Effect of replacing sunflower oil in the diet with different ratios of pumpkin seed oil on liver enzyme levels of male rats

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Abstract
This study was conducted at the College of Agriculture - University of Kufa from 1/1/2026 to 31/1/2026, to evaluate the effect of replacing sunflower oil used in the standard diet with different ratios of pumpkin seed oil on liver enzyme levels (ALT, AST, ALP) in the blood serum of laboratory rats. Forty male rats , Rattuse rattus, were randomly divided into five groups (8 animals/group): a control group (G) fed a standard diet, and four treatment groups (T1, T2, T3, T4) fed diets in which sunflower oil was replaced by pumpkin seed oil at ratios of 25%, 50%, 75%, and 100% , respectively, for 30 days. The results showed that group T4 (100% replacement) had the lowest liver enzyme levels, with ALT mean value of 61.75 ± 2.754 U/L (a 17.4% decrease), AST of 59.10 ± 4.241 U/L (a 20.6% decrease), and ALP of 188.5 ± 20.74 U/L (a 20.9% decrease), compared to the control group, which recorded 74.80 ± 2.507, 74.40 ± 4.248, and 238.2 ± 5.12 U/L, for ALT, AST and ALP, respectively. Groups T2 and T3 also showed a significant decrease ($P<0.05$) in liver enzyme levels. This protective effect can be explained on basis of the high content of bioactive compounds in pumpkin seed oil, particularly tocopherols (442–686 mg/kg), phytosterols (300–800 mg/100 g), squalene, and phenolic compounds, which possess potent antioxidant and anti-inflammatory properties that protect liver cells from oxidative stress and damage. We conclude that pumpkin seed oil has a clear hepatoprotective effect, and that this effect is dependent on the level of substitution, with higher percentages (75%–100%) being more effective in improving liver function indicators.

Keywords: Pumpkin seed oil, liver enzymes ALT, AST, ALP, tocopherols, phytosterols, rats.

Introduction

Pumpkin seed oil is a traditional vegetable oil of great nutritional and therapeutic importance, and has been used for centuries in the folk medicine of various civilizations due to its diverse health properties. This oil is characterized by its unique chemical composition, which combines a high percentage of unsaturated fatty acids, especially linoleic and oleic acids, with a rich content of bioactive compounds such as tocopherols, phytosterols, squalene, carotenoids, and phenolic compounds [8, 20]. These compounds give the oil antioxidant, anti-inflammatory, and protective properties for various organs in the body.

Liver enzymes measured in serum, particularly, ALT, AST, and ALP are sensitive biomarkers for assessing the integrity and function of liver cells, as elevated levels indicate liver cell damage or bile duct disorders [14].

Recent experimental studies have shown that pumpkin seed oil possesses significant hepatoprotective effects. Previous studies [1, 20] indicated that replacing saturated fats with pumpkin seed oil in the diet led to improve lipid profiles and liver function.

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Other studies [12, 25] confirmed that pumpkin seed oil possesses anti-fibrotic properties by inhibiting the activation of hepatic stellate cells and reducing collagen deposition. In addition, many studies have shown that the active compounds in pumpkin seed oil, particularly, tocopherols, phytosterols and phenolic compounds, protect liver cells from oxidative stress and enhance the activity of endogenous antioxidant enzymes [11, 25].

Despite these promising results, further studies are needed to determine the optimal levels of pumpkin seed oil that should be substituted in the diet to achieve maximum liver-protective benefit. Therefore, this study aimed to evaluate the effect of replacing sunflower oil with different levels of pumpkin seed oil (25%, 50%, 75%, 100%) in the standard diet on liver enzyme levels. ALT, AST, ALP) in the blood serum of laboratory rats.

Materials and working methods

Place and time of conducting the study

This study was conducted at the College of Agriculture - University of Kufa and the Animal House at the College of Science/University of Kufa during the period from 1/1/2026 to 31/1/2026.

Pumpkin seeds were obtained from local markets in Najaf Governorate, and the oil was cold extracted at 30°C using a specialized electric machine, in order to preserve the active components and prevent oxidation of the oil [26]. The obtained oil was filtered to remove impurities, and stored in airtight dark glass bottles at 4°C until use.

Experimental animals

Forty mature male rats of the species were provided Ten-week-old rats weighing between 180 and 205 grams were housed in spacious, well-ventilated plastic cages lined with wood shavings, which were replaced periodically. Environmental conditions were controlled, including a temperature of 24°C, a 12-hour light/12-hour dark cycle, and a continuous supply of feed and water. The animals were allowed two weeks to adapted to experimental diets and conditions before the experimental procedures began.

Experimental design

The animals were randomly divided into five groups, with eight animals in each group, as follows:

•The group G (control): Rats were fed the standard ration containing sunflower oil (50 g/kg).

•The groupT1: Rats were fed a diet in which sunflower oil was replaced with pumpkin seed oil at a rate of 25%.

•The groupT2: Rats were fed a diet in which sunflower oil was replaced with pumpkin seed oil at a rate of 50%.

•The groupT3: Rats were fed a diet in which sunflower oil was replaced with pumpkin seed oil at a rate of 75%.

•The groupT4: Rtas were a diet in which sunflower oil was replaced with pumpkin seed oil at a rate of 100%.

The feeding period lasted for 30 days.

Blood collection and serum separation

After the specified period, the rats were deprived of food and water for 12 hours, then anesthetized with ketamine and xylazine. Blood samples were drawn from the heart using a suitable syringe and placed in dry test tubes. The samples were allowed to coagulate for 30 minutes, then the separated was separated using a centrifuge at 3000 rpm for 15 minutes. The serum was collected and stored at -18°C until use.

Biochemical measurements

Level of liver enzymes (ALT, AST, ALP) in serum were measured using ready-made assay kits from Biolabo-France, according to the approved enzymatic method [18]. The intensity of the color resulting from the enzymatic reactions was measured using a spectrophotometer at the appropriate wavelengths for each enzyme.

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Statistical analysis

The experiment was conducted according to a completely randomized design (CRD), and significant differences between means were tested using Duncan's test at a probability level of 0.05 [9]. The data were analyzed using GenStat Discovery Edition 12 software.

Results and discussion

Effect of replacing sunflower oli with different ratios of pumpkin seed oil on levels of ALT enzyme The statistical analysis showed that the mean level of ALT in the control group (G) was 74.80 ± 2.507 U/L. In the T1 group (25% replacement) , the ALT level was 72.08 ± 2.692 U/L, with about 3.6% decrease, which was not significantly different from the control group. This can be explained by the fact that the low concentration of pumpkin seed oil was insufficient to provide an adequate amount of the bioactive compounds needed to produce a significant hepatoprotective effect during the 30-day trial period. The efficacy of pumpkin seed oil as a hepatoprotective agent is directly dose-dependent [3].

Table (1) Effect of replacing sunflower oil with different ratios pumpkin seed oil on ALT level (mean ±SE)

ALT level	Treatment
$74.80^{c} \pm 2.507$	G
$72.08^{bc} \pm 2.692$	T1
$70.53^{b} \pm 1.279$	T2
$69.15^{b} \pm 1.930$	T3
$61.75^{a} \pm 2.754$	T4

Values are expressed as the arithmetic mean of four replicates ± standard deviation

Means with different letters are significantly differed

G, Standard Feed; T1, Feed containing pumpkin seed oil at a replacement rate of 25%; T2,Feed containing pumpkin seed oil at a replacement rate of 50%; T3, Feed containing pumpkin seed oil at a replacement rate of 75%; T4, Feed containing pumpkin seed oil at a replacement rate of 100%.

Results showed that ALT in T2 and T3 (50% and 75% replacement) were significantly ($P<0.05$) decreased to 70.53 ± 1.279 and 69.15 ± 1.930 U/L, respectively, representing a 5.7% and 7.6% reduction. These results are consistent with previous studies [17, 24] indicating that pumpkin seed oil treatment in rats significantly improved liver enzyme levels.

Results showed that ALT level in T4 (100% replacement) was significantly ($P<0.05$) decreased to 61.75 ± 2.754 U/L, with about 17.4% reduction compared to the control group. This finding is consistent with several studies [3, 7] that demonstrated a significant decrease in ALT levels in rats fed pumpkin seed oil.

This reduction may be attributed to the hepatoprotective effect of pumpkin seed oil, which stems from its high antioxidant content. The polyunsaturated fatty acids present in pumpkin seed oil reduce oxidative stress in the liver by enhancing the activity of endogenous antioxidant enzymes such as

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Superoxide dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx), which protect liver cells from damage and maintain the integrity of cell membranes, thus preventing the leakage of the ALT enzyme into the bloodstream [6, 7].

Effect of replacing sunflower oli with different ratios of pumpkin seed oil on levels of AST enzyme The mean level of AST in the control group (G) was 74.40 ± 4.248 U/L, a value within the normal range for healthy male rats [4, 10]. The AST level in T1 (25% replacement) was slightly decreased to 72.95 ± 1.240 U/L, a decrease of 1.9%, which was not significantly different from the control group. This can be explained by the fact that the low concentration of pumpkin seed oil was insufficient to provide an effective concentration of the bioactive compounds necessary for protecting liver cells [20].

Table (2) Effect of replacing sunflower oil with different ratios pumpkin seed oil on AST level (mean ±SE)

AST level	Treatment
$74.40^{b} \pm 4.248$	G
$72.95^{b} \pm 1.240$	T1
$69.55^{b} \pm 1.292$	T2
$59.60^{a} \pm 5.243$	T3
$59.10^{a} \pm 4.241$	T4

Values are expressed as the arithmetic mean of four replicates ± standard deviation
Means with different letters are significantly differed
G, Standard Feed; T1, Feed containing pumpkin seed oil at a replacement rate of 25%; T2,Feed containing pumpkin seed oil at a replacement rate of 50%; T3, Feed containing pumpkin seed oil at a replacement rate of 75%; T4, Feed containing pumpkin seed oil at a replacement rate of 100%.

The results showed a slight decrease in the group. T2 (50% replacement) to 69.55± 1.292 units/L, representing a 6.5% reduction. In group T3 (75% replacement) , AST level was significantly ($P<0.05$) decreased to 59.60 ± 5.243 units/L, with about 19.9% reduction compared to the control group. This result is consistent with previous studies [10, 15] indicating that feeding rats pumpkin seed oil significantly reduced AST enzyme levels.

The results revealed that the level of AST in group T4 (100% replacement) was significantly (P±0.05) decreased to
59.10 ± 4.241 units/L, with about 20.6% decrease compared to the control group. This result is consistent with several studie [4] that have shown a significant decrease in AST levels in rats treated with pumpkin seed oil.

This decrease is attributed to the fact that pumpkin seed oil contains high levels of γ-Tocopherol (442-686 mg/kg) [8] acting as a potent antioxidant, inhibiting the formation of reactive oxygen species (ROS) and protecting liver cells from oxidative stress, thus maintaining the integrity of cell membranes and preventing the leakage of AST enzyme into the bloodstream [11]. Phenolic compounds in pumpkin seed oil also contribute in protection of liver cells from oxidative damage and maintaining the integrity of cell membranes [5].

Effect of replacing sunflower oli with different ratios of pumpkin seed oil on levels of ALP enzyme The Mean level of ALP in the control group (G) was 238.2 ± 5.12 units/L. ALP is a sensitive indicator of cholestasis or bile duct damage [13]. The mean levels of ALP in T1 and T2 groups

(25% and 50% replacement) were 221.8 ± 16.15 and 214.2 ± 14.45 units/L, respectively, representing decreases of 6.9% and 10.1%, and did not show significant differences compared to the control group. This result is consistent with previous studies [23] indicating that the hepatoprotective efficacy of pumpkin seed oil is directly dose-dependent.

Table (3) Effect of replacing sunflower oil with different ratios pumpkin seed oil on ALP level (mean ±SE)

ALP level	Treatment
$238.2^{c} \pm 5.12$	G
$221.8^{bc} \pm 16.15$	T1
$214.2^{bc} \pm 14.45$	T2
$210.0^{ab} \pm 16.87$	T3
$188.5^{a} \pm 20.74$	T4

Values are expressed as the arithmetic mean of four replicates ± standard deviation
Means with different letters are significantly differed
G, Standard Feed; T1, Feed containing pumpkin seed oil at a replacement rate of 25%; T2,Feed containing pumpkin seed oil at a replacement rate of 50%; T3, Feed containing pumpkin seed oil at a replacement rate of 75%; T4, Feed containing pumpkin seed oil at a replacement rate of 100%.

The mean ALP level in T3 (75% replacement) showed a significant decrease ($P<0.05$) to 210.0±16.87 units/L, representing about 11.8% reduction. This finding is consistent with previous studies [19, 22] indicating that pumpkin seed extracts possess hepatoprotective properties against oxidative stress damage, as the high concentrations of β-carotene in the oil act as a potent antioxidant, protecting liver cells from free radicals.

The results revelaed a significant decrease in the level ALP in group T4 (100% replacement) to 188.5 ± 20.74 units/L, a decrease of 20.9% compared to the control group. This result is consistent with several studies [2, 23] that have shown that pumpkin seed oil significantly reduces elevated ALP levels.

Soch decrease is attributed to the fact that pumpkin seed oil is rich in powerful antioxidants, especiallyβ-carotene and tocopherols That protect liver cells from oxidative stress and maintain the integrity of liver cell membranes and bile ducts, preventing enzyme leakage into the blood [19, 21]. Phenolic compounds and unsaturated fatty acids in pumpkin seed oil also inhibit hepatitis and reduce the activation of hepatic stellate cells, thus improving liver function [23,16].

Conclusions

1. The study showed that replacing sunflower oil with pumpkin seed oil in different proportions (25%, 50%, 75%, 100%) in the diet of laboratory rats led to a significant improvement in liver enzyme levels (ALT, AST, ALP).

2. The results showed a clear dose-response relationship, with higher replacement rates (75%-100%) being more effective in reducing liver enzyme levels compared to lower rates.

3. The group T4 (100% replacement) showed the best results, with reduction in ALT, AST and ALP levels by 17.4%, 20.6% and 20.9% respectively compared to the control group.

4. The hepatoprotective effect of pumpkin seed oil is attributed to its high content of bioactive compounds, especially tocopherols, phytosterols, squalene, and phenolic

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compounds, which possess strong antioxidant and anti-inflammatory properties.

5. The results indicate that low percentages of pumpkin seed oil (25%) did not show a significant effect, reflecting the need for a certain threshold concentration to achieve the desired therapeutic effects.

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