EFFECT OF *CHICORIUM INTYBUS* EXTRACT ON HISTOLOGICAL CHANGES IN LIVER AND KIDNEY OF HYPERCHOLESTEROLEMIC RABBITS

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ABSTRACT

In this study, the experiment was designed to investigate the effect of daily oral administration of (0.5gm/kg. B.W) of chicory (*Chicorium intybus*) for one month on weight and histology of kidney and liver of hypercholesterolemic rabbits (induced by oral administration of 0.5 gm/kg of cholesterol dissolved in 3ml Soya bean oil) and compared this effect with medicial therapy used for atherosclerosis treatment .

The results revealed a significant increase ($P < 0.05$) the weights of livers and kidneys in hypercholesterolemic group when compared with other groups ,while the histopathological results showed histological changes in the livers and kidneys of hypercholesterolemic rabbits and also in chicory treated group when compared with other groups.

INTRODUCTION

Cholesterol is a fat-like substance found in the bloodstream and in all the cells. It is used in the body in the synthesis of the cell membranes, protecting nerves, digesting dietary fats. It is a precursor of certain hormones such as the sex hormones; around 75% of cholesterol of the body needs is made naturally by the liver and other cells of the body and can be affected by heredity while the 25% comes from foods such as eggs, meats, butter, and dairy products (1).

Normal healthy adults synthesize cholesterol at a rate of approximately 1gm /day and consume approximately 0.3 gm /day. A relatively constant level of cholesterol in the body (150-200 mg/dL) is maintained primarily by controlling the level of *de nove* synthesis. The level of cholesterol synthesis is regulated in part by the dietary intake of cholesterol (2). The type and amount of carbohydrate feeding is associated with changes in the levels, composition and metabolism of serum lipoproteins(3), triglycerides (4), and other lipids (5).

Chicory (*Chicorium intybus*) belong to the family Asteraces (Compositae), it is used medicinally as far back as tenth and eleventh centuries. The chicory is reported to contain resin, essential oil, pectose, inulin, levulin, wax, enzyme, fatty acids like mellisic, sitoserol stigmasterol, saponine (6). Today it is employed for dyspepsia, indigestion, bile stimulation and as general stimulant, especially for kidney and liver disorders (7). Chicory may be hepato-chemoprotective. It may increase antioxidant liver enzymes, reduce lipid peroxidation and normalize blood lipid levels (8).

MATERIALS AND METHODS

Preparation of Chicory Alcoholic Extract:

Fifty gm of dried leaves obtained from local market in Basrah were refluxed with (500 ml) of 70% ethanol, and then concentrated by rotary evaporator (PUCHI Rotavpor RE, Switzerland) at 50°C.

Experimental animals:

Twenty four adult male rabbits were brought from local market/ Basrah. The animals were acclimatized for four weeks, and then divided into 4 groups equal number (6 rabbit/group). The animals maintained on concentrated food and alfalfa was *ad libitum*.

Experiment design: 3 groups of animals in this experiment were orally administrated (0.5 gm/kg BW.) with cholesterol dissolved in 3ml soy bean oil for two weeks, after that they were divided into four groups as follows:

The first group(hypercholesterolemic group) : which received 3ml /kg B.W. of normal saline (0.9% NaCl). The second group (chicory extract treated group): was administrated (0.25gm/ kg B.W./day) of ethanolic extract of chicory leaves dissolved in 3ml/kg BW of normal saline for one month. The third group (Atorvastatin treated group): received (0.4 mg/kg B.W./day) of atrovastatin (medicinal therapy for atherosclerosis case) dissolved in 3ml of normal saline for one month. Fourth group (normal control group): received 3ml/kg B.W. of normal saline for one month.

For histological study, the rabbits were killed by cervical dislocation. Immediately after death, the organs (Liver and Kidney) were removed. These organs were examined grossly for the presence of any changes then fixed in 10 % formalin until the preparation for histological section.

The preparation of slides for these organs and stained by Routine stain (Hematoxyline and Eosin stains) (9).

Statistical Analysis:

The data was analyzed on the basis of one way ANOVA test by using SPSS version 9.0. All data are expressed as mean ± SD. (using a significant level of $P<0.05$). The differences between specific groups were determined by least significant difference (LSD).

RESULTS

1-Determination of LD_{50} of alcoholic extract of *Chicorium intybus* Leaves:

The doses of alcoholic extract and mortality percentage in albino mice are presented in Table (1). The results in this table showed no mortality rate from ascending doses until 3 gm/kg B.W.

Table (1): Number of dead mice after (24 hrs) post oral administration of different doses of alcoholic extract of *Chicorium intybus* on albino mice.

Groups	Dose gm/Kg B. W.	No. of mice used	No. of mice dead	Mortality
1	0.9%NaCl	6	0	0%
2	0.25	6	0	0%
3	0.5	6	0	0%
4	1.0	6	0	0%
5	1.5	6	0	0%
6	2.00	6	0	0%
7	2.5	6	0	0%
8	3.00	6	0	0%

2- Effect of alcoholic extract of *Chicorium intybus* leaves on organs weight (Kidneys and Liver):

Highly significant increased ($P < 0.05$) in weight of kidneys and liver in hypercholesterolemic group when compared with treated groups these results shown in table (2).

Table (2): Effect of *Chicorium intybus* **extract of leaves on Kidneys and Liver weights in hypercholesterolemic rabbits:**

Groups	Liver	Kidney	
		right	left
Hypercholesterolemia rabbits	**98.78* ± 1.98**	**9.42* ± 0.57**	**9.70* ± 0.25**
chicory extract treated rabbits	**83.31 ± 0.85**	**6.69 ± 0.44**	**6.98 0.21**
Atorvastatin treated rabbits	**86.55 ± 1.15**	**6.70 0.11**	**6.65 0.15**
Normal control rabbits	**85.57 ± 1.43**	**7.00 ± 0.34**	**6.95 ± 0.29**

*** Denote the highly significant difference between groups (P<0.05)**

3- Histopathological Effect alcoholic extract of *Chicorium intybus* **leaves of Kidney and Liver tissues:**

Kidney of hypercholesterolemic group showed fatty infiltration and occasional areas with minimal renal tubules (medulla tubules) dilation, fatty and hydropic degeneration in the cells of renal tubules with hemorrhage (Fig.2).

In group treated with chicory alcoholic extract, there were peripheral areas of vacuolated renal tubules, several prominent areas of dilated medulla tubules and dilated proximal convoluted tubules (Fig.3). In atorvastatin treated group, occasional areas of minimal dilated renal tubules and hyperemia (medulla tubules) showed in fig.4.

The null lesions can be seen in the section of liver in normal control group (fig.5). There were cloudy swelling and hyperatrophic hepatocytes with hardly apparent sinusoid appeared in this section of liver in hypercholestermic rabbits (Fig.6), while in liver of chicory treated group, showed normal liver cord, sinusoid easily can be seen and with null lesions (fig.7). In atorvastatin group showed congestion of central vein with inflammatory cells (Fig.8)

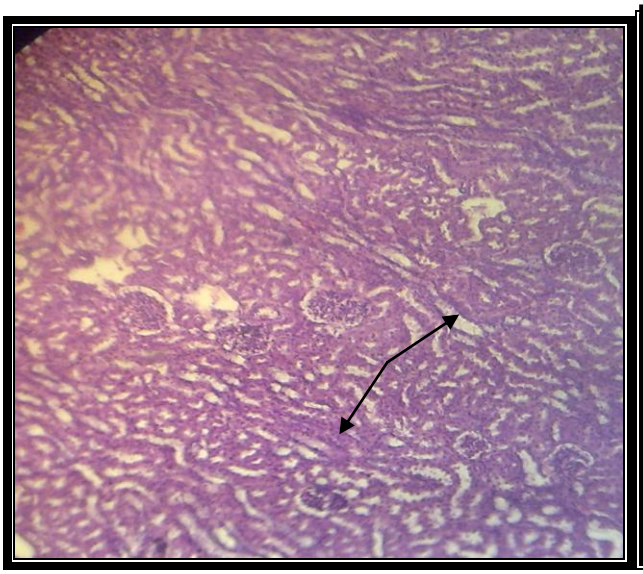

Fig. (1): Kidney in normal control rabbit (Normal renal tubules)(H&E stain) 400X

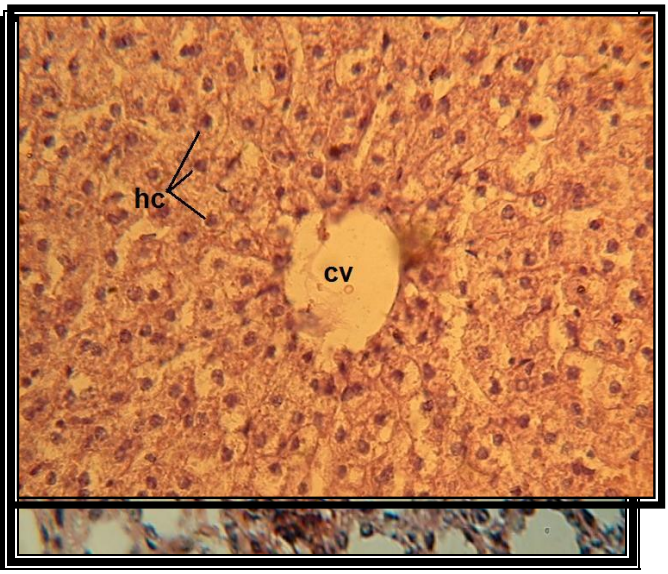

Fig.(2): Kidney in Hypercholestermic rabbit (A-fatty infiltration , B-dilated renal tubules) (H&E stain) (400X).

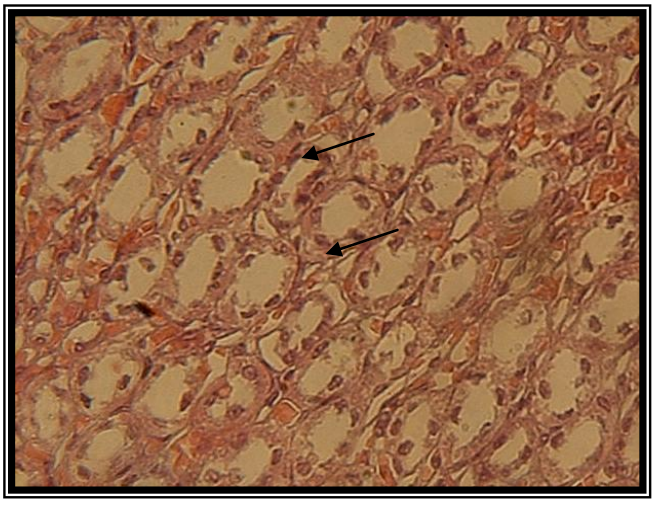

Fig.(3): Kidney in hypercholesterolemic rabbit treated with extract (dilated renal tubules) (H&E stain) 400X

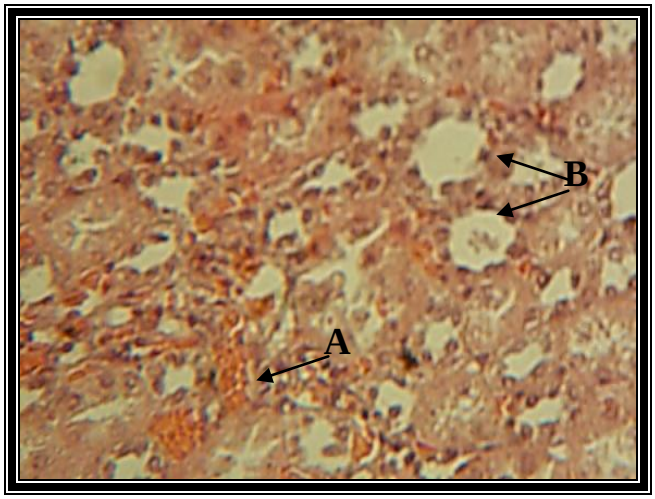

Fig. (4): Kidney in hypercholesterolemic rabbit treated with drug (A-hyperemia. B-minmial dilated tubules (H&E stain) 400X.

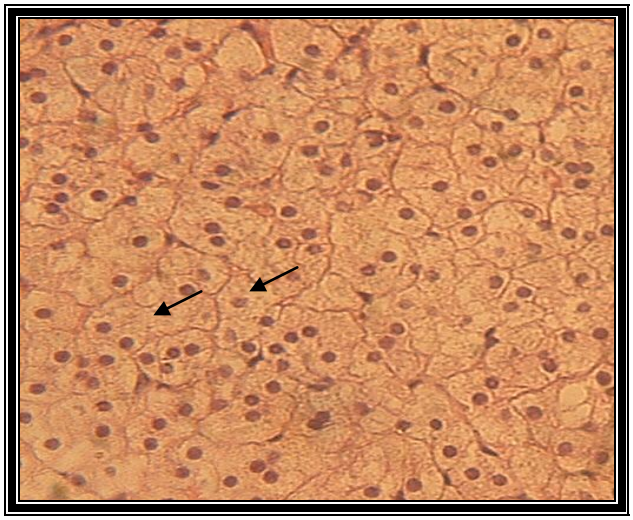

Fig. (5): Liver in normal control rabbit (normal shape and size of hepatic cells =hc ,cv=centeral vein) (H&E stain) 400X

Fig.(6): Liver in Hypercholestermic rabbits (hyperatrophic hepatocytes with hardly apparent sinusoid)(H&E stain) 400X

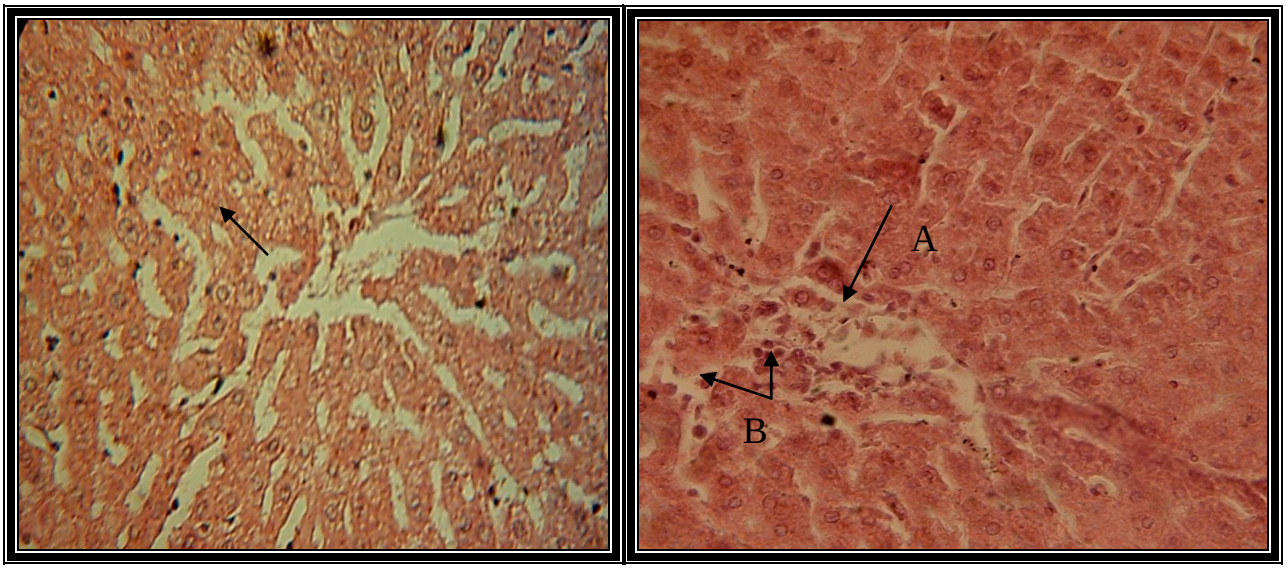

Fig. (7): Liver in hypercholesterolemic rabbit treated with extract (within normal limits) (H&E stain) 400X.

Fig. (8): Liver in hypercholesterolemic rabbit treated with atorvastatin (A=congestion of central vein with inflammatory cells=B) (H&E stain) 400X.

DISCUSSION

In hypercholesterolemia rabbits in this study, the weight of liver and kidney increased significantly when compared with normal control and treated groups these results may be due to accumulation of fat in the cells of the liver and kidney due to high level of cholesterol and other fats in the blood, its uptake by HDL and return to the liver to metabolism and excretion with urine and fecal. These results were an agreement with Jennings *et al.* (10) whose observed that 1% cholesterol feeding increased total liver lipids almost three folds and liver cholesterol concentration almost 10-folds which significantly increase in liver weight.

While the weights of livers and kidneys in the groups treated with chicory and atorvastatin, no significant different when compared with normal control ,these results may be due to the plant (chicory) have soluble fiber, its have a hypolipidemic effect and not hydrolyzed by digestive enzyme.. These results agreement with Jennings *et al.* (10) who reported the addition of 1% or 5% of chicory water extract to the diet of hypercholesterolemic rats decreased the weight of liver and no significant different in weight of liver in treated group when compared with normal control group. Rumessen *et al.* (11) and Bosello *et al.* (12) revealed that the most soluble fibers decreased the absorption of lipids in the proximal intestine and increased the absorption in the mid-intestine, which might alter the size and composition of lipoproteins secreted by the intestine.

Histological examination of liver tissues section of hypercholesterolemic rabbits revealed hyperatrophic hepatocytes, hardly apparent sinusoids with fatty infiltration these may be due to increase the level of cholesterol and other lipids in the blood and therefore the liver uptake these increase in levels to oxidation and metabolism to decrease these levels and increase excreted with urine and feces. The liver participates in uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides (13). Lipid structure, composition and configuration in addition to excessive fat and cholesterol consumption affect the lipid profile in plasma (14), as well as fat tissue deposition (15) and gene expression of lipoproteins and their receptors (16). In this context, the intake of diets containing a high proportion of saturated fatty acids, mainly myristic, lauric and palmitic acids has been associated with hypercholesterolemia (17). These alterations appear to be due to impaired catabolism rather than increased synthesis (18). Cholesterol accumulates in cells and extracellular spaces of the wall endothelial lining in arteries (19 & 20). In the kidney treated with *Chicory intybus* showed dilated tubules because this plant have diuretic actions as described by Baldwin (21) *Chicorium intybus* have diuretic, tonic and slightly laxative. It is a general

stimulant to system, but specially to the urinary organs, and is chiefly used in kidney and liver disorder.

These results disagreement with Al-Asside (22) which used galactomannan in treated of hypercholesterolemic rabbits. The liver showed irregular lobular and disappear of lobulation and hepatocytes missing the cuboidal shapes when compared with control group. While in the kidney of treated group the cells appears in small size and capillary vessels shrinkage and less in number, degeneration and irregular cortex.

Conclusion, the results of this study suggest the importance of the oral administration of cholesterol on the weight and histological changes of the liver and kidney, as well as, effects of oral administration of alcoholic extract of *Chicorium intybus* on weight and histopathological changes in liver and kidney as protective and treatment actions because this plant have not toxicity effect.

تأثير نبات الطرخشقون على التغيرات النسيجية لكبد وكلى أرانب فرط الكوليسترول

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الخلاصة

في هذه الدراسة صممت التجربة لملاحظة تأثيرا لتجريع الفموي اليومي لـ(0.5غم/ كغم من وزن الجسم) للمستخلص الكحولي لنبات الطرخشقون (*Chicory intybus*) لشهر واحد على وزن ونسيج كبد وكلى للارانب المستحدث فيها فرط الكوليسترول (بواسطة التجريع الفموي لـ 0.5 غم/كغم من الكوليسترول مذاب في 3 مل من زيت فول الصويا) ومقارنة هذا التأثير مع الدواء الطبي الاتروفاستاتين المستخدم لعلاج تصلب الشرايين.

اظهرت النتائج زيادة معنوية ($P<0.5$) في وزن كبد وكلى الارانب المستحدث فيها فرط الكوليسترول مقارنةً بالمجاميع الأخرى. بينما اظهرت النتائج المرضية النسيجية تغيرات نسيجية في كبد وكلى أرانب فرط الكوليسترول وكذلك المعالجة بمستخلص الطرخشقون مقارنةً بالمجاميع الأخرى.

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