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Utilization of Marrubium Vulgare Extract as a Therapeutic to Hepatic Damage Induced by Carbon Tetrachloride in Rats

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ABSTRACT

Marrubium vulgare is used as popular medicine in many countries. The aim of this study was to evaluate the essential oils as natural antioxidants. Moreover, lipid profile and antihepatotoxic properties of different concentrations of the extract against carbon tetrachloride (CCl4) which cause liver damage in rats are examined. Different concentrations of the extract were taken orally at levels of 100, 200, 300 and 400 mg /kg-1 body weight from Marrubium vulgare leaves separately in normal saline 5 ml/ kg-1 body weight, four times per week for four weeks along with CCl4 started at the fourth week of induction of hepatotoxicity. The antihepatotoxic activity was assessed by measuring aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) as well as histopathological examination. Different concentrations of extract showed significant antihepatotoxic effects by reducing the levels of AST and ALT significantly; meanwhile, ALP level was non-significantly decreased. Regarding the antioxidant activity, the extract exhibited significant effects by increasing the decreased glutathione GSH, superoxide dismutase (SOD) and the lowered production of MDA. Furthermore, different concentrations of the extract of Marrubium vulgare protect the rats' livers against CCl4- induced hepatotoxicity. This effect may be attributed to the antioxidant activities of these extracts and also, these extracts had contained flavonoid compounds and phenolic acids as natural antioxidants.
In conclusion, the results obviously supported the possible antihepatic effects of Marrubium vulgare extracts against CCl4-induced hepatic damage in rats. Antihepatic damage effect might be due to the fact that the essential oil has natural antioxidants.

Keywords: *Marrubium vulgare, phytochemically, biochemical parameters, antioxidant enzyme, histopathological*

INTRODUCTION

The plant kingdom is a source of theraby for various pathologies. Numerous research groups to date direct their efforts to produce natural medicinal products and plant extracts to prevent or treat different diseases [1]. As reported by the World Health Organization (WHO), approximately 80% of people in many countries use traditional medicine for their health care [2].

Marrubium vulgare L. (M. vulgare, Lamiaceae), commonly known as ‘White Horehound’, is a robust perennial herb, with dense cottony stems and white flowers. Several studies have reported that treatment with low doses of M. vulgare, has many beneficial effects such as : anti-bacterial effects [3] and antioxidant actions [4,5]. It was also

proved that this plant lowers blood pressure in rats when used in traditional Moroccan medicines (hypotensive activity) [6]. Other effects are hypoglycemic and hypolipidemic effects that have been proved by the administration of Marrubium vulgare in diabetic rats with 500 mg/kg/ d [7]. Scientific research about M. vulgare has shown that the treatment with different doses, in adult rats, induces a slight increase in body weight over time [8]. Also, the treatment improves liver's functions in rats [9,10].

Hepatotoxicity is one of the dangerous diseases which depends on the fast food as well as exposure to environmental contaminations and heavy intake of medications. Various xenobiotics are known to cause liver damage, one among them is carbon tetrachloride (CCl4) that may cause peroxidation of lipids [11,12].

Herbal medicines have attracted the interest of more scientific researchers as alternative natural therapies. There has been upward trend in the use of phytomedicines in Europe and the USA [13,14]. Marrubium vulgare is a medicinal plant used as a popoular medicine in many countries. Marrubium vulgare is used for the treatment of different diseases, including inflammatory, gastroenterical and respiratory disorders [15].

Phytochemically, Marrubium vulgare is described by the presence of different compounds, and used as natural antioxidants such as polyphenols, tannins, flavonoids, diterpenes and saponins [16,17]. The Marrubium vulgare's extract has occured to have damaging effects on the rats' livers. The antioxidant properties of Marrubium vulgare's extract have been confirmed by its ability to inhibit lipid peroxidation [18].

The antioxidants have a definte role in the injury healing process. Many compounds from natural antioxidants have properties to increase the tissues' adhesion during the injury healing, stimulating re-epithelization and ripening of the extracellular matrices [19]. Thus, the antioxidant and antimicrobial properties may represent therapeutic tools to accelerate injury healing process [20]. Several natural antioxidants and antimicrobials showed healing effects, and are able to promote one or more mechanisms of the reparative process [21]. Therefore, numerous herbs and plant extracts have been employed to promote injury healing to succeed, and in some cases, led to the reduction of diseases [22].

Marrubium vulgare L. (White horehound), a perennial herb is used as a traditional medicine to treat bronchitis, coughs and colds. The leaves and flowering stems are used as diuretic, antispasmodic, antiseptic, antidiabetic [23]. Essential oils are volatile, aromatic oily liquids; natural products with terpene structure described by an intense smell, and are constituted of aromatic plants as secondary metabolites. Essential oils are important tools, and have a significant function in the protection of the plants as antibacterials, antivirals, antifungals, insecticides [24]. Diversity in the chemical contents of essential oils have been referred to many factors, such as environment, biotic stress, genetic heritance and the phenological stages of the plants [25].

This work aimed to investigate the potential antihepatotoxic effects of the leaves' extract of Marrubium vulgare on carbon tetrachloride-induced hepatotoxicity and liver damage in rats. Some biochemical parameters of liver functions were measured in the blood serum. In addition, the antioxidant enzymes' (SOD, GSH, and MIDA) activities were determined in liver and also, the histopathology was studied.

MATERIALS AND METHODS

MATERIALS:

Marrubium vulgare L**.** leaves were collected from Taif governorate, Saudi Arabia. The sample was grounded by household grinding machine.

Liquid paraffin and carbon tetrachloride were purchased from Merck (Darmstadt, Germany. Kits of liver functions and other parameters were obtained from Bicon Diagnosemittel GmbH and Co. KG Hecke 8 made in Germany.

METHODS:

Distillation of essential oil:

Marrubium vulgare leaves were dried in the shade in natural air far from moisture and all pollutants for a fortnight in the room temperature. The dried leaves were ground prior to the operation and then 100 g of ground Marrubium vulgare were submitted to water distillation for 4 hrs using a Clevenger apparatus. The distilled essential oils were dried over anhydrous sodium sulfate, filtered and the obtained essential oil was stored in sealed glass vial at 4-5°C prior to analysis of GC/MS.

Gas chromatography and mass spectrometry (GC/MS):

Gas chromatography and mass spectrometry analyses were performed on Perkin-Elmer mass spectrometer using BPX5 column (30 m×0.25 mm×0.25µm phase thickness). An electron ionization system with ionization energy of 70 eV was used for GC/MS detection. The carrier gas was helium with a flow rate of 1.3 ml/min. Injector, and MS

transfer line temperatures were set at 230 °C and 250 °C, respectively. The oven temperature was the same as with GC analysis. Diluted samples (1/10 in acetone, v/v) of 1.0\μL were injected in the split/split less (5:1 split) mode according to van Den Dool and Kratz[26].

Preparation of the Marrubium vulgare extract:

100g of Marrubium vulgare leaves was added to one liter ethanol 80% at room temperature, and the prepared infusion was filtered in order to carry out biological tests on the experimental animals.

Biological investigation:

Male Wister rats, weighing 200-250g were used in this study in accordance with the guidelines of the Biochemical and Research Ethical Committee at King Abdulaziz University, Jeddah, Saudi Arabia. Animals were purchased from the animal house of King Fahd Medical Research Center, King Abdulaziz University. Animals were housed in a well-ventilated, temperature-controlled room at 22 ± 3°C with 12 h light dark cycle and fed on basal diet for eight days. The basal diet consisted of corn starch 70%, casein 10% corn oil 10%, salt mixture 4%, vitamin mixture 1% and cellulose 5% according to AOAC [27]. After feeding on basal diet for eight days, the rats were divided into six groups. The first group was considered as the normal control, fed on basal diet and received paraffin oil 3ml/kg^{-1} body weight, subcutaneous (Sc) two times per week, for eight weeks and normal saline 5 ml/ kg^{-1} body weight, orally (Po) four times per week, for eight weeks**.** The second group, CCl_4, was considered as the positive control, fed on basal diet, and received carbon tetrachloride 40% in paraffin oil (3ml/kg^{-1} body weight, Sc) two times per week, for eight weeks, and normal saline (5 ml/ kg^{-1} body weight, Po) four times per week for eight weeks**.** The third, fourth, fifth and sixth groups were fed on basal diet and received carbon tetrachloride 40% in paraffin oil (3ml/kg^{-1} body weight, Sc), two times per week for eight weeks and 100, 200, 300 and 400 mg /kg^{-1}, Po body weight from Marrubium vulgare leaves' extract separately in normal saline 5 ml/ kg^{-1} Po body weight, four times per week for eight week**s.** Each rat was weighted every two days, and the food consumption was calculated.

At the end of the experiment, twenty four hours after dosing of vehicle, CCl_4 or plant extracts, blood samples were collected from the orbital sinus. The serum was separated by centrifugation at 3500 rpm and kept under -7°C for determination of liver enzymes. Animals were anesthetized with diethyl ether and sacrificed by cervical dislocation for separation of the liver. The livers were dissected out, divided into two parts. One part was kept in liquid nitrogen for determination of antioxidant status, and the other part was immediately fixed in formaldehyde solution 10% and was used for histopathological examination.

Serum glucose, total lipids, total cholesterol and triglycerides were determined according to Tietz [28], knight et al. [29], Allain et al. [30] and Fossati and Prencipe [31], respectively. High and low density lipoprotein- cholesterol in serum was determined according to Burstein [32] and Fruchart [33].

Serum Aspartate aminotransferease (AST) and alanine aminotransferease (ALT) were determined colorimetrically following Schumann and Klauke [34], while Serum alkaline phosphatase (ALP) was detected according to Belifield and Goldberg [35].

Hepatic superoxide dismutase (SOD) activity was determined according to the method described by Sun and Zigman [36]. Reduced glutathione (GSH) level was measured according to the chemical method described by Moron et al. [37], while lipid peroxidation products were determined by measuring malondialdehyde (MDA) content in tissue homogenates, according to the method of Uchiyama and Mihara[38].

Histopathological examination

Liver pieces preserved in 10% formaldehyde solution were used for histopathological study. The liver tissues were placed in plastic cassettes and immersed in neutral buffered formalin for 24 h. The fixed tissues were processed routinely, embedded in paraffin, cut into 4 mm-thick sections and stained with hematoxylin and eosin (H&E). The extent of carbon tetrachloride-induced hepatic damage was evaluated by assessing the morphological changes in the liver sections

Statistical analysis:

The obtained data were exposed to analysis of variance. Duncan's multiple range tests at ($P \leq 0.05$) level was used to compare the means. The analysis was carried out using the PRO ANOVA procedure of Statistical Analysis System [39].

RESULTS AND DISCUSSION

Identification and quantification of the oil components of Marrubium vulgare:

Table (1) showed that the constituents of the essential oils of Marrubium vulgare had contained exactly 18 compounds, mostly the aromatic ones were identified, representing 97.94 % in the oil of Marrubium vulgare. The

major constituents were α-Pinene (19.45%), Camphene (13.50%), β- Bisabolene (13.10%), β-Pinene (11.01%), β-myrcene (5.37%), linalool (4.76%), α-Terpinene (4.62%) and α-limonene (3.88%). Other important compounds were Trans- sabinene hydrate (3.85), Trans-caryophyllene (3.79%), Caryophyllene (3.55), Bicyclogermacrene (2.55%), Germacrene (2.35%), Terpinen-4-ol (2.18%), Sabinene (2.09%) and β-Farnesene (1.89%), respectively. These results are in agreement with [40**]** who reported that the α-Pinene and β-Pinene, the main constituents of the essential oil of M. vulgare L., have pharmaceutical and medicinal properties**.** Total synthesis of α-Pinene exhibiting the antimicrobial activity was achieved [41]. A research revealed that the main constituents of α-Pinene and β-Pinene of essential oils from Ferula microcolea exhibited the antioxidant activity [42]. An essential oil exhibited the antibacterial activities against food spoilage pathogens including the α-Pinene and β-Pinene as major compounds. Xu et al. [43] presented the double effects of α-pinene on turpentine beetle, Dendroctonus valens: α-pinene inhibited the feeding activities of bark beetle, and the bark beetle exploited to produce pheromones.

Table 1. Chemical composition of the Marrubium vulgare L. essential oil analyzed by gas chromatography-mass spectrometry

Major compounds	Oil composition %	Other compound	Oil composition %
α-Pinene	19.45	Trans- sabinene hydrate	3.85
Camphene	13.50	Trans-caryophyllene	3.79
β- Bisabolene	13.10	Caryophyllene	3.55
β-Pinene	11.01	Bicyclogermacrene	2.55
β- myrcene	5.37	Germacrene	2.35
linalool	4.76	Terpinen-4-ol	2.18
α-Terpinene	4.62	Sabinene	2.09
α-limonene	3.88	β-Farnesene	1.89

Biological experiment:

The effects of Marrubium vulgare extract on initial, final body weight, and feed efficiency ratio in rats:

The results from Table (2) indicated the effect of Marrubium vulgare extract on initial, final body weight and feed efficiency ratio in rats. From the resultants, it could be observed that the normal negative control group fed on basal diet had the highest final body weight (194.5 g, increased in gain body weight 27.0 g) and feed efficiency ratio (5.84%) at the end of the experimental period (eight weeks). While, the positive control group fed on basal diet, significantly increased the final body weight (177.0 g increased 6.7g about initial body weight) and feed efficiency ratio was 1.92%. Meanwhile, in the rat groups orally fed on Marrubium vulgare extract two times per week at levels of 400 mg/ kg, no significant changes in the final body weight and total food intake between them were observed. It means that the rats fed on different parts of Marrubium vulgare extract at ratio of 400 mg/kg were not affected on food intake and body weight may be because the Marrubium vulgare extract had contained rich amounts of essential oils as natural antioxidants.

Table 2. Initial, final body weight and feed efficiency ratio in rats fed orally of Marrubium vulgare extract:

Groups	Initial body weight	Final body weight	Gain body weight	Total food intake	Feed efficiency ratio
Control negative	167.5 ±7.6 ^{b}	194.5 ±6.6 ^{a}	27.0	462.7 ±26.0 ^{a}	5.84 ±0.2 ^{a}
Control CCl_4	170.3 ±8.0 ^{ab}	177.0 ±7.0 ^{b}	6.7	348.6 ±28.3 ^{b}	1.92 ±0.6 ^{d}
100 mg M. vulgare	181.7 ±8.6 ^{a}	191.8 ±7.9 ^{a}	10.1	450.4 ±31.3 ^{a}	2.24 ±0.2 ^{c}
200 mg M. vulgare	182.3 ±9.1 ^{a}	197.8 ±8.2 ^{a}	15.5	445.6 ±31.1 ^{a}	3.48 ±4.7 ^{b}
300 mg M. vulgare	175.8 ±7.8 ^{b}	195.8 ±8.1 ^{a}	20.0	435.0 31.2 ^{a}	4.60 ±0.2 ^{d}
400 mg M. vulgare	173.2 ±7.6 ^{b}	198.3 ±9.5^{a}	25.1	430.4 ±15.4 ^{a}	5.83 ±0.4 ^{a}

The effects of Marrubium vulgare xtract on glucose level and lipid parameter in rats:

Table (3) showed the effect of Marrubium vulgare extract at 100, 200, 300 and 400 mg/kg b.wt ratios on glucose level, total lipids, triglycerides and total cholesterol fractions in rats during eight weeks. The results showed the significant decrease of serum total lipid, when the rats fed orally on 400 mg/kg b.wt to 350.5 mg/dl than the control CCl_4 which was 605 mg/dl after eight weeks. On the same parallel, when rats orally fed on diets containing 400 mg/kg from Marrubium vulgare extract, the serum total cholesterol and triglycerides were significantly decreased to 130.0 and 136.8 mg/dl, respectively comparing to the control CCl_4 in which they were 238.8 and 235.8 mg/dl. Results showed that the Marrubium vulgare extract was more effective on serum lipid patterns may be due to the fact that Marrubium vulgare extract was rich in polyphenols and flavonoids contents. Also, the effectiveness of Marrubium vulgare extract was clear in triglycerides more than in the total cholesterol.

The results of HDL- cholesterol and LDL- cholesterol in hyperlipidemic and diabetic rats orally fed with Marrubium vulgare extract at 100, 200, 300 and 400 mg/kg b.wt ratios are reported in the same table. From the resultants, it could be observed that the HDL- cholesterol was significantly increased in rats orally fed on Marrubium vulgare extract at 300 and 400 mg/dl ratios. However, in the rats orally fed on Marrubium vulgare extract at 300 and 400 mg/kg b. wt ratios, LDL- cholesterol from 168.3 mg/dl in control positive was significantly lowered to 67.3 and 62.7 mg/dl, respectively. Marrubium vulgare has been used in the traditional medicine [44]. Due to that reason, in rats injected with CCl_4, there were improvements in the total lipid, triglyceride and total cholesterol when the rats were fed orally by Marrubium vulgare extract at 100, 200, 300 and 400 mg/kg b.wt.

Liver has an important role in the metabolism of lipids, carbohydrates, and proteins. Injection of CCl4 caused a significant increase in different lipid parametrs, and HDL showed a significant decrease. The protein synthesis decreased, and the metabolism of phospholipids which involved abnormal lipoprotein levels stopped. Meanwhile, the increase in the cholesterol levels might increase the esterification of fatty acids, the inhibition of fatty acid β-oxidation, and decrease the excretion of cellular lipids [45]. Carbon titra chloride was stimulated to transfer acetate into liver cells, and lead to an increase in cholesterol synthesis. It also increases the synthesis of fatty acids and triglyceride of acetate and increased lipid esterification [46]. The accumulation of triglyceride in liver might occur due to the inhibition of lipase enzyme activity [47]. Marrubium vulgare extracts have antihyperglycemic effects, and are attributed to have an ability to get back the function of pancreatic tissues by increasing insulin inhibit in the intestinal absorption of glucose or to the simplification of metabolites in insulin dependent processes [48,49].

From the results, it could be noticed that in the rats fed orally on 300 and 400 mg/kg b. wt ratios of Marrubium vulgare extract, the serum glucose blood level was significantly decreased to145.2 and 140.3 mg/dl, followed by the rats orally fed on 200 and 100 mg/kg b. wt ratios of Marrubium vulgare extract (171.5 and 222.3 mg/dl). It is clear that feeding orally by Marrubium vulgare extract at 300 and 400 mg/kg b. wt ratios reduce the serum glucose level. This decrease may be caused by the high amount of essential oils as natural antioxidants, from Marrubium vulgare extract has the capacity to keep the blood sugar levels safe and also, lower insulin that can improve serious problems of diabetics.

From the obvious results, it could be noticed that the Marrubium vulgare extract is rich in the phenolic acids, flavonoids compounds and essential oils as natural antioxidants. These contents may be, could prevent and lower the diabetic levels and lipid parameters.

Table 3. The effect of Marrubium vulgare extract on glucose level and lipid parameters in rats (mg/dl)

Groups	Total lipids	Triglycerides	Total cholesterol	HDL cholesterol	LDL cholesterol	Total glucose
Control negative	326.3 ±17.3 [d]	103.8 ±8.6 [d]	120.8 ±8.3 [d]	49.5 ±5.8 [a]	60.5 ±3.0 [d]	128.0 ±8.0 [d]
Control CCl_4	605.0 ±25.7 [a]	235.8 ±15.4 [a]	238.8 ±14.3 [a]	23.0 ±1.9 [b]	168.3 ±8.5 [a]	250.0 ±16.5 [a]
100 mg M. vulgare	566.0 ±23.9 [b]	212.0 ±8.3 [b]	172.5 ±8.1 [b]	27.3 ±1.9 [b]	102.9 ±5.3 [b]	222.3 ±12.2 [b]
200mg M. vulgare	471.3 ±22.5 [c]	194.8 ±8.9 [c]	163.0 ±8.2 [c]	37.5 ±1.8 [c]	86.6 ±5.4 [c]	171.5 ±11.7 [c]
300mg M. vulgare	390.2 ±19.2 [d]	145.3 ±7.5[d]	135.2 ±5.9[d]	40.2 ±2.1[a]	67.3 ±4.3[d]	145.2 ±8.3[d]
400mg M. vulgare	350.5 ±21.9 [d]	136.8 ±8.6 [d]	130.0 ±8.0 [d]	42.0 ±2.6 [a]	62.7 ±4.3 [d]	140.3 ±12.3 [d]

The effect of Marrubium vulgare extract on serum AST, ALT and ALP parameters:

Table (4) shows the effects of Marrubium vulgare on liver function indices of rats. Feeding with the M. vulgare at different concentrations of extract had significantly increased the activity of alanine transaminase (ALT) (23.50, 25.00, 27.98 and 29.33 IU/L, respectively) when the concentration M. vulgare gradually increases. The activity of aspartate transaminase (AST) was significantly reduced in rats orally fed at concentrate of 100mg M. vulgare (34.72 IU/L), and increased significantly to the group orally fed at concentrate of 400mg M. vulgare (48.21 IU/L), and nearly to the negative control (51.04 IU/L). Rats orally fed on different concentrations of M. vulgare's extract had a significant decrease in ALP (198.51, 185.70, 180.32 and 175.46 IU/L, respectively) compared to the control CCl_4 rats (225.00 IU/L). Changes in the activities of these enzymes indicated injury to organelles such as mitochondria leading to the release of soluble enzymes like AST [50].

Properties of Marrubium vulgare extracts against carbontetra chlorid ($CC1_4$)-induced liver damage in rats showed significant antihepatotoxic effect via significantly reducing AST, ALT, and LDH 9. In other studies, the aqueous extract of the whole plant was studied for antihepatotoxic activity against carbontetra chlorid ($CC1_4$) -induced liver damage in male rats. The extract was taken orally by rats at 500 mg/kg body weight dose for 7days, and was compared with the standard drug Silymarin at 10 mg/kg body weight dose. The aqueous extract had significant antihepatotoxic activity and reduced the elevated levels of serum enzymes, and increased the total protein [51,52].

Table 4. The effects of Marrubium vulgare extract on carbon tetrachloride CCl_4-treated induced alterations in serum hepatic enzymes including aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP)

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control negative	51.04±5.54^{b}	30.00±3.73^{b}	172.05± 2.54^{d}
Control CCl_4	72.33±3.88^{a}	40.04± 5.44^{a}	225.00±5.50^{a}
100mg M. vulgare	34.72±4.22^{c}	23.50±4.52^{c}	198.51±6.60^{b}
200mg M. vulgare	35.54±4.10^{c}	25.00±5.55^{c}	185.70±8.44^{c}
300mg M. vulgare	41.88±6.36^{b}	27.98±8.43^{b}	180.32±4.62^{c}
400mg M. vulgare	48.21±6.35^{b}	29.33±7.54^{b}	175.46±6.31^{d}

Data presented as mean (n=6 rats) ± standard deviation, values with different superscripts within the columns are significantly different at $P<0.05$, while those which are partially similar are not significant.

The effect of Marrubium vulgare's extract on liver GSH, SOD and MDA parameters:

Results presented in table (5) showed that the injection of CCl4 induced a significant increase in the enzyme level of GSH activities when increasing the concentration of M. vulgare extract was 275.67, 282.52, 295.28 and 310.55 µ/g, respectively, compared with CCl_4 content (188.73 µ/g). On other hand, it increased the MDA level in liver tissues (8.54, 8.99, 9.32 and 10.54nmol/g, respectively) compared to the normal control values (8.02nmol/g). However, CCl4 did not affect the activity of SOD in liver tissues. These results concurred with Akther et al.[18] and Zarei and Shivanandappa[53] who suggested that in the rats pre-treated with M. vulgare, the perturbation was significantly attenuated, which was indicated by reduction in lipid peroxidation level and enhancement of antioxidant states (SOD, GPx and CAT) when compared with those of normal rats.

Superoxide dismutase (SOD), catalase (CAT) and the enzyme of the glutathione redo cycle i.e. glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Rd) are the primary intracellular antioxidants, and are considered to be preventive or primary antioxidants as they prevent free radical chain reaction by decreasing the available concentrations of free radical to initiate the process [54].

Table 5. The effect of Marrubium vulgare extract on liver GSH, SOD and MDA parameters

Groups	GSH µ/g	SOD µ/g	MDA nmol/g
Control negative	330.54 ± 18.54^{ab}	600.52 ± 32.43^{c}	8.02 ± 1.30^{c}
Control CCl_4	188.73 ± 12.74^{c}	470.63 ± 27.42^{d}	14.20 ± 1.98^{a}
100mg M. vulgare	275.67 ± 18.47^{b}	658.44 ± 33.65^{b}	8.54 ±1.21^{c}
200mg M. vulgare	282.52 ± 16.40^{b}	673.40 ± 35.55^{ab}	8.99 ± 1.54^{c}
300mg M. vulgare	295.28±15.34 ^{ab}	698.27 ±31.29^{a}	9.32 ± 1.89^{b}
400mg M. vulgare	310.55 ± 20.22^{a}	711.00 ± 38.90^{a}	10.54 ± 1.62^{b}

Data are presented as mean (n=6 rats) ± standard deviation, values with different superscripts within the column are significantly different at P<0.05, while those which are partially similar are not significant.

Histopathological experiment:

The effect of Marrubium vulgare extract on histopathological disorders:

One of the four primary categories that have proved useful for evaluation of the experimental hepatic injury in laboratory animals is the histological analysis of the liver injury, thus the biochemical findings in this study emphasized on the pathological changes of the liver.

The light microscopic examination of normal control group's liver sections (Fig. 1) have shown the normal structure of the liver. The unit of liver tissue is the classic hepatic lobule (H. I.), which is surrounded by the connective tissues. In the center of hepatic lobule, there is a central vein (C. V.). The plates are radiating from the central vein towards the periphery of the hepatic lobule.

It is clear that from figure (2) which represents the light microscopic examination of the injured group which received CCl_4 two times per week, for eight weeks, there were extensive severe vascular degenerative changes in the hepatocytes. Few hepatocytes show granular degenerative changes. Another section of the hepatocytes of the same group in figure (3) shows congestion of portal blood vessels accompanied by the severe degenerative changes in hepatocytes, is vacuolated and enlarged, and cytoplasm appears faintly stained and the nuclei are shrunken. These results may be caused by the CCl_4 which increased the production of free radicals which can directly bind to the hepatocellular membrane protein and lipids leading to alkylation reactions and possible enzyme inactivation [55].

Figures (4 and 5) show the light microscopic examination of liver sections which received carbon tetrachloride 40% in paraffin oil (3ml/kg^{-1} body weight, Sc), two times per week for eight weeks and 100 and 200 mg /kg^{-1} of Po body weight from Marrubium vulgare leaves' extract separately in normal saline 5 ml/ kg^{-1} Po body weight, four times per week for eight weeks, that some hepatocytes show necrosis, others were suffering from granular derivative changes.

Figures (6 and 7) represented the light microscopic examination of liver sections which received carbon tetrachloride 40% in paraffin oil (3ml/kg^{-1} body weight, Sc) two times per week, for eight weeks, and 300 and 400 mg /kg^{-1}, Po body weight from Marrubium vulgare leaves' extract separately in normal saline of 5 ml/ kg^{-1} Po body weight, four times per week for eight weeks, that hepatocytes show slight granular derivative changes, others were necrotic, shown in figure (6). Whereas figure (7) represents that hepatocytes showed the slightly diffused vascular degenerative changes, and others were necrotic.

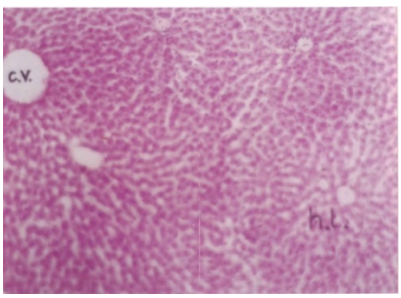

Fig 1. Liver of rats from negative control, untreated group showing normal entral vein and normal hepatocytes.

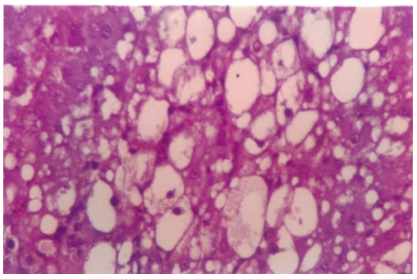

Fig 2. Liver of rats from positive control receiving 40% CCl4/paraffin oil, showing granular degenerative changes (H. E. X:650).

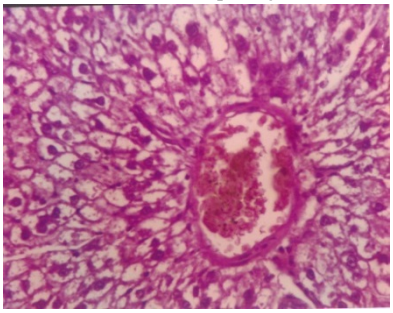

Fig 3. Liver of rats in positive control showing congestion of portal blood vessels, accompanied by severe changes of hepatocytes (H.EX:400).

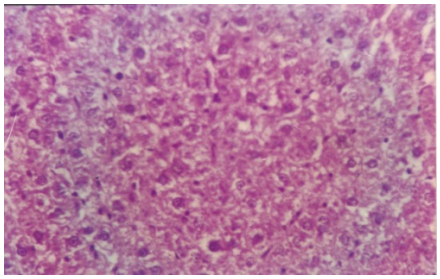

Fig 4. Liver of rats received 100 mg /kg^{-1}, Po body weight from Marrubium vulgare extract showing necrosis of some hepatocytes (HEX: 400).

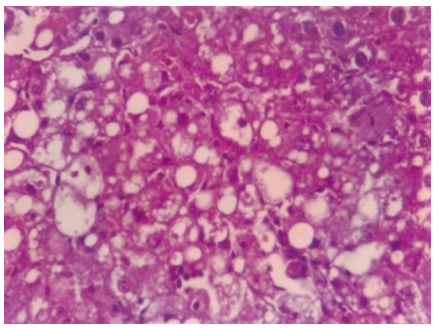

Fig 5. Liver of rats received 200mg /kg^{-1}, Po body weight from Marrubium vulgare leaves' extract showing vascular changes of some hepatocytes (H. E. X:650).

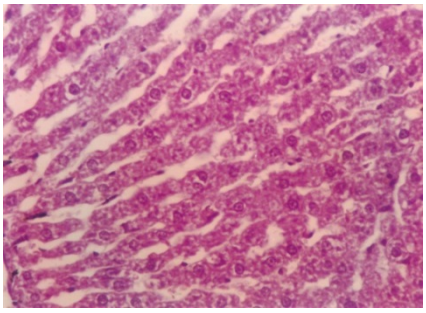

Fig 6. Liver of rats received 300 mg /kg^{-1}, Po body weight from Marrubium vulgare leaves' extract showing infiltration of edema between the hepatocytes (H. E. X: 400).

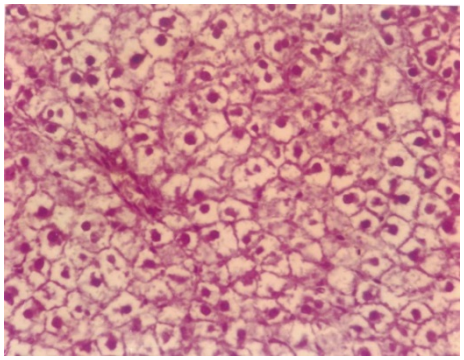

Fig 7. Liver of rats received c 400 mg /kg^{-1}, Po body weight from Marrubium vulgare leaves' extract showing diffused vascular degenerative changes of hepatocytes , and others were necrosis (H. E. X:400).

CONCLUSION

This study concluded that the essential oil of Marrubium vulgare leaves' extract has natural antioxidant effects, and it can protect liver against damages of carbon tetrachloride-induced hepatotoxicity and liver damages in rats. The results also demonstrated that the concentrations of this Marrubium vulgare's extract were at levels of 100, 200, 300 and 400 mg /kg-1, the body weight can decrease the abnormally increased levels of cholesterol and triglyceride which indicate hypolipidemic parameters.

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