Research Article

Hepatoprotective Effects of London Rocket (*Sisymbrium irio* L) Extract against CCL_4 induced Hepatotoxicity in Albino Rats.

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Received: 29-05-2017; **Revised:** 18-07-2017; **Accepted:** 22-08-2017.

ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of methanolic extract and ethyl acetate extract of S. irio L against CCl4 induced liver damage in albino rats. The methanolic extract of *S. irio* L (1000mg/kg) and ethyl acetate extract (100mg/kg) were administered orally to the experimental animals with CCl4 induced hepatotoxicity (1 ml /kg). Silymarin (50 mg/kg) was given as reference standard. There were found that The plant extract was effective in protecting the liver against the injury induced by CCl4 in rats. Such hepatoprotective effects were evident from significant reduction in serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin. Furthermore, the result of the current study declared that the methanolic extract and ethyl acetate extract of S.irio L possesses hepatoprotective activity against CCl4 induced hepatotoxicity in rats. Histopathological studies revealed the post treatment of S. irio clearly exhibited the significant protection of liver cells.

Keywords: *Sisymbrium irio* L, CCl4, hepatoprotective, hepatotoxicity.

INTRODUCTION

Siymbrium irio L, known as London rocket[1]. Is an annual herb of family Brassicaceae distributed in Iraq. The stems erect, 15-50 cm tall, branched throughout or above the middle. Lower leaves long, gradually reduced upward, upper ones sessile, petals 2.5-4 mm long, pale yellow. Seeds usually in one series per loculeca, oblong, minutely papillose [2].

S. irio is used in treating coughs and chest congestion, rheumatism and to detoxify liver and spleen, reduce swelling and clean wounds[3]. *S. irio* has many uses in folk medicine in treatment of inflammation and rheumatism [4].

S. irio can be used for dietary purposes[5]. Seeds are used as expectorant, febrifuge and used in treatment of voice disorders[6].*S. irio* has antipyretic, analgesic, anti-microbial and antioxidant potential [7]. The Bedouin use the leaf of London Rocket as a tobacco substitute[8].Phytochemical screening analysis revealed that the plant contained secondary metabolites like flavonoids, alkaloids, oils and glycosides[9].The 70% ethanolic extract was subjected to different qualitative chemical tests to find out the presence of different phytoconstituents i.e. alkaloids, glycosides, carbohydrate, phenolics and tannins, phytosterols, fixed oils, fats, proteins and amino acids, flavonoids, saponins, gums and mucilage by means of detection methods of (Trease & Evans 2002)[10]. Saudi Arabia species of *Sisymbrium irio* Linn from aerial parts isolated ten flavonoids with anti-oxidant properties[11].

Figure: 1: *Sisymbrium irio* L whole plant

Hepatoprotective Activity

Carbon Tetra Chloride (CCl4) is a prototype of chemical used to induce the hepatotoxicity widely. CCl4 induced hepatotoxicity is mainly due to its intermediate reactive metabolites such as trichloromethyl radical (CCl3•) and its derivative trichloromethylperoxy radical (CCl4OO•), generated by cytochrome P4502E1 (CYP450 2E1). In addition, CCl4 intermediates also induce the production of reactive oxygen species (ROS), which play an important role in pathogenesis of different degenerative disease like atherosclerosis, liver disorders, lung, aging and diabetes mellitus. Carbon tetrachloride (CCl4) induced hepatotoxicity characterized by increasing in the liver enzymes like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and γ-glutamyltransferase (γ-GT) and histopathological changes like steatosis and

centrilobular necrosis due to membrane lipid peroxidation[12].

Numerous flavonoids such as apigenin, catechin, rutin, quercetin, naringenin, and kaempferol have well-recognized hapatoprotective activities[13].

Moreover, several studies stated that chronic diseases such as diabetes may lead to development of hepatic problems. Flavonoids like anthocyanins have a preventive effect against various hepatic diseases by affecting certain enzymes like glutamate cyseine ligase. A study demonstrated that anthocyanin cyanidin-3-O-β-glucoside causes a rise in hepatic Gene expression by increasing cAMP levels to activate protein kinase-A which regulates cAMP response element binding protein phosphorylation to enhance DNA binding and raise transcription. Similarly, treatment with certain enzyme lowers hepatic lipid peroxidation, prevents the release of pro-inflammatory cytokines, and protects against the development of hepaticstenosis[14].

MATERIALS AND METHODS

Plant material

The aerial parts of plant *S. irio*L. were collected from Al-Jadriya area at the University of Baghdad and was authenticated by the National herbarium in Abu-Ghraib and identified by prof. Ali Al-Musawi, in Department of Biology/College of Sciences, of Baghdad University.

Plant extract

Air dried powder of the aerial parts of *S. irio* L. (200 gm) were defatted with hexane (1000 ml) by Soxhlet apparatus till exhaustion then the marc was extracted by Soxhlet with methanol (90%, 1000 ml) till exhaustion. The extract was filtered to get rid of plant ashes, then the filtrate was concentrated to 400 ml. and divided into two fractions; the first part was evaporated by rotary evaporator to dryness and taken as the total methanolic extract (TME). The other fraction was taken and 100ml. of distilled water is added, the suspension was partitioned with ethyl acetate (3x 100ml).The combined ethyl acetate layers (EAE) were dried with anhydrous sodium sulphate, filtered, evaporated under vacuum and weighted. TLC made for extracts, by compare it with kaempferol, rutin, quercetin standard in three solvent systems (Methanol: chloroform 10:90), respectively. Toluene: chloroform: acetone (40: 25: 35) respectively formic acid: Chloroform: acetone (16.5:75:8.5) respectively, and the spots detected by UV.

The aqueous layer collected and undergo to reflex for three hour with little volume of dilute HCL, this hydrolysis with HCL led to open the glycoside linkage and produce glycon part (sugar part) and aglycon part (kaempferol)[15].

The upper layer, aglycon part (kaempferol), investigated by TLC in 3solvent systems (Methanol: chloroform 10:90) respectively Toluene: chloroform: acetone (40: 25: 35) respectively formic acid: Chloroform: acetone (16.5:75:8.5) respectively. The lower layer collected (sugar part), investigated by benedict test and TLC in the solvent system (Methanol: chloroform 10:90) respectively.

Hepatoprotective activity

Animals

Forty adult albino rats *Ratusratus* of both sexes weighing between250-300 g was used in this study. The experiment was done at the animal House of Al-Nahrain University. The animals were kept at standard Conditions and relative humidity in plastic cages, and were provided food and water and *add libitum* with a 12 hours and 12 hours light and dark cycle. All animals were acclimatized for 1 week before start of the study[16].

Experimental Design

Animals were divided into the five groups, consisting of eight animals per each group as followed:

1- Group I: Normal control (2% CMC 5 ml/kg, orally)

2- Group II: rats were treated with CCl4 (1.0 ml/kg, orally) [16].

3- Group III: rats were treated with CCl4 (1.0 ml/kg, orally) then total methanolic extract (TME) (500 mg/kg, orally)

4- Group IV: CCl4 (1 ml/kg, orally) then Ethyl acetate extract (EAE)(mg/kg, orally)

5- Group V: CCl4 (1.0 ml/kg, orally) then Silymarin (50 mg/kg)[17]. All the groups were treated for 21 days[16].

Induction of Experimental Hepatotoxicity

Hepatotoxicity was induced by administration of CCl4 at a dose of 1ml/kg, p.o of body weight at first day of the study[18].

Preparation of Solutions

CCl4 was diluted with olive oil in 1:1 ratio, and TME, EAE were suspended in 2% CMC solution.

Calculation of Dose of Plant Extract given:

Acute toxicity studies on rats were performed and found to be2000mg/kg dose 20.0% mortality recorded. Half of these doses (1000mg/kg) of the TME wasgiven[19].

1000mg/kg =250 mg/rat suspended in 1ml of 2%CMC and given for group III once daily orally for 21 days.

1000mg/kg of TME equal to 100mg/kg EAE.

100mg/kg = 25 mg/rat suspended in 1ml of 2%CMC and given for group (IV) once daily orally for 21 days.

(Each 100g of TME give 10g of EAE).

Liver Enzymes Assay

After that the blood samples were collected using direct cardiac puncture under light ether anesthesia and serum was separated for the estimations of serum AST, ALT, alkaline phosphatase (ALP) and total bilirubin all kits were from AGAPPE DIGNOSTICS SWITZERL and GmbH

Histopathological Examination

A portion of liver tissue samples was collected from the each group and preserved in 10% neutral formalin solution for the histopathological examination. Liver samples were sectioned at 5µm and hematoxylin and eosin (H&E) staining were used. The liver section identified by Dr.Kefah at Digestive and liver diseases hospital.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to evaluate the results of different parameters involved in the study. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant[20].

RESULTS AND DISCUSSION

Hepatoprotective Activity

The animals treated with CCl4 exhibited a significant (p< 0.01) rise in serum AST, ALT levels and incase of ALP, total bilirubin levels as well increased significantly (p<0.001) when compared to control. The level of hepatic enzymes (AST, ALT, ALP and Bilirubin) were significantly (p<0.01) reduced after treatment with total methanolic extract (TME) and with ethyl acetate extract (EME) compared to CCL4. Silymarin (50 mg/kg) treated animals also showed significant decrease (P<0.01) in AST, ALT, ALP and bilirubin (P<0.05) levels when compared to group II (Table 1).

Table 1: Effect of different groups on serum hepatic enzymes levels.

The Group	Mean ± SE			
	AST (IU/L)	ALT(IU/L)	ALP(IU/L)	Total Bilirubin (mg/dl)
G1: Normal	117.86 ±5.68	54.33 ±3.24	261.22 ±33.95	0.269 ±0.07
G2: CCl4	368.92 ±43.41 +++	201.00 ±18.04 +++	514.98 ±33.69 +++	0.486 ±0.06 +++
G3: Total extract	252.90 ±12.78 ***	111.32 ±9.82 ***	269.81 ±34.79 ***	0.386 ±0.04 *
G4: Ethyl acetate extract	186.18 ±4.60 ***	96.66 ±5.84 ***	273.36 ±32.54 ***	0.314 ±0.04 **
G5: Silymarin drug	166.28 ±15.08 ***	77.12 ±4.23 ***	266.83 ±32.47 ***	0.274 ±0.05 **

Statistical comparisons of the data for groups followed by the Tukey's test. Values are mean ± SE (n= 8 rats per each group) * (P<0.05), ** (P<0.01), *** (P<0.001) significantly different from the group treated with CCl_4.+++ (P<0.001) significantly different from the control.

Table (1) showed serum AST, ALP, ALP, and bilirubin levels and their levels are mainly increased in rats belonging to Group II. The hepatotoxic properties of the CCl4 are mainly due to its active metabolite, trichloromethylradical[21]. These activated radicals were bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. So, this process leads to excessive formation and accumulation of lipids in tissues such as liver[22].

High levels of serum AST indicate liver damage, like what shown during cardiac infarction and muscle injury and in viral hepatitis. AST catalyzes the conversion of alanine to pyruvate and glutamate and is released in similar manner. So, ALT is more specific to the liver, and thus a better parameter for detecting liver damage. The elevated serum enzymes revealed the presence of cellular leakage and loss of functional integrity of cell membrane in liver[23]. Serum ALP levels on the other hand are associated to the function of hepatic cell. Rise in serum level of ALP may due to increased synthesis in presence of biliary pressure[24].

The reverse of increased serum enzymes in CCl4 that induced liver damage by the *S. irio* treated groups may be owing to the prevention of the leakage of intracellular enzymes by its membrane stabilizing action. So this is in contrast with the usually accepted opinion that serum levels of transaminases return to normal with the therapeutic of hepatic parenchyma and the regeneration of hepatocytes[25].

One of the most suitable clinical signs to the severity of necrosis is the bilirubin and its increase is a measure of binding, conjugation and excretory capacity of hepatocyte. Reduction in serum bilirubin later the treatment with the extract in liver damage, showed the efficiency of the extract in normal functional status of the liver [22].

Decreased values of these enzymes levels in *S. irio* treated groups showed their ability to normalize the status of

hepatic damage might be due to the presence of flavonoids.

The effectiveness of any hepatoprotective drug may dependent on its abilityin reducing the harmful effect or repairing the normal hepatic physiology that has been impaired by a hepatotoxin. Silymarin and the *S. irio* extract both reduced CCl4-induced elevated enzyme levels in tested groups, this shows the protection of structural integrity of hepatocyte membrane or renewal of damaged liver cells, which may be due to the presence of flavonoids.

Histopathological Examination:

Histopathological study of the liver of the normal control group showed the normal architecture of hepatocytes with portal vein figure (2).

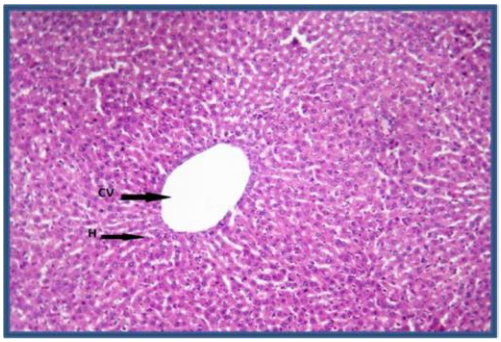

Figure-2: Section of liver in control rat showing normal architecture of liver sample, (CV) central vein (H) hepatocyte, (100X, H&E)

Liver sections of rats belonging to Group II, which are treated with CCL4showedcentrilobular necrosis, mononuclear cells infiltration (interface hepatitis) with portal inflammations show in figure (3).

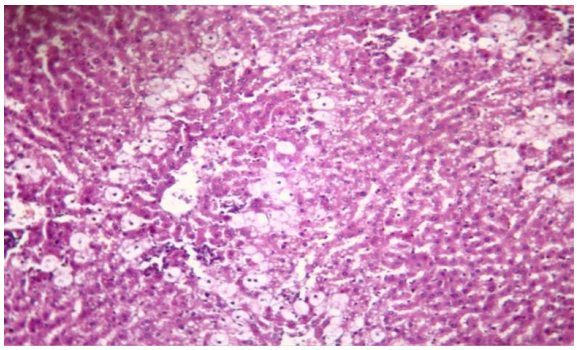

Figure 3: Section of liver in rat treated with CCL4 (Group II) showing (N) centrilobular necrosis, (C) congestion, (100X magination, H&E stain used).

The liver of rats from group III, IV which treated with TME, EAE respectively had been show congested central vein with mild mononuclear cells infiltration around ventral vein and mild portal inflammation and Group IV with minimal necrotic and inflammatory changes figures (4) and (5).

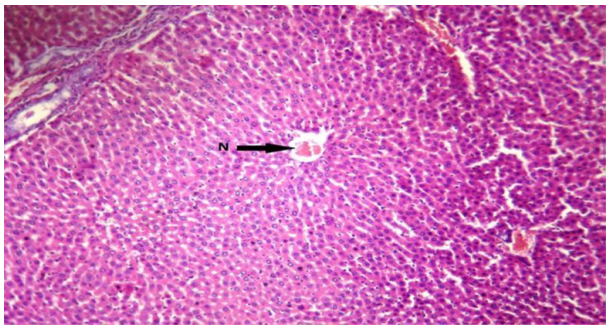

Figure 4: Section of liver in rat treated with EAE (Group III) showing congestion and mild portal inflammation, normal hepatocyte (100Xmagination, H&E stain used).

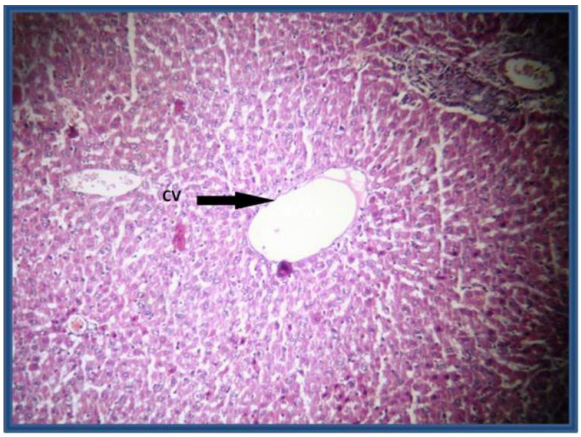

Figure (5): Section of liver in rat treated with TME (Group IV) showing, (CV) central vein with minimal portal inflammation (100X magination, H&E stain used)

Rat's liver from Group V treated with silymarin showed preserved architecture with no significant pathological change had been seen (Figure 6).

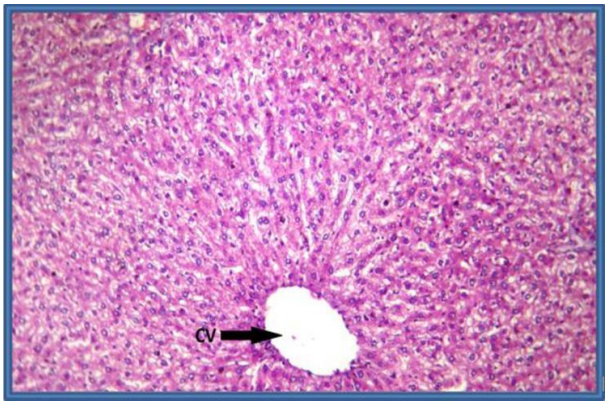

Figure 6: Section of liver in silymarin treated rats (Group VI) showing normal architecture of liver sample, no pathological change, (CV) central vein (100X magination, H&E stain were used).

This could be attributed to the formation of highly reactive oxygen species, caused by the administration of CCl4. But, post treatment with *S. irio* reduced these effects. Hence, histopathological studies revealed the post treatment of *S.irio* clearly exhibited the significant protection of liver cells, which confirmed the above

values. It is well known that plants or their extracts contain a number of chemicals belonging to different classes. Most of them are of pharmacological importance such as flavonoids, steroids, triterpenoids and their glycosides, alkaloids etc. So, the extract *S. irio*was subjected to preliminary to detect the different classes of compounds present in it. The results of the study revealed that the flavonoidal, glycosides and phenolic compounds are present in *S. irio* which can control liver diseases. Therefore, the hepatoprotective activity of *S. irio* may is attributed to aforesaid classes of compounds present in it[26].

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Source of Support: Nil, **Conflict of Interest:** None.