

## The effect of pistachio Oil on Important Body Organs in Female Wistar Rats: an Experimental Study with Histopathological Evaluations

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Information	Abstract
<p><b>Article Type:</b> Original Article</p>	<p><b>Introduction:</b> Pistachios are a rich source of antioxidants and anti-inflammatory agents, which are widely used in the treatment of diseases affecting the kidneys, liver, heart, and respiratory system. This study aimed to investigate the effects of pistachio oil as a dietary supplement on important bodily organs.</p> <p><b>Methods:</b> Twenty-one female rats were used in a randomized design, which included three groups (n=21). Group 1: rats received a normal diet; Groups 2 and 3: rats received a normal diet supplemented with 10 mg/kg and 50 mg/kg of pistachio oil via gavage for 21 days, respectively. Blood samples were collected for biochemical assessments, including blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose (Glu), triglycerides (Tg), high-density lipoprotein cholesterol (HDL), and total cholesterol (Chol). The liver, kidneys, ovaries, uterus, and brain were harvested for histological evaluations. A significance level of P&lt;0.05 was considered statistically significant.</p> <p><b>Results:</b> Our results indicated that the administration of pistachio oil at both doses (10 and 50 mg/kg) significantly increased biochemical parameters, including Glu, Tg, HDL, AST, and ALT (P&lt;0.001). Additionally, both doses of pistachio oil significantly decreased Chol compared to the control group (P&lt;0.001). Chronic consumption of pistachio oil did not induce pathological lesions in any of the examined organs.</p> <p><b>Conclusion:</b> Our results demonstrated that the consumption of pistachio oil is suitable because it is effective and safe without any organ toxicity.</p>
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## 1. Introduction

Pistachio (*Pistacia vera*) seeds belong to the family Anacardiaceae. They are a rich source of various bioactive compounds, including antioxidants and anti-inflammatory agents such as gallotannins, gallic acid, myricetin, and quercetin [1]. Different varieties of pistachios exhibit excellent health-promoting properties. Historically, pistachios have been utilized by ancient civilizations to treat diseases affecting the kidneys, liver, heart, and respiratory system [2, 3]. Numerous studies have confirmed these effects, including the nephroprotective properties of pistachio hydroalcoholic extract against cisplatin- or gentamicin-induced nephrotoxicity [4-6]. Furthermore, pistachio extract or oil has been shown to attenuate neurocognitive complications in ovariectomized animals [7, 8]. Additionally, hydroalcoholic extracts of pistachio mitigate seizures induced by pentylenetetrazole [9]. Previous research indicates that pistachios possess anti-cancer properties and may reduce cancer risk [10]. The liver plays a critical role in the detoxification of nutrients, and these nutrients can influence liver health in various ways. Almost all food substances in the digestive system are processed and enter the liver through the portal vein, where large toxins are removed [11]. Subsequently, toxins are transported to the kidneys for excretion from the body. The high volume of toxins that must be eliminated can lead to their accumulation in the renal tubules, resulting in renal toxicity [12]. When the kidneys fail to adequately filter toxins from the body, these substances accumulate in the blood instead of being excreted in urine [13]. Therefore, when a compound is added to the diet or food products to create a healthy and balanced diet, it is essential to ensure that this compound does not have toxic effects on various

organs. Consequently, the present study aimed to evaluate the effects of pistachio oil on key bodily organs in female Wistar rats through biochemical and histopathological assessments.

## 2. Materials and Methods

### 2.1. Plant material

In the present investigation, seeds of *Pistacia vera* cv. Akbari were used, which were identified by a horticultural specialist. A voucher sample (No. AK212) was kept at the Pistachio Research Center, Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rafsanjan, Iran. For oil extraction, 50 g of pistachio powder was combined with 240 ml of hexane as a solvent. [14].

### 2.2. Animals and experimental design

The experiments were performed on 21 adult and virgin female Wistar rats weighing approximately 250 to 300 g. The study was approved by the ethics committee of Rafsanjan University of Medical Sciences (IR.RUMS.REC.1396.99). The polycarbonate cages were used for animals (four per cage) at room temperature ( $21 \pm 1$  °C) with a 12-hour light/dark cycle and free access to food and water. The rats were equally divided into three groups: Group 1 (Control group; N=7): rats receiving a normal diet; Group 2 (N=7): rats receiving a normal diet plus 10 mg/kg of pistachio oil by gavage for 21 days; and Group 3 (N=7): rats receiving a normal diet plus 50 mg/kg of pistachio oil by gavage for 21 days. [15, 16].

### 2.3. Sample collection

On the 22nd day of the experiment, the animals were euthanized via intraperitoneal injection of ketamine. Blood samples were collected from the heart into EDTA tubes. Serum samples were also obtained, and the following

biochemical parameters were measured using a biochemical autoanalyzer (MINDRAY, Guangzhou, China) with respective commercial kits (ParsAzmoon Co., Tehran, Iran): blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose (Glu), triglycerides (Tg), high-density lipoprotein cholesterol (HDL), and total cholesterol (Chol).

#### 2.4. Histopathological evaluations

After collecting blood samples from the hearts, the animals were euthanized, and liver, kidney, brain, ovary, and uterus tissues were harvested and fixed in 10% paraformaldehyde for 24 hours. These tissues were then processed for histopathological analysis and stained with hematoxylin and eosin. Sections were evaluated using a light microscope at different magnifications, and microscopic images were captured with a camera attached to the microscope. The following parameters were measured: necrosis and inflammation in the kidneys; endometrial linings, endometrial and myometrial thickness, epithelial cell height, and atretic follicles in the ovaries; hepatocyte degeneration, megalocytosis, and sinusoidal dilatation in the liver; as well as inflammation, degeneration of neuronal cells, and necrosis in the brain.

#### 2.5. Statistical analysis

The SPSS software version 26 was analyzed collected data. Data are presented as mean  $\pm$  standard deviation (SD). Differences between groups were assessed using one-way ANOVA, followed by the Dunnett post hoc test. A p-value of  $<0.05$  was considered statistically significant.

### 3. Results

#### 3.1. Biochemical and functional tests

According to the results presented in Table 1, chronic administration of both doses of pistachio

oil (10 and 50 mg/kg) for 21 days significantly increased biochemical parameters such as Glu (both doses:  $P<0.001$ ), Tg (both doses:  $P<0.001$ ), and HDL (10 mg/kg:  $P=0.020$ ; 50 mg/kg:  $P<0.001$ ) compared to the control group. Conversely, chronic administration of both doses of pistachio oil significantly decreased Chol (both doses:  $P<0.001$ ) compared to the control group. Additionally, a significant difference was observed between the two doses of pistachio oil regarding changes in biochemical tests (Glu:  $P=0.006$ ; Tg:  $P<0.001$ ; HDL:  $P=0.009$ ). Table 2 shows the effects of pistachio oil treatment for 21 days on kidney and liver function tests. Administration of both doses of pistachio oil significantly increased kidney and liver function tests, including AST (both doses:  $P<0.001$ ) and ALT (10 mg/kg:  $P=0.007$ ; 50 mg/kg:  $P<0.001$ ) compared to the control group. Furthermore, a significant difference was noted between the two doses of pistachio oil in changes to kidney and liver function tests such as AST ( $P<0.001$ ) and ALP ( $P=0.028$ ). There was no significant difference between the administration of both doses of pistachio oil and the control group regarding BUN, Cr, and ALP

#### 3.2. Histopathological results

Hematoxylin and eosin (H&E) staining was used to evaluate the toxic effects of chronic administration of pistachio oil on various organs in female rats. Histological examination of kidney sections from the treatment groups showed no evidence of necrosis or inflammation, and the lumens of the collecting tubules were clearly visible compared to the control group. Moreover, the glomeruli and urinary spaces of the Bowman capsule appeared normal (Figure 1). The consumption of pistachio oil supplements at both doses did not induce pathological changes such as hepatocyte degeneration, megalocytosis, or sinusoidal

dilatation in liver slides. Additionally, fibrosis and inflammation were not observed in portal fields compared to the control group (Figure 2). Histological evaluations of the ovaries and uterus in the treated groups revealed no pathological lesions. The endometrial linings were inactive and compact, containing blood vessels and connective tissue cells. Furthermore, epithelial cell height, as well as endometrial and myometrial thickness, were similar in both control and treated animals. Histological analysis of the ovaries in the control and experimental groups demonstrated a comparable number of atretic follicles (Figure 3). The results also indicated no inflammation, degeneration of neuronal cells, or necrosis in the brain slides of animals treated with pistachio oil compared to the control group (Figure 4).

#### 4. Discussion

The results of the current study showed that administration of pistachio oil for 21 days at both doses (10 and 50 mg/kg) increased biochemical parameters such as Glu, Tg, HDL, AST, and ALT, while decreasing Chol compared to the control group. Moreover, chronic consumption of pistachio oil did not induce pathological lesions in various organs of female rats, including the kidneys, liver, ovaries, uterus, and brain.

The nutritional profile of pistachios varies depending on the climate in which they are grown [17]. Compared to other nuts, pistachios contain lower fat and higher amounts of lutein and linoleic acid, which may help reduce fat accumulation in the body. They are also rich in protein, vitamin A,  $\beta$ -carotene, vitamin C, calcium, iron, monounsaturated fat, and polyunsaturated fat. Additionally, pistachios contain antioxidant compounds that reduce the risk of various disorders [18, 19]. A previous study reported that a pistachio diet significantly

decreased glucose, LDL, Chol, and Tg, while also reducing serum interleukin-6, total oxidant status, and malondialdehyde levels, and increasing superoxide dismutase levels [20]. Hamed *et al.* (2021) demonstrated the beneficial effects of the external green hull of pistachio in mitigating nephro- and hepatotoxicity caused by cisplatin and carbon tetrachloride in rats. They reported that the external hull reduced nephro- and hepatotoxicity by decreasing creatinine (Cr), urea, uric acid, AST, ALT, and ALP levels while normalizing the plasma lipid profile (Chol, Tg, LDL, HDL) through increased superoxide dismutase and glutathione peroxidase activities in both tissues [21]. Iso found that pistachio oil increased biochemical parameters such as Glu, Tg, HDL, AST, and ALT. In our previous investigations, we demonstrated the reno- and hepatoprotective effects of pistachio hydroalcoholic extract against cisplatin- or gentamicin-induced nephrotoxicity via biochemical and histological tests [4-6]. Furthermore, we found that hydroalcoholic extracts of pistachio have antioxidant effects by reducing malondialdehyde levels while increasing catalase, superoxide dismutase, and glutathione peroxidase activities in renal tissue [6]. Therefore, pistachio oil appears to be a powerful agent with no toxic effects on hepatic and renal function or tissues.

Women have sought to enhance their fertility using herbal medicine; however, few investigations have focused on their use for gynecological care. It is essential for women to ensure the safety and efficacy of herbal remedies [22]. Some plants such as licorice root [23], Aloe vera [24], Carica papaya [25], and Ziziphus jujuba [26] used to treat gynecological diseases affecting fertility, contraception, irregular bleeding, menstrual issues, and abortion.. A Mediterranean diet rich in various nuts has been

shown to reduce the incidence of infertility and pregnancy-related complications [27]. In traditional medicine, pistachio oil has been used to alleviate menopausal symptoms due to its antioxidant properties that help maintain the structure and health of the female reproductive system. Previous studies indicated that both pistachio oil and hydroalcoholic extract reduced behavioral manifestations of menopause in ovariectomized rats [7, 8]. Our results showed that chronic consumption of pistachio oil had no pathological effect on the ovaries or uterus of female rats; however, further studies with larger sample sizes are needed to determine whether fertility is affected by such diets. The present study demonstrated that pistachio oil had no adverse effects on the brains of female rats. Alturfan *et al.* (2009) reported that pistachio supplementation can improve blood lipid profiles and may therefore be used to prevent cardiovascular problems [28]. Additionally, Nuzzo *et al.* (2020) reported the neuroprotective effects of pistachio consumption in preventing high-fat diet-induced neuronal apoptosis in mice [29]. Another study indicated that pistachio oil

reduced anxiety-like behaviors in rats; this neuroprotective effect may be mediated through improvements in lipid profiles [30]. T

Based on these studies, our results indicate that pistachio oil has no adverse effects on major organs and can be used safely without concern.

## 5. Conclusions

Based on the results of the present study, it can be concluded that pistachio oil has no toxic effects on kidney, brain, liver, uterus, or ovarian functions. The use of pistachio oil is considered an important supplement for women due to its safety profile.

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## Conflict of interest

All authors declare that they have no conflict of interest.

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