2025, Scienceline Publication
World's Veterinary Journal
World Vet J, 15(2): 487-497.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2025.wvj49
PII: S232245682500049-15

Effects of Gallic Acid on Testicular Dysfunction Induced by Topiramate in Rats

Rana Ramadan[1,2]*, El-Said El-Sherbini[1], Gehad Elsayed[1], and Mohamed El-Adl[1]

[1]*Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt*
[2]*Department of Basic Veterinary Science, Faculty of Veterinary Medicine, Delta University for Science and Technology, Mansoura, Egypt*
*Corresponding author's Email: ranar6152@gmail.com

ORIGINAL ARTICLE
Received: March 02, 2025
Revised: April 26, 2025
Accepted: May 24, 2025
Published: June 30, 2025

ABSTRACT

Topiramate (TPM), a widely used anticonvulsant, has been documented to induce testicular dysfunction through its pro-oxidant properties, leading to cellular damage and hormonal abnormalities in the testes. This damage is characterized by increased malondialdehyde (MDA) levels and decreased activity of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD), which are essential for reducing oxidative stress. This study aimed to evaluate the effects of TPM and Gallic Acid (GA) on reproductive health in male rats. Forty mature Sprague-Dawley rats, aged 16 to 18 weeks and weighing between 180 and 200 g, were divided into four experimental groups (10 rats each): a control group, a TPM-treated group, a TPM + GA-treated group, and a GA-only group. The rats received TPM (18 mg/kg) orally for 60 days, with or without GA (50 mg/kg) administered orally for the same period. Testicular tissues were examined for oxidative stress markers (MDA, SOD, CAT), sperm motility, hormonal concentrations (Testosterone, gonadotropin-releasing hormone [GnRH], and 17β-hydroxysteroid dehydrogenase type 3 [17β-HSD3]), and histological changes. The results showed that TPM significantly increased MDA levels while decreasing CAT and SOD activity, indicating oxidative stress compared to the control group. Sperm motility was also impaired in the TPM-treated group. However, GA treatment led to a notable reduction in MDA levels and restored antioxidant enzyme activity toward normal levels. Hormonal analysis revealed that TPM affected testosterone and GnRH levels, although GA partially mitigated these changes. Immunohistochemical and histological assessments demonstrated considerable testicular damage in the TPM group, whereas the GA-treated group showed slight improvements in testicular histopathology and reduced cellular death. In conclusion, GA (50 mg/kg) exhibited a protective effect against TPM-induced testicular dysfunction.

Keywords: Anti-inflammatory, Antioxidant, Anti-apoptotic, Gallic acid, Topiramate, Testicular dysfunction

INTRODUCTION

Topiramate (TPM) induces testicular dysfunction and infertility through multiple mechanisms that affect both cellular and systemic dimensions of male reproductive health (Kadhem and Majhwol, 2020). The fundamental mechanism involves the generation of oxidative stress in testicular tissue. Topiramate elevates the generation of reactive oxygen species (ROS) beyond the limits of the antioxidant defense system, resulting in oxidative damage to testicular cells (El Makawy et al., 2019). This oxidative stress impacts cellular structures in the testes, particularly the seminiferous tubules, which are crucial for sperm generation. The impairment of these tubules disrupts spermatogenesis, resulting in diminished sperm quality, lower motility, and heightened morphological abnormalities (El Makawy et al., 2019). Moreover, oxidative stress induces apoptosis, or programmed cell death, in germ cells, hence intensifying the decline in sperm count and overall fertility (Jafari et al., 2021).

Endocrine disruption represents another significant pathway through which TPM affects male fertility. It interferes with the hypothalamic-pituitary-gonadal (HPG) axis, which regulates reproductive hormones. TPM reduces the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both of which are crucial for facilitating spermatogenesis and testosterone production. Furthermore, the medication directly impairs Leydig cells, which are responsible for testosterone production. Decreased testosterone levels adversely affect sperm production and result in lower desire and other secondary sexual functions (El Makawy et al., 2019). Numerous investigations have identified this hormonal imbalance as a significant contributor to the reproductive toxicity associated with TPM (Kadhem and Majhwol, 2020).

The Gallic acid (GA), a natural phenolic compound known as 3,4,5-trihydroxybenzoic acid, is recognized for its anti-inflammatory and antioxidant properties (Al Zahrani et al., 2020). Found in numerous plants, vegetables, nuts, and fruits, GA exhibits significant pharmacological capabilities, such as anti-tumor, anti-bacterial, anti-diabetic, anti-obesity,

To cite this paper: Ramadan R, El-Sherbini ES, Elsayed G, and El-Adl M (2025). Effects of Gallic Acid on Testicular Dysfunction Induced by Topiramate in Rats. *World Vet. J.*, 15(2): 487-497. DOI: https://dx.doi.org/10.54203/scil.2025.wvj49

anti-microbial, and anti-myocardial ischemia effects (Hadidi et al., 2024). Inflammation, a protective physiological and pathological reaction triggered by external stimuli or tissue damage, often involves the synthesis of inflammatory mediators such as interleukin-1β, tumor necrosis factor-α, and prostaglandin E2. Although acute or chronic inflammation is crucial for defense, excessive inflammation can result in significant health complications, such as cardiovascular illnesses, septic shock, atherosclerosis, and immunological dysfunction (Medzhitov, 2021).

The current experimental study aimed to investigate the beneficial effects of GA on the testicular tissue of TPM-treated rats by evaluating its influence on several parameters. Their antioxidant efficacy against TPM was evaluated by measuring malondialdehyde (MDA) levels and assessing catalase (CAT) and superoxide dismutase (SOD) activities in testicular tissue. Furthermore, an immunohistochemistry screening for caspase 3 and interleukin (IL)-6 was performed on the testicular tissue of the treated rats.

MATERIALS AND METHODS

Ethical approval

This study complies with national and international regulations. The study obtained permission from the Research Ethics Committee at Mansoura University (MU-ACUC, Ph. D. 66).

Animals

Forty mature Sprague-Dawley rats, aged 16 to 18 weeks and weighing between 180 and 200 g, were acquired from the animal facility at the Faculty of Veterinary Medicine, Mansoura University, Egypt. The study was conducted in the laboratory research facility at the Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt. The rats were maintained in controlled environmental conditions, with a 12-hour light-dark cycle, a temperature of 22 ± 4 °C, and humidity levels of 5 ± 10%. Each rat was allocated a floor space of 450 cm^2 (about 70 in^2) within cages that had a height of 18 cm (7 inches). During a 7-day acclimatization period, the rats were granted unrestricted access to water and a commercial pellet diet. The commercial pellet diet contains a metabolizable energy (ME) of 3.8 kcal/g and a crude protein (CP) content of 24%.

Experimental design

Forty rats were randomly divided into four groups of ten rats each, with four replicates per group, to evaluate the effects of GA on testicular dysfunction associated with TPM therapy in rats. The rats were provided with a conventional control pellet meal and water for one week as the acclimatization period. During the subsequent 60 days, the initial cohort, designated as the placebo control group receiving distilled water, was provided only with a standard control diet, without any medication. The second group, identified as TPM, received an oral dosage of TPM (18 mg/kg body weight) (obtained from BAL Pharma Limited, Bangalore, Karnataka) via a stomach tube (John and Anitha, 2015). The third group received TPM (18 mg/kg) and GA (50 mg/kg/24 hrs) orally (purchased from Sigma-Aldrich Chemical Co., USA). The fourth group was administered GA (50 mg/kg/24 hrs) orally (Moradi et al., 2021). After a 60-day study period, the rats in the four groups were anaesthetized with 20 mg kg-1 of thiopental sodium (Moradi et al., 2021), and the testes were harvested for subsequent hormonal, immunohistochemical, and histological analyses. Blood samples (1.5 mL) were collected from each rat for further investigation.

Collection of testicular tissue samples

The testes were divided into two segments. A 50 mg portion of fresh tissue was preserved in normal saline and subsequently homogenized in a cold potassium phosphate buffer (pH 7.5). The homogenate was subsequently centrifuged at 4°C to isolate the supernatant, which was meticulously separated and preserved at -20°C to avert enzymatic degradation and oxidation. The supernatant was then used for ELISA analysis (USA) to measure pro-inflammatory cytokines, including interleukin-6 (IL-6), as well as catalase (CAT) activity, superoxide dismutase (SOD) activity, and malondialdehyde (MDA) concentrations. The remaining portion was excised and preserved in 10% formalin for histological evaluation and immunohistochemical examination of caspase 3 and IL-6. The remaining testicular segment was immediately preserved in 10% neutral buffered formalin to maintain tissue morphology for future histological evaluation. The fixation technique preserved cellular and structural integrity, facilitating precise microscopic analysis. The fixed tissue was subsequently treated utilizing normal histological methods, including dehydration, paraffin embedding, sectioning, and staining. Additionally, immunohistochemistry analysis was conducted to assess the expression of caspase-3 (an apoptosis marker) and IL-6 (a pro-inflammatory cytokine), adhering to the protocol published by Hsu et al. (1981). This method facilitated the identification and measurement of these markers within the tissue, yielding significant insights into inflammation and apoptotic activity.

Semen collection and evaluation

Spermatozoa were obtained from the epididymal tail using compression. Sperm motility was evaluated using the wet mount technique. The process entailed placing a diluted semen sample on a pre-warmed microscope slide, examining the sample under a microscope at ×400 magnification, and assessing the proportion of motile sperm within the population (Seed et al., 1996).

Oxidant and antioxidant defense markers

The activity of testicular catalase was determined based on Aebi (1984), wherein the generated hydrogen peroxide reacted with a potassium permanganate solution (0.01N KMNO4), and the residual permanganate was quantified at 480 nm. Nishikimi et al. (1972) studied the activity of testicular superoxide dismutase (SOD) by measuring the reduction capacity of superoxide radicals in the presence of nitroblue tetrazolium, using phenazine methosulfate as a mediator. The levels of MDA, a marker of lipid peroxidation in testicular tissues, were measured calorimetrically, utilizing thiobarbituric acid (Ohkawa et al., 1979).

Hormonal analysis

Microplate immunoenzymometric assay kits obtained from Cayman Chemical (USA) were utilized to quantify testosterone (Adebayo et al., 2020). The Testosterone ELISA Kit from Calbiotech, Inc. (CBI), USA (Catalogue No. TE373S) was utilized to quantify testosterone levels, while the gonadotropin-releasing hormone (GnRH) ELISA Kit from Elabscience®, UK (Catalogue No: E-EL-0071) and the 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) ELISA Kit (Sandwich ELISA) from LSBio, USA (Catalogue No: LS-F49574) were employed to assess GnRH and HSD hormones, respectively. The testing protocols for each kit were executed according to the published instructions.

For this experiment, 25 µL of calibrators, controls, and samples were allocated into appropriately labeled anti-hormone-coated microtiter wells. The plates were evaluated after a 60-minute incubation at room temperature with a conjugate (monoclonal anti-hormone antibodies conjugated with horseradish peroxidase) at a concentration of 10 µL and mixed for 20-30 seconds via centrifugation. The contents of the microtiter wells were discharged onto paper towels and blotted dry. A 1:25 dilution of the concentrated washing solution and distilled water was prepared in a separate container, followed by the addition of 300 µL of the reconstituted washing solution to each well, which was subsequently decanted and blot-dried. Following four rinses of the plates, 100 µL of TMB substrate was added to each well, and the plates were incubated at room temperature in a dark cabinet for 15 to 20 minutes. The microtiter wells were analyzed using an ELISA reader (Elx 800, BioTek, England) after the addition of 150 µl of the stopping reagent (0.2 M sulphuric acid solution) at consistent intervals.

The hormone concentration of each sample was determined using a 4-parameter calibration curve, with optical densities/absorbance values plotted on the Y-axis and calibrator concentration values on the X-axis. All test validation criteria for each assay were satisfied as per the manufacturer's instructions for different kits. Coefficients of variation (% CV) were computed from replicates and numerous test plates after homogenization. Based on assessing the data in accordance with the manufacturer's specifications, intra-assay and inter-assay CVs were observed below 6% and 9%, respectively (Adebayo et al., 2020).

Histopathological and immunohistochemistry analysis

Paraffin sections were stained following the protocol suggested by Hsu et al. (1981) utilizing specific antibodies for caspase-3 at a dilution of 1:500, Anti-IL-6 Receptor 1 antibody at a ratio of 1:1000, and Anti-ErbB2/HER2 antibody [H2Mab-139] (ab264541) at a dilution of 1:100 (Abcam, Cambridge, UK). Tissue sections (5 µm) from all experimental groups were affixed to charged slides, dewaxed, hydrated, and then subjected to antigen retrieval according to the manufacturer's instructions. After overnight incubation of the sections with primary antibodies at 4 °C in a humidified chamber, the slides were washed with phosphate-buffered saline and then incubated with the secondary antibody (Expose mouse and rabbit specific horseradish peroxidase/3,3'-diaminobenzidine detection kit, Abcam; ready-to-use; Cat. #: ab80436, United Kingdom) for 15 minutes at room temperature in a humidified chamber. The slides were then rinsed with phosphate-buffered saline. Staining was conducted via the 3,3'-diaminobenzidine chromogenic agent, followed by counterstaining with haematoxylin, dehydration, and mounting in a synthetic media. Three immuno-labeled sections were examined for each antigen per animal. For each antigen per animal, three immuno-labeled sections were analyzed by selecting three non-overlapping tissue slices from each testis to ensure representative sampling. The slices were meticulously affixed to glass slides, deparaffinized, and rehydrated prior to antigen retrieval to improve epitope accessibility.

The removed testicular tissue was initially preserved in a 4% paraformaldehyde solution and later embedded in paraffin for sectioning. Glass slides were produced from these paraffin-embedded tissues. Xylene was employed in the

deparaffinization procedure to remove the paraffin. A 3% hydrogen peroxide solution was utilized to inhibit endogenous peroxidase activity. The slides underwent pepsin treatment at 42°C for 5 minutes to amplify the signal. They were subsequently incubated overnight at 4°C with mouse anti-caspase and IL-6 antibodies (Abcam Limited, UK). The detection technique was implemented using Peroxidase/3,3′-Diaminobenzidine (Collins, 2007). Image analysis classified staining intensity into four levels: negatively stained (0-10%), mildly stained (10-25%), moderately stained (26-50%), and strongly stained (51-100%) (Hsu et al., 1981).

Statistical analysis

Statistical differences in the mean of biochemical variables between control and treated rats were evaluated using one-way ANOVA, followed by post hoc Tukey's test. Normality was assessed using the Shapiro–Wilk test. All statistical analyses were conducted using GraphPad Prism for Windows version 5.0 (GraphPad Software, Inc., San Diego, CA, USA). A significance criterion of $p < 0.05$ was set for all tests. Data were expressed as means ± standard deviation (SD), and a significance level of $p < 0.05$ was set for all the tests.

RESULTS

Evaluation of sperm motility

Sperm motility in the control group (G1) was measured at 84.7% ± 0.65 ($p < 0.05$). However, no motility was observed in the groups administered Topiramate (G2), and Topiramate + GA (G3, Table 1).

Oxidant and antioxidant defense markers

The findings indicated a non-significant reduction in testicular malondialdehyde (tMDA) levels (8.08 nmol/g.tissue ± 0.50) in the GA-treated group (G4), alongside a significant elevation in tCAT activity (2.39 ± 0.035U/g. tissue) and tSOD activity (179.34 ± 3.99U/g. tissue) compared to the control group, suggesting an improved antioxidant capacity in testicular tissues ($p < 0.05$). In the Topiramate-treated group (G2), tMDA levels were significantly increased (23.40 ± 1.20nmol/g.tissue), suggesting elevated oxidative stress, while tCAT (0.87 ± 0.08U/g. tissue) and tSOD (80.90 ± 5.096U/g. tissue) levels were significantly decreased, signifying impaired antioxidant activity ($p < 0.05$). Co-administration of Topiramate and GA (G3) markedly decreased tMDA levels (16.22 ± 0.81nmol/g.tissue) and partially reinstated tCAT (1.44 ± 0.045U/g. tissue) and tSOD (109.88 ± 2.77U/g. tissue) activity in comparison to the Topiramate group ($p < 0.05$). The control group (G1) exhibited consistent levels of tMDA (9.68 ± 0.30nmol/g. tissue), tCAT (2.15 U/g. tissue ± 0.067), and tSOD (149.74 ± 1.92U/g. tissue), providing a baseline for comparison (Figure 1, $p < 0.05$).

Hormonal parameters in testicular tissue homogenate

Hormonal analyses revealed notable discrepancies among the groups ($p < 0.05$). The Topiramate-treated group (G2) demonstrated the lowest testosterone levels (0.57 ± 0.044ng/ml) and 17βHSD activity (139.80 ± 2.48pg/ml), along with the highest GnRH levels (63.91 ± 6.60pg/ml), indicating hormonal dysregulation. Conversely, the Topiramate+GA group (G3) exhibited improved testosterone levels (0.85 ± 0.045ng/ml) and GnRH levels (35.80 ± 1.42pg/ml) approaching normal values, although 17βHSD activity (143.82 ± 2.41pg/ml) remained elevated ($p > 0.05$). The GA-only group (G4) displayed reduced testosterone levels (0.59 ± 0.057ng/ml) and 17βHSD activity (98.48 ± 3.57pg/ml) while sustaining GnRH levels (25.08 ± 0.87pg/ml) comparable to the control group (G1: testosterone 1.42 ± 0.043ng/ml, GnRH 24.58 ± 0.96pg/ml, and 17βHSD 220.14 ± 13.74pg/ml , Figure 2).

Immunohistochemical analysis

Figure 3 illustrates the immunohistochemical expression of caspase in testicular sections across different treatment groups: the control group, showed few immunopositive stained germ epithelial cells (A, B); the GA group, showed minimal immunostained seminiferous tubular epithelial cells (C, D); the TDM group, showed highly intense immunopositive staining in damaged germ epithelial cells (E, F, G, H, I, J); and the TDM-GA group, showing mild immunopositive stained tubular epithelial cells (K, L, M, N).

Figure 4 shows the IHC expression of IL-6 in testicular sections across different treatment groups: the control group, showed negative immunopositive stained germ epithelial cells (A, B); the GA group, showed negative immunostained seminiferous tubular epithelial cells (C, D); the TDM group, showed moderate faint immunopositive staining in damaged germ epithelial cells (E, F, G); and the TDM-GA group, showed negative immunopositive stained tubular epithelial cells (H, I).

Histopathology of testicular tissues

Figure 5 displays testicular sections from different treatment groups: the control group, showed normal seminiferous tubular epithelial architecture (A, B); the GA group, showed normal architecture of germ epithelial lining of seminiferous tubules (C, D); the TDM group, showed tubular necrosis with loss of most germinal epithelium and formation of intraluminal spermatid giant cells (E, F); the TDM group, showed degenerative seminiferous tubules with diffuse cystic vacuolation lined with one cell line (G, H); the TDM group, showed diffuse degenerative to completely loss of germinal epithelium or clumped, shrinkage epithelial lining (atrophied tubules) (I, J); and the TDM-GA group, showed mild tubular disordered with tubular vacuolation (K, L, M, N).

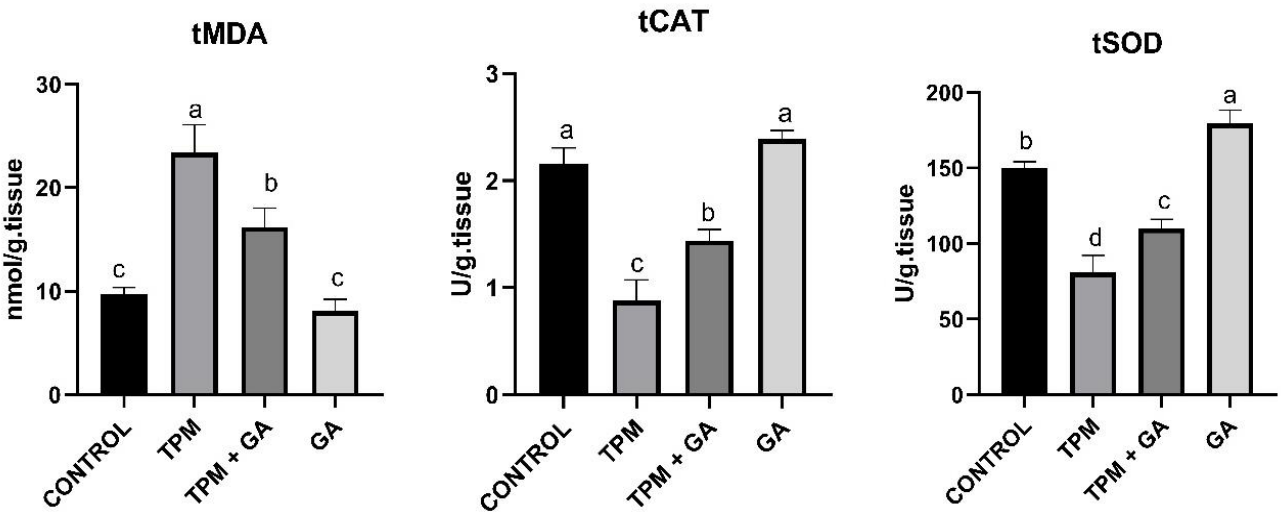

Figure 1. Testicular oxidant and antioxidant defense markers in rats intoxicated with Topiramate (TPM) and supplemented with Gallic acid. tMDA: testicular malondialdehyde, tCAT: testicular catalase, tSO: testicular sodium dismutase. Results are expressed as means ± SD. Different superscript letters in each diagram shows significant differences ($p < 0.05$). Control group, TPM: Topiramate group, TPM + GA: Topiramate + Gallic acid group, GA: Gallic acid group.

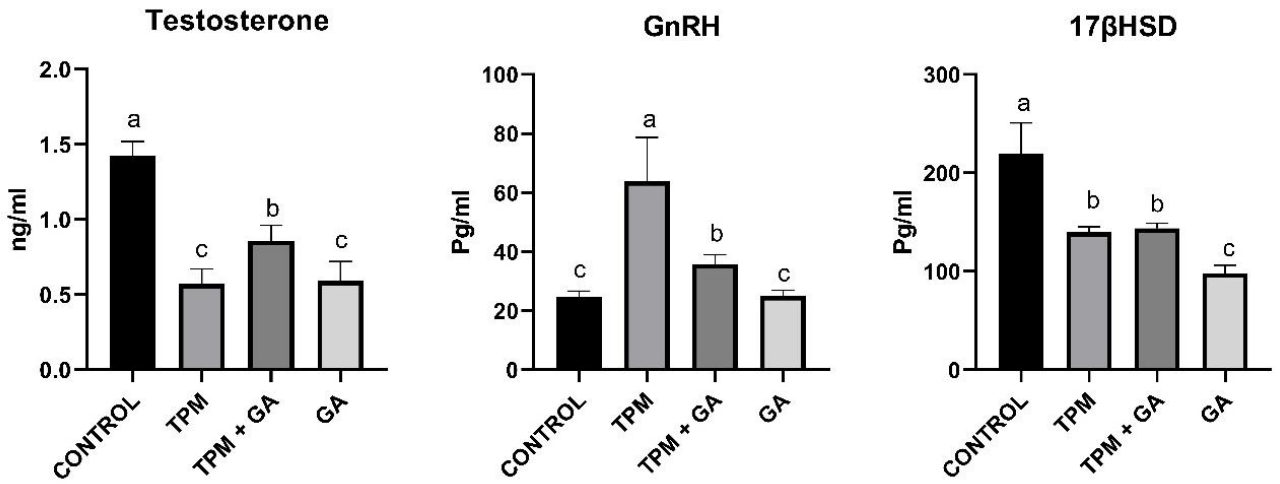

Figure 2. Effects of gallic acid on topiramate-induced testicular impairment on hormonal analysis. Results are expressed as means ± SD. Different superscript letters in each diagram shows significant differences ($p < 0.05$). CONTROL: Control group, TPM: Topiramate group, TPM + GA: Topiramate + Gallic acid group, GA: Gallic acid group. GnRH: Gonadotropin-releasing hormone, 17β-HSD3: 17β-hydroxysteroid dehydrogenase type 3

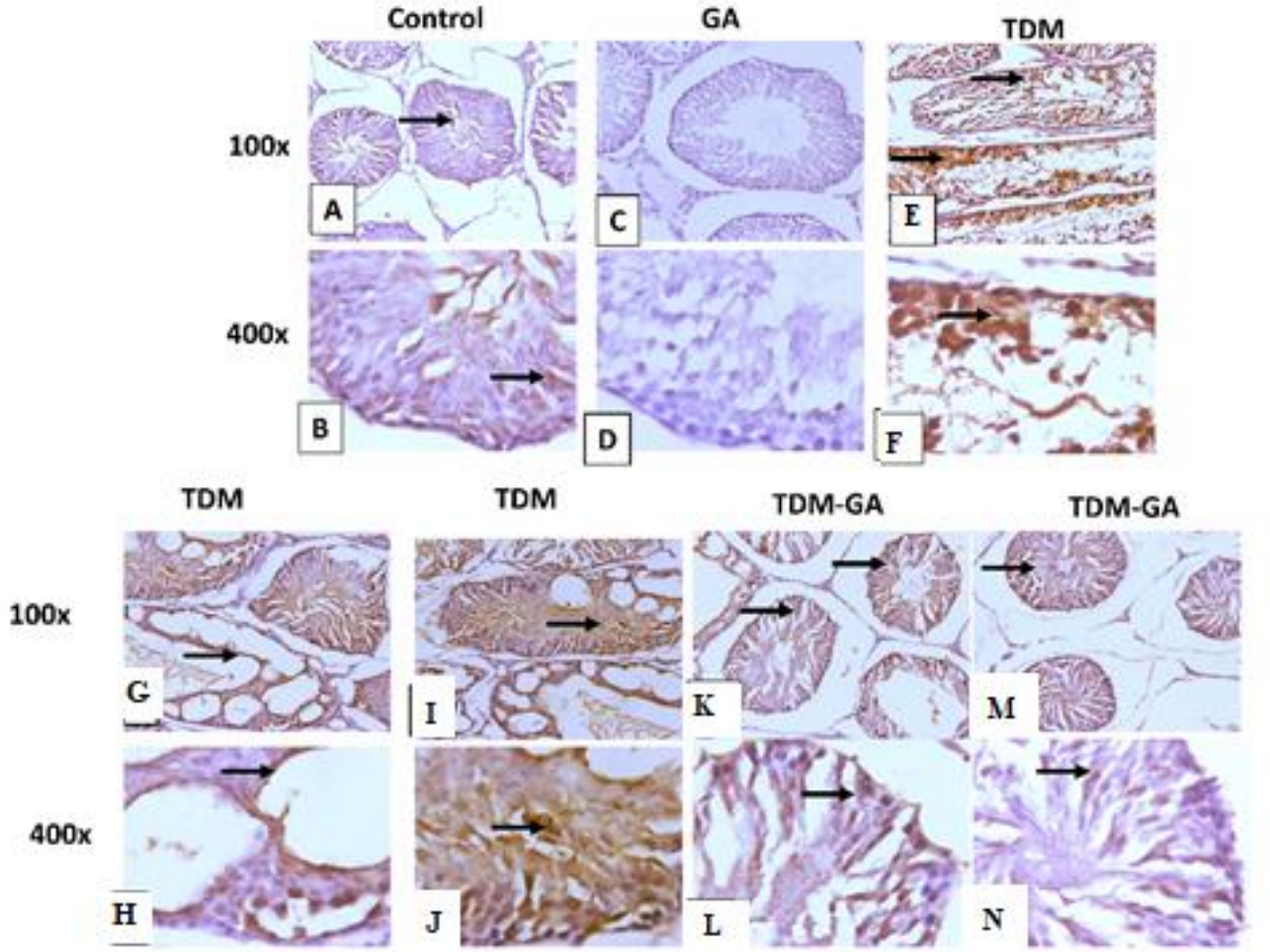

Figure 3. The immunohistochemistry of caspase-3 expression in testicular sections across different treatment groups. **A, B:** control group, showing few immunopositive stained germ epithelial cells (black arrow). **C, D:** GA group, showing minimal immunostained seminiferous tubular epithelial cells. **E, f, G, H, I, J:** TDM group showing highly intense immunopositive staining in damaged germ epithelial cells. **K, l, M, N:** TDM-GA group, showing mildly immunopositive stained tubular epithelial cells. The black arrow indicates a few immunopositive-stained germ epithelial cells. Image magnification=100x, 400x.

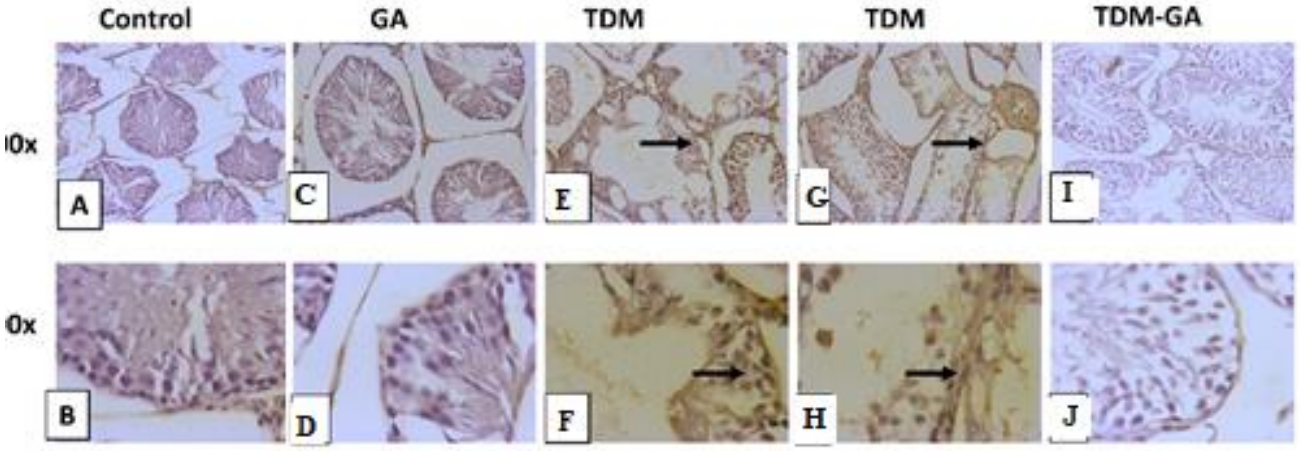

Figure 4. The immunohistochemistry of IL-6 expression in testicular sections across different treatment groups. **A, B:** Control group, showing negative immunopositive stained germ epithelial cells. **C, D:** GA group, showing negative immunostained seminiferous tubular epithelial cells. **E, F, G:** TDM group, showing moderate faint immunopositive staining in damaged germ epithelial cells. **H, I:** TDM-GA group, showing negative immunopositive stained tubular epithelial cells. Thin arrow = Positive immunostained germ epithelial cells. Image magnifications = 100x, 400x.

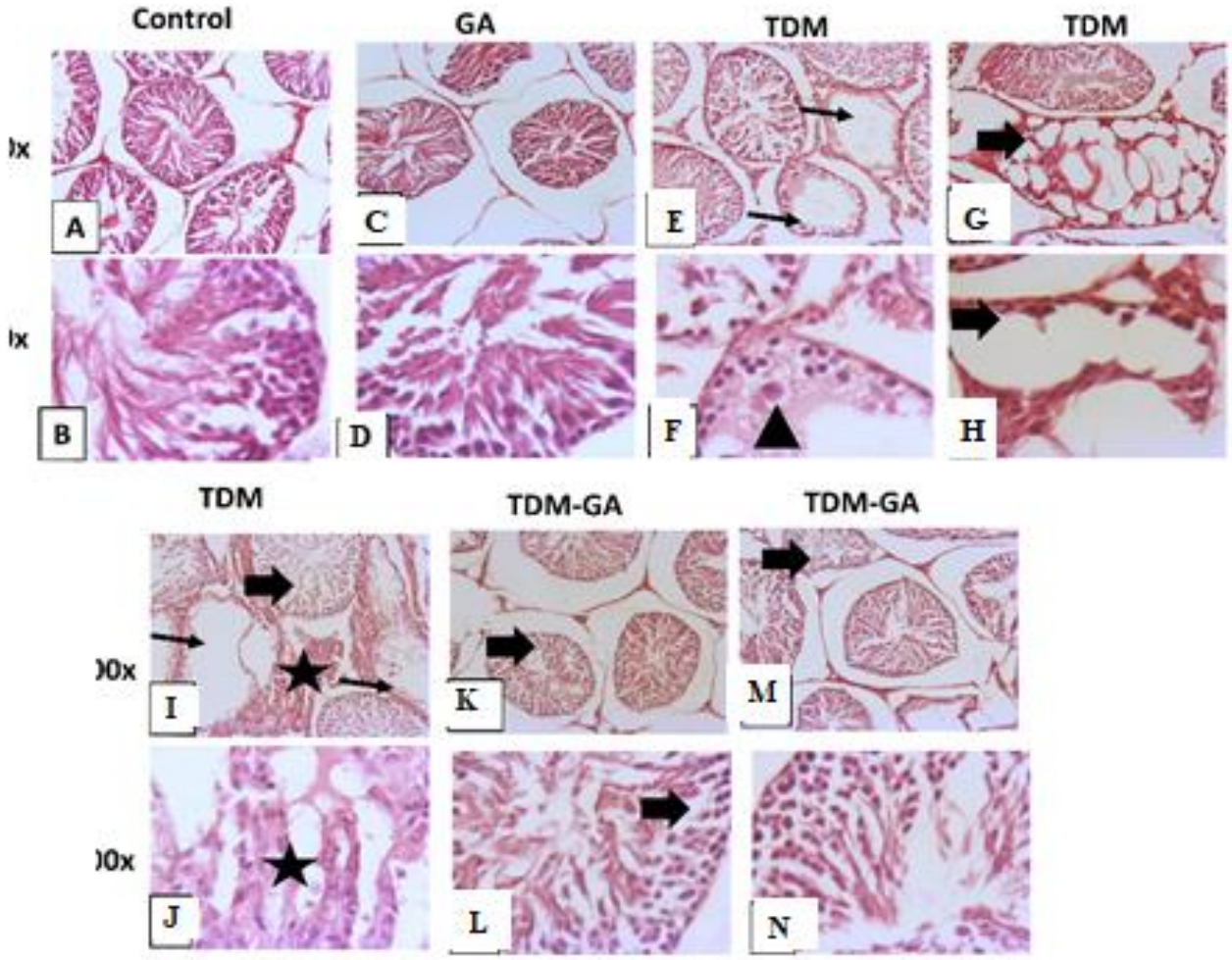

Figure 5. The testicular sections across different treatment groups. **A, B:** Control group, showing normal seminiferous tubular epithelial architecture. **C, D:** GA group, showing normal architecture of germ epithelial lining of seminiferous tubules. **E, F:** TDM group, showing tubular necrosis with loss of most germinal epithelium and formation of intraluminal spermatid giant cells. **G, H:** TDM group, showing degenerative seminiferous tubules with diffuse cystic vacuolation lined with one cell line. **I, J:** TDM group, showing diffuse degeneration to complete loss of germinal epithelium or clumped, shrinkage of epithelial lining (atrophied tubules). **K, L, M, N**: TDM-GA group, showing mild tubular disorder with tubular vacuolation. Thin arrow indicates Strophied tubules; Thick arrow indicates Tubular vacuolation; star indicates tubular epithelial shrinkage; arrowhead indicates intraluminal giant cells. Image magnifications: 100x, 400x.

Table 1. Effects of gallic acid on topiramate-induced testicular impairment on sperm motility in rats

Group	Sperm motility (%)
Control	84.7 ± 0.65a
Topiramate	No semen, no sperm
Topiramate+GA	No semen, no sperm
GA	86.3 ± 0.39a

Means with different letters are significantly different ($p \leq 0.05$); GA: Garlic acid

DISCUSSION

Topiramate, a widely used antiepileptic drug, has demonstrated protective effects against various types of organ damage, including oxidative stress-related injuries (Jafari et al., 2021). The GA, a natural antioxidant, has shown promising protective effects in ameliorating testicular damage. This study aimed to evaluate the combined impact of topiramate and GA on reproductive function in rats.

Table 1 illustrates substantial differences in sperm motility across the experimental groups. The rats in the control group (G1) displayed a normal motility of 84.7 % ± 0.65, but no sperm motility was observed in the rats in groups G2, G3, and G4. This finding aligns with those of Bahey and Elkelany (2023), who demonstrated that topiramate-induced oxidative stress can hinder spermatogenesis. The complete loss of motility observed in these groups is in agreement with their findings. On the other hand, Jafari et al. (2021) revealed that topiramate has oxidative and anti-inflammatory effects on ischemia-reperfusion injury, which lends more credence to the idea that topiramate plays a role in interrupting normal testicular function.

Several investigations have shown ample evidence that GA can mitigate the adverse effects of testicular toxicity. Demir et al. (2024), for instance, established that GA alleviates testicular damage by decreasing oxidative stress pathways such as HMGB1/NF-κB. The improved motility observed in the GA-treated group (G4) corroborates the findings of the GA-treated group in rats. Similarly, Waly et al. (2024) and Behairy et al. (2024) demonstrated that GA is effective in reversing the inhibition of spermatogenesis caused by hazardous substances. Moreover, Abarikwu et al. (2022) highlighted GA's ability to reduce inflammation and oxidative damage in the testes.

Furthermore, Jalili et al. (2021) emphasized the antioxidant properties of GA in protecting sperm parameters, including motility, against drug-induced toxicity. Recent studies by Novin et al. (2020) and Hosseinzadeh et al. (2022) further support GA's protective role in preserving the quality of sperm and reducing DNA fragmentation. Taking into consideration these data, it appears that the antioxidant and anti-inflammatory mechanisms of GA play a significant role in the maintenance of reproductive function in rats.

Increased lipid peroxidation, a characteristic feature of oxidative stress, is reflected in the elevated levels of MDA that were identified in G2 (the TPM-treated group). Through their research, Steele et al. (2024) discovered that MDA is a valid biomarker for oxidative damage caused by the breakdown of cellular membranes. GA's powerful antioxidant qualities, which can reduce oxidative damage by scavenging free radicals, are demonstrated by the considerable reduction in MDA levels that occurred in G4 (the GA-treated group). These results are consistent with the findings of Asci et al. (2017), who highlighted the role of GA in reducing MDA levels in methotrexate-induced testicular dysfunction in rats. This is accomplished by neutralizing reactive oxygen species and stabilizing cellular membranes.

Catalase is a crucial antioxidant enzyme that decomposes hydrogen peroxide into water and oxygen, thus protecting cells from the damage induced by oxidative stress. The CAT activity in G2 is decreased, which indicates that antioxidant defense has been compromised as a result of topiramate-induced oxidative stress. This finding aligns with that of Jafari et al. (2021), who observed diminished CAT activity in ischemia-reperfusion injury models. On the other hand, the fact that GA therapy led to a considerable increase in CAT activity in G4 demonstrates that it is capable of restoring the function of antioxidant enzymes by enhancing the enzymatic defense system against oxidative stress and reducing free radical accumulation. Similar findings were reported by Demir et al. (2024), who found that GA increases CAT activity in models of torsion/detorsion-induced testicular damage. This finding suggests that GA plays a protective function in decreasing oxidative load levels. In addition, Waly et al. (2024) highlighted the fact that GA is responsible for the upregulation of CAT expression, which serves as a contributor to the protective effects that GA has against oxidative stress in damaged tissues, such as the testicular tissues.

Reduced SOD activity in G2 indicates oxidative damage and impaired cellular antioxidant defense, as observed by Arab et al. (2022) in cadmium-induced testicular dysfunction in rats. Superoxide dismutase (SOD) is an essential enzymatic defense against oxidative stress, as it catalyzes the dismutation of superoxide radicals into hydrogen peroxide. On the other hand, the significant increase in SOD activity observed in G4 demonstrates that GA can restore endogenous antioxidant systems. Behairy et al. (2024) reported similar findings, suggesting that GA dramatically boosts SOD activity by lowering oxidative stress and increasing enzymatic efficiency in the reproductive system.

The findings of Sheweita et al. (2018) indicated that topiramate-induced oxidative stress was demonstrated by the elevated levels of MDA and decreased levels of CAT and SOD activity. As demonstrated by Hashemzaei et al. (2020) and Abarikwu et al. (2014), GA could dramatically restore antioxidant enzyme activities. These studies demonstrate the effectiveness of GA in preventing oxidative damage in testicular tissues.

Testosterone, a hormone crucial for male sexuality, plays a vital role in reproductive and metabolic processes. However, endocrine disturbances have been shown to reduce testosterone levels. In this study, topiramate's disruption of the hypothalamic-pituitary-gonadal (HPG) axis led to significantly lower testosterone levels in G2, measured at 0.57 ng/ml ± 0.044, compared to previous levels. This indicates that topiramate has a negative impact on male reproductive hormones. In contrast, G3 demonstrated increased testosterone levels after receiving GA treatment, highlighting the protective function that GA plays in reproductive health outcomes. The findings of this study are consistent with the hypothesis that GnRH is an essential regulator of the HPG axis. It does so by promoting the release of LH and FSH, which in turn regulate the production of testosterone. These findings corroborate those of Arab et al. (2022), who showed lower GnRH expression following topiramate exposure. It is clear that the suppression of GnRH in G2 is a reflection of the disruption of the HPG axis of the cell. As a result of administering GA in G3, GnRH levels were restored, which demonstrates the role that GA plays in preserving the integrity of the HPG axis. These results are supported by Demir et al. (2024), who found that GA protects against torsion/detorsion-induced GnRH depletion by reducing oxidative stress and maintaining hypothalamic function.

As a crucial enzyme in the process of testosterone biosynthesis, 17βHSD is responsible for the conversion of androstenedione to testosterone in Leydig cells. According to Waly et al. (2024), who established that GA upregulates 17βHSD expression and enzymatic activity, thereby counteracting the inhibitory effects of oxidative stress on

testosterone synthesis, a decrease in 17βHSD activity in G2 implies reduced steroidogenic function. This was previously documented by Waly et al. (2024).

Consistent with the findings of Jalili et al. (2022), topiramate caused a disruption in the maintenance of hormonal equilibrium by lowering testosterone levels and raising GnRH levels. Behdarvand-Margha et al. (2021) and Rotimi et al. (2024) revealed that co-treatment with GA resulted in an increase in testosterone levels and a normalization of GnRH. These findings demonstrate that, from a physiological standpoint, GA has protective effects on the hypothalamic-pituitary-gonadal axis by regulating hormone levels, reducing oxidative stress, and preserving testicular function.

Caspase is a principal activator of the intrinsic apoptotic pathway, activated by the release of mitochondrial cytochrome c. Increased caspase-9 levels in G2 suggest mitochondrial dysfunction and oxidative stress, consistent with the findings of El Makawy et al. (2022), who linked topiramate-induced tissue damage to intrinsic apoptosis. GA treatment in G3 significantly reduced caspase-9 expression, highlighting its ability to stabilize mitochondrial membranes and prevent cytochrome c release. Treatment with GA reduced caspase-8 expression in G3, underscoring its potential to protect cellular integrity by modulating apoptotic signaling pathways. Demir et al. (2024) demonstrated GA's role in downregulating caspase-8, thereby preserving testicular function in torsion/detorsion-induced injury. GA treatment in G3 significantly reduced caspase-3 levels, suggesting its anti-apoptotic effect through the mitigation of oxidative stress. This finding is in line with that of Behairy et al. (2024), who reported GA's ability to suppress caspase-3 activation in arsenic-induced testicular injury.

The Bcl-2/Bax ratio is a critical determinant of cell survival or apoptosis. A decreased Bcl-2/Bax ratio in G2 reflects enhanced pro-apoptotic signaling, as reported by Varzandeh et al. (2024) in topiramate-induced colitis. However, GA treatment in G3 restored the Bcl-2/Bax balance, indicating its role in inhibiting apoptotic pathways.

The testicular tissue in G2 exhibited disrupted seminiferous tubules, reduced spermatogenesis, and increased interstitial edema, all of which are indicative of testicular oxidative stress and apoptosis. These observations are consistent with those of Arab et al. (2022), who documented similar testicular damage in rats exposed to topiramate. The involvement of mitochondrial dysfunction, as well as the activation of the caspase cascade in testicular cell apoptosis, was also reported by Jafari et al. (2021).

The GA-treated groups demonstrated preserved testicular architecture, improved spermatogenic cell layers, and reduced edema in the seminiferous tubules and interstitial spaces. Waly et al. (2024) further supported these findings, showing that GA protects against tartrazine-induced testicular dysfunction by enhancing antioxidant defense and modulating inflammatory markers. Additionally, Abarikwu et al. (2024) emphasized GA's role in restoring hormonal balance and preventing spermatogenesis dysfunction in busulfan-induced testicular injury.

Immunohistochemical analysis showed topiramate-induced overexpression of caspase-3 and IL-6 in testicular tissues, consistent with the findings of Apaydın et al. (2023). GA treatment mitigated these changes, reducing caspase expression and restoring tissue architecture. Furthermore, histopathological findings of organ necrosis and fibrosis in topiramate-treated rats, as reported by Olayinka et al. (2015) and Elmorsi et al. (2023), were ameliorated by GA treatment.

CONCLUSION

The findings of the current study underscore the protective effects of GA at a dose of 50 mg/kg, administered orally for 60 days, in mitigating topiramate-induced testicular dysfunction. Specifically, GA improves sperm motility as well as histological and hormonal parameters. Further research is required to investigate the medicinal potential of this substance.

DECLARATIONS

Funding
The current study did not receive any funding aids or grants from any organization.

Competing interests
The authors declare that there is no conflict of interest.

Authors' contributions
Rana Ramadan contributed to study design, data collection, and manuscript drafting. El-Said El-Sherbini assisted in methodology, statistical analysis, and interpretation of results. Gehad Elsayed conducted the data validation. Mohamed El-Adl played a key role in the literature review, manuscript revision, and final approval of the study. All authors declare that consent to participate was obtained where applicable. All authors read and approved the final edition of the manuscript.

Availability of data and materials

The data of the current manuscript is available upon reasonable request from the corresponding author.

Ethical considerations

The authors have checked and accepted necessary ethical considerations, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

REFERENCES

Abarikwu SO, Akiri OF, Durojaiye MA, and Alabi AF (2014). Combined administration of curcumin and Gallic acid inhibits Gallic acid-induced suppression of steroidogenesis, sperm output, antioxidant defenses and inflammatory responsive genes. The Journal of Steroid Biochemistry and Molecular Biology, 143: 49-60. DOI: https://www.doi.org/10.1016/j.jsbmb.2014.02.008

Abarikwu SO, Mgbudom-Okah CJ, Njoku RC, Okonkwo CJ, Onuoha CC, and Wokoma AFS (2022). Gallic acid ameliorates busulfan-induced testicular toxicity and damage in mature rats. Drug and Chemical Toxicology, 45(4): 1881-1890. DOI: https://www.doi.org/10.1080/01480545.2021.1892949

Abarikwu SO, Okonkwo CJ, Ezim OE, Obinna VC, Nebeolisa CE, Ndufeiya-Kumasi LC (2024). Testicular weight deficits, altered variables of antioxidant defense system, spermatogenesis impairment, and inflammation induced by busulfan injection are ameliorated by Gallic acid administration in rats. Naunyn-Schmiedeberg's Archives of Pharmacology. Available at: https://link.springer.com/article/10.1007/s00210-024-03699-z

Adebayo AO, Akinloye AK, Oke BO, and Taiwo VO (2020). Relationship between Body Mass Index (BMI) and testicular and hormonal parameters of sexually active male greater cane rats (*Thryonomys swinderianus*). Animal Reproduction, 17(1): e20190026. DOI: https://www.doi.org/10.21451/1984-3143-AR2019-0026

Aebi H (1984). Catalase *in vitro*. Methods in enzymology. Academic Press, 105: 121-126. DOI: https://www.doi.org/10.1016/S0076-6879(84)05016-3

Al Zahrani NA, El-Shishtawy RM, and Asiri AM (2020). Recent developments of Gallic acid derivatives and their hybrids in medicinal chemistry: A review. European Journal of Medicinal Chemistry, 204: 112609. DOI: https://www.doi.org/10.1016/j.ejmech.2020.112609

Apaydın FG, Kalender S, Baş H, and Kalender Y (2023). Protective role of Gallic acid against fenitrothion-induced hepatotoxicity and nephrotoxicity via oxidative stress, histopathological and biochemical alterations. Research Square, pp. 1-21. DOI: https://www.doi.org/10.21203/rs.3.rs-2699186/v1

Arab HH, Abd El Aal HA, Alsufyani SE, El-Sheikh AA, Arafa ESA, Ashour AM, Kabel AM, and Eid AH (2022). Topiramate reprofiling for the attenuation of cadmium-induced testicular impairment in rats: Role of NLRP3 inflammasome and AMPK/mTOR-linked autophagy. Pharmaceuticals, 15(11): 1402. DOI: https://www.doi.org/10.3390/ph15111402

Asci H, Ozmen O, Ellidag HY, Aydin B, Bas E, and Yilmaz N (2017). The impact of Gallic acid on the methotrexate-induced kidney damage in rats. Journal of Food and Drug Analysis, 25(4): 890-897. DOI: https://www.doi.org/10.1016/j.jfda.2016.12.010

Bahey NG and Elkelany MM (2023). Structural alterations in the rat cornea and retina induced by topiramate: A histological and immunohistochemical study. Egyptian Journal of Histology, 46(3): 1376-1392. DOI: https://www.doi.org/10.21608/ejh.2023.30590

Behairy A, Hashem MM, Abo-EL-Sooud K, El-Metwally AE, Soliman AM, Mouneir SM, Hassan BA, and Abd-Elhakim YM (2024). Mitigating effect of Gallic acid on zinc oxide nanoparticles and arsenic trioxide-induced spermatogenesis suppression, testicular injury, hormonal imbalance, and immunohistochemical changes in rats. Naunyn-Schmiedeberg's Archives of Pharmacology, 397(12): 9859-9875. DOI: https://www.doi.org/10.1007/s00210-024-03228-y

Behdarvand-Margha Z, Ahangarpour A, Shahraki M, Komeili G, and Khorsandi L (2021). The effects of Gallic acid and metformin on male reproductive dysfunction in diabetic mice induced by methylglyoxal: An experimental study. International Journal of Reproductive Biomedicine, 19(8): 715-726. DOI: https://www.doi.org/10.18502/ijrm.v19i8.9619

Collins TJ (2007). ImageJ for microscopy. Biotechniques, 43(1): S25-S30. DOI: https://www.doi.org/10.2144/000112517

Demir S, Kazaz I, Kerimoglu GÖ, Alemdar N, Colak F, Arici T, Mentese A, and Aliyazicioglu Y (2024). Gallic acid attenuates torsion/detorsion-induced testicular injury in rats through suppressing of HMGB1/NF-κB axis and endoplasmic reticulum stress. Revista Internacional de Andrología, 22(1): e100123. DOI: https://www.doi.org/10.1016/j.androl.2023.100123

El Makawy AI, Ibrahim FM, Mabrouk DM, Ahmed KA, and Fawzy Ramadan M (2019). Effect of antiepileptic drug (Topiramate) and cold pressed ginger oil on testicular genes expression, sexual hormones and histopathological alterations in mice. Biomedicine & Pharmacotherapy, 110: 409-419. DOI: https://www.doi.org/10.1016/j.biopha.2018.11.104

El Makawy AI, Mabrouk DM, Ibrahim FM, and Ahmed KA (2022). Genotoxic, biochemical and histopathological studies to assess the topiramate hepatorenal toxicity in mice. Drug and Chemical Toxicology, 45: 103-112. DOI: https://www.doi.org/10.1080/01480545.2020.1765980

Elmorsi RM, El Saadany AA, Kabel AM, and Abou El-Seoud SH (2023). The protective effects of topiramate and spirulina against doxorubicin-induced cardiotoxicity in rats. Human & Experimental Toxicology, 42: 09603271231198624. DOI: https://www.doi.org/10.1177/09603271231198624

Hadidi M, Liñán-Atero R, Tarahi M, Christodoulou MC, and Aghababaei F (2024). The potential health benefits of Gallic acid: Therapeutic and food applications. Frontiers in Nutrition, 13(8): 1001. DOI: https://www.doi.org/10.3389/fnut.2024.01001

Hashemzaei M, Tabrizian K, Alizadeh Z, Pasandideh S, Rezaee R, Mamoulakis C, Tsatsakis A, Skaperda Z, Kouretas D, and Shahraki J (2020). Resveratrol, curcumin and Gallic acid attenuate glyoxal-induced damage to rat renal cells. Toxicology Reports, 7: 1571-1577. DOI: https://www.doi.org/10.1016/j.toxrep.2020.11.008

Hosseinzadeh A, Mehrzadi S, Siahpoosh A, Basir Z, Bahrami N, and Goudarzi M (2022). Gallic acid ameliorates di-(2-ethylhexyl) phthalate-induced testicular injury in adult mice. Human & Experimental Toxicology, 41: 09603271221078867. DOI: https://www.doi.org/10.1177/09603271221078867

Hsu SM, Raine L, and Fanger HX (1981). Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. Journal of Histochemistry & Cytochemistry, 29(4): 577-580. DOI: https://www.doi.org/10.1177/29.4.6166661

Jafari A, Ghasemnejad-Berenji H, Nemati M, and Ghasemnejad-Berenji M (2021). Topiramate: A novel protective agent against ischemia reperfusion-induced oxidative injury after testicular torsion/detorsion. The American Journal of Emergency Medicine, 44: 257-261. DOI: https://www.doi.org/10.1016/j.ajem.2020.03.060

Jalili C, Korani M, Pazhouhi M, Ghanbari A, Zhaleh M, Davoudi S, and Rashidi I (2021). Protective effect of Gallic acid on nicotine-induced testicular toxicity in mice. Research in Pharmaceutical Sciences, 16(4): 414-424. DOI: https://www.doi.org/10.4103/1735-5362.319579

Jalili C, Abdolmaleki A, Roshankhah SH, and Salahshoor MR (2022). Effects of Gallic acid on rat testopathy following morphine administration: An experimental study. Journal of Herbmed Pharmacology, 9(1): 61- 67. DOI: https://www.doi.org/10.15171/jhp.2020.09

John P and Anitha L (2015). Nephrolithiatic potential of topiramate in male albino rats. Asian Journal of Research in Pharmaceutical Sciences and Biotechnology, 3(4): 93-100. Available at: https://scholar.archive.org/work/f3cug3vdsngrvidnp2boajkugq

Kadhem WM and Majhwol EM (2020). Effect some of antiepileptic drugs (AEDs) on some male reproductive parameters in white rats. EurAsian Journal of BioSciences, 14(1): 1925.

Medzhitov R (2021). The spectrum of inflammatory responses. Science, 374(6571): 1070-1075. DOI: https://www.doi.org/10.1126/science.abi5200

Moradi A, Abolfathi M, Javadian M, Heidarian E, Roshanmehr H, Khaledi M, and Nouri A (2021). Gallic acid exerts nephroprotective, anti-oxidative stress, and anti-inflammatory effects against diclofenac-induced renal injury in male rats. Archives of Medical Research, 52(4): 380-388. DOI: https://www.doi.org/10.1016/j.arcmed.2020.12.005

Nishikimi M, Rao NA, and Yagi K (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications, 46(2): 849-854. DOI: https://www.doi.org/10.1016/S0006-291X(72)80218-3

Novin MG, Golmohammadi MG, Sagha M, Ziai SA, Abdollahifar MA, and Nazarian H (2020). Protective effect of Gallic acid on testicular tissue, sperm parameters, and DNA fragmentation against toxicity induced by cyclophosphamide in adult NMRI mice. Urology Journal, 17(1): 78-85. DOI: https://www.doi.org/10.22037/uj.v17i1.4858

Ohkawa H, Ohishi N, and Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2): 351-358. DOI: https://www.doi.org/10.1016/0003-2697(79)90738-3

Olayinka ET, Ore A, Ola OS, and Adeyemo OA (2015). Ameliorative effect of Gallic acid on cyclophosphamide-induced oxidative injury and hepatic dysfunction in rats. Medical Sciences, 3(3): 78-92. DOI: https://www.doi.org/10.3390/medsci3030078

Rotimi DE, Komolafe C, Iyobhebhe M, Akinduko AA, Durojaiye T, Oluwayemi ET, Akinsanola B, and Olaolu TD (2024). Therapeutic role of Gallic acid in male infertility. International Conference on Science, Engineering and Business for Driving Sustainable Development Goals, Omu-Aran, Nigeria, pp. 1-9. DOI: https://www.doi.org/10.1109/SEB4SDG55512.2024.1234567

Seed J, Chapin RE, Clegg ED, Dostal LA, Foote RH, Hurtt ME, Klinefelter GR, Makris SL, Perreault SD, Schrader S, Seyler D et al. (1996). Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: A consensus report. Reproductive Toxicity, 10(3): 237-244. DOI: https://www.doi.org/10.1016/0890-6238(96)00028-7

Sheweita SA, Almasmari AA, and El-Banna SG (2018). Tramadol-induced hepato-and nephrotoxicity in rats: Role of curcumin and Gallic acid as antioxidants. PloS One, 13(8): e0202110. DOI: https://www.doi.org/10.1371/journal.pone.0202110

Steele JW, Krishnan V, and Finnell RH (2024). Mechanisms of neurodevelopmental toxicity of topiramate. Critical Reviews in Toxicology, 54(7): 465-475. DOI: https://www.doi.org/10.1080/10408444.2024.2368552

Varzandeh R, Khezri MR, Esmaeilzadeh Z, Jafari A, and Ghasemnejad-Berenji M (2024). Protective effects of topiramate on acetic acid-induced colitis in rats through the inhibition of oxidative stress. Naunyn-Schmiedeberg's Archives of Pharmacology, 397(2): 1141-1149. DOI: https://www.doi.org/10.1007/s00210-024-02345-6

Waly H, Ezz El-Arab RF, Abou Khalil NS, Hassanein KM, Al-Salahy MB, and Saleh SM (2024). Gallic acid counteracts tartrazine-induced testicular dysfunction in rats: Biochemical, histopathological and ultrastructural evidences. The Journal of Basic and Applied Zoology, 85(1): 24. DOI: https://www.doi.org/10.1186/s41936-024-00370-0