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Mitigating Action of Korean Red Ginseng Extract against Antidepressant Agent (Duloxetine)-Induced Oxidative Insult, Inflammation, and Apoptosis in Rat Male Reproductive System

Esraa M. Fahmy[1], Abdelhamid E. Abdelhamid[1], Doaa A. Mansour[2], Sara M. Baraka[3], Reda M. S. Korany [4,5], Asmaa A. Abdallah[6], Aya A.Fahmy[1*], Reham H. Alattar[7] and Ahmed Abdou Said[1]

[1] *Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Egypt.*

[2] *Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt.*

[3] *Chemistry of Natural Compounds Department, National Research Centre, Giza 12622, Egypt.*

[4] *Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.*

[5] *Pathology Department, Faculty of Veterinary Medicine, Egyptian Chinese University, Egypt.*

[6] *Theriogenology Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.*

[7] *Pharmacy **R**esearch fellow at General **A**dministration for **M**edical **A**ffairs*

Abstract

THE long term use of antidepressant drugs is linked to several negative impacts on the liver, kidney, and reproductive system. Red ginseng (GE) is highly recognized as a rich saponins plant with valuable biological properties. From this point, the current investigation focused on the potential safeguarding properties of (GE) aqueous extract against the degenerative effects of duloxetine (Dulo, a well-known antidepressant drug) on the male reproductive system. In a male rat model, Dulo was given at 2 doses (2.7 and 5.4 mg/kg BW orally), while GE was concurrently orally gavaged at 100 mg/kg BW for 2 consecutive months. Assessments of semen quality, relative testes weight, serum sex hormones, testicular oxidant/antioxidant status, and histopathological and immuno-histochemical changes in both seminal vesicle and testes were utilized. Dulo administration dose-dependently ($P < 0.05$) was associated with a significant loss of sperm motility % and sperm cell concentration, with a marked drop in the live-to-dead sperm ratio, and a considerable % of sperm abnormalities. Furthermore, a significant decline ($P < 0.01$) in the relative testes weight, coupled with disturbances in serum sex hormones viz. total testosterone, luteinizing hormone, and follicle-stimulating hormone, along with marked repression in the testicular antioxidant defence mechanism. Additionally, several structural changes and inflammatory-stimulated apoptosis reactions were noticed in the testes and seminal vesicles of rats treated with Dulo in a dose-dependent manner. All these alterations accompanied by Dulo administration at the 2 adopted doses were significantly ($P < 0.01$) ameliorated by GE administration through preserving semen quality and relative testes weight, balance of serum sex hormones, and alleviating oxidative stress in the testicular tissues. Moreover, GE significantly well-maintained the testicular and seminal vesicle structures, and dampened the over-expression of tumour necrosis factor α, and BAX, with up-regulating BCL2 expression in the testes and seminal vesicles ($P < 0.05$). It could be concluded that GE possesses considerable mitigating actions against the toxic impacts of Dulo on male gonads through exerting antioxidant, anti-inflammatory, and anti-apoptotic properties, making it a target for future studies as an effective agent in preserving the male reproductive system.

Keywords: Duloxetine, Ginseng extract, Testicular damage, Semen, Oxidation, Apoptosis, Rats.

Introduction

Depression is one of the most prevalent and debilitating mental illnesses worldwide. This disorder is characterized by impaired memory, motivation, motor function, emotional management, cognitive function, and neuro-vegetative symptoms. In addition to these basic effects, this illness can also result in several secondary impairments that undermine social and economic functioning globally [1]. Antidepressant medications are categorized based on their chemical composition and mechanism of action [2]. Thus, antidepressant medications are divided into 5 classes: monoamine oxidase inhibitors,

*Corresponding authors: Aya A. Fahmy, E-mail: aya30090@gmail.com Tel.: +201064292952
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tricyclic antidepressants, serotonin-norepinephrine reuptake inhibitors (SNRIs), selective serotonin reuptake inhibitors (SSRIs) and non-typical antidepressants. [3].

Even though SSRIs are the primary class of antidepressants, SNRIs, which include venlafaxine and duloxetine have recently become well-known because of their unique properties and the dual reuptake of serotonin and noradrenaline, which makes them useful therapeutic alternatives, especially in cases of severe depression. Additionally, SNRIs are well known for having large tolerance and safety margins [4]. Clinical trials have shown that Dulo improves pain tolerance, reduces pain perception, and raises serotonin and norepinephrine levels in the brain and spinal cord [5]. Dulo is most frequently recommended for the following conditions: generalized anxiety disorder, fibromyalgia, musculoskeletal pain, osteoarthritis, diabetic peripheral neuropathy pain, and severe depression [6].

It is commonly recognized that antidepressants can adversely affect body organs, particularly sexual organs in humans [7]. The most noticeable effect of duloxetine toxicity has been seen in the gastrointestinal and neurological systems, with hepatic cell destruction [8]. Ebrahem et al., [9] claimed that Dulo may negatively impact male fertility by changing the histological characteristics of testicular tissues and producing a discernible decrease in sperm concentration, viability, and motility when testosterone levels are lowered [9]. Furthermore, it was reported that duloxetine produced negative effects that exacerbated oxidative stress [10]. An imbalance between reactive oxygen species (ROS) generation and antioxidant system activity causes oxidative stress [11]. Overproduction of ROS damages sperm since they are highly susceptible to oxidation. Both an abundance of unsaturated fatty acids in the membrane and a deficiency of cytoplasmic antioxidant enzymes are thought to contribute to this awareness [12].

Medicinal plants are used to treat a range of disorders and preserve health [13]. Because of its various health advantages, low risk of side effects, and reasonable cost, herbal therapy has become more and more popular [14]. One of the most potent herbal remedies, ginseng extract (GE) (Korean GE, Panax ginseng Meyer; Araliaceae) has been used for generations to improve longevity, immunological function, and physical performance [15]. Ginsenosides are the primary bioactive components in ginseng; they are steroidal saponins that have a variety of pharmacological effects [16]. GE contains a wider range of ginsenosides than fresh or white ginseng root [17]. Scientifically, GE has a multiple forms of biological activity, including anti-inflammatory [18], antioxidant [19], hepatorenal protection [20, 21], neuroprotection [22], radioprotection [23], anticancer effects [24], immunologic advantages [25], and anti-diabetic [26]. Ad-additionally, GE and its ginsenoside had antiviral properties against the human coronavirus strain OC43 [27]. GE was administered to increase libido and shield rats against testicular damage and infertility [28,29].

Because of its useful potential, including its affordability, accessibility, and safety, ginseng extract has attracted the attention of researchers. However, no previous research has looked into GE's possible ability to prevent testicular damage brought on by the antidepressant medication duloxetine. To determine whether GE could, in general, reduce the effects of duloxetine on testicular impairment and spermatogenesis disruption, the current study examined testes and seminal vesicles tissues histopathologically and immunohistochemically, monitored oxidative stress markers, and measured changes in serum sex hormones after 2 months of daily administration.

Material and Methods

Drugs and chemicals

Dulo 60 mg and Dulo 30 mg were supplied from Pharco Company (Alexandria, Egypt). The ginseng was bought in a commercial form of Korean GE (100% natural Panax ginseng Roots Powder) (Haraz, Cairo, Egypt).

Animals and ethics

Twenty-five adult male albino Wistar rats (150–200 grams) were received from the laboratory Animal Unit, Zagazig University's Faculty of Veterinary Medicine. The rats were housed under standard conditions (25 ºC, 40–60 % relative humidity, 12 h light and 12 h dark cycle). They were given a standard rodent diet from the local market with tap water was available ad lib for two weeks before be-ginning the experiment to acclimatize them to the environment. This research was carried out. Following ethical guidelines and political factors approved by Animal Care at Zagazig University's Faculty of Veterinary Medicine in Zagazig, Egypt (approval no. ZU-IACUC/2/F/226/2024).

Preparation of Korean GE extract

The Korean extract (GE) was prepared by mixing the powder of Ginseng with distilled water overnight yielding a dark brown extract [30]. Then, the extract was concentrated under reduced pressure. The GE was prepared in distilled water at a concentration of 20 mg/ml to be given per os to rats (1ml/200 g rat Bwt) [31].

Rational of dose selection of treatments

The dose of GE was chosen according to previous studies [32] which verified the efficacy and high safety properties of GE at dose of one hundred mg/kg

rat body weight. Meanwhile, the doses of Dulo (2.7 and 5.4 mg/kg rat BW) were calculated based on the body surface area conversion against human dose 30 and 60 mg/kg BW, respectively [33].

Experimental design

Following the end of the acclimatization period, rats were divided into 5 equal groups randomly; (5 rats per group): Group I (control) received DW (5mL/Kg BW), group II (Dulo 2.7) received 2.7 mg/ kg BW of Dulo per os once daily for 60 days, group III (Dulo 5.4) received 5.4 mg/kg BW Dulo per os once daily for 60 days .group VI (Dulo 2.7+ GE) received 2.7 mg/kg BW Dulo per os once daily plus GE (100 mg/kg bw/orally/once/60 days), and group V (Dulo 5.4 + GE) received 5.4 mg/kg BW Dulo per os once daily plus GE as in group VI. GE was administered one h before Dulo exposure.

Sample preparation

The rats were weighed and blood samples were taken from abdominal under sodium thiopental anesthesia (50 mg/kg BW intraperitoneal) 24 h after the last drug delivery [32]. Serum samples were then separated using a cooling centrifuge (3000 rpm for 15 min) (Laboren Zentrifugen, 2K15 Sigma, Germany). Serum samples for hormonal assay such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were stored for biochemical examination at -80 °C. Rats were decapitated, and the relative weights of the testes were determined by collecting and weighing the tissues. For additional biochemical tests, portions of the testicular tissues were stored at -80 °C. The seminal vesicle and remaining testicular tissues were stored in 10% buffered formalin for histological and immunohistochemical investigations. The relative testes weight (%) was calculated as follows: = [organ weight (g)/final body weight (g)] * 100.

Semen analysis

After being extracted from the epididymis, the semen was diluted in 37 °C heated physiological saline [34]. One drop of semen was applied concurrently to a dry, clean slide that had been prewarmed and stained with Eosin-Nigrosin stain to determine the sperm's vitality (dead to live percent). The percentage of dead and live sperms was then computed [35]. Sperm abnormalities were identified by combining one drop of diluted semen with an Eosin stain, estimating the percentage of abnormal and normal sperms, and then dilution with formalin and sodium bicarbonate solution to estimate the concentration of epididymal sperm, followed by sperm counting with a Neuberger hemocytometer [36].

Biochemical assessment

Determination of serum hormonal profile

The frozen serum was thawed to measure serum testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) by enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions [37-39] (Catalog NO. (TE187S), (FS232F), and (LH231F) (Calbio tech, El Cajan, CA, USA), respectively), using a microplate reader (Infinite 50, Männedorf, Switzerland) at wavelength 450 nm.

Determination of the testicular tissue oxidative biomarkers

According to Behairy et al. [40], the homogenate of testicular tissue was made in cold phos-phate-buffered Saline. (pH 7, P.B.S, 0.011 mol/L). The glass homogenizer (1:9 W/V) was used to homogenise each rat's testis. To remove TES tissue debris for hormone assessment and oxidative stress, the resultant homogenates were centrifuged for five minutes at 5000 ×g, and the supernatants were filtered using a Millipore filter (0.45 µm). Total antioxidant capacity (TAC) and malondialdehyde (MDA) levels were estimated using kit reagents according to the methods described by [41].

Histopathological examination

Samples of seminal vesicles and testes were gathered, preserved in 10% neutral buffered formalin, cleaned, dehydrated, and embedded in paraffin. For histopathological examination, the paraffin-embedded blocks were cut into sections that were 5 microns thick and stained with hematoxylin and eosin [42].

Histopathological lesion scoring

Testicular and seminal vesicle histopathological changes were graded as follows: no change (0), mild (1), moderate (2), and severe (3) changes. The grading was done by percentage: mild changes were those that were less than 30%, moderate changes were those that were less than 30% - 50%, and severe changes were those that were greater than 50% [43].

Immunohistochemistry

Immunohistochemistry was carried out following the method [44], following xylene deparaffiniza-tion tissue fragments were rehydrated using different alcohol grades. For 20 min, sections were pretreated citrate buffer with a pH equal 6 in order to retrieve antigens. In a humidified chamber, sections were incubated for two hours with anti-TNF-α (ab270264; Abcam, Cambridge, UK; 1:100 dilution rate), rabbitppolyclonal anti-Bcl-2nantibody at a concentration of 01:50 (ab59348; Abcam, Cambridge, UK), and rabbit monoclonal anti-Bax antibody [E63] at a concentration of 1:250 (ab32503; Abcam, Cambridge, UK). Goat anti-rabbit IgG H&L (HRP) (ab205718; Abcam, Cambridge, UK) was used to incubate the sections in addition to 3,3′-diaminobenzidine tetra hydrochloride (DAB, Sigma)

as a chromogen. After counterstaining the slides with hematoxylin, they were finally mounted using DPX. The primary antibodies were swapped out for PBS on the negative control slides.

Assessment of Bax, Bcl-2 and TNF-α immunostaining

Five tissue slices from each group were analyzed to determine the quantitative immuno-reactivity of Bax, Bcl-2, and TNF-α. Immuno-reactivity was analyzed in 10 microscopical fields per section under a high-power microscopic field (x 400). Using color de-convolution image J 1.52 p software (Wayne Rasband, National Institutes of Health, USA), the % of positively stained cells (%) was estimated [45].

Statistical analysis

The normal data distribution at $P > 0.05$ has been checked using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons were used to examine the collected data then the data was displayed as means ± SE. Statistical analyses were conducted utilizing the Statistical Package for Social Science (SPSS, version 17.0, SPSS Inc., Chicago, IL, USA). Statistical differences were set at $P < 0.05$.

Results

Effects of GE on semen quality parameters in Duloxetine-treated rats

Duloxetine administration at the wo dose levels 2.4 and 5.7 mg/kg was associated with significant loss of sperm motility % (39.56% and 66.59%, respectively), the ratio of live to dead sperms (41.33% and 66.23%, respectively), and SCC (43.35% and 57.80%, respectively), along with a marked increase in sperm abnormalities % (640.25% and 944.41%, respectively) in comparison to those of the control group ($P < 0.01$). While concurrent administration of GE with Dulo significantly antagonized the loss of SCC improved the percentage of sperm motility and live/dead ratio and decreased the occurrence of sperm abnormalities as illustrated in (Fig.1).

The effects of GE on relative testes weight and serum hormonal profile in Duloxetine-treated rats

A significant reduction in rats' relative testes weights, approximately 33.47%, and 67.35%, has been noticed in rats administered Dulo at doses of 2.7 and 5.4 mg /kg BW, respectively compared to the control group (Table 1). By contrast, oral administration of GE to rats intoxicated with Dulo 2.7 and 5.4 mg/kg BW showed marked preservation of relative testes weight compared to the Dulo-treated groups. In addition, a profound disturbance of serum sex hormones has been documented in Dulo-treated rats at doses of 2.4 and 5.7 mg/kg as indicated by significant repression of total testosterone (64.08% and 83.55%, respectively), LH (72.59% and 88.09%, respectively), and FSH (65.55% and 86.13%, respectively) in relative to those level of control rats ($P < 0.01$). In comparison to the control rats and dulo-treated groups, a partial restoration in serum testosterone, LH, and FSH levels was recorded in animals treated with GE which highlights its potential androgenic effects (Table 1).

Effects of GE on testicular oxidative and antioxidant biomarkers in Duloxe-tine-treated rats

The rats that received Dulo at doses of 2.4 and 5.7 mg/kg BW showed a high incidence of lipid peroxidation, along with a decline in antioxidant capacity ($P < 0.01$), as verified by a significant increase in MDA (3.09 folds and 11.43 folds, respectively) levels, coupled with a decrease in TAC concentration (54.71% and 86.61%, respectively) in rat testicular tissue compared to the control ones (Table 2). Con-versely, GE administration to rats challenged with Dulo (2.4, and 5.7 mg/kg) significantly diminished the liberation of MDA by 56.46% and 49.82%, respectively, and enhanced TAC levels (80.67% and 167.90%, respectively) in testicular tissues when compared to their respective values of Dulo-treated groups, sig-nifying its antioxidant capacity (Table 2).

Histopathological finding

Haematoxylin and eosin stain

Testis:

The control group showed normal histological structure of seminiferous tubules (Fig. 2a). Dulo 2.7 treated group showed necrosis and vacuolation of spermatogenic cells (Fig. 2b) with the occurrence of sperm coagulum (Fig.2c). Dulo 5.4 treated group showed massive necrosis of spermatogenic cells (Fig. 2 d) with coagulation of sperms in seminiferous tubule lumen (Fig. 2e). Dulo 2.7 +GE treated group showed normal spermatogenic cells (Fig. 2 f) with normal sperm formation in the seminiferous tubule lumen (Fig.2g). Dulo 5.4 +GE treated group showed nearly normal seminiferous tubules (Fig. 2 h).

Seminal vesicle: Normal histological structure was shown in the control group (Fig. 3a). Dulo 2.7 treated group showed mild infiltration of muscles with mononuclear inflammatory cells (Fig. 3b). Dulo 5.4 treated group showed severe destruction and necrosis of smooth muscles of the muscles layer with infiltration of mononuclear inflammatory cells (Fig. 3c), Edema and a significant infiltration of mononuclear inflammatory cells in the tunica adventitia were also seen in this group. (Fig. 3 d). Dulo 2.7 +GE treated group showed normal structure of seminal vesicle (Fig.3e). Dulo 5.4 +GE treated group showed mild mononuclear infiltration some inflammatory cell in muscles (Fig. 3 f). Testes and seminal vesicle lesions were recorded and scored according to severity in Table 3.

Findings for Immunohistochemistry for Bax, Bcl-2 and TNF-α

Bax and TNF-α expression in testes and seminal vesicles showed weak immune-reactive cells in the control group, but Bcl-2 showed strong expression (Figs. 4 & 5 a). Testes and seminal vesicles of the Dulo 2.7 treated group showed moderate immuno-expression of Bax and TNF-α markers and weak Bcl-2 (Figs. 4 & 5 b.). Groups treated with Dulo 5.4 exhibited strong expression of TNF-α also Bax, and low expression of Bcl-2 (Figs. 4 & 5 c). Dulo 2.7 +GE treated group showed a reduction in immune-reaction of TNF-α and Bax and restoration of Bcl-2 immuno-reactivity (Figs. 4 & 5 d). Dulo 5.4 + GE treated group showed low TNF-α and Bax but strong Bcl-2 immuno-reactivity (Figs. 4 & 5 e). Bax , Bcl-2., and TNF-αa area % expression in testes and seminal vesicles were illustrated in Figs. 6, 7, 8 and 9.

Discussion

It is believed that infertility and psychological problems are tightly linked [46]. Sexual dysfunction affects between 58% and 70% of people using SNRIs, which include venlafaxine, levomilnacipran, du-loxetine, and desvenlafaxine [3]. In several inflammatory and neuropathic pain models, Dulo, a strong SNRI, decreases allodynia and is commonly used to treat depression [47]. Dulo's negative impact on the male fertility has been documented by Ebrahem et al., [9]. Because of this, the current study was conducted to examine the potential protective effect of GE against testicular toxicity caused by duloxetine.

The present investigation found that exposure to Dulo at both doses, particularly the high dose, was associated with a significant change in sperm characteristics, such as a decrease in sperm concentration, motility, and viability, as well as an increase in sperm abnormalities. In addition, a significant reduction in the relative weight of the testes was noted after Dulo administration, which could contribute to the drop in androgen concentration and fluid of the seminiferous tubules. Dulo-treated rats have been shown to exhibit a severe disruption of spermatogenesis-related serum sex hormones, as seen by a marked suppression of total testosterone, LH, and FSH levels compared to normal rats.

In the same respect, Kaur et al., [48] found that venlafaxine (VH), an SNRI, significantly altered the testicular histoarchitecture and diminished sperm concentration and motility. Additionally, VH reduced the antioxidant defence system enzymes SOD, CAT, and GSH-Px while significantly increasing ROS levels and lipid peroxidation in contrast to the control animal. This might be a result of VH's direct suppression of antioxidant enzymes [48].

According to previous studies, antidepressants may have negative impacts on the reproductive system of male [49]. Although the precise mechanism by which antidepressant drugs impact the reproductive system is still unknown, it has been suggested that they may disrupt the Hypothalamic-Pituitary-Gonadal axis by inhibiting dopamine receptors, including dopamine reuptake, and/or increasing prolactin secretions, which prevent gonadotropin from being released, resulting in a decrease in FSH and LH [50]. Additionally, it is commonly believed that the inhibitory effects of SSRIs and SNRIs on male reproduction are caused by their lowered dopamine neurotransmissions, which boost male prolactin levels, causing spermatogenesis, which suppresses defective sperm motility and changes the quality of sperm [51-53].Furthermore, high prolactin levels were connected to some cases of gynecomastia associated with the prescription of fluoxetine and venlafaxine [54].

Male fertility is often measured using the levels of testosterone, FSH, and LH. Sex hormones and antidepressants are tightly related [55]. Dulo can cause endocrine imbalance by inhibiting the activity of the cytochrome CYP17 enzyme as reported by in vitro research. This is because most tissue that produces steroids depends on the function of cytochrome CYP17 (P450 17), a key enzyme upstream in the steroidogenic pathway [56], which is confirmed by our findings that revealed a significant decline of total testosterone in the Dulo-treated rats relative to those recorded in the control rats. Testosterone and sperm production levels are closely associated with testicular size [57]. So, our data revealed that the high dose of Dulo caused reduction in relative weight of both testes, which might be due to the low levels of serum testosterone.

Furthermore, Oxidative stress is acknowledged to be the major contributor to reproductive ab-normalities, which may interfere with the process of sperms synthesis and resulted in male infertility [58] and [59]. Excessive ROS production is associated with Dulo toxicity, which can result in oxidative stress and cell damage. Spermatozoa are especially susceptible to peroxidative, high levels of polyunsaturated fatty acids, which can lead to ROS attacks. Increased ROS and LPO levels affect normal sperm quality (motility, viability, function, and concentration) through their interactions with lipid membranes, proteins, nuclear, and mitochondrial DNA [60, 61]. Through its interference with cyclic adenosine monophosphate in leydig cells, ROS can lead to complications associated with testosterone synthesis [62]. Our results are consistent with the findings of El-Din and Abd-ElAty [10], who investigated the effects of Dulo on male rats' testes and found that it significantly decreased the levels of FSH, LH, and testosterone. In addition to producing oxidative stress, which showed up as increased MDA and lower levels of GSH, SOD, and CAT, it also

reduced germ cell apoptosis, which led to testicular degeneration [10].

Consequently, an excess of ROS initiates a series of signals inside cells that encourage the expression of proinflammatory cytokines [63]. In the tissues of the testicles and seminal vesicles, a high dose of Dulo caused a substantial production of the proinflammatory cytokine TNF-α. Through sperm membrane destruction and spermatogenesis reduction, TNF-α contributes to spermatogenesis impairment. Furthermore, TNF-α decreases sperm DNA integrity, sperm motility and viability and the % of apoptotic spermatozoa. It also encourages mitochondrial dysfunction [64]. Likewise to our results, Lynda et al., [65] found that a course of treatment of fluoxetine significantly increased the levels of IL-1β and TNF-α in the testicular tissues, demonstrating its inflammatory effect [65].

Moreover, ROS can cause more severe and permanent cell deterioration, which can lead to necrosis or apoptosis and finally cell death [66]. The process of apoptosis is strictly regulated and primarily initiated by pro- and anti-apoptotic agents [67]. Bcl-2 is thought to protect cells against apoptosis through its anti-apoptotic qualities, whereas Bax protein promotes damaged cells to undergo programmed cell death [68]. Parallel to the results of this research, Kaur et al., [48] stated that the overproduction of ROS caused by VH therapy led to the release of pro-apoptotic proteins, which intern destroyed the mice's testicles. The intrinsic machinery/apoptotic pathway was activated, as evidenced by a significant rise in the expression of the proteins caspase-9, caspase-3, and Bax and a decline in Bcl-2. [48].

Further supporting the biochemical results were our histological observations, which showed Dulo's detrimental effects on the rat testicular tissue. In line with previous research, Ebrahem et al., [9] found that Dulo caused thickening of the testicular wall, necrosis of some seminiferous tubules, angiopathic changes like thrombosis of some interstitial blood vessels, accumulation of degenerated cellular debris in seminiferous tubules with atrophy and misshaping, and a decrease in the number of layers of germinal epithelium and vacuole degeneration [9]. Recently, the usage of herbal medicines to enhance male functioning has gained popularity due to their antioxidant properties and capacity to promote sexual hormones. Since ancient times, ginseng, one of the most well-known medicinal herbs in the world, has been utilized in traditional Chinese medicine [69].

Based on the obtained results, the co-administration of GE to Dulo-intoxicated rats, enhanced the testicular function and improved the male rat's fertility by marked improvement of the testicular histo-pathological architecture, preserved the relative testes weight, antagonized the loss of SCC, improved the % of sperm motility, and live/dead ratio decreased occurrence of sperm abnormalities, also elevated level of serum FSH, testosterone and LH which highlighting its potential androgenic effects, in addition, diminished the liberation of MDA along with enhancing TAC concentration in testicular tissues, as well showed a reduction in immune-reaction of the proinflammatory cytokine (TNF-α) and apoptotic marker (Bax) with the restoration of antiapoptotic marker (Bcl-2) in seminal vesicles and testicular tissues, when compared to their respective values of the Dulo-treated group, signifying its antioxidant, anti-inflammatory and anti-apoptotic abilities, respectively.

These findings are consistent with a large body of research that has demonstrated that GE has a an effect for protection and prevention of testicular injury caused by several conditions, such as the use of anticancer drugs [70-72], cell phone radiation [73], streptozotocin-induced diabetes [74], toxins [75], and aging-induced testicular damage [76].

Similarly, Ismail et al., [31] demonstrated that KRG extract significantly enhanced the histological testicular architecture, animal and testis weights, sperm count, and sperm motility. It also restored the levels of TH, FSH, and LH in the serum and improved testicular deterioration by lowering inflammatory responses and oxidative stress in the testis tissues of groups that were intoxicated by radiation (R), cyclophosphamide (CP), and R/CP [31]. Moreover, Lee et al., [29] reported that GE restored the testis weight, sperm impairment, sex hormone receptors, and antioxidant-related enzymes in rats' testes after they were immobilized under stress [29]. According to Hassan et al., [77] supplementation may also have an impact on spermatogenesis activity; increase the concentration of sperm with normal morphology, mass activity, and progressive motility, since ginseng stimulates the expression of the protein Catsper, which increase sperm motility [78]. Moreover, Linjawi [79] stated that ginseng reduced DNA damage, elevated levels of serum LH, FSH also free testosterone and improved the expression of reproductive genes [79].

Accordingly, ginseng's cytoprotective, anti-inflammatory, and antioxidant qualities are primarily responsible for its protective efficacy against Dulo-mediated testicular damage. These features further support our findings and may be able to repair testicular and sperm damage [80]. Ginseng promotes testicular function because of its high quantity of amino acids, vitamins A, E, C, B2 and B12 as well as metals including salt calcium, phosphorus, iodine, copper, iron, zinc, selenium and potassium [81]. Also, ginseng contains saponin components (ginsenosides) that include triterpene and sugar moieties, along with nonsaponin compounds made up of polysaccharides in variable amounts.

Quercetin, kaempferol, gentisic, catechin, and rutin are among the flavonoids in ginseng that have strong antioxidant qualities [82]. Gin-senosides share structural similarities with steroid hormones, such as androgens, which are essential for regulating spermatogenesis and preserving and improving male sexual characteristics [83]. In fact, via ac-tivating steroid receptors found in spermatozoa and male genital tissue, ginsenosides affect spermatogenesis and male sexual functions [84, 85].

For sperm to perform physiologically normally, including capacitation, hyper activation, acrosome responses, and signalling pathways that ensure proper fertilization, the natural balance between ROS and antioxidants is essential [86]. In line with our results, Aslan et al., found that ginseng utilization dramatically increased plasma TAC and decreased total levels of oxidative stress in testicular damage caused by cisplatin [72]. Also, According to De Freitas et al., ginseng metabolite (GIM-1) increased SOD, CAT, and GPx levels in human Sertoli cells, reducing oxidative stress and apoptosis. [87].

Further evidence that the anti-apoptotic and anti-inflammatory activities of ginseng includes the effective alleviation of seminal vesicle and testicular histopathological alterations, restoration of spermatogenesis, reduction of the immune response to the proinflammatory cytokine (TNF-α), and restriction of apoptosis through the restoration of the balance between BAX and Bcl-2 includes the effective alleviation of seminal vesicle and testicular histopathological alterations, restoration of spermatogenesis, reduction of the immune response to the proinflammatory cytokine (TNF-α), and restriction of apoptosis through the restoration of the balance between BAX and Bcl-2 [88] . Similarly, Ghamry et al., [88] observed that ginseng's potent affinity for Bcl-2, caspase, and inflammatory cytokine receptors may be the cause of suppressing the apoptotic and inflammatory mechanism associated with exposure to cypermethrin [89].

Conclusion

In the current work, an animal model of testicular injury caused by duloxetine and an intervention using ginseng extract was constructed. Long-term Dulo usage in rats resulted in testicular toxicity through oxidative stress and inhibited testosterone synthesis, which may have an impact on reproductive processes. However, due to its antioxidant, anti-inflammatory and anti-apoptotic properties combination therapy of Dulo and GE was more effective in protecting the testicles. As a result, using GE is recommended anytime antidepressant drugs such as Dulo are taken. Further study is needed to clarify its mode of action and protective benefits.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval:

Animal Care at Zagazig University's Faculty of Veterinary Medicine in Zagazig, Egypt, authorized this study in accordance with ethical standards and political coniderations (approval no. ZU-IACUC/2/F/226/2024).

TABLE 1. Mitigating potentials of GE against Duloxetine-induced alterations in relative testes weight, serum testosterone, LH, and FSH levels in male rats.

Treatments	Relative testes weight (%)	Total testosterone (ng/mL)	LH (u IU –mL)	FSH (u IU –mL)
Control	2.45 ± 0.017 [a]	5.29 ± 0.25 [a]	24.19 ± 1.19 [a]	4.47 ± 0.33 [a]
Dulo 2.7	1.63 ± 0.08 [b]	1.90 ± 0.15 [d]	6.63 ± 0.37 [d]	1.54 ± 0.11[cd]
Dulo 5.4	0.80 ± 0.17 [c]	0.87 ± 0.14 [e]	2.88 ± 0.14 [e]	0.62 ± 0.04 [d]
Dulo 2.7 + GE	1.63 ± 0.14 [b]	4.16 ± 0.36 [b]	20.15 ± 0.79 [b]	3.09 ± 0.25 [b]
Dulo 5.4 + GE	1.55 ± 0.03 [b]	3.24 ± 0.16 [c]	12.74 ± 0.69 [c]	2.21 ± 0.27 [bc]
P –value	0.001	0.001	0.001	0.001

Values are depicted as mean ± SE. a-e Different superscript in the same column indicating significance different at $P < 0.05$.3.3. Cardiac enzymes

TABLE 2. Mitigating the potential of (GE) on testicular oxidative and antioxidant biomarkers in Duloxetine-treated rats.

Treatments	MDA (nmol/mg)	TAC (ng/mg)
Control	0.75 ± 0.05 [d]	6.05 ± 0.33 [a]
Dulo 2.7	2.32 ± 0.15 [c]	2.74 ± 0.16 [c]
Dulo 5.4	8.57 ± 0.28 [a]	0.81 ± 0.04 [d]
Dulo 2.7 + GE	1.01 ± 0.05 [d]	4.95 ± 0.16 [b]
Dulo 5.4 + GE	4.30 ± 0.32 [b]	2.17 ± 0.16 [c]
P-value	0.001	0.001

Values are depicted as mean ± SE. a-e Different superscripts in the same column indicate a significant difference at $P < 0.05$.

TABLE 3. Histopathological lesion scoring in testes and seminal vesicles of the experimental groups

Lesions	Control	Dulo 2.7	Dulo 5.4	Dulo 2.7 + GE	Dulo 5.4 + GE
Testes					
Degeneration of spermatogenic cells	0	2	3	1	1
Necrosis of spermatogenic cells	0	2	3	0	0
Sperm coagulum	0	2	3	0	1
Seminal vesicle					
Muscolosa degeneration	0	1	3	0	1
Muscolosa mononuclear infiltration	0	1	3	0	1
Adventitia edema and mononuclear infiltration	0	00	3	0	1

The scoring system was designed as 0 = absence of the lesion in all rats of the group (n= 5), 1= (<30%), 2= (<30% – 50%), and 3= (>50%).

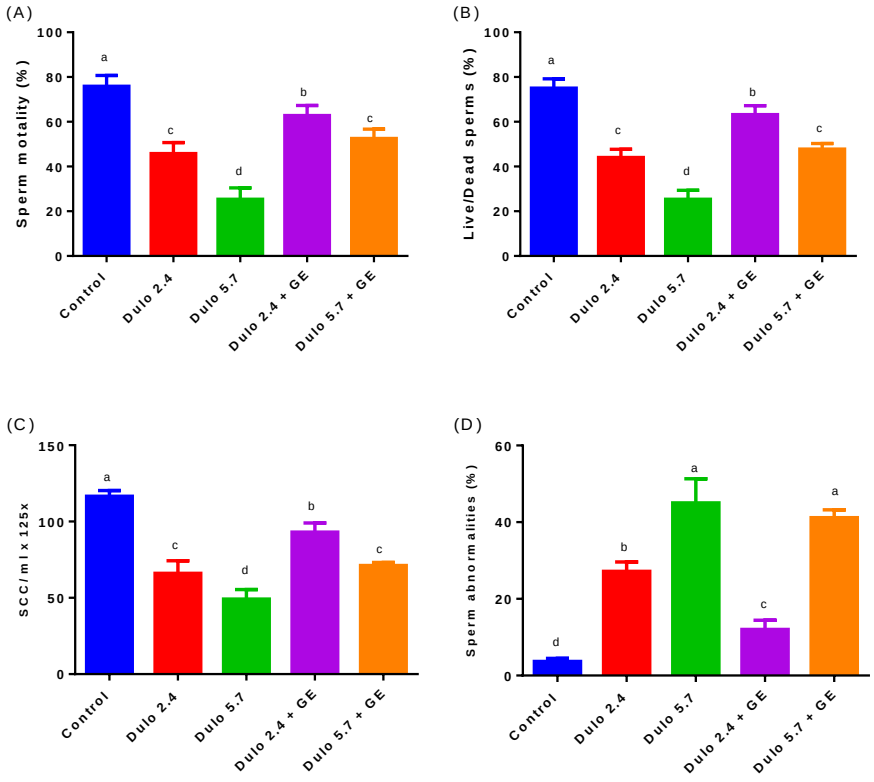

Fig. 1. GE's effects on the measures of semen quality in rats treated with duloxetine, where A stands for sperm motility %, B for live/dead sperm ratio, C for SCC, and D for sperm abnormalities %. The values are shown as mean ± SE. A significant difference at $P < 0.05$ is indicated by different superscripts.

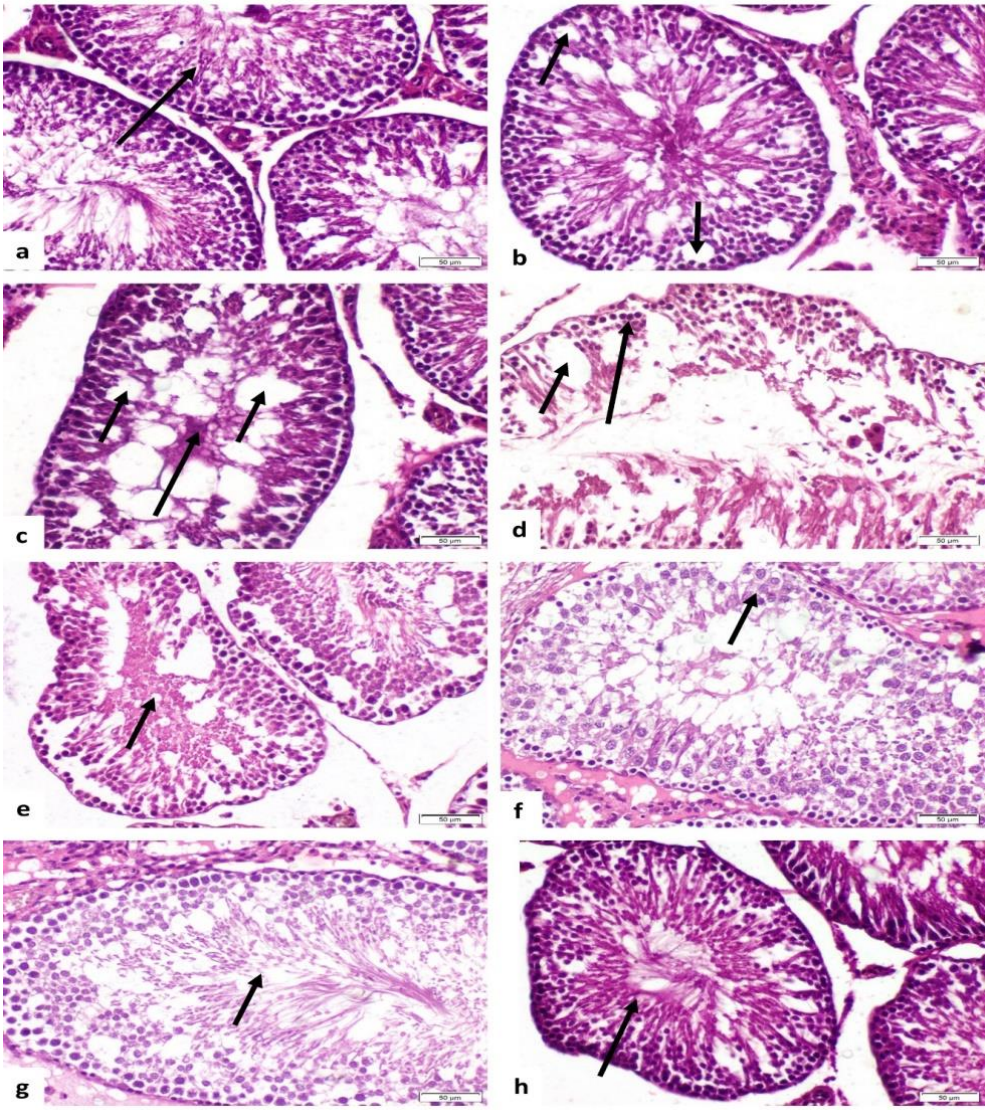

Fig. 2. Photomicrograph, rat testes (Sections stained with H&E, scale bar 50 µm). (a) control group showing a normal histological structure of seminiferous tubules (arrow). (b) Dulo 2.7 treated group showed necrosis and vacuolation of spermatogenic cells (arrows). (c) Dulo 2.7 treated group showed the presence of sperm coagulum (long arrow) and vacuolation of spermatogenic cells (short arrow). (d) Dulo 5.4 treated group exhibited massive necrosis of spermatogenic cells (long arrow) and vacuolation of spermatogenic cells (short arrow). (e) Dulo 5.4 treated group showing coagulation of sperms in seminiferous tubules lumen (arrow). (f) Dulo 2.7 +GE treated group showed normal spermatogenic cells (arrow). (g) Dulo 2.7 +GE treated group showed normal sperm formation in seminiferous tubules lumen (arrow). (h) Dulo 5.4 +GE treated group showed nearly normal seminiferous tubules (arrow).

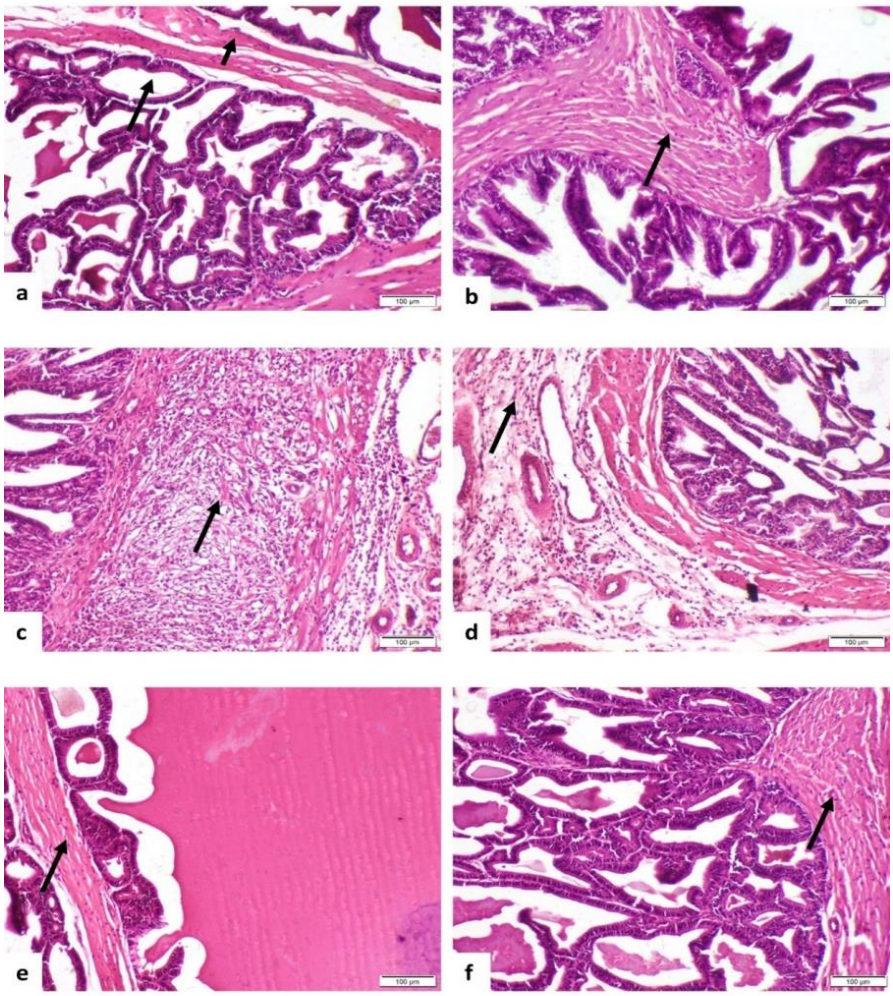

Fig. 3. **Photomicrograph, rat seminal vesicle (Sections stained with H&E, scale bar one hundered µm).** (a) long arrow indicate normal histological structure of mucosa and short arrow indicate normal histological structure muscolosa. (b) Dulo 2.7 treated group showing mild infiltration of muscolosa with mononuclear inflammatory cells (arrow). (c) Dulo 5.4 treated group showing severe destruction and necrosis of smooth muscles of muscolosa layer with infiltration of mononuclear inflammatory cells (arrow). (d) Dulo 5.4 treated group showing edema and severe mononuclear inflammatory cells infiltration in tunica adventitia (arrow). (e) Seminal vesicle structure is normal (arrow) in the Dulo 2.7 +GE treated group. (f) The muscolosa of the Dulo 5.4 + GE-treated group exhibits a little infiltration of mononuclear inflammatory cells (arrow).

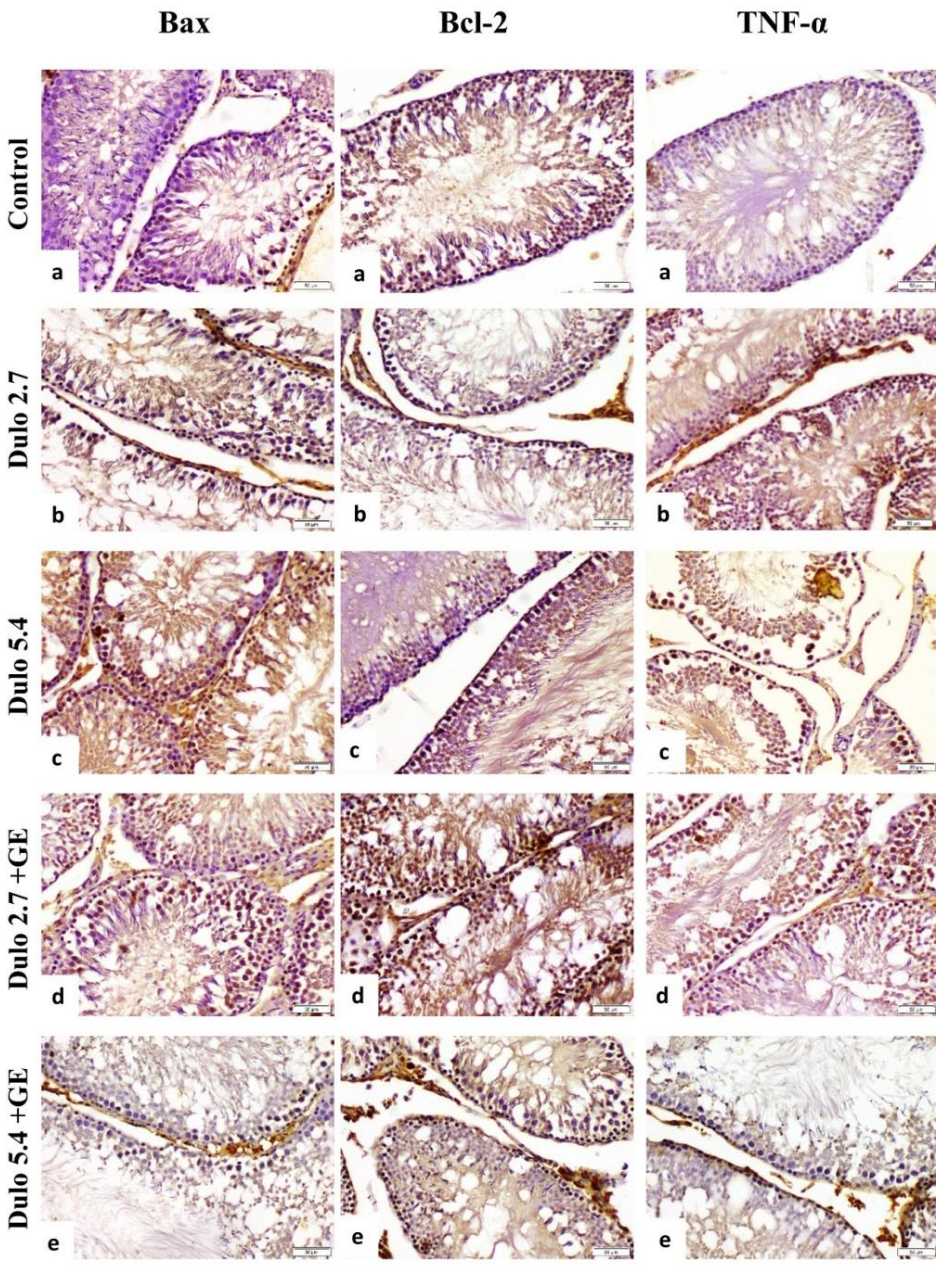

Fig. 4. Testicular immunostaining of BAX, Bcl-2, and TNF-α (sections stained with BAX, Bcl-2, and TNF-α, scale bar 50 µm). (a) Bcl-2 expression was robust while Bax and TNF-α expression was very poor in the control group. (b) The group treated with Dulo.2.7 exhibited weak Bcl-2 expression and moderate immuno-expression of TNF-α and Bax markers. (c) The group treated with Dulo 5.4 had weak Bcl-2 expression and significant Bax and TNF-α expression. (d) The group treated with Dulo 2.7 + GE exhibited significant Bcl-2 expression and a poor immunological response to Bax and TNF-α. (f) The group treated with Dulo 5.4 + GE exhibited robust Bcl-2 immuno-reactivity and modest Bax and TNF-α.

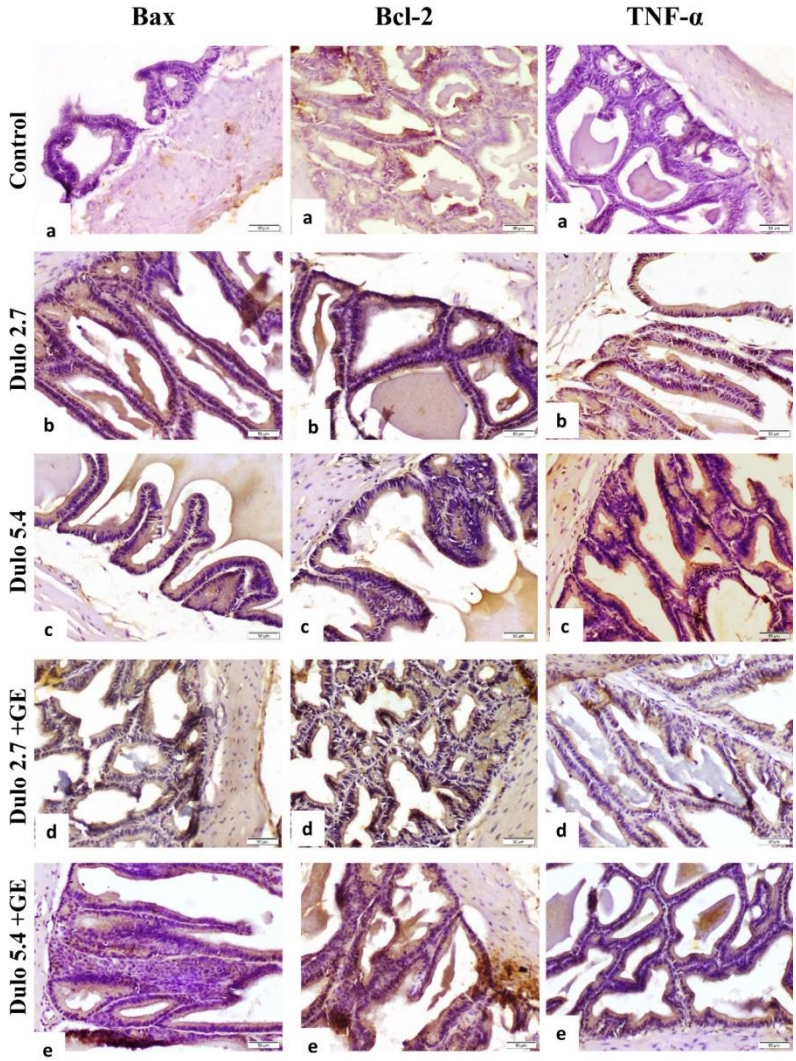

Fig. 5. Seminal vesicle immunostaining of BAX, Bcl-2, and TNF-α (sections stained with BAX, Bcl-2, and TNF-α, scale bar 50 µm). (a) Bcl-2 expression was robust, while Bax and TNF-α expression was very poor in the control group. (b) The group treated with Dulo 2.7 exhibited poor Bcl-2 and moderate immuno-expression of TNF-α and Bax. (c) The group treated with Dulo 5.4 had weak Bcl-2 expression and significant Bax and TNF-α expression. (d) The group treated with Dulo 2.7 + GE exhibited a substantial expression of Bcl-2 immuno-reactivity and a decrease in Bax and TNF-α immune-reactivity. (f) The group treated with Dulo 5.4 + GE exhibited robust Bcl-2 immunoreactivity and modest Bax and TNF-α.

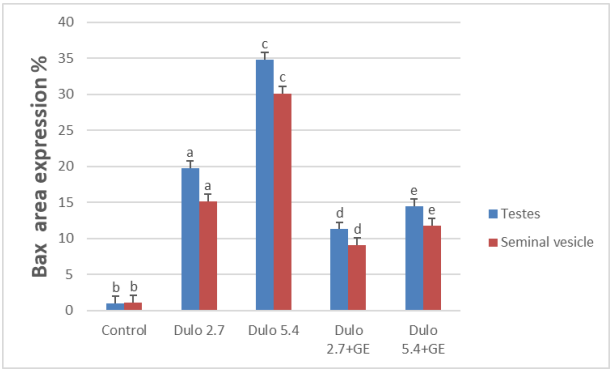

Fig. 6. Immunostaining expression of BAX proteins area % in testes and seminal vesicles of the experimental groups. The data was presented as mean ±SE, where a significant difference at $P < 0.05$ was indicated by a distinct letter.

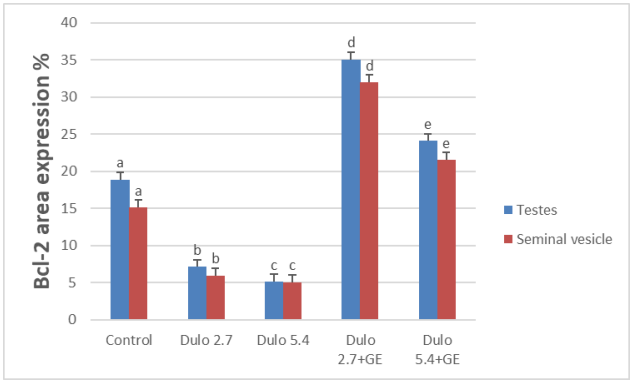

Fig. 7. Immunostaining expression of Bcl-2 proteins area % in testes and seminal vesicles of the tested groups. Mean ± SE was used to express the data, and alphabetical letters denoted significant differences at $P < 0.05$.

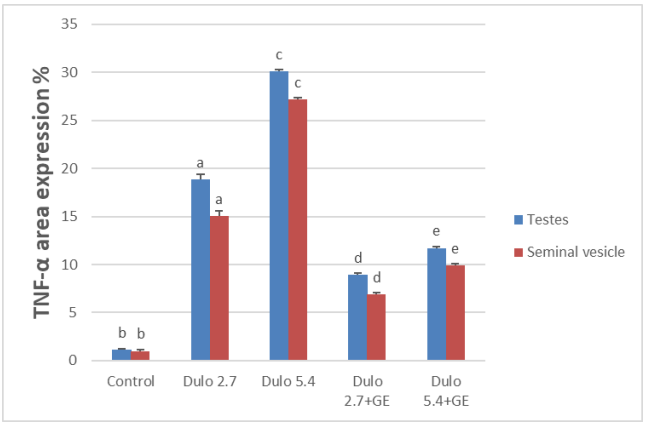

Fig. 8. Immunostaining expression of TNF-α area % in testes and seminal vesicles of different groups. Data was expressed as mean ±SE, with different letters indicating significant differences at $P < 0.05$.

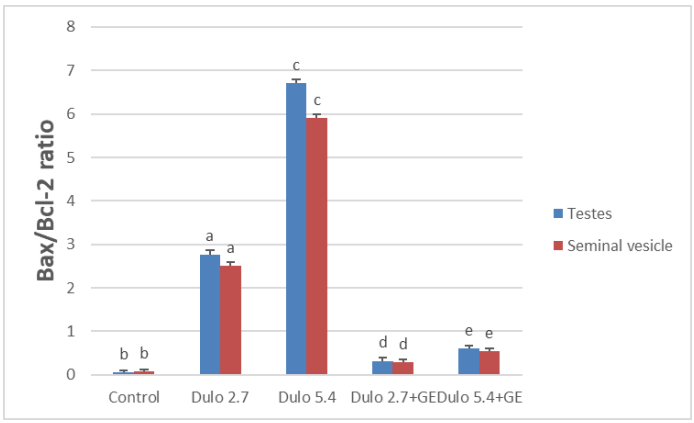

Fig. 9. BAX/Bcl-2 ratio of the experimental groups. The mean ± SE was used to express the data. Significant differences at $P < 0.05$ are indicated by different letters..

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التأثيرات المخففة لمستخرج الجينسنغ الكوري الأحمر ضد الأضرار التأكسدية، والالتهابات، والموت الخلوي الناتجة عن العامل المضاد للاكتئاب (دولوكستين) في الجهاز التناسلي الذكري للفئران

إسراء محمد فهمي[1]، عبد الحميد السيد عبد الحميد[1]، دعاء منصور[2]، سارة بركة[3]، رضا قرني[45]، أسماء عبد الله[6]، آية أشرف فهمي[1]، ريهام العطار[7] وأحمد عبده سعيد[1]

[1] قسم الفارماكولوجيا، كلية الطب البيطري، جامعة الزقازيق، مصر.
[2] قسم الكيمياء الحيوية وكيمياء التغذية، كلية الطب البيطري، جامعة مدينة السادات، مدينة السادات، مصر.
[3] قسم مركبات الطبيعة الكيميائية، المركز القومي للبحوث، الجيزة 12622، مصر.
[4] قسم الباثولوجيا، كلية الطب البيطري، جامعة القاهرة، القاهرة، مصر.
[5] قسم الباثولوجيا ، كلية الطب البيطري، الجامعة المصرية الصينية، مصر.
[6] قسم التوليد والتناسل، كلية الطب البيطري، جامعة الزقازيق، مصر.
[7] زميل بحثي في الصيدلة بالإدارة العامة للشؤون الطبية، مصر.

الملخص

يرتبط الاستخدام طويل الأمد للأدوية المضادة للاكتئاب بالعديد من التأثيرات السلبية على الكبد والكلى والجهاز التناسلي. يُعتبر الجينسنغ الأحمر نباتًا غنيًا بالصابونين ويُعرف بخصائصه البيولوجية القيمة. استهدفت هذه الدراسة التحقيق في التأثير الوقائي للمستخلص المائي من الجينسنغ الأحمر ضد التأثيرات التنكسية لدواء دولوكسيتين - أحد الأدوية المضادة للاكتئاب المعروفة - على الجهاز التناسلي الذكري. تم إعطاء دولوكسيتين للفئران الذكور بجرعتين (٢.٧ و٥.٤ مليغرام لكل كيلوغرام من وزن الجسم عن طريق الفم)، بينما تم إعطاء الجينسنغ الأحمر بجرعة ١٠٠ مليغرام لكل كيلوغرام من وزن الجسم عن طريق الفم لمدة شهرين متتاليين. تم تقييم جودة السائل المنوي، ووزن الخصيتين النسبي، ومستويات هرمونات الجنس في المصل، وحالة الأوكسيد/مضاد الأوكسيد في الخصيتين، بالإضافة إلى التغيرات الهيستولوجية والمناعية الكيميائية في الحويصلات المنوية والخصيتين. أظهرت النتائج أن تناول دولوكسيتين تسبب في انخفاض كبير في حركة الحيوانات المنوية، وتركيزها، مع زيادة في نسبة التشوهات، بالإضافة إلى انخفاض ملحوظ في وزن الخصيتين واضطراب في هرمونات الجنس في المصل، مع تثبيط آلية الدفاع المضادة للأوكسيد في الخصيتين. كما لوحظت تغيرات هيكلية وتفاعلات التهابية في الخصيتين والحويصلات المنوية. ومع ذلك، أدى إعطاء الجينسنغ الأحمر إلى تحسن كبير في جميع هذه التغيرات، حيث حافظ على جودة السائل المنوي، ووزن الخصيتين، وتوازن هرمونات الجنس، كما خفف من التوتر التأكسدي في الأنسجة الخصوية. علاوة على ذلك، ساعد الجينسنغ الأحمر في الحفاظ على الهياكل الطبيعية للخصيتين والحويصلات المنوية، وقلل من التعبير الزائد لعامل نخر الورم ألفا وبي إيه إكس، بينما زاد من التعبير عن بي سي إل ٢ في الخصيتين والحويصلات المنوية. في الختام، يمكن الاستنتاج أن الجينسنغ الأحمر يمتلك تأثيرات وقائية قوية ضد التأثيرات السامة لدولوكسيتين على الغدد التناسلية الذكرية، مما يجعله مرشحًا مثاليًا للدراسات المستقبلية كعلاج فعال لحماية الجهاز التناسلي الذكري .

الكلمات الدالة: دولوكسيتين، مستخلص الجينسنغ، تلف الخصيتين، السائل المنوي، الأوكسدة، الاستماتة، الفئران.