

**Protective Effect of *Linum usitatissimum* Aqueous Extract against Hepatotoxicity Induced by Monosodium Glutamate in Male Rats**

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ABSTRACT

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Linum usitatissimum is a nutrient-rich seed and its liver function improving effect has long been studied. This study investigated the impact of *Linum usitatissimum* seeds extract on monosodium glutamate-induced physiological changes in albino rats. Dry seeds of *Linum usitatissimum* were procured in the Kerbala market in Iraq. The seeds were milled and the aqueous extract was prepared and used for the study. Forty albino rats were randomized into 4 groups of ten animals each. Group-1 (control) were orally administered 2 mL of normal saline, group-2 were orally administered with 14 mg/kg body weight of monosodium glutamate, group-3 were orally administered with 400 mg/kg body weight of *Linum usitatissimum* aqueous extract, group-4 were orally administered 14 mg/kg body weight of monosodium glutamate, after 4 hours of receiving 400 mg/kg body weight of *Linum usitatissimum* aqueous extract, daily for 30 days.

The animals were sacrificed after 30 days of treatment. Blood samples were obtained in all the groups and analyzed for hepatic function indices. There was an increase in hepatic function indices of Alanine transaminase (ALT), Aspartate transaminase (AST), and Alkaline phosphatase (ALP) serum levels in group-2, compared to the control group-1 and group-3. The enzyme levels normalized after treatment with *Linum usitatissimum* aqueous extract (group-4). In conclusion, *Linum usitatissimum* aqueous extract affected some physiological parameters induced by monosodium glutamate. The present findings suggest that the phytochemical constituents of *Linum usitatissimum* such as flavonoids, alpha-linolenic acid, and secoisolariciresinol diglucoside (SDG) may play a role in improving liver function.

Keywords: Monosodium glutamate, *Linum usitatissimum*, Flavonoids, Secoisolariciresinol diglucoside, alpha-linolenic acid.

Introduction

Medicinal plants have important physiological effect such as their protective role against oxidative damage and prevention of oxidative stress-induced diseases such as diabetes, Alzheimer's disease, and cancer.^{1,2} They have also been used to increase the production of digestive enzymes and to improve liver, pancreatic, and small intestinal functions.³ Some Medicinal plants including *Linum usitatissimum* have been used in folk medicine in the management of hypertension.^{4,5}

Linum usitatissimum, L. (Flaxseeds) belong to Linaceae family.⁶ It is one of the richest known sources of Lignans such as secoisolariciresinol diglucoside (SDG).⁷ which has been shown to prevent cell proliferation and reduce tumor growth in experimental animal models perhaps by modifying estrogen receptor signaling.⁸ One of the three main sources of phytoestrogens and among the richest sources of alpha-linolenic acid, soluble and insoluble fibers.⁹ It is a rich source of essential fatty acids with wound healing properties.¹⁰ Flaxseeds contain flavonoids which has been shown to exhibit numerous pharmacological effects such as anticancer, anti-

convulsant, antiulcer, analgesics, hepatoprotective, and anti-oxidants activities *in vitro* and *in vivo*.¹¹⁻¹⁴ As a powerful antioxidant, flaxseed has a role in protecting the liver and kidneys from strong oxidants,¹⁵ and prevent oxidative stress-induced diseases such as renal failure, liver failure, hyperlipidemia, and diabetes.¹⁶ Monosodium glutamate (MSG) was used in Japan as a flavour enhancer in many types of food, producing a flavour called umami meaning savory.^{17,18} It is also used in disguised forms, such as in flavoured natural flavours, yeast extract, hydrogenated protein, and in soy protein. Each of these substances contains a percentage of free glutamate, a harmful component of monosodium glutamate.¹⁹ Although it is widely used as a food flavour and catalyst for taste and appetite, studies have indicated that MSG is toxic to humans and laboratory animals especially at high doses.²⁰⁻²² Therefore, the present study is aimed at evaluating the hepatic effect of aqueous extract of flaxseed alone and in ameliorating MSG-induced hepatotoxicity in rats.

Materials and Methods*Plant seeds collection and extraction*

Dry seeds of *Linum usitatissimum* were obtained from the Kerbala market, Iraq. The seeds were identified and authenticated by assists Prof. Nibal M. Tarrad at the Biology Department, University of Kerbala, Iraq. The seeds were cleaned and dried for 48 hours at room temperature. The dried seeds were milled to a fine powder using a mechanical grinder.

A 100-gram portion of the powdered seeds sample was suspended in 500 mL of distilled water for 24 hours at room temperature and filtered. The filtrate was then poured into a stainless plate and the

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extract was dried in the oven at 30°C for 24 hours. The concentrated extract was stored in the refrigerator until needed for the study.

Experimental animals

Ethical permission was granted by the Research and Experimental Ethics Committee of College of Education for Pure Sciences - Department of Life Sciences under ethical approval number 231 in October 2019. Forty male albino Rats, ages between 12 to 14 weeks old, weight between 275 – 400 g, were obtained from the Animal House, College of Pharmacy, University of Kerbala. Animals were handled and maintained following the International Animal Care and Use Guidelines.²³ The rats were randomly divided into 4 groups (1-4) of 10 rats each. They were kept in separate cages and left for 2 weeks to acclimatize. The animals were fed with standard rodent chow based on the semi-purified diet of the American Institute of Nutrition (AIN),²⁴ with access to water *ad libitum*.

Group 1: negative control, were treated with 2 mL of normal saline.

Group 2: positive control, in which hepatotoxicity was developed, by administration of monosodium glutamate orally at a dose of 14 mg/kg body weight per day.¹⁹⁻²⁶

Group 3: were orally administered with the aqueous extract of flaxseeds at a dose of 400 mg/kg per day according to previous studies which found that the dose of 400 mg/kg was more effective.²⁷⁻²⁹

Group 4: were administered with MSG at a dose of 14 mg/kg body weight after 4 hours of treatment with the aqueous extract of flaxseeds, orally at a dose of 400 mg/kg per day.

All treatment were carried out for 30 days. During the 30 days experiment period, environmental conditions were controlled; the humidity was 55 to 60% and the room temperature was 22 ± 2°C, with 12-h light exposure.

Serum liver enzymes analysis

The animals were euthanized using a carbon dioxide inhalation chamber method.³⁰ Blood samples of six rats in each group were taken from the cardiac aorta of anesthetized animals and transferred to non-heparinized vacuum blood collection tubes. The tubes were held stationary until the blood separated into 2 layers. Blood serum layers were centrifuged (3000 rpm for 15 min at 25°C), and the supernatants were used for the liver enzymes assay. AST, ALT enzymes (Randox Laboratories kit), and ALP enzyme (Bio Merieux kit) test kits were used for the assay.^{31,32}

Statistical analysis

All data were analyzed using one-way ANOVA using the IBM SPSS Statistics 20 software. A least significant difference (LSD) test was performed to compare any significant differences ($P < 0.05$) in variables between groups, and data were expressed as mean ± standard deviation (SD).³³

Results and Discussion

Effect of monosodium glutamate on liver enzymes

Table (1), displays the mean serum levels of the enzymes AST, ALT, and ALP in the rats after 30 days of treatment compared to the control. From the differences in the means of the enzymes levels, it was observed that the levels of the enzymes in rats in the MSG group (Group-2) increased significantly ($p < 0.05$) compared to the group-1 (control group).

This observation was consistent with the studies of Alshubaily *et al.* and Ahmed *et al.*^{25,34}

However, the studies reported that the elevation of the liver enzymes; AST, ALT and ALP varied with the dose of MSG as well as the duration of administration.

Perhaps the high level of liver enzymes in group-2 can be attributed to the destructive effect of MSG on the hepatocytes. As more liver cells are destroyed, more enzymes are released into the blood.^{35,36} This effect could be explained by MSG-induced free radical production which reacts with polyunsaturated fatty acids of the cell membrane leading to the impairment of mitochondrial and plasma membranes which ultimately cause enzyme leakage.³⁷ High levels of liver enzymes in MSG treated rats could also be explained by the accumulation of glutamine in the liver caused by the breakdown of monosodium glutamate into Na⁺ and L-glutamate, which is converted to the liver-damaging glutamine.³⁸ Levels of the circulating liver enzymes are indices for liver function test.³⁹

Effect of aqueous extract of flaxseed on liver enzymes

As shown in Table (1), the mean serum levels of enzymes AST, ALT and ALP in rats treated with 400 mg/kg body weight of the aqueous extract of flaxseeds (group-3) decreased significantly ($p < 0.05$) compared to the control (group-1). This result is consistent with the works of Moula, 2011,⁴⁰ and Wahba and Ibrahim, 2013.⁴¹ Previous study attributed the hepatoprotective effect of flaxseed extract to its high levels of Omega-3 fatty acids and : Alpha-Linolenic Acid ALA, which can protect against oxidative damage to the liver membranes by free radical removal.⁴² Flaxseeds have also been shown to protect against hepatotoxicity induced by some toxic substances such as cadmium and Thiocloprid.^{43,44} Apart from being rich in polyunsaturated fatty acids as previously mentioned, as plant vegetable, flaxseeds are rich source of phenolics and flavonoids, which are responsible for its anti-inflammatory and antioxidant properties.⁴⁵ The improved levels of liver enzymes in flaxseed treated group may be due to an increase in the level of glutathione peroxidase (GPx) as flaxseed oil has been shown to increase the levels of antioxidant enzymes such as glutathione peroxidase.^{46,47} Similarly, sugars isolated from flaxseed have shown to have antioxidants and anti-inflammatory properties.⁴⁸

Effect of aqueous extract of flaxseed on MSG-induced liver toxicity

As presented in Table (1), the serum levels AST, ALT and ALP of rats in group-4, which were treated with 14 mg/kg body weight of MSG, after 4 hours of receiving 400 mg/kg body weight of flaxseeds aqueous extract, show no significant differences ($P \geq 0.05$) compared to the control group. This finding is also consistent with findings from previous studies were the hepato- and reno-protective effect of flaxseed oil have been observed.^{40,41,43,49,50}

Studies have also shown the cardioprotective effect of flaxseed oil which has been attributed to its high content of antioxidant fatty acids; Omega-3 and alpha-linolenic acid (ALA).^{51,52} Shakir and Madhusudhan observed that feeding laboratory animals with 15% flaxseed prevented carbon tetrachloride (CCl₄)-induced hepatotoxicity by maintaining the normal levels of AST, ALT, ALP and hepatic lipid peroxide.⁵³ The hepatoprotective effect of flaxseed has been greatly attributed to its antioxidant property due to the presence of phenols, flavonoids and other phytochemicals such as Secoisolariciresinol Diglucoside SDG that may possess properties of reducing oxidative stress and inflammation.⁵⁵

Table 1: Comparison of means of liver enzymes levels AST, ALT, and ALP in the serum of albino rats in the treatment groups with the control

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Group-1 (Control)	46.32 ± 0.63	53.36 ± 0.78	62.40 ± 1.99
Group-2 (MSG treated)	74.02 ± 1.43*	67.30 ± 1.03*	77.12 ± 1.99*
Group-3 (flaxseeds treated)	31.40 ± 0.98*	36.00 ± 1.38*	36.48 ± 1.12*
Group-4 (flaxseeds & MSG treated)	48.66 ± 0.32	16.53 ± 0.32	16.61 ± 0.32
LSD	2.81	2.87	4.56

Values represent Mean SEM. * $P < 0.05$ (significant difference between test and control).

Key: ALT = Alanine transaminase, AST = Aspartate Transaminase, ALP = Alkaline Phosphatase.

Conclusion

This study demonstrated that the aqueous extract of *Linum usitatissimum* (flaxseed) has protective effect against MSG-induced hepatotoxic damage. The hepato-protective effect of the extract may have occurred by augmenting the antioxidant defense mechanisms.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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