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Research Article

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HEPATOPROTECTIVE EFFECT OF *Carthamus tinctorius* L. AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The hepotaprotective activity of methanolic extract of flowers of *Carthamus tinctorius* was investigated in carbon tetrachloride-induced liver injury in rats. The rats were divided into four groups (I, II, III and IV) of eight each, Group I (served as normal control) received distilled water orally, Group II received CCl_4 1ml/kg as single oral dose, group III received 200mg/kg of CT extract orally for 30 successive days, Group IV received 200mg/kg of CT extract orally for 30 successive days prior to CCl_4 administration. CCl_4 trea- ted rats showed a significant elevation ($p<0.05$) in the serum level of aspartate aminotranferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TSB), and also elevation in tissue malondialdehyde (MDA) content, serum tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) with reduction of tissue glutathione (GSH) content, serum superoxide dismutase (SOD) and catalase (CAT). Pretreatment of rats with *Carthamus tinctorius* extract for 30 successive days prior to CCl_4 administration produced a significant reduction ($p<0.05$) in the level of biochemical markers, aminotransferases, TSB, lipid peroxidation product, and inflammatory cytokines with elevation in enzymatic and non enzymatic antioxidants compared with CCl_4 treated rats. Histopathological changes in liver samples were compared with control and CCl_4 treated group. The present results indicated the hepatoprotective (antioxidadant and anti-inflammatory) effect of *Carthamus tinctorius* against CCl_4-induced hepatotoxicity in rats as judged from measured serum and liver tissue biochemical parameters, and histopathological finding.

Keywords: *Carthamus tinctorius*, safflower, carthamin, safflower yellow A, hepatoprotective.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a member of the family *Compositae* or *Asteraceae.* It is an important annual industrial crop. The stem, leaves, seeds, and flowers are used for different purposes.[1] Carthamin extracted from its flowers was used in form of infusion for treatment of circulatory system related diseases.[2] Traditionally, safflower has been used for purgative and alexipharmic effects, as well as in a medicinal oil to promote sweating and cure fevers in the Middle East, India and Africa.[1] It is widely used in the treatment of many disorders and diseases including menstrual problems, cardiovascular diseases, pain and swelling associated with trauma, chronic and atrophic gastritis, rheumatism, and chronic nephritis. It has also used mainly for the treatment of cardiovascular disease because it invigorates the circulation and reduces blood cholesterol levels.[3] The major constituents of *Carthamus tinctorius* (CT) flowers are carthamin, precarthamin, anhydrosafflower yellow b, safflower yellow b, hydroxy safflower yellow a, safflomin c, in addition to flavonoids quercetin, kaempferol, and their related hydroxyl derivatives and glycosides.[4]

An *in-vivo* study for hepatoprotective activity was employed using CCl_4 as hepatotoxic. Carbon tetrachloride is reported to produce trichloromethyl radical (CCl_3*), which can initiate the oxidation of macromolecules and lipids leading to oxidative stress.[5,6] Reactive oxygen species (ROS) generated by mitochondria or from other intracellular or extracellular sites can cause cell damage and initiate various degradation processes.[7,8] The reverse of this phenomenon can be considered as the index of hepatoprotective activity.

Unfortunately, conventional or synthetic drugs used in treatment of liver diseases are inadequate and sometimes can have serious side effects. In absence of a reliable liver protective drug in modern medicine, there are number of medicinal preparation recommended for treatment of liver disorders.[9] In view of sever undesirable side effects of synthetic agents, there is growing focus to follow systematic research and to evaluate scientific basis for the traditional herbal medicines that are supposed to possess hepatoprotective activity, therefore, the methanolic extract of CT has been taken up in the present study to screen hepatoprptective agents from simple plant extract.

MATERIALS AND METHODS

Collection of plant material

The plant was cultivated in the garden of medicinal plants in the college of pharmacy / Baghdad University. The flowers were collected and dried in shade, and a voucher

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sample was kept in the department of pharmacognosy and therapeutic plant of the college.

Extraction & isolation

150 gm of the dried petals of *Carthamus tinctorius* were extracted by soxhlet using petroleum ether, ethyl acetate, and methanol successively, 1.5L from each solvent was used to extract the petals for 15 hours; each fraction was filtered and evaporated to dryness using rotary vacuum evaporator.[10]

Test Animals

Thirty two white Albino rats of both sexes, weighing 200-250 gm were used randomly in this study; they were obtained from and maintained in the Animal House of the Pharmacy College, University of Baghdad under conditions of controlled temperature. The animals were fed commercial pellets and tap water/*ad libitum*.

Drugs and Chemicals

A total of 32 rats were equally divided into 4 groups (eight rats in each group). Group I, which served as normal control, received distilled water orally. Group II, received 200mg/kg of CT extract orally for 30 successive days[11], group III, received CCl_4 1ml/kg as single dose.[12] Group IV, received 200mg/kg of CT extract orally for 30 successive days prior to CCl_4 administration.

Blood samples were collected after 24 hours from the end of each treatment by direct cardiac puncture and the serum obtained was used for biochemical parameters assay. The rats were sacrificed by cervical dislocation, the liver were removed immediately, washed with ice-cold saline and a 10% homogenate prepared for the estimation of malondialdehde (MDA), the end product of lipid peroxidation and glutathione (GSH).

Biochemical analysis

Serum level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TSB), superoxide dismutase (SOD), catalase (CAT), tumer necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were estimated using readymade kit.

Malondialdehyde level was estimated in liver tissue homogenate by a reaction with thiobarbituric acid (TBA) solution according to method of Beuge and Aust in 1978.[13] The absorbance was read at 535 nm. Glutathione level was estimated in liver tissue homogenate by a reaction with [5, 5-dithiobis-(2-nitrobenzoic acid)] (DTNB) reagent according to method of Ellman in 1959.[14] The absorbance was read at 412 nm.

Histopathological examination

Animals were sacrificed on the day of blood collection and liver was removed, sliced, and washed in saline. Liver pieces were preserved in 10% formaldehyde solution for histopathological study; tissue was prepared according to the method of Jonquiere L C *et al* in 1995[15] using paraffin sections technique. Sections made were about 4-6 µm in thickness, were stained with heamatoxylin (H) and eosin (E) then photographed to be examined under microscope.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA). P value <0.05 was considered significant.

RESULTS AND DISCUSSION

Biochemical parameters

Rats treated with CT extract alone, showed no significant change in all biochemical parameters that have been measured.

In order to probe the possible antioxidant mechanism by which CT prevents hepatic damage caused by CCl_4, investigation on tissue level of MDA and GSH were carried out. MDA level was found to be significantly elevated (158.82%, $p<0.05$) in CCl_4 treated rats while GSH level was significantly decreased (42.39%, $p<0.05$) in this group. Rats treated with CT extract prior to CCl_4 administration, showed significant decrease in MDA level, 55.39% and significant increase in GSH level, 54.4% (table **1**).

The level of antioxidant enzymes, SOD and CAT was significantly decreased (43.4% and 47.7% respectively, $p<0.05$) in CCl_4 treated rats. These enzymes level was significantly increased, 67.24% and 70.6% respectively in rats treated with CT prior to CCl_4 administration (table **1**). There was significant increase ($p<0.05$) in serum hepatic enzyme levels (AST, ALT, ALP) and TSB; 373.68%, 551.8%, 56.77%, 118.18% respectively in CCl_4 treated rats, which were significantly reduced; 75.8%, 81.07%, 49.7%, 60% respectively in rats treated with CT extract prior to CCl_4 administration (table **1**).

Table 1. The effect of carthamus tinctorius extract on plasma and liver tissue level of biochemical parameters of rats treated with CCl_4 to induced hepatotoxicity.

Parameters	Group I Control (n=8)	Group II CT (n=8)	Group III CCl_4(n=8)	Group IV CCl_4+CT (n=8)
MDA(mol/g)	34±2.7	32.87±2.03	88±5.78 ^{a*}	39.25±7.22 ^{b}
GSH(Mmol/g)	41.87±3.23	42.65±3.87	24.12±3.85 ^{a*}	37.25±4.53 ^{b}
SOD(u/ml)	76.87±4.18	88.25±5.75	43.5±5.47 ^{a*}	72.75±5.92 ^{b}
CAT(u/l)	64.87±3.83	68.12±4.79	33.25±3.77 ^{a*}	56.75±4.89 ^{b}
AST(u/l)	19±1.45	16.7±2.12	90.37±8.84 ^{a*}	21.75±3.1 ^{b}
ALT(u/l)	12.87±12.45	12.125±7.85	78.75±8.5 ^{a*}	14.9±7.05 ^{b}
ALP(u/l)	48.5±4.72	44.12±2.95	76±8.11 ^{a*}	50.25±4.16 ^{b}
TSB(mg/dl)	0.55±0.09	0.51±0.08	1.64±0.14 ^{a*}	0.66±0.01 ^{b*}
TNF-α(pg/ml)	19.75±5.07	21.57±5.07	87.12±5.07 ^{a*}	23.87±5.07 ^{b*}
IL-6(pg/ml)	40.86±12.55	41.37±12.55	155.33±12.55 ^{a*}	50.25±12.5 ^{b*}

Each value represents Mean ± standard deviation; (*): Significantly different with respect to control group ($p< 0,05$); Values with non-identical superscripts (a, b) with the same parameter; n= Number of animals.

For estimation anti inflammatory effect of CT extract, serum level of TNF- α and IL-6 was measured. The level of these cytokines were found to be significantly elevated (341.11% and 280.15% respectively, $p<0.05$) in CCl_4 treated rats, these cytokines level was significantly decreased, 72.60% and 67.64% respectively in serum of rats treated with CT extract prior to CCl_4 administration (table **1**).

Histopathological examination

Section of rat's liver treated with CCl_4 showed inflammatory cell infiltration, sinusoidal dilatation with kupffer cell (kc) hyperplasia, severs steatosis, central vein deformity, ballooning degeneration and necrosis compared with control group (figure **3**). Treatment with CT extract prior to CCl_4 administration, showed normal central vein and normal hepatocytes, mild degree of

steatosis, and dispersed necrotized cells (figure **4**) compared with control and CCl_4 treated group.

Figure 1. Liver tissue of control rats showing normal histology. (H & E, x10)

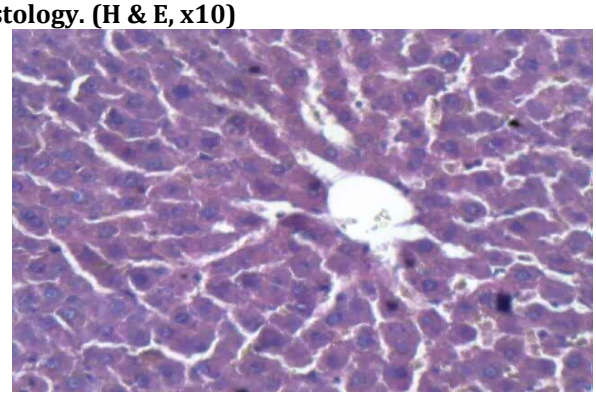


Figure 2. Liver tissue of rats treated with CT showing normal histology. (H & E, x10)

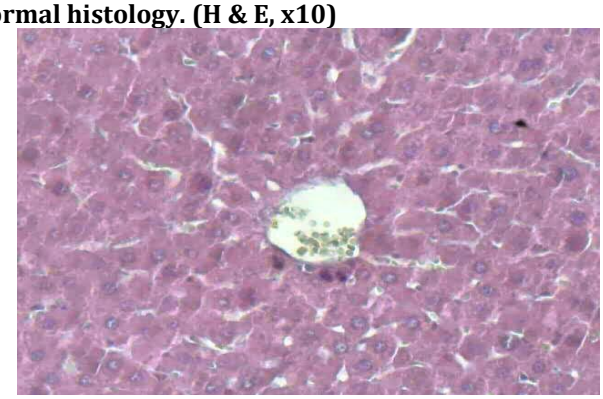


Figure 3. Liver tissue of CCl_4 treated rats showing central vein deformity, sever steatosis and inflammotary cell infiltration, and sinusoidal dilatation with KC hyperplasia. (H & E, x10).

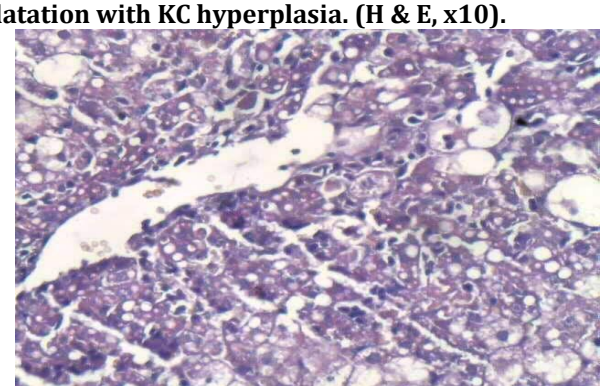
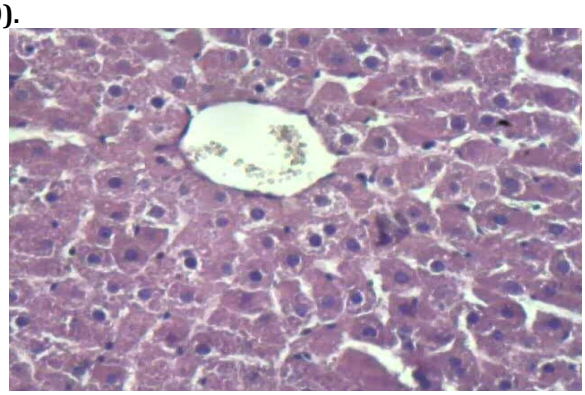


Figure 4. Liver tissue of CT and CCl_4 treated rats showing normal central vein and necrosis, hepatocyte, dispersed necrotized cells, and mild steatosis. (H & E, x10).



Hepatotoxicity of CCl_4

Carbon tetrachloride is one of the most commonly toxin used in the experimental study of liver diseases, liver injury induced are the best characterized system of xinobiotic induced hepatotoxicity and used for screening hepatoprotective activities of drugs. The basis of CCl_4 induced hepatotoxicity lies in its biotransformation by cytochrome-p450 system to two free radicals, CCl_3* and trichloromethyl peroxy radical (CCl_3OO*).[16] In the present study it has been observed that CCl_4 induced a significant elevation in MDA level in liver tissue homogenate. Increase of MDA is an index used to identify free radicals-induced injuries[17] (table **1**). The activated radicals of CCl_4 binds covalently to the macromolecules and induced per oxidative degradation of membrane lipid of endoplasmic reticulum (ER) which is rich in polyunsaturated fatty acids, this lead to formation of lipid peroxide which in turn gives MDA as end product of peroxidation.[18]

Carbon tetrachloride free radicals initiate the peroxidation of membrane poly-unsaturated fatty acids which results in the generation of reactive oxygen species which induced oxidative stress, when oxidative stress reaches a certain limit, a defense mechanism against ROS become insufficient[19], leading to a decrease in the intracellular concentration of GSH and antioxidant enzymes include SOD and CAT[20] (table **1**).

These free radicals, CCl_3* and CCl_3OO* induced cellular damage recognized by an increase in serum levels of AST, ALT, and ALP which indicates liver cell damage and leakage of enzymes from cells.[21] Total serum bilirubin was increased significantly in CCl_4 treated rats, this could be possibly be as a result of increased production of bilirubin, or decreased conjugation and secretion in hepatocytes due to hepatic cellular damage[22] (table **1**).

TNF-α and IL-6 was significantly raised in rat's serum samples in the present study. TNF-α is important pro-inflammatory cytokine synthesized by KC and released into to the blood stream either in response to necrosis or as a direct action by activated hepatotoxins, it mediated CCl_4 induced hepatic injuries and initiated cascade of cytokines includes IL-6[23,24] (table **1**), these facts support the present work and they are in consistent with previous studies.[25-27]

Hepatoprotective effects

Carthamus tinctorius L. is valuable herbal plant; it is widely used for the treatment of many cardiovascular, cerebrovascular, and gynecological diseases. Its flower contains several active phytochemical; for example, carthamin, carthamidine, isocarthamidine, safflower yellow A, safflower yellow B, safflomin C, hydroxyl safflower yellow A, tinctoramine. In addition to the flavone luteolin, lauric acid, myristic acid, palmitic acid.[28]

In the present study it was demonstrated that the treatment of rats with extract of CT resulted in decreased lipid peroxidation product (MDA) and increased GSH level with concomitant increase in antioxidant enzymes (SOD, CAT)(table **1**).

The present result showed that pretreatment with extract of CT at dose 200mg/kg restored the biochemical parameters (ALT, AST, ALP, total bilirubin) near the normal level compared with CCl_4 treated group (table **1**),

The level of inflammatory mediator TNF-α and IL-6 was significantly decreased in animals pretreated with extract of CT compared with CCl_4 treated rats (table **1**).

Histopathological finding in this study agrees with earlier reports which improved that CCl_4 causes necrosis, mononuclear cell infiltration, steatosis, foamy degeneration of hepatocytes[29] (figure **3**). Liver tissue of rats treated with extract of CT at dose 200mg/kg for 30 successive days prior to CCl_4 administration showed an

apparently normal organ with few necrotized hepatocytes and mild steatosis (figure **4**).

Generally, the hepatoprptective effect of CT occur as a result of increased level of enzymatic and non enzymatic antioxidant defense mechanisms, scavenging free radical, modulating signal transduction, these activities are responsible for antioxidant, and anti-inflammatory effects of polyphenols[30] contained in safflower flowers such as flavonoids includes carthamin, quercetin, kaempferol and phenolic acids includes caffeic acid.[31-33]

The antioxidant effect of polyphenolic compounds is mediated by scavenging free radicals generated by microsomal reduction of CCl_4[34], peroxide, and hydroperoxide thus inhibit the oxidative stress that lead to degenerative diseases.[35] The antioxidant mechanism of polyphenolics was not related to the direct scavenging activity only, but due to an increase in both tissue GSH synthesis[36], and GSH antioxidant enzymes glutathione peroxidase (GPX) and glutathione reductase (GR)[37] which are in turn lead to increase the total thiol status inside the tissue, these mechanisms are responsible for decline in MDA level and elevation in level of GSH, SOD, CAT and also responsible for decline in the level of AST, ALT, ALP, TSB due to stabilizing cell membrane.

Oxidative stress induced inflammation is mediated by the activation of nuclear factor- KB (NF-KB) which allow the expression of TNF-α, IL-6, and induced nitric oxide synthase (iNOS), Therefore, inflammatory mediators (TNF-α and IL-6) level decreased by antioxidant and anti-inflammatory effects of dietary polyphenolic compounds which mediated by inhibition of NF-KB transactivation[30]; inhibiting prostaglandin synthesis, IL-6 production, and iNOS protein synthesis.[38]

These facts related to CT are consistent with other study which proofed that medicinal plants contains phenolic compounds like flavonoids have heptoprotective effect against toxins induced liver injury.[39-41]

CONCLUSION

The present data suggest that the efficiency of *carthamus tinctorius* as hepatoprotective plant is due to presence of phenolic compounds which have antioxidant and anti inflammatory effects that have the ability to protect liver against CCl_4 induced hepatotoxicity.

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