The Protective Effect Of Hawthorn (*Crataegus Sinaica* extract) In Reducing The Pathological Changes Of Cholesterol In Albino Rats

Zeinab M. Labib and *Nahla A. G. Ahmed Refat
Animal Health Research Institute-Benha branch
*Pathology Dept. Faculty of Vet. Med. Zagazig University

ABSTRACT

Crataegus (Hawthorn) has long been used as a folk medicine and is utilized in some pharmaceutical preparations because of its neuro and cardio sedative actions and its low toxicity. This study was aimed to investigate the efficacy of Crataegus when administered to hypercholesterolemic rats. Twenty five male albino rats were equally divided into 5 groups (Gps). Group1 served as control without any treatment. Group2 was given 0.5ml/100gm B.wt. from a fresh mixture of 4 parts of distilled water and 5 parts of alcohol. Group 3 was received 0.5ml/100gm B.wt. Crataegus. Group 4 was given 0.5 gm/kg B.wt. cholesterol to induce hyperlipidemia. Group 5 intubated with 0.5ml/100gm B.wt. Crataegus and 0.5 gm/kg B.wt. cholesterol. All treatments were given by stomach tube daily for sex weeks(the period of the experiment). Necropsy was performed at the end of the experiment and all macroscopic abnormalities were recorded. Cholesterol induced degenerative and necrotic changes in the hepatocytes and renal epithelia. Congestion of hepatic sinusoids and hepatic and renal blood vessels was observed. These blood vessels showed thickening of their walls. Cholangitis and thickening of hepatic capsule were seen. The lungs showed thickening of interalveolar septa with compensatory focal alveolar emphysema and desquamation of the lining epithelium of some bronchi beside perivascular edema. The heart revealed congestion, hyalinization and Zenker's necrosis in the cardiac myofibers. The pulmonary and cardiac blood vessels showed medial hypertrophy and narrowing of their lumina.

The pathological changes in rats of on group 5 which, simultaneous administered both cholesterol and Crataegus, were mild in comparison to cholesterol group alone.

Finally, it could be concluded that, the Crataegus is highly effective in alleviation the pathological changes induced by cholesterol.

INTRODUCTION

Hypercholesterolemia (hyperlipidemia) induces severe degenerative changes in the liver like hyperemia, cell swelling, vacuolar and fatty changes *(1)*. On the same context hypercholesterolemia is the major risk factor in the initiation and progression of arteriosclerosis and cardiovascular diseases *(2,3)*.

There is an increase demand for using plants in therapy (Back to nature) instead of using synthetic drugs which have many adverse effects that may be more dangerous than the disease itself *(4)*. Hawthorn is a member of the *Rosaceae* family, has yellow, yellow green, orange or red berries with one, two or three nut lets. Red berries are the most common one used *(5)*. Hawthorn flowering tops are harvested in late spring and early summer, the berries in September and October *(6)*. It is known that tincture of Crataegus (TCR), an alcoholic extract of the berries of Hawthorn, *Crataegus oxyacantha, Crataegus rosaceae,* is widely used today in herbal medicine as a heart tonic *(7,8)*, in heart failure and cardiovascular disease *(9,10)*. TCR decreases lipid deposits in liver and aorta. Also produced remarkable reduction in the increased levels of cholesterol, triglycerides and phospholipids *(1)*. On the same line TCR is said to have a solubilising effect on the crustaceous and calcareous deposits in arteries *(11)*. Additionally TCR is used in the treatment of

digestive ailments, dyspnea, kidney stones, cardiovascular disorders, hypertention, angina pectoris, arthritis, rheumatism, antispasmodic, sedative, insomnia, prevent arteriosclerosis, ability to increase the integrity of the blood vessel wall, improve coronary blood flow and positive effects on oxygen utilization *(1,12)*.

The objective of the present study was to investigate the role of Crataegus extract in the protecting liver, kidneys, heart and lungs against lesions induced by cholesterol.

MATERIALS AND METHODS

Animals

The experiment was conducted on 25 male albino rats. The rats were obtained from Zagazig Vet Med. Animal House Colony. Animals weight ranged from150- 200gm, housed in stainless steel cages under hygienic condition and fed one week on basal diet for adaptation. After the period of adaptation the animals were divided into 5 equal groups, each contains 5 rats.

The first group: Received no treatment and kept as negative control group. The second group: Intubated with 0.5ml/100gm B.wt./day fresh mixture of 4 parts distilled water + 5 parts ethanol.

The third group: Intubated with 0.5ml/100gm B.wt./day from diluted Crataegus extract *(1,9)*.

The fourth group: Intubated with 0.5gm/kg B.wt./day cholesterol *(13)*.

The fifth group: intubated with diluted Crataegus extract (0.5ml/100gm B.wt./day) and cholesterol (0.5gm/kg B.wt./day).

After 6 weeks post-treatment ,animals were subjected post mortem (PM) examination as well as histopathological investigation. Specimens were obtained from heart and its blood vessels, liver, kidneys and lungs then fixed in 10% neutral formaline buffered solution. Paraffin sections of 5µ. thickness were prepared and stained with Hematoxylin (H) and and Eosin (E) for histopathological examination according to *(14)*.

Cholesterol: Pure cholesterol–Prolabo–Paris (El-Nasr Pharmaceutical Chemical Company).

Hawthorn plant: The *Egyptian Crataegus Sinaica* was kindly obtained from Enviromental Search Center of Santi-Cathrine, Suiz Canal University. The tincture of Crataegus (TCR) is prepared by mixing 100 gm fresh pulp of the ripe berries of Hawthorn with 635 ml of ethanol. This extract is diluted by taking 1 part of the extract with 4 parts of distilled water and 5 parts of ethanol (1).

RESULTS

Cholesterol group(4 th group)

The liver was macroscopically slightly congested and enlarged.

Microscopically, congestion in the central veins and portal blood vessels with hydropic degeneration of hepatic cells besides few leukocytic infiltrations were seen (fig. 1). Dilated hepatic sinusoids with pressure atrophy of the hepatic cords were observed (fig. 2). The hepatic blood vessels showed thickening and vacuolation of their wall. Cholangitis was seen and represented by proliferation of lining epithelium of the bile duct besides few leukocytic infiltration and fibrous connective tissue and smooth muscles fibers proliferation around these ducts. Hyalinized media of the hepatic arteriole could be seen in some portal area (fig. 3). Thickening of the hepatic capsule by fibrous connective tissue proliferation was detected (fig. 4) The hepatic cells showed cloudy swelling, vacuolar and hydropic degeneration besides coagulative necrosis of some hepatocytes represented by karyolysis.

The kidneys were macroscopically enlarged. Microscopically, hypercellularity of some glomeruli, cloudy swelling and coagulative necrosis of convoluted tubular epithelium, represented by karyolysis were observed (fig. 5). Hyaline and cellular casts were reported (fig. 6). The desquamated epithelium in the cellular casts become mineralized with production of basophilic

stones (fig. 7). Some glomeruli revealed proliferation of endothelial cells and thickening of Bowman's capsule. Perivascular edema of the renal blood vessel was seen (fig. 8). The lungs were macroscopically apparently normal. Microscopically, the lungs showed thickening of interalveolar septa by congestion and round cell infiltration (fig. 9). Compensatory focal alveolar emphysema was seen particularly adjacent the thickened interalveolar septa. The bronchial epithelium was slightly hyperplastic and desquamated. The adjacent pulmonary arterioles showed perivascular edema (fig. 10) and medial hypertrophy which represented by marked thickening of the media due to enlargement and proliferation of smooth muscle cells and narrowing of their lumina (fig. 11). The heart was macroscopically, apparently normal. Microscopically, the heart showed focal hyaline degeneration in the cardiac muscle fibers and Zinker's necrosis. Edema among cardiac muscles fibers (fig. 12) represented by widely separated cardiac muscle fibers and perivascular edema (fig. 13) were seen . The myocardial blood vessels and capillaries were congested (fig. 14). The wall of some cardiac blood vessels was thickened. Myomalecia cordis, represented by focal disappearance of muscle fibers was noticed.

Cholesterol and crataegus group (5th group)

The macroscopic picture didn't show any abnormalities. Microscopically, the liver, revealed few round cell infiltrations mainly lymphocytes in the interstitial tissue (fig. 15). The kidneys, showed cloudy swelling and hydropic degeneration in the epithelial lining of some renal tubules (fig. 16). The lungs, revealed few round cells infiltrations particularly in the peribronchial tissue. The adjacent alveolar septa were slightly thickened by congested perialveolar capillaries and round cell infiltration (fig. 17). The heart, showed focal hyaline degeneration in the cardiac muscle fibers (fig. 18).

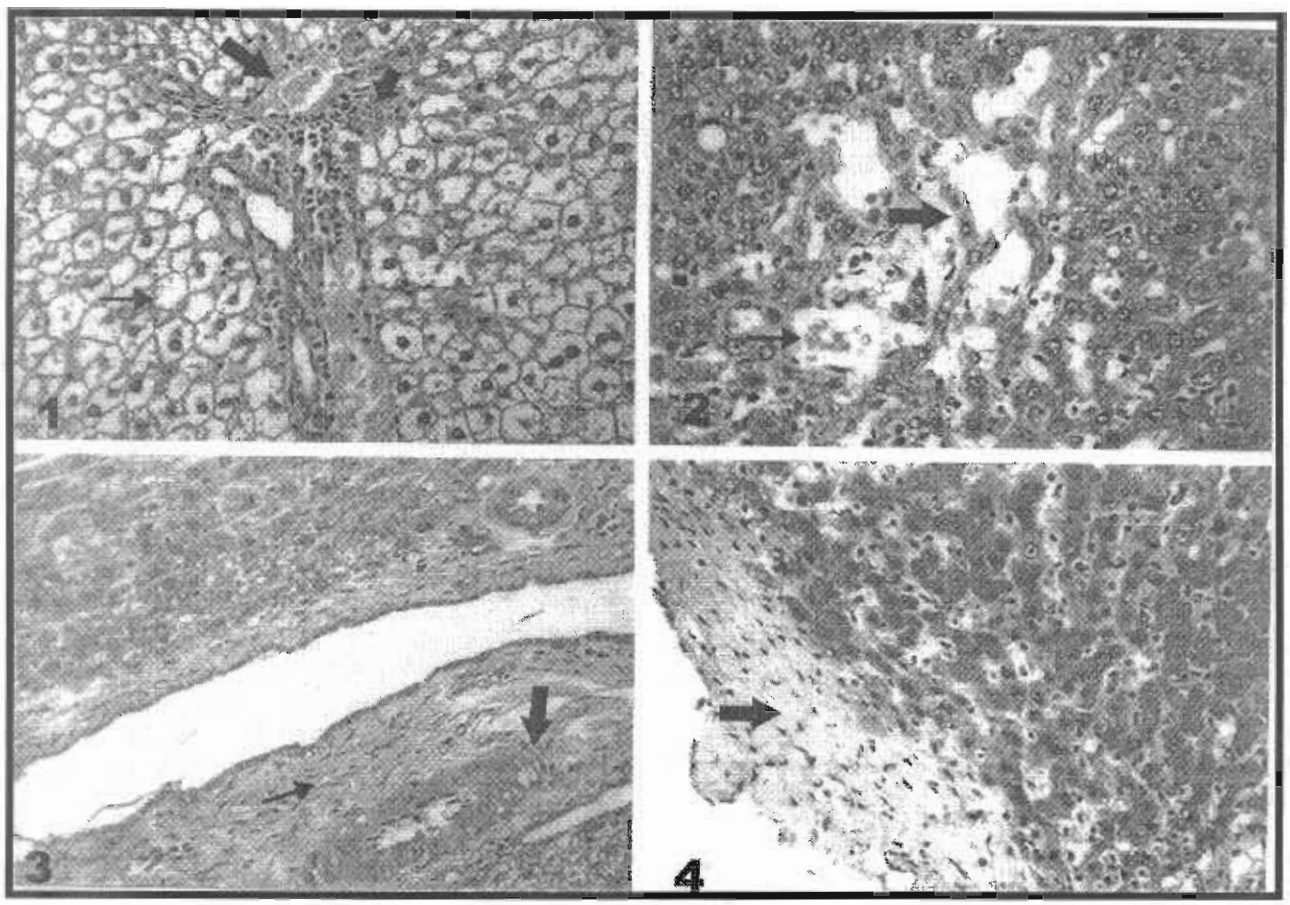
Gps. 1,2 and 3

Neither macroscopically nor microscopically changes were detected in these groups.

DISCUSSION

In this study, the cholesterol induced pathological lesions in liver, kidneys, heart, lungs and blood vessels. The main adverse effects were degenerative and necrotic changes in the hepatocytes and renal tubules. Congestion of hepatic sinusoids and renal blood vessels were observed. These blood vessels showed thickening and vacuolation of their walls. Cholangitis and thickening of hepatic capsule by fibrous tissue were seen. The previous lesions are in partial agreement with *(1)*, who noticed severe degeneration changes and congestion of the liver of rats fed on cholesterol. The lungs revealed thickening of interalveolar septa with compensatory focal alveolar emphysema. Desquamation of the lining epithelium of some bronchi and perivascular edema were seen. The heart showed congestion, hyalinization and Zenker's necrosis in the cardiac myofibers. The latter showed edema among them. The pulmonary and cardiac blood vessels showed mild hypertrophy and narrowing of their lumina . The achieved results are in partial concurrence with *(15)* who found that hypercholesterolemia (1% cholesterol- rich diet during 2 month) induced considerable lesions and atherosclerosis in the cardiovascular system of rabbit.

The previous lesions in liver, kidneys, heart, lungs and blood vessels may be induced via tissue oxidative damage of cholesterol *(16,17)*, reported that the cholesterol enriched diet significantly reduced the catalase and superoxide dismutase activities and increased the formation of oxygen- derived free radicals in rats. The myocardial and pulmonary lesions usually followed by hepatic and renal lesions *(18)*. The lesions of group 5, simultaneously administered cholesterol and Crataegus to rats, were effective in protecting the rats against harmful effect of cholesterol, the lesions were milder compared with those received cholesterol only.



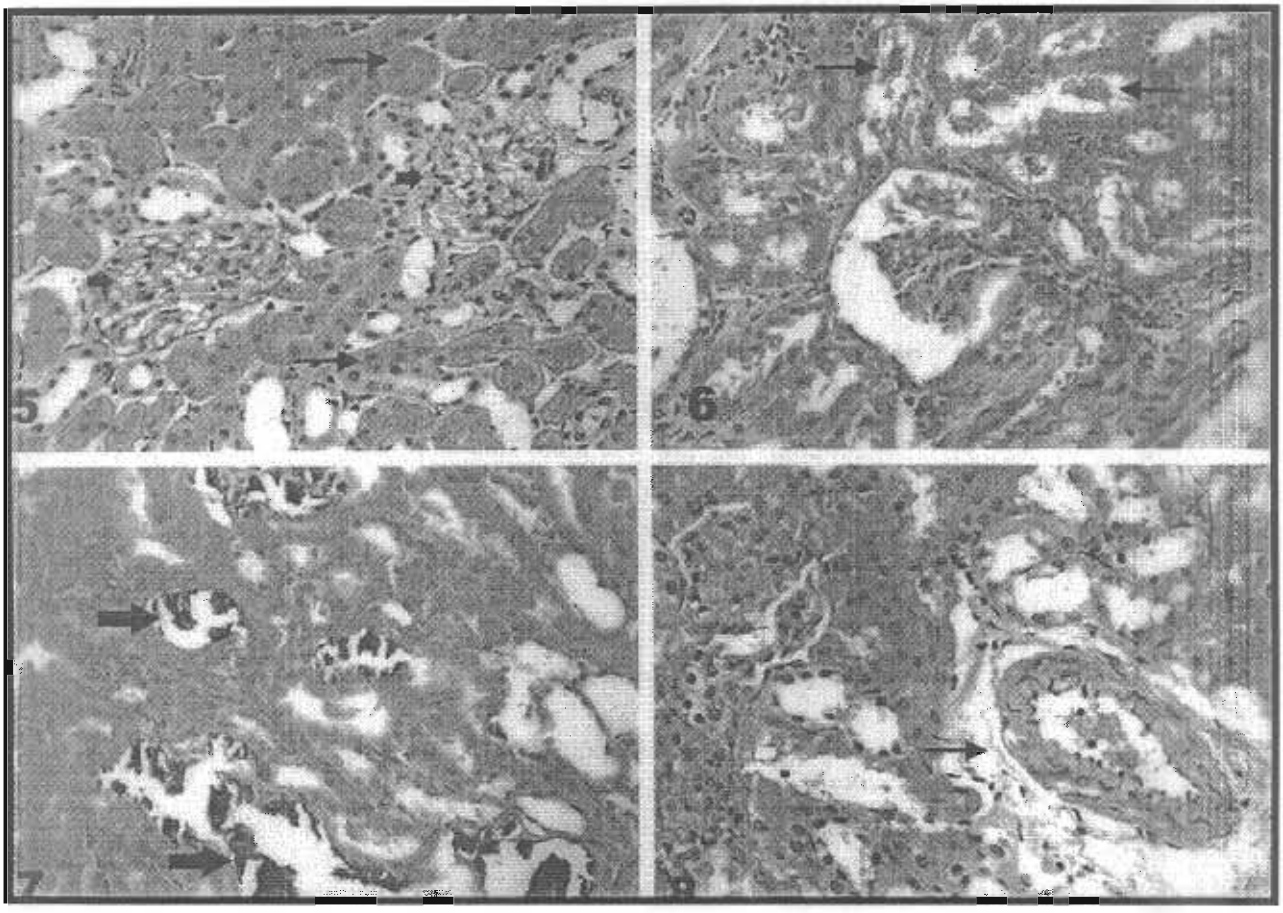
Figs(1-4): Liver of cholesterol group showing:

1-Hydropic degeneration of hepatocytes (thin arrow) and congested blood vessel with few leukocytic infiltration in the portal area (thick arrow), H&E., x 300.

2-Congested hepatic sinusoids (thin arrow) with pressure atrophy of some hepatic cords(thick arrow), H&E.,x300.

3-Portal area showing smooth muscle fibers proliferation(thin arrow) and hyalinized media of the hepatic arteriole (thick arrow), H&E.,X300.

4-Thickening of the hepatic capsule by fibrous connective tissue proliferation (arrow), H&E.,x300.



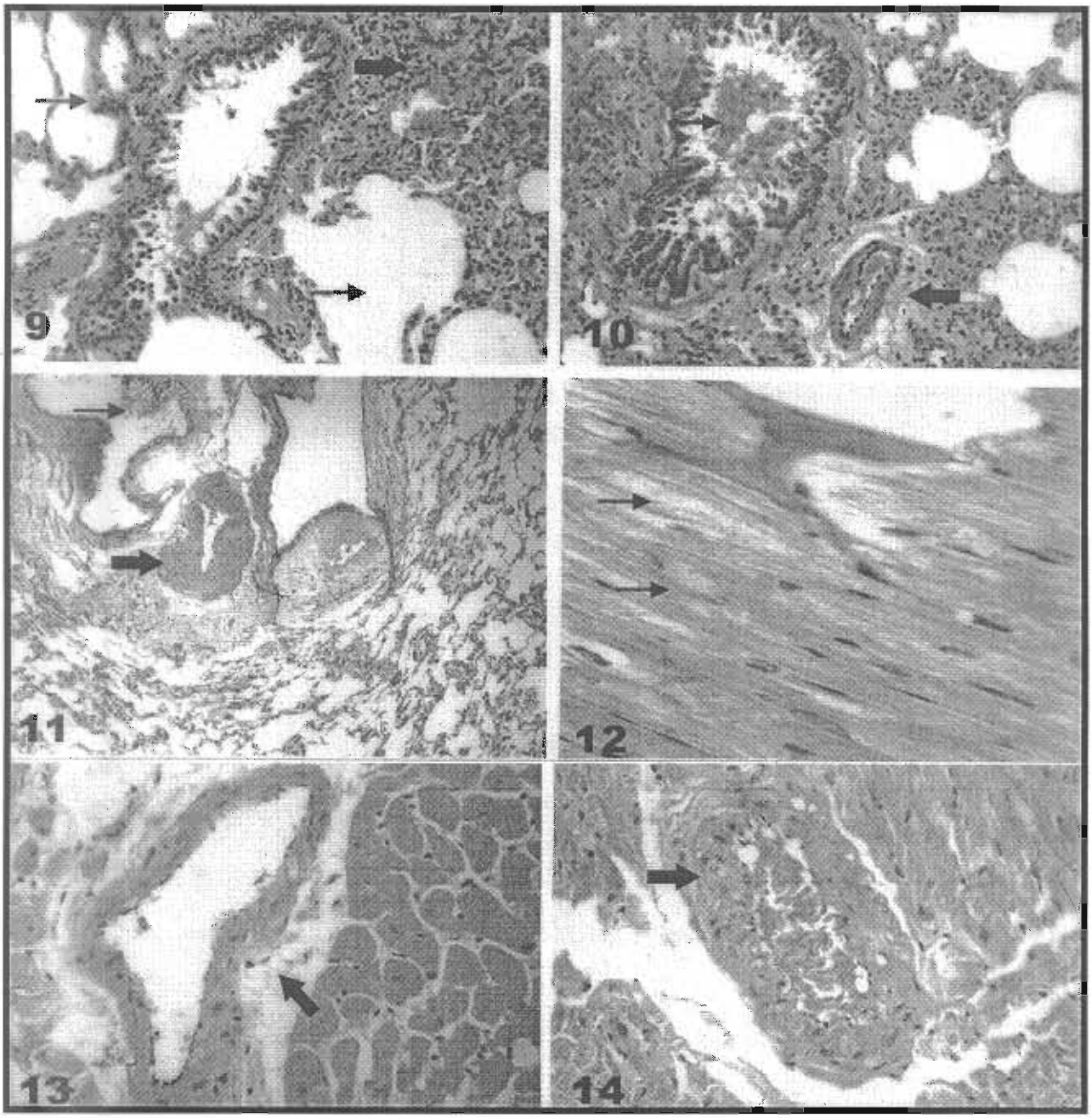
Figs(5-8): Kidney of cholesterol group showing :

5-Hypercellularity of some glomeruli, cloudy swelling and coagulative necrosis of some renal tubules (thin arrows) represented by karyolysis,H&E.,x300

6- Cellular casts inside the lumina of some renal tubules (arrow) , H&E.,X300.

7-Calcification of cellular casts(arrows),H&E.,x300.

8-Perivascular edema, H&E.,x300.



Figs (9-11): Lung of cholesterol group showing :

9-Thickening of interalveolar septa by red blood cells and leukocytes (thick arrow) and compensatory focal alveolar emphysema(thin arrow) H&E.,X300.

10-Perivascular edema (arrow),H&E.,x300.

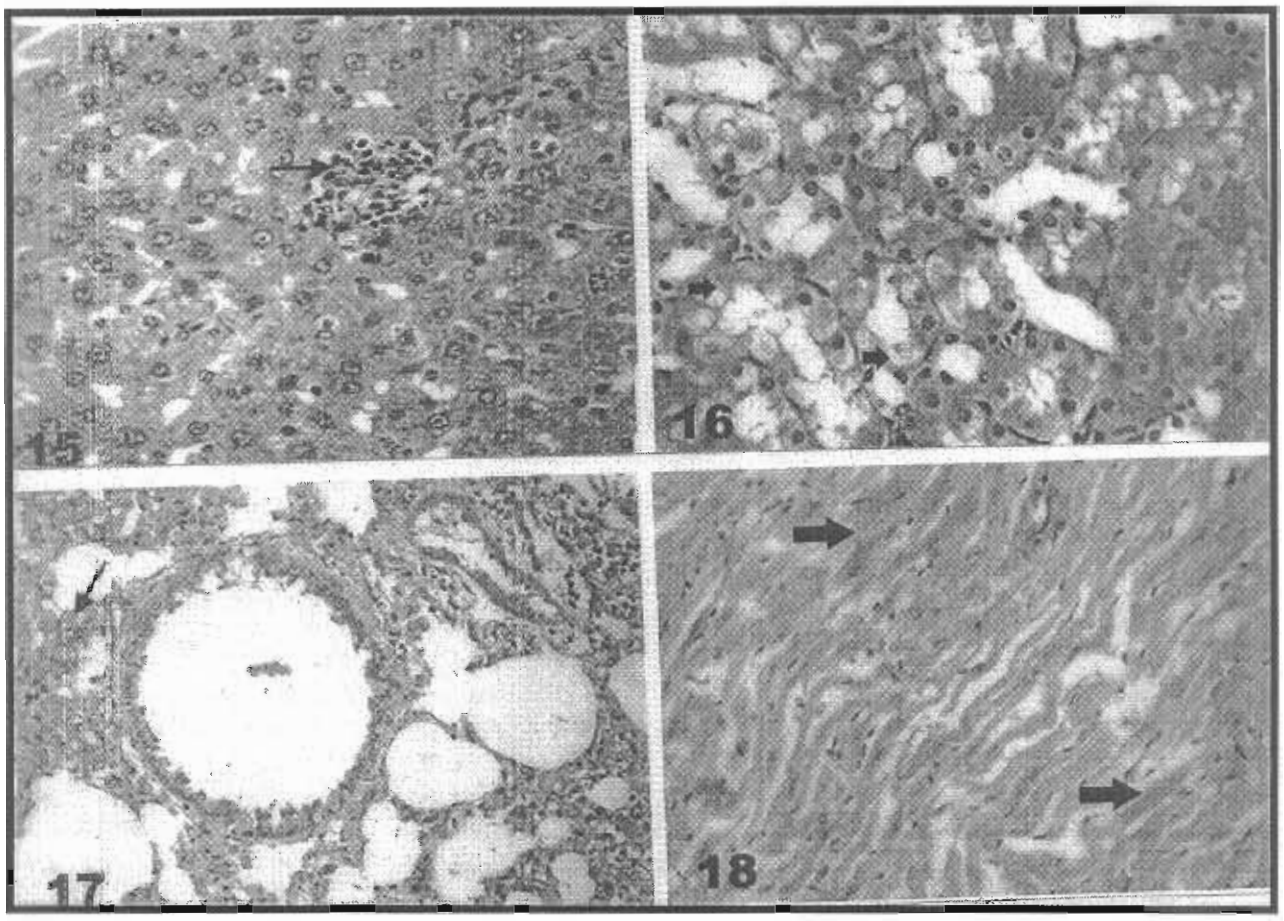
11-Medial hypertrophy of peribronchial blood vessel,H&E.,x300 .

Figs (12-14): Heart of cholesterol group showing :

12-Edema among cardiac muscle fibers (arrows) represented by widely separated cardiac muscle fibers , H&E.,x300.

13-Perivascular edema(arrow),H&E.,x300.

14- Congested blood vessel(arrow),H&E.,X300.



Figs(15-18): Cholesterol and Crataegus group showing :

15-Liver:Interstitial infiltration with round cells (arrow),H&E., x300.

16-Kidney:Hydropic degeneration of some renal tubules(arrow) ,H&E.,x300.

17-Lung :Slightly thickened interalveolar septa by few round cells and congested perialveolar capillaries (arrow), H&E.,x300.

18-Heart:Focal hyaline degeneration (arrows) of cardiac muscle fibers,H&E.,x300.

These results are coincided with *(1)*, who reported that Crataegus ameliorating the damage effect of hyperlipidemic diet (HLD) on liver and circulatory system of rats. On the same line *(19)* showed that the pretreatment of rats with Crataegus in a dose 50-200 mg/kg by gavage for 5 days reduced liver lesions induced by lipopolysaccharied . Also our result proved *(12,20)*, which showed that TCR protects against pathological changes induced by isoproterenol in rat heart and recorded that the Crataegus is used in the treatment of kidney stones, cardiovascular disorder, arteriosclerosis and can increase the integrity of the blood vessel walls. The ameliorating effect of Crataegus on hypercholesterlemic lesions may be attributed mainly to its flavonoids content as procyanidin, aromatic acid and cardiotonic amines *(10)*, and these beneficial effects may in part be due to the presence of antioxidant flavonoid components *(21)*. On the same line, Crataegus has antioxidant potentials (effective inhibitor of oxidative processes, efficient scavenger of superoxide O2- and increase GSH biosynthesis) *(22)*. Crataegus was also shown to increase bile acid excretion and depress hepatic cholesterol synthesis in atherogenic diet fed rats *(2)*. The authors added that Crataegus also prevents the accumulation of cholesterol in the liver by enhancing cholesterol degradation to bile acids and by simultaneously suppressing cholesterol biosynthesis. In addition, the Crataegus

maintained mitochondrial antioxidant status, prevented mitochondrial lipid peroxidative damage *(9)*.

Finally, it could be concluded that the Crataegus is highly effective in alleviation the pathological changes induced by cholesterol.

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الملخص العربى

التأثير الوقائى لخلاصة أشجار الزعرور فى تقليل التغيرات المرضية للكولسترول فى الجرذان البيضاء

زينب محمد لبيب و نهلة عبد الغفار أحمد رفعت*

معهد بحوث صحة الحيوان – فرع بنها

و قسم الباثولوجيا كلية الطب البيطرى جامعة الزقازيق*

تعتبر أشجار الزعرور من الأشجار التى يستخدم ثمارها منذ زمن قديم فى علاج كثير من الأمراض و على رأسهم تصلب الشرايين و علاج فرط التوتروحصوات الكلى. وازداد استخدامه فى الآونة الأخيرة نظرا لعدم وجود آثار جانبية له. وقد هدف هذا البحث معرفة الدور الوقائى لخلاصة اشجار الزعرور على التغيرات المرضية للكولسترول على كبد و كلى و قلب ورئة الجرذان البيضاء البالغة. وقد اجريت هذه الدراسة على عدد ٢٥جرذ أبيض قسمت إلى ٥ مجموعات متساوية. ولقد أخذت هذه المجموعات على التوالى:

المجموعة الاولى: مجموعة ضابطة سالبة.

المجموعة الثانية: أعطيت ٥ مل/١٠٠جرام من وزن الجسم خليط من الماء المقطر و الكحول.المجموعة الثالثة: أعطيت٥ مل /١٠٠جرام من وزن الجسم مستخلص أشجار الزعرور.

المجموعة الرابعة: أعطيت ٥جرام/كيلو جرام من وزن الجسم كولسترول .

المجموعة الخامسة: أعطيت كلا من مستخلص أشجار الزعرور و الكولسترول بنفس النسب السابقة.

ولقد أعطيت الجرعات الدوائية لجميع المجموعات عن طريق الفم بواسطة الأنبوبة المعدية يوميا و لمدة ٦ أسابيع. وفى نهاية التجربة تم ذبح الجرذان من كل مجموعة لإجراء الصفة التشريحية وأخذ عينات من الكبد والكلى و الرئة و القلب لفحصها مجهريا .

ولقد أظهر الفحص المجهرى أن المجموعة التى تناولت الكولسترول وجد بها تنكسات مختلفة ونخر فى خلايا الكبد و الأنابيب الكلوية، كما وجد احتقان فى الأوعية الدموية الكبدية والكلوية و وذمة. وفى الرئة وجد زيادة فى سمك الحواجز ما بين الحويصلات الهوائية مع وجود تقشر فى الأنسجة المبطنة للشعيبات الهوائية. و فى القلب وجد احتقان و تنكسات و نخر بسيط فى عضلاته، و لقد وجد سمك فى جدار الأوعية الدموية الكبدية و الكلوية و الرئوية و القلبية مع ضيق فى تجويفها .أما المجموعة الخامسة الأساسية فلقد كانت التغيرات المرضية بسيطة عند مقارنتها بمجموعة الكولسترول.

مما سبق نستنتج من هذه الدراسة أن مستخلص ثمرة أشجار الزعرور لها تأثير فعال فى الوقاية من الآفات المجهرية التي أحدثتها مجموعة الكولسترول.