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Influence of crude extract of Hawthorn *crataegus oxyacantha* on some physiological aspects in mature male Rats exposed to hydrogen peroxide over load.

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Summary

This study was carried out to investigate the protective effects of 70% ethanolic alcohol extract of hawthorn (*crataegus oxycantha*) on some physiological functions of male rats exposed to 1% H_2O_2. Fifteen mature male Newzeland rats were randomly divided into three groups:- control group (C) ,two groups treated with 1% H_2O_2 alone (G1) or 1%H_2O_2 with crude extract of hawthorn(G2) orally daily for 30 days .Blood samples were taken at zero time and 30 days of the experiment .The present study declared an alteration in the lipid profile of the treated group (G2) at the end of treatment (30 days) manifested by asignificant reduction ($p<0.05$) in serumTC,TAG,LDL-C, VLDL-C concentrations. And elevation ($p<0.05$) in serum, HDL-C, as compared to the treated group (G1). Antioxidant status also exhibited significant ($p<0.05$) changes characterized by an elevation of serum GSH in group (G2). Histological study revealed that oral treatment with 1% H_2O_2 caused congestion of blood vessels of the heart with infiltration of inflammatory cells and odema between muscle fibers. It is concluded that treatment with hawthorn showed no clear pathological lesions.

Key words: Hawthorn , crataegus oxyacantha, hydrogen peroxide, Rat.

تأثيرالمستخلص الخام لثمار الزعرور على بعض الصفات الفسلجية الناتجة من فرط بيروكسيد الهيدروجين في ذكور الجرذان البالغة

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الخلاصة

أجريت هذه الدراسة لمقارنة الدور الوقائي للمستخلص الكحولي(70% من الكحول الأثيلي) لثمار الزعرور في بعض المؤشرات الفسلجية في ذكور الجرذان المعرضة للأجهاد التاكسدي بواسطة 1% من بيروكسيد الهيدروجين . تم أستخدام خمسة عشر جرذا نيوزيلانديا من الذكور البالغة قسمت عشوائيا الى ثلاثة مجاميع متساوية:- مجموعة سيطرة(C) أعطيت ماء الشرب الأعتيادي, ومجموعتين معالجة أعطيت ماء الشرب الأعتيادي مضافا اليه بيروكسيد الهيدروجين بتركيز 1% لوحده (G1) أو مضافا أليه المستخلص الكحولي لثمار الزعرور(G2) لمدة ثلاثين يوما . تم سحب عينات الدم للفترات 0 و 30 يوم من التجربة. أظهرت الدراسة الحالية تغيرا ملحوظا في دهون الدم للمجموعة المعاملة الثالثة بعد 30 يوما من المعالجة والتي تميزت بأنخفاض معنوي اكبر من 0.05 في الكوليسترول الكلي والدهون الثلاثية وكوليسترول الشحوم البروتينية واطئة الكثافة وكوليسترول الشحوم البروتينية ذات الكثافة الواطئة جدا , بالأضافة الى الأرتفاع المعنوي في تركيز الكلوتاثيون في مصل الدم . بينت نتائج الفحص النسيجي حدوث احتقان الأوعية الدموية في قلب الجرذان المعاملة ب 1% من بيروكسيد الهيدروجين مع وجود ارتشاحات التهابية خلوية ووذمة بين الألياف العضلية . لم تظهرالمعاملة بالمستخلص الكحولي للزعرور اي أفات مرضية .

مفاتيح الكلمات:- ثمار الزعرور, بيروكسيد الهايدروجين, الصفات الفسلجية, ذكور الجرذان

Introduction

Antioxidants can be defined as substances able to inhibit or delay the oxidative damage of protein, nucleic acid and lipid caused by dramatic increase of reactive oxygen species (ROS) (1) by inhibiting the initiation or propagation of oxidizing chain reactions (2). Antioxidants can be classified into endogenous antioxidants including Superoxide Dismutases (SOD)(3) Catalase(4), Peroxiredoxins (5), Thioredoxin and Glutathione system (6) which were involved in the neutralization of ROS species (7) and non-enzymaticExogenous antioxidants contained in a wide spectrum of herbs, fruits, and vegetables (8).

Plants have been used for several years as a source of traditional medicine to treat various diseases and conditions (9). A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can provide therapeutic effect (10). Phytochemical, especially Phenolics, in fruits and vegetables are suggested to be the major bioactive compounds for health benefits. Phenolics are one of the groups of nonessential dietary components that have been associated with the inhibition of atherosclerosis and cancer. The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipooxygenase, and scavenge free radicals (11). The leaves, flowers, and berries of hawthorn contain a variety of bioflavonoid-like complexes, including oligomericprocyanidins (OPCc), vitexin, quercetin, and hyperoside (12, 13,14and15). The recommended daily dose of hawthorn is 160-900 mg of a native water-ethanol extract of the leaves or flowers (equivalent to 30-169 mg of epicatechin or 3.5-19.8 mg of flavonoids) (16 and 17). Its fruit has been used over the course of time as a diuretic, for dyspnea, and renal calculi. There are also studies that show its sedative and anxiolytic effects (18), and cardiotonic properties (19). So the antioxidant and the hypolipidemic activity of crataegus oxycantha was the aim of this study.

Materials and Methods

The fresh fruits of hawthorn were extracted with 70% ethanol according to (20). Fifteen mature (3-5 months) adult AlbinoWistar male Rats were randomly divided into three groups (each of 5) and treated as follows for 30 days :- Animals in group one had free access to food and water and served as control, group two(G_1) animals were subjected to ad libitum supply of drinking watercontaining 1% H_2O_2 (35% of hydrogen peroxide solution was diluted with water ,group three (G_2) animals were subjected to ad libitum supply of drinking water containing 1% H_2O_2 and received 300 mg / kg B.W. of crude ethanolic extract of Crataegus Oxyacantha dissolved in distilled water . Blood samples were collected by heart puncture technique at 0 time and 30 days of the experiment, serum collection by centrifugation (3000 rpm) for 15 minutes and frozen at -20C°until analysis. Serum samples were used for measuring the following parameters: - serum total cholesterol (TC) concentration was enzymaticlly measured using enzymatic assay kit (spain react) (21). Enzymatic estimation of serum triacylglycerol (TAG) concentration and serum high density lipoprotein cholesterol concentrations (HDL-C) were measured enzymatically using enzymatic kit) Linear chemicals) (22). Serum low density lipoprotein- cholesterol (LDL-C) concentration and serum very low density lipoprotein-cholesterol (VLDL-C)

concentration were calculated according to (23). Determination of Serum Reduced glutathione concentration (GSH) depended on the action of sulfhydryl groups (24). Stock standard solution of GSH (0.001M) was prepared by dissolving 0.0307 gm of GSH standard in a final volume of 100 ml of 0.4M Tris-EDTA-Na buffer (pH8.9). from stock solution 2, 5, 10, 40, 50, and 60 µM of standard GSH were prepared by following formula$N_1V_1=N_2V_2$ (Figure 1). The animals were then sacrificed for a histological examination, heart tissue sections were prepared according (25). Differences between experimental groups were statistically evaluated using two way analysis of variance (ANOVA) (26).

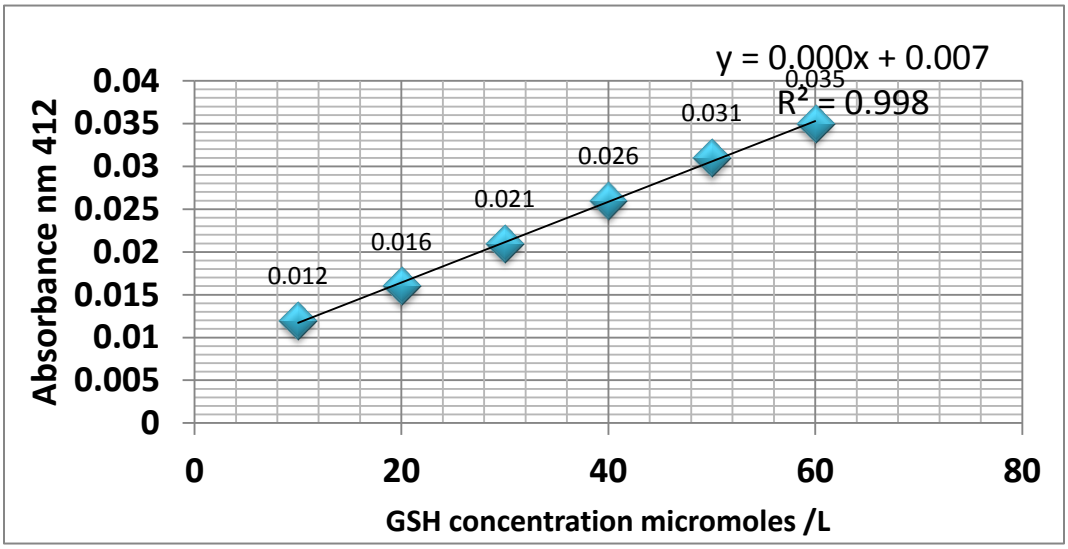

Figure (1) Standard curve of GSH concentration.

Results and Discussion

The effect of 70% alcoholic extract of *Crataegus oxyacantha* on lipid profile and antioxidant activity in mature male rats was shown in tables (1 and 2). Data pertaining to total cholesterol , TAG, HDL, LDL, VLDL concentration of rats in control group , group (G1) treated with 1% H_2O_2 and group (G2) treated with 1%H_2O_2plus 300 mg/ kg B.W. crud extract of *Crataegus oxyacantha* are depicted in table (1). The results showed after 30 days of treatment, significant (P< 0.05) decrease in serum TC concentration in the group (G2) as compared to the treated group (G1). There was significant (P<0.05) decrease in serum TAG in group (G2) as compared to the treated group (G1) and control group. There was significant (P< 0.05) increase in serum HDL concentration in group (G2) as compared to the treated group (G1), while there was significant (p<0.05) decrease in serum VLDL concentraton in group (G2) as compared to treated group (G1) and control. The results showed significant (P< 0.05) decrease in serum LDL concentration in treated group (G2) as compared to the treated group (G1). The mean values of serum reduced glutathione concentration in the control and treated groups along the experimental period are depicted in table (2). The statistical analysis indicated that the mean value of treated group (G2) significantly (P< 0.05) increased after 30 days of treatment as compared to control and treated group (G1). The result of the current study showed that oral administration of 1% H_2O_2 in drinking water for 30 days to adult male rats comparing to *Crataegus oxyacantha*, caused a case of dyslipidemia table (1) manifested by significant elevation in serum TC, TAG, LDL, VLDL concentration and significant depression in serum HDL concentration

which were similar to the findings on rat (27) and on japaness Quail (28). The potent oxidative effect of H_2O_2 which caused an oxidative damage manifested by free radicals and this lead to asubsequent complication and development of atherosclerosis (29 and 30). The present study demonstrated clearly that crude extract of hawthorn fruit possessed hypolipidemic activity, Hawthorn extract has been reported to lower plasma cholesterol concentration and other lipid profile in rats (31and 32) and rabbits (33). The mechanism for the hypocholesterolemic activity of hawthorn fruit may be either due to its inhibition of cholesterol and bile acid absorption or increased excretion of these neutral and acidic sterols (34). The beneficial effect of hawthorn was through reduction of intestinal cholesterol absorption via inhibition of the intestinal ACAT activity (35). These results, taken together, provide scientific evidence that hawthorn could be a useful natural ingredient for lowering plasma cholesterol concentrations in humans (36).

The elevation of serum TAG level in H_2O_2 treated group may contribute to the diffiency of lipoprotein lipase (the key enzyme determining the removal rate of TAG from plasma), associated with increased output of lipoprotein from the liver (37). Tincture of hawthorn inhibited oxidation of LDL and VLDL (38). Crataegus up- regulates hepatic LDL receptors, resulting in greater influx of plasma LDL – cholesterol into the liver by preventing the accumulation of cholesterol in the liver and cholesterol degradation to bile acids , promoting bile flow and suppressing cholesterol biosynthesis (32and39) .On the other hand, the investigation showed depression in the HDL concentration in group (G1) treated with 1% H_2O_2 while group (G2) treated with 1% H_2O_2 plus 300 mg / kg B.W. crude extract of hawthorn showed significant increase in this parameter. High density lipoprotein (HDL) plays an essential role in plasma lipid transport. It provides a reservoir of C, a lipoproteins, which are required for the metabolism of chylomicrons and very low density lipoproteins (VLDL), and acts as a scavenger of surplus unesterified cholesterol from these lipoproteins. HDL is also the major vehicle for the transport of cholesterol from peripheral cells to the liver for excretion and catabolism (40). Supplementation of hawthorn (4 weeks) with high cholesterol diet resulted in marked decrease in total cholesterol and LDL-lipoprotein, and more importantly with an increase in HDL-lipoprotein (41).

The current study showed decrease in the concentration of reduced glutathione (GSH) in group (G1) treated with 1% H_2O_2 and significant elevation in group (G2) administrated 1% H_2O_2 with hawthorn. Hyperlipidemia induced oxidative stress lead to decreased level of GSH in blood (42). The oxidative stress lead to increase oxidation of GSH into bisulphoric phase GSSG by inhibition pathway of pentose phosphate shunt which limits NADPH production that is necessary for activity of glutathione reductase enzyme which is important in GSH reproduction from oxidized phase (GSSG) (43). Hawthorn contains abundant amount of antioxidants such as chlorogenic acid, epicatechin, hyperoside and quercetin (54) which may be useful in alleviating the adverse effects associated with low-density lipoprotein (LDL)-cholesterol oxidation in atherosclerosis (45). Many studies show that hawthorn exhibited antioxidant activity associated with its flavonoids, polyphenol, procyanidin and this activity may be attributed to its effective inhibition of oxidative processes, efficient scavenging of O2- and possible increasing GSH biosynthesis (46 and 47). The histological structure of normal heart of control group is show in figure (2). After 30 days of oral gavage of 1% H_2O_2 the treated group (G1) showed pathological changes in the heart manifested as congested myocardial blood vessels with neutrophils in their lumen as well as odema between the myocardial muscle fibers , it also showed inflammatory cells between muscle fibers and lumen of blood vessels (figures 3and 4) .The histological

changes in the heart of animals of group (G2) treated with 1% H_2O_2 plus 300 mg/ kg B.W. of crude extract of *Crataegus oxyacantha* showed no clear pathological lesions (figure 5).

Table (1):-Effect of crude extract of *crataegus oxyacantha* on lipid profile in male rat (mg/dl)

	Group Treatment Orally for 30 days	Control Group(Distilled water)	G1 1% H_2O_2 with water	G2 1% H_2O_2+300mg/ kg B.W. of plant extract with water
Zero time	Total cholesterol conc.	67.61 ± 2.89 Aa	73.03 ±1.97 Aa	67.96 ± 3.11 Aa
	Triacylglycerol conc.	66.58 ± 2.23 Aa	65.26 ± 2.19 Ba	66.64 ± 2.44 Aa
	HDL conc.	51.43 ± 3.19 Aa	46.96 ± 1.31 Aa	47.71 ± 2.05 Aa
	LDL conc.	29.50 ± 5.06 Aa	39.12 ± 2.53 Aa	33.58 ± 2.34 Aa
	v-LDL conc.	13.32 ± 0.45 Aa	13.05 ± 0.44 Ba	13.33 ± 0.49 Aa
After 30 days treatment	Total cholesterol conc.	50. 85 ± 1.24 Bb	64.61 ± 3.37 Ba	49.86 ± 3.38 Bb
	Triacylglycerol conc.	65.29 ± 3.54 Ab	124.01± 6.91 Aa	49.84 ± 3.32 Bc
	HDL-c conc.	56.67 ± 1.12 Aa	42.63 ± 3.56 Ab	53.20 ± 5.52 Aa
	LDL- c conc.	7.24 ± 2.15 Bb	46.79 ± 5.36 Aa	14.38 ± 4.68 Bb
	v-LDL –c conc.	13.06 ± 0.71 Ab	24.80 ± 1.38 Aa	9.97 ± 0.66 Bc

Values expressed as means ± SE.n=5 / group
Small letters denote between groups differences, p< 0.05 vs cnotrol.
Capital letters denote within groups differences, p<0.05 vs control.

Table (2):- Effect of crude extract of *Crataegus oxyacantha* on glutathione concentration (µmol/l)

Group	Zero time	30 days treatment
Control	30.40 ± 1.60 Aa	27.20 ± 1.36 Ab
G1	30.00 ± 1.90 Aa	27.60 ± 0.75 Ab
G2	29.60 ± 1.60 Ba	41.20 ± 0.80 Aa

Values expressed as means ± SE.n=5 / group
Small letters denote between groups differences, p< 0.05 vs control.
Capital letters denote within groups differences, p< 0.05 vs control.

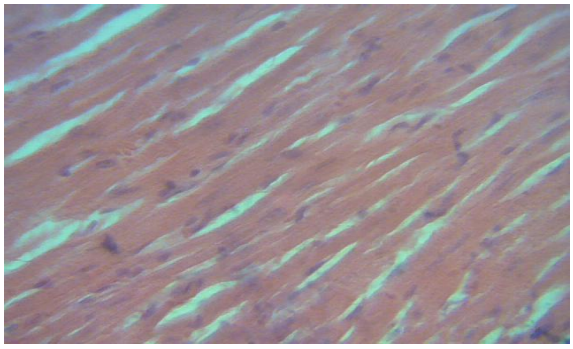

Figure 2:- Histological section of normal heart of rat (H and E, 40^X)

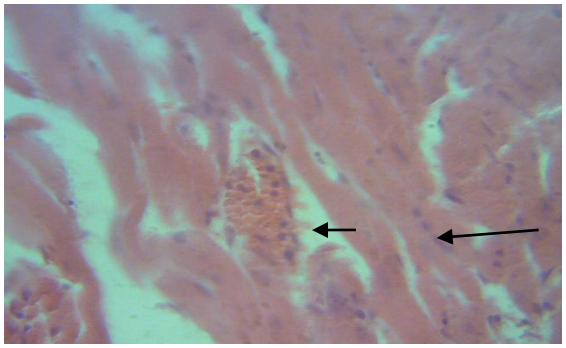

Figure 3:- Histological section of heart of rat shows congested blood vessels with neutrophils in their lumen (⟶) (H and E, 40^X)

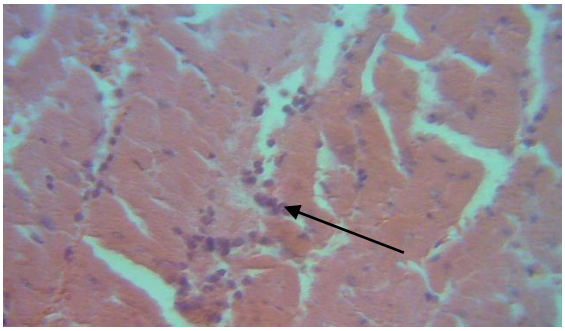

Figure 4:- Histological section of heart of rat shows inflammatory cells between the muscle fibers and lumen of blood vessels (⟵) (H and E, 40^X)

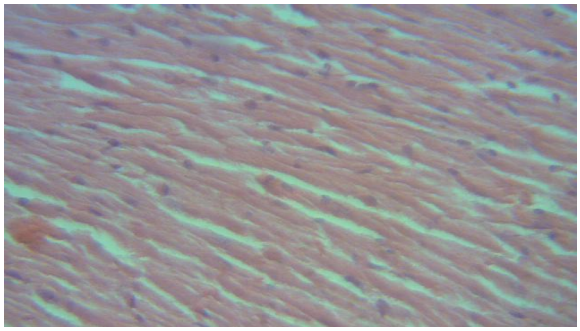

Figure 5:- Histological section of heart of rat treated with1% H_2O_2+300mg/kg B.W. of *crataegus oxyacantha* shows no clear pathological lesion (H and E, 40^X)

The histological changes in the heart showed congested myocardial blood vessels with neutrophils in their lumen as well as odema between the myocardial muscle fibers, but no lesion was observed in group treated with hawthorn. It could be concluded from this study that *Crataegus oxyacantha* has hypolipidemic and antioxidant activity.

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