

## Research Paper:

# Hepatorenal Protective Effects of Sesame Seeds Oil, Flaxseed Oil and their Mixture against Methotrexate Toxicity in Rats



Hagar Farid Elbakry<sup>1</sup> , Hoda Abdel Rahman Abdel Salam<sup>2</sup> , Sherein Saeid Abdelgayed<sup>3</sup> , Doha A. Mohamed<sup>1\*</sup> 

1. Department of Nutrition and Food Sciences, National Research Centre, Dokki, Cairo, Egypt.

2. National Nutrition Institute, Cairo, Egypt.

3. Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.



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### \*Corresponding author:

**Doha Mohamed, PhD.**

**Address:** Department of Nutrition and Food Sciences, National Research Centre, Dokki, Cairo, Egypt.

**E-mail:** [dohamohamed@yahoo.com](mailto:dohamohamed@yahoo.com)

## ABSTRACT

**Background:** Methotrexate (MTX) is an anti-metabolite drug used in the treatment of many cancers and autoimmune diseases.

**Methods:** This study investigated the protective effect of flaxseed oil, sesame seed oil, and their mixture on the MTX-induced hepatorenal toxicity. Thirty rats divided into five groups of: normal control, MTX control, and flaxseed oil, sesame seed oil, and the mixture groups. The oils were administered to rats orally (2 ml/kg) for nine consecutive days followed by a methotrexate injection intraperitoneally (20 mg/kg) on the 9th day. Blood samples, liver and kidney tissues were collected from all rats for biochemical studies and histopathological assessments. The total phenolic content and fatty acid profiles of the oils were also determined.

**Results:** Methotrexate induced hepatorenal toxicity as evident by the histopathological assessments of liver and kidneys, elevation of liver and kidney functions' biomarkers, and increased plasma and liver oxidative stress associated with a rise in the tumor necrosis factor-alpha, as an inflammatory marker. Administration of flaxseed oil, sesame seed oil or the mixture prevented the MTX-toxicity at varying degrees as shown by reduced oxidative stress and inflammatory response, and improved liver and kidney functions. The mixture was the most efficient treatment associated with the histopathological improvements in the liver and kidney tissue samples, and all biochemical parameters tested.

**Conclusion:** Flaxseed oil, sesame seeds oil and the mixture may be used therapeutically to prevent hepatorenal toxicity induced by MTX. The effect is likely due to the presence of phenolic compounds and polyunsaturated fatty acids in the oils with antioxidant and anti-inflammatory properties.

**Keywords:** Biomarkers, Kidneys, Liver, Methotrexate, Oxidative stress, Plant oils

## Introduction

**M**ethotrexate (MTX) is an anti-metabolite drug that interferes with the metabolism of folic acid. It is used to treat cancers and autoimmune diseases, such as rheumatoid arthritis. However,

MTX is associated with hepatorenal toxicity, a major health condition that involves both liver and kidneys concurrently [1, 2]. Liver is a critical bodily organ that is essential in the digestive tract homeostasis. Having a healthy liver is crucial to the human health as it plays an essential role not only in the metabolism but also in the detoxification of xenobiotics [3].

There are numerous ecological and chemical agents, which cause hepatic injury or toxicity [4]. Despite much scientific advances made in the area of hepatology; the management of liver diseases is still a challenge to modern medicine [5]. Kidneys are considered the main organ that preserve the equilibrium and are responsible for excreting metabolic waste products from the blood [6]. Both oxidative stress and inflammatory conditions play important roles in the promotion, development and progression of kidney and liver diseases, especially hepatorenal toxicity [7-10]. Excessive production of Reactive Oxygen Species (ROS) leads to an imbalance between the body's enhanced pro-oxidant and deficient antioxidant capacities that develop during the course of kidney diseases [8]. Dietary interventions with anti-inflammatory and antioxidant properties, such as polyunsaturated fatty acids and phenolic compounds, may be used as an alternative treatment in the management of hepatorenal toxicity [5, 11, 12].

Polyunsaturated fatty acids are categorized as  $\omega$ -3 and  $\omega$ -6 fatty acids. Omega-3 fatty acids are extracted from animal products and fish oil. They contain Eicosapentaenoic Acid (EPA) ( $\omega$ -3:20:5) and Docosahexaenoic Acid (DHA) ( $\omega$ -3:22:6), and plant sources, such as flaxseed [13]. Flaxseed oil is the richest source of  $\alpha$ -linolenic acid (ALA), which is the metabolic precursor of EPA and DHA [14]. Flaxseed oil has multiple biological activities, due to the considerable content of  $\omega$ -3 and  $\omega$ -6 fatty acids [13]. It contains phenolic compounds rich in lignans, which possess anti-inflammatory, antioxidant and anti-androgenic activities [15, 16].

Sesame seed oil contains linoleic acid (C18:2,  $\omega$ -6) and oleic acid (C18:1,  $\omega$ -9), which account for 80% of all fatty acids [17]. Sesame oil, rich in valuable food components, is widely used in foods and is considered as major source of sesamol, with ample health benefits [18, 19]. The phytochemicals present in sesame seed oil are considered protective and serve synergistically as antioxidant, antihypertensive, antimutagenic, anti-inflammatory, antithrombotic, and cardio-protective agents [17, 20-22]. The optimal ratio of essential fatty acids  $\omega$ -3:  $\omega$ -6 are reported to range between 1:1 and 1:4 [23]. There is no naturally occurring oil that contains the ideal ratio of omega-3 and omega-6 fatty acids. Therefore, natural oils must be blended to obtain an ideal ratio of omega-3 and omega-6 fatty acids.

The current research aimed to investigate the protective effect of flaxseed oil, sesame seeds oil and their mixture on the MTX-induced hepatorenal toxicity. To prepare an omega-3 and omega-6 fatty acids mixture at an optimal

ratio, we blended flaxseed oil and sesame seed oil at a 1:1 ratio. Then, the total phenolic contents and fatty acid profiles of the oils were analyzed.

## Materials and Methods

**Ethical clearance:** Animal methods were accomplished in agreement with the Ethics Committee of the National Research Center and obeyed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

**Plant materials and oil extraction:** Flaxseed (*Linum usitatissimum* L.) and sesame seed (*Sesamum indicum*) were obtained from Agriculture Research Center, Giza, Egypt. Flaxseed and sesame seeds were crushed and compressed in a screw-press machine at 15rpm and 35°C. For this purpose, we used a Carver hydraulic press (Thomas Scientific, New York, USA) under 10,000 lb/square inch pressure for 1hr at room temperature according to the method of Üstun et al. [24].

**Fatty acids profile of flaxseed and sesame seeds oils:** The fatty acid methyl esters constituents of the studied oils were determined according to the Methods of AOAC [25]. The material was subjected to Gas Liquid Chromatography (GLC) analysis of the fatty acids. The identification and assessment of the methyl ester were carried out by the same experimental condition published by Mohamed et al. [26].

**Total phenolic contents of flaxseed and sesame seeds oils:** The total phenolic contents of the flaxseed and sesame oils were estimated on a spectrophotometer at 765nm, using Folin-Ciocalteu reagent [27]. The total phenolic contents were expressed as Gallic Acid Equivalents (GAE) in mg/g of the oils, and the data were expressed as the Means $\pm$ SD's.

**Preparation of the oral dose of the oils:** Flaxseed oil, sesame seed oil and their 1:1 mixture were dispersed individually in water using Tween-80 as the suspending agent to be administered orally to the rats. For the controls, the vehicle was prepared through mixing the same volume of Tween-80 in water.

**Animals:** Thirty male, Sprague Dawley rats, weighing 150 $\pm$ 8.1g were purchased from the animal house of National Research Center, Cairo, Egypt. Rats were kept in stainless steel cages and had free access to pellet food and water *ad libitum*. The experimental procedures on the animals were in agreement with the guidelines of

Ethics Committee of the National Research Centre and obeyed the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

**Diet:** All rats were fed a balanced diet (10% protein, 10% corn oil, 23.5% sucrose, 47% maize starch, 5% cellulose, 3.5% salt mixture and 1% vitamin mixture) throughout the study duration.

**Experimental design:** The rats were divided into five groups of six rats each as follows:

1. Group one was the normal control for nine days.
2. Group two was MTX control for nine days (hepatorenal toxicity).
3. Groups 3, 4 and 5 were given a daily oral dose (2 ml/kg) of either flaxseed oil (Group 3), or sesame seeds oil (Group 4) or the oils' mixture (Group 5), respectively, for nine days.

On the 10<sup>th</sup> day, rats in all groups except those in the normal controls, were injected intraperitoneally with methotrexate (20 mg/kg) for the induction of hepatorenal toxicity [28]. Rats in all groups were fed a balanced diet during the experimental period. At the completion of the study, blood samples were collected from all of the fasting rats. The plasma samples were used for the determination of urea [29], creatinine [30] as the kidney function indicator, alanine transaminase (ALT), aspartate transaminase (AST) [31], as the liver function indicator, Malondialdehyde (MDA) [32], as the lipid peroxidation indicator, Catalase (CAT) [33], and Glutathione Peroxidase (GPx) (ELISA kit, Cat No.SL1033Ra, Sunlong®) as the antioxidant enzymes. Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) was determined using ELISA kit (Cat No. SL1341Ra, Sunlong®) as an inflammatory marker. The plasma Total Cholesterol (T-Ch) [34], Triglycerides (TG) [35], High-Density Lipoprotein Cholesterol (HDL-Ch) [36], total proteins [37], albumin [38] were also determined, and the globulin and Albumin to Globulin ratio (A/G) were calculated. After collecting blood samples, the rats were sacrificed, and their liver and kidneys were removed. A portion of the liver tissue was immediately frozen from each sample for the determination of MDA [32] and CAT [33] in the tissue homogenates.

**Histopathological assessments:** Upon sacrificing the animals, the liver and kidneys were removed and fixed in Bouin's fluid. Twenty-four hours later, these tissue samples were washed three times with ethanol 70%,

dried by serially graded ethanol and then embedded in paraffin wax. For light microscopic examination, slices of paraffin sections were stained with haematoxylin and eosin, examined and photographed using a digital microscope (Olympus BX50, Japan) [39].

**Statistical analyses:** Data were expressed as the Mean $\pm$ SE and analyzed by one-way ANOVA followed by the Tukey's multiple comparison tests, using SPSS software, v. 22. The differences among the means were considered statistically significant at  $P \leq 0.05$ .

## Results

**Phenolic compounds and fatty acid profiles of flaxseed and sesame seeds oils:** The total phenolic contents of flaxseed and sesame seeds oils were  $0.85 \pm 0.108$  and  $9.27 \pm 0.531$  mg GAE/g oil, respectively. Sesame seeds oil was richer in its phenolic content than flaxseed oil.

The fatty acids profile of both flaxseed and sesame seed oils are shown in Table 1. The fatty acid profile of the flaxseed oil revealed that linolenic acid (C18:3, w-3) was its main unsaturated fatty acid constituent. Linoleic acid (C18: 2, w-6) was one of the essential polyunsaturated omega-6 fatty acids in the tested oils. Sesame seeds oil was rich in linoleic (w-6) and oleic (w-9) fatty acids, present at 41.9% and 40.37%, respectively. Palmitic acid was the major saturated fatty acid present in flaxseed oil at 5.14% and in sesame seeds oil at 10.05%.

**Protective effects against methotrexate hepatorenal toxicity:** Rats in MTX control group showed significant elevation of aminotransferase enzymes (AST & ALT). The plasma levels of creatinine and urea, as indicators of liver and kidney functions, were elevated compared to those of the normal controls (Table 2). These parameters confirmed the biochemical signs of hepatorenal toxicity. The administration of flaxseed and sesame seeds oils, and the mixture attenuated the liver and kidney functions compared to those of the control rats treated with MTX (Table 2).

The administration of MTX led to the reductions in plasma of total proteins, albumin, globulin and albumin/globulin ratio, compared to that of the normal controls (Table 3). The oral administration of flaxseed oil, sesame seeds oil and their mixture caused a significant elevation in the plasma proteins.

The plasma TNF- $\alpha$ , as an inflammatory marker, showed a significant increase in the control rats treated with MTX compared to those of the normal controls

**Table 1.** Fatty acid contents of flaxseed and sesame seeds oils

Fatty Acids	Flaxseed Oil	Sesame Seed Oil
Myristic acid: C14 (0)	0.075	0.17*
Palmitic acid: C16 (0)	5.14	10.05
Stearic acid: C18 (0)	4.48	5.73
Oleic acid: C18 (1)	15.8	40.37
Linoleic acid: C18(2)	15.31	41.9
Linolenic acid: C18 (3)	58.85	0.95
Arachidic acid: C20 (0)	0.12	0.68
Total identified saturated fatty acids	9.82	16.63
Total identified unsaturated fatty acids	89.96	83.22
Omega-3/Omega-6	3.84	0.023

\*Values are expressed as relative area percentage of total fatty acids.

(Table 4). There were an elevation of plasma and liver MDA, as the lipid peroxidation marker, and a reduction in the antioxidant enzymes, i.e., GPX and CAT in both the plasma and the liver tissue samples. The various treatments resulted in significant improvements in all of the studied parameters to varying degrees (Table 4). The MTX Treatment induced signs of hepatorenal toxicity, as evident by the elevation of the oxidative stress and inflammatory markers in the present research.

The rats in the MTX-treated group showed significant rises in the plasma T-Ch and TG, and a reduction in the plasma levels of HDL-Ch (Table 5). The administration of the various oil samples, as the therapeutic interventions, improved the plasma levels of T-Ch, TG and HDL-Ch to varying degrees.

### Histological effect

**Kidneys:** Histologically, the kidneys' microscopic examinations of the normal control rats showed signs of healthy glomeruli and renal tubules (Figure 1-A). The kidneys in the MTX-treated controls showed signs of vacuolar degeneration of the glomerular epithelia with deposition of crystals (Figure 1-B), along with congestion of the peri-tubular blood capillaries and necrosis of the renal tubules (Figure 1-C). All rats that received the oral administration of the flaxseed oil, sesame seeds oil and their mixture showed varying degrees of improvement and recovery of the examined tissues compared to those in the MTX-treated controls. The rats that received oral administration of flaxseed oil showed mild histological improvement in kidneys (Figure 1-D). These kidneys showed minimal changes including congestion in

**Table 2.** Liver and kidney functions in the different experimental groups

Groups	Mean±SE			
	AST (IU/L)	ALT (IU/L)	Creatinine (mg/dl)	Urea (mg/dl)
Normal control	53.83±1.33 <sup>a</sup>	5.83±0.65 <sup>a</sup>	0.61±0.04 <sup>a</sup>	36.67±1.20 <sup>a</sup>
MTX control	82.28±2.57 <sup>b</sup>	12.90±0.73 <sup>b</sup>	1.33±0.12 <sup>b</sup>	48.37±1.03 <sup>b</sup>
Flaxseed oil	70.75±1.47 <sup>c</sup>	7.99±0.58 <sup>cd</sup>	0.89±0.04 <sup>c</sup>	38.83±0.75 <sup>ac</sup>
Sesame oil	73.10±1.81 <sup>c</sup>	8.50±0.43 <sup>c</sup>	0.77±0.10 <sup>ac</sup>	41.50±1.15 <sup>c</sup>
Oil mixture	69.75±2.11 <sup>c</sup>	6.50±0.67 <sup>ad</sup>	0.85±0.05 <sup>c</sup>	37.17±0.48 <sup>a</sup>

Values with different superscript letters in the same columns are significantly different at P<0.05 level. MTX-control: Methotrexate control; ALT: alanine transaminase; AST: aspartate transaminase.

**Table 3.** Plasma total protein, albumin, globulin and albumin/globulin ratio of the different experimental groups

Groups	Mean±SE			
	Total Protein	Albumin	Globulin	Albumin/Globulin
Normal control	7.4±0.11 <sup>a</sup>	3.8±0.08 <sup>a</sup>	3.6±0.06 <sup>a</sup>	1.97±0.02 <sup>a</sup>
MTX control	5.3±0.31 <sup>b</sup>	2.2±0.21 <sup>b</sup>	3.1±0.20 <sup>b</sup>	0.71±0.08 <sup>b</sup>
Flaxseed oil	7.1±0.06 <sup>a</sup>	3.8±0.09 <sup>a</sup>	3.3±0.12 <sup>ab</sup>	1.87±0.05 <sup>a</sup>
Sesame oil	6.9±0.17 <sup>a</sup>	3.5±0.06 <sup>a</sup>	3.4±0.19 <sup>ab</sup>	1.98±0.06 <sup>a</sup>
Oil mixture	7.2±0.16 <sup>a</sup>	3.7±0.08 <sup>a</sup>	3.5±0.09 <sup>ab</sup>	1.96±0.02 <sup>a</sup>

Values with different superscript letters in the same columns are significantly different at P<0.05 level. MTX-control: Methotrexate control.

the glomerular and peri-tubular capillaries, and perivascular edema (Figure 1-D). The rat groups that received the oral dose of sesame seed oil showed moderate histological improvement in the kidneys, along with minimal congestion in the interstitial blood vessels (Figure 1-E). The rats that received the oil mixture revealed marked improvements with complete regression of kidneys' lesions (Figure 1-F). These kidneys showed apparently healthy renal parenchyma with normal renal glomeruli and tubules (Figure 1-F).

**Liver:** The liver tissue samples in the normal controls showed healthy polyhedral hepatocytes and central veins (Figure 2-A). The liver in the MTX-treated controls (hepatorenal toxicity) revealed congested hepatoportal blood vessels, hyperplastic bile ducts, and newly formed bile ductuli (Figure 2-B). These pathologic changes were together with large focal areas of hepatocytic necrosis plus mononuclear cells infiltration (Figure 2-C). All of the rats that received oral administration of flaxseed

oil, sesame seeds oil and their mixture showed varying degrees of improvements and tissue lesions regression compared to those in the MTX-treated controls. The rats that received the oral dose of flaxseed oil showed mild histological improvement of the liver (Figure 2-D) together with slight dilations of the hepatoportal blood vessels and hyperplasia of the bile ducts (Figure 2-D). The rats that received the oral dose of sesame seeds oil showed moderate histological improvement in the liver with minimal congestion of the central veins (Figure 2-E). The rats that received the oil mixture exhibited marked improvements and complete lesions regression in the liver with healthy hepatic parenchyma, normal hepatocytes and the central veins (Figure 2-F).

## Discussion

The sesame seeds oil, tested in this study, had a higher content of phenolic compounds compared to the flaxseed oil. Consistent with our findings, flaxseed oil has been

**Table 4.** Plasma tumor necrosis factor-alpha and oxidative stress markers in plasma and liver tissue of the different experimental groups

Parameters	Mean±SE					
	Normal Control	MTX Control	Flaxseed Oil	Sesame Oil	Oil Mixture	
Liver tissue	MDA (nmol/g)	16.6±0.60 <sup>a</sup>	33.4±0.93 <sup>b</sup>	25.4±0.45 <sup>c</sup>	27.0±0.68 <sup>c</sup>	25.2±0.91 <sup>c</sup>
	Catalase (U/g)	1.90±0.06 <sup>a</sup>	1.31±0.07 <sup>b</sup>	1.62±0.04 <sup>cd</sup>	1.52±0.04 <sup>c</sup>	1.71±0.04 <sup>d</sup>
Plasma	MDA (nmol/ml)	7.40±0.29 <sup>a</sup>	17.02±0.72 <sup>b</sup>	10.57±0.23 <sup>c</sup>	10.99±0.54 <sup>c</sup>	10.03±0.33 <sup>c</sup>
	Catalase (U/L)	488.3±8.33 <sup>a</sup>	247.0±8.70 <sup>b</sup>	386.7±8.82 <sup>c</sup>	375.5±5.62 <sup>c</sup>	395.0±4.83 <sup>c</sup>
	GPX (IU/mL)	48.00±0.89 <sup>a</sup>	38.7±2.51 <sup>b</sup>	44.8±1.11 <sup>ac</sup>	42.0±1.15 <sup>bc</sup>	46.0±1.46 <sup>ac</sup>
	TNF-α (ng/mL)	20.50±1.09 <sup>a</sup>	29.5±2.13 <sup>b</sup>	23.5±0.76 <sup>a</sup>	24.2±1.05 <sup>a</sup>	22.5±0.89 <sup>a</sup>

Values with different superscript letters in the same rows are significantly different at P<0.05 level. MTX-control: Methotrexate control; MDA: Malondialdehyde; GPX: Glutathione peroxidase; TNF-a: Tumor necrosis factor-a.

**Table 5.** Plasma total cholesterol, triglycerides and HDL-Ch in various experimental groups.

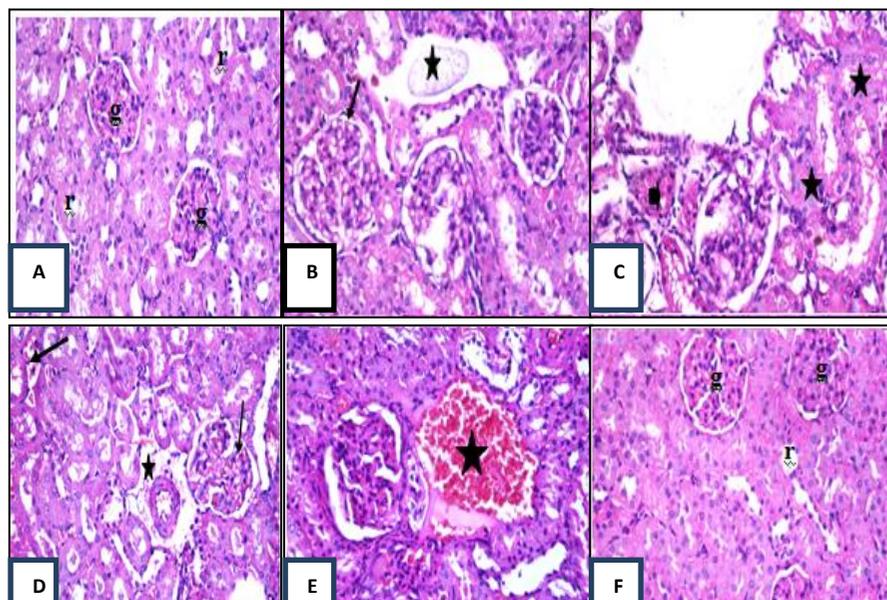
Groups	Mean±SE		
	T-Ch (mg/dl)	HDL-Ch (mg/dl)	TG (mg/dl)
Normal control	77.3±2.53 <sup>a</sup>	39.8±0.95 <sup>a</sup>	67.0±2.13 <sup>a</sup>
MTX control	83.3±1.96 <sup>b</sup>	23.5±1.71 <sup>b</sup>	100.8±2.30 <sup>b</sup>
Flaxseed oil	79.2±1.70 <sup>ab</sup>	38.7±0.88 <sup>a</sup>	74.3±1.63 <sup>ac</sup>
Sesame oil	81.2±0.87 <sup>ab</sup>	35.3±0.49 <sup>c</sup>	77.5±1.48 <sup>c</sup>
Oil mixture	78.7±1.86 <sup>ab</sup>	38.3±0.76 <sup>a</sup>	70.2±2.15 <sup>a</sup>

Values with different superscript letters in the same column are significantly different at  $P < 0.05$  levels. MTX-control: Methotrexate control; T-Ch: Total cholesterol; TG: triglycerides; HDL-Ch: High-density lipoprotein cholesterol.

poor in its phenolic contents as also reported by other studies [40, 41]. It has been reported that sesame seeds oil contains 1-2% phenolic compounds [17, 22]. Phenolic compounds are very important factors in the quality of oils, since they play significant roles in the oils' nutritional value, oxidation stability and protective quality [40]. Therefore, blending flaxseed oil with sesame seeds oil enhances the concentration of the phenolic compounds in the oil mixture compared to that of flaxseed

oil alone. The blending increases the stability of flaxseed oil due to reducing its oxidative properties.

The flaxseed oil profile revealed that linolenic acid (C18:3, w-3) was its major unsaturated fatty acid. The sesame seeds oil was rich in both linoleic (w-6) and oleic (w-9) fatty acids, as reflected in Table 2. Our results of the fatty acids profile for flaxseed are consistent with those reported by previous studies [13, 42, 43], while our results of the sesame seeds oil are in agreement with

**Figure 1.** Photomicrographs of the kidney tissue samples; magnification at 400x; H&E stained

A: Normal control; B & C: MTX control; D: Flaxseed oil group; E: Sesame seed oil; F: Mixture of both oils.

**A:** Kidneys with normal glomeruli (g) and renal tubules (r). **B:** Kidneys with vacuolar degenerated glomerular epithelium (arrow) and crystals deposition (\*). **C:** Kidneys with congestion in the peri-tubular blood capillaries (arrow head) and necrosed renal tubules (\*). **D:** Kidneys from rats given oral dose of flaxseeds oil showed mild improved parenchyma with minimal changes including; congestion in the glomerular capillaries (thin arrow) and peri-tubular blood capillaries (thick arrow), together with perivascular edema (\*). **E:** Kidneys from rats given oral dose of sesame seeds oil showed moderate improved parenchyma with minimal changes of congested interstitial blood vessel (\*). **F:** Kidneys from rats given oral dose of both oils mixture showed apparently healthy renal parenchyma with normal glomeruli (g) and renal tubules (r).

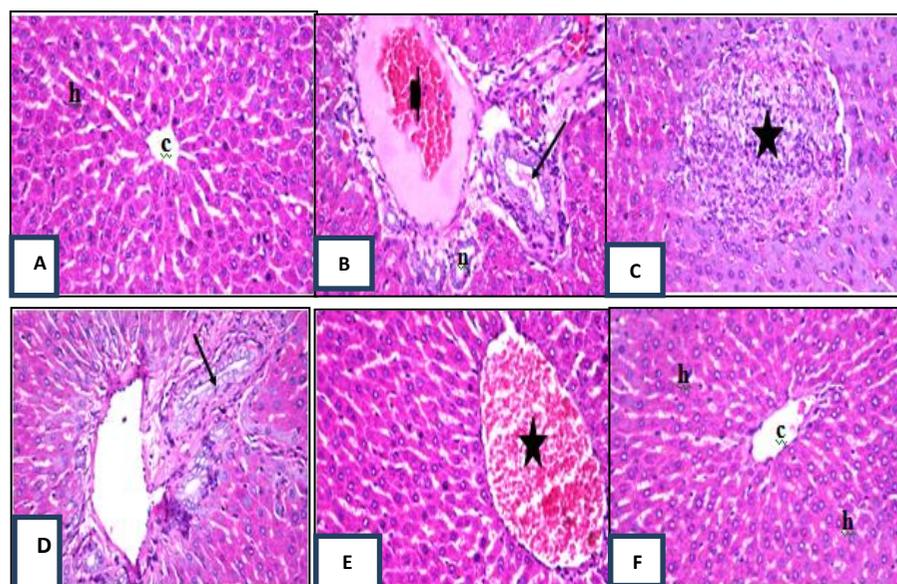
those reported by Rashed et al. [15] and Wan et al. [17]. Based on our results, we suggest that the flaxseed and sesame seeds oils are rich in w-3 and w-6 fatty acids, respectively. Both w-3 and w-6 are essential fatty acids that people need to consume in their daily diets. The optimal ratio of these essential fatty acids, i.e., w-3 & w-6, are reported to range between 1:1 and 1:4 [40]. Therefore, blending the two oils at 1:1 ratio will bring the final ratio of the two fatty acids to 1.4: 1. Otherwise, the fatty acid content in flaxseed would be 3.84 and in sesame seeds 0.023, if we do not mix them at the suggested ratio (Table 1). This is a very important point to consider from the nutritional perspective.

Injection of the rats with MTX led to histopathological changes in the liver and kidneys. It elevated the markers of oxidative stress and inflammatory response, and the biochemical signs of dyslipidemia, as evident by high plasma T-Ch, TG and reduced HDL-Ch. Our findings indicate that MTX induces hepatorenal toxicity in rats. The administration of the oils individually or the mixture caused significant improvements in the histopathological alterations of the liver and kidneys, and other biochemical parameters that were examined.

The elevations of the liver aminotransferase enzymes and kidneys' creatinine and urea were consistent with

those reported earlier by Rizk et al. [28] and Samdanci et al. [44] who suggested that MTX induced hepatorenal toxicity in rats. The elevation of aminotransferases is considered the reliable marker of hepatic cells' damage as these enzymes are released when the hepatocytes are injured [45]. The hepatorenal toxicity of MTX is due to its ability to bind with dihydrofolic reductase, which subsequently prevents the conversion of folic acid to its active form, folinic acid, leading to the inhibition of nucleic acids and proteins synthesis. This causes the hepatocyte's organelles and cell membranes release their enzymes into the blood stream, and the kidney function rises [46]. The renal toxicity observed in the present study due to MTX administration, as shown by elevated urea and creatinine, is due to the low solubility of MTX at acidic pH, so it precipitates in renal tubules and induces injuries [47]. Also, MTX is mainly filtered through the kidneys, hence the reason for the observed disturbance in the kidney function [48]. The flaxseed oil has also shown preventive effect against kidney dysfunction induced in rats by cisplatin, which is a chemotherapy agent [42]. Further, Eraky et al. have suggested that the omega-3 fatty acids in these oils protect against liver and kidneys toxicity induced by acetaminophen overdose [49].

The administration of MTX led to reduced plasma proteins as compared to that noted in the normal control rats.



**Figure 2.** Photomicrographs of liver tissue samples; magnification at 400x; H & E stained

A: Normal group; B & C: MTX control group; D: Flaxseed oil group; E: Sesame seed oil; F: Mixture of both oils. A: Liver with normal polyhedral hepatocytes (h) and normal central vein (c). B: Liver with congested hepatoportal blood vessel (arrow head), hyperplastic bile duct (arrow), and newly formed bile ductuli (n). C: Liver with large focal area of hepatic necrosis infiltrated with mononuclear cells infiltration (\*). D: Liver from rats given oral dose of flaxseed oil showed mild improved parenchyma with slight dilatation in the hepatoportal blood vessel and hyperplasia in the bile duct (arrow). E: Liver from rats given oral dose of sesame seeds oil showed moderate improved parenchyma with minimal changes of congested central vein (\*). F: Liver from rats given oral dose of both oils mixture showed apparently healthy hepatic parenchyma with normal hepatocytes (h) and central vein (c).

In liver failure, the albumin concentration in the serum and its function are reduced. Also, albumin infusion led to reduction in the plasma creatinine with the subsequent reduction in mortality, thus, a useful step in the management of patients with hepatorenal syndrome [50, 51]. The hepatic proteins' levels are linked to nutritional status, and are useful indicators of the severity of liver illness [52]. Reductions in plasma proteins, albumin, globulin and albumin/globulin ratio, which is associated with the administration of MTX might be attributed to their reduced reabsorption by the damaged kidney tubules, as evident by the histopathological changes documented in this study. In this context, our findings are consistent with those reported by previous studies [28, 44].

The plasma TNF- $\alpha$ , as an inflammatory marker, was elevated significantly in the rats treated with MTX compared to those of the normal controls (Table 4). These changes were associated with an elevation of MDA in the plasma and liver, indicative of lipid peroxidation, and reductions in the antioxidant enzymes, such as GPX in plasma, and CAT in both plasma and liver. The elevation of MDA together with the reduction in the antioxidant enzymes are suggestive of disturbances due to the oxidative stress. It has been reported previously that MTX administration is associated with elevation of oxidative stress due to the subsequent rise in the levels of ROS, hydrogen peroxide and hydroxyl radicals [28, 44, 53]. These pathological alterations lead to lipid peroxidation and reduced antioxidant enzymes' activities.

The MTX-induced hepatorenal toxicity caused the elevated oxidative stress and inflammatory response [54], as documented in the present research. Malondialdehyde is the end product of lipid peroxidation, thus it is a valuable indicator of tissue damage [54]. Improvement in the oxidative stress and inflammation, as observed in rats that received flaxseed oil, sesame seeds oil and the mixture, may be attributed to the reduced production of ROS secondary to the antioxidant and anti-inflammatory activities of the phenolic compounds present in the oils. The anti-inflammatory effect of these oils may be attributed to the presence of unsaturated fatty acids, especially w-3 and w-9 fatty acids.

It has been reported that monounsaturated fatty acids (oleic acid, w-9) and polyunsaturated fatty acids (linoleic acid, w-3) exhibit anti-inflammatory properties [55]. In the present study, the rats in the MTX control group showed significant elevation of plasma total cholesterol and triglycerides together with the reduced plasma HDL-Ch (Table 5). The administration of our oil treatments improved the plasma T-Ch, TG and HDL-Ch level

to varying degrees. It has also been shown previously that hepatorenal pathology induced significant elevation of T-Ch along and reduction in HDL-Ch [56]. Improvement in plasma levels of T-Ch, TG and HDL-Ch after the oils administered suggests the lipid lowering effects of the phenolic compounds and unsaturated fatty acids present in the oils used in this study. Consistently, other studies have previously reported that flaxseed and sesame seeds oils provide hypocholesterolemic effects [15, 57-59]. The histopathological changes observed in the present research are in agreement with those reported by Rizk et al. [28] who suggested that MTX induced major histopathological changes in both liver and kidneys.

## Conclusions

The administration of flaxseed and sesame seeds oils, and their mixture could potentially be used to prevent or reduce MTX-induced hepatorenal toxicity. The results of this study provided experimental evidence that the oils reduced inflammation, oxidative stress and improvement in liver and kidney functions, and improved the histopathological alterations caused by MTX. The antioxidant and anti-inflammatory activities of the studied oils may be attributed to the presence of phenolic compounds and unsaturated fatty acids, especially w-3 and w-9. Blending of flaxseed and sesame seeds oils is recommended for obtaining the ideal w-3/w-6 fatty acids contents. This mixture showed promising protective effect against MTX-induced hepatorenal toxicity.

## Ethical Considerations

### Compliance with ethical guidelines

Animal methods were accomplished in agreement with the Ethics Committee of the National Research Centre and obeyed the commendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

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### Authors' contributions

All authors equally contributed to preparing this article.

### Conflict of interest

The authors declared no conflict of interest.

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