Biomedicine & Pharmacotherapy 110 (2019) 409–419

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Effect of antiepileptic drug (Topiramate) and cold pressed ginger oil on testicular genes expression, sexual hormones and histopathological alterations in mice

Aida I. El Makawy[a], Faten M. Ibrahim[b], Dalia M. Mabrouk[a], Kawkab A. Ahmed[c], Mohamed Fawzy Ramadan[d,*]

[a] National Research Center, Cell Biology Dept., 33 El Bohouth St., Dokki, Giza, P.O. 12622, Egypt
[b] National Research Centre, Medicinal and Aromatic Plants Research Dept., 33 El Bohouth St., Dokki, Giza, P.O. 12622, Egypt
[c] Pathology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[d] Agricultural Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig, 44519, Egypt

ARTICLE INFO

Keywords:
Antiepileptic
Cold pressed oils
Antioxidants
Histopathology
Immunohistochemistry

ABSTRACT

Sexual dysfunction in the epileptic patient is difficult to confirm whether it is ailment or therapy related. Antiepileptic drugs often use in reproductive age, through reproductive progress and maturation. On the other side, cold-pressed oils are rich in bioactive phytochemicals with health-promoting traits. The target of this work was to appraise the sexual dysfunction of antiepileptic Topiramate (**TPM**) and cold pressed ginger oil (**CPGO**) as antiepileptic alternative medicine in male mice. Fifty-four adult male albino mice were divided into nine groups (n = 6 mice). One group given saline and used as negative control; another one was given corn oil as vehicle. Six groups administered orally with TPM or CPGO at 100, 200 and 400 mg/kg. Moreover, group of animals co-administrated orally CPGO with TPM (400 mg/kg) to study their interaction. Fatty acid profile and tocols composition of CPGO were determined. *in vitro* assays were undertaken to evaluate radical scavenging traits of CPGO utilizing sable 1,1-diphenyl-2-picrylhydrazyl (DPPH·) and galvinoxyl radicals. The study investigated antioxidant and oxidative stress markers, sexual hormones levels, mRNA levels of vascular endothelial growth factor (Vegfa), synaptonemal complex protein (Sycp3), Wilms tumor gene (Wt1) as well as histopathological and immunohistochemical examination. Strong radical scavenging potential of CPGO against stable DPPH· and galvinoxyl radicals was recorded. The results revealed that TPM caused a dose-dependent reduction in the antioxidant activities and testosterone content, while, malonaldehyde (MDA) and nitric oxide (NO) as oxidative stress markers were elevated. Vegfa and Sycp3 mRNA expression down-regulated at all Topiramate tested doses, but Wt1 up-regulated at 400 mg/kg. TPM (400 mg/kg) revealed histological alterations associated with strong positive Bax immune reactive spermatogoneal and Leydig cells. Ginger oil elevated the CAT and SOD (antioxidant enzymes), serum testosterone and diminished the oxidative stress, up regulated the expression of Vegfa and Sycp3 and down-regulated the Wt1 expression. Meanwhile, CPGO revealed no histopathological alterations and no Bax immune-reactive cells. CPGO co-administration with TPM (400 mg/kg) attenuated the TPM toxicity. High doses of TPM may exhibit sexual dysfunction but CPGO is safe and has androgenic property. CPGO co-administration could protect the antiepileptic patient from the TPM sexual dysfunction.

1. Introduction

Epilepsy is a central nervous system disorder characterized by a repeated transient attack of disturbed brain function that results in convulsive and seizure episodes [1]. Reproductive toxicity occurs in epilepsy patients due to the drugs used in treatment, as well as the disease itself. It is recommended that seizures and antiepileptic drugs alter hormone levels and cause reproductive dysfunction by affecting the hypothalamic-pituitary-gonadal axis. Epilepsy affects approximately 50 million people worldwide [2]. Antiepileptic drugs (AEDs) are a diverse group of medications that progressively used for disorders other than epilepsy, such as migraine prophylaxis, and neuropathic

* Corresponding author.
E-mail addresses: aelmakawy@yahoo.com (A.I. El Makawy), fatenmibrahim@gmail.com (F.M. Ibrahim), dalia.mamdouh@ymail.com (D.M. Mabrouk), kawkababdelaziz@yahoo.com (K.A. Ahmed), hassanienmohamed@yahoo.com (M. Fawzy Ramadan).

https://doi.org/10.1016/j.biopha.2018.11.146
Received 22 October 2018; Received in revised form 28 November 2018; Accepted 28 November 2018

pain [3]. Antiepileptic drug adverse effects stay a leading reason for treatment failure and the main determinant of an impaired health-related feature of epileptic people life. Adverse effects could develop acutely after beginning handling and could affect the body organ. Several efforts carried out to minimize the onus of AEDs toxicity [4].

Topiramate **(TPM)** is a psychotropic drug that used as an antiepileptic drug and now used for many indications, including migraine prophylaxis [5]. TPM is synthesized from D-fructose and contains a sulfamate moiety that is crucial for its pharmacological action [6]. Tauboll et al. [7] showed that many AEDs affect reproductive function. The TPM side effects for pregnant women are a permanent concern, since epileptic women often require continuing use of the drug during pregnancy for seizure control [8]. Teratogenic impacts and hazard of inborn malformations promoted by TPM are reported. Literature confirmed that TPM causes skeletal anomalies [9], pathological neurotoxicity in the cerebral cortex [10], birth weight decrease and increase in spontaneous abortion rate [11].

Alternative medicine plays a vital function in the epilepsy cure and it is a centuries-old practiced medical type in various cultures [12]. The herbal medical mores are time-honored and based on complicated medical theories undergo long-term repetitive confirmation. Herbal medications are the most customary approach to alternative medications, which participate in the therapy to control AEDs complications [13].

Alternative medicine plays a vital function in the epilepsy cure and it is a centuries-old practiced medical type in various cultures [12]. The herbal medical mores are time-honored and based on complicated medical theories undergo long-term repetitive confirmation. Herbal medications are now the most customary approach to alternative medications, which participate in the therapy to control AEDs complications [13].

Ginger, the rhizome of Zingiber officinale Roscoe (family, Zingiberaceae), is a crop widely used as a dietary supplement with human health benefits [14]. The interest in ginger is endorsed to its several biologically active compounds content such as gingerol, shogaols, gingerdiol, gingerdione, α-zingiberene, curcumin, and β-sesquiphellandrene [15]. *Zingiber officinale* used as a condiment in several countries but also it acts as a treatment for ailments; for instance, gastrointestinal disorders, colds, arthritis, hypertension and migraines [16–18]. Ginger was found to be effective in antioxidant, anti-inflammatory and antimicrobial activities [19–21]. Maghbooli et al. [22] confirmed the efficiency of ginger powder in the therapy of common migraine attacks and its similarity to the antiepileptic drug. The bulk of the studies highlight ginger antioxidant activity due to their ability to inhibit the oxidation of free radicals [20,23,24], were associated to the prevention of many diseases [25–27].

It is therefore very imperative to find out the testicular effects of TPM ingestion and CPGO as alternative medicine as well as their interaction. Thus, the objective of in-progress study was to estimate the impact of TPM and CPGO in male mice. The study was conducted using the expression pattern of some testicular genes, biochemical assessment of testicular antioxidant enzymes, oxidative stress markers and sexual hormones. In addition, histological and immunohistochemical studies were carried out to check the changes in mice testes.

2. Materials and methods

2.1. Chemicals

Topiramate tablet (100 mg) was purchased from Kahira pharmaceutical company (Cairo, Egypt). Each tablet contains 100 mg of TPM. CPGO was purchased from EL-Captain Company, Egypt. ELISA kits for testosterone, follicle stimulating hormone (FSH) and Leutinizing hormone (LH) were purchased from Sunlong Biotech Co (Zhejiang, China). Antioxidant parameters kits were purchased from Bio diagnostic (Egypt). All chemicals and reagents used were of analytical grade.

2.2. Gas chromatography (GC) of fatty acids methyl esters (FAME) in CPGO

Fatty acids in CPGO were transesterified to FAME by N-trimethylsulfoniumhydroxide (Macherey-Nagel, Germany) according to Arens et al. [28]. Shimadzu GC-14 A equipped with flame ionization detector (FID) and C-R4 AX chromatopac integrator (Kyoto, Japan) was used. The flow rate of the carrier gas helium was 0.6 mL/min and the split value with a ratio of 1:40. A sample of 1 µL was injected on a 30 m x 0.25 mm x 0.2 µm film thickness Supelco SPTM-2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperature was set at 250 °C. The initial column temperature was 100 °C programmed by 5 °C/min until 175 °C and kept 10 min at 175 °C, then 8 °C/min until 220 °C and kept 10 min at 220 °C. A comparison between the retention times of the samples with those of an authentic standard mixture(Sigma, St. Louis, MO, USA; 99% purity specific for GC), run on the same column under the same conditions, was made to facilitate identification.

2.3. High-performance liquid chromatography (HPLC) of tocols in CPGO

A solution of 250 mg of CPGO in 25 mL *n*-heptane was directly used for the HPLC [29]. The HPLC analysis was conducted using a Merck Hitachi low-pressure gradient system, fitted with an L-6000 pump, a Merck-Hitachi F-1000 Fluorescence Spectrophotometer (The detector wavelength was set at 295 nm for excitation, and at 330 nm for emission) and a D-2500 integration system; 20 µL of the samples were injected by a Merck 655-A40 Autosampler onto a Diol phase HPLC column 25 cm 94.6 mm ID (Merck, Darmstadt, Germany) using a flow rate of 1.3 mL/min. The mobile phase used was *n*-heptane/*tert*-butyl methyl ether (99:1, v/v).

2.4. Radical scavenging activity (RSA) of CPGO toward DPPH

RSA of CPGO was assayed with $DPPH^{\cdot}$ radical dissolved in toluene according to [30]. Toluene solution of $DPPH^{\cdot}$ radicals was prepared at a concentration of 10^{-4} M. Ten mg of CPO (in 100 µL toluene) was mixed with 390 µL toluene solution of $DPPH^{\cdot}$ radicals, and vortexed for 20 s at ambient temperature. Against a blank of toluene (without $DPPH^{\cdot}$), the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 1, 30, and 60 min of mixing using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward $DPPH^{\cdot}$ radicals was estimated from the differences in absorbance of the $DPPH^{\cdot}$ solution with or without sample (control) and the inhibition percent was calculated from the following equation:

$$\% \text{ Inhibition} = [(\text{absorbance of control} - \text{absorbance of test sample})/\text{absorbance of control}] \times 100.$$

2.5. Radical scavenging activity of CPGO toward galvinoxyl radical

A miniscope MS 100 ESR spectrometer (Magnettech GmbH; Berlin, Germany) was used according to [30]. Conditions of the experiment were: microwave power, 6 db; measurement at room temperature; centerfield, 3397 G, receiver gain 10, sweep width 83 G, and modulation amplitude 2000 mG. Ten mg of CPGO (in 100 µL toluene) was reacted with 100 µL of a toluene solution of galvioxyl (0.125 mM). The mixture was stirred for 20 s then transferred into a 50 µL micro-pipette. The amount of galvinoxyl radical inhibited was measured after 60 min of the addition of the galvinoxyl radical solution. Signal intensities were evaluated by the peak height of galvinoxyl signals against a control. Estimation of the radical concentration was calculated by evaluating the decrease of the ESR signals in arbitrary units after 60 min incubation using the Kinetik Show 1.06 Software program (Magnettech GmbH, Germany). Reproducibility was *ca.* 5% as usual for kinetic parameters.

2.6. Animals

Adult male Swiss albino mice (5–6 weeks of age and 20–25 g weight) used in this experiment, were obtained from the animal breeding house of the National Research Centre (Dokki, Egypt). The animals were maintained at a constant temperature (22 ± 2 °C) on a 12 h light/dark cycle with free access to food and water. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Centre and the National Institutes of Health (NIH publication 86-23 revised 1985). The Ethics Committee of the National Research Centre (Approval No. 18039) and Institutional Animal Care and Use Committee, Cairo University (CU-IACUC) (Approval No. CU/II/F/51/18), approved the research

2.7. Experimental design

Fifty-four adult male albino mice were divided into nine groups (n = 6 mice). Group 1 of animals was given saline solution (0.25 mg/mice/day) via oral gavages and used as negative control. Group 2 were given corn oil (0.25 mg/mice/day) and used as a vehicle for CPGO. Groups 3, 4 and 5 were orally gavaged by TPM doses (100, 200 and 400 mg/kg/mice/day) for four weeks. Groups 6, 7 and 8 were orally gavaged by CPGO at the same doses and period of administration, which selected according to Rashidian et al. [31]. Group 9 were administrated orally with the high dose of both TPM and CPGO (400 mg/kg/mice/day) for the same period.

2.8. Biochemical analysis

2.8.1. Blood samples

At the end of the experimental period, the animals were lightly anesthetized for blood sample collection from the orbital sinus in clean dry tubes and left to clot, then centrifuged at 4000 rpm for 10 min at 4 °C (Sigma Laborzentifugen, 2K15, Germany, GmbH) to separate sera. Sera aliquots were frozen at −80 °C for analysis of sexual hormones.

2.8.2. Testes tissue collection

The animals of each treated group were sacrificed by neck vertebra luxation. Testes tissues were isolated and washed with ice-cold saline to carry out the gene expression, biochemical analysis and histopathology studies. The right testis from each animal was collected and kept frozen at −80 °C for gene expression analysis. One g of testes were homogenized in 9 volumes of phosphate buffer saline then centrifuged at 4000 rpm for 15 min. The supernatant was separated off and used for assessment of antioxidant enzymes and oxidative stress biomarkers.

2.8.3. Testicular antioxidant enzymes, oxidative stress markers and sexual hormones

Protein concentration was measured as described by Sedlack and Lindsay [32]. Superoxide dismutase (SOD) was measured at 560 nm [33] as the reduction suppression rate of nitrotetrazolium blue and for 1 unit of activity. The amount of protein was taken which provided 50% inhibition of nitrotetrazolium blue reduction under standard conditions. Catalase (CAT) activity was measured spectrophotometrically at room temperature by monitoring the decrease in absorbance at 240 nm resulting from the decomposition of H_2O_2 according to the method of Aebi [34]. Malondialdehyde levels in testes tissue were assayed using the thiobarbituric acid reaction method [35]. Quantification of the thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to the standard curve of MDA equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. Nitric oxide (NO) concentration was determined using an indirect method based on the measurement of nitrite concentration in testes tissue according to Montgomery and Dymock [36]. Follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels were determined using competitive immunoassay technique [37].

2.9. Testicular genes expression analysis

Total RNA was isolated from the right testis of experimental animals using Easy red total RNA extraction kit (Intronbio, Korea) according to the manufacturer's instructions. Isolated RNA was stored at −80 °C. The purity and quality were measured with a NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific, USA). One μg of RNA was treated with DNase kit (Thermo Fisher Scientific, USA) then cDNA was synthesized using a RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The obtained cDNA was stored at −20 °C. cDNA (1 μL)was mixed with 1 μL of 10 pM/μL forward primer, 1 μL of 10 pM/μL reverse primer, 2 μL dNTPs, 0.5U Go Taq Polymerase (Promega, USA), 5 μL 10X buffer and 14.8 μL H_2O. PCR conditions were 95 °C for 2 min, 35 cycles of 95 °C for 15 s, 63 °C for 15 s, and 72 °C for 15 s. RT-PCR reactions were performed in triplicate and glyceraldehyde-3-phosphate dehydrogenase (GADPH) used as an internal standard of mRNA expression. Primers used are shown in Table 1. An aliquot of the RT-PCR products (7 mL) was separated on a 1.2% ethidium bromide stained agarose gel and visualized under UV light. The density of each PCR product was measured using Quantity One software. The ratio between the levels of the target gene amplification product was calculated by normalizing against GAPDH.

2.10. Histopathological studies

At the end of the experimental period, specimens from testes of all animals were collected and fixed in formalin buffered saline (10%), washed, dehydrated, cleared and embedded in paraffin. Paraffin blocks were sectioned at 4–5 micron thickness and stained with Haematoxylin and Eosin for histopathological examination [38]. Ten microscopic fields per section/mice (n = 6) were examined by a light microscope (Olympus BX50, Japan) under high power magnification (x 400). Spermatogenesis was measured on histologic sections using modified Johnsen's score. This method applies a score from 1 (no seminiferous epithelium) to 10 (full spermatogenesis recorded) for each seminiferous tubule cross-section [39]. The germinal epithelium of at least 50 tubules was assessed for each testis and the mean Johnsen's score per mouse was calculated. An experienced investigator blinded to treatments performed the histologic evaluation.

Table 1
Primers used for RT-PCR.

Gene	Accession no.	Nucleotide sequence 5′–3′	Size of PCR product (bp)
Sycp3	NM_011517.2	GACAGCGACAGCTCACCGG GGTGGCTTCCCAGATTTCCCAGA	90
Vegfa	NM_001025257.3	TGCTCTCTTGGGTGCACTGGAC GACGGCAGTAGCTTCGCTGGT	147
Wtl	NM_144783.2	GGCGCTTTGAGGGGTCCGAC AAAGTGGGCGGAGCACCGAC	205
GAPDH	NT_166349.1	CAAGGTCATCCATGACAACTTTG GTCCACCACCCTGTTGCTGTAG	469

2.11. Immunohistochemical staining and evaluation of apoptosis through Bax expression

Bax protein products were detected by specific monoclonal antibodies. From each testes block, 4-pm-thick sections were cut for immunohistochemical (Bax) staining. Bax expression level in testes was examined according to Martín-Burriel et al. [40]. Sections were incubated with primary antibodies against Bax (1:100 dilution) (Santa Cruz Biotechnology Inc., Dallas, TX, USA). The immune reaction was visualized using diaminobenzidine tetrachloride (DAB, Sigma, St. Louis, MO, USA). The positive immune reactive cells showed brown-stained cytoplasm. Staining intensity and its distribution were graded as negative (no staining), weak, moderate, or strong intensity. Quantification of Bax apoptotic protein was estimated by measuring the area % expression from 5 randomly chosen fields in each section and averaged using image analysis software (Image J, version 1.46a, NIH, Bethesda, MD, USA).

2.12. Statistical analysis

The results were expressed as mean ± SE of different groups. The differences between the mean values were evaluated by one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS version 16). Duncan's Post-Hoc test was performed to determine significant differences between groups. Correlations were done to test for linear relations between variables using Pearson correlation test. Values of $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Fatty acids composition, tocols profile and antiradical traits of CPGO

Ginger, a medicinal herb rich in bioactive components, is widely used. This study reports for the first time on the bioactive components in CPGO. Fig. 1**A** presents the relative percentages of fatty acids in CPGO. Linoleic and oleic acids were the major fatty acids accounting for almost 81% of total identified FAME, wherein palmitic and stearic were the major saturated fatty acids **(SFA)**, accounting for almost 14% of total identified FAME. The amounts of C16:0, C18:0, C18:1*n*-9, C18:2 and C18:3 were 8.81%, 5.40%, 39.5%, 42.6%, 0.45%, respectively. CPGO contained high amounts of monounsaturated fatty acids (**MUFA**, 41.0% of the total identified FAME) and polyunsaturated fatty acids **(PUFA**, 43.1% of the total identified FAME). A striking feature of CPGO was the high levels of PUFA and MUFA.

Fig. 1**B** presents the percentages of tocols in CPGO under study. In general, CPGO contained high levels of unsaponifiable (3.2 g/kg oil). The amounts of α-, γ- and *δ*-tocopherols were 3.87, 500.8 and 13.7 mg/kg oil, respectively. In our study β- tocopherol was not detected. Furthermore, the levels of α-, β-, γ- and *δ*-tocotrienols were 167.4, 9.11, 3.87 and 190.9 mg/kg oil, respectively. In CPGO, 56.2% of the total tocols amount was as γ-tocopherol followed by *δ*-tocotrienol (*ca.* 21.4%) and α-tocotrienol (*ca.* 18.8%). Other tocols were measured in lower amounts.

Antiradical traits of the CPGO were compared using stable DPPH· and galvinoxyl radicals. Fig. 1**C** showed that CPGO had high RSA against DPPH·. After 1 min, 30 min and 60 min of incubation with DPPH· radicals, 13%, 17% and 28% of DPPH· radicals were quenched by CPGO, respectively. ESR measurements showed the same pattern. After 1 min, 30 min and 60 min of incubation with galvinoxyl radicals, 9%, 14%, and 19% of galvinoxyl radicals were quenched by CPGO, respectively (Fig. 1C).

3.2. Testicular antioxidant enzymes and oxidative stress markers

The measured SOD and CAT activities as well as MDA and NO levels in the mice testes are illustrated in Table 2. SOD and CAT levels of TPM group showed a dose-dependent significant decrease ($p \leq 0.05$), while CPGO significantly elevated their levels in a dose-dependent manner. Co-administration of TPM and CPGO (400 mg/kg) attenuated the SOD and CAT approximately to control. However, TPM significantly elevated the MDA and NO in a dose-dependent pattern compared with control. Meanwhile, CPGO showed a dose-dependent decrease ($p \leq 0.05$) in their levels than control. As regards to TPM and CPGO co-administration, the MDA and NO levels were reduced significantly than those of TPM-treated animals (Table 2).

3.3. Sexual hormones analysis

The results of testosterone, FSH and LH are presented in Fig. 2. Testosterone data analysis revealed that TPM exhibited a significantly dose-dependent decrease in serum testosterone ($p \leq 0.05$) than control. CPGO bring about significantly dose-dependent increased ($p \leq 0.05$) in testosterone. In the interim, no significant differences were found in the serum FSH, LH levels of three-doses TPM-treated animals and control. However, CPGO (200 and 400 mg/kg) significantly raised the serum FSH and LH ($p \leq 0.05$) over the control. The co-administration of TPM and CPGO (400 mg/kg) significantly raised the testosterone level ($p \leq 0.05$) but the rise in the FSH and LH was not significant.

3.4. Gene expression analysis

Results showed that TPM decreased the mRNA expression level of germinal cell linked genes; Vegfa gene that expressed in spermatogonial cells and synaptonemal complex protein (Sycp3) gene that expressed in spermatocytes. Mainly, significantly downregulation of Vegfa gene expression was observed in all TPM treated groups, while, Sycp3 expression was down-regulated significantly in animals exposed to a dose of 200 and 400 mg/kg bw. In meantime, the expression change of Sycp3 in animals exposed to the dose of 100 mg/kg was not significant. Regarding to CPGO, data revealed that it up regulated the Vegfa gene expression. The upregulation was none significant in 100 and 200 mg/kg doses but significant in 400 mg/kg compared to control. Meanwhile, CPGO up regulated the Sycp3 gene expression in dose-dependent pattern as in Fig. 3. In the case of the Wt1 gene that expressed in Sertoli cells, results pointed out that TPM caused expression down-regulation in testes of 100 and 200 mg/kg groups and up-regulation in 400 mg/kg group than control. However, CPGO showed down-regulation in the Wt1 expression in animals of 200 and 400 mg/kg treated groups compare to TPM and control. While ginger oil 100 mg/kg showed non-significant change. Moreover, CPGO decreased the mRNA expression as compared to TPM. Regarding the interaction between TPM and CPGO 400 mg/kg, results revealed that ginger oil modulated the expression of three tested genes than in TPM group as shown in Fig. 3.

3.5. Histopathology

Results of modified Johnson scoring are shown in Table 3. According to the histological grading of spermatogenesis, there was a significant histopathologic decrease in Johnsen's score ($p \leq 0.05$) of the testes of 200 and 400 mg/kg TPM-treated groups with a mean values 7.50 ± 0.223 and 5.16 ± 0.477, respectively as compared to control group (mean value, 10.00 ± 0.00). On the other hand, the groups treated with three doses of CPGO and the group of CPGO and TPM showed normal score (10.00 ± 0.00) as control.

Moreover, microscopically, testes from the negative control group and corn oil vehicle group revealed the seminiferous tubules normal histological structure with normal spermatogonial cells and complete spermatogenesis with sperm production (Fig. 4A). Testes of TPM (100 mg/kg) group revealed no histopathological alterations (Fig. 4B). Conversely, mild histopathological changes were noticed in examined sections from 200 mg/kg group. These changes described as small diameter seminiferous tubules with spermatogonial cells in seminiferous tubules degeneration (Fig. 4C). Necrosis and desquamation of

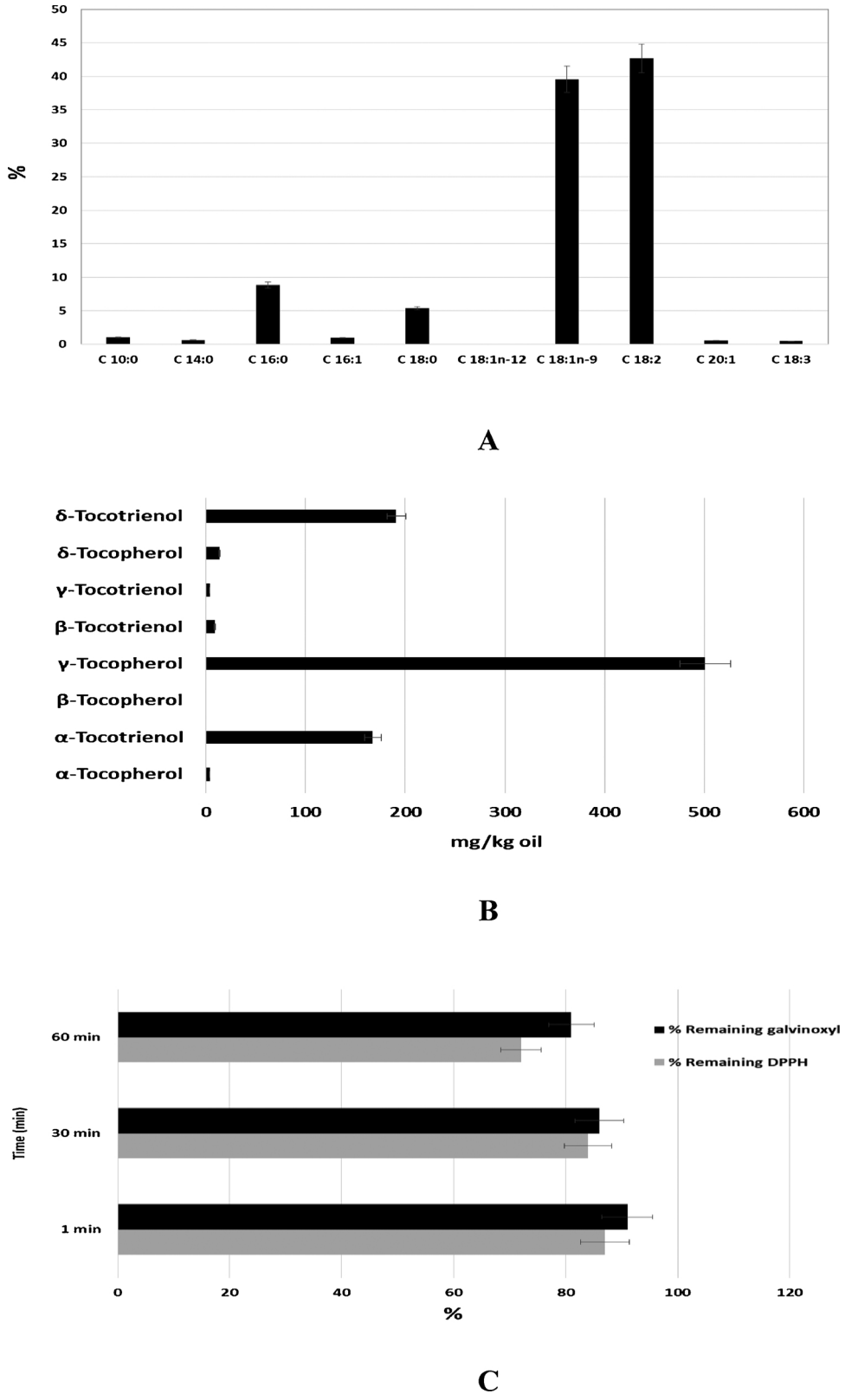


Fig. 1. (A) Fatty acids profile (%), (B) tocols composition (mg/kg oil), and (C) antiradical activities of CPGO. Error bars show the variations of determinations in terms of standard deviation.

spermatogonial cells lining seminiferous tubules were observed in most examined sections. Moreover, severe histopathological alterations were recorded in examined sections from mice administered a high dose of TPM (400 mg/kg). All examined sections from this group revealed atrophy of seminiferous tubules, marked degeneration of spermatogonial cells with formation of spermatid giant cells, interstitial edema and Leydig cells necrosis (Fig. 4D). Marked necrosis and the entire absence of germinal cells inside the seminiferous tubules was noticed in some

Table 2
Effect of TPM, CPGO and their interaction on antioxidant status in mice testes.

GR	Control		100 mg/kg		200 mg/kg		400 mg/kg		400 mg/kg TPM & CPGO
	-ve control	Corn oil	TPM	CPGO	TPM	CPGO	TPM	CPGO	
SOD (U/min/mg protein)	20.58 + 0.25^{d}	20.27 + 0.27^{d}	13.92 + 0.18^{f}	24.79 + 0.49^{c}	7.85 + 0.15^{g}	32.85 + 0.48^{b}	4.58 + 0.25^{h}	43.68 + 0.42^{a}	15.89 + 0.06^{e}
CAT (U/min/mg protein)	35.92 + 0.16^{d}	36.06 + 0.14^{d}	22.05 + 0.27^{f}	40.96 + 0.12^{c}	12.15 + 0.36^{g}	51.25 + 0.26^{b}	7.26 + 0.12^{h}	74.59 + 0.36^{a}	30.55 + 0.50^{e}
MDA (mmol/g tissue)	8.84 + 0.03^{e}	8.41 + 0.07^{f}	16.21 + 0.12^{c}	7.29 + 0.09^{g}	28.25 + 0.14^{b}	5.43 + 0.08^{h}	39.98 + 0.12^{a}	4.22 + 0.09^{i}	12.20 + 0.11^{d}
Nitric oxide (μmol/g tissue)	40.41 + .0.43^{e}	34.35 + 0.08^{f}	168.90 + 06^{c}	34.51 + 0.62^{f}	207.29 + 0.15^{b}	31.49 + 0.38^{g}	242.66 + 0.19^{a}	28.12 + 0.21^{h}	70.14 + 0.44^{d}

Results are expressed as the mean ± SEM.
Different superscripts within the same row designate significant differences ($p \leq 0.05$).
SOD = Superoxide dismutase activity, CAT = Catalase activity, MDA = Lipid peroxidation.

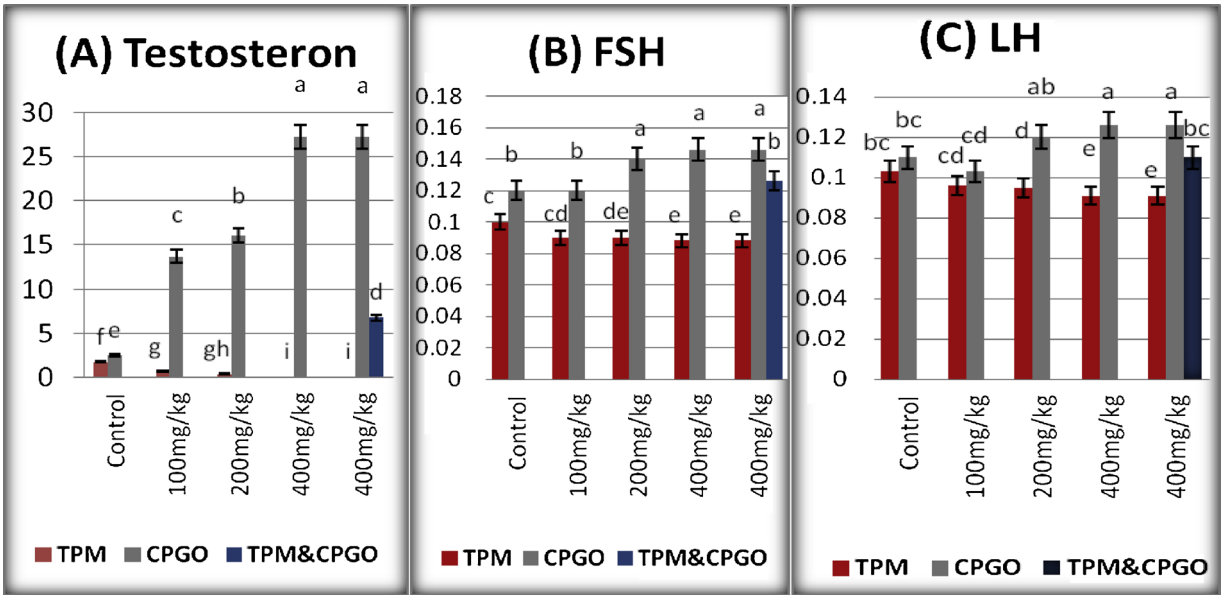


Fig. 2. Effect of TPM, CPGO and their interaction as antiepileptic drugs on (A) testosterone (ng/mL), (B) FSH, and (C) LH hormones (mIU/mL) in testes. Data shown as mean ± SEM; error bars show the variations of determinations in terms of standard deviation. One – way analysis of variance was used for data analysis (n = 6), mean values with unlike superscript letters were significantly different ($p \leq 0.05$).

examined sections (Fig. 4E). In contrary, testes of mice gavage CPGO (at three doses) revealed no histopathological alterations with complete spermatogenesis (Figs. 4F, G and H). Testes of co-administered orally with the high dose of TPM and CPGO group revealed normal diameter seminiferous tubules, normal spermatogonial cells and complete spermatogenesis with sperm production (Fig. 4I).

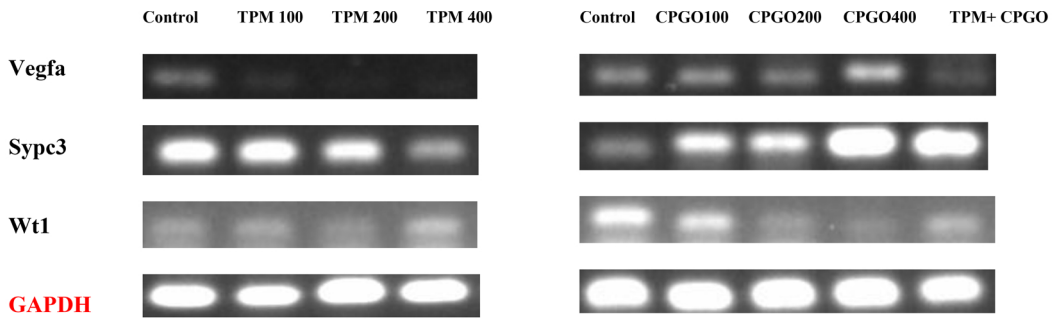
3.6. Immunohistochemistry

Different degrees of the apoptotic index in testes were recorded using Bax as an indicator. Immunohistochemistry revealed no Bax immune-reactive cells in the testes of control, corn oil vehicle and low-dose TPM (100 mg/kg bw) groups (Fig. 5A & B). Testes of TPM (200 mg/kg bw) group showed weak Bax immune-reactive spermatogonial cells (Fig. 5C). In contrary, the strong positive immune reaction of spermatogonealand Leydig cells was observed in mice administered 400 mg TPM (Fig. 5**D** & **E**). In contrast, examined sections from mice administered three doses of CPGO revealed no Bax immune-reactive cells (Fig. 5**F–H**). Moreover, testes of mice co-administered orally with the high dose of TPM and CPGO (400 mg) group revealed no Bax immune-reactive cells (**Fig. 5I**). Fig. 5J revealed the evaluation of immunostaining expression of Bax % (apoptotic indicator) in the mice from different experimental groups.

4. Discussion

The epileptic sexual dysfunction is often difficult to verify whether the dysfunction is ailment or treatment correlated. There is a scarcity of data on the incidence of sexual dysfunction between the population as a whole and among those handling AEDs [41]. Sexual dysfunction linked to AEDs is common. Case reports of gabapentin-induced sexual dysfunction suggest that the minimum total daily dose required for sexual dysfunction is 900 mg [42]. Gabapentin-induced total sexual dysfunction (loss of libido, anejaculation, anorgasmia, and impotence) at a total daily dose of only 300 mg [43]. Valproate may produce high incidences of decreased libido. Topiramate, pregabalin and gabapentin may cause sexual dysfunction [44]. Therefore, the target of the study was to inspect the testicular dysfunction of TPM, CPGO and their interaction.

Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants [45]. The present study indicated that TPM appears to cause an oxidative status alteration in testis tissue of mice. Growing evidence indicated that AEDs therapy could cause an increase in oxidant markers. Valproate (VPA) has been estimated in several studies. Sobaniec et al. [46] found elevations of MDA concentrations in patients treated with VPA. Karabiber et al. [47] reported that antiepileptic efficiency of VPA may work



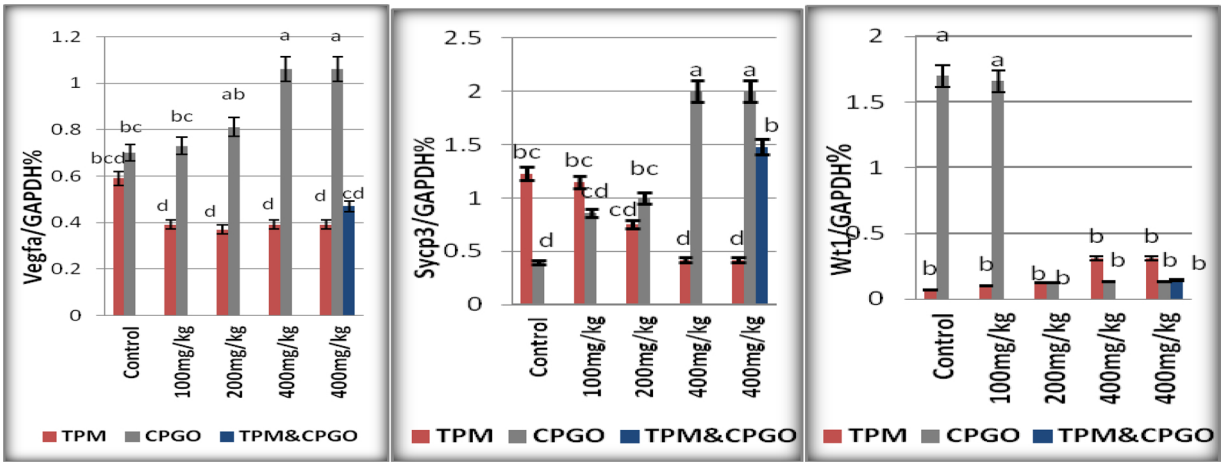


Fig. 3. Semi – quantitative PCR analysis of Vegfa, Sycp3 and Wt1 genes expressed in testis tissue. Data shown as mean ± SEM; error bars show the variations of determinations in terms of standard deviation. One – way analysis of variance was used for data analysis (n = 6), mean values with unlike superscript letters were significantly different ($p \leq 0.05$).

through its ability to release NO. Peker et al. [48] confirmed elevation in serum NO in children receiving VPA. The oxidative stress caused sperm abnormality proposed to be an important contributing factor in most cases of infertility [49]. Cold pressed ginger oil improved the oxidative status of testes cells, wherein it increased the SOD and CAT activities and minimized the MDA and NO. Ghasemzadeh et al. [50] reported that ginger contains high phenolics and flavonoids content, dependable for its high antioxidant traits. The antioxidant activity of ginger was due to its major bioactive compounds namely zingerone, zingiberene, gingerdiol, gingerols, and shogoals [51]. Herve et al. [52] discovered that ginger oil could be used in quails to diminish the reproductive cells lipid peroxidation and enhance fertility with no adverse effects on growth performances. The antioxidant trait of ginger has been endorsed to the protective actions of ginger bioactive substances against free radical attack [53]. Herve et al. [52] reported that the antioxidant action of ginger essential oil subsequently reduced the lipid peroxidation responsible for apoptosis in spermatogenic cells. The testicular histology in ginger oil treated quails revealed structural improvements compare to control birds that showed mild necrosis. Ghlissi et al. [54] indicated that dietary ginger decreased levels of MDA and testosterone, enhanced semen count and motility, and modulated the SOD, and CAT activities.

Sex hormones levels contribute to sexual dysfunction as well as other reproductive disorders and these levels could be changed due to the consumption of AEDs [55]. The male sexual hormones (FSH, LH, and testosterone) have a task in the maintenance of reproductive functions. It recognized that LH and FSH are outflows under the hypothalamic gonadotropin-releasing hormone control. LH stimulates the testosterone inflow from Leydig cells, and testosterone is required for the secondary sexual characteristics growth, spermatogenesis, and the storage of spermatozoa in the epididymis. FSH regulates the spermatozoa production in Sertoli cells [56]. It has been shown that

Table 3
The histological grading of spermatogenesis according to Johnsen's score.

GR	Control		100 mg/kg		200 mg/kg		400 mg/kg		400 mg/kg TPM & CPGO
	-ve control	Corn oil	TPM	CPGO	TPM	CPGO	TPM	CPGO	
Johnsen's score	$10.00^{a} \pm 0.00$	$10.00^{a} \pm 0.0$	$10.00^{a} \pm 0.00$	$10.00^{a} \pm 0.00$	$7.50^{b} \pm 0.223$	$10.00^{a} \pm 0.00$	$5.16^{c} \pm 0.477$	$10.00^{a} \pm 0.00$	10.00^{a} 0.00

Values are expressed as means ± SEM. n = 6 in each group.
Means carrying different superscripts (a, b, c) are significantly different at ($p \leq 0.05$).

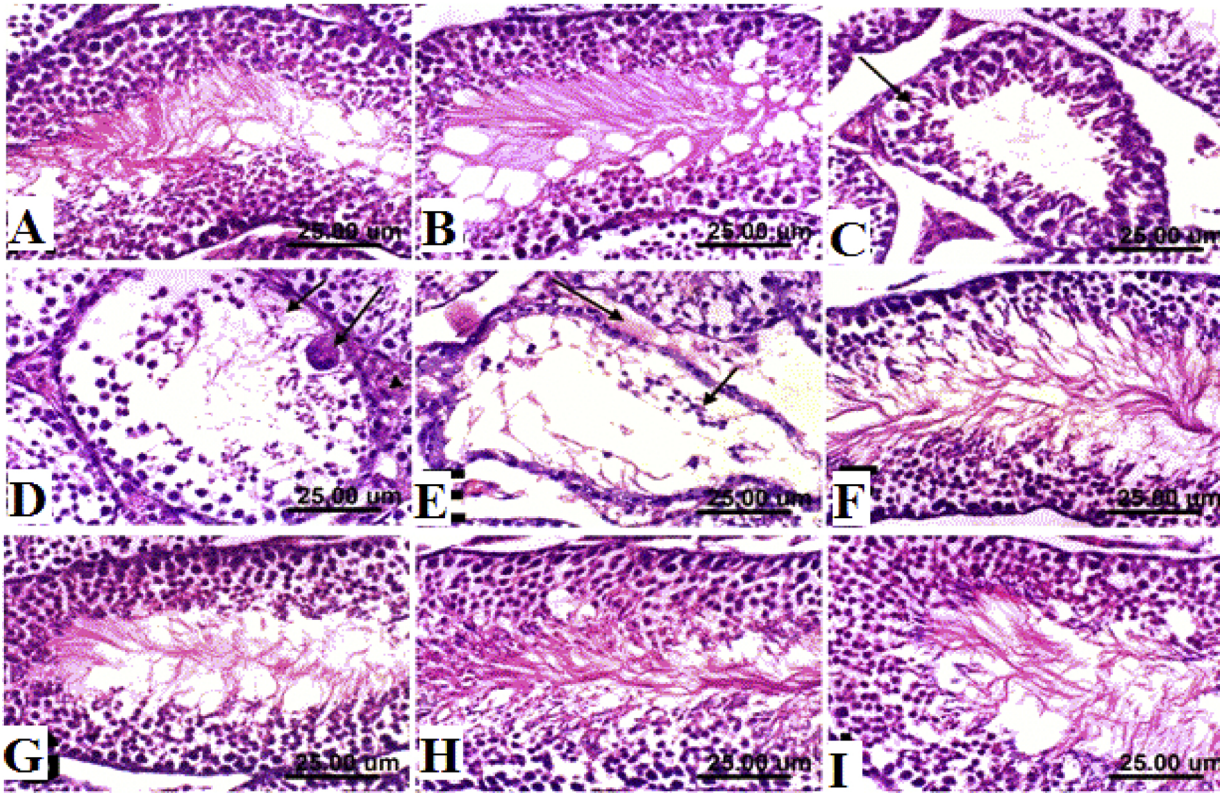


Fig. 4. Testis of mice (H & E scale bar 25 um), **A**) from control, untreated group showing the normal histological structure of seminiferous tubules with normal spermatogoneal cells and complete spermatogenesis with sperm production, **B**) treated with TPM (100 mg/kg b.w.) showing no histopathological alterations, **C**) treated with TPM (200 mg/kg b.w.) showing small diameter seminiferous tubules with degeneration of spermatogoneal cells lining seminiferous tubules (arrow), **D** & **E** treated with TPM (400 mg/kg b.w.), **D**) showing marked degeneration of spermatogoneal cells lining seminiferous tubules (short arrow) with formation of spermatid giant cells (long arrow), interstitial oedema and necrosis of leydig cells (arrow head), **E**) showing marked necrosis and complete absence of germinal cells lining seminiferous tubules (short arrow) and interstitial oedema (long arrow). **F**, **G** & **H** treated with CPGO (100 mg, 200 mg and 400 mg, respectively) showing no histopathological alterations. **I**) Co-treated with TPM 400 mg and CPGO 400 mg showing normal diameter seminiferous tubules, normal spermatogoneal cells and complete spermatogenesis with sperm production.

antiepileptic drugs influence hypothalamic-pituitary gonadal axis and cause reproductive dysfunction [57]. In our study, TPM decreased the testosterone with the dose-dependent pattern, while the decrease in FSH and LH was non-significant. This result was in concord with several studies performed using different AEDs to search their effects on sex hormones. Najaf et al. [58] epileptic patients received carbamazepine (CBZ), sodium valproate (VPA) and lamotrigine (LTG) had significantly reduced testosterone than the control. Baysal et al. [2] reported that Levetiracetam (LEV, 300 mg/kg) significantly decreased testosterone, FSH, and LH levels. Osuntokun et al. [59] confirmed that Gabapentin or carbamazepine male rat's chronic administration for 28 days exhibited a significant reduction in testosterone. In the meantime, the result of the testis histopathological examination revealed high dose of TPM showed marked atrophy of seminiferous tubules, seminiferous tubules spermatogonial cells degeneration with spermatid giant cells, interstitial edema and necrotic Leydig cells. These interpret the minimization in sex hormone levels, where the population of Leydig cells maintains levels of testosterone under luteinizing hormone control [60]. Svalheim et al. [61] reported that AEDs handling could change the levels of different sex hormones. TPM lift up the hepatic synthesis of sex hormone binding globulin (SHBG) that lessens the accessibility of bioactive testosterone that may cause sexual troubles and weak down the fertility.

In contrary, results clarified that CPGO causes a significant increase in the testosterone, FSH and LH hormones. Meanwhile, testes of CPGO treated groups revealed no histopathological alterations with complete spermatogenesis. This finding was in accordance with Memudu et al. [62], who demonstrated that administration of 200 mg/kg ginger increased testosterone level. In addition, in the ginger-treated group, testis histological architecture appears normal with the presence of spermatids and spermatozoa. This follow assume that ginger has strong antioxidant and androgenic potentials at a dose of 200 mg/kg. It also has no harmful effects on spermatogenesis in the testis and hence has a strong fertility property. Morakinyo et al. [63] indicated that *Zingiber Officinale* extract possesses pro-fertility properties in male rats that might be the product of its powerful antioxidant and androgenic activities. Mohammadi et al. [64] indicated that ginger could cause a significant increase in testosterone, germ cells numbers and Sertoli cells in seminiferous tubules that might be reasons for the significant boost of spermatogenesis.

Vegfa is the first tissue-specific angiogenic molecule that selectively induces the proliferation and survival of endothelial cells of testes and it might favor the entry of hormones in the vascular system [65]. Mice that are deficient in Sycp3 fail to establish synapsis, resulting in meiotic arrest during spermatogenesis in male [66]. In our study, TPM down regulated the Vegfa and Sycp3 expression at all tested doses. Wt1 expression in testes of 100 and 200 mg/kg was down regulated, while in 400 mg/kg was up regulated. In addition, histological examination of testes administered TPM (400 mg/kg) revealed atrophy of seminiferous tubules, marked degeneration of spermatogonial cells inside the seminiferous tubules with the formation of spermatid giant cells, interstitial edema and necrosis of Leydig cells. The Vegfa isoforms secreted by Sertoli cells and germ cells are needed for the preservation of spermatogonium, sperm counts, and normal fertility [67]. These clarify the link between TPM histological alteration in testes tissue and the down-regulation of Vegfa and Sycp3 expression. The Vegfa also affects

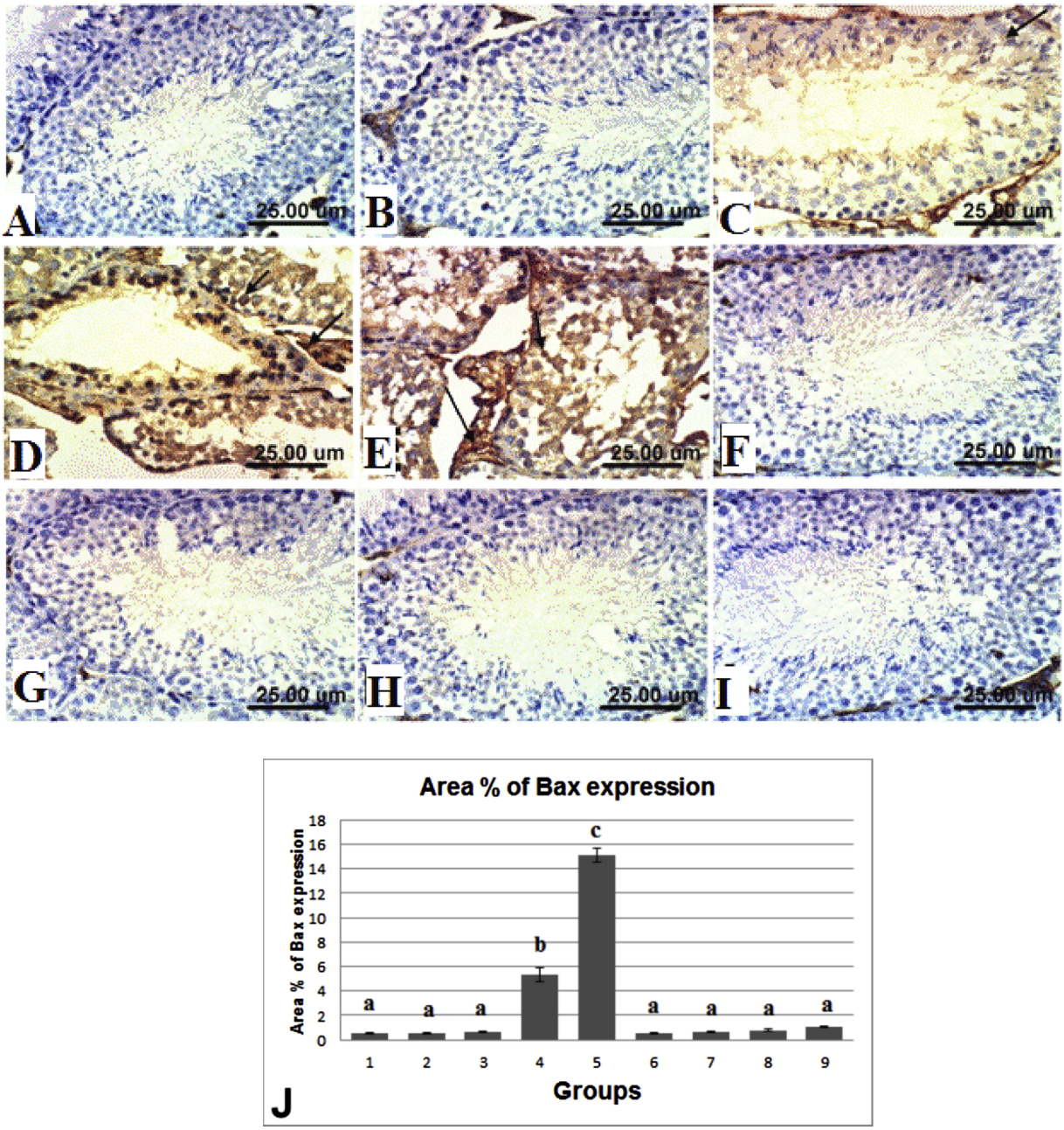


Fig. 5. Immunostaining for Bax protein in the testes (**scale bar 25 um**). **A**) Control mice showing no Bax immune-reactive cells, **B**) mice treated with TPM (100 mg/kg b.w.) showing no Bax immune-reactive cells, **C**) mice treated with TPM (200 mg/kg b.w.) showing weak Bax immune-reactive spermatogoneal cells (arrow). **D** & **E**) mice treated with TPM (400 mg/kg b.w.) showing strong positive immune reaction of spermatogoneal cells (short arrow) and leydig cells (long arrow). **F**, **G** & **H**) mice treated with CPGO (100 mg, 200 mg and 400 mg, respectively) showing no Bax immune-reactive cells. **I**) mice co-treated with TPM 400 mg and CPGO 400 mg showing no Bax immune-reactive cells. **J**) immunostaining area (%) of Bax expression. Data shown as mean ± SE; error bars show the variations of determinations in terms of standard deviation. One – way analysis of variance was used for data analysis (n = 6), mean values with unlike superscript letters were significantly different ($p \leq 0.05$).

the balance between proapoptotic and antiapoptotic proteins, and it has up-regulated the BcL2 compare to Bax, implicating their function in germ cell survival. Thus, regulation of apoptosis alteration might be related to impaired spermatogenesis, leading to infertility [68]. This finding supported our immunohistochemistry results, since strong positive immune reactive spermatogonial and Leydig cells were observed in testes for 400 mg/kg TPM group. Owing to somatic and germ cells complex nature, the interaction required for germ cell differentiation, alteration of numerous cell types' activity might lead to changes in germ cell production. Also, the role of Wt1 in murine testes differentiation is confirmed by the distraction of seminiferous tubules and the absence of germ cells upon conditional Wt1 deletion in Sertoli cells, where it is crucial for the maintenance of Sertoli cells and seminiferous tubules in the developing testes [69].

Regarding CPGO administration to male mice, results revealed that CPGO up-regulated the mRNA expression of tested genes compare to TPM. Histological and immunohistochemical analyses indicated that there were no histopathological alterations and no Bax immune-reactive cells in testes of animals treated with the three doses of CPGO. This finding was in agreement with several studies on ginger. Arash et al. [70] found the testes of ginger treated groups showed normal seminiferous tubule with normal germinal epithelium morphology.

Memudu et al. [62] confirmed that in animals administered ginger (200 mg/kg), the testicular histology showed no abnormality and spermatogenic cell lineage is normal containing all cells.

Finally, CPGO co-administration with TPM (400 mg/kg) improved the antioxidant activities, oxidative stress parameters, sexual hormones levels, mRNA expression of Vegfa, Sycp3 and Wt1, histological as well as the immunohistochemical alterations induced by TPM. Arash et al. [70] reported that ginger significantly increased the sperm physical characters and serum total testosterone in rats. Ginger rhizome found to beat the reproductive toxicity of gentamicin through the elevation of testosterone levels [71]. The histopathology of our study agreed with Nashwa et al. [72], where testis showed marked necrosis of spermatogonial cells, seminiferous tubules germ cells degeneration due to the administration of Ciprofloxacin, while ginger co-administration showed normal seminiferous tubules. Sakr and Shalaby [73] demonstrated that co-administration of ginger aqueous extract enhanced the histological and histochemical alterations induced by fungicide metalaxyl in mice testes. In addition, ginger extract co-administration with arsenite was found to defend against undesirable change in the reproductive organ, attenuate the decline in sperm functions, and improve the level of the reproductive hormone with increased antioxidants actions and peroxidation reduction [74]. Hosseini et al. [75] verified that ginger was effective in decreasing the sperm DNA fragmentation percentage in infertile men. It was established that the mechanism of ginger protection is related to its antioxidant activity [73].

5. Conclusion

From the above-mentioned results, we could concluded that the antiepileptic drug TPM caused male sexual dysfunction in mice. TPM caused reduction in sexual hormones content, alteration in testes genes mRNA expression and spermatogoneal and leydig cells as well as histological and immunohistochemical changes. On the other hand, CPGO improved the androgenic property and sexuality. In addition, CPGO co-administration with TPM could attenuate or protect from the sexual dysfunction that may harm the epilepsy patients as a result of consuming the antiepileptic drugs. It could be recommended adherence to therapeutic doses antiepileptic drugs, where the excessive doses consumption could lead to weak sexuality. In addition, it is recommended that epilepsy men could use ginger through antiepileptic medications to minimize the impact of these medications on their fertility.

Declarations of interest

None.

References

[1] S. Bernard, M.D. Chang, H. Daniel, M.D. Lowenstein, New Eng. J. Med. Epilepsy 349 (2003) 1257–1266.
[2] M. Baysal, S. Ilgin, G. Kili, V. Kilic, S. Ucarcan, O. Atli, Reproductive toxicity after levetiracetam administration in male rats: evidence for role of hormonal status and oxidative stress, PLoS One 12 (2017) e0175990.
[3] H. Patel, D.C. Toe, S. Burke, R.S. Rasu, Anticonvulsant use after formulary status change for brand-name second generation anticonvulsants, Am. J. Manag. Care 16 (2010) e197–e204.
[4] P.I. Perucca, F.G. Gilliam, Adverse effects of antiepileptic drugs, Lancet Neurol. 11 (2012) 792–802.
[5] M. Khalkhali, Topiramate-induced persistent eyelid Myokymia, Case Rep. Psychiatry (2016) 7901085.
[6] S. Otoom, H. Batieneh, Z. Hassan, A. Daoud, Effects of long-term use Topiramate on fertility and growth parameter in adult male rats, Neuroendocrinol Lett. 25 (2004) 351–355.
[7] E. Tauboll, S.L. Roste, S. Svalheim, L. Gjerstad, Disorders of reproduction in epilepsy-What can we learn from animal studies, Seizure 17 (2008) 120–126.
[8] S. Hernandez-Díaz, C.R. Smith, A. Shen, R. Mittendorf, W.A. Hauser, M. Yerby, L.B. Holmes, Comparative safety of antiepileptic drugs during pregnancy, Neurology 78 (2012) 1692–1699.
[9] R.A. Fadel, R.P. Sequeira, M. Obeidat, A.H.A. Salem, Effect of prenatal administration of therapeutic doses of topiramate on ossification of ribs and vertebrae in rat fetuses, Rom. J. Morphol. Embryol. 53 (2012) 321–327.
[10] H.A. Hashish, Histopathologic effect of prenatal Topiramate exposure on rat cerebral cortex and hippocampus, J. Interdisciplinary Histopathol. (Ankara) 2 (2014) 61–68.
[11] A. Ornoy, N. Zvi, J. Arnon, R. Wajnberg, S. Shechtman, O. Diav-Citrin, The outcome of pregnancy following Topiramate treatment: a study on 52 pregnancies, Reprod. Toxicol. 25 (2008) 388–389.
[12] W. Liu, G. Tongtong, P. Zhenxiang, L. Yashu, L. Jiayin, L. Bingjin, The effects of herbal medicine on epilepsy, Oncotarget 8 (2017) 48385–48397.
[13] S.C. Schachter, Botanicals and herbs: a traditional approach to treating epilepsy, Neurotherapeutics 6 (2009) 415–420.
[14] K. An, D. Zhao, Z. Wang, J. Wu, Y. Xu, G. Xiao, Comparison of different drying methods on Chinese ginger (*Zingiber officinale* Roscoe): changes in volatiles, chemical profile, antioxidant properties, and microstructure, Food Chem. 197 (2016) 1292–1300.
[15] X. Zhao, B. Zingiber, W.R. Yang, Y. Yang, S. Wang, Z. Jiang, G.G. Zhang, Effects of ginger root (*Zingiber officinale*) on laying performance and antioxidant status of laying hens and on dietary oxidation stability, Poult. Sci. 90 (2011) 1720–1727.
[16] B. White, Ginger: an overview, Am. Family Phys. 75 (2007) 1689–1691.
[17] K.R. Shanmugam, K. Mallikarjuna, N. Kesireddy, K. Sathyavelu Reddy, Neuroprotective effect of ginger on antioxidant enzymes in streptozotocin-induced diabetic rats, Food Chem. Toxicol. 49 (2011) 893–897.
[18] A. Hosseini, N. Mirazi, Alteration of pentylenetetrazole-induced seizure threshold by chronic administration of ginger (*Zingiber officinale*) extract in male mice, Pharm. Biol. 53 (2015) 752–757.
[19] S. Dugasani, M.R. Pichikac, V.D. Nadarajahc, M.K. Balijepalli, S. Tandra, J.N. Korlakunta, Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol, J. Ethnopharmacol. 127 (2010) 515–520.
[20] A.H. El-Ghorab, M. Nauman, F.M. Anjum, S. Hussain, M. Nadeem, A comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*), J. Agric. Food Chem. 58 (2010) 8231–8237.
[21] S. Noori, F. Zeynali, H. Almasi, Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets, Food Cont. 84 (2018) 312–320.
[22] M. Maghbooli, F. Golipour, M.A. Esfandabadi, M. Yousefi, Comparison between the efficacy of ginger and sumatriptan in the ablative treatment of the common migraine, Phytother. Res. 28 (2014) 412–415.
[23] M.C. Mesomo, A.P. Scheer, E. Perez, P.M. Ndiaye, M.L. Corazza, Ginger (*Zingiber officinale* R.) extracts obtained using supercritical CO_2 and compressed propane: kinetics and antioxidant activity evaluation, J. Supercrit. Fluids 71 (2012) 102–109.
[24] G. Oboh, A.J. Akinyemi, A.O. Ademiluyi, Antioxidant and inhibitory effect of red ginger (*Zingiber officinale* var. rubra) and white ginger (*Zingiber officinale* Roscoe) on Fe^{2+} induced lipid peroxidation in rat brain, In vitro. Exp. Toxicol. Pathol. 64 (2012) 31–36.
[25] S.K. Gupta, A. Sharma, Medicinal properties of *Zingiber officinale* Roscoe-a review, J. Pharm. Biol. Sci. 9 (2014) 124–129.
[26] W. Liu, C.L. Zhou, D. Chen, Q.H. Li, Optimized microwave-assisted extraction of 6-gingerol from *Zingiber officinale* roscoe and evaluation of antioxidant activity in vitro, Acta Sci. Pol. Technol. Aliment. 13 (2014) 155–168.
[27] M. Przygodzka, D. Zielinska, Z. Ciesarová, K. Kukurová, H. Zielinski, Comparison of methods for evaluation of the antioxidant capacity and phenolic compounds in common spices, LWT-Food Sci. Technol. 58 (2014) 321–326.
[28] M. Arens, E. Schulte, K. Weber, Fatty acid methyl diester, transesterification with Trimethylsulfoniumhydroxid (Schnellverfahren), Fat Sci. Technol. 96 (1994) 67–68.
[29] M.F. Ramadan, M.M.S. Asker, M. Tadros, Lipid profile, antiradical power and antimicrobial properties of *Syzygium aromaticum* oil, Grasas y Aceites 64 (2013) 509–520.
[30] M.F. Ramadan, S.G. Kinni, M. Seshagiri, J.-T. Mörsel, Fat-soluble bioactives, fatty acid profile and radical scavenging activity of *Semecarpus anacardium* seed oil, J. Am. Oil Chem. Soc. 87 (2010) 885–894.
[31] A. Rashidian, S. Mehrzadi, A.R. Ghannadi, P. Mahzooni, S. Sadr, M. Minaiyan, Protective effect of ginger volatile oil against acetic acid-induced colitis in rats: a light microscopic evaluation, J. Integ. Med. 12 (2014) 115–120.
[32] J. Sedlack, R.H. Lindsay, Estimation of total, protein bound and non sulphydryl groups in tissue with Ellman's reagent, Anal. Biochem. 25 (1968) 192–205.
[33] I. Fridovich, Superoxide dismutases, Adv. Enzymol. Relat. Areas Mol. Biol. 41 (1974) 35–97.
[34] H. Aebi, Catalase in vitro, Methods Enzymol. 105 (1984) 121–126.
[35] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351–358.
[36] H.A.C. Montgomery, D.F. Dymock, The determination of nitrite in water, Analyst 86 (1961) 414–416.
[37] V. Sharma, J. Boonen, N.S. Chauhan, M. Thakur, B. De Spiegeleer, V.K. Dixit, *Spilanthes acmella* ethanolic flower extract: LC-MS alkylamide profiling and its effects on sexual behavior in male rats, Phytomed. 18 (2011) 1161–1169.
[38] J.D. Bancroft, M. Gamble, J.D. Bancroft, M. Gamble (Eds.), Theory and Practice of Histological Techniques, 6th edition, Churchill Livingstone, Elsevier, Philadelphia, PA, 2006.
[39] S.G. Johnsen, Testicular biopsy score count-A method for registration of spermatogenesis in human testis: normal values and results in 352 hypogonadal males, Hormones 1 (1970) 1–24.
[40] I. Martín-Burriel, N.O. Roome, O. Dorchies, A. Prenez, Histopathological and molecular changes during apoptosis produced by 7H-dibenzo [c,g]-carbazole in mouse liver, Toxicol. Pathol. 32 (2004) 202–211.

[41] M. Atif, M. Azeem, R.M. Sarwar, Potential problems and recommendations regarding substitution of generic antiepileptic drugs: a systematic review of literature, Springerplus 5 (2016) 182.
[42] A. Dalal, L. Zhou, Gabapentin and sexual dysfunction: report of two cases, Neurologist 14 (2008) 50–51.
[43] K.R. Kaufman, P.J. Struck, Gabapentin-induced sexual dysfunction, Epilepsy Behav. 21 (2011) 324–326.
[44] Y. Yang, X. Wang, Sexual dysfunction related to antiepileptic drugs in patients with epilepsy, Expert Opin. Drug Saf. 15 (2016) 31–42.
[45] Z. Durackova, Free radicals and antioxidants for non-experts, Systems Biology of Free Radicals and Antioxidants, Springer, Berlin, Germany, 2014, pp. 3–38.
[46] W. Sobaniec, E. Solowiej, W. Kulak, L. Bockowski, J.S.K. Kuzia, B. Artemowicz, Evaluation of the influence of antiepileptic therapy on antioxidant enzyme activity and lipid peroxidation in erythrocytes of children with epilepsia, J. Child Neurol. 21 (2006) 558–562.
[47] H. Karabiber, C. Yakinci, Y. Durmaz, I. Temel, N. Mehmet, Serum nitrite and nitrate levels in epileptic children using VPA or carbamazepine, Brain Dev. 26 (2004) 15–18.
[48] E. Peker, S. Oktar, M. Ari, R. Kozan, M. Dogan, E. Cagan, S. Söğüt, Nitric oxide, lipid peroxidation, and antioxidant enzyme levels in epileptic children using valproic acid, Brain Res. 1297 (2009) 194–197.
[49] P. Gharagozloo, A. Gutierrez-Adan, A. Champroux, A. Noblanc, A. Kocer, A. Calle, S. Perez-Cerezales, E. Pericuesta, A. Polhemus, A. Moazamian, J.R. Drevet, R.J. Aitken, A novel antioxidant formulation designed to treat male infertility associated with oxidative stress: promising preclinical evidence from animal models, Hum. Reprod. 31 (2016) 252–262.
[50] A. Ghasemzadeh, E.Z.H. Jaafar, A. Rahmat, M.E.P. Wahab, R.M. Abd Halim, Effect of different light intensities on total phenolics and flavonoids synthesis and antioxidant activities in young ginger varieties (*Zingiber officinale* Roscoe), Inter. J. Mol. Sci. 11 (2010) 3885–3897.
[51] I. Stoilova, A. Krastanov, A. Stoyanova, P. Denev, S. Gargova, Antioxidant activity of a ginger extract (*Zingiber officinale*), Food Chem. 102 (2007) 764–770.
[52] T. Herve, K.J. Raphael, N. Ferdinand, L.T.F. Vitrice, A. Gaye, M.M. Outman, W.M.N. Marvel, Growth performance, serum biochemical profile, oxidative status, and fertility traits in male Japanese quail fed on ginger (*Zingiber officinale*, Roscoe) essential Oil, Vet. Med. Int. (2018) 7682060.
[53] A. Haksar, A. Sharma, R. Chawla, R. Kumar, R. Arora, S. Singh, J. Prasad, M. Gupta, R.P. Tripathi, M.P. Arora, F. Islam, R.K. Sharma, *Zingiber officinale* exhibits behavioral radioprotection against radiation-induced CTA in a gender-specifc manner, Pharmacol. Biochem. Behav. 84 (2006) 179–188.
[54] Z. Ghlissi, R. Atheymen, A.M. Boujbiha, Z. Sahnoun, M.F. Ayedi, K. Zeghal, A. El Feki, A. Hakim, Antioxidant and androgenic effects of dietary ginger on reproductive function of male diabetic rats, Int. J. Food Sci. Nutr. 64 (2013) 974–978.
[55] M.J. Morrell, K.L. Flynn, S. Doñe, E. Flaster, L. Kalayjian, A.M. Pack, Sexual dysfunction, sex steroid hormone abnormalities, and depression in women with epilepsy treated with antiepileptic drugs, Epilepsy Behav. 6 (2005) 360–365.
[56] P. Wisniewski, R.M. Romano, M.M. Kizys, K.C. Oliveira, T. Kasamatsu, G. Giannocco, M.I. Chiamolera, M.R. Dias-da-Silva, M.A. Romano, Adult exposure to bisphenol A (BPA) in Wistar rats reduces sperm quality with disruption of the hypothalamic-pituitary testicular axis, Toxicology 329 (2015) 1–9.
[57] S. Kumar, G. Kaur, Second generation anti-epileptic drugs adversely affect reproductive functions in young non-epileptic female rats, Eur. Neuropsychopharmacol. 24 (2014) 1709–1718.
[58] R.M. Najafi, B. Ansari, M. Zare, F. Fatehi, A. Sonbolestan, Effects of antiepileptic drugs on sexual function and reproductive hormones of male epileptic patients, Iran. J. Neurol. 11 (2012) 37–41.
[59] O.S. Osuntokun, G. Olayiwola, A. Oladele, I. Ola, O. Ayoka Abiodun, Chronic administration of gabapentin and a gabapentin-carbamazepine combination reversibly suppress testicular function in male Wistar rats (*Rattus norvegicus*), Pathophysiology 24 (2017) 63–69.
[60] D. Rebourcet, P.J. O'Shaughnessy, A. Monteiro, L. Milne, L. Cruickshanks, N. Jeffrey, F. Guillou, T.C. Freeman, R.T. Mitchell, L.B. Smith, Sertoli cells maintain Leydig cell number and peritubular myoid cell activity in the adult mouse testis, PLoS One 9 (2014) e105687.
[61] S. Svalheim, L. Sveberg, M. Mochol, E. Tauboll, Interactions between antiepileptic drugs and hormones, Seizure 28 (2015) 12–17.
[62] A.E. Memudu, I.D. Akinrinade, O.M. Ogundele, E. Duru, Investigation of the androgenic activity of ginger (*Zingiber officinale*) on the histology of the testis of adult Sprague Dawley rats, J. Med. Medical Sci. 3 (2012) 697–702.
[63] A. Morakinyo, O. Adeniyi, A. Arikawe, Effects of *Zingiber officinale* on reproductive functions in the Male rat, Afr. J. Biomed. Res. 11 (2008) 329–334.
[64] F. Mohammadi, H. Nikzad, M. Taghizadeh, A. Taherian, A. Azami-Tameh, S.M. Hosseini, A. Moravveji, Protective effect of *Zingiber officinale* extract on rat testis after cyclophosphamide treatment, Andrologia 46 (2014) 680–686.
[65] P. Hoffmann, Y. Saoudi, M. Benharouga, C.H. Graham, J.-P. Schaal, C. Mazouni, J.-J. Feige, N. Alfaidy, Role of EG-VEGFA in human placentation: physiological and pathological implications, J. Cell. Mol. Med. 13 (2009) 2224–2235.
[66] M. Tsutsumi, H. Kogo, H. Kowa-Sugiyama, H. Inagaki, T. Ohye, H. Kurahashi, Characterization of a novel mouse gene encoding an SYCP3-like protein that relocalizes from the XYbody to the nucleolus during prophase of male meiosis I, Biol. Reprod. 85 (2011) 165–171.
[67] L.U. Ningxia, M.K. Sargent, T.D. Clopton, E.W. Pohlmeier, M.V. Brauer, M.R. McFee, S.J. Weber, N. Ferrara, W.D. Silversides, S.A. Cupp, Loss of Vascular Endothelial Growth Factor A (VEGFAA) Isoforms in the testes of male mice causes subfertility, reduces sperm numbers, and alters expression of genes that regulate undifferentiated spermatogonia, Endocrinology 154 (2013) 4790–4802.
[68] D. Jaiswal, S. Trivedi, K.N. Agrawal, K. Singh, Dysregulation of apoptotic pathway candidate genes and proteins in infertile azoospermia patients, Fertil. Steril. 104 (2015) 736–743.
[69] F. Gao, S. Maiti, N. Alam, Z. Zhang, J.M. Deng, R.R. Behringer, C. Lecureuil, F. Guillou, V. Huff, The Wilms tumor gene, Wt1, is required for Sox9 expression and maintenance of tubular architecture in the developing testis, Proc. Nat. Acad. Sci. U. S. A. 103 (2006) 11987–11992.
[70] K. Arash, F. Fatemeh, N. Mohammad, A.K. Amir, C.O. Chelar, G. Marefat, H. Mohammad, The effects of ginger on spermatogenesis and sperm parameters of Rat, Iran. J. Reprod. Med. 7 (2009) 7–12.
[71] A. Zahedi, A. Khaki, H.R. Ahmadi-Ashtiani, H. Rastegar, S. Rezazadeh, *Zingiber officinale* protective effects on gentamicin's toxicity on sperm in rats, J. Med. Plants 9 (2010) 93–98.
[72] A.A. Nashwa, A.A. Kawkab, M.M. Samar, The protective effect of ginger and N-acetyl cysteine on ciprofloxacin-induced reproductive toxicity in male rats, J. Am. Sci. 7 (2011) 741–752.
[73] A.S. Sakr, Y.S. Shalaby, Ginger extract protects metalaxyl-induced histomorphological and histochemical alterations in testes of albino mice, Int. J. Appl. Pharm. Sci. Res. 1 (2011) 36–42.
[74] A.O. Morakinyo, P.U. Achema, O.A. Adegoke, Effect of *Zingiber officinale* (Ginger) on sodium arsenite induced reproductive toxicity in male rats, Afr. J. Biomed. Res. 13 (2013) 39–45.
[75] J. Hosseini, M.A. Mamaghani, H. Hosseinifar, M. Ali, S. Gilani, F. Dadkha, M. Sepidarkish, The influence of ginger (*Zingiber officinale*) on human sperm quality and DNA fragmentation: a double blind randomized clinical trial, Int. J. Reprod. BioMed. 14 (2016) 533–540.