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The Effect of Giresun Hazelnut Oil Against Formaldehyde Damage in Rat Testicular Tissues

Efecto del Aceite de Avellana Giresun Contra el Daño Inducido por Formaldehído en Tejidos Testiculares de Rata

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SUMMARY: The aim of this study was to determine the toxic effects of formaldehyde on testicular tissue and the protective effect of Giresun hazelnut oil against these effects. In the study, 40 Wistar albino male rats were used. Rats in Group I were injected intraperitoneally with formaldehyde every other day. Rats in Group II were designated as the control group. Rats in Group III were intragastrically administered Giresun hazelnut oil 100 mg/kg and rats in Group IV were administered Giresun hazelnut oil 200 mg/kg every other day with formaldehyde injection. At the end of the 30-day experimental period, all rats were euthanized under high dose anesthesia after blood sampling. Biochemical and histologic evaluations were performed on the testicular tissues. Lipid peroxidation (LPO) and myeloperoxidase enzyme activity (MPx) were examined in rats treated with formaldehyde. Compared to the control, LPO levels and MPx enzyme activity were found to be very high in the formaldehyde group. Testosterone levels were very low in the formaldehyde group. The applied Giresun hazelnut oil brought this ratio to the level of healthy tissues. Testosterone levels and interstitial cell (Leydig cell) numbers were also found to be very low in tissues exposed to formaldehyde. Formaldehyde caused harmful effects on testicular tissue and water extract of Giresun hazelnut oil showed a significant protective effect against these effects.

KEY WORDS: Formaldehyde; Rat; Testis; Giresun hazelnut oil.

INTRODUCTION

Formaldehyde (FA) is a ubiquitous environmental pollutant and a simple aldehyde compound (CH_2O) characterized by its colorless appearance, high water solubility, and distinctive irritating odor (Smith, 1992). This low molecular weight and highly reactive aldehyde has extensive applications across various industries and scientific disciplines. FA is commonly found in textiles, dyes, plastics, and household cleaning products. In healthcare settings, it serves multiple purposes including as a component in dental coatings, a treatment for intractable cystitis, and in hemodialysis solutions. Due to its exceptional preservative properties, FA remains the predominant chemical utilized in anatomy laboratories for long-term cadaver and organ preservation. Additionally, it is widely employed for tissue fixation in histology and pathology laboratories (Evren *et al*., 2010).

The pervasive use of FA in numerous applications results in constant human exposure in daily life. Anatomists performing dissections and students studying cadavers are particularly susceptible to unavoidable FA contact. Experimental investigations have conclusively demonstrated that FA, now recognized as a carcinogen, exerts detrimental effects on multiple physiological systems including respiratory, nervous, reproductive, and digestive systems (Monteiro-Riviere & Popp, 1986). Regarding the reproductive system specifically, FA exposure has been linked to infertility. Animal studies have reported that FA induces oxidative damage in testicular tissue, reduces serum testosterone levels, and adversely affects sperm quality through testicular impairment (Majumder & Kumar, 1995; Sarsılmaz & Özen, 2000; Özen *et al*., 2003).

Recent research has further elucidated the mechanisms underlying FA-induced reproductive toxicity. Duong *et al*. (2011), conducted a comprehensive review revealing that FA exposure significantly decreases succinate

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dehydrogenase (SDH) activity in testicular tissue, establishing SDH as a potential biomarker for testicular damage (Duong *et al*., 2011). Han *et al*. (2015) demonstrated that FA exposure triggers autophagy in testicular tissues, suggesting that autophagy may be a critical factor responsible for male reproductive impairment following FA exposure. Additionally, Wang *et al*. (2015), reported that FA vapor exposure at concentrations of 10 and 20 ppm destroyed testicular structure and decreased sperm concentration, further confirming FA's reproductive toxicity.

Infertility represents a significant global health concern affecting approximately 15% of couples attempting to conceive, with male factors contributing to 20-50% of cases (Birmingham, 2015). It is estimated that 37% of couples seeking assisted reproductive technology interventions present with male-origin infertility. The etiology of male infertility encompasses genetic factors, environmental exposures, and varicocele. Among these, compromised sperm quality emerges as the predominant issue in male infertility, primarily resulting from oxidative stress (OS) (Birmingham, 2015).

OS has been identified as one of the principal mediators of male infertility through its detrimental effects on sperm function (Agarwal *et al*., 2014a). OS represents a state characterized by increased cellular damage triggered by oxygen and oxygen-derived free radicals known as reactive oxygen species (ROS). This condition occurs when augmented ROS production overwhelms the body's antioxidant defense mechanisms. While minimal ROS levels are essential for normal sperm function, disproportionate concentrations can negatively impact spermatozoa quality and impair overall fertilizing capacity. Tremellen (2008) established that OS plays a central role in sperm dysfunction, representing a significant pathological mechanism underlying male infertility. The pathophysiology of OS-induced male infertility involves multiple mechanisms. ROS and their metabolites can attack DNA, lipids, and proteins; alter enzymatic systems; produce irreparable cellular alterations; cause cell death; and ultimately lead to declining semen parameters associated with male infertility (8m). Specifically, OS causes lipid peroxidation of sperm cell membranes, which are rich in polyunsaturated fatty acids, making them particularly vulnerable to oxidative damage. This results in decreased sperm motility, reduced sperm-oocyte fusion capability, and DNA fragmentation (Takeshima *et al*., 2021). For normal spermatozoa functioning, ROS must be balanced by antioxidants. Antioxidants of enzymatic origin are naturally present in seminal fluid, while non-enzymatic antioxidants are derived from dietary sources. Dietary antioxidants have gained significant attention for their potential protective effects against reproductive damage. Hazelnuts, predominantly cultivated in Turkey's Black Sea Region (including Giresun, Ordu, Trabzon, and Samsun provinces), represent a rich source of antioxidant compounds, carbohydrates, proteins, minerals, and vitamin E. This nutritional profile has prompted numerous investigations into hazelnuts' potential benefits for various health conditions including anemia, bone development, cardiac rhythm regulation, cholesterol management, sexual function enhancement, and skin rejuvenation. Recent studies have specifically examined hazelnuts' effects on reproductive health. Kara *et al*. (2021a), demonstrated that hazelnut supplementation significantly improved testicular antioxidant function and semen quality in both young and old male rats. Their research revealed that hazelnut-supplemented diets enhanced histopathological variables, sperm quality, seminal plasma and plasma oxidative stress markers, seminal plasma vitamin E levels, and plasma testosterone concentrations. Kara *et al*. (2021b), further investigated hazelnuts' protective effects against doxorubicin-induced reproductive damage, concluding that hazelnuts may exert positive effects on reproductive system damage through their high antioxidant activities, which reduce cell membrane oxidation.

The present study aims to investigate the protective effects of Giresun hazelnut oil against formaldehyde-induced damage in rat testicular tissues. Given the established reproductive toxicity of formaldehyde and the promising antioxidant properties of hazelnuts, we hypothesized that hazelnut extract administration would mitigate the adverse effects of formaldehyde on testicular tissue through its antioxidant mechanisms. This investigation employed both biochemical and histological methodologies to comprehensively evaluate the potential protective effects of hazelnut extract against formaldehyde-induced testicular damage.

MATERIAL AND METHOD

Experimental Animals

The present study utilized forty (40) male Wistar albino rats weighing 250-300 g, obtained from the Giresun University Animal Experiments Laboratory. Prior to experimental procedures, the animals were allowed a one-week acclimatization period to adapt to their new environment. Throughout the study, rats were housed under controlled environmental conditions (temperature: 20-22 °C, relative humidity: 60±5 %) with a standardized 12-hour light/12-hour dark cycle. The animals were maintained in polycarbonate cages with stainless steel wire tops and provided with standard rat pellet feed and tap water *ad libitum*. The water was refreshed daily, and no dietary restrictions were imposed during the experimental period.

All animal handling and experimental procedures were conducted in strict accordance with institutional guidelines for the care and use of laboratory animals. The study protocol received approval from the Giresun University Animal Experiments Local Ethics Committee (approval date: 19.07.2023, decision number: 2023/1).

The formaldehyde solution (37% w/v) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and diluted with sterile saline to achieve the required concentration. Vitamin E (a-tocopherol acetate) was also procured from Sigma-Aldrich. All substances were prepared fresh on the day of administration under aseptic conditions.

Preparation of Hazelnut Extract:

Giresun oil hazelnuts were sourced from the hazelnut orchard of Ferhat Önal Grup A.Ş. located in the Espiye District of Giresun Province, Turkey. The extraction procedure was performed according to a modified protocol based on methods described by Yuan *et al*. (2018a), with adaptations to optimize the extraction of bioactive compounds. The hazelnuts were initially shelled and carefully inspected to remove any damaged kernels. To enhance extraction efficiency, the hazelnuts were finely chopped using a commercial food processor (Waring Commercial, Torrington, CT, USA) to increase the surface area and minimize water content. The resulting hazelnut powder was then subjected to a drying process in a vacuum evaporator (Heidolph Laborota 4000, Schwabach, Germany) for 12 hours at 45 °C under reduced pressure to remove residual moisture.

Following desiccation, the dried hazelnut powder was suspended in distilled water at a ratio of 1:10 (w/v) and subjected to continuous agitation using an orbital shaker (IKA KS 4000, Staufen, Germany) at 150 rpm for seven days at 65 °C. This extended extraction period was implemented to maximize the yield of water-soluble bioactive compounds, particularly phenolic antioxidants. The extraction temperature of 65°C was selected based on previous optimization studies demonstrating enhanced extraction of phenolic compounds from hazelnut without significant degradation.

After the extraction period, the mixture was filtered through Whatman Grade 1 filter paper to remove solid particulates, followed by a second filtration through a 0.45 mm membrane filter (Millipore, Bedford, MA, USA) to ensure removal of fine particles. The resulting filtrate was concentrated using a rotary evaporator (Heidolph Laborota 4000) at 40 °C under reduced pressure until a final concentration of 100 mg/ml was achieved. The extract was stored in amber glass bottles at 4 °C until use, with stability confirmed for up to 30 days based on preliminary studies.

Experimental Design and Treatments

The current study was planned in light of other research conducted in the literature. Formaldehyde was chosen for this study because it is used to preserve cadavers in anatomy and specimens in histopathology, and we believe that hazelnuts, with their high antioxidant levels and a widespread use in our region, will mitigate this damage. Groups were formed accordingly. While specifying the groups and adjusting the doses and experimental duration, support was taken from the literature mentioned below.

Following acclimatization, the rats were randomly allocated into five experimental groups (n=8 per group) using a computer-generated randomization sequence. Each group was housed in two separate cages (4 rats per cage) to minimize stress and ensure adequate space. The experimental groups were designed as follows:

- Group I (Formaldehyde group, FD): Rats received intraperitoneal (i.p.) injections of formaldehyde at a dose of 10 mg/kg (diluted 1:10 in sterile saline) at the same time every day for 30 consecutive days. This dosage was selected based on previous studies demonstrating significant testicular toxicity without excessive systemic effects (Sarsılmaz & Özen, 2000; Özen *et al*., 2003).

- Group II (Control group): Animals were administered equivalent volumes of sterile saline via i.p. injection at the same time every day for 30 consecutive days serving as the negative control.

- Group III (FD+Hazelnut 100 mg/kg): Rats received i.p. formaldehyde (10 mg/kg, diluted 1:10 in saline) at the same time every day for 30 consecutive days. concurrent with daily oral administration of hazelnut extract (100 mg/kg) via orogastric intubation in a maximum volume of 1 ml.

- Group IV (FD+Hazelnut 200 mg/kg): Rats received i.p. formaldehyde (10 mg/kg, diluted 1:10 in saline) at the same time every day for 30 consecutive days. concurrent with daily oral administration of hazelnut extract (200 mg/kg) via orogastric intubation in a maximum volume of 1 ml.

- Group V (FD+Vitamin E): Rats received i.p. formaldehyde (10 mg/kg, diluted 1:10 in saline) at the same time every day for 30 consecutive days. concurrent with daily oral administration of vitamin E (a-tocopherol acetate, 50 mg/kg) via orogastric intubation. This group served as a positive control, as vitamin E is a well-established antioxidant with documented protective effects against formaldehyde-induced testicular damage (Vosoughi *et al*., 2013).

Experimental Procedures and Sample Collection

Throughout the 30-day experimental period, all animals were monitored daily for clinical signs of toxicity, changes in behavior, and body weight fluctuations. Body weights were recorded at baseline and weekly intervals using a calibrated digital scale (Sartorius, Göttingen, Germany).

At the conclusion of the experimental period, all rats were fasted overnight (12 hours) with free access to water. The following morning, animals were anesthetized using a combination of ketamine hydrochloride (90 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) (Ketasol, Richter Pharma AG, Wels, Austria; Rompun, Bayer, Leverkusen, Germany). Once deep anesthesia was confirmed by the absence of pedal withdrawal reflexes, blood samples were collected via cardiac puncture using 5 ml syringes with 21G needles. Blood samples were immediately transferred to serum separator tubes and allowed to clot at room temperature for 30 minutes. The samples were then centrifuged at 3,000 × g for 15 min at 4 °C to separate serum. The resulting serum was aliquoted into microcentrifuge tubes and stored at -80 °C until biochemical analysis. Following blood collection, the animals were euthanized by cervical dislocation under deep anesthesia. The abdominal cavity was immediately accessed via a midline laparotomy, and both testes were carefully excised along with the epididymides. The testes were gently cleaned of adhering connective tissue and fat, weighed using an analytical balance (Sartorius, Göttingen, Germany), and processed for subsequent analyses. For each animal, the right testis was fixed in Bouin's solution for histological examination, while the left testis was divided into two portions: one was immediately flash-frozen in liquid nitrogen and stored at -80°C for biochemical analyses, and the other was homogenized for immediate assessment of oxidative stress parameters.

Histological Analysis

The testicular tissues fixed in Bouin's solution were processed according to standard histological procedures as described by Mazaud-Guittot *et al*. (2011) with minor modifications. After 24 hours of fixation, the tissues were dehydrated through an ascending series of ethanol concentrations (70 %, 80 %, 90%, and 100 %), cleared in xylene, and embedded in paraffin wax. Serial sections of 5-6 μm thickness were obtained using a rotary microtome (Leica RM2255, Wetzlar, Germany). The sections were mounted on glass slides, deparaffinized with xylene, rehydrated through a descending series of ethanol concentrations, and stained with hematoxylin and eosin (H&E) according to the protocol described by Akbas *et al*. (2023). Briefly, the sections were immersed in Harris hematoxylin solution for 5 min, rinsed in running tap water for 5 min, counterstained with eosin Y solution for 2 min, dehydrated through an ascending ethanol series, cleared in xylene, and mounted with DPX mounting medium (Sigma-Aldrich).The stained sections were examined under a light microscope (Olympus BX51, Tokyo, Japan) equipped with a digital camera (Olympus DP74) at various magnifications (100×, 200×, and 400×). For each animal, a minimum of 20 randomly selected seminiferous tubules from different regions of the testis were evaluated for histomorphological parameters, including:

1. Seminiferous tubule diameter (μm)
2. Seminiferous epithelium height (μm)
3. Presence of degenerative changes (vacuolization, epithelial sloughing, giant cell formation)
4. Interstitial space characteristics
5. Interstitial cell (Leydig cell) count per interstitial area

All measurements were performed using calibrated image analysis software (Olympus cellSens, Tokyo, Japan). The histological evaluations were conducted by two independent observers blinded to the experimental groups to minimize bias.

Biochemical Analyses

Tissue Homogenization. Testicular tissue samples stored at -80°C were thawed on ice and homogenized in ice-cold phosphate-buffered saline (PBS, pH 7.4) at a ratio of 1:10 (w/v) using a tissue homogenizer (Ultra-Turrax T25, IKA, Staufen, Germany). The homogenates were centrifuged at 10,000 × g for 15 min at 4 °C, and the resulting supernatants were collected for biochemical analyses. The protein concentration in each sample was determined using the Bradford method (Bradford, 1976) with bovine serum albumin as the standard.

Lipid Peroxidation (LPO) Determination. Lipid peroxidation levels in testicular tissue were assessed by measuring malondialdehyde (MDA) content using the thiobarbituric acid reactive substances (TBARS) method as described by Ohkawa *et al*. (1979), with minor modifications. Briefly, 0.5 g of testicular tissue was homogenized in an appropriate buffer to obtain a homogenate. The homogenate was centrifuged at 5,000 × g for 20 min at 4 °C. The reaction mixture consisted of 0.2 ml of tissue supernatant, 0.2 ml of 8.1 % sodium dodecyl sulfate (SDS), 1.5 ml of 20 % acetic acid (pH 3.5), and 1.5 ml of 0.8 % thiobarbituric acid (TBA). The mixture was heated at 95 °C for 60 min, then cooled on ice. After cooling,

1.0 ml of distilled water and 5.0 ml of n-butanol/pyridine mixture (15:1, v/v) were added, and the solution was vortexed vigorously. The mixture was centrifuged at 4,000 × g for 10 min, and the absorbance of the organic layer was measured spectrophotometrically at 532 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The MDA concentration was calculated using a standard curve prepared with 1,1,3,3-tetraethoxypropane and expressed as nanomoles of MDA per milligram of protein (nmol/mg protein).

Myeloperoxidase (MPx) Activity Assay. Myeloperoxidase enzyme activity was determined according to the method described by Bradley *et al*. (1982), which measures hypochlorous acid (HOCl) production in tissues. Briefly, 0.1 g of testicular tissue was homogenized in 10 ml of 50 mM phosphate buffer (pH 6.0). The homogenate was centrifuged at 10,000 × g for 15 min at 4 °C to obtain the supernatant. The reaction mixture was prepared by adding 100 ml of the supernatant to 1.9 ml of 10 mM phosphate buffer (pH 6.0) and 1 ml of o-dianisidine hydrochloride solution (0.167 mg/ml) containing 0.0005 % hydrogen peroxide. The change in absorbance was recorded at 460 nm at 30-s intervals for a total of 5 min using a UV-visible spectrophotometer (Shimadzu UV-1800). MPx activity was calculated using the molar extinction coefficient of oxidized o-dianisidine (11.3×10^3 M^{-1} cm^{-1}) and expressed as units per gram of tissue (U/g tissue), where one unit of MPx activity was defined as the amount of enzyme degrading 1 mmol of hydrogen peroxide per minute at 25 °C.

Testosterone Analysis. Serum testosterone levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Cayman Testosterone EIA Kit 582701, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. The assay is based on the competition between testosterone and a testosterone-acetylcholinesterase (AChE) conjugate for a limited number of testosterone-specific rabbit antiserum binding sites. Briefly, 50 ml of each serum sample was added to the appropriate wells of the ELISA plate, followed by the addition of testosterone AChE tracer and testosterone antiserum. The plate was incubated at room temperature for 2 hours on an orbital shaker. After incubation, the wells were washed five times with wash buffer, and Ellman's reagent (containing the substrate for AChE) was added. The plate was incubated in the dark for 60-90 minutes, and the absorbance was measured at 412 nm using a microplate reader (Thermo Multiskan FC, Waltham, MA, USA). Testosterone concentrations were calculated using a standard curve generated with known concentrations of testosterone and expressed as picograms per milligram of protein (pg/mg protein).

Statistical Analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using the Shapiro-Wilk test. Since the data followed a normal distribution, parametric statistical methods were employed for all analyses. Differences between experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons. All results were expressed as mean ± standard deviation (SD). A p-value less than 0.05 was considered statistically significant. For histomorphometric analyses, the intra- and inter-observer variabilities were assessed using intraclass correlation coefficients (ICCs). Power analysis was performed using G*Power software (version 3.1.9.7, Heinrich-Heine-Universität Düsseldorf, Germany) to ensure adequate sample size for detecting significant differences between groups with a power of 0.8 and an alpha level of 0.05.

RESULTS

Biochemical Results

LPO levels and MPx activities, which are oxidative damage markers, were measured spectrophotometrically in testicular tissues. As indicated in the graphs, the LPO level was found to be significantly higher in the testicular tissues administered formaldehyde compared to the healthy group ($p < 0.05$). A significant decrease was detected in the groups given hazelnut extracts and vitamin E. Again, there is a very high increase in MPx enzyme activities in the formaldehyde applied group compared to the healthy group. In the treatment groups, this increase was reduced almost to the healthy group ($p< 0.05$) (Fig.1). Testosterone levels were determined in serum analyzes of the same tissues. As expressed in the graph; Testosterone level in the formaldehyde group was determined as 0.2500±0.003. It was measured as 0.4000±0.002, 0.2610±0.002, 0.3510±0.001 and 0.3870±0.002 pg/mg in both doses of healthy, hazelnut extracts and vitamin E groups, respectively. It has been shown that there are statistically significant differences between groups ($p< 0.05$) (Fig. 2).

Histological Results

In the current study, testicular tissues were examined to express histological changes. It was observed that the seminiferous tubules and interstitial area in the control group testes had a normal histological appearance. It has been observed that the seminiferous tubule wall consists

of sustentacular cells (Sertoli cells) and spermatogenic cells at different stages of development. It was determined that there were blood vessels of Leydig cells in the interstitial area and the general structure was regular and compact. In the formaldehyde group, degeneration and vacuolization were observed in the seminiferous tubules. Additionally, separations were determined in the basal lamina. It is noteworthy that the integrity is also disrupted in the interstitial area. It was determined that there was a decrease in the number of interstitial cells compared to the control group. It appears that hazelnut extracts reduce the toxic effect of formaldehyde in tissues. It was determined that degeneration decreased in the seminiferous tubules. Depending on the dose, it can be said that the toxic effect is more effective in tissues at 200 mg/kg. It was determined that the testicles of rats given vitamin E had a structure close to the control. It was observed that the seminiferous tubule structure was similar to the control group and that there was integrity in the seminiferous tubule and interstitial area compared to the formaldehyde group. The presence of sustentacular cells and germ cells in the seminiferous tubules is noteworthy (Figs. 2 and 3).

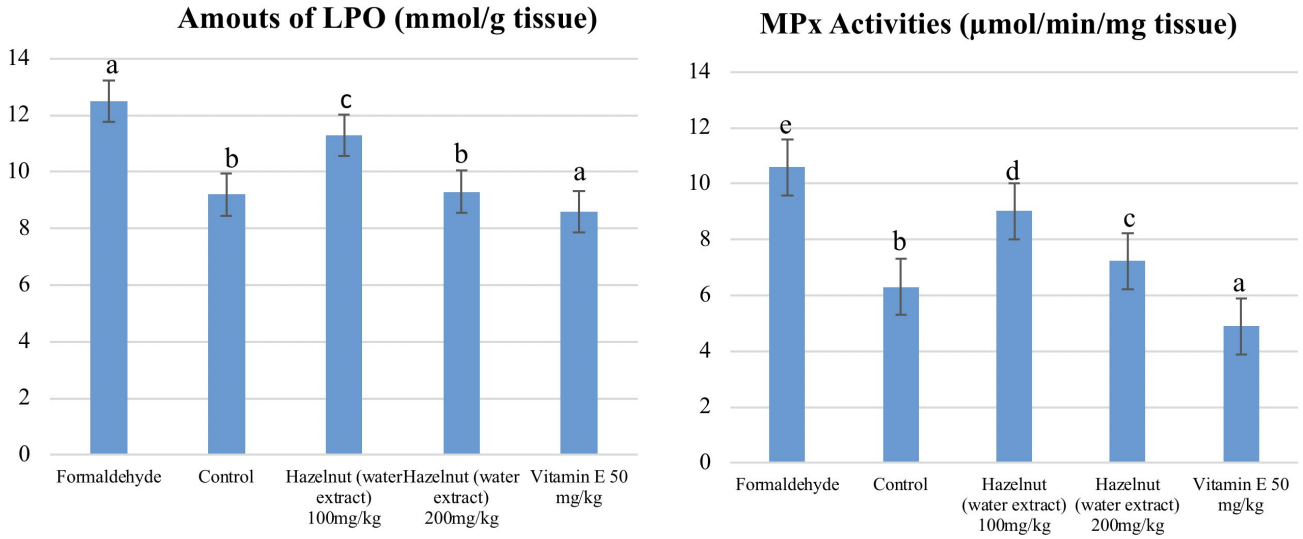

Fig. 1. The results of LPO levels and MPx activities in testis tissues, the testosterone levels in serum analyzes of the obtained from formaldehyde, control, hazelnut extracts and vitamin E.

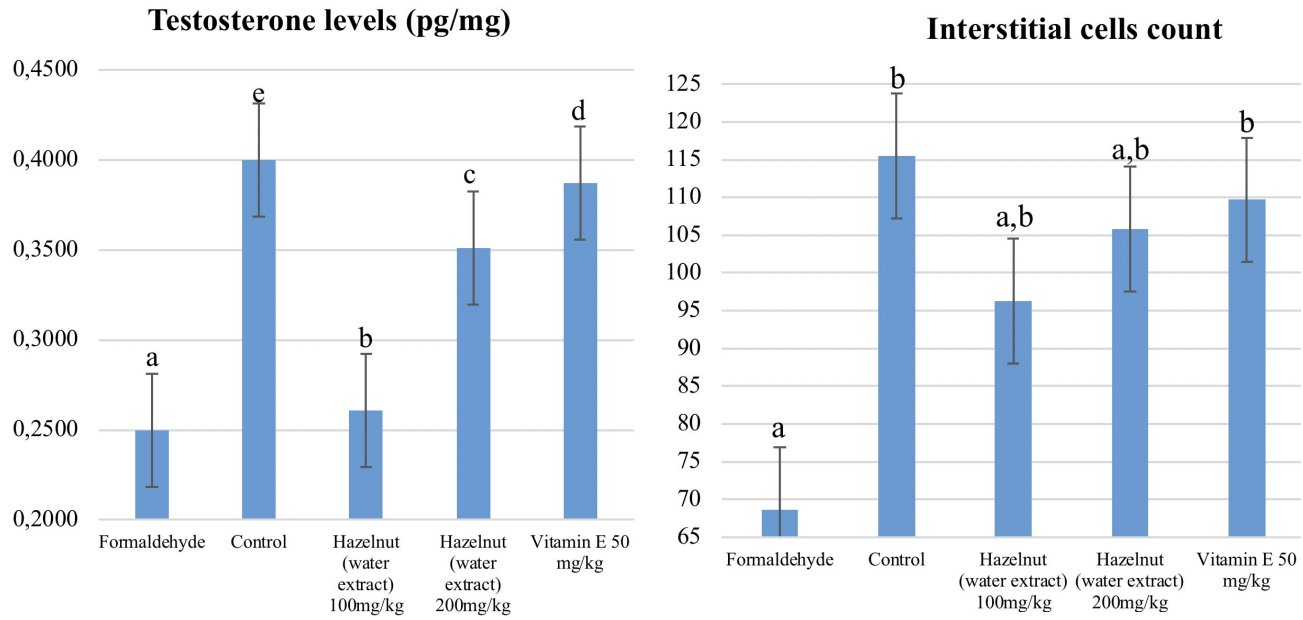

Fig. 2. The results of the testosterone levels in serum analyzes and interstitial cell count of the obtained from formaldehyde, control, hazelnut extracts and vitamin E.

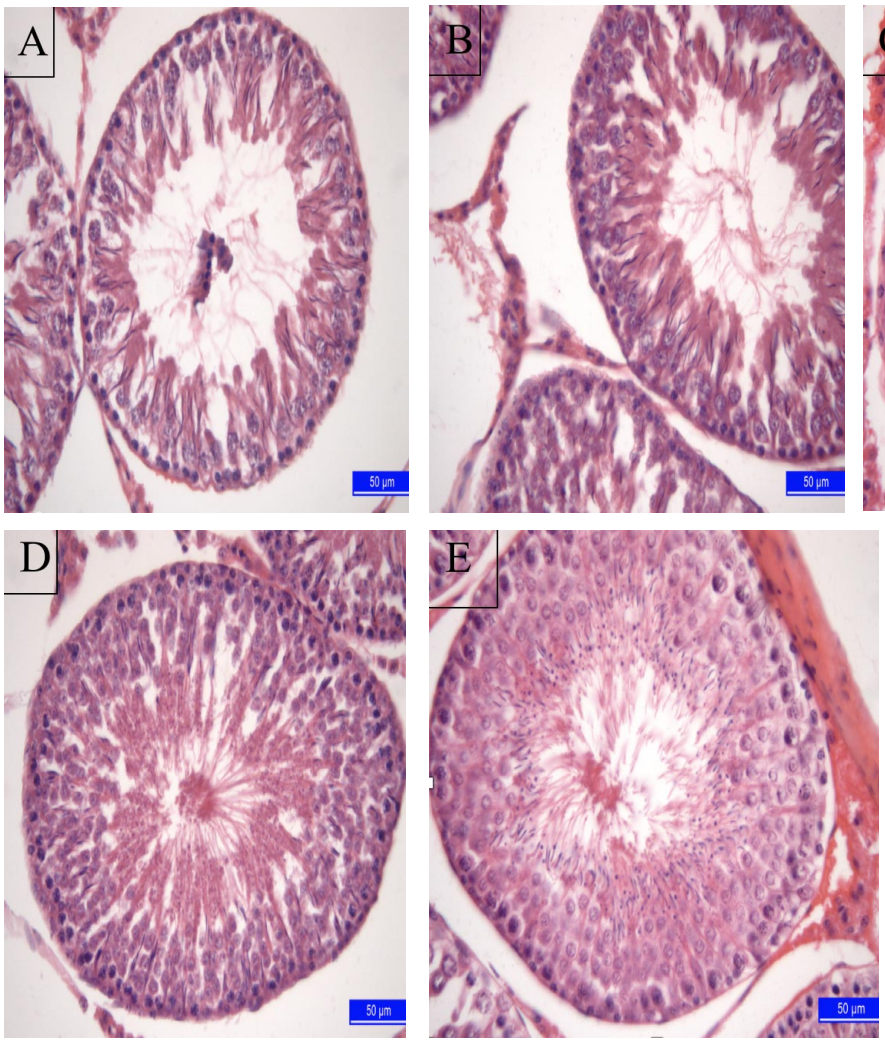

Fig. 3. The rat testicular tissues treated with formaldehyde, Giresun hazelnut oil, water extract and vitamin E (A- formaldehyde, B- Giresun hazelnut oil100 mg/kg, C- Giresun hazelnut oil 200 mg/kg, D- vitamin E, E-control group)

DISCUSSION

Formaldehyde (FA) is a ubiquitous environmental pollutant with well-documented toxic effects on various organ systems. The present study investigated the potential protective effects of Giresun hazelnut oil extract against FA-induced testicular damage in rats. Our findings demonstrated that FA exposure resulted in significant testicular toxicity, characterized by elevated oxidative stress markers, decreased testosterone levels, and histopathological alterations. Importantly, administration of hazelnut extract, particularly at the higher dose (200 mg/kg), substantially mitigated these adverse effects, suggesting a protective role of hazelnut-derived antioxidants against FA-induced reproductive toxicity.

Formaldehyde-Induced Testicular Damage and Oxidative Stress

The current study revealed that FA administration significantly increased lipid peroxidation (LPO) levels and myeloperoxidase (MPx) enzyme activity in testicular tissues compared to the control group. These findings align with previous research demonstrating FA's capacity to induce oxidative stress in reproductive tissues. Razi *et al*. (2013), reported that long-term FA exposure causes significant oxidative damage in testicular tissue, leading to impaired spermatogenesis and reduced sperm quality. Similarly, Asadi *et al*. (2017), identified oxidative stress as a critical factor in the development of male infertility, particularly in tissues with high rates of cell division and mitochondrial oxygen consumption, such as the testes.

The mechanism underlying FA-induced testicular damage involves multiple pathways. The environmental exposures to toxicants like FA can disrupt the Sertoli-Sertoli cell-mediated blood-testis barrier (BTB), compromising the

protective microenvironment essential for spermatogenesis. Our histological findings corroborate this mechanism, as we observed significant degeneration and vacuolization in the seminiferous tubules, along with separations in the basal lamina in the FA-exposed group. These structural alterations likely contribute to the observed reduction in testosterone levels and interstitial cell numbers.

The increased MPx activity observed in our study suggests enhanced neutrophil infiltration and inflammatory responses in testicular tissue following FA exposure. This finding is consistent with Betancourt-Martínez *et al*. (2023), who demonstrated that FA induces both oxidative stress and inflammatory responses in testicular tissue. The inflammatory cascade triggered by FA exposure may further exacerbate oxidative damage through the release of pro-inflammatory cytokines and additional reactive oxygen species (ROS), creating a self-perpetuating cycle of tissue injury (Betancourt-Martínez *et al*., 2023).

Furthermore, our results showed significantly decreased serum testosterone levels in FA-exposed rats, consistent with previous studies. Zang *et al*. (2017), reported that FA exposure inhibits sexual behavior, causes reproductive organ atrophy, and impairs spermatogenesis in male mice, effects that are partially mediated by reduced testosterone production. The decreased testosterone levels observed in our study may be attributed to the direct toxic effects of FA on interstitial cells, as evidenced by the reduced interstitial count in histological examinations. Testosterone is essential for normal spermatogenesis and maintenance of secondary sexual characteristics; thus, its reduction following FA exposure has significant implications for male reproductive health (Zang *et al*., 2017).

Protective Effects of Hazelnut Extract Against Formaldehyde-Induced Testicular Damage

A notable finding of our study was the significant protective effect of Giresun hazelnut oil extract against FA-induced testicular damage. Administration of hazelnut extract, particularly at the higher dose (200 mg/kg), markedly reduced LPO levels and MPx activity in testicular tissues, restored testosterone levels, and improved histological parameters compared to the FA-only group. These protective effects were comparable to those observed with vitamin E, a well-established antioxidant.

The protective mechanisms of hazelnut extract can be attributed to its rich antioxidant content. Hazelnuts contain various bioactive compounds, including vitamin E, phenolic acids, flavonoids, and phytosterols, which possess potent antioxidant and anti-inflammatory properties (Yuan *et al*., 2018b). These compounds can neutralize free radicals, inhibit lipid peroxidation, and enhance endogenous antioxidant defense systems, thereby mitigating oxidative stress-induced tissue damage (Yuan *et al*., 2018b).

Our findings align with those of Kara *et al*. (2019), who demonstrated that hazelnut supplementation significantly improves testicular antioxidant function and semen quality in both young and old male rats (Kara *et al*., 2019). Their study showed that hazelnut-supplemented diets enhanced histopathological variables, sperm quality, and reduced oxidative stress markers in seminal plasma. Similarly, Kara *et al*. (2021a), reported that hazelnut supplementation mitigated doxorubicin-induced damage to the reproductive system in male rats, further supporting the protective role of hazelnuts against reproductive toxicants.

The dose-dependent protective effect observed in our study suggests that the higher concentration of antioxidant compounds in the 200 mg/kg dose provided more effective protection against FA-induced oxidative damage. This observation is consistent with previous studies demonstrating dose-dependent antioxidant effects of plant extracts against various toxicants (Abarikwu *et al*., 2022). The comparable efficacy of the 200 mg/kg hazelnut extract to vitamin E, a standard antioxidant, highlights the potential of hazelnut-derived antioxidants as natural alternatives for protecting against reproductive toxicity.

Histopathological Improvements with Hazelnut Extract Treatment

The histopathological findings in our study provide further evidence for the protective effects of hazelnut extract against FA-induced testicular damage. FA exposure resulted in significant structural alterations in testicular tissue, including degeneration and vacuolization of seminiferous tubules, separations in the basal lamina, and disruption of the interstitial area. These observations are consistent with previous studies reporting FA-induced histopathological changes in testicular tissue (Ozen *et al*., 2008; Tesfaye *et al*., 2021).

Administration of hazelnut extract, particularly at the higher dose (200 mg/kg), substantially improved these histopathological parameters. The seminiferous tubules showed reduced degeneration, and the integrity of both the seminiferous tubules and interstitial areas was better preserved compared to the FA-only group. These improvements were comparable to those observed in the vitamin E-treated group, which showed a structure close to the control group.

The preservation of testicular architecture by hazelnut extract is likely mediated by its antioxidant and anti-inflammatory properties, which protect cellular membranes and organelles from oxidative damage. The maintenance of seminiferous tubule integrity is particularly important for normal spermatogenesis, as it provides the specialized microenvironment required for germ cell development. Similarly, the preservation of interstitial cells in the interstitial area is crucial for testosterone production, which regulates spermatogenesis and maintains male reproductive function.

Clinical Implications and Translational Significance

The findings of our study have several important clinical implications. Formaldehyde is a ubiquitous environmental and occupational toxicant, with exposure occurring in various settings, including industrial workplaces, medical facilities, and even residential environments through building materials and consumer products. Chronic exposure to FA has been associated with adverse reproductive outcomes in humans, including reduced semen quality and fertility issues (Lv *et al*., 2022).

Duong *et al*. (2011), conducted a systematic review of FA's reproductive and developmental toxicity, reporting associations between occupational FA exposure and adverse reproductive outcomes, including menstrual disorders, endometriosis, and decreased fertility. The Centers for Disease Control and Prevention (CDC, 2024) has also acknowledged that working with FA may increase the risk of fertility problems or miscarriage (Duong *et al*., 2011). These findings underscore the public health significance of identifying protective interventions against FA-induced reproductive toxicity (Duong *et al*., 2011).

Our study suggests that dietary supplementation with hazelnut or hazelnut-derived antioxidants may offer protection against FA-induced reproductive damage. This finding is particularly relevant for individuals with occupational exposure to FA, such as anatomists, pathologists, embalmers, and industrial workers. Dietary interventions represent a practical, accessible, and potentially cost-effective approach to mitigating the adverse reproductive effects of environmental toxicants.

Furthermore, the protective effects of hazelnut extract observed in our study may extend to other reproductive toxicants that induce oxidative stress through similar mechanisms. Oxidative stress is a common pathway in the reproductive toxicity of various environmental chemicals, including heavy metals, pesticides, and industrial solvents (Duong *et al*., 2011). Therefore, the antioxidant properties of hazelnuts may provide broader protection against a range of reproductive toxicants, although this hypothesis requires further investigation (Agarwal *et al*. 2014b).

Study Limitations and Future Research Directions

While our study provides valuable insights into the protective effects of hazelnut extract against FA-induced testicular damage, several limitations should be acknowledged. First, the study focused primarily on biochemical and histological parameters, without directly assessing sperm parameters or fertility outcomes. Future studies should include comprehensive spermatological analyses, including sperm count, motility, morphology, and DNA integrity, as well as fertility assessments through mating experiments.

Second, the molecular mechanisms underlying the protective effects of hazelnut extract were not fully elucidated in the current study. Future research should investigate the effects of hazelnut extract on specific signaling pathways involved in oxidative stress, inflammation, and apoptosis in testicular tissue. Techniques such as gene expression analysis, immunohistochemistry, and Western blotting could provide deeper insights into the molecular mechanisms of protection.

Third, our study used a relatively short-term exposure model (30 days), which may not fully capture the effects of chronic FA exposure that occurs in occupational settings. Longer-term studies are needed to evaluate the protective efficacy of hazelnut extract against chronic FA exposure and to determine the optimal duration and timing of supplementation for maximal protection.

Fourth, while our study demonstrated the protective effects of whole hazelnut extract, it did not identify the specific bioactive compounds responsible for these effects. Future studies should isolate and characterize the active components of hazelnut extract and evaluate their individual and synergistic effects against FA-induced reproductive toxicity. This approach could lead to the development of more targeted interventions with enhanced efficacy.

Finally, the translational significance of our findings to human populations requires further investigation. Clinical studies are needed to evaluate the effects of hazelnut consumption on reproductive parameters in individuals with occupational exposure to FA or other reproductive toxicants. Such studies should consider factors such as dose, duration, and timing of hazelnut consumption, as well as individual variations in metabolism and susceptibility to reproductive toxicants.

CONCLUSION

In conclusion, our study demonstrates that Giresun hazelnut oil extract provides significant protection against formaldehyde-induced testicular damage in rats, likely through its antioxidant and anti-inflammatory properties. The protective effects were particularly pronounced at the higher dose (200 mg/kg) and were comparable to those of vitamin E, a standard antioxidant. These findings suggest that dietary supplementation with hazelnuts or hazelnut-derived antioxidants may represent a promising strategy for mitigating the adverse reproductive effects of formaldehyde exposure.

The results contribute to the growing body of evidence supporting the beneficial effects of plant-derived antioxidants against environmental reproductive toxicants. Further research is warranted to elucidate the molecular mechanisms underlying these protective effects, identify the specific bioactive compounds responsible, and evaluate the translational significance of these findings to human populations. Such investigations could lead to the development of evidence-based dietary recommendations or nutraceutical interventions for individuals at risk of formaldehyde-induced reproductive toxicity.

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CONFLICT OF INTERESTS. The authors have not declared any conflict of interests.

KESKIN, A.; BERKTAS AYDIN, O.; BOYDAK, M. & UNDAG, I. Efecto del aceite de avellana Giresun contra el daño inducido por formaldehído en tejidos testiculares de rata. *Int. J. Morphol., 44(1)*:239-249, 2026.

RESUMEN: El objetivo de este estudio fue determinar los efectos tóxicos del formaldehído en el tejido testicular y el efecto protector del aceite de avellana Giresun contra estos efectos. En el estudio se utilizaron 40 ratas Wistar albinas macho. A las ratas del Grupo I se les inyectó formaldehído cada dos días por vía intraperitoneal . Las ratas del Grupo II se designaron como grupo control. A las ratas del Grupo III se les administró aceite de avellana Giresun (100 mg/kg) por vía intragástrica, y a las ratas del Grupo IV se les administró aceite de avellana Giresun (200 mg/kg) cada dos días junto con la inyección de formaldehído. Al finalizar el periodo experimental de 30 días, todas las ratas fueron sacrificadas bajo anestesia general tras la extracción de muestras de sangre. Se realizaron evaluaciones bioquímicas e histológicas de los tejidos testiculares. Se examinó la peroxidación lipídica (LPO) y la actividad de la enzima mieloperoxidasa (MPx) en ratas tratadas con formaldehído. En comparación con el grupo control, los niveles de LPO y la actividad de la enzima MPx fueron muy elevados en el grupo tratado con formaldehído. Los niveles de testosterona fueron muy bajos en este grupo. La aplicación de aceite de avellana Giresun normalizó esta proporción, equiparándola a la de los tejidos sanos. Los niveles de testosterona y el número de células intersticiales también fueron muy bajos en los tejidos expuestos al formaldehído. El formaldehído causó efectos nocivos en el tejido testicular, mientras que el extracto acuoso de aceite de avellana Giresun mostró un efecto protector significativo contra estos efectos.

PALABRAS CLAVE: Formaldehído; Rata; Testículo; Aceite de avellana Giresun.

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