Hepatoprotective Effects of *Moringa Oleifera* Seed Oil and Pumpkin Seed Oil on CCL_4 Induced Hepatotoxicity in Rats

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Abstract

This study was designed to estimate the hepatoprotective and antioxidant effects of *Moringa oleifera* seed oil (MOSO) and pumpkin seed oil (PSO) against the hepatic toxicity induced by CCL4 in rats. Sixty male albino rats (120 ± 10 g) were randomly divided into 6 equal groups. Group 1 was the control group and was orally given corn oil once daily for 28 days. Group 2 was given MOSO at a dose of 0.2 ml/rat, while group 3 received PSO (300 mg/kg b. wt.) once daily for 28 days. Group 4 received CCL4 i/p at a dose of 2.5 ml/kg b. wt. on days 13 and 14 of the experiment and was designated as a positive control group. Groups 5, and 6 received MOSO and PSO at the same dose, route, and duration as groups 2 and 3 and were injected with CCL4 i/p at a dose of 2.5 ml/kg b. wt. on days 13 and 14 of the experiment. The results showed that oral administration of MOSO and PSO to CCL4-intoxicated rats on day 28 of the experiment significantly reduced the elevated levels of liver enzymes and bilirubin and elevated serum levels of total protein, albumin, and globulin, as well as a significant improved in lipid profile compared to the CCL4-intoxicated group. Furthermore, GSH and CAT levels were significantly higher in the treated groups, while MDA levels were significantly lower compared to the CCL4 intoxicated group. The histopathological examination of liver sections in rats pretreated with MOSO and PSO showed favorable changes in the degenerative changes induced by CCL4. Our results propose that oral administration of *Moringa oleifera* and pumpkin seed oils have potent antioxidant and hepatoprotective effects against CCL4 toxicity in rats.

Keywords: carbon tetrachloride, *Moringa oleifera*, pumpkin seed, hepatotoxicity, rats.

Introduction

The liver is a multipurpose organ that is crucial in controlling the body's internal chemical environment. It serves as a hub for the metabolism of nutrients like lipids, carbohydrates, and proteins as well as waste products, detoxification, the removal of medicines and xenobiotics from the body ***(Roy et al., 2012)***. The major cause of the liver disorder is exposure to environmental pollutants and xenobiotics such as alcohol, carbon tetrachloride, and paracetamol that cause liver damage by producing reactive oxygen species, which are extremely toxic ***(Al-Seeni et al., 2016).***

Carbon tetrachloride (CCL_4) is widely used to induce hepatotoxicity in experimental animals. CCl_4 belongs to the class of hepatotoxins, which act after metabolic activation. It is metabolized in the endoplasmic reticulum by cytochrome P450 enzymes (mostly CYP2E1) to the highly reactive trichloromethyl radical (CCl3•). CCl3• rapidly reacts with oxygen to form the highly reactive trichloromethyl peroxyl radical (CCl3OO•), which rapidly reacts with lipids to form lipid peroxidation products. CCL_4 hepatotoxicity is characterized by hepatocellular necrosis with fat deposition ***(Adewale et al., 2014)***.

The majority of synthetic medications used to treat liver lesions can result in severe liver damage ***(Saleem et al., 2010)***. Phytochemicals are one of the finest options for treating hepatotoxicity, particularly those brought on by free radicals. It was determined that phytochemical polyphenol extract reduced CCL_4 hepatotoxicity ***(El-bakry et al., 2016)***.

Moringa oleifera (MO) belongs to the *Moringaceae* family. MO is one of the most beneficial trees in the world since almost every component of the tree has useful qualities. Young leaves are consumed as a salad, while shade-dried leaves are utilized as a natural source of vitamin A, C, and E, calcium, protein, amino acids, polyphenols, and antioxidants. As a result, it is utilized as a natural source of growth promotors, nutritional supplements, and antioxidant agents ***(Olatosin et al., 2013; Singh et al., 2014)***.

The pumpkin belongs to the *Cucurbitaceae* family ***(Bardaa et al., 2016).*** Pumpkin seed oil (PSO) has high amounts of antioxidant vitamins such as α- and γ-tocoferol, β-carotene and vitamin E. Additionally, it has high concentrations of lutein and selenium as well as phenolic substances such as vanillic acid, tyrosol, and vanillin. It has higher levels of squalene than other seed oils, a carbon organic molecule with numerous industrial applications. It is a rich source of proteins and phytosterols. The presence of all these constituents in PSO, clarify their beneficial and valuable effects on human health ***(Stevenson et al., 2007; Ali and Abdelzaher, 2017)***.

This study examined the hepatoprotective effects of *Moringa oleifera* seed oil and pumpkin seed oil in terms of serum biochemical parameters such as liver enzymes, bilirubin levels, proteinogram, lipid profile, as well as histopathological examination against CCL4-induced hepatotoxicity in rats.

Materials and Methods

Chemicals

biochemical parameters were obtained from Sigma Pharmaceuticals (USA) and Roche Diagnostics (Germany), respectively, while malondialdehyde (MDA), reduced glutathione (GSH), and catalase (CAT) kits were obtained from Bio-diagnostic (Giza, Egypt).

Plant oil

Moringa olifera seed oil (MOSO) was obtained from Genera Nutrients Private Limited (India). Pumpkin seed oil (PSO) was obtained from Imtenan (Egypt).

Animals

Sixty male Wister rats weighing 120±10 g were obtained from the Laboratory Animals house at Zagazig University's Faculty of Veterinary Medicine in Egypt. The animals were kept in a controlled laboratory environment with a temperature of 20-25 °C, a 12h light/dark cycle, and 50% humidity, as well as free access to food and water. They were housed in plastic cages and acclimatized for 10 days prior to the experiment. The experimental design was approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt (approval no. 201906). They were given human care, and the experiment was carried out in accordance with institutional guidelines.

Experimental design

The sixty rats were randomly divided into six equal groups. Group 1 received vehicle (corn oil) orally every day for 28 days and served as the control group. Group 2 (MOSO group) received *Moringa oleifera* seed oil (MOSO) orally at a dose of 0.2 ml/rat daily for 28 days ***(Al-Said et al., 2012).*** Group 3 (PSO group) received pumpkin seed oil (PSO) orally at a dose of 300 mg /kg b. wt. daily for 28 days ***(Elmeligy et al., 2019)***. Group 4 (CCL_4 group) received an intraperitoneal injection of CCL_4 (2.5 ml/kg body weight) dissolved in corn oil (30%) on days 13 and 14 of the experiment ***(Elmeligy et al., 2019)***. Group 5 (MOSO+CCL_4 group) and group 6 (PSO+CCL_4 group) received MOSO and PSO daily for 28 days at the same doses as groups 2 and 3, as well as an i/p injection of CCL_4 on days 13 and 14 of the experiment.

Blood sampling

Five overnight-starved rats from each group were randomly selected on days 15 and 28 of the experiment. Isoflurane was used to anesthetize the rats, and blood samples were taken from their hearts in sterile, clean tubes without anticoagulants. The blood samples were left to clot at room temperature for 30 minutes

then centrifuged for 10 minutes, at 3000 rpm. Sera were collected and stored at -20 °C until the biochemical analysis.

Tissue specimens

Liver specimens were collected, washed with iced buffer saline several times, and plotted with filter paper, then divided into two parts. The first part was fixed in 10% formalin for histopathological examination. The second part of the liver was homogenized with cooled phosphate buffer solution using a homogenizer, then centrifuged (2000 rpm for 5 min) at 4°C using a cooling centrifuge to get rid of cell debris according to ***Lin et al. (1998)***. The homogenate was preserved in -20 °C until determination of MDA, GSH, and CAT.

Hepatic enzymes and bilirubin assay

Serum ALT, AST, and ALP activities were estimated calorimetrically by the method of ***Bakker and Mücke (2007).*** Total and direct bilirubins were estimated calorimetrically by the methods of ***Bakker and Mücke (2007)*** and ***Löhr et al. (2009)*** respectively. Indirect bilirubin was calculated according to ***(Water and Gerade, 1970)***.

Proteinogram assessment

Serum total protein was measured according to ***Bakker and Mücke (2007)***. Albumin was assayed according to ***Junge et al. (2007)***. Globulin concentration was calculated by subtracting the albumin from the total protein ***(Busher, 1990)***. Then the albumin/globulin ratio was calculated ***(Duran et al., 2014).***

Lipid profile

Serum total cholesterol, triglycerides and HDL-cholesterol were measured by the method of ***Bakker and Mücke (2007), Dastych et al. (2014)*** and ***Rohatgi et al. (2014)*** respectively. Serum LDL cholesterol was calculated according to the Friedewald formula (LDL-cholesterol = total cholesterol – HDL – VLDL) ***(Sibal et al., 2010)***. Serum VLDL-cholesterol was calculated by the equation (VLDL-cholesterol = triglycerides/5) ***(Meeusen et al., 2014)***.

Hepatic MDA and antioxidant biomarkers

The levels of the hepatic MDA, GSH, and CAT activity were assessed according to ***Ohkawa et al. (1979)***, ***Beutler (1963),*** and ***Aebi (1984)*** respectively.

Histopathological examination

Liver specimens were rinsed in saline and then fixed in 10% buffered formalin. After fixation, samples were impregnated with molten paraffin wax, then embedded and blocked out. The 5-µm thick paraffin sections were cut and stained with haematoxylin and eosin for microscopical examination ***(Suvarna; et al., 2013)***.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20. Quantitative data was described using mean and standard error. The significance of the obtained results was judged at the 5% level. F-test

one-way analysis of variance (ANOVA) was used to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons ***(Landau and Everitt, 2003)***.

Results

Tables 1-2 show the effects of different treatments on serum liver enzymes, bilirubin and proteinogram on days 15 and 28 of the experiment. Oral administration of MOSO or PSO revealed no significant differences in liver enzymes, bilirubin levels, and proteinogram parameters compared to the control rats. However, the CCL_4 intoxicated group had significantly higher liver enzymes, total, direct, and indirect bilirubin levels, compared to the control group. On day 15, CCL_4-intoxicated group showed a significant reduction in total protein, albumin, and A/G ratio with non-significant change in globulin level, while on day 28, a significant decrease in serum levels of total protein, albumin, and globulin with non-significant change in A/G ratio compared to the control. The group 5 and group 6 showed a significant reduction in serum liver enzymes activity and bilirubin levels with marked elevation in proteinogram parameters compared to CCL_4-intoxicated rats (gp.4).

Tables 3-4 illustrate the influence of different treatments on serum lipid profile on days 15 and 28 of the experiment. No significant variations were recorded in lipogram group 2 &3 on days 15 and 28 post-treatments. However, a significant elevation in cholesterol, TG, LDL, and VLDL levels with a significant decrease in serum HDL level were recorded in CCL_4-intoxicated rats in contrast to control. Meanwhile, on day 15 of the experiment MOSO+CCL_4 group and PSO+CCL_4 group showed significant decrease in the cholesterol and LDL with significant increase in HDL and non-significant change in TG, and VLDL comparatively with CCL_4-intoxicated group. After 28 days, MOSO+CCL_4 group significantly decreased cholesterol, LDL, and increased serum HDL levels with non-significant changes in TG, and VLDL when compared to intoxicated group. Moreover, PSO+CCL_4 group revealed significant decrease in cholesterol, TG, LDL, VLDL, with a significant increase in HDL compared to the intoxicated group.

Tables 5-6 show how different treatments affected hepatic MDA and antioxidant biomarkers on days 15 and 28 of the experiment. MOSO and PSO groups showed no significant differences in MDA, GSH, and CAT levels on days 15 and 28. When compared to the control, the CCL4-intoxicated group had significantly higher MDA levels and significantly lower GSH and CAT levels. On the other hand, administration of MOSO or PSO to CCL_4-intoxicated rats resulted in a

significant reduction in MDA and a significant increase in GSH and CAT tissue levels compared to CCL_4-intoxicated rats.

Histopathological results:

Normal control group liver tissue revealed normal hepatic structure (Fig. 1A). MOSO or PSO liver tissue with normal hepatic structure and no pathological lesion (Fig.1 B&C). CCL_4-intoxicated liver tissue showed degenerative and necrotic changes, as well as moderate fibroblastic proliferation and early collagen deposition, resulting in the formation of distinctive fibrous strands surrounding individual or groups of hepatic lobules (Fig. 1D). The liver tissue of the MOSO+CCL_4 group revealed mild congestion in the portal vein, as well as a few inflammatory cells infiltrating the portal area with large hyperchromatic nuclei (Fig. 1E). The liver tissue of the PSO+CCL_4 group revealed mild effects of CCL_4 toxicity in the form of biliary profiliration, portal vascular dilatation, and mild vacuolation of the hepatic cells with prominent centrally located nuclei (Fig. 1F).

Table (1): Changes in liver function tests in different treated groups after 15 days:

Groups	ALT (U/	AST (U/	ALP (U/	Total bilir	Direct bilir	Indirect bilir	Total pro	Albumin	Globulin	A/G
Control	40.20^{c}±	185.0^{c}±	235.0^{c}±	0.11^{d}±	0.04^{cd}±	0.08^{d}±	6.38^{ab}	4.18^{a}±	2.20^{ab}±	1.92^{a}±
MOSO	49.20^{bc}±	189.8^{c}±	229.8^{c}±	0.12^{d}±	0.04^{cd}±	0.08^{d}±	6.50^{ab}	4.24^{a}±	2.24^{ab}±	1.91^{a}±
PSO	39.0^{c} ±	184.2^{c}±	232.0^{c}±	0.10^{d}±	0.02^{d}±	0.08^{d}±	6.58^{a}±	4.30^{a}±	2.28^{a}±	1.90^{a}±
CCL_4	80.0^{a}±	308.6^{a}±	325.6^{a}±	0.52^{a}±	0.26^{a}±	0.25^{a}±	4.12^{d}±	2.24^{c}±	1.86^{b}±	1.20^{b}
MOSO +CCL_4	56.20^{b}±	224.6^{b}	247.8^{bc}±	0.28^{b}±	0.13^{b}±	0.15^{b}±	6.08^{bc}	3.90^{ab}±	2.18^{ab}±	1.79^{a}±
PSO+ CCL_4	58.80^{b}±	244.0^{b}	267.8^{b}±	0.19^{c}±	0.07^{c}±	0.12^{c}±	5.70^{c}±	3.68^{b}±	2.02^{ab}±	1.84^{a}±

Data are expressed as means ± SEM (n = 5)

Means in the same column with common having different superscript letters are significant at $p \leq 0.05$

MOSO= *Moringa oleifera* seed oil PSO= pumpkin seed oil

CCL_4= Carbon tetrachloride.

ALT= Alanine aminotransferase AST= Aspartate aminotransferase

ALP=Alkaline phosphatase

Table (2): Changes in liver function tests in different treated groups after 28 days

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G
Control	**$49.60^{c} \pm 2.11$**	**$175.4^{cd} \pm 5.54$**	**$190.0^{d} \pm 5.28$**	**$0.13^{c} \pm 0.00$**	**$0.04^{c} \pm 0.00$**	**$0.09^{c} \pm 0.00$**	**$6.36^{a} \pm 0.05$**	**$4.28^{a} \pm 0.05$**	**$2.08^{a} \pm 0.02$**	**$2.06^{a} \pm 0.04$**
MOSO	**$60.0^{bc} \pm 2.83$**	**$170.2^{d} \pm 2.94$**	**$180.0^{d} \pm 3.03$**	**$0.13^{c} \pm 0.00$**	**$0.04^{c} \pm 0.00$**	**$0.09^{c} \pm 0.00$**	**$6.54^{a} \pm 0.07$**	**$4.30^{a} \pm 0.08$**	**$2.24^{a} \pm 0.04$**	**$1.92^{a} \pm 0.07$**
PSO	**$54.80^{bc} \pm 2.78$**	**$181.4^{cd} \pm 3.61$**	**$199.0^{d} \pm 3.27$**	**$0.11^{c} \pm 0.00$**	**$0.02^{c} \pm 0.00$**	**$0.09^{c} \pm 0.00$**	**$6.58^{a} \pm 0.09$**	**$4.36^{a} \pm 0.07$**	**$2.22^{a} \pm 0.04$**	**$1.95^{a} \pm 0.04$**
CCL_4	**$82.40^{a} \pm 2.06$**	**$326.8^{a} \pm 6.18$**	**$323.0^{a} \pm 7.53$**	**$0.45^{a} \pm 0.01$**	**$0.23^{a} \pm 0.01$**	**$0.22^{a} \pm 0.01$**	**$4.21^{c} \pm 0.09$**	**$2.70^{c} \pm 0.06$**	**$1.50^{b} \pm 0.04$**	**$1.81^{a} \pm 0.06$**
MOSO+CCL_4	**$61.0^{b} \pm 3.21$**	**$203.6^{b} \pm 4.47$**	**$225.6^{c} \pm 3.14$**	**$0.27^{b} \pm 0.01$**	**$0.13^{b} \pm 0.01$**	**$0.15^{b} \pm 0.01$**	**$6.30^{ab} \pm 0.04$**	**$4.18^{ab} \pm 0.07$**	**$2.12^{a} \pm 0.10$**	**$1.95^{a} \pm 0.13$**
PSO +CCL_4	**$52.20^{bc} \pm 1.66$**	**$195.0^{bc} \pm 4.39$**	**$270.2^{b} \pm 5.74$**	**$0.27^{b} \pm 0.01$**	**$0.12^{b} \pm 0.01$**	**$0.15^{b} \pm 0.01$**	**$6.02^{b} \pm 0.06$**	**$3.90^{b} \pm 0.07$**	**$2.12^{a} \pm 0.04$**	**$1.86^{a} \pm 0.07$**

Data are expressed as means ± SEM (n = 5)
Means in the same column with common having different superscript letters are significant at $p \leq 0.05$
MOSO= *Moringa oleifera* seed oil PSO= pumpkin seed oil
CCL_4= Carbon tetrachloride
ALT= Alanine aminotransferase AST= Aspartate aminotransferase
ALP=Alkaline phosphatase

Table (3): Changes in serum lipid profile in different treated groups after 15 days:

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	$105.8^{c} \pm 2.71$	$51.0^{b} \pm 3.75$	$41.0^{a} \pm 2.70$	$54.60^{b} \pm 2.49$	$10.20^{b} \pm 0.75$
MOSO	$111.1^{bc} \pm 2.87$	$66.20^{b} \pm 3.35$	$42.0^{a} \pm 1.64$	$55.88^{b} \pm 2.99$	$13.24^{b} \pm 0.67$
PSO	$104.6^{c} \pm 2.42$	$60.60^{b} \pm 2.60$	$39.80^{a} \pm 1.74$	$52.68^{b} \pm 3.79$	$12.12^{b} \pm 0.52$
CCL_4	$188.4^{a} \pm 11.03$	$96.60^{a} \pm 4.30$	$30.40^{b} \pm 0.93$	$138.7^{a} \pm 10.81$	$19.32^{a} \pm 0.86$
MOSO+CCL_4	$111.4^{bc} \pm 4.04$	$89.20^{a} \pm 3.51$	$41.0^{b} \pm 3.21$	$52.56^{b} \pm 2.63$	$17.84^{a} \pm 0.70$
PSO+CCL_4	$130.4^{b} \pm 2.91$	$85.40^{a} \pm 3.25$	$43.20^{a} \pm 1.39$	$70.12^{b} \pm 2.12$	$17.08^{a} \pm 0.65$

Data are expressed as means ± SEM (n = 5)
Means in the same column with common having different superscript letters are significant at $p \leq 0.05$

MOSO= *Moringa oleifera* seed oil PSO= pumpkin seed oil
CCL_4= Carbon tetrachloride.
HDL= High density lipoprotein LDL= Low density lipoprotein
VLDL= Very low density lipoprotein

Table (4): Changes in serum lipid profile in different treated groups after 28 days:

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	$106.8^{d}\pm4.81$	$65.0^{c}\pm3.29$	$41.40^{a}\pm2.25$	$52.40^{c}\pm2.55$	$13.0^{c}\pm0.66$
MOSO	$111.0^{cd}\pm2.35$	$63.0^{c}\pm3.05$	$40.60^{ab}\pm1.50$	$57.80^{c}\pm2.39$	$12.60^{c}\pm0.61$
PSO	$115.6^{bcd}\pm2.99$	$71.80^{bc}\pm1.39$	$44.0^{a}\pm2.0$	$57.24^{c}\pm3.18$	$14.36^{bc}\pm0.28$
CCL_4	$145.0^{a}\pm4.46$	$84.80^{a}\pm2.31$	$33.60^{b}\pm1.81$	$94.44^{a}\pm3.15$	$16.96^{a}\pm0.46$
MOSO+CCL_4	$129.4^{b}\pm3.76$	$77.40^{ab}\pm2.99$	$41.40^{a}\pm1.69$	$72.44^{b}\pm2.28$	$15.48^{ab}\pm0.60$
PSO+CCL_4	$122.8^{bc}\pm2.15$	$67.40^{bc}\pm3.36$	$42.60^{a}\pm0.93$	$66.72^{bc}\pm1.94$	$13.48^{bc}\pm0.67$

Data are expressed as means ± SEM (n = 5)
Means in the same column with common having different superscript letters are significant at $p \leq 0.05$
MOSO= Moringa oleifera seed oil PSO= Pumpkin seed oil CCL_4= Carbon tetrachloride
HDL= High density lipoprotein LDL= Low density lipoprotein VLDL= Very low density lipoprotein

Table (5): Changes in MDA and antioxidants in different treated groups after 15 days:

Groups	MDA (nmol/gm)	GSH (mg/gm)	CAT (u/mg)
Control	$0.54^{b} \pm 0.04$	$108.0^{a} \pm 5.29$	$17.71^{a} \pm 0.47$
MOSO	$0.61^{b} \pm 0.07$	$91.63^{ab} \pm 3.79$	$14.30^{ab} \pm 1.71$
PSO	$0.71^{b} \pm 0.06$	$95.52^{ab} \pm 2.48$	$14.02^{ab} \pm 0.69$
CCL_4	$23.17^{a} \pm 3.20$	$13.74^{d} \pm 0.67$	$0.48^{c} \pm 0.07$
MOSO+CCL_4	$3.36^{b} \pm0.46$	$73.25^{c} \pm 4.25$	$11.74^{b} \pm 0.79$
PSO+CCL_4	$2.42^{b} \pm0.66$	$83.27^{bc} \pm 3.38$	$10.55^{b} \pm 0.44$

Data are expressed as means ± SEM (n = 5)
Means in the same column with common having different superscript letters are significant at $p \leq 0.05$
MOSO= Moringa oleifera seed oil PSO= Pumpkin seed oil CCL_4= Carbon tetrachloride.
MDA= Malondialdehyde GSH= Glutathione CAT=Catalase

Table (6): Changes in MDA and antioxidants in different treated groups after 28 days:

Groups	MDA (nmol/gm)	GSH (mg/gm)	CAT (u/mg)
Control	$0.74^{d} \pm 0.04$	$113.0^{a} \pm 4.59$	$15.43^{a} \pm 1.40$
MOSO	$0.92^{d} \pm 0.01$	$100.9^{ab} \pm 5.0$	$14.90^{a} \pm 1.40$
PSO	$1.0^{d} \pm 0.01$	$106.3^{ab} \pm 2.0$	$11.11^{ab} \pm 0.12$
CCL_4	$18.86^{a} \pm 0.16$	$21.74^{d} \pm 5.29$	$1.42^{c} \pm 0.10$
MOSO+CCL_4	$9.11^{b} \pm 0.68$	$89.29^{b} \pm 0.46$	$10.09^{b} \pm 0.99$
PSO+CCL_4	$5.94^{c} \pm 0.63$	$70.19^{c} \pm 4.03$	$9.35^{b} \pm 0.47$

Data are expressed as means ± SEM (n = 5)

Means in the same column with common having different superscript letters are significant at $p \leq 0.05$

MOSO= Moringa oleifera seed oil PSO= Pumpkin seed oil CCL_4= Carbon tetrachloride

MDA= Malondialdehyde GSH= Glutathione CAT=Catalase

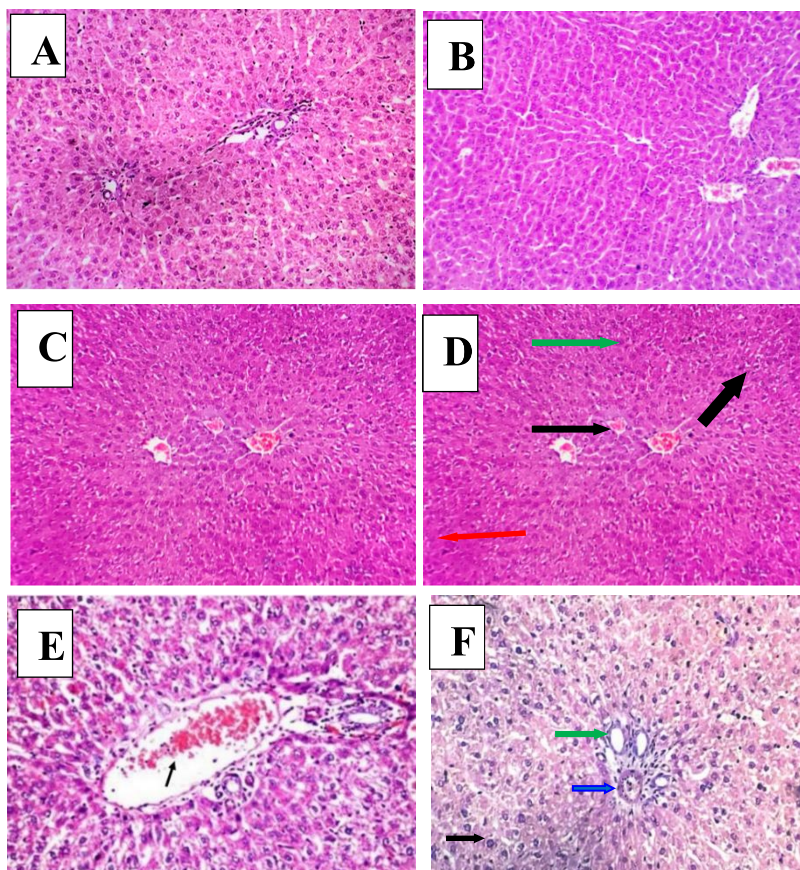

Figure 1. A: showing normal hepatic structure in control group (gp.1)**. B:** showing normal hepatic tissue of MOSO treated group (gp.2). **C:** showing no pathological lesion in hepatic tissue of PSO treated group (gp.3). **D:** showing

fibroblastic proliferation and formation of fibrous strands around hepatic lobules (black arrow), and vascular dilatation (green arrow) ballonoing degeneration (red arrow) in hepatic tissue of CCL_4-intoxicated group (gp.4). **E**: showing mild congestion in the portal vein (black arrow) associated with few inflammatory cells infiltration in the portal area in MOSO+CCL_4 group (gp.5). **F**: showing biliary profiliration (blue arrow), portal vascular dilatation (green arrow), and mild vacuolation of the hepatic cells with prominent centrally located nuclei (black arrow) in PSO+CCL_4 group (gp.6) (H&E) (all the photos X 200 except E X400).

Discussion

Natural remedies derived from the herbal plants are considered a safe and efficient choice of hepatic injury treatment ***(Olatosin et al., 2014).*** The popularity of herbal plants is rising worldwide, but more research is needed to establish their scientific efficacy, safety, and dose guidelines ***(Saleem et al., 2010)***.

CCl_4 toxicity causes a significant elevation in AST, ALT, ALP, and bilirubin with a significant reduction in proteinogram parameters as compared with control rats. Many researchers have used AST, ALT, ALP and bilirubin as useful hallmarks of CCL_4 hepatotoxicity ***(Abdel-Moneim et al., 2015)***. The toxicity of CCL_4 caused the hepatocyte membrane to lose its functional integrity, which allowed ALT & AST enzymes to leak into the blood stream ***(Oshiobugie et al., 2018)***. Elevated ALP level in serum may be attributed to increase its production due to biliary pressure ***(Taj et al., 2014)***. The increase in the bilirubin levels is considered an indicator of cholestasis, hepatic insufficiency, and increase hemoglobin destruction that produce bilirubin ***(Bishayi et al., 2002)***. The significant reduction in serum total protein, albumin, and globulin may be due to nephritic syndrome and glomerulonephritis caused by CCL_4 toxicity which led to albuminuria ***(Tokofai et al., 2021)***. Hypoproteinemia attributed to hepatic damage caused by CCL_4 metabolites that lead to protein synthesis inhibition as the liver is the main organ that responsible for synthesis of most proteins ***(Singh et al., 2014)***. Our results were confirmed by the histopathological examination which revealed periportal hepatocytes degenerative and necrotic changes, intense portal inflammatory reaction in the hepatic tissue from CCL_4-intoxicated rats. These results came in line with ***Althnaian et al. (2013)***, ***Singh et al. (2014)*** and ***Madkour et al. (2014)***. Oral administration of MOSO in CCL_4-intoxicated rats showed a marked reduction in serum ALT, AST, ALP and bilirubin levels with a significant increase in proteinogram parameters compared to CCL_4-intoxicated group. This improvement in the biochemical

parameters may be due to MO seeds constituents such as flavonoids, tannin, alkaloids and saponin that are mentioned to have antioxidant properties, inhibit cytochrome p-450 aromatase, scavenge free radicals, and inhibit ROS interaction with cell DNA, and cell membrane that inhibit lipid peroxidation, and prevent cell death ***(Biomy et al., 2018)***. These biochemical results were confirmed by histopathological examination which showed few of vacuolar degeneration and macrosteatosis, and regenerative changes in the form of cytoplasmic basophilia and large hyperchromatic nuclei. Our results were in harmony with ***Biomy et al. (2018)***, and ***Ali (2022)***. Oral administration of PSO in CCL_4-intoxicated rats demonstrate a marked amelioration in liver enzymes activity, bilirubin levels, and proteinogram matching to CCL_4- intoxicated group. This improvement may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. Furthermore, PSO's hepatoprotective effects due to its phenolic and flavonoid antioxidant activity by inhibiting cytochrome P-450 aromatase, acting as free radical scavenger that restore normal functional status of the hepatocytes and restore serum proteins levels in close to control rats**.** Serum total bilirubin reduction by PSO indicated decreased cell swelling and obstruction of bile pathway induced by CCL_4 ***(Elmeligy et al., 2019)***. Our data were confirmed by the histopathological examination, represented by a few hepatocellular hydropic degenerations, and mild biliary proliferation, with regenerative changes. Our findings agreed with earlier one ***(Abou-Zeid et al., 2018; Atasever et al., 2020)***.

Regarding to lipid profile on days 15 and 28 of the experiment, significant elevation in serum cholesterol, TG, LDL, and VLDL levels with a significant decrease in serum HDL level was recorded in CCL_4-intoxicated rats compared to the normal rats. Oxidative stress reduced the rate of lipolysis and the activity of lipolytic enzymes ***(Tokofai et al., 2021)***. The main metabolic defect caused by CCL_4 is reduction of hepatic triacylglycerols release outward resulting in TAGs accumulation in the liver, increase cholesterol synthesis, decreases TAGs hydrolysis and fatty acids ß-oxidation that leads to more fatty acids ready for esterification ***(Brai et al., 2020)***.

MOSO treatment for suppress the changes in lipid profile induced by CCL_4 toxicity by a significant decrease in serum cholesterol and LDL levels with significant increase in HDL. These results may be attributed to active phenolic components of MOSO that reduce blood lipid levels by decreasing intestinal absorption of cholesterol, LDL-C receptor expression promotion, deactivation of lipid synthesis, lipoprotein production

and increase in cholesterol disposal through bile acids ***(Tokofai et al., 2021)***. Co-administration of PSO + CCL_4 showed a significant decrease in serum cholesterol and LDL levels with a significant increase in HDL on day 15 of the experiment comparing to group with CCL_4 toxicity, but serum TG, and VLDL levels did not show any significant change compared to the CCL_4 group which need more time to return to normal values. While on day 28 of the experiment, PSO + CCL_4 group revealed a significant decrease in total cholesterol, TG, LDL, and VLDL with significant increase in HDL level compared to the CCL_4 group. PSO effect on lipid profile might be attributed to its constituent of polyunsaturated fatty acids, phytosterols, tocopherols and B-carotene. Pumpkin seed fatty acids increase the liver ability to remove bad cholesterol through proliferation of LDL- receptors in the liver ***(Ayman et al., 2019).*** Analogous results were obtained by ***Negm*** *(2018)*.

The intoxication of rats with CCL_4 revealed an increase in lipid peroxidation (MDA level) and a reduction in the antioxidants (GSH and CAT) levels in liver tissue. CCL_4 metabolism generates free radicals which cause lipid, protein, and DNA oxidative damage ***(Messarah et al., 2010)***. MDA is one of the lipid peroxidation end products that is markedly increased in rats who have consumed CCL_4 ***(Yang et al., 2015)***. GSH, a key component of the overall antioxidant defense system, decreased in the CCL_4 treated group due to its utilization by the excessively generated quantity of free radicals in the hepatocytes ***(Le et al., 2016)***. CAT is another endogenous antioxidant that participates in enzymatic protective mechanism. CAT takes the responsibility of transformation of H_2O_2 into water ***(Mantzarlis et al., 2017)***. Treatment with CCL_4 decreased the mRNA expression of the genes for antioxidant enzymes ***(Manubolu et al., 2014)***. Our outcome agrees with those of ***Al-Said et al. (2012)*** and ***Elmeligy et al. (2019)***. MOSO or PSO administration to CCL_4-intoxicated rats resulted in a significant reduction in MDA and a significant increase in GSH and CAT tissue levels compared to CCL_4-intoxicated group. *Moringa oleifera* administration significantly improved MDA as a result of its free radical scavenging and antioxidant activities due to the presence of phenolic compounds, which are multifactorial antioxidants. However, the subsequent recovery of GSH in rats treated with MO extract might be due to *de-novo* GSH synthesis or GSH regeneration (GSSG to GSH) ***(Singh et al., 2014)***. *Moringa oleifera* phenolic compounds are able to protect the cell from emptying GSH through glutathione reductase activation ***(Ali, 2022)***. Our results came in parallel to ***Olatosin et al. (2014)***. The improvement in the PSO

+ CCL_4 group might be attributed to PSO antioxidant property as it rich in essential fatty acids that preserve cell membrane fluidity ***(El-Adawy and Taha, 2001)***. Moreover, PSO is a rich source of β-carotene that has been reported to have anti-inflammatory and antioxidant activities, and vitamin E that considered as a potent antioxidant that stop free radicals' formation ***(Schafer et al., 2002)***.

Conclusion

In conclusion, the present study proved that MOSO and PSO are able to significantly alleviate the oxidative stress induced by CCL_4 in rats. Additionally, these findings demonstrated that MOSO and PSO have therapeutic effects in treating some health issues related to toxicities status induced by CCL_4, and this was demonstrated by their favorable effects on some biochemical and histopathological parameters of the rats.

References

Abdel-Moneim, A. M., Al-Kahtani, M. A., El-Kersh, M. A. & Al-Omair, M. A. (2015). Free radical-scavenging, anti-inflammatory/anti-fibrotic and hepatoprotective actions of taurine and silymarin against CCl4 induced rat liver damage. *J PLoS One, 10*(12), e0144509.

Abou-Zeid, S. M., Abubakr, H. O., Mohamed, M. A. & El-Bahrawy, A. (2018). Ameliorative effect of pumpkin seed oil against emamectin induced toxicity in mice. *J Biomedicine Pharmacotherapy, 98*, 242-251.

Adewale, O., Adekeye, A., Akintayo, C., Onikanni, A. & Sabiu, S. (2014). Carbon tetrachloride (CCl4)-induced hepatic damage in experimental Sprague Dawley rats: Antioxidant potential of Xylopia aethiopica. *The Journal of Phytopharmacology, 3*(2), 118-123.

Aebi, H. (1984). Catalase in vitro. Methods in enzymology. Elsevier.

Al-Seeni, M. N., El Rabey, H. A., Zamzami, M. A. & Alnefayee, A. M. (2016). The hepatoprotective activity of olive oil and Nigella sativa oil against CCl4 induced hepatotoxicity in male rats. *J BMC Complementary Alternative Medicine, 16*(1), 1-14.

Al-Said, M. S., Mothana, R. A., Al-Yahya, M. A., Al-Blowi, A. S., Al-Sohaibani, M., Ahmed, A. F., et al. (2012). Edible oils for liver protection: Hepatoprotective potentiality of Moringa oleifera seed oil against chemical-induced hepatitis in rats. *Journal of Food Science, 77*(7), T124-T130.

Ali, A. H. (2022). The Protective Effect of Moringa Oleifera Seeds Extract on Liver Damage in Mice. *J Egyptian Academic Journal of Biological Sciences, B. Zoology,* 14(1), 239-247.

Ali, D. & Abdelzaher, W. (2017). Possible protective effect of pumpkin seed oil against sodium nitrite in rats. *Int J Clin Pharmacol Toxicol, 6*(2), 262-269.

Althnaian, T., Albokhadaim, I. & El-Bahr, S. M. (2013). Biochemical and histopathological study in rats intoxicated with carbontetrachloride and treated with camel milk. *J SpringerPlus, 2*(1), 1-7.

Atasever, A., Senturk, M., Ekebas, G., Yaman Gram, D. & Eren, M. (2020). The Effects of Pumpkin Seed Oil on Carbon Tetrachloride Induced Chronic Hepatic Damage in Rats and Detection of Hepatic Apoptosis by Caspase Activity. *J Indian J Anim Res.*

Ayman, Z. E. E., Ayman, S. F. & Khalid, M. F. (2019). Effect of pumpkin seed oil on lipid metabolism in experimental hyperlipidemic rats. *Benha Veterinary Medical Journal, 36*(1), 302-309.

Bakker, A. J. & Mücke, M. (2007). Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. *J Clinical Chemistry and Laboratory Medicine, 45*(9).

Bardaa, S., Ben Halima, N., Aloui, F., Ben Mansour, R., Jabeur, H., Bouaziz, M., et al. (2016). Oil from pumpkin (Cucurbita pepo L.) seeds: evaluation of its functional properties on wound healing in rats. *J Lipids in health disease, 15*(1), 1-12.

Beutler, E. (1963). Improved method for the determination of blood glutathione. *J. lab. clin. Med., 61*, 882-888.

Biomy, E. S., El-Sayed, M. G. & El-Komy, A. A. (2018). Phytochemicals and protective effects of Moringa oleifera seed extract on CCl4-induced hepatotoxicity and hemotoxicity in rats. *J International Journal of Basic Clinical Pharmacology, 7*(12), 1.

Bishayi, B., Roychowdhury, S., Ghosh, S. & Sengupta, M. (2002). Hepatoprotective and immunomodulatory properties of Tinospora cordifolia in CCl4 intoxicated mature albino rats. *J The Journal of toxicological sciences, 27*(3), 139-146.

Brai, B., Falode, J., Adisa, R. & Odetola, A. (2020). Effects of aqueous leaf extract of avocado (Persea americana) on total cholesterol, triacylglycerols, protein and haematological parameters in CCl4-intoxicated rats. *J Clinical Phytoscience, 6*(1), 1-6.

Busher, J. (1990). Clinical Methods: The History, Physical, and Laboratory Examinations Walker, Hall and Hurst, Butterworths Boston.

Dastych, M., Wiewiorka, O. & Benovská, M. (2014). Ethamsylate (Dicynone) interference in determination of serum creatinine, uric acid, triglycerides, and cholesterol in assays involving the Trinder reaction; in vivo and in vitro. *J Clinical laboratory, 60*(8), 1373-1376.

Duran, A. O., Inanc, M., Karaca, H., Dogan, I., Berk, V., Bozkurt, O., et al. (2014). Albumin-globulin ratio for prediction of long-term mortality in lung adenocarcinoma patients. *J Asian Pacific Journal of*

Cancer Prevention, 15(15), 6449-6453.

El-Adawy, T. A. & Taha, K. M. (2001). Characteristics and composition of different seed oils and flours. *J Food chemistry, 74*(1), 47-54.

El-Bakry, K., Toson, E. S., Serag, M. & Aboser, M. (2016). Hepatoprotective effect of Moringaoleifera leaves extract against carbon tetrachloride-induced liver damage in rats. . *World J Pharm PharmSci, 5*, 76-89.

Elmeligy, M. H., Farid, A. S. & Fararh, K. (2019). Antioxidant and hepatoprotective effect of pumpkin seed oil in CCl4-intoxicated rats. *J Benha Veterinary Medical Journal, 36*(2), 77-89.

Junge, W., Bossert-Reuther, S. & Klein, G. (2007). Reference Range Study for Serum Albumin using different methods. *J Clin Chem Lab Med, 45*, 194.

Landau, S. & Everitt, B. S. (2003). *A handbook of statistical analyses using SPSS*, Chapman and Hall/CRC.

Le, L., Huynh, L. & Do, T. (2016). Study on hepatoprotective effect of Moringa oleifera Lam. leaves to prevent hepatocyte lesion induced by CCl. *J Pharm Sci, 43*(3), 153-163.

Lin, C., Hsu, Y., Lin, T., Hsu, F. & Hsu, H. (1998). Antioxidant and hepatoprotective activity of punicalagin and punicalin on carbon tetrachloride-induced liver damage in rats. *Journal of Pharmacy Pharmacology, 50*(7), 789-794.

Löhr, B., El-Samalouti, V., Junge, W., Maatouk, H., Halabi, A., Fahle, A., et al. (2009). Reference range study for various parameters on Roche clinical chemistry analyzers. *J Clinical laboratory, 55*(11-12), 465-471.

Madkour F.F., Khalil W. F., and Dessouki A. A. (2012). Protective Effect of Sargassum dentifolium (Phaeophyceae) in Carbon Tetrachloride Induced Hepatitis in Rats. *International Journal of Pharmacy and Pharmaceutical Sciences, 4* (3).

Mantzarlis, K., Tsolaki, V. & Zakynthinos, E. (2017). Role of oxidative stress and mitochondrial dysfunction in sepsis and potential therapies. *J Oxidative medicine cellular longevity, 2017.*

Manubolu, M., Goodla, L., Ravilla, S., Thanasekaran, J., Dutta, P., Malmlöf, K., et al. (2014). Protective effect of Actiniopteris radiata (Sw.) Link. against CCl4 induced oxidative stress in albino rats. *Journal of ethnopharmacology, 153*(3), 744-752.

Meeusen, J. W., Lueke, A. J., Jaffe, A. S. & Saenger, A. K. (2014). Validation of a proposed novel equation for estimating LDL cholesterol. *J Clinical chemistry, 60*(12), 1519-1523.

Messarah, M., Boumendjel, A., Chouabia, A., Klibet, F., Abdennour, C., Boulakoud, M. S., et al. (2010). Influence of thyroid dysfunction on liver lipid peroxidation and antioxidant status

in experimental rats. *J Experimental Toxicologic Pathology, 62*(3), 301-310.

Negm, S. H. (2018). Effect of Pumpkin (Cucurbita pepo L.) on Immune System and Liver Functions of Rats Induced with Liver Cirrhosis. *Journal of Research in the Fields of Specific Education, 17*, 273- 292.

Ohkawa, H., Ohishi, N. & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *J Analytical biochemistry, 95*(2), 351-358.

Olatosin, T., Akinduko, D. & Uche, C. (2013). Evaluation of the hepatoprotective efficacy of Moringa oleifera seed oil on CCl4-induced liver damage in Wistar albino rats. *J Int J Eng Sci, 2*, 13-18.

Olatosin, T. M., Akinduko, D. S. & Uche, C. Z. (2014). Antioxidant capacity of Moringa oleifera seed oil against CCl4-induced hepatocellular lipid peroxidation in wistar albino rats. *J European Journal of Experimental Biology, 4*(1), 514-518.

Oshiobugie, M., Olaniyi, A. & Raphael, A. (2018). Experimental and mathematical model for the hepatoprotective effect of methanolic extract of Moringa oleifera leaf against CCl4-induced hepatotoxicity in Sprague Dawley male albino rats. *J Adv Med Med Res, 26*, 1-14.

Rohatgi, A., Khera, A., Berry, J. D., Givens, E. G., Ayers, C. R., Wedin, K. E., et al. (2014). HDL cholesterol efflux capacity and incident cardiovascular events. *New England Journal of Medicine, 371*(25), 2383-2393.

Roy, S. D., Das, S., Shil, D. & Dutta, K. N. (2012). Herbal hepatoprotective agents: a review. *J World J. Pharma. Res, 1*(2), 87-99.

Saleem, M., Nazir, M., Ali, M. S., Hussain, H., Lee, Y. S., Riaz, N., et al. (2010). Antimicrobial natural products: an update on future antibiotic drug candidates. *J Natural product reports, 27*(2), 238-254.

Schafer, F. Q., Wang, H. P., Kelley, E. E., Cueno, K. L. & Buettner, S. M. (2002). Comparing β-carotene, vitamin E and nitric oxide as membrane antioxidants. *Biological Chemistry, 383*(3-4).

Sibal, L., Neely, R., Jones, A. & Home, P. (2010). Friedewald equation underestimates low-density lipoprotein cholesterol at low concentrations in young people with and without Type 1 diabetes. *J Diabetic medicine, 27*(1), 37-45.

Singh, D., Arya, P. V., Aggarwal, V. P. & Gupta, R. S. (2014). Evaluation of antioxidant and hepatoprotective activities of Moringa oleifera Lam. leaves in carbon tetrachloride-intoxicated rats. *J Antioxidants, 3*(3), 569-591.

Stevenson, D. G., Eller, F. J., Wang, L., Jane, J.-L., Wang, T. & Inglett, G. E. (2007). Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. *J Journal of agricultural food chemistry, 55*(10), 4005-4013.

Suvarna;, S. K., Christopher Layton & Bancroft, J. D. (2013).

Bancroft's theory and practice of histological techniques, [Oxford] : Churchill Livingstone Elsevier.

Taj, D., Khan, H., Sultana, V., Ara, J. & Ehteshamul-Haque, S. J. P. J. P. S. (2014). Antihepatotoxic effect of golden berry (Physalis peruviana Linn.) in carbon tetrachloride (CCl4) intoxicated rats. *J Pak J Pharm Sci, 27*(3), 491-494.

Tokofai, B. M., Idoh, K., Oke, O. E. & Agbonon, A. (2021). Hepatoprotective Effects of Vernonia amygdalina (Astereaceae) Extract on CCl4-Induced Liver Injury in Broiler Chickens. *J Animals, 11*(12), 3371.

Water, M. & Gerade, H. (1970). A colorimetric method for determination bilirubin in serum and plasma. *Micro Chem J, 15*, 231-236.

Yang, B.-Y., Zhang, X.-Y., Guan, S.-W. & Hua, Z.-C. (2015). Protective effect of procyanidin B2 against CCl4-induced acute liver injury in mice. *J Molecules, 20*(7), 12250-12265.

الملخص العربى

التأثيرات الواقية للكبد لزيت بذور المورينجا و زيت بذور اليقطين على السمية الكبدية برابع كلوريد الكربون فى الفئران

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صممت هذه الدراسة لتقييم التأثيرات الواقية لزيت بذورالمورينجا أوليفيرا وزيت بذور اليقطين في ذكور الفئران البيضاء ضد سمية رابع كلوريد الكربون. تم استخدام ستون ذكر فأرأبيض و تقسيمهم إلى 6 مجموعات متساوية. المجموعة (1): و هى مجموعة ضابطة. المجموعة (2): تلقت 0.2 مل من زيت بذور المورينجا أوليفيرا / فار عن طريق الفم يومياً لمدة 4 أسابيع. المجموعة (3): تناولت زيت بذور اليقطين بجرعه 300 مجم / كجم من وزن الجسم عن طريق الفم يومياً لمدة 4 أسابيع. المجموعه (4): حقنت الفئران برابع كلورايد الكربون في التجويف البرتونى بجرعة 2.5 مل / كجم من وزن الجسم في اليومين 13 و 14 من التجربة . اما المجموعتين الخامسة والسادسة فتم علاجهما بنفس جرعات المجموعة (2) و (3) لمدة ٤أسابيع و في اليومين 13 و 14 من التجربه تم حقن رابع كلوريد الكربون. تم تجميع مصل الدم لعمل الفحوصات البيوكيميائية ، و اختبارات الاجهاد التأكسدى في انسجه الكبد والفحص المرضي لأنسجة الكبد. أظهرت النتائج أن تناول زيت بذور المورينجا وزيت بذور اليقطين في الفئران المسممه برابع كلورايد الكربون لمده ٤ أسابيع أدى إلى انخفاض ملحوظ في المستويات المرتفعه من البيليروبين وانزيمات الكبد وارتفاع ملحوظ في البروتين الكلي ، الألبومين ، والجلوبيولين في مصل الدم و الانزيمات المضاده للاكسده في نسيج الكبد وانخفاض مستوى مالونديالديهيد في نسيج الكبد مقارنه بالمجموعه المسممه. كما اظهر الفحص النسيجى للمجموعات المعالجه بالمورينجا و اليقطين تحسنا ملحوظا مقارنه بالمجموعه المسممه. و قد خلصت هذه الدراسة الى إلى أن تناول زيت المورينجا أوليفيرا وزيت بذور اليقطين لهما تأثير قوي مضاد للأكسدة ووقائي للكبد ضد التسمم برابع كلورايد الكربون في الفئران و قد يكون مفيدًا للمرضى الذين يعانون من الإجهاد التأكسدي و امراض الكبد.