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Studying the Chemical Composition and Hepatoprotective Activity Of *Capparis Sinaica* veill towards CCl_4 Injury in Albino Rats

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Abstract

The medicinal plant, *Capparis sinaica* veill. growing in Al-Tofaha valley, Saint Catherine, South Sinai, Egypt , it belongs to the family Capparaceae ,which has a few purposes in folk medicine for a long time. The preliminary phytochemical screening of the plant, revealed the presence of tannins, flavonoids, glycosides , sterol, saponins and alkaloids. In this study, the methanolic extract of aerial part of *Capparis sinaica* veill. was carried out by using HPLC analysis which revealed the presence of 15 bioactive phenolic compounds. The rutin is the major compound in the plant. Moreover, the ameliorative effect of the methanolic extract from *C. sinaica* towards the [500 miroliter of CCl_4]-induced hepatotoxicity in male Wistar rats were investigated. The CCl_4-treated rats showed a significant decline in the serum levels of high-density lipoprotein (HDL), albumin (ALB) as well as the hepatic levels of glutathione (GSH) and activities of catalase (CAT), superoxide dismutase (SOD) , glutathione reductase (GR), in addition to elevation in the levels of total lipids (TL), triglycerides (TG), total cholesterol (TC), low-density lipoproteins (LDL), globulin (G), total bilirubin (TBil) , alanine and aspartate aminotransferase and alkaline phosphatase (ALAT and ASAT, ALP) and the hepatic levels of malondialdehyde (MDA) were recorded. The administration of methanolic extract of the aerial part of *Capparis sinaica*, notably improved all the studied parameters. This study showed that CCl_4 administration to Wistar rats, at a high dose, could induce a hepatic injury in addition to certain metabolic alterations. The study was extended to investigate tissue histopathology.

In conclusion, the methanolic extract of aerial part of *Capparis sinaica* may function as a good candidate for the treatment or prevention of liver failure. However, further investigations are required to unveil the molecular identification of the active ingredients and elucidation of the mechanisms involved in the effect.

keywords

Capparis sinaica veill., Capparaceae, phenolic, flavonoids, HPLC, Hepatotoxicity. induced by CCl_4

1. Introduction

The *Capparis sinaica* veill**.** species has a great interest in the field of traditional medicine for its bioactive compounds. *Capparis sinaica* veill belongs to the family Capparaceae, that has several uses in the Egyptian folk medicine for many years [1]. The Capparaceae family composed of 45 genera and approximately 1000 disseminated particularly in the tropical and subtropical locale, particularly East Africa and South America [2] , It is the most plant of this family contains many active constituents such as alkaloids, tannins, saponins, steroids, terpenoids, flavonoids, phenolic constituents and glycosides [3,4]. In the Arabian folk medicine, several *Capparis* species have many uses [5]. The total herb of *C. sinaica* is utilized for cerebral pain, loss of motion, snakebite and enlarging. Moreover, the leaves of *C. spinosa* are used for treating ear ache, coughs, expelling stomach worms and for diabetes [6].

The main objectives of this study were to estimate the phytochemical characterization of the methanolic extract of the aerial part of *Capparis* by HPLC and investigate the hepatoprotective properties towards carbon tetrachloride (CCl_4) induced injury in rats liver. [7,8]

Material and Methods

Plant material and Extraction

Samples of *C. sinaica* veil **,** were collected from Al-Tofaha valley, Saint Catherine, South Sinai, Egypt in September (2019) . It was identified by Dr. Alaaeldin Sayed Sayed Ewase, Ministry of Environment Nature

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The aerial parts (80 g) were crushed into small pieces and then extracted by 70% MeOH: H_2O (*v*:*v*) at ambient temperature for one week. The solution was filtered then concentrated till dryness using rotavapor (Heizbad Hei-VAP, Heidolph, Germany), yielding (30 g) , stored at 4°C for phytochemical screening, HPLC, and hepatotoxicity detection.

HPLC analysis

The phytochemical analysis of *C. sinaica* veill. 70% MeOH extract was performed using high -performance liquid chromatography analysis (HPLC). The HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Kromasil C_{18} column (4.6 mm x 250 mm i.d., 5 µm) at room temperature.

The mobile phase is water (A) with 0.05% trifluoroacetic acid and acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a gradient as follows: 0-3 min (80% A); 3-8 min (60% A); 8-12 min (40% A); 12-15 min (10% A) and 15-30 min (80% A). The multi-wavelength detector was monitored at 280-420 nm. The injection volume was 10 µl for each of the sample solutions. Each compound was identified by its retention time and by spiking with standards under the same conditions.

Assessment of Biological Activities

In *vivo* Hepatoprotective Activities

Animal

Male Wistar albino rats (120 to 150 g) were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. All animals were kept in controlled environment of air and temperature with access of water and diet ad libitum. Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt.

Experimental design

Rats were divided into 3 groups consisting of 6 rats each as follows:

Group 1, served as normal healthy control rats.

Group 2 (Intoxicated group): Rats were intraperitoneally injected with 500 microliters of CCl_4 diluted 1: 9 (v/v) in olive oil (0.1 ml) twice a week for six consecutive weeks**.**

Group 3 (treated group): Rats were given orally *C. sinaica* methanolic extract (200 mg/kg body weight) in a dose of 1 mL as a treatment for two weeks after (i.p.) single injection with the same assigned dose of intoxicated group.

Study of some Biochemical Parameters

Sample Preparations

Blood was collected from each animal by puncture of sublingual vein. Blood samples were collected into dry test tubes and then centrifuged at 3000 rpm in order to separate serum. The sera were kept at -20°C for further biochemical analysis. In order to collect the hepatic tissues, rats were immediately dissected. The liver was homogenized with 10% w/v ratio in ice-cold 50 mM Tris HCl buffer at pH 7.4 and then centrifuged at 10,000 rpm for 20 min at 4° C. The supernatant was collected and kept in deepfreeze at 20° C for further analyses.

Estimation of Serum Biochemical Parameters

In the serum of all the experimental groups, the levels of total lipids (TL), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total proteins (TP), albumin (A), globulin (G), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), total bilirubin (TBil) and direct bilirubin (DBil) were measured calorimetrically using Biodiagnostics kits (Dokki, Giza, Egypt).

Non-enzymatic and Enzymatic Antioxidant Assay

Lipid peroxide assay: The level of malondialdehyde (MDA) in the liver homogenate was assayed according to the described technique **[9].** The principle of this method depends on the reaction of the liberated MDA after lipid peroxidation (LPO) of the cell membranes with thiobarbituric acid in acidic medium.

The concentrations of non-enzymatic (glutathione, GSH) as well as enzymatic (catalase, CAT, superoxide dismutase, SOD, glutathione reductase, GR) antioxidants were estimated in the homogenate of the liver of control and treated rats. The method by which GSH content was measured was based on the reaction of 5, 5'-Dithiobis-2-nitrobenzoic acid with GSH **[10]**. The CAT activity was estimated in accordance to the method described **[11]**. The SOD activity assessment was based on the ability of SOD to inhibit the reduction reaction of nitrobluetetrazolium dye mediated by phenazine methosulphate **[12]**. The principle for measuring the GR activity was based on its ability to catalyze the reduction of glutathione (GSSG) **[13]**.

Histopathological Study

Liver tissues were excised from sacrificed animals, individually weighed, and, from them, 5 µm thickness slices were cut, fixed in 10% paraformaldehyde, and embedded in paraffin wax blocks. Tissue sections of 5 µm thick were stained with hematoxylin and eosin (H&E).

Statistical analysis

Data were statistically analyzed by the aid of Statistical Package of the Social Sciences, SPSS version 23 (copyrighted by IBM SPSS software, USA). Data were expressed as a mean ± standard error of mean (SEM).

Results and Discussion

Phytochemical Screening*:*

The *C. sinaica*. showed that the plant are rich with phenolics, flavonoids, glycosides, alkaloids, and also, contains: tannins, sterols, and saponines, this is in agreement with previous reports [14,15].

HPLC analysis

The methanolic extract. of aerial part of *C. sinaica*. was analyzed by HPLC (Fig 1 and Table 1). The analysis led to tentative identification of 15 bioactive phenolic compounds structure (Fig.2).

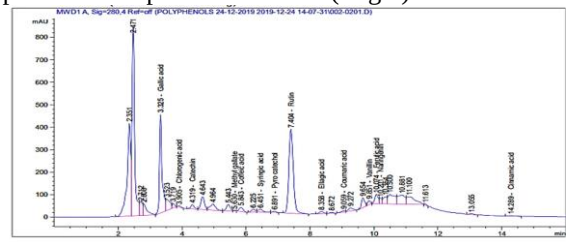

Figure 1: HPLC analysis of the methanolic extract of aerial part of *C. sinaica veil* The HPLC peaks are gallic acid, chlorogenic acid, catechin, methyl gallate, coffeic acid, syringic acid, pyro catechol, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, taxifolin and cinnamic acid respectively.

From the HPLC analysis, it identify that rutin compound is the major compound with higher concentration then gallic acid and finaly ferulic acid, this idicate the phenolic compound is responcible for hepatotoxicity induced by CCl_4 through scavenging free radicals of bioactive phenolic compounds which improved liver functions, and normalizing the liver histopathological architecture.

Hepatoprotective activities

Effect on serum biochemical parameters

The lipid profile of the experimental animals as affected by the administration of CCl_4 alone, and *C. sinaica* methanolic extract plus CCl_4 are shown in Table 2. The serum levels of TL, TC, TG, LDL-C and HDL-C of the rats were markedly influenced by the type of treatment. In comparison to control group, all the studied lipid profile parameters of CCl_4-treated group were significantly elevated except the levels of HDL-C that were notably reduced. On the other hand, rats treated E. retusa extracts ME plus CCl_4 exhibited a marked reduction in the levels of TL, TC, TG and LDL-C, as compared with the CCl_4-treated group. The results of the present study have also established that CCl4 treatment could have affected the lipid metabolism of liver (triglyceride and cholesterol levels). This is evidenced from the present observations in which CCl_4 caused a significant ($p < 0.05$) increase in the levels of lipid parameters. In this connection Muller et al. [16] stated that CCl_4 intoxication is similar to hepatitis in case of the triglycerides catabolism. This situation could be also attributed to the reduction of lipase activity, which could lead to decrease in triglyceride hydrolysis [17]. On the other hand, it can be assumed that hypercholesterolemia in CCl_4 intoxicated rats was resulted from damage of hepatic parenchymal cells that lead to disturbance of lipid metabolism in liver [18]. However, rats treated with *E. retusa* extracts ME showed a significant ($p < 0.05$) decline in triacylglycerol and cholesterol values compared to CCl_4 -intoxicated rats. The mechanism of lipid lowering effects of *C. sinaica* methanolic extract might be attributed to an inhibitory activity on microsomal acyl coenzyme A: cholesterol acyltransferease in *vitro*. This enzyme is responsible for acylation of cholesterol to cholesterol esters in liver [19].

Moreover, serum protein profile of different groups of rats in Table (2) was noticeably affected by the type of treatment as rats administered CCl_4 alone exhibited marked reductions in the levels of albumin simultaneous with a significant increase in the levels of globulin, as compared to the controls. Thus, the A/G ratio of this group was remarkably reduced. On the other hand, the rats of *C. sinaica* methanolic extract plus CCl_4 -treated groups displayed a marked increase in the levels of albumin and A/G ratio but a marked decrease in the levels of globulin, as compared to the CCl_4-treated group.

In this study the significant ($p < 0.05$) decrease in serum albumin of rats treated with CCl_4 as compared to control may indicates poor liver functions or impaired synthesis, either primary as in liver cells damage or secondary to diminished protein intake and reduced absorption of amino acids caused by a malabsorption syndromes or malnutrition, or loss protein in urine, due to nephritic syndrome and chronic glomerulonephritis [20]. On the other hand, a significant ($p < 0.05$) increase in concentration of serum albumin was observed in rats received *E. retusa* extracts plus CCl_4 in comparison to rats received CCl_4 alone. The increase of albumin concentration after treatment with *C. sinaica* methanolic extract may be attributed to the decrease in lipid peroxidation processes and increase in the activities of plasma protein thiols as a result of the treatment [21].

Also, liver function markers, as influenced by the administration of CCl_4- *C. sinaica* methanolic extract alone and mixed, were presented in Table (2). The activities of ASAT, ALAT and ALP and TBil, in serum of rats were significantly affected by the type of treatment, whereas the serum levels of DBil were not affected by any of the studied factors. In comparison to the controls, the CCl_4-treated rats showed significant elevations in the activities of ASAT and ALAT and ALP as well as the levels of TBil. On the contrary, the activities of ALP, ASAT and ALAT as well as the levels of TBil and DBil of *C. sinaica*

methanolic extract plus CCl_4 -treated rats were not significantly different from those of the control group.

Table 1: Identification Of Bioactive Phenolic Compounds By Using HPLC Analysis

Methanolic extract of aerial part of *Capparis sinaica* veill					
No	Compounds	R_t min	Area	Conc.(µg/ml = µg/ 22mg)	Conc. (µg/g)
1	Gallic acid	3.325	2421.85	174.07	7912.42
2	Chlorogenic acid	3.905	40.96	2.96	134.65
3	Catechin	4.319	151.11	25.56	1161.86
4	Methyl gallate	5.630	13.53	0.26	12.02
5	Coffeic acid	5.843	156.01	5.19	235.94
6	Syringic acid	6.451	128.48	4.23	192.24
7	Pyro-catechol	6.891	32.14	2.85	129.54
8	Rutin	7.404	3598.23	460.76	20943.82
9	Ellagic acid	8.358	88.42	4.80	218.09
10	Coumaric acid	9.059	43.77	0.96	43.56
11	Vanillin	9.851	109.99	2.54	115.43
12	Ferulic acid	10.074	288.55	9.86	448.23
13	Naringenin	10.410	146.78	8.22	373.84
14	Taxifolin	10.881	0.00	0.00	0.00
15	Cinnamic acid	14.289	20.73	0.24	10.84

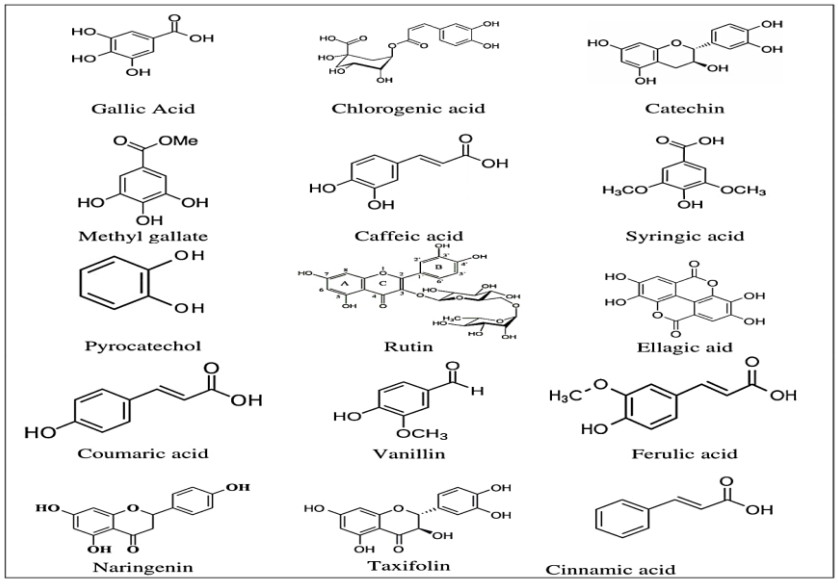

Figure .2 Structure of bioactive phenolic compounds structure of methanolic extract of aerial part of *C. sinaica*.

Table (2): Effect of injection of CCl_4 alone or with *oral administration of C. sinaica* methanolic extract, on some biochemical parameters of male albino rats

Parameters	Experimental groups		
	Control	CCl_4	*C. sinaica* methanolic extract + CCl_4
TL ($mgdL^{-1}$)	512.04 ± 43.06	673.2 ± 50.1	623.5 ± 46.4
TC ($mgdL^{-1}$)	118.20 ± 12.97	236.4 ± 26.5	184.6 ± 18.1
TG ($mgdL^{-1}$)	104.40 ± 7.34	166.9 ± 17.3	140.5 ± 13.4
LDL-C ($mgdL^{-1}$)	61.20 ± 9.87	145.8 ± 14.5	135.7 ± 9.7
HDL-C ($mgdL^{-1}$)	36.60 ± 6.40	27.4 ± 3.1	32.1 ± 4.3
TP (g d L^{-1})	6.68 ± 0.22	6.09 ± 0.7	6.39 ± 0.8
A (g d L^{-1})	4.42 ± 0.13	3.5 ± 0.1	4.2 ± 0.2
G (g d L^{-1})	2.46 ± 0.24	4.1 ± 0.3	3.5 ± 0.4
A/G ratio	1.72 ± 0.16	1.03 ± 0.05	1.5 ± 0.05
ASAT (U L^{-1})	33.02 ± 1.30	121.4 ± 22.6	94.1 ± 8.4
ALAT (U L^{-1})	25.60 ± 1.50	74.9 ± 10.5	49.2 ± 5.3
ALP (U L^{-1})	55.30 ± 3.84	82.7 ± 10.3	70.1 ± 6.2
TBil (mg d L^{-1})	0.66±0.02	0.99 ± 0.07	0.81 ± 0.07
DBil (mg d L^{-1})	0.11 ± 0.005	0.16 ± 0.008	0.14 ± 0.007

The increased serum levels of hepatic markers have been attributed to the liver injury, because these enzymes are found in cytoplasmic area of the cell and they are released into circulation in case of cellular damage [24]. On the other hand, treatment with *C. sinaica* methanolic extract plus CCl_4 was found to suppress ($p < 0.05$) the increase of serum AST and ALT activities. In accordance with the present results, many other plant extracts were reported to have considerable therapeutic effects on liver injury induced by chemical agents, for example, administration of poly phenolic extracts from chicory (Cichorium intybus) resulted in wholly normalization of the serum AST and ALT levels in mice exposed to thioacetamide, a hepatotoxic organosulfur compound [25]. Rafiei et al. [26] have also reported similar effects from barberry extract upon administration to CCl_4 induced hepatotoxic animals. These finding implies that challenge to protect liver tissue from CCl_4 injury.

Non-enzymatic and Enzymatic Antioxidant Assay

The effects of CCl_4 alone or with *C. sinaica* methanolic extract administrations on the levels of hepatic MDA and GSH and the activities of endogenous antioxidant enzymes were shown in Table (3). The hepatic levels of MDA and GSH as well as the activities of CAT, SOD and GR were significantly influenced by the type of treatment. In the liver of rats administered CCL4 alone, there was a meaningful elevation in the levels of MDA accompanied by a marked reduction in the GSH content, SOD and GR activities as compared to those of controls. In the rats of *C. sinaica* methanolic extract plus CCl_4 -treated groups, the mean values of hepatic MDA concentration were significantly lower than those of CCl_4-treated rats and were not significantly different from those of the controls. On the other hand, the mean values of hepatic GSH content of *C. sinaica methanolic extract* plus CCl_4 -treated rats were significantly higher than those of CCl_4 -treated group. As compared to the CCl_4-treated group, the rats administered *C. sinaica* methanolic extract plus CCl_4 showed a marked elevation in the activities of CAT and SOD and GR, that did not significantly differ from those of the controls.

Table (3): Effect of oral administration of CCl_4 alone or with various *C. sinaica* methanolic extract, on the levels of some antioxidants of male albino rats

Parameters	Experimental groups		
	Control	CCl4	*C. sinaica* methanolic extract + CCl4
MDA (nmol g-1 liver)	4.48 ± 0.11	10.2 ± 0.4	7.1 ± 0.4
GSH (mg g-1 liver)	40.04 ± 5.10	23.2 ± 8.3	30.7 ± 7.5
CAT (U g-1 liver)	104.3 ± 17.1	50.6 ± 8.5	79.6 ± 16.8
SOD (U g-1 liver)	9.56 ± 0.17	4.9 ± 0.4	6.9 ± 0.2
GR (U g-1 liver)	73.20 ± 2.71	39.1 ± 1.9	61.6 ± 3.2

Data are represented as mean ± standard error

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Data of the present study is in accordance with the findings of other workers such as Park et al. [27] who reported that hepatotoxic effects by CCl_4 are lipid peroxidation origin, and are largely due to its active metabolite CCl_3 (This metabolite can abstract hydrogen from fatty acids, initiating the lipid peroxidation), lead to cell injury, and finally liver damage. Moreover, Palanivel et al. [28], stated that 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. In this connection, the present study revealed that E. retusa extracts BT or ME decreased ($p < 0.05$) 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. As previously noted and similar to the results achieved for other plants in the literature [29-30], our observations and findings can be attributed to the antioxidant ingredients of *C. sinaica* methanolic extract that probably inhibit lipid peroxidation and consequently inhibition of oxidative stress. Therefore, the cell membranes remain intact and as a result cells are prevented to enter the necrosis step.

Histopathological results

Microscopic examinations of sections of liver from normal control rats show the normal architecture of hepatic lobules. The central veins lies at the center of the lobules surrounded by cords of hepatocytes. Between the strands of hepatocytes, the hepatic sinusoids are seen (Figure 3). Histopathological investigation of liver from rats administered with CCl_4 alone showing disruption of the liver tissue with loss of lobular arrangement, bridging fibrosis with collagenous septa formation expanded portal tract to central vein with mononuclear cells, vacuolar degeneration and necrosis of hepatocytes (Figure 4). Liver sections of rats administered with CCl_4 and C. sinaica methanolic extract showing mild inflammatory cells infiltrations around central vein, vacuolar degeneration, and necrosis of hepatocytes. Binucleated and activated Kupffer cells were noticed (Figure 5).

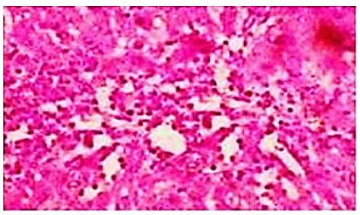

Figure 3 : Photomicrograph of section in liver of control rat shows *normal histological structure of hepatic lobules central vein, hepatocytes, blood sinusoids, and nuclei (H&E, ×400).*

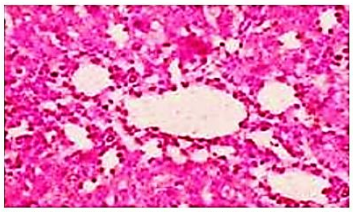

Figure 4: Photomicrograph of section in liver of rat administered with CCl_4 alone showing *disruption of the liver tissue with loss of lobular arrangement, bridging fibrosis with collagenous septa formation expanded portal tract to central vein with mononuclear cells, vacuolar degeneration and necrosis of hepatocytes. Dilated and congested central vein was observed and pyknotic nuclei (H&E, ×400).*

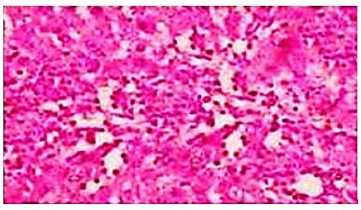

Figure 5: Photomicrograph of section in liver of rat administered with CCl_4 and *C. sinaica* methanol extract showing *mild inflammatory cells infiltrations around central vein, vacuolar degeneration, and necrosis of hepatocytes. Binucleated and activated Kupffer cells were noticed (H&E, ×400).*

In the present investigation, the biochemical findings were also confirmed by histpathological observations. The changes mostly include hepatocellular necrosis or apoptosis, fatty accumulation, inflammatory cells infiltration and other histological manifestations which were also consistent with the findings of other authors **[29,30].**

Conclusion

Hepatoprotective effects of *C. sinaica* methanolic extract on CCl_4-induced hepatic damage in male Wistar rats were observed in the present study. Probably, antioxidative properties of the extract helped hepatic cells to obviate CCl_4-induced necrosis and inflammation which can be also observed in histopathological findings. The results obtained here and the reports from previous studies suggest that *C. sinaica* methanolic extract may function as a good candidate for the treatment or prevention of liver failure.

However, further investigations are required to unveil the molecular identification of the active ingredients and elucidation of the mechanisms involved in the effect.

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