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Original Article

Potential Effect of *Marrubium vulgare L.* extracts on CCL_4 model induced hepatotoxicity in albino mice

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ABSTRACT

Marrubium vulgare L. (white horehound) belongs to *Lamiaceae* family. The plant is formerly much esteemed in hepatic affections and in phthisism. This work was conducted to investigate hepatoprotective and therapeutic effects of *Marrubium vulgare* aqueous ethanolic and petroleum ether extracts on CCL_4 induced liver cell toxicity in mice. The aerial parts of *Marrubium vulgar* were defatted and then were extracted with 70% aqueous ethanol. Liver and kidney functions, catalase activity as well as lipid peroxidation were measured. Silymarin in a recommended dose was used as a reference drug. Malonaldialdehyde concentration in *Marrubium* extracts treated mice was considerably decreased as compared to CCL_4 or silymarin treated mice. Liver and kidney function parameters remained in the normal levels in extracts treated groups. Administration of *Marrubium* ethanolic extract significantly enhanced superoxide dismutase and catalase activity also total antioxidant capacity with significant reduction in lipidperoxide conc. when extract used as protective or therapeutic agents. *Marrubium vulgare L* extracts produced potent therapeutic activity than hepatoprotective activity and it was more effective than silymarin in two cases.

Key words: Hepatoprotective, Antioxidant, *Marrubium*, CCL_4, Therapeutic

INTRODUCTION

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical [1]. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity of CCl_4 [2]. This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP and decrease in protein.

Marrubium vulgare L. (white horehound) belongs to the *Lamiaceae* family is a perennial, herbaceous medicinal plant native to temperate regions. This plant was frequently employed as folk medicine to treat a variety of ailments, exhibits antispasmodic and antinociceptive effects. It possesses tonic, aromatic, stimulant, expectorant, diaphoretic and diuretic properties. It is helpful for bronchial asthma and nonproductive cough. It was formerly much esteemed in various uterine, visceral and hepatic affections and in phthisis [3]. The plant is reported to possess hypoglycemic [4], antibacterial [5], antidiabetic [6] , Gastroprotective activity [7] and many other reported biological activities. Essential oils extracted by distillation from aromatic plants are appreciated for their bioactive efficacy as fungicides, bactericides [8], antioxidant [9] and other biological activities. Marrubiin is diterpenoid lactone that constitutes the bitter principle of the horehound and many other medicinal plants of the family *Lamiaceae*, which are used in several countries to treat different pathologies [10]. Extensive pharmacological studies have demonstrated that marrubiin displays a suite of activities including antinociceptive [11] , antioxidant, antigenotoxic [12], cardioprotective [13], vasorelaxant [14], gastroprotective [15],antispasmodic [16], immunomodulating [17], antioedematogenic [18] , analgesic [19, 20] and antidiabetic properties [21].The crude extract of *Marrubium vulgare* is widely used as

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antihypertensive treatment in traditional medicine. It has been shown to decrease systolic blood pressure in spontaneously hypertensive rats and to inhibit KCl-induced contraction in rat aorta[22] .

The essential oil and the extract obtained from the aerial parts of *Marrubium vulgare* have been shown to have strong antimicrobial and antioxidant activities [23].The main active ingredient that is produced and accumulated in the aerial parts of the plant is a diterpenoid known as marrubiin [24]. A substantial antioxidant, anticoagulant, antiplatelet and anti-inflammatory effects have been attributed to marrubiin [25]. *Marrubium vulgare*, also contains marrubenol and phenylpropanoid esters which have been shown to exhibit L-type calcium channel blocking and cyclooxigenase (COX) inhibitors activities [14, 26] .Further, phenylpropanoids have been proved to protect cardiomyocyte against hypoxia-induced death [27]. This work aimed to evaluate hepatoprotective and therapeutic effects of *Marrubium vulgare L* aqueous ethanolic and petroleum ether extracts on CCL_4 induced hepaotoxicity in mice.

MATERIALS AND METHODS

Plants extract preparation: The aerial parts of *Marrubium vulgare* were collected from plants cultivated in the Expermintal Farm of the National Research Centre, Nobaria, El-Bihara Governrate (150 Km Northern South of Cairo), Egypt, air dried and fine-powdered. The fine powder of *Marrubium vulgare* aerial part was defatted using petroleum ether then the defatted powder was extracted with 70% aqueous ethanol. The crude ethanolic and petroleum ether extracts were concentrated using rotary evaporator under reduced pressure at 45^{o}C then the concentrated extracts were lyophilized.

Animals: Male albino mice were obtained from animal house of National Research Centre. Male albino mice weighting 30± 35g were housed in polypropylene cages, each cage was contained ten mice in case of LD_{50} assessment while it was contained eight mice in case of hepatoprotective study. Animals were fed on standard diet, temperature through the housing was controlled at 24ºC, relative humidity 65±5% and light/dark cycles (12/12hrs).

Experimental design: Animals were kept for one day under the condition of experiment then they were gavaged with 0.5ml of different solution used in experiment. Animals were divided into three main groups includes negative control group, normal group treated with extracts or silymarin and treated group. Each subgroup contained eight mice. The control group was administered saline solution (0.5ml/ day/ 5 days). The second main group is the healthy normal group which was divided into five subgroups, the first was administered corn oil while the second and the third groups were gavaged with extracts of medicinal plant prepared in corn oil (0.5ml of 1/10 extract LD_{50}/ day/ 5days), the fourth group was gavaged with silymarin in a recommended dose (25mg/ kg) for 5days prepared in 0.5ml corn oil. The fifth group was orally administered with CCL_4. The third main group was treated group which contain six subgroups, the first one was treated with alcoholic extract (0.5ml of 250mg/ kg/ day/ 5days) then was administered with CCL_4, however, the second group was gavaged with petroleum ether (0.5ml of 200mg/ kg/ day/ 5days) then was administered with CCL_4. The third group was orally administered silymarin in a recommended dose (0.5ml/ day/ 5days) then was administered with CCL_4. The fourth, fifth and sixth groups are therapeutic treated group which administered CCL_4 followed with administration of alcoholic extract and petroleum ether extract as well as silymarin using the same doses mentioned above. Blood samples, livers were collected after 48hrs of CCL_4 in case of hepatoprotective experiment and after 48 hrs from the last extract and silymarin dose.

Biochemical assessment: At the end of the experiment, animals were fasted for 12 h and blood samples were taken from treated and control mice by puncturing retro orbital plexus. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum were measured with kits [28]. After collection of blood samples mice's livers were collected, then immediately excised and rinsed in ice cold normal saline. Liver homogenate (5%) was prepared in bidistilled water using potter-Elvehjem homogenizer with Teflon pestle. Protein concentration was measured as described**,** by [29]. superoxide dismutase (SOD) was measured at 560nm [30] as the reduction suppression rate of nitrotetrazolium blue and for 1 unit of activity, the amount of protein was taken which provided 50% inhibition of nitrotetrazolium blue reduction under standard conditions. Catalase activity was spectrpphotometrically according to [31]. Malondyaldehyde (MDA) determination in liver was assayed by spectrophotometric method at 534nm [32]. All kits were purchased from Biodiagnostic, Egypt.

Statistical Analysis: The obtained results were expressed in median± SD (standard deviation). The levels of statistic significance (P<0.05) were calculated based one-way ANOVA test for comparisons among means.

RESULTS

Marrubium vulgare aqueous ethanolic and petroleum ether extracts were investigated for their hepatoprotective and therapeutic effects on CCL_4 induced liver cell damage model. Administration of CCL_4 elevated Glutamic-oxalacetic transaminase and Glutamic-oxalacetic transaminase activities (100.05 and 100.23 IU/L, respectively) while administration of *Marrubium* extracts as controls did not affect liver function, they save GOT and GPT activities at the same of those untreated group animals as shown in Table (1). Ethanolic extract showed potent protective effect against the damage caused by CCL_4 administration, it reduced GOT and GPT activities (65.54 and 54.2 IU/L, respectively) but its protective effect remained lower than the silymarin effect (60.05 and 47.36 IU/L for GOT and GPT, respectively). On the other hand, petroleum ether extract significantly reduced GOT and GPT activities as compared to CCL_4 animals (68.23 and 47.22 IU/L). Regarding kidney function, administration of CCL_4 produced a high disturbance in kidney function exhibited as significant increments in all determined parameters. Oral administration of ethanol extract significantly reduced creatinine, uric acid and urea determined in animals'sera (2.65 mg/dl, 6.32 mg/dl and 1.71 mg/dl, respectively). Petroleum ether extract showed the same effect and data of ethanol extract with uric acid and urea, while creatinine was reduced by administration of petroleum ether by more than ethanol extract (2.35 mg/dl). Silymarin significantly reduced creatinine and urea as pet. ether extract and it was most efficient in saving uric acid at the same control level (1.69 mg/dl) as shown in Table (1). All mentioned data indicated that ethanol extract was more efficient as hepatoprotective agent followed by silymarin then pet. Ether extract.

Regarding the therapeutic impact of the two extracts, petroleum ether extract exhibited a promising effect in treating the adverse effect of CCL_4 on liver and kidney functions as compared to ethanol extract or silymarin. It significantly lowered GOT and GPT activities (61.23 and 42.24 IU/L) more than ethanol extract (68.54 and 44.62 IU/L) and silymarin (65.62 and 49.31 IU/L), (**Table, 2).** On the other hand, administration of pet. ether extract significantly inhibited production of creatinine (2.00 mg/dl), urea (4.01mg/dl) and uric acid (1.41 mg/dl) as compared to CCl_4 (3.78 mg/dl, 7.28 g/dl and 2.36 mg/dl for ceatinine, urea and uric acid, respectively). However, silymarin was more effective than ethanol extract in determined kidney function parameters. Oral administration of ethanol ectract significantly reduced creatinine, urea and uric acid concentration in sera (3.05 mg/dl, 6.59 mg/dl, 1.38 mg/dl for creatinine, urea and uric acid respectively) while silymarin showed the same results of pet. Ether extract on creatinine and uric acid (2.00 mg/dl and 1.49 mg/dl).

CCL_4 administration significantly inhibited superoxide dismutase (5.11 U/ min/ mg prot.) while ethanolic and pet. Ether extracts, as $^{+}$ve control groups, significantly induced it (11.18 and 28.05 U/ min/ mg prot., respectively) and they were more efficient than sylimarin (19.17 U/ min/ mg prot.)(**Table, 3).** Marrubium extracts repaired the adverse effect of CCL_4 on SOD activity. Alcoholic extract administration as protection treatment induced SOD (14.50 U/ min/ mg prot.), however, pet. ether induced \sod by more than alc. extract (18.38 U/ min/ mg prot.). The same trend of results was observed with catalase, pet.ether was the most effective inducer (30 U/ min/ mg prot.) as compared to alc. extract or sylimarin (21 and 24.24 18.38 U/ min/ mg prot., respectively) which was decreased by CCL_4 administration (11.81 18.38 U/ min/ mg prot.).

The recorded increments in SOD and CAT were accompanied with significant increments ($P \leq 0.05$) in Total antioxidant capacity (TAC). TAC was enhanced when animals administered the marrubium alcoholic and pet. ether extracts as vehicle control groups (3.66 and 4.41 mmol/ g tissue, respectively). The lowest TAC were this of animals treated with CCL_4 (0.44 mmol/ g tissue) while administration of alc. and pet. ether extracts significantly increased the liver TAC (1.44 and 3.11 mmol/ g tissue, respectively) also it was enhanced with sylimarin admin. (2.14 mmol/ g tissue).

The induction of SOD, CAT and TAC produced by methanolicand pet. extracts ameliorated the lipid peroxide level determined in liver tissue. CCL_4 admin. increased lipid peroxide conc. in tissue (28.64 mmol/ g tissue) which was reduced with methanolic extract and pet. ether extract administration (6.23 and 7.12 mmol/ g tissue) as compared to –ve control group (7.15 mmol/ g tissue).

The petroleum ether extract administration was the most potent therapeutic treatment as compared to alcoholic extract and sylimarin. It caused valuable enhancement in determined antioxidant parameters. It increased SOD activity (19.15 U/ min/ mg) as compared to CCL_4 group (5.11 U/ min/ mg) and catalase activity (30.23 U/ min/ mg) with significant amelioration in TAC (3.71 mmol/ g tissue) which produce significant reduction in tissue lipid peroxide conc. (5.99 mmol/ g tissue) as shown in **Table (4).**

On the other hand, ethanol extract administration showed the same effect on antioxidant parameters as sylimarin treatment. It enhanced SOD activity (17.29 U/ min/ mg), CAT activity (28.53 U/ min/ mg) and TAC (2.04 mmol/ g tissue) with decreasing lipidperoxides (13.11 mmol/ g tissue) in liver tissue.

DISCUSSION

In the assessment of liver damage by CCl_4, the determination of enzyme levels such as AST, ALT is largely used. Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. High levels of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury. AST catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [33]. Serum ALP and total protein levels on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure [34].

Administration of CCl_4 caused elevated liver enzyme levels, AST, ALT. There was a restoration of these enzyme levels on administration of the herb extract of *Marrobium vulgar* in a dose dependent manner and also by silymarin at a dose of 25 mg/kg. The reversal of increased serum enzymes in CCl_4-induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [35].

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Both silymarin and the plant extract decreased CCl_4 induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells.

The increase in lipid peroxidation level in liver induced by CCl_4 suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Treatment with *M. vulgar*\ significantly reverses these changes. Hence it is likely that the mechanism of hepatoprotection of *M. vulgar* is due to its antioxidant effect.

Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury [36], SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. In *M. vulgar* causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, and the highest activity is found in the red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [37]. Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide.

Preliminary phytochemical studies of *M vulgare* as an important medicinal plant, shows strong antioxidant properties due to presence of flavonoids, terpenes, and phenols [38, 39]. The observed antioxidant and hepatoprotective activity may be due to the presence of flavanoids.

CONCLUSION

Marrubium petroleum ether and ethanolic extracts exhibited potent hepatoprotective and therapeutic effect better than sylimarin as they ameliorated liver antioxidant status in two cases.

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Table (1). Effect of *Marrubium vulgare L* extracts as hepatoprotective agent on liver and kidney functions in mice injured with CCL_4

Group	Control			Silymarin		Ethanolic extract		Petroleum ether	
Parameter	-ve control	Corn oil	CCL_4	+ve	Treated	+ve	Treated	+ve	Treated
Glutamic - oxalacetic Transaminase IU/L	50.83± 0.79[a]	48.73± 2.63	100.05 ±0.85	53.96±0.75	60.05±0.83	51.91±1.78[a]	65.54±1.34	50.53±0.69[a]	68.23±1.34
Glutamic-pyruvic Transaminase IU/L	44.33± 0.63[b]	45.27± 2.54[b]	100.23 ±3.01	45.45±0.56[b]	47.36±0.68[c]	47.91±1.58[c]	54.22±2.41	44.62±2.11[a]	47.22±2.61[c]
Creatinine Mg/dL	2.23± 0.11[e]	2.29± 0.81[e]	3.78± 0.34	2.41±0.97[d]	2.37±0.34[d]	1.82±0.22	2.65±0.41	1.62±0.34	2.35±0.35[d]
Urea mg/dL	5.34± 2.13[f]	4.17± 0.45	7.28± 0.61	6.12±0.51	6.72±0.57[g]	5.14±0.48[f]	6.32±0.57[s]	5.68±0.33[f]	6.55±0.72[gs]
Uric acid mg/dL	1.68± 0.24[m]	1.34± 0.23[n]	2.36± 0.03	2.18±0.03	1.69±0.44[m]	1.32±0.21[n]	1.71±0.31[q]	1.69±0.51[m]	1.72±0.12[q]

Data are presented as the means ± S.D Groups have the same letter had insignificant difference between them

Table (2): Effect of *Marrubium vulgare L* extracts as therapeutic agent on liver and kidney functions in mice injured with CCL_4

Group	Control			Silymarin		Ethanolic extract		Petroleum ether	
Parameter	-ve Control	Corn oil	CCL_4	+ve	Treated	+ve	Treated	+ve	Treated
Glutamic-oxalacetic Transaminase (IU/L)	50.83±0.79[a]	48.73±2.63	100.05±0.85	53.96±0.75	65.62±1.27	51.91 ±1.78[a]	68.54± 1.34	50.53± 0.69[a]	61.23± 1.34
Glutamic-pyruvic transaminase (IU/L)	44.33±0.63[b]	45.27±2.54[b]	100.23±3.01	45.45±0.56[b]	49.31±0.63[c]	47.91 ±1.58[c]	48.33± 3.01[c]	44.62± 2.11[b]	42.24± 1.67
Creatinine mg/dl	2.23±0.11[e]	2.29±0.81[e]	3.78±0.34	2.41±0.97	2.00±0.49[d]	1.82± 0.22	3.05± 0.65	1.62± 0.34	2.00± 0.43[d]
Urea mg/dl	5.34±2.13	4.17±0.45[f]	7.28±0.61	6.12±0.51	7.59±0.38	5.14± 0.48	6.59± 0.58	5.68± 0.33	4.01± 1.48[f]
Uric acid mg/dl	1.68±0.24[k]	1.34±0.23[h]	2.36±0.03	2.18±0.03	1.49±0.035	1.32± 0.21[h]	1.38± 0.34	1.69± 0.51[k]	1.41± 0.23

Data are presented as the means ± S.D Groups have the same letter had insignificant difference between them

Table (3): Effect of *Marrubium vulgare L* extracts as hepatoprotective agent on antioxidant status in liver mice injured with CCL_4

Group	Control			Silymarin		Ethanolic extract		Petroleum ether	
Parameter	-ve Control	Corn oil	CCL_4	+ve	Treated	+ve	Treated	+ve	Treated
Superoxide dismutase activity U/ min/ mg protein	15.14± 0.72[a]	15.00± 3.66[a]	5.11±1.87	19.17±0.66[b]	15.37±0.82[a]	22.18±1.40	14.50±2.43[a]	28.05 ±1.05	18.38± 1.89[b]
Catalase activity U/ min/ mg protein	28.52± 3.08[c]	27.54± 2.84[c]	11.81±2.61	30.32±4.41[e]	24.24±1.15	33.40±2.85	21.00±2.43	36.11 ±3.26	30.24± 1.41[e]
Total antioxidant capacity mmol/ g tissue	3.00± 1.01[d ah]	2.97± 1.34[d]	0.44±0.02	3.71±1.51[g]	2.14±0.64	3.66±0.99[g]	1.94±0.72	4.41± 1.00	3.11± 0.77[h]
Lipidperoxidation mmol/ g tissue	7.15± 1.18[m]	6.63± 1.19	28.64±2.87	5.99±0.79[n]	7.12±1.08[m]	6.11±1.66[k]	6.23±0.86[k]	6.31± 0.54[k]	5.88± 0.91[n]

Data are presented as the means ± S.D; Groups have the same letter had insignificant Diffrence between them

Table (4): Effect of *Marrubium vulgare L* extracts as therapeutic agent on antioxidant status in liver mice injured with CCL_4

Group	Control			Silymarin		Ethanolic extract		Petroleum ether	
Parameter	-ve Control	Corn oil	CCL_4	+ve	Treated	+ve	Treated	+ve	Treated
Superoxide dismutase activity U/ min/ mg protein	15.14±0.72 [a]	15.00±3.66 [a]	5.11±1.87	19.17±0.66 [c]	18.78± 0.89 [bc]	22.18 ±1.40	17.29± 1.98 [b]	28.05 ±1.05	19.15± 2.61[c]
Catalase activity U/ min/ mg protein	28.52±3.08 [d]	27.54±2.84 [d]	11.81±2.61	30.32±4.41[e]	26.62± 2.13 [d]	33.40 ±2.85	28.53± 3.05[d]	36.11 ±3.26	30.34± 1.41[e]
Total antioxidant capacity mmol/ g tissue	3.00±1.01[f]	2.97±1.34[f h]	0.44±0.02	3.71±1.51[g]	2.41± 1.08	3.66± 0.99[g]	2.04±0. 31	4.41± 1.00	2.91±0. 97[h]
Lipidperoxidation mmol/ g tissue	7.15±1.18	6.63±1.19	28.64±2.87	5.99±0.79 [n]	12.45± 3.45[m]	6.11± 1.66[n]	13.11± 2.66 [m]	6.31± 0.54	10.02± 1.94

Data are presented as the means ± S.D Groups have the same letter had insignificant difference between them

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