

Article

Effect of Pumpkin Seed Oil and Vitamin E on Histological Changes Liver of Rabbite Liver

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Abstract: The study evaluated the histological and biochemical effects of pumpkin seed oil (PSO), with or without vitamin E, on the liver of male rabbits. Twelve four-month-old rabbits were allocated to three groups (n = 4) and treated for three weeks. Serum ALT and AST activities and hepatic histology were assessed. PSO alone increased ALT (42.53 ± 0.78 U/L) and AST (46.63 ± 4.50 U/L) compared with controls (34.83 ± 0.60 and 33.90 ± 0.21 U/L, respectively), whereas vitamin E supplementation reduced these activities to 27.70 ± 3.15 and 34.70 ± 0.30 U/L. Histologically, PSO induced alterations in liver appearance and central vein diameter, while vitamin E supplementation was associated with reduced biochemical alterations. These findings suggest that vitamin E may mitigate PSO-associated hepatic changes, potentially through antioxidant activity.

Keywords: Pumpkin seed oil; Vitamin E; Rabbit liver; Liver enzymes; Histological

Introduction

Pumpkin, a member of the Cucurbitaceae family, is well-known for being delicious as well as delicious food. Pumpkin seeds are utilized as a useful source of eatable oil with several beneficial qualities. Pumpkin seed oil (PSO) was observed to decrease free Radicals and was helpful in opposition to arthritis in rats [1]. The oil contains a lot of phytoestrogens Rats' plasma lipid levels and cardiovascular outcomes are inhibited following supplementation [2]. PSO administration demonstrated a potent anti-atherogenic effect in hypercholesterolemic rats and hepatoprotective effects [3]. PSO has been shown to be beneficial against colon and breast cancer's fast cell growth [4] as well as against wound animals' ability to heal [5,6]. Demonstrated the high antioxidant and genoprotective activity of pumpkin seed extract, making it a highly advantageous dietary supplement. PSO is abundant in a number of bioactive substances, including phytosterols and tocopherols [7], carotenoids [8], and phenolic compounds [9]. The oil is rich in oleic acid and linoleic acid [10]. The liver is one of the largest organs in the body and the main site of metabolism and intensive secretion [11]. The liver plays a major role in the removal of toxins and excretion of many internal and external compounds and any damage or impairment of liver function may lead to many effects on health [12]. Because rabbits and a humans share the hepatic vascular system shares a number of morphological similarities, rabbits are frequently used as research models [13]. When compared with a human liver, the rabbit's liver anatomy differs in a few significant ways. There are four major lobes in a rabbit's liver [14]. These are the caudate lobe and three cranial

lobes, comprising the right, medial left and lateral left lobes. The caudate and cranial lobes of the rabbit liver are distinct from one another, which makes it possible to study each region separately. In the portal venous system, the most prevalent anatomical pattern, referred to as "conventional," is the presence of an original portal vein (OPV), which is created when the mesenteric and gastrosplenic veins converge [15].

Materials and Methods

Study plan

Twelve male rabbits weighing about 1.5-200 kg and four months of age were used. The rabbits were kept in standard cages at 20–22°C, 25–30% humidity, and a 12-hour light/dark cycle. They had unlimited access to fresh water and standard commercial food. The rabbits were divided into three groups, each comprising 4 rabbits. Group 1 received pumpkin seed oil as dose 1 ml /day by syringe, group 2 received pumpkin seed oil and vitamin E at a dose 0.85 ml/day for three weeks, and group 3 was the control group and was not given pumpkin seed oil or vitamin E. Liver enzyme chemical analysis tests were performed (ALT, AST, ALP, Bilirubin)

Liver preparation

All animals were anesthetized by intramuscular injection of ketamine at 29.1 mg/kg and xylazine at 4.3 mg/kg. Subsequently, euthanasia was performed by intravenous injection of 600 mg of ketamine. After euthanasia, the liver was removed, thoroughly washed with normal saline, and fixed in 10% neutral formalin for 24–48 hours at pH 7.2 ± 0.5 [16].

Histological examination

After fixation in 10% neutral formalin, the tissues underwent routine histological processing, and 5- μ m-thick sections were obtained using a rotary microtome. Staining techniques, including hematoxylin and eosin (H&E), were applied to analyze tissue structure. The stained slides were then examined under a light microscope (Olympus), and images were captured for further analysis. The central vein electrode was measured using the ImageJ program [17].

Statistical Analysis:

The Statistical Packages of Social Sciences-SPSS (SPSS, 2019) program was used to detect the effect of different groups in study parameters. Least significant difference (LSD) test was used to significantly compare means in this study" [18].

Results

Anatomical results

The results of the anatomical study in the control group and the group receiving Vitamin E-rich pumpkin seed oil showed that the liver appeared normal in color (reddish-brown) and shape, with no abnormalities (Fig. 1A).

However, in the group receiving pumpkin seed oil extract, the liver was not entirely normal, exhibiting specific changes. Its color was paler than in the first group, with slight or pronounced differences in surface area (Fig. 1B)...

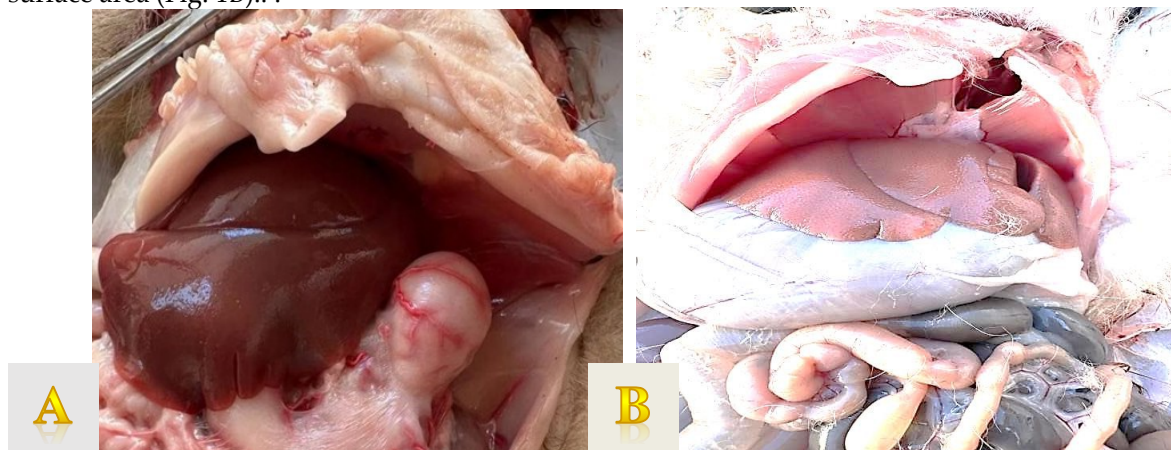
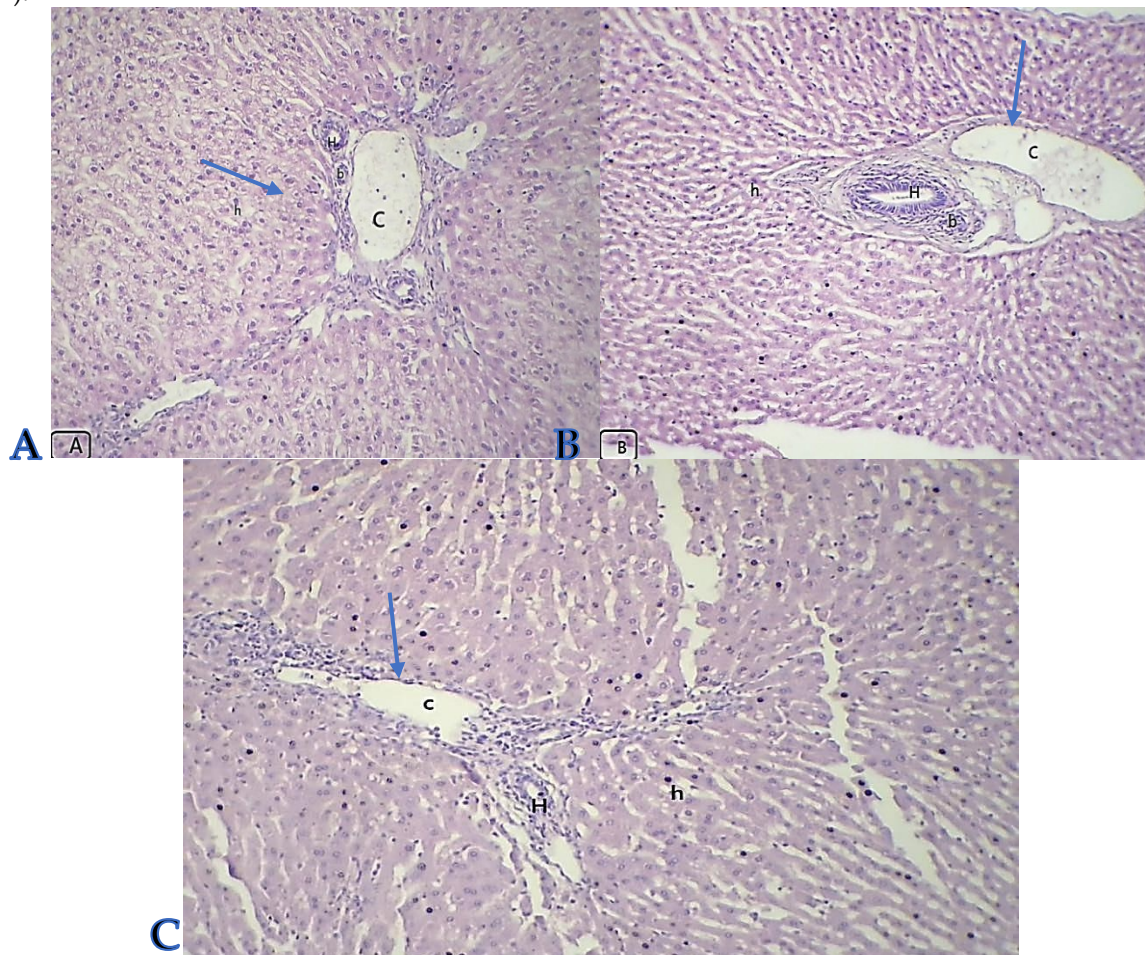


Figure 1. The photography of the liver (A) Rabbits that were given doses of pumpkin seed oil with vitamin E. (B) Rabbits that were given doses of pumpkin seed oil only liver appeared normal in color (reddish-brown) and shape, with no abnormalities (Fig. 1A). in the group receiving pumpkin seed oil extract, the liver was not entirely normal, exhibiting specific changes. Its color was paler than in the first group, with slight or pronounced differences in surface area (Fig. 1B).

Histological results

Histological examination of the liver tissue from the rabbit used as the control group after three weeks of the experiment showed normal hepatocytes arranged in cords around the central vein, as recorded in (Fig. 2C). In rabbits administered pumpkin seed oil, moderate centrilobular congestion was observed, with normal hepatocytes, mild dilatation of the central vein, and inflammatory cell infiltration, as recorded in (Fig. 2A). In rabbits administered pumpkin seed oil and vitamin E, dilatation of the central vein and hepatic artery was observed, as recorded in (Fig. 2B).

After measuring the diameter of the central vein using the ImageJ program, the result showed significant differences in the diameter of the central vein among the studied groups. The pumpkin seed oil group with vitamin E recorded the highest average (244.69 ± 45.25), compared with the pumpkin seed oil group (159.54 ± 10.98), whereas the control group recorded the lowest average (100.76 ± 17.92) (Table 1).



(Figure 2) (A). Photomicrograph of the liver in local rabbits illustrates the control group. C, central vein; b, bile duct; H, hepatic artery; h, hepatocyte. (B): The effect of pumpkin seed oil and vitamin E on liver histology: C, central vein; b, bile duct; H, hepatic artery; h, hepatocyte. (C): This figure shows the histology of the liver in the oil group. C, central vein; b, bile duct; H, hepatic artery; h, hepatocyte. (H&E stain, 100X).

Table 1. Comparison of Central Vein Diameter among Different Groups

Group	Central vein diameter (Mean $\pm$ SE, μ m)
Pumpkin seed oil	159.54 $\pm$ 10.98 ^{ab}
Pumpkin seed oil + vitamin E	244.69 $\pm$ 45.25 ^a
Control	100.76 $\pm$ 17.92 ^b
LSD (0.05)	68.769
P-value	0.010

Results of biochemical analyses (ALT, AST, ALP, Bilirubin)

The results showed significant differences among the groups in some liver enzymes. The pumpkin seed oil group had the highest ALT level (42.53 $\pm$ 0.78) U/L, compared with the control group (34.83 $\pm$ 0.60) U/L. However, the enzyme level decreased significantly in the pumpkin seed oil with vitamin E group, reaching (27.70 $\pm$ 3.15) U/L. The p-value was (P = 0.0045), indicating a significant difference between the groups.

The results also showed significant differences in AST enzyme levels. The pumpkin seed oil group achieved the highest value at 46.63 $\pm$ 4.50 units/L, followed by the pumpkin seed oil with vitamin E group at 34.70 $\pm$ 0.30 units/L. The control group recorded the lowest value at 33.90 $\pm$ 0.21 units/L, with a p-value of 0.0234. As for total bilirubin, the results did not show significant differences between the groups, with values ranging from 0.733 $\pm$ 0.12 to 0.800 $\pm$ 0.05 units/L, and a p-value of 0.8424.

Similarly, no significant differences were observed in alkaline phosphatase (ALP) levels among the groups, with values of 242.67 $\pm$ 12.66, 239.67 $\pm$ 17.33, and 251.67 $\pm$ 1.66 U/L for the pumpkin seed oil, pumpkin seed oil with vitamin E, and control groups, respectively, and a p-value of 0.7848 (Table 2).

Table 2. Comparison of liver enzyme activities and total bilirubin levels among the experimental groups

Group	ALT (U/L)	AST (U/L)	Total bilirubin (mg/dL)	ALP (U/L)
Pumpkin seed oil	42.53 $\pm$ 0.78 ^a	46.63 $\pm$ 4.50 ^a	0.800 $\pm$ 0.05	242.67 $\pm$ 12.66
Pumpkin seed oil + vitamin E	27.70 $\pm$ 3.15 ^c	34.70 $\pm$ 0.30 ^b	0.766 $\pm$ 0.03	239.67 $\pm$ 17.33
Control	34.83 $\pm$ 0.60 ^b	33.90 $\pm$ 0.21 ^b	0.733 $\pm$ 0.12	251.67 $\pm$ 1.66
LSD	6.588**	9.019*	0.275 NS	43.021 NS
P-value	0.0045	0.0234	0.8424	0.7848

Discussion

The anatomical results showed a change in liver color after administration of pumpkin seed oil, while this change was not observed when the oil was administered with vitamin E. This can be explained by the nature of vegetable oils and the effect of oxidative stress on liver tissue. Pumpkin seed oil is high in unsaturated fatty acids, which are more susceptible to oxidation within the body, leading to the formation of lipid peroxidation products. Studies have shown that increased lipid peroxidation leads to damage to cell membranes and histological and chemical changes in the liver, such as discoloration and elevated markers of oxidative stress like MDA and ALT [19,20]. In contrast, vitamin E is one of the most important fat-soluble antioxidants. It works by halting the chain reaction of free radicals within cell membranes, thus reducing lipid peroxidation and protecting cells from oxidative damage. Numerous studies have confirmed that vitamin E reduces lipid peroxidation and improves liver tissue integrity in animal models [21]. Experimental studies on vegetable oils also indicate that their effect on the liver is highly dependent on the oxidative state and the presence of concomitant antioxidants. The addition of vitamin E significantly reduces the toxic effects of oxidation and preserves liver tissue structure [22].

The results shown in Table 2 indicate a significant increase in the central diameter of the liver in the pumpkin seed oil group with vitamin E and the control group, while the pumpkin seed oil group showed moderate values. This is attributed to the antioxidant effects of both pumpkin seed oil and vitamin E, as these compounds reduce oxidative damage and improve blood flow within liver tissue.

The results of the current study are consistent with those of [23], who demonstrated that vitamin E acts as an active antioxidant, partly in protecting muscle tissue and resisting the damaging effects of free radicals, thus improving liver tissue condition and maintaining vascular integrity. They also noted that reducing oxidative stress contributes to improved circulation and reduced damage and swelling. These findings are consistent with the study by [24], which demonstrated that pumpkin seed oil possesses remarkable properties for the liver due to its potent, expert-like activity in the absence of phenolic compounds. Compounds help reduce viral replication and the emergence of tissue-like structures within the virus. The study also demonstrated the use of loose-cell analysis to activate tissue structure and its detrimental effects on viruses. [25] Also noted that pumpkin seed oil contains a high percentage of tocopherols and antioxidants, which contribute to protecting vital tissues from reduced replication processes. This may explain the improvement observed in central Qatar in the current study.

The results showed statistically significant differences between the studied groups in some liver enzymes. A study by [24] demonstrated that pumpkin seed oil possesses antioxidant properties and may play a protective role against liver damage caused by oxidative stress, due to its content of active compounds such as unsaturated fatty acids and phenolic compounds. This study supported the interpretation of the findings regarding changes in liver enzyme levels in the current research. Furthermore, a study by [25] showed that pumpkin seed oil contains high levels of tocopherols and antioxidants, which explains the bioactive effects the oil exhibits in improving certain physiological functions and reducing the harmful effects of free radicals. A study by [23] focused on the vital role of vitamin E as one of the most important natural antioxidants, protecting cell membranes from oxidation and reducing cell damage. This is consistent with the findings of the current study, which showed a decrease in ALT and AST enzyme levels with vitamin E supplementation. Furthermore, [26] showed that the use of antioxidants leads to improved liver function and reduced signs of liver damage in laboratory animals, which supports the scientific interpretation of the results obtained in this study.

Conclusion

The present study indicates that supplementation with vitamin E alongside pumpkin seed oil improved certain hepatic histological features, particularly vascular integrity and blood flow within the liver tissue. These findings support the potential role of natural antioxidants, such as vitamin E, in mitigating oxidative stress-related tissue damage.

Conflict of interest:

The authors have nothing to say about a conflict of interest.

Ethical Approval:

The study was approved by the Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq (Approval No. Vet Medicine/238; May 2026).

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