Int. J. Morphol.,
43(4):1230-1239, 2025.

The Ameliorative Effect of Carvacrol Against Pregabalin-Induced Testicular Injury in Adult Male Albino Rats: Histopathological and Immunohistochemical Study

Efecto Reparador del Carvacrol Contra la Lesión Testicular Inducida por Pregabalina en Ratas Albinas Macho Adultas: Estudio Histopatológico e Inmunohistoquímico

Marwa S. Badawi[5]; Awwad Alenezy[2]; Safya E. Esmaeel[3,6]; Naglaa A. Bayomy[1,8]; Naglaa Mokhtar[4,7]; Eslam K. Fahmy[3,6]; Saad H. Elshafey[1] & Reda H. Elbakary[8]

BADAWI, M. S.; ALENEZY, A.; ESMAEEL, S. E.; BAYOMY, N. A.; MOKHTAR, N.; FAHMY, E. K.; ELSHAFEY, S. H. & ELBAKARY, R. H. The ameliorative effect of carvacrol against pregabalin-induced testicular injury in adult male albino rats: Histopathological and immunohistochemical study. *Int. J. Morphol., 43(4)*:1230-1239, 2025.

SUMMARY: Pregabalin (PGB) is an anti-convulsant drug that is indicated in epilepsy, neuropathic pain and anxiety. Pregabalin is considered an analogue to the neurotransmitter gamma amino butyric acid (GABA). A phytochemical called carvacrol is obtained from aromatic plants in the Oregano genus. It is well recognized for its anti-inflammatory and antioxidant properties, as well as its ability to stop the growth of many cancer cells. The purpose of this study was to assess Carvacrol's antioxidant capacity in relation to PGB-induced testicular injury. Forty male albino rats were randomly divided into 4 groups with 10 rats in each group. Group I (control group). Group II animals received Carvacrol orally in a dose of 40 mg/kg/day for 4 weeks. Group III animals received PGB orally in a dose of 1200 mg/kg/day for 4 weeks. Group IV animals received (PGB + Carvacrol). The testes were taken out, weighed, and sampled for biochemical, immunohistochemical, and histopathological analyses at the end of the experiment. In order to analyze the semen, the epididymes were also removed. PGB administration at a dose of 1200 mg/kg raised FSH and LH levels while decreasing serum testosterone levels. Additionally, it led to an increase in sperm abnormalities and a decrease in sperm motility, count, and viability percentage. Additionally, there was a decrease in GSH, GPx, and SOD and an increase in MDA, which suggests testicular oxidative damage. Testicular histology revealed morphological alterations. Carvacrol administered concurrently was able to undo the negative effects of PGB. This was seen in the revised levels of the mentioned measures, which were almost normal when compared to the PGB and control groups. Carvacrol ameliorated PGB-induced testicular damage, according to the current study's biochemical, histological, and immunohistochemical results.

KEY WORDS: Pregabalin; Testicular injury; Oxidative stress; Testosterone; Sperm parameters; Carvacrol.

INTRODUCTION

The most prevalent long-term neurological condition worldwide is thought to be epilepsy. Convulsions result from the central nervous system's disrupted natural balance between excitation and inhibition (Asadi-Pooya *et al*., 2012). Through several ways, antiepileptic medications naturally balance the excitatory and inhibitory postsynaptic potential (Deckers *et al*., 2000).

Pregabalin is frequently used in primary care, neurology, and psychiatry. It is an anticonvulsant medication that is six times more powerful than gabapentin despite having a comparable structure. Pregabalin is frequently used to treat neuropathic pain, notably in diabetic patients, fibromyalgia, anxiety, and epilepsy (Baldwin *et al*., 2013; Loftus & Wright, 2014; Evoy *et al*., 2017). PGB was

[1]Department of Anatomy, College of Medicine, Northern Border University, Arar, Saudi Arabia.
[2]Department of Family and Community Medicine, College of Medicine, Northern Border University, Arar, Saudi Arabia.
[3]Department of Physiology, College of Medicine, Northern Border University, Arar, Saudi Arabia.
[4]Department of Medical Biochemistry, College of Medicine, Northern Border University, Arar, Saudi Arabia.
[5]Department of Anatomy, College of Medicine, Sohag University, Egypt.
[6]Department of Physiology, College of Medicine, Zagazig University, Egypt.
[7]Department of Medical Biochemistry and Molecular Biology, College of Medicine, Mansoura University, Egypt.
[8]Department of Histology, College of Medicine, Tanta University, Egypt.
FUNDING. Northern Border University, Arar, KSA, project number "NBU-FFR 2025-230-01

Received: 2025-03-01 Accepted: 2025-05-25

displayed for the first time on the market by Pfizer under the trade name Lyrica. The Food and Drug Administration (FDA) authorized it in 2004 (Al-Zubaidi *et al*., 2015).

According to Martinotti *et al*. (2013), pregabalin exerts its analgesic and anxiolytic effects by binding to voltage-gated calcium channels. It participates in the dopaminergic reward system and possesses characteristics similar to those of GABA (Cai *et al*., 2012). Pregabalin has been misused by some abusers to replace other common prohibited drugs like opium and tramadol (Baird *et al*., 2014).

The ability of PGB to diminish the release of excitatory neurotransmitters like glutamate and substance P, as well as peptides such as calcitonin gene-related peptide, accounts for its wide range of therapeutic effects, which include lowering neuropathic pain and anxiety and managing epilepsy (Foroutan & Nikvarz, 2016). By doing this, the overstimulated neurons in the central nervous system revert to their original positions (Baidya *et al*., 2011).

Preclinical animal studies demonstrated that pregabalin (PGB) temporarily impaired sperm quality, reducing sperm count and motility while increasing abnormalities and negatively affected reproductive function by decreasing fertility and raising rates of pre-implantation embryo loss (Etemad, *et al*., 2013; Ding *et al*., 2017). According to certain clinical research, pregabalin causes erectile dysfunction, delayed ejaculation, and changes sexual desire (Calabrò & Bramanti, 2010; Bucur & Jeczmien, 2011). Unlikely, previous clinical research showed that PGB 600 mg/day for 12 weeks did not significantly change spermatogenesis or blood levels of FSH and testosterone in healthy men (Sikka *et al*., 2015).

The so-called Mediterranean diet often includes plants from the Lamiaceae family, including sage (*Salvia* spp.), oregano (*Oregano vulgare*), and thyme (*Thymus vulgaris*) (Guidi & Landi, 2014). One of the active components that contribute significantly to the essential oils of Lamiaceae plants is the monoterpenoid phenol carvacrol (5-isoropyl-2-methylphenol) (Gunes-Bayir *et al*., 2019; Ahmad *et al*., 2021).

Carvacrol has antioxidant properties both in vitro and in vivo. The presence of a hydroxyl group (OH•) covalently bonded to an aromatic ring is associated with these effects (Guimarães *et al*., 2010; Aristatile *et al*., 2015). Carvacrol has been approved by the Federal Drug Administration (FDA) for use as a food preservative and is thought to be safe in small amounts (Ghorani *et al*., 2021). According to studies, carvacrol possesses biological activities as an immunological and nerve impulse modulator, as well as antibacterial, bactericidal, anti-inflammatory, anticancer, antioxidant, antifungal, and depressive qualities (Silva *et al*., 2018).

The anti-inflammatory, cytoprotective, and antioxidant properties of CRV have been linked to its ability to lessen kidney damage brought on by bilateral renal ischemia-reperfusion (I/R) (Ozturk *et al*., 2018). Another study shown that CRV protected male rat testicles from cisplatin-induced reproductive impairment (Aksu *et al*., 2016).

Therefore, this study aimed to investigate whether carvacrol could mitigate testicular injury resulting from pregabalin administration in adult male albino rats.

MATERIAL AND METHOD

Animals. Forty healthy adult male albino rats weighing between 200 and 250 g were used in this study. They were purchased from Tanta University's animal house, Tanta Faculty of Medicine, Egypt. During the experiment, the rats were housed in polypropylene cages with normal illumination and a temperature-controlled environment (25 ± 2 °C), and they were allowed unlimited access to laboratory food and water. They had at least two weeks to acclimate to their new environment before the study started. All animal procedures were approved by the Research Ethics Committee of the Faculty of Medicine, Tanta University, Egypt, Approval Code: 36264PR1079/2/25

Experimental design. Following the time of acclimatization, the rats were randomly split into four groups, each consisting of ten rats:

Group I (Control group): received distilled water daily for four weeks through oral gavage.

Group II: Received carvacrol orally (Sigma-Aldrich Chemical Co. St. Louis, MO, USA) at a dose of 75 mg/kg/day for 4 weeks (Bagheri *et al*., 2023).

Group III: Received pregabalin dissolved in distilled water orally at a dose of 1200 mg/kg/day for 4 weeks (Badawi, 2023).

Group IV: Received a combination of pregabalin (1200 mg/kg/day) and carvacrol (75 mg/kg/day) orally for 4 weeks.

An intraperitoneal injection of sodium pentobarbital (35 mg/kg bw) was used to anesthetize the rats twenty-four hours following the last medication regimen. The heart was examined by cutting into the chest wall. To extract serum, 5

ml of intracardiac blood was extracted and centrifuged for 15 min at 3000 rpm. In order to measure the serum concentrations of FSH, LH, and testosterone, the samples were stored at -20°C. Radioimmunoassay was used to measure hormones (RIA).

The animals were then sacrificed by decapitation, and opening the abdomen and scrotum of the rats was done to extract both testicles and epididymes. For biochemical analysis, the appropriate testes were promptly snap frozen in liquid nitrogen and kept at -80 °C. The testicular tissues' concentrations of reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) were then assessed. The left testes were fixed in 10 % neutral buffered formalin (NBF) and weighed and sampled for histopathological examinations.

Serum hormonal assay. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were assessed by radioimmunoassay using a commercial kit (AccuBind ELISA Kits, California, USA). Analyses were conducted in accordance with the manufacturer's instructions.

Semen analysis. The caudal portion of the epididymis was cut and placed at 37 ºC in a Petri dish with 2 ml of regular saline (0.9 % NaCl). The tissue is left for 30 to 60 seconds to allow the sperm to seep from the tubules. The resulting fluid will be handled exactly like semen and will have a whitish or grayish tint. The fluid should be collected in an Eppendorf tube.

Sperm count. After adding 0.5 ml of the semen to 1 ml of the semen diluting fluid (sodium bicarbonate 5 g, formalin 1 ml, and distilled water 99.0 ml), the mixture was thoroughly mixed. In the hemocytometer chamber, a single drop of sperm suspension was placed. And for ten minutes, the hemocytometer was placed in a humid area to allow the sperm to settle. Spermatozoa in the hemocytometer's squares were counted under a microscope. [The number of spermatozoa per ml = No. of sperms × dilution factor × depth factor /No. of areas counted] (Oyedeji *et al*., 2013).

Sperm motility. On a slide that had been preheated, two drops of heated 2.9 % sodium citrate and one drop of semen were mixed together. The slide was then examined under a microscope at × 400 magnification after being covered with a heated cover slip. Ten randomly selected microscope fields were used to test the motility of ten sperm. Consequently, the motility of 100 sperm was assessed at random. To determine the percentage of motile sperm, the number of motile sperms was divided by the total number of counted sperm (i.e., 100) (Oyedeji *et al*., 2013).

Sperm viability (Life/dead ratio). On a preheated slide, two drops of heated Eosin/Nigrosin stain were mixed with one drop of semen to form a homogeneous smear, which was then left to air dry. The stained slide was then immediately examined at X400 magnification using a microscope. The live sperm cells did not absorb the stain, while the dead sperm cells did. The proportion was calculated after the number of stained and unstained sperm was counted (Oyedeji *et al*., 2013).

Sperm morphology. On a preheated slide, two drops of warmed Eosin/Nigrosin stain were mixed with one drop of semen to form a homogeneous smear, which was then left to air dry. The stained slide was then immediately examined at × 400 magnification using a microscope. A random selection of five microscope fields was made, and the number of abnormal spermatozoa was calculated from the total number of spermatozoa in each field. The percentage of abnormal spermatozoa was then expressed (Oyedeji *et al*., 2013).

Assessment of oxidative stress. To get rid of any remaining blood clot, the right testes were rinsed with 10 % cold phosphate buffered saline (PBS) solution (PH 7.4). To gather the supernatant fluids, tissues were weighed, homogenized in PBS, and centrifuged for 15 min at 4 °C at 8000 rpm. The intended indicators of oxidative stress were measured using these supernatant components.

Using spectrophotometry (PD-303 Spectrophotometer; APEL CO., LTD., Japan), the tissue MDA level (a marker for lipid peroxidation) was analyzed. Based on the Ohkawa *et al*. (1979) method, MDA concentration was determined spectrophotometrically by measuring the absorbance of the thiobarbituric acid reactive substances (TBARS) complex at 535 nm using a commercial assay kit (Cayman Chemical Company, Ann Arbor, USA, Cat. No. 10009055). Results were quantified as nanomoles of MDA per gram of tissue protein (Ahmida, 2012).

Antioxidant activity was evaluated by measuring GSH, GPx, and SOD levels. GSH concentration was determined spectrophotometrically at 412 nm using a kinetic assay based on the Ellman (1959) method, performed with a commercial Glutathione Assay Kit (Cayman Chemical, Cat. No. 703002). Data were reported as μmol GSH per gram of tissue protein (Soltani *et al*., 2018). Glutathione peroxidase (GPx) activity was determined following Paglia & Valentine (1967) protocol using a commercial assay kit (abcam, UK; Cat. ab102530), with spectrophotometric measurements taken at 340 nm. Results were reported as units per gram of tissue protein (Soltani *et al*., 2018). Superoxide dismutase (SOD) activity was assessed via the xanthine/xanthine oxidase method (Sun *et al*., 1988) using a Cayman Chemical assay kit (Item 706002), by measuring the amount of reduced nitro blue

tetrazolium (NBT) with one unit of SOD (defined as the amount of protein that inhibits the rate of NBT reduction by 50 %). SOD was expressed as units/mg tissue protein (Soltani *et al*., 2018).

Histological and immunohistochemical examination. Each animal's left testis is preserved for 24 hours in 10 % neutral buffered formalin. After that, it is treated and embedded in paraffin wax, and a microtome is used to cut pieces that are 4 µm thick. To find histological alterations, certain paraffin sections were stained with hematoxylin and eosin (H&E) and seen under a light microscope (Bancroft & Layton, 2014). Proapoptotic protein (Bax) immunoexpression was evaluated by immunohistochemically stained paraffin sections. Proteinase K (20 µg/ml) was used to break down the protein in the tissue after the paraffin sections had been deparaffinized using xylene and ethanol. Endogenous peroxidase activity was then inhibited for 10 min by treating them with a 3 % hydrogen peroxidase solution. After that, sections were treated for 15 min with prediluted mouse monoclonal antibodies (10 µg/ml) against Bax (Dako, Carpinteria, California, USA), then for another 15 min with a rabbit anti-mouse antibody and another 15 min with the streptavidin-biotin-peroxidase complex. After five minutes of diaminobenzidine (DAB) staining, the specimens were counterstained for two to five minutes using Mayer's hematoxylin. Brown precipitation-showing cells were regarded as having positive Bax expressions (Singh *et al*., 2015).

Statistical Analysis. All data were analyzed using SPSS software (version 26; IBM Corp., Armonk, NY, USA). Results are presented as mean ± standard deviation (SD). The unpaired Student's t-test was used to compare means between two independent groups with statistical significance set at $p < 0.05$.

RESULTS

Over the course of the 4-week experiment, none of the experimental rats passed away.

Testicular weight evaluation. The PGB group's testis weight at the end of the experiment was considerably lower than that of the PGB+Carvacrol group and the control group ($P<0.05$). In contrast, there was no noticeable variation in testis weight between the PGB+Carvacrol, Carvacrol, and control groups (Table I).

Serum hormonal assay. PGB-treated rats showed a significant increase in FSH and LH hormones and a significant decrease in testosterone hormone concentrations when compared to the control and PGB+Carvacrol groups ($P<0.05$). However, there was no significant difference in the concentration of serum hormones between the control, Carvacrol, and PGB+Carvacrol groups (Table II).

Semen analysis. While there was no significant difference between the control, Carvacrol, and PGB+Carvacrol groups, there was a significant decrease in sperm motility, count, and percent of viability as well as an increase in sperm abnormalities in PGB-treated rats when compared to the control and PGB+Carvacrol groups ($P<0.05$) (Table III).

Assessment of oxidative stress. The MDA level was higher in the PGB-treated rats than in the control group ($P<0.05$), but the activity of the antioxidant enzymes GSH, GPx, and SOD was significantly reduced. PGB-induced increases in renal MDA and decreases in renal GSH, GPx, and SOD were significantly inhibited by the administration of carvacrol in conjunction with PGB ($P < 0.05$) (Table IV).

Table I. Testis weight of rats in the various groups under study.

Parameters	Group I (Control)	Group II (Carvacrol)	Group III (PGB)	Group IV (PGB+ Carvacrol)
Testis weight (g)	1.73±0.04	1.70±0.02	0.97± 0.02 [a]	1.69±0.06 [b]

The mean ± standard deviation is used to express the data. The student's t test was used to statistically assess the results at $P < 0.05$. a $p < 0.05$ in relation to group I, the control group. b $p < 0.05$ in contrast to group III, the PGB group.

Table II. Serum levels of testosterone, FSH and LH hormones in the various groups under study.

Parameters	Group I (Control)	Group II (Carvacrol)	Group III (PGB)	Group IV (PGB+ Carvacrol)
Testosterone(ng/ml)	4.82±1.08	4.80±0.025	2.43±1.29 [a]	4.75±0.005 [b]
FSH (mIU/ml)	8.69±0.04	8.73±1.09	13.44±0.35 [a]	9.11±1.09 [b]
LH (mIU/ml)	5.07±0.012	5.16±1.03	8.38±0.002 [a]	5.74±1.03 [b]

The mean ± standard deviation is used to express the data. The student's t test was used to statistically assess the results at $P < 0.05$. a $p < 0.05$ in relation to group I, the control group. b $p < 0.05$ in contrast to group III, the PGB group.

Table III. Sperm parameters in the various groups under study.

Parameters	Group I (Control)	Group II (Carvacrol)	Group III (PGB)	Group IV (PGB+Carvacrol)
Sperm count	106.3±5.3	101.4±2.05	63.3±3.43[a]	99.3±2.75[b]
Motility (%)	91.3±2.8	89.2±1.09	48.44±1.6[a]	87.4±2.33[b]
Viability (%)	94.6±3.45	92.23±1.03	62.81±2.13[a]	90.4±2.15[b]
Abnormalities (%)	2.43±0.13	2.47±0.48	9.64±0.15[a]	2.52±0.24[b]

The mean ± standard deviation is used to express the data. The student's t test was used to statistically assess the results at $P < 0.05$. ap < 0.05 in relation to group I, the control group. bp < 0.05 in contrast to group III, the PGB group.

Table IV. Malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) levels in the various groups under study.

Parameters	Group I (Control)	Group II (Carvacrol)	Group III (PGB)	Group IV (PGB+Carvacrol)
MDA (nmol/g tissue protein)	78.62±4.32	75.33±4.56	123.13±6.74[a]	83.15±3.73[b]
GSH (µmol/g tissue protein)	12.41±0.39	12.14±0.73	4.32±1.12[a]	11.69±0.85[b]
GSH-Px (units/g tissue protein)	29.53±1.88	29.14±1.45	12.92±2.89[a]	25.91±1.37[b]
SOD (units/mg tissue protein)	7.35±0.04	7.28±0.03	3.14±0.08[a]	7.11±0.02[b]

The mean ± standard deviation is used to express the data. The student's t test was used to statistically assess the results at $P < 0.05$. ap < 0.05 in relation to group I, the control group. bp < 0.05 in contrast to group III, the PGB group.

Histological findings. The testis of control rats of H&E-stained sections showed seminiferous tubules surrounded by connective tissue, containing many rounded or polygonal interstitial cells (Leydig cells). Immediately surrounding each tubule were flattened myoid cells. Inside the tubule, there was sustentacular cells (Sertoli cells), and germ cells of the spermatogenic lineage. Prominent among the latter were spermatogonia, located near the basement membrane, and primary spermatocytes closer to the lumen of the tubule. Sperm were located in the center of the tubule (Fig. 1).

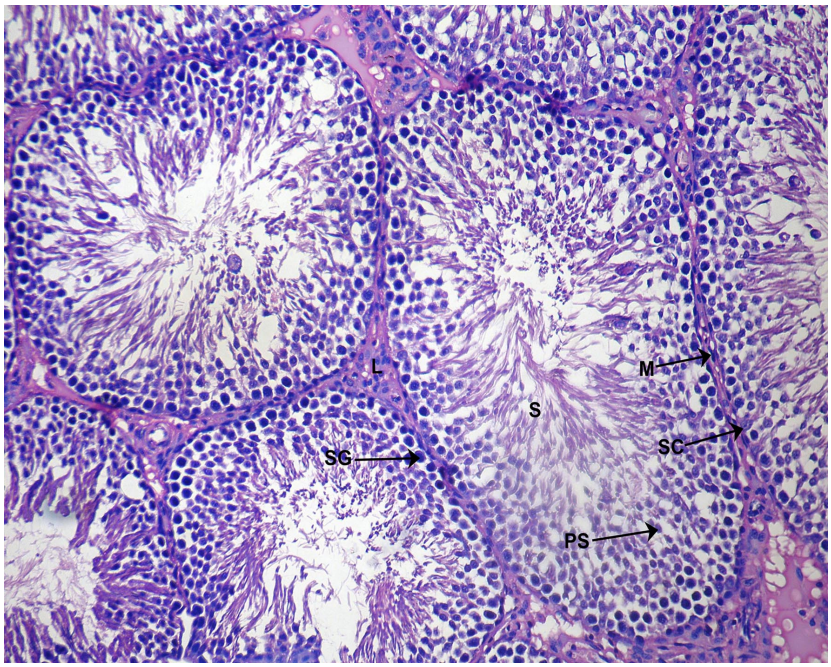
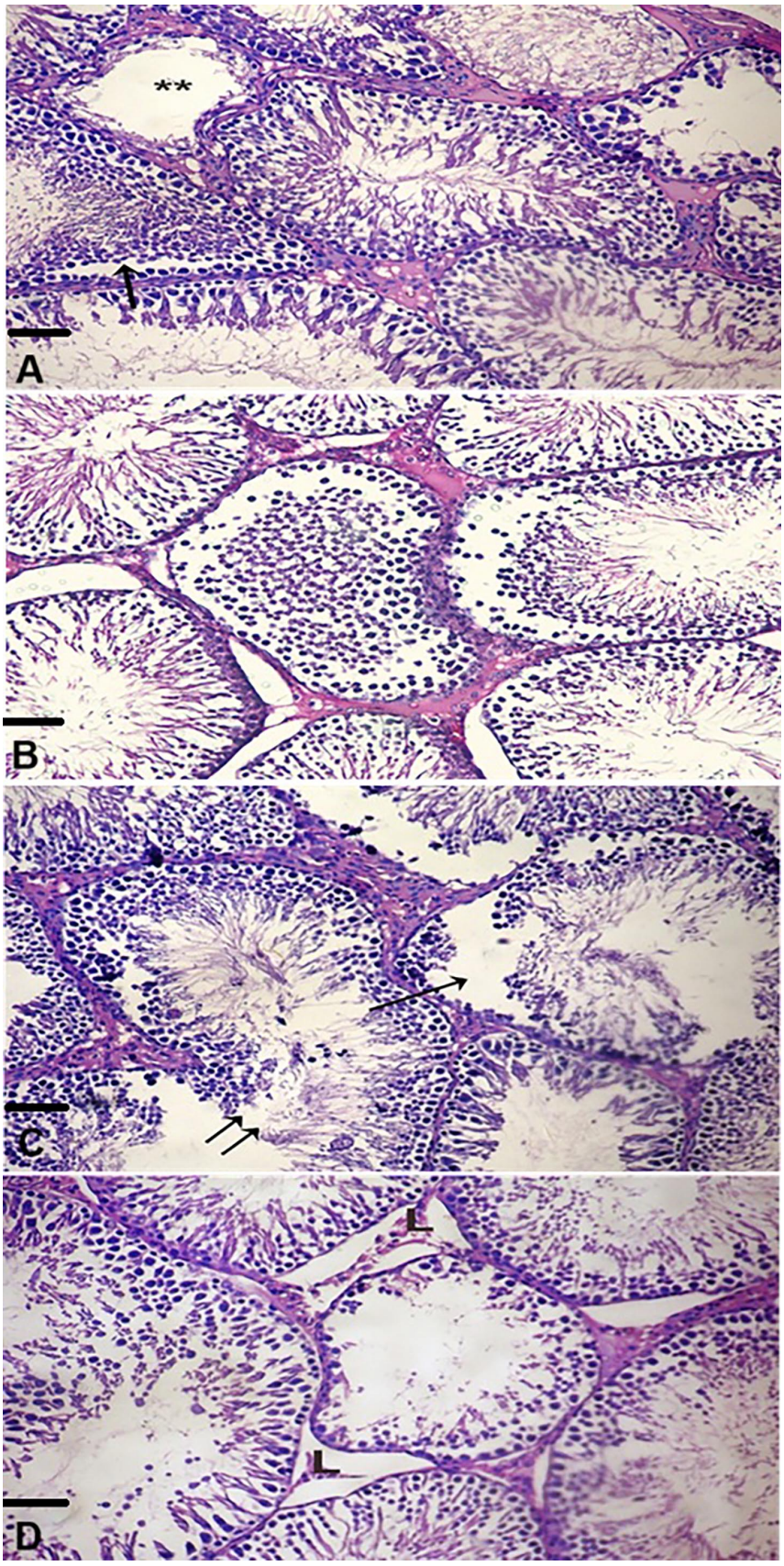


Fig. 1. A photomicrograph of normal architecture of the testis in the control group showing seminiferous tubules surrounded by connective tissue, containing many rounded or polygonal interstitial cells (L). Immediately surrounding each tubule were flattened myoid cells (M). Inside the tubule, a unique seminiferous epithelium composed of columnar sustentacular cells (SC), and germ cells of the spermatogenic lineage is seen. Prominent among the germ cells are spermatogonia (SG), located near the basement membrane, and primary spermatocytes (PS) closer to the lumen of the tubule. Sperm are located in the center of the tubule (S) (H&E, scale bars = 40 µm).

In PGB-treated group, H&E-stained sections showed distortion of seminiferous tubules, with irregular and wrinkled basement membrane and spermatogonia's separation from the basement membrane. There was sperm loss in the cores of some tubules (Fig. 2A). Other tubules showed predominance of spermatogonia on the expense of other germ cells (Fig. 2B). Some tubules showed destruction and loss of part of the basement membrane (double arrows). Loss of continuity of the chain of spermatogenesis was seen in some tubules (Fig. 2C). The interstitium showed shrunken and destroyed interstitial cells (Fig. 2D).

In rats receiving PGB+Carvacrol, H&E-stained sections showed a histological structure which was more or less similar to normal (Fig. 3).



Immunohistochemical findings

Bax antigen immunostaining. The testicular tissues in the control group showed a negative Bax immunostaining reaction (Fig. 4a). Rats given PGB showed dark brown granules in the cytoplasm of the majority of their testicular cells (Fig. 4b). The testicular tissues of the PGB+carvacrol-treated group exhibited Bax activity that was essentially identical to that of the control group (Fig. 4c).

Fig. 2. (A-D) Photomicrographs of testis in PGB-treated rats. (A) Distortion of seminiferous tubules, with irregular and wrinkled basement membrane and detachment of spermatogonia from the basement membrane (→). Some tubules showed loss of sperms in their centers (**). (B) Other tubules showed predominance of spermatogonia on the expense of other germ cells. (C) Some tubules showed destruction and loss of part of the basement membrane (double arrows). Loss of continuity of the chain of spermatogenesis was seen in some tubules (→) (D) The interstitium shows shrunken and destroyed interstitial cells (L) (H&E, scale bars = 40 μm).

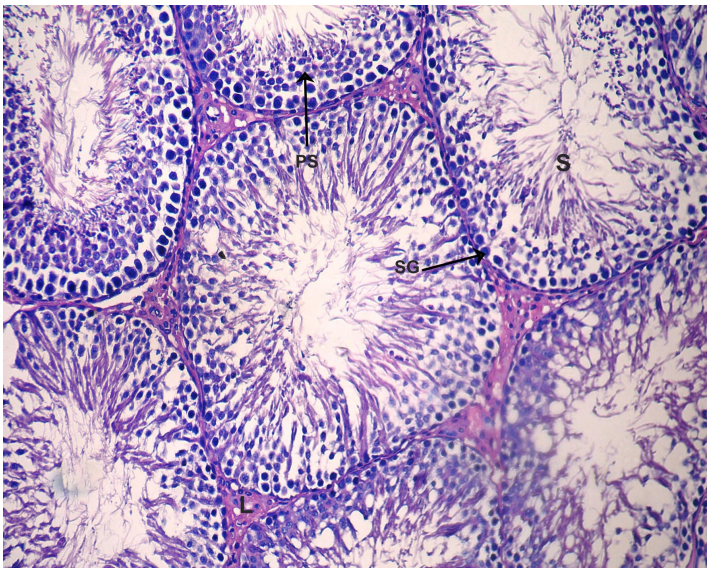


Fig. 3. A photomicrograph of testis in rats treated with PGB+carvacrol showing normal looking testicular tissue more or less similar to normal. (H&E, scale bars = 40 μm).

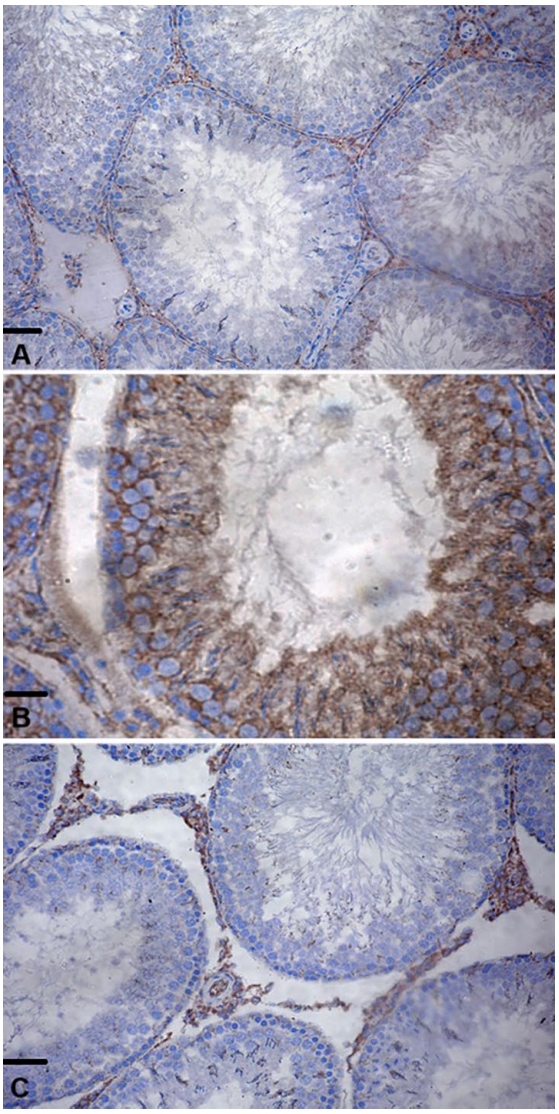


Fig. 4. (A) Negative immunostaining reaction of Bax in the testicular tissue of the control group. (B) Strong positive immunostaining reaction of Bax in the testicular tissue of the PGB-treated group. (C) Negative immunostaining reaction of Bax in the testicular tissue of group treated with PGB+carvacrol (Bax immunostaining, scale bars = 40 μm).

DISCUSSION

The spermatogenesis phase is thought to be a time of significant cellular change that is extremely vulnerable to outside stimuli. Exposure to harmful substances has varying effects on different stages of germ cells. Chemical agents are more commonly thought of as spermatocyte poisons, however spermatogonia have been identified as ionizing radiation target cells (Griswold, 2016).

Although gabapentinoids, including pregabalin and gabapentin, are frequently utilized in primary care, psychiatry, and neurology, there is growing evidence that they may be abused (Liliana *et al*., 2015). Many fragrant herbs, including oregano and thyme, produce carvacrol, a monoterpenic phenol (Suntres *et al*., 2013). Its ability to scavenge radicals can help reduce damage caused by oxidative stress. It increases the activity of enzymatic antioxidants such as glutathione peroxidase (GPx), reduced glutathione (GSH), and superoxide dismutase (SOD) (Evazalipour *et al*., 2021).

In this study, we aimed to determine whether carvacrol may protect adult male albino rats from testicular damage caused by pregabalin.

Testicular weight significantly decreased after PGB treatment, which may indicate detrimental effects on testicular function. The reduction of spermatogenesis, which results in fewer spermatogenic and interstitial cells, may be the cause of this drop in testicular weight. This finding was corroborated by Kamel & Khalifa (2015), who found that pregabalin 20 mg/kg for 65 days significantly reduced the weights of the testicles, seminal vesicles, and prostate glands. Carvacrol effectively reduced the testicular damage brought on by PGB treatment, as evidenced by the fact that the testicular weight in the group treated with both PGB and carvacrol did not differ substantially from the control group. Another study that found that giving carvacrol to rats inebriated with MTX improved their testis weight corroborated this finding (Ibrahim *et al*., 2023).

Comparing the PGB-treated group to normal rats, the blood hormonal assay showed a considerable increase in FSH and LH levels and a significant drop

in serum testosterone levels. These results were consistent with those of Hareedy *et al*. (2020), who found that testosterone levels greatly dropped and FSH and LH levels significantly increased when PGB was given for two months compared to the control group. Another study found that when taken orally for 28 days, pregabalin raised levels of FSH and LH while decreasing levels of serum testosterone (Bostanian *et al*., 2016). The concurrent administration of carvacrol with PGB significantly restored the normal hormonal levels, supporting its protective effects. Ibrahim *et al.* (2023), showed an increase in testosterone levels in rats cotreated with carvacrol with MTX, and attributed this improvement to the antioxidant and cytoprotective effects of carvacrol.

Sperm metrics showed that the PGB-treated group had significantly more sperm abnormalities and significantly lower sperm motility, count, and viability percentage. Pregabalin reduces the activity and division of the testes' epithelial cells by increasing prolactin secretion; as a result, fewer spermatogenic cells should be present after PGB treatment (Bostanian *et al*. 2016). These findings concurred with those of Kamel & Khalifa (2015), who reported that oral treatment of pregabalin 20 mg/kg for 65 days resulted in a significant rise in sperm abnormalities and a notable decline in sperm viability percentage, motility, and count. Another study found that sperm agglutination increased significantly and sperm count, motility, and morphologically normal sperms significantly decreased after 35 days of daily exposure to PGB 600 mg/kg (Al-Zubaidi *et al*., 2015). On the other hand, the sperm parameters returned to normal in the group that received both PGB and carvacrol. Bagheri *et al*. (2023), similarly reported this outcome, finding that carvacrol administration helped diabetic rats' sperm parameters return to normal.

Additionally, the study's measurements of oxidative stress markers verified that PGB results in oxidative stress and testicular injury. Malondialdehyde (MDA) levels in the testicles significantly increased, whereas Superoxide dismutase (SOD), reduced glutathione (GSH), and glutathione peroxidase (GPx) levels significantly decreased. However, following carvacrol treatment, there was a notable decrease in level of MDA and a restoration of SOD GSH, GSH-Px, and levels. This illustrated how its antioxidant qualities can lessen the oxidative damage brought on by PGB. These findings were documented in several previous investigations (Shoorei *et al*., 2019; Bagheri *et al*., 2023).

Rats given PGB showed changes in their testicular histological architecture, including distortion of seminiferous tubules, with irregular and wrinkled basement membrane and spermatogonia's separation from the basement membrane. A portion of the basement membrane was destroyed and lost in certain tubules. There were fewer primary spermatocytes, spermatids, and sperm in other tubules, with undifferentiated spermatogonia predominating. Shrunken and damaged interstitial cells were visible in the interstitium. Another study that found deteriorated seminiferous tubules in the PGB-treated group corroborated the current study's findings. About 30 % of seminiferous tubules exhibit late spermatid, whereas the majority exhibit early spermatid spermatogenesis (Hareedy *et al*., 2020). According to a third study, the groups treated with PGB had less spermatogonia, primary spermatocytes, and spermatids, as well as interstitial cell loss and death (Bostanian *et al*., 2016). The majority of the other spermatogenic cells are overpopulated by spermatogonia, according to Al-Zubaidi *et al*., (2015). Therefore, the proportion of primitive germ cells was higher than that of more advanced ones.

The PGB+carvacrol group's testicular tissue showed a noticeable histological improvement, demonstrating the protective benefits of carvacrol. Carvacrol showed regeneration of testicular histological architecture with varying degrees of injury, according to other research that reported similar findings (Daggulli *et al*., 2014; Araghi *et al*., 2017; Shoorei *et al*., 2019; Bagheri *et al*., 2023).

Immunohistochemical results presented a clear relationship between apoptosis and PGB exposure. The testicular tissues in PGB group revealed positive immunostaining reaction for Bax (pro-apoptotic proteins). On the other hand, combining carvacrol with PGB strikingly reduced Bax activity, indicating a suppression in the apoptotic process and suggesting protective mechanisms. Another study confirmed these results by demonstrating that carvacrol therapy significantly decreased the expression of Bax in diabetic rats' testicular tissue (Shoorei *et al*., 2019).

It could be concluded that carvacrol offers significant protective effects against PGB-induced testicular injury through various mechanisms, including reducing oxidative stress and modulating apoptosis. These results imply that carvacrol may be a useful supplemental treatment in clinical settings involving PGB treatment, warranting further investigation into optimal dosing and mechanisms of action. Future research should also aim to translate these findings into clinical trials to assess the safety and effectiveness of combining carvacrol with PGB in epileptic patients.

ACKNOWLEDGEMENTS

The author extend appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA for funding this research work through the project number “NBU-FFR 2025-230-01”.

BADAWI, M. S.; ALENEZY, A.; ESMAEEL, S. E.; BAYOMY, N. A.; MOKHTAR, N.; FAHMY, E.K.; ELSHAFEY, S. H. & ELBAKARY, R. H. Efecto reparador del carvacrol contra la lesión testicular inducida por pregabalina en ratas albinas macho adultas: Estudio histopatológico e inmunohistoquímico. *Int. J. Morphol., 43(4)*:1230-1239, 2025.

RESUMEN: La pregabalina (PGB) es un fármaco anticonvulsivo indicado para la epilepsia, el dolor neuropático y la ansiedad. Se considera un análogo del neurotransmisor ácido gamma amino butírico (GABA). El carvacrol, un fitoquímico, se obtiene de plantas aromáticas del género Oregano. Es bien reconocido por sus propiedades antiinflamatorias y antioxidantes, así como por su capacidad para detener el crecimiento de muchas células cancerosas. El propósito de este estudio fue evaluar la capacidad antioxidante de carvacrol en relación con la lesión testicular inducida por PGB. Cuarenta ratas albinas macho se dividieron aleatoriamente en 4 grupos con 10 ratas en cada grupo. Grupo I (grupo control). Los animales del grupo II recibieron carvacrol por vía oral en una dosis de 40 mg/kg/día durante 4 semanas. Los animales del grupo III recibieron PGB por vía oral en una dosis de 1200 mg/kg/día durante 4 semanas. Los animales del grupo IV recibieron (PGB + carvacrol). Los testículos fueron extraídos, pesados y muestreados para análisis bioquímicos, inmunohistoquímicos e histopatológicos al final del experimento. Para analizar el semen, también se extirparon los epidídimos. La administración de PGB a una dosis de 1200 mg/kg elevó los niveles de FSH y LH, a la vez que disminuyó los niveles séricos de testosterona. Además, provocó un aumento de las anomalías espermáticas y una disminución de la motilidad, el recuento y el porcentaje de viabilidad espermáticos. Asimismo, se observó una disminución de GSH, GPx y SOD, así como un aumento de MDA, lo que sugiere daño oxidativo testicular. La histología testicular reveló alteraciones morfológicas. La administración concomitante de carvacrol logró contrarrestar los efectos negativos de la PGB. Esto se observó en los niveles revisados de las medidas mencionadas, que fueron casi normales en comparación con los grupos de PGB y control. El carvacrol mejoró el daño testicular inducido por PGB, según los resultados bioquímicos, histológicos e inmunohistoquímicos del presente estudio.

PALABRAS CLAVE: Pregabalina; Lesión testicular; Estrés oxidativo; Testosterona; Parámetros espermáticos; Carvacrol.

REFERENCES

Ahmad, A.; Saeed, M. & Ansari, I. A. Molecular insights on chemopreventive and anticancer potential of carvacrol: implications from solid carcinomas. *J. Food Biochem., 45(12)*:e14010, 2021.

Ahmida, M. H. S. Protective role of curcumin in nephrotoxic oxidative damage induced by vancomycin in rats. *Exp. Toxicol. Pathol., 64(3)*:149-53, 2012.

Aksu, E. H.; Kandemir, F. M.; Altun, S.; Küçükler, S.; Çomaklı, S. & Ömür, A. D. Ameliorative effect of carvacrol on cisplatin-induced reproductive damage in male rats. *J. Biochem. Mol. Toxicol., 30(10)*:513-20, 2016.

Al-Zubaidi, A. M.; Al-Salihi, A. R. & Al-Dujaily, S. S. The effect of pregabalin (Lyrica) on the spermatogenic cells in rat. *Int. J. Embryo Infertil. Res., 5(1)*:9-13, 2015.

Araghi, A.; Tabari, M. A. & Golshahi, H. Protective effect of carvacrol on ketamine-induced testicular damage in mouse model of schizophrenia. *J. Herbmed Pharmacol., 6(3)*:100-6, 2017.

Aristatile, B.; Al-Numair, K. S.; Al-Assaf, A. H.; Veeramani, C. & Pugalendi, K. V. Protective effect of carvacrol on oxidative stress and cellular DNA damage induced by UVB irradiation in human peripheral lymphocytes. *J. Biochem. Mol. Toxicol., 29(11)*:497-507, 2015.

Asadi-Pooya, A. A.; Nikseresht, A. R. & Yaghoubi, E. Old remedies for epilepsy: Avicenna's medicine. *Iran. Red Crescent Med. J., 14(3)*:174-7, 2012.

Badawi, M. S. A study on the effect of different doses of pregabalin on the testis in adult male albino rat. *Int. J. Sci. Dev. Res., 8(12)*:860-7, 2023.

Bagheri, R. B.; Shadman, J. & Khaki, A. A. The effect of carvacrol plus treadmill exercise on testis structure and oxidative stress in streptozotocin-induced diabetic rats. *Crescent J. Med. Biol. Sci., 10(3)*:144-50, 2023.

Baidya, D. K.; Agarwal, A.; Khanna, P. & Arora, M. K. Pregabalin in acute and chronic pain. *J. Anaesthesiol. Clin. Pharmacol., 27(3)*:307-14, 2011.

Baird, C. R. W.; Fox, P. & Colvin, L. A. Gabapentinoid abuse in order to potentiate the effect of methadone: a survey among substance misusers. *Eur. Addict. Res., 20(3)*:115-8, 2014.

Baldwin, D. S.; Ajel, K.; Masdrakis, V. G.; Nowak, M. & Rafiq, R. Pregabalin for the treatment of generalized anxiety disorder: an update. *Neuropsychiatr. Dis. Treat., 9*:883-92, 2013.

Bancroft, J. D. & Layton, C. X. *The Hematoxylin and Eosin*. In: Bancroft, J. D. & Layton, C. X. (Eds.). Theory and Practice of Histological Techniques. 7th ed. London, Churchill Livingstone, , 2014. pp.173-86.

Bostanian, S.; Shariati, M. & Zamanpoor, M. Effect of pregabalin on pituitary-gonad axis and testis histological changes in adult rat. *J. Appl. Environ. Biol. Sci., 6(3)*:165-71, 2016.

Bucur, M. & Jeczmien, P. Pregabalin and libido - Case reports. *Open Neuropsychopharmacology J., 4*:8-9, 2011.

Cai, K.; Nanga, R. P.; Lamprou, L.; Schinstine, C.; Elliott, M.; Hariharan, H.; Reddy, R. & Epperson, C. N. The impact of gabapentin administration on brain GABA and glutamate concentrations: a 7T ∂H-MRS study. *Neuropsychopharmacology, 37(13)*:2764-71, 2012.

Calabrò, R. S. & Bramanti, P. Pregabalin-induced severe delayed ejaculation. *Epilepsy Behav., 19(3)*:543, 2011.

Daggulli, M.; Dede, O.; Utangac, M. M.; Bodakci, M. N.; Hatipoglu, N. K.; Penbegul, N.; Sancaktutar, A. A.; Bozkurt, Y.; Türkçü, G. & Yüksel, H. Protective effects of carvacrol against methotrexate-induced testicular toxicity in rats. *Int. J. Clin. Exp. Med., 7(12)*:5511-6, 2014.

Deckers, C. L.; Czuczwar, S. J.; Hekster, Y. A.; Keyser, A.; Kubova, H.; Meinardi, H; Patsalos, P. N.; Renier, W. O. & Van Rijn, C. M. Selection of antiepileptic drug polytherapy based on mechanism of action: the evidence reviewed. *Epilepsia, 41(11)*:1364-74, 2000.

Ding, J.; Shang, X.; Zhang, Z.; Jing, H.; Shao, J.; Fei, Q.; Rayburn, E. R. & Li, H. FDA-approved medications that impair human spermatogenesis. *Oncotarget, 8(6)*:10714-35, 2016.

Etemad, L.; Mohammad, A.; Mohammadpour, A. H.; Mashhadi, N. V. & Moallem, S. A. Teratogenic effects of pregabalin in mice. *Iran. J. Basic Med. Sci., 16(10)*:1065,2013.

Evazalipour, M.; Moayedi, S.; Safarzadeh Kozani, P. & Zamani, E. The protective effects of carvacrol on diphenhydramine-induced genotoxicity in human peripheral blood lymphocytes. *Res. J. Pharmacogn., 8(1)*:19-27, 2021.

Evoy, K. E.; Morrison, M. D. & Saklad, S. R. Abuse and misuse of pregabalin and gabapentin. *Drugs, 77(4)*:403-26, 2017.

Foroutan, N. & Nikvarz, N. Role of pregabalin in management of pruritus: a literature review. *J. Pharm. Pharm. Sci., 19(4)*:465-74, 2016.

Ghorani, V.; Alavinezhad, A.; Rajabi, O.; Mohammadpour, A. H. & Boskabady, M. H. Safety and tolerability of carvacrol in healthy subjects: a phase I clinical study. *Drug Chem. Toxicol., 44(2)*:177-89, 2021.

Griswold, M. D. Spermatogenesis: the commitment to meiosis. *Physiol. Rev., 96(1)*:1-17, 2016.

Guidi, L. & Landi, M. *Aromatic Plants: Use and Nutraceutical Properties.* In: Gurib-Fakim, A. (Ed.). Novel Plant Bioresources: Applications in Food, Medicine and Cosmetics. Hoboken, Wiley-Blackwell, 2014. pp.303-45.

Guimarães, A. G.; Oliveira, G. F.; Melo, M. S.; Cavalcanti, S. C.; Antoniolli, A. R.; Bonjardim, L. R.; Silva, F. A.; Santos, J. P.; Rocha, R. F.; Moreira, J. C.; et al. Bioassay-guided evaluation of antioxidant and antinociceptive activities of carvacrol. *Basic Clin. Pharmacol. Toxicol., 107(6)*:949-57, 2010.

Gunes-Bayir, A.; Kiziltan, H. S. & Kocyigit, A. *Plant Family, Carvacrol, and Putative Protection in Gastric Cancer*. In: Watson, R. R. & Preedy, V. R. (Eds.). Dietary Interventions in Gastrointestinal Diseases. Cambridge (MA), Academic Press, 2019. pp.3-18.

Hareedy, M. S.; Tawfik, K. M.; Badary, D. M.; Mahmoud, W. A. & Mohamed, E. E. M. Pregabalin administration and withdrawal affect testicular structure and functions in rats. *Andrologia, 52(11)*:e13808, 2020.

Ibrahim, D. E.; Lebda, M. A.; Hashem, A. E. & Elfeky, M. S. The antioxidative effect of carvacrol on methotrexate-induced testicular damage in rats. *Alex. J. Vet. Sci., 76(2)*:614, 2023.

Kamel, D. & Khalifa, H. A. Alpha 2-delta (a2-d) ligand pregabalin could adversely affect male fertility. *Int. J. Pharm. Sci., 5(5)*:1226-9, 2015.

Liliana, M. T.; Lacramioara, O.; Elena, L. C.; Andra Sabina, N. V.; Gabriela, R. & Gratiela, P. The effects of pregabalin on psycho-motor abilities and cognitive processes in mice. *Rev. Med. Chir. Soc. Med. Nat. Iasi, 119(1)*:185-92, 2015.

Loftus, H. & Wright, A. Potential misuse of pregabalin and gabapentin. *BMJ, 348*:g1290, 2014.

Martinotti, G.; Lupi, M.; Sarchione, F.; Santacroce, R.; Salone, A.; De Berardis, D.; Serroni, N.; Cavuto, M.; Signorelli, M.; Aguglia, E.; et al. The potential of pregabalin in neurology, psychiatry and addiction: a qualitative overview. *Curr. Pharm. Des., 19(35)*:6367-74, 2013.

Ohkawa, H.; Ohishi, N. & Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem., 95(2)*:351-8, 1979.

Oyedeji, K. O.; Bolarinwa, A. F. & Adigun, A. K. Effect of aspirin on reproductive functions in male albino rats. *IOSR J. Pharm. Biol. Sci., 4(6)*:49-54, 2013.

Ozturk, H.; Cetinkaya, A.; Duzcu, S. E.; Tekce, B. K. & Ozturk, H. Carvacrol attenuates histopathogic and functional impairments induced by bilateral renal ischemia/reperfusion in rats. *Biomed. Pharmacother., 98*:656-61, 2018.

Paglia, D. E. & Valentine, W. N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med., 70(1)*:158-69, 1967.

Shoorei, H.; Khaki, A.; Khaki, A. A.; Hemmati, A. A.; Moghimian, M. & Shokoohi, M. The ameliorative effect of carvacrol on oxidative stress and germ cell apoptosis in testicular tissue of adult diabetic rats. *Biomed. Pharmacother., 111*:568-78, 2019.

Sikka, S. C.; Chen, C.; Almas, M.; Dula, E.; Knapp, L. E. & Hellstrom, W. J. Pregabalin does not affect sperm production in healthy volunteers: a randomized, double-blind, placebo-controlled, noninferiority study. *Pain Pract., 15(2)*:150-8, 2015.

Silva, E. R.; de Carvalho, F. O.; Teixeira, L. G.; Santos, N. G. L.; Felipe, F. A.; Santana, H. S. R.; Shanmugam, S.; Quintans Júnior, L. J.; de Souza Araújo, A. A. & Nunes, P. S. Pharmacological effects of carvacrol in in vitro studies: a review. *Curr. Pharm. Des., 24(29)*:3454-65, 2018.

Singh, L.; Pushker, N.; Saini, N.; Sen, S.; Sharma, A.; Bakhshi, S.; Chawla, B. & Kashyap, S. Expression of pro-apoptotic Bax and anti-apoptotic Bcl-2 proteins in human retinoblastoma. *Clin. Exp. Ophthalmol., 43(3)*:259-67, 2015.

Soltani, M.; Moghimian, M.; Eivari, S. H. A.; Shoorei, H.; Khaki, A. & Shokoohi, M. Protective effects of Matricaria chamomilla extract on torsion/detorsion-induced tissue damage and oxidative stress in adult rat testis. *Int. J. Fertil. Steril., 12(3)*:242-8, 2018.

Sun, Y.; Oberley, L. W. & Li, Y. A simple method for clinical assay of superoxide dismutase. *Clin. Chem., 34(3)*:497-500, 1988.

Suntres, Z. E.; Coccimiglio, J. & Alipour, M. The bioactivity and toxicological actions of carvacrol. *Crit. Rev. Food Sci. Nutr., 55(3)*:304-18, 2015.

Corresponding author:
Dr. Marwa Sayed Badawi
Anatomy Department
Faculty of Medicine
Northern Border University
4894 Iyath Al Antaki
Al Musaidiyah
Arar
Northern borders
SAUDI ARABIA

E-mail: Yousef_yomna@yahoo.com