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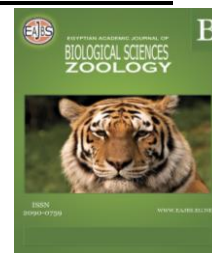


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The Protective Effect of *Moringa Oleifera* Seeds Extract on Liver Damage in Mice

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ABSTRACT

The current study sought to assess the antioxidant and hepatoprotective properties of *Moringa oleifera* seed extract versus carbon tetrachloride (CCl₄)-stimulated liver damage. **Materials & Methods:**(40) Male albino mice were separated into four groups for the investigation The control group (A) was given distilled water (DW), whereas the other three groups (B, C, and D) were administered CCl₄ intraperitoneally (IP) (0.5 ml/kg) mixed with corn oil (vol/vol), for 8 weeks, twice a week. Group (C) was given *Moringa* seed extract (1g/kg) orally every day for 8 weeks, while group (D) was given corn oil orally, and the groups (C) and (D) were compared to group (B) which was given CCl₄ IP. The researchers measured liver functions, blood-reduced glutathione (GSH), total antioxidant capacity (TAC), and malondialdehyde (MDA) in erythrocytes. Moreover, catalase enzymes in liver tissue homogenate were also assayed and superoxide dismutase (SOD), in addition to liver histopathology. **Results:** Following a CCl₄ injection, liver enzymes and MDA levels were elevated. Conversely, TAC, serum albumin, total protein, catalase, tissue SOD, and blood glutathione levels were all decreased. Administration to *Moringa oleifera* seeds extract reduces MDA levels and enzyme activity in the liver while increasing antioxidant activity. Simultaneously, after treatments with this extract, the histological damage effects of CCl₄ intoxication were also improved. **Conclusion:** Finally, *Moringa oleifera* seeds extract increased innate antioxidant activity and reduced CCl₄-induced liver damage, suggesting that it might be employed as a hepatoprotective medication in the future.

INTRODUCTION

The liver is important in the control of numerous physiological processes in our bodies. These include metabolic, and storage activities and secretory (Kumar *et al.*, 2011). It is also involved in the detoxification of a number of medicines and xenobiotics. In this instance, the liver becomes vulnerable to the toxicity of these drugs because the metabolic output of detoxifying responses might be harmful to the liver when rising (Mohammed, 2020). Carbon tetrachloride (CCl₄) is commonly used as an experimental example of liver injury. Trichloromethyl (CCl₃) and trichloromethyl peroxy (CCl₃O₂) radicals are reductive dehalogenation products that result from CCl₄ hepatotoxicity (Haytham *et al.*, 2019). These radicals may bind to proteins and fats, as well as flush out the hydrogen atom of unsaturated fatty acids, causing fat oxidation and liver damage (Amani *et al.*, 2020). Hepatocyte damage activates Kupffer cells, which release strong

early inflammatory mediators like reactive oxygen species (ROS), particularly superoxide anions, which are responsible for the generation of hydrogen peroxides (H₂O₂) and peroxy nitrates, resulting in oxidative stress (Bagali *et al.*, 2020). Antioxidants may reduce oxidative damage produced by free radicals in two ways: indirectly by improving the cell's natural defenses and/or straightway by scavenging free radicals (Kingsley, 2020). Antioxidants like superoxide dismutase (SOD) could scavenge superoxide anions, whilst glutathione reduced (GSH) is in charge of removing H₂O₂ via glutathione peroxidase. H₂O₂ is also absorbed by catalase's activity (Elgebaly *et al.*, 2018). In this way, antioxidant activity is vital in guarding versus CCl₄-induced liver damage. Treatment with phytochemicals has recently emerged as one of the greatest options for treating hepatotoxications, which are controlled by a process of eliminating free radicals (Soliman S & Soliman M, 2019). Through a free radical scavenging action, the polyphenol extract of all these phytochemicals was reported to reduce CCl₄-induced liver damage (El-seedi *et al.*, 2019). *Moringa oleifera* (MO) is a member of the Moringaceae plant family. Its leaves are high in phenolic acids, polyphenols, carotenoids, flavonoids, vitamins, and alkaloids (Qian *et al.*, 2022). As a result, the MO plant is utilized as a nutritional supplement for both animals and humans (Trigo *et al.*, 2022). It has long been utilized as a traditional medicinal source and was used to heal a variety of ailments, earning it the moniker "wonder tree." Following that, the leaves were investigated for anti-inflammatory, anticancer, hepatoprotective, and antifungal effects (Mollel *et al.*, 2021). Previous research has shown that an ethanolic extract of MO seeds may successfully protect hepatic tissue against antitubercular medicines and acetaminophen-induced tissue damage (Islam *et al.*, 2021). As a result, it is now well accepted that CCl₄-mediated hepatotoxicity is caused in part by the formation of reactive oxygen species, and that MO seeds ethanolic extract contain potent antioxidative compounds. The main goal of this study is to explore if the seed extract of this plant may help with CCl₄-induced liver damage.

MATERIALS AND METHODS

The seeds of MO were collected from Baghdad University, Jadiriya, the sample was identified and classified by the University of Baghdad grassland, college of science.

Chemicals:

Sigma Aldrich (Germany), supplied carbon tetrachloride (CCl₄). Aspartate transaminase (AST) and Alanine transaminase (ALT) activities in serum were assayed (Reitman & Frankel, 1957). While Alkaline Phosphatase (ALP) serum level is estimated by the principle of (Tietz, 1976). Serum albumin and total protein were performed by the method (Doumas *et al.*, 1971). and (Gornall *et al.*, 1949). Total antioxidant capacity (TAC) is purchased from Sigma Aldrich (Germany). Other compounds employed in this study were of the highest analytical quality available.

Preparation of Extract:

Two kilos of dried seeds were extracted for 48 hours with 2 L of 70% (v/v) ethanol. The extract was then purified and dried at 40 °C in a rotary evaporator beneath low pressure. The extract was made by icing the concentration and then draining it into a powder (Kadry & Toson, 2016). The yield per 1000 g of dehydrated seeds was 65 g. (6.5 %). A sample of 1 g of MO crude extract was pendent in 10 ml of DW before administration.

Experimental Animals:

A total of 40 male mice were used in this study were albino at the age of (8-10) weeks and average weight (21±6 g), the mice were housed in polypropylene cages under

controlled conditions at temperature (25-28) C° with a 12/12 hr light/ dark cycle. Mice were acclimatized condition for 14 days before the commencement of the experiment.

Experimental Design:

Forty albino mice were randomly divided into four groups (10 / group).

Group A: The control group has administered 0.1 ml of DW daily for 8 weeks.

Groups B, C, and D: mice of these Groups were injected i.p. CCl₄ (0.5ml CCl₄/kg b.wt) mixed in corn oil v/v twice a week for 8 weeks (Nandakishor, 2014).

Group (C) orally treated with MO seed extract (1g/kg) daily for 8 weeks (Kou *et al*, 2020). and group (D) orally treated with corn oil via a stomach tube and compared groups (C) and (D) to group (B) was treated IP. with CCl₄.

Samples:

After the trial, all animals were starved for 12 hours before being slaughtered under chloroform anesthesia. In clean, dry tubes with or without EDTA, all blood was taken. Glutathione reduction (GSH) was determined in all blood. Plasma and Serum samples were then collected by centrifugation for 15 min. at 4000 rpm and they were preserved in Eppendorf tubes and stocked at -20 ° C until wanted for check of biochemical parameters. The determination of ALT, AST, ALP, and MDA, while, total protein, albumin, and total antioxidant capacity were made using the lower erythrocyte coat in the EDTA tubes assayed in mice serum. For histological examinations, large segments of the liver were taken, washed in normal saline, and preserved in 10% formalin. At the same time, the specific weight of liver tissue of each mouse was washed in physiological saline and then mixed thoroughly in ice-cold phosphate buffer (50 mM, pH 7.5). The mixture (10 percent, w/v) was centrifuged at 12000 rpm for 20 minutes at 4 °C in a cooling centrifuge, and the supernatant was reaped and stored at -20 °C for subsequent biochemical assays,

Statistical Analysis:

The Statistical Analysis System (SAS, 2012). Was used to affect different factors in study parameters. A low significant difference (LSD) test at (P<0.01) was used to significant contrast between means in this study.

RESULTS AND DISCUSSION

Effect of MO Treatment on the Liver Functions:

Table 1 shows the effect of CCl₄ which caused a significant increase (p<0.001) In comparison to the MO group, there was a substantial decrease in serum ALP, ALT, and AST activity, as well as a significant drop in total proteins and serum albumin levels. When compared to mice given CCl₄, MO exhibited big improvements in each of these liver function tests. Carbon tetrachloride (CCl₄) is among the most often utilized hepatotoxins in investigations looking into the effects of free radicals and oxidative stress on the liver (Vani, *et al*, 2019). It's because CCl₄ metabolism starts with the creation of the trichloromethyl free radical (CCl₃), which is produced by the blended function of the cytochrome P450 oxygenase system. This free radical combines quickly with oxygen to form the trichloromethyl peroxy radicals, which respond quickly (CCl₃OO) (Vidičević *et al.*, 2019). In the present study, the serum activities of AST, ALT, and ALP were elevated after CCl₄ intoxication, a procedure that involves the release of cytosolic enzymes such as AST, ALT, and ALP, as well as an increase in serum levels. As a result, measuring the activity of these serum enzymes may aid in the assessment of liver functioning (Kumar *et al*, 2009). Furthermore, the decrease in serum total protein and albumin concentrations following CCl₄ intoxication might be attributable to significant liver damage caused by cellular membrane inflammation, lipid

peroxidation, regression, and/or inactivation of synthetic functions. The latter might be owing to trichloromethyl free radical coupling with the cell membrane (Rahmat, & Choudhary, 2014). Any hepatoprotective drug's effectiveness is determined by its ability to either diminish or restore normal average liver physiology that has been disrupted by CCl₄ and/or additional hepatotoxicants (Dineshkumar *et al.*, 2013). After CCl₄ intoxication, MO treatment results in considerable improvements in blood levels of AST, ALT, ALP, total protein, and albumin. The decrease in the activities of AST, ALT, and ALP resulting from MO seeds extract leads to an early improvement in the liver cell's cellular membrane safety, which is clear evidence of the drug's anti-hepatotoxic effect. The results of this investigation contradicted those of earlier studies, which revealed that an extract of MO seeds reduces high levels of AST, ALT, and ALP in mice inebriated with acetaminophen, Diclofenac, cadmium chloride, and alcohol. (Páramo *et al.*, 2019). The plant extract (D) protects the hepatocellular membrane's integrity of the structure and prevents the enzymes from leaching into the bloodstream. Our findings support the widely held belief that transaminase rates return to their normal with the repair of hepatic parenchyma and the renewal of hepatocytes (Guo *et al.*, 2020).

Table 1: Comparison between different groups in Liver Enzymes, Albumin, and Total protein

Group	Mean ± SE (gm)				
	ALP	ALT	AST	Albumin	T.Protein
Control (A)	79.80±3.47 ab	40.28 ±5.11 b	31.22 ±4.83 b	4.46 ±0.30 a	7.42 ±0.51 a
CC14 (B)	201.2± 0.72 c	79.80 ±3.47 a	66.06 ±7.42 a	2.16 ±0.26 b	3.90 ±0.37 b
MO (C)	81.17±3.25 b	37.07 ±5.31 b	33.31±2.65 b	4.34 ±0.32 a	7.12±0.55 a
corn oil (D)	209.55 ±4.04 c	69.99 ±2.19 a	63.85 ±3.62 a	2.29±0.38 b	3.62 ±0.46 b
LSD value	10282 **	12.657 **	14.885 **	0.961 **	1.434 **
P-value	0.0001	0.0001	0.0001	0.0001	0.0001
This means having the different letters in the same column differed significantly. ** (P≤0.01).					

Effect of MO Treatment on The Mean Activities of MDA, TAC, and SOD, GSH, as well as Catalase Enzymes:

Table 2 showed that the intoxication of mice with CCl₄ induce a significant (P<0.001) increase in MDA level, a significant (P<0.01) decrease in TAC concentration, and activities of both hepatic SOD and GSH as well as catalase enzymes content compared with those of MO group. The administration of MO extract resulted in significant improvement (P<0.01) in all of these parameters compared with those of mice administered CCl₄. The process might entail the latter's involvement in the cells' scavenging abilities, and hence the decreases in their mean values induced MDA overproduction. These findings were in line with (Tili *et al.*, 2015). In hepatic tissue, CCl₄ treatment resulted in a considerable increase in MDA and a decrease in GSH content as well as the activity of antioxidant enzymes, catalase enzymes, and SOD. The preventive properties of MO seed extract may be mediated by antioxidants. The findings of antioxidant enzymes both enzymatic (catalase and SOD) and nonenzymatic (GSH), as well as overall antioxidant properties, which were shown to be increased following

treatment of CCl₄-intoxicated mice with this extract demonstrated. During the same period, the concomitant decreases in MDA levels following the latter enzymatic increases verify MO's antioxidant-mediated mechanism. Furthermore, earlier research has demonstrated. The antioxidant action of MO leaves is mostly due to their high level of phenolic compounds, which include kaempferol, quercetin, chlorogenic acid, rutin, rhamnetin, and apigenin (Oldoni *et al.*, 2021). These phenolic chemicals protect against oxidative damage in several ways. This is because they could operate as reducing agents by donating hydrogen atoms and scavenging singlet oxygen, resulting in the preservation of the generated free radicals, resulting in stable combinations that do not begin or spread oxidation (Xu *et al.*, 2019). Furthermore, the anti-inflammatory and analgesic properties of such phenolic compounds in MO must be included in the protective processes. Furthermore, phenolic substances, such as flavonoids, can conserve cells from emptying reduced glutathione by stimulating glutathione reductase and raising the action of other antioxidant enzymes, both of which are beneficial to hepatoprotection (Kalinová *et al.*, 2021).

Table 2: Comparison between different groups in Anti-Oxidant

Group	Mean ± SE				
	MDA	TAC	SOD	GSH	Catalase
Control (A)	6.19 ±0.22 c	0.858 ±0.19 a	19.05 ±1.14 a	157.94 ±130.83 a	120.19 ±7.35 a
CC14 (B)	12.99 ±0.42 a	0.034 ±0.005 b	12.19 ±0.98 c	65.51 ±47.16 c	74.09 ±3.63 c
MO (C)	6.43 ±0.42 c	1.050 ±0.011 a	21.19 ±0.43 a	156.51 ±94.97 a	137.17 ±7.21 a
corn oil (D)	11.02 ±0.52 b	0.636 ±0.23 a	16.42 ±0.67 b	97.92 ±75.38 b	98.29 ±3.76 b
LSD value	1.244 **	0.455 **	2.569 **	27.59 **	17.327 **
P-value	0.0001	0.0001	0.0001	0.0001	0.0001
This means having the different letters in the same column differed significantly. ** (P≤0.01).					

Histopathological Study:

The histopathological variations in the liver were studied in H&E-stained sections. In the group that was given CCl₄, histopathological variations included hepatic architecture disorganization with acute fatty degradation of liver cells and inflammatory cells infiltration especially macrophages, as well as the number of necrotic cells and oedema. (Fig. 1) When compared to the control group's liver, it revealed typical liver anatomy, including normal hepatocytes, normal central vein, and normal sinusoids. (Fig. 2) In the corn oil mouse group, there were binucleated hepatocytes, mononuclear cells (mainly lymphocytes), and apoptosis. (Fig 3) Using MO extract, however, showed improvement in liver histology, with most regions appearing to have healed, hepatocytes well intact, and no region of necrosis (Fig. 4). This study discovered that MO had a great protective role in mice against CCl₄-stimulated liver fibrosis. Histological studies corroborated this outcome. Hepatic fibrogenesis is widely established to be linked to liver cells necrosis and inflammation (Ogaly *et al.*, 2018). Chronic liver cells inflammation is commonly related to the evolution of hepatic fibrosis, and inflammatory cells such as lymphocytes and macrophages have been implicated in fibrosis production (Essawy *et al.*, 2012). The anti-inflammatory action of MO was validated in this work

by a reduction in ALT, ALP, and AST activity in the liver, as well as a reduction in inflammatory cell infiltrations in histological examination. As a result, MO's anti-inflammatory activity might be one of the processes behind the anti-fibrotic effect of CCl₄-induced liver fibrosis. The hepatotoxicity of CCl₄ is caused by its metabolism via cytochrome P-450, which produces extremely reactive trichloromethyl free radicals, causing membrane damage and lipid peroxidation (Popoola *et al.*, 2020). Currently, the treatment of MO seed extract prevents the rise in MDA scales and improves the depletion of SOD efficacy in the liver. This suggests that the antioxidant activity of MO extract could be the key mechanism of treatment versus CCl₄-induced toxicity and fibrosis. MO seeds contain essential bioactive substances like glucosinolates, thiocarbamates, isothiocyanates, and flavonoids, which may explain their antioxidant properties (Zhao *et al.*, 2019). These substances neutralize ROS, bind metal ions, and replenish membrane-bound antioxidants. This conclusion is consistent with prior research that found MO extract to have antioxidant properties (Adighije *et al.*, 2020). Finally, the current study indicated that MO seed extract could protect from CCl₄-stimulated fibrosis in mice through its antioxidant and anti-inflammatory effects.

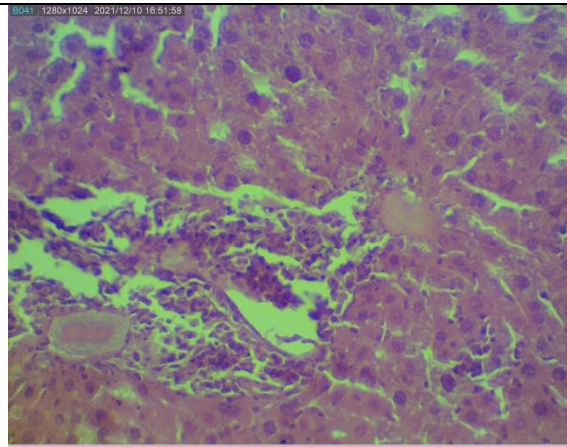


Fig. 1: Histological section in mice liver of CCl₄ group showed **a:** Odema, **b:** necrosis, **c:** macrophages cells (H&E steam) (X400)



Fig. 2: Histological section in mice liver of control group showed **a:** normal central vein, **b:** hepatocyte, **c:** sinusoids (H&E steam) (X400)

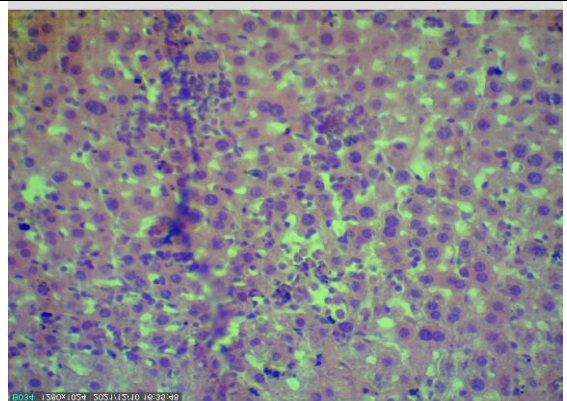


Fig. 3: Histological section in mice liver of corn oil group showed **a:** apoptosis, **b:** lymphocytes cells (H&E steam) (X400)

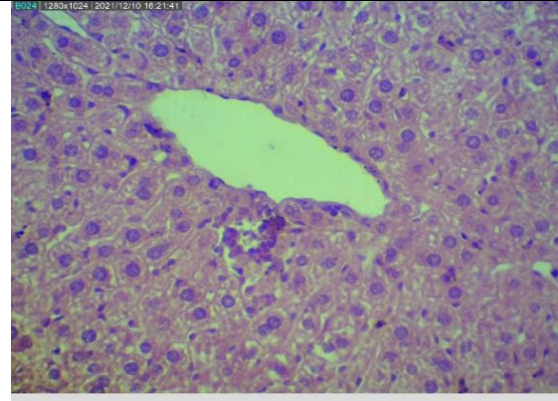


Fig. 4: Histological section in mice liver of MO extract group showed **a:** normal central vein, **b:** normal hepatocytes, **c:** slight cellular swelling (H&E steam) (X400)

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