركيز الكولسترول الكلي (TC) والكليسريدات الثلاثيه (TAG) والكلولسترول في البروتينات الشحميه ذات الكثافه العاليه (HDL–C) والواطئه (LDL–C) والواطئه جدا(VLDL–C) بالاضافه الى اخذ مقاطع نسيجيه للابهر والعضله القلبيه .اظهرت نتائج هذه الدراسه ان تعرض الحيوانات الى كلوريد الكاميوم بتركيز 50 جزء بالبليون في ماء الشرب لمده 60 يوما قد تسبب حدوث تلف في القلب تمثل بزياده معنويه في (TC,LDL–C,VLDL–C) .بينما ساهم تجريع الفلافونيدات مع الكاميوم (T2) في حصول تغيرات ايجابيه معنويه في المعاير التي اشير اليها سابقا والتي تمثلت بحصول زياده معنويه في تركيز الHDL–C ،بالاضافه الى حصول انخفاض معنوي في تراكيز كل من VLDL–C ,LDL–C,TC,TAG

في مصل الدم . اظهرت نتائج المقاطع النسيجيه للقلب والابهر في المجموعه المعرضه للكاميوم T1 في وجود مؤشرات اوليه لافه التصلب العصيدي تجلت بصوره رئيسيه بارتشاح الخلاليا الالتهابيه (الخلاليا العدله والالتهابيه) على جدار الابهر وارتشاح الدهون في الطبقه المصليه بالاضافه الى ارتشاح خلاليا وحيده النواه في منطقه ال intima layer بينما ادت المعامله بالفلافونيدات المستخلصه من اوراق البقدونس (T2) الى قله ظهور التلف في القلب .لقد اكدت نتائج هذه الدراسه التاثير الوقائي والدور المانع للاكسده للفلافونيدات المستخلصه من اوراق البقدونس ضد التلف الحاصل في القلب تحت تاثير الكادميوم.

الكلمات المفتاحيه : كلوريد الكادميوم ، ابجنين ،صورة الدهون ،القلب ، المعدنوس .

Introduction

Cadmium (Cd)is an environmental pollutant that is released naturally from minerals ,forest fires and volcanic emission (12) cigarette smoke ,tap and well water ,food ,fungicides and seafood are regarded as important sources of pollution with Cd (4,15) .Besides Cd is a product of zinc and lead mining and smelting ,which are important sources of environmental pollution (11). On the other head , a major role of cd intake (for nonsmoker) is ingested ,this is largely attributed to the presence of trace level of cd in food stuff of natural origin e.g. cereals ,beans ,carrots, tomatoes , beverage coffee and tea (34,50). This heavy metals is now have numerous undesirable effects on health in experimental animals and humans (7) , including kidney (51), bone (24), liver(54) and nervous system (27) . It has been suggested that the exposure of cd has been associated with wide variety of cardiovascular disease such as atherosclerosis (36),heart failure (46) and cardiomyopathy (3,41). Flavonoids are a diverse group of low molecular mass polyphenolic compounds widely distributed in plants ,they occur naturally in broad range of fruits ,vegetable ,nuts ,seeds ,herbs, spices, stems ,flowers and beverages such as green tea and red wine (25,32,52,53) ,there are more than 8000 different compounds (58). Addition to its effects on reproductive system (18), flavonoids have been reported to exert multiple biological effects , including antioxidant, free radicals scavenging abilities (19), diuretics (8), anti-inflammatory and anti-carcinogenic activity (9).Apigenin is natural flavonoids present abundantly in common fruits and vegetable such as parsley (30,33,42) ,onion , orange ,tea, chamomile wheat, sorouts, apples broccoli(16,20). This study was undertaken to assess the relative efficacy of apigenin extracted from parsley on some biomarkers related to cardiac functions of cadmium treated rats.

Materials and methods

Extraction of flavonoids from parsley (*Petroselineum sativum*) leaves

Crude flavonoids were extracted from parsley leaves according to the method of harborne (17) modified by AL-Kawary (2). Then extracted flavonoids were subjected to further purification through solubilization in 85% ethanol and gel filtration on Sephadex LH-20 . Dried flavonoids fraction then was subjected to further analysis using thin layer chromatography technique.

Experimental Design:

Thirty adult (between 2.5-3 months) Albino male rats (175-225gm) were randomly and equally and divided into three groups (10 rat/ group) and were treated daily for 60 days as follows: 1.Group C (control). 2. Group T1:Rats of these group were allowed to ad libitum supply of drinking water containing 50 ppb of CDCL2 .3. Group T2: Rats of these group were received 150mg/kg B.W. of flavonoids (apigenin) extracted from parsley orally, in addition to 50ppb of cdcl2 in drinking water . fasting blood samples were collected by cardiac puncture technique at 0,30,60 days of experiment for measuring the following parameters .Serum (TAG) ,(TC) and (HDL-C) using enzymatic kit (linear chemicals Barcelona, spian) , (LDL-C) and (VLDL-C) concentration (13). Besides, sections from heart and aorta were taken at the end of experiment for histological study (26). Statistical analysis of data was performed on the basis of Two –Way Analysis of Variance (ANOVA) using SAS® software (SAS, 13) at a significant level of ($P< 0.05$). Differences were determined using least significant differences (LSD) (55).

Results

The yield of crude flavonoids extracted from samples revealed that out of each 100 gm. of dry ground leaves parsley was approximately 2.68 g. of crude flavonoids was obtained .Three peaks were found in the supernatant of the extract (figure-1), (p1,p2 and p3) on UV 330 nm and the proportion of these peaks were 14.06, 83.43 and 3.5% respectively .The major peak (p2) was collected and dried under vacuum then subjected to thin layer chromatography analysis on silica gel (figure-2) ,and the RF of purified fraction was 0,81 . Such value was approximately the same of apigenin as recorded by Harborne (17), under similar diagnostic conditions.

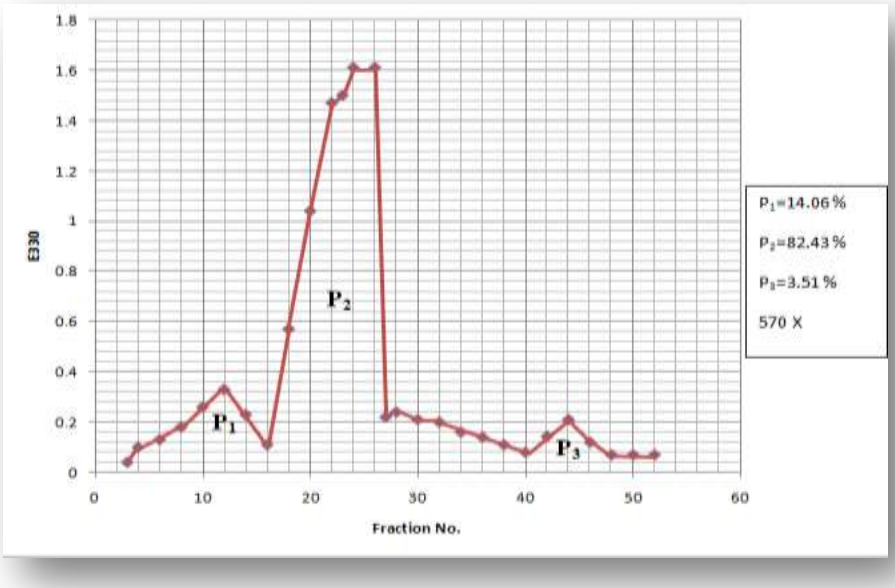

Figure -1 : Purification of crude flavonoids from parsley on Sephadex LH-20 column (38 × 1. 6) ID. Ethanol (85 %) was used as an eluent at flow rate of 30 ml/hr. , fraction size: 4 ml.

Figure -2: TLC analysis of flavonoids extracted by Harborne (1973).
1. Purified apigenin on Sephadex LH-20. 2. Crude apigenin .

Lipid profile testes:

Serum TC,TAG,LDL-C and VLDL-C concentration showed a significant increase ($p< 0.05$) at the day 60 of treatment (tables 1-4) in rats received 50ppb of cdcl4 compared with control and T2 groups where oral intubation of extracted flavonoids (T2group) significant suppressed ($p< 0.05$) the elevated previously mentioned parameters and the values tend to normalize that of the control. On the other hand a significant increase ($p< 0.05$) in serum HDL-C concentration (table 5) was recorded in T2 group as compared To the T1 treated group at the san=me treated period (after 60 days) .

Table -1: Effect of flavonoids extracted from (*Petroselineum sativum*) leaves on serum total cholesterol (mg/dl) concentration in control and cadmium treated rats.

Groups / Days	C	T1	T2
Zero	93.8 ± 3.10 A a	94.5 ± 2.41 A a	96.5 ± 2.41 A a
30	93 ± 3.12 A a	95.5 ± 3.38 A a	91.6 ± 2.05 A a
60	95.6 ± 3.04 A a	110.3 ± 3.56 B b	94.1 ± 3.18 A a

Values are expressed as mean ± SE, n = 6 each group

C: control group. T1: Animals received 50 ppb of $CdCl_2$ in drinking water.

T2: Animals received 50ppb of $CdCl_2$ and 150mg/kg B.W. of flavonoids extracted from parsley.

Capital letters denote differences between groups, P<0.05 vs. control.

Small letters denote differences within group, P<0.05 vs. control.

Table-2: Effect of flavonoids extracted from (*Petroselineum sativum*) leaves on serum triacylglycerol (mg/dl) concentration in control and cadmium treated rats.

Groups / Days	C	T1	T2
Zero	87.3 ± 3.54 A a	88.3 ± 4.01 A a	89.6 ± 3.14 A a
30	88.5 ± 4.34 A a	89.8 ± 3.59 A a	84.8 ± 2.42 A a
60	88.8 ± 3.37 A a	105.3 ± 3.06 B b	86.6 ± 3.15 A a

Values are expressed as mean ± SE, n = 6 each group

C: control group. T1: Animals received 50 ppb of $CdCl_2$ in drinking water.

T2: Animals received 50ppb of $CdCl_2$ and 150mg/kg B.W. of flavonoids extracted from parsley.

Capital letters denote differences between groups, P<0.05 vs. control.

Small letters denote differences within group, P<0.05 vs. control.

Table-3: Effect of flavonoids extracted from (*Petroselineum sativum*) leaves on serum low density lipoprotein-cholesterol (LDL-C) (mg/dl) concentration in control and cadmium treated rats.

Groups / Days	C	T1	T2
Zero	42.4 ± 3.17 A a	41.3 ± 5.22 A a	44.4 ± 1.48 A a
30	40.9 ± 3.55 A a	45.7 ± 5.22 A a	41.6 ± 1.94 A a
60	42.4 ± 2.31 A a	64.4 ± 5.11 B b	45.6 ± 3.93 A a

Values are expressed as mean ± SE, n = 6 each group

C: control group. T1: Animals received 50 ppb of $CdCl_2$ in drinking water.

T2: Animals received 50ppb of $CdCl_2$ and 150mg/kg B.W. of flavonoids extracted from parsley.

Capital letters denote differences between groups, P<0.05 vs. control.

Small letters denote differences within group, P<0.05 vs. control.

Table -4: Effect of flavonoids extracted from (*Petroselineum sativum*) leaves on serum very low density lipoprotein-cholesterol (VLDL-C) (mg/dl) concentration in control and cadmium treated rats.

Groups / Days	C	T1	T2
Zero	17.4 ± 0.71 A a	17.7 ± 0.79 A a	17.9 ± 0.63 A a
30	17.7 ± 0.86 A a	17.9 ± 0.77 A a	16.9 ± 0.44 A a
60	17.7 ± 0.67 A a	21.06 ± 0.61 B b	17.3 ± 0.63 A a

Values are expressed as mean ± SE, n = 6 each group

C: control group. T1: Animals received 50 ppb of $CdCl_2$ in drinking water.

T2: Animals received 50ppb of $CdCl_2$ and 150mg/kg B.W. of flavonoids extracted from parsley.

Capital letters denote differences between groups, P<0.05 vs. control.

Small letters denote differences within group, P<0.05 vs. control.

Table-5: Effect of flavonoids extracted from (*Petroselineum sativum*) leaves on serum high density lipoprotein-cholesterol (HDL-C) (mg/dl) concentration in control and cadmium treated rats.

Groups / Days	C	T1	T2
Zero	34 ± 1.48 A a	35.5 ± 1.73 A a	34.16 ± 0.87 A a
30	32.6 ± 1.33 A a	31.8 ± 1.58 A a	33.2 ± 2.33 A a
60	35.5 ± 1.84 A a	24.8 ± 1.74 B b	30.6 ± 2.04 C a

Values are expressed as mean ± SE, n = 6 each group

C: control group. T1: Animals received 50 ppb of $CdCl_2$ in drinking water.

T2: Animals received 50ppb of $CdCl_2$ and 150mg/kg B.W. of flavonoids extracted from parsley.

Capital letters denote differences between groups, P<0.05 vs. control.

Small letters denote differences within group, P<0.05 vs. control.

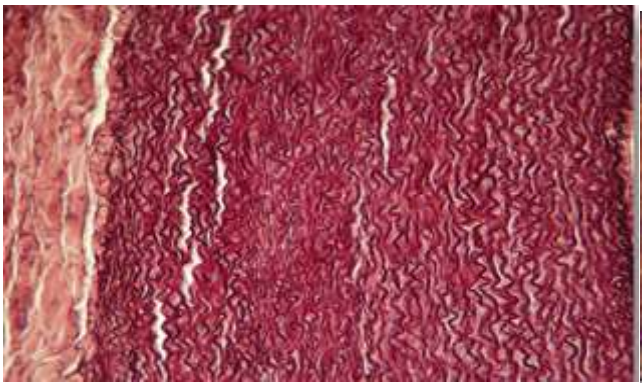

Figure -3: Histological section of normal aorta of rat (H and E, X40).

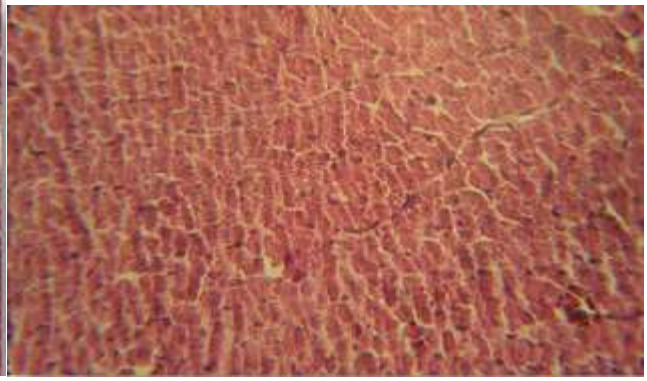

Figure -3: Histological section of normal aorta of rat (H and E, X40).

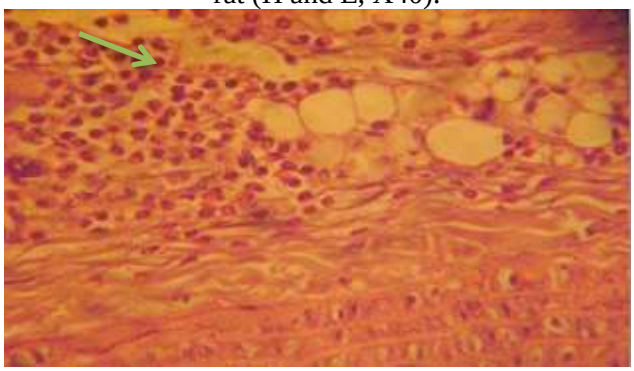

Figure-5: Histological section of aorta of rat treated with $CdCl_2$ (50 ppb) in drinking water (T1). Note: severe inflammatory cell infiltration mainly, neutrophil and macrophage around the aorta (arrow). (H and E, X40)

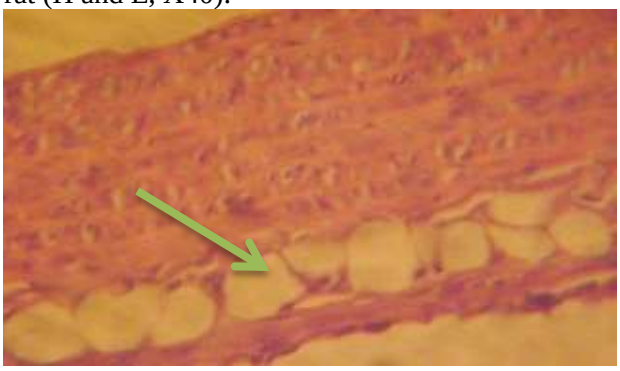

Figure-6: Histological section of aorta of rat treated with $CdCl_2$ (50ppb) in drinking water (T1). Note: fatty infiltration in serosal layer (arrow). (H and E, X40).

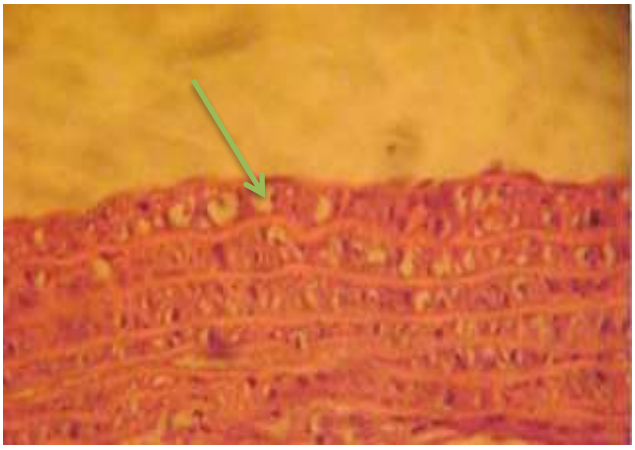

Figure -7: Histological section of aorta of rat treated with $CdCl_2$ (50ppb) in drinking water (T1). Note: vacuolation on mononuclear cell infiltration in the intima of the aorta (arrow). (H and E, X40).

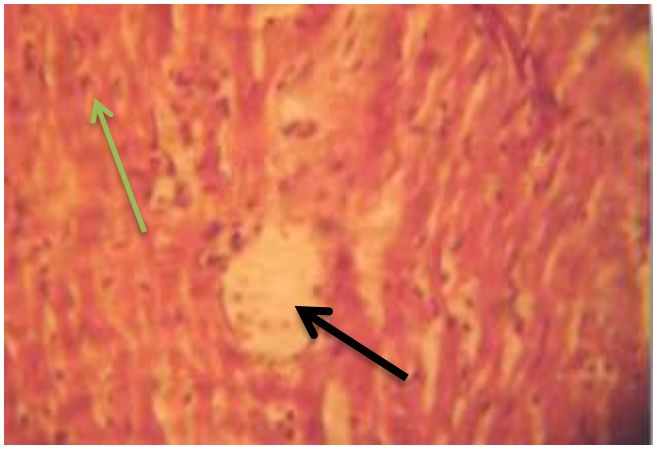

Figure-8: Histological section of heart of rat treated with $CdCl_2$ (50ppb) in drinking water (T1). Note: inflammatory cell infiltration between the muscle fiber (→) and congestion of blood vessels (→) (H and E, X40).

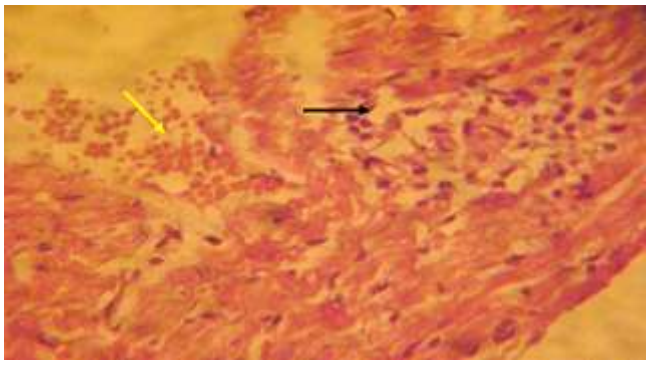

Figure-9: Histological section of heart of rat treated with $CdCl_2$ (50ppb) in drinking water (T1). Note: Inflammatory cell infiltration mainly neutrophil and macrophage in the atrium (→), and congestion of blood vessels (→). (H and E, X40).

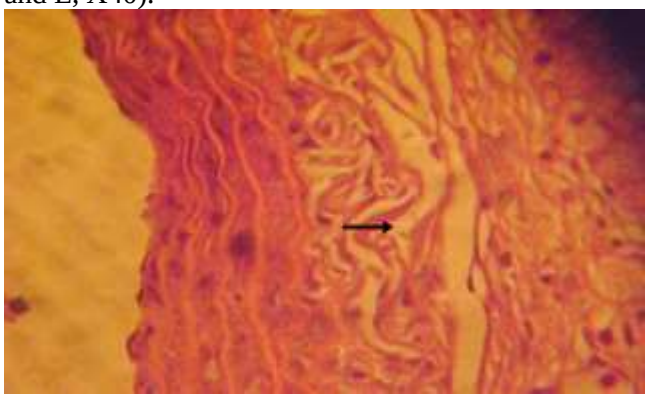

Figure-10: Histological section of aorta of rat treated with $CdCl_2$ (50ppb) in drinking water plus flavonoids 150 mg/kg B.W. (T_2). Note: partial regression of the lesion with few inflammatory cell infiltration between the muscle fibers (arrow) (H and E, X40).

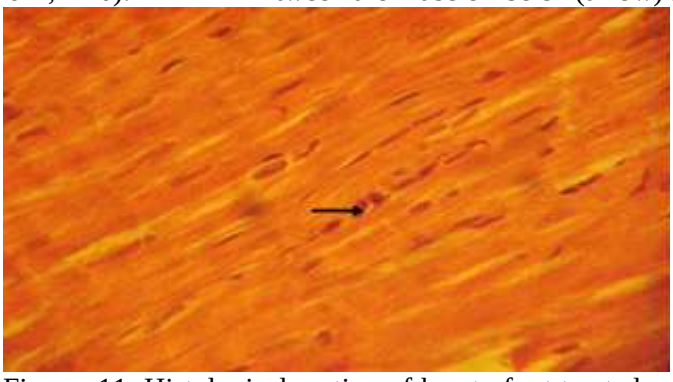

Figure -11: Histological section of heart of rat treated with $CdCl_2$ (50ppb) in drinking water plus flavonoids 150 mg/kg B.W. (T_2) Note: there is few inflammatory cells infiltration between the muscle fiber (arrow) (H and E, X40).

Discussion:

Hyperlipidemic effect of cadmium is agreement with (14,39,44,47) . The observed dyslipidemia after Cd exposure may reflect the suppression of lipid metabolism and reduction in antioxidant enzymes duo to Cd induced oxidative stress Disturbing the antioxidant defense system after Cd exposer as result of increase quenching enormous free radicals (FRs) produced under such condition (22,40,49) may be responsible for such depression in HDLC concentration and elevation in LDL-C (10). This suggestion is documented in our previous study (23), where significant decrease in GSH (non-enzymatic antioxidant) and elevation in MDA concentration were observed after Cd exposure. Olisekodiaka and his colleagues,(38) confirmed that Cd can inversely affect lipoprotein and lipid profile via lipid peroxidation.

The results also explained the lowering effect of parsley extract on TC, TAG, LDL-C concentration , which was documented in vivo (21,56,57). Parsley hypolipidemic effects may be attributed to its flavonoids content , mainly apigenin (42), that play a role in lowering activity of both HMG-CoA reductase and acetylCoA cholesterol-o-acetyl transferase (ACAT) (31), Flavonoids decrease activity of rate limiting enzyme of cholesterolgenesis by phosphorylation of enzyme directly (60), leading to hyper-cholesterolemia . Besides, flavonoids possess beneficial effect on cardiovascular risk factor such as lipoprotein oxidation , dyslipidemia, endothelial dysfunction and regulation of serum lipid profile (28). As well as , the free radicals scavenging activities of parsley (29) and apigenin (43) may reduce LDL-C oxidation and depressed its transportation to different tissues.

Elevation in HDL-C concentration after parsley extract , could be due to stabilizing effect of its polyphenols on plasma lipoprotein or due to systemic effect of flavonoids to modulate various enzymatic activity that can affect lipoprotein leading to augmentation of HDL-C. Such elevation in HDL-C is in agreement with (1,5) , where increase HDL-C facilitate transport of cholesterol from serum to liver, where its catabolized and excreted from bodies (59). Besides high HDL-C concentration is correlated with decrease risk of cardiovascular disease (48).

Histopathological observation of aorta and heart tissue of animal in group T1 (figures-3,4,5) also confirmed the cardio toxic effect of CdCl2 (35,45) comparing to control (figure-2). Sections of muscle (fingures-6,7) manifested by occurrence of atheromatous lesion indicating injury of vascular endothelium after cadmium exposure (46). Which may be due to the function role of hydrogen radical generation in the endothelium (37) .These radical have been documented to be responsible for endothelial dysfunction and atheroma (6). The present study also confirm the curative effect of apiginin which causes regeneration of cardiac cell(figure-8,9). This may be due to its anti-oxidant properties .

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