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Protective Effect of Ginseng against Dexamethasone-Induced Testicular Injury in Adult Male Rats

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Abstract.The current study was carried out to evaluate the toxic effect of dexamethasone (DEX) on the testis of adult male rats and the possible protective effect of the ginseng (GIN) against DEX-induced toxicity. Twenty-four adult male albino rats were used and divided into four equal groups. Group I: control rats, group II: rats received GIN only, group III: rats received DEX only and group IV: rats received DEX plus GIN. At the end of the treatment period, all rats were sacrificed under anesthesia. Sera and testicular tissues were collected for various biochemical, histopathological and immunohistochemical examinations. Obtained results showed that, treatment with DEX caused a significant decrease in both serum concentration of testosterone and levels of testicular antioxidant markers including reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities. On contrary, DEX produced increase in testicular content of malondialdehyde (MDA). In parallel, treatment with DEX induced destructive effects on the testicular tissue as reduced spermatogenic cells, degeneration of Leydig cells, appearance of necrotic areas, deposition of collagen fibers, and trigger apoptosis through decreasing Bcl-2 and increasing Bax. Treatment with GIN attenuated DEX-induced adverse changes in serum testosterone levels, tissue antioxidants, apoptosis and histology of testis. These results suggested the ability of GIN to protect testis against DEX-induced tissue injury and dysfunction in adult male rats.

keywords: Dexamethasone, Ginseng, Testis, Oxidative stress, Apoptosis

1. Introduction

Dexamethasone (DEX) is one of the glucocorticoid drugs that commonly used as anti-inflammatory and immunosuppressive drug all over the world [1,2]. However, excessive consumption of DEX inhibits male reproduction because Leydig cells in the testis are one of the targets of glucocorticoid drugs which in turn lead to inhibition of testosterone secretion that are essential for the onset of male puberty and spermatogenesis [3]. In clinical studies, children with systemic lupus erythematosus who received a cumulative dose of DEX greater than 400 mg/kg had a puberty delay [4]. Also, maternal treatment with DEX during lactation has been reported to affect adversely the development of the reproductive system in offspring [5]. It has been reported that, oxidative stress and production of free radicals were implicated in DEX-induced testicular tissue injury and dysfunction [1,6].

Ginseng (GIN) has been used as a traditional remedy for many thousand years, and it became one of the most famous and precious herbal medicines consumed worldwide [7]. The medicinal properties of GIN include its anticancer, antidiabetic and immunomodulatory effects; and its ability to relief oxidative stress [8,9]. The pharmacological properties of GIN have been attributed to the bioactive components in this herb, which include ginsenosides, saponins, flavonoids, polyphenols and volatile oils [8-10]. Previous studies showed that GIN helps to improve the reproductive function and increase sperm quality in patients with fertility problems [11]. In addition, administration of red GIN in old-aged rats had a beneficial role in age-induced dysfunction of spermatogenesis [12]. Improvement of reproductive function and male

fertility by GIN might be due to its potent antioxidant ability [9], direct effect of GIN on the pituitary gland as it reduces prolactin production or an effect on the central nervous system leading to increase dopaminergic action that causes reduction in the production of prolactin [13]. It is also suggested that ginsenosides may have an effect at different levels of the hypothalamus-pituitary-testis axis [14,15].

The present study was conducted to evaluate both the toxic effects of DEX on the testis and the potential protective effects of GIN against biochemical, histopathological and immunohistochemical alterations induced by DEX in adult male rats.

2. Materials and methods

Chemicals

DEX was manufactured by Amriya for pharmaceutical industries, Alexandria, Egypt. GIN was manufactured by EIPICO (10^{th} of Ramadan City, Egypt). All other chemicals used were of analytical grade.

Experimental animals

Twenty-four male Wistar albino rats weighing between 130 and 150g were purchased from the animal house of VACSERA (Cairo, Egypt) and used in the present study. Rats were housed in stainless cages under controlled environmental conditions of a temperature (23 ± 2 °C) and photoperiod of 12 h light/12 h dark cycle, in the animal house of the Faculty of Science, Mansoura University. All animals fed on a standard laboratory diet and given water ad libitum. Under these controlled conditions, rats were kept for one week before the beginning of the experiment for acclimatization. All policies and procedures were approved by the Animal Care and Institutional Ethics Committee of Mansoura University, Egypt.

Animal groups and treatments

Rats were divided into four equal groups, 6 rats each, as follows:

Group 1: rats received no treatment and served as control.

Group 2: rats given GIN orally at a dose of 200 mg/kg b. wt. [16].

Group 3: rats given DEX (7 mg/kg b.wt) via ip injection [17].

Group 4: rats given GIN orally (200 mg/kg b.wt) and DEX (7mg/kg b.wt) via ip injection.

All treatments were given for 10 consecutive days.

Rats were sacrificed under ether anesthesia at the end of the treatment period. Blood samples were collected and left to clot then, centrifuged for 15 min at 3000 rpm. Serum was collected in clean tubes and stored at -20 °C for biochemical analysis. Meanwhile, the testes were quickly removed and washed. The right testis was homogenized in cold distilled water and centrifuged at 3000 rpm for 15 min. The clear homogenates were collected and stored at -20 °C for subsequent biochemical assays. The left testis of each rat was instantly fixed in 10% neutral buffered formalin for histological and immunohistochemical examinations.

Biochemical analysis

Serum level of total testosterone was assayed by ELISA according to Rosner *et al*. [18]. MDA level was determined in testis homogenate according to the method of Ohkawa *et al*. [19]. Testicular content of GSH was evaluated according to the method of Beutler *et al.* [20]. The activities of testicular CAT and SOD were measured on basis of the method of Aebi [21] and Nishikimi *et al.* [22], respectively.

Histopathological study

Slices of fixed testis in 10% neutral buffered formalin were dehydrated in ascending series of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Then, thin sections were cut (5μm) for each section and stained with hematoxylin/eosin (H&E) and Masson's trichrome. The stained sections were examined under the light microscope for histopathological studies.

Immunohistochemical study

In brief, paraffin sections of the testis (5μm) were de-paraffinized, rehydrated and then treated with citrate buffer solutions (0.01 mol/L, pH 6.0). Slides were incubated with primary antibodies (Bax and Bcl-2) at 4°C overnight. Diaminobenzidine was used for visualization. The slides were counterstained with hematoxylin staining and examined using

a light microscope.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5.0 software (San Diego, California, USA). Statistical comparisons were performed by one way analysis of variance (ANOVA), followed by Neuman-Keuls post-hoc test. Differences were considered statistically significant at $P \leq 0.05$.

3. Results

Obtained results in Fig. (1) show that exposure of male rats to DEX in a dose of 7mg/kg/day for 10 consecutive days caused significant decreases in the concentration of testosterone in the serum, as compared to control group. However, treatment with GIN (200 mg/kg/day) along with DEX for the same period markedly increased serum testosterone levels, as compared to DEX-treated group.

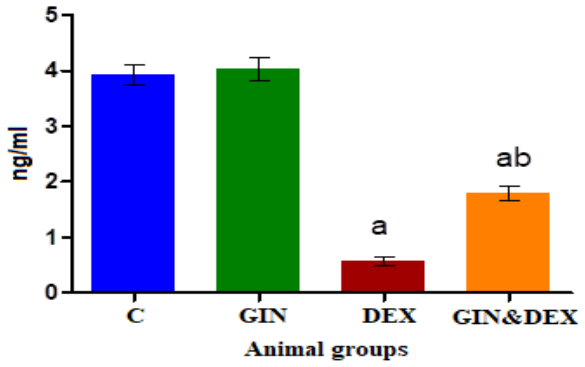

Fig. 1. Mean serum of testosterone concentration in experimental groups. C: control, GIN: ginseng, DEX: dexamethasone; Values were expressed as means ± SE (n= 6); (a, b) indicate the significant change at $P \leq 0.05$.

a = significant change when compared to control; b = significant change when compared to DEX group.

Table 1: Effect of ginseng on dexamethasone-induced changes in testicular oxidative stress markers.

Indices	Control	GIN	DEX	GIN& DX
MDA (nmol/g)	258.30±4 .83	257.90 ±4.26	368.70 ±14.96[a]	308.00± 9.02[ab]
GSH (nmol/g)	16.40 ±0.85	17.39 ±0.86	9.43 ±0.45[a]	12.72 ±0.45[ab]
SOD (U/g)	245.80± 2.35	247.30 ± 1.75	181.70 ± 3.36[a]	223.20 ±2.29[ab]
CAT (U/g)	144.80±1 .72	147.30 ±2.21	109.30 ±2.64[a]	129.50 ±1.95[ab]

GIN: ginseng, DEX: dexamethasone, MDA: malondialdehyde, GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase.

Values were expressed as means ± SE (n= 6).

(a, b) indicate the significant change at $P \leq 0.05$.

a = significant change when compared with control, b = significant change when compared with DEX group.

In table (1), treatment of rats with DEX significantly increased the testicular content of MDA and decreased testicular contents of GSH as well as the activities of SOD, and CAT enzymes in comparison with the control group. Meanwhile, combined treatment with GIN and DEX significantly lowered the concentration of MDA and raised the levels of antioxidant parameters (GSH, SOD and CAT) in testis homogenate, when compared to DEX-treated group.

Histopathological examination of control testis sections stained with H & E in Fig. (2A) showed normal testicular architecture without any pathological alternations. Seminiferous tubules are composed of different types of germ cells including spermatogonia (SG), spermatocytes (SP), spermatids (SD) and spermatozoa (SZ). Sertoli cells (SC) were distinguished between the spermatogenic cells and lumen (L) appeared in the middle of the seminiferous tubules. The interstitial tissue between the seminiferous tubules contains typical Leydig cells (LC).

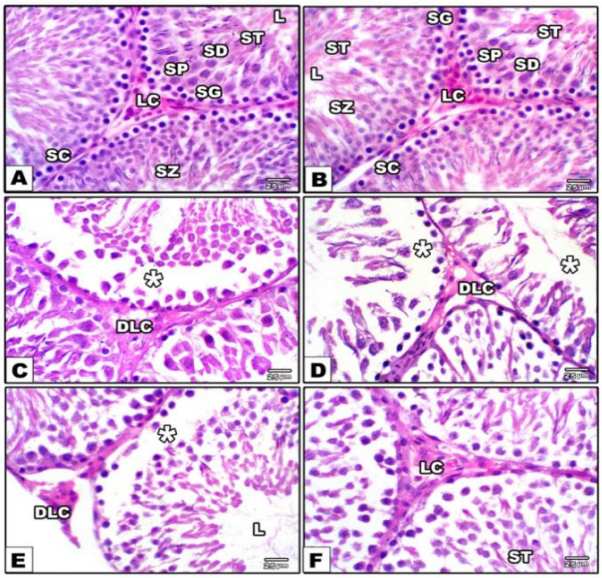

Fig. 2. Photomicrographs of rat testis

sections in the different experimental groups stained with haematoxylin and eosin (H&E). (A): Section from testis of control group. (B): Section from testis of GIN group. Both (A) and (B) sections reveal normal structure of the testicular tissue. (C & D & E): Sections from testis of DEX group mainly show a marked loss of germ cells and existence of wide separation between germ cells inside the seminiferous tubules (asterisk), and degeneration of Leydig cells (DLC). (F): Section from testis of group treated with DEX plus GIN simultaneously exhibit marked improvement of the histological architecture of testicular tissue.

Examination of the testis sections of GIN group showed the same normal structure of testicular tissue as in control group (Fig. 2B). As shown in Fig. (2C, D, E), DEX administration caused severe histological abnormalities including degenerative germ cells, marked loss of various spermatogenic cells, presence of necrotic areas, breakup between germs cells (asterisk) and degenerated Leydig cells (DLC). Treatment of DEX group with GIN simultaneously led to attenuation of the tissue damaging effect of DEX. So, GIN appeared to be effective in the protection of testicular tissue against DEX-induced histopathological alterations in adult male rats (Fig. 2F).

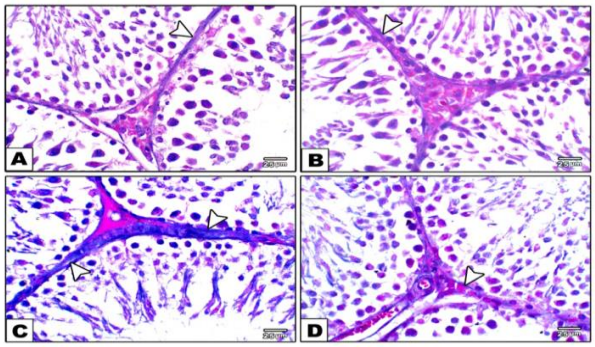

Fig. 3. Photomicrographs of rat testis sections in the different experimental groups stained with Masson's trichrome. (A): Section from testis of control group. (B): Section from testis of GIN group. Both (A) and (B) sections reveal normal pattern of collagen fibers deposition (arrow head). (C): Section from testis of DEX group display marked accumulation of collagen fibers around seminiferous tubules (arrow head). (D): Section from testis of group treated with DEX plus GIN simultaneously show reduction in collagen fibers deposition around seminiferous tubules (arrow head).

Masson's trichrome-stained sections of control group visualized a normal pattern of collagen fibers deposition as a thin layer around the seminiferous tubules (Fig. 3A). GIN group showed the same normal pattern of collagen fibers deposition as in control group (Fig. 3B). However, Masson's trichrome-stained sections of the DEX group revealed a marked increase in the collagen fibers deposition between seminiferous tubules (Fig. 3C). Co-treatment of DEX and GIN displayed a marked decrease in collagen fibers deposition between seminiferous tubules, as compared to the DEX group, suggesting ability of GIN to protect against DEX-induced testicular fibrosis (Fig. 3D).

Immunohistochemical examinations showed intense positive Bcl-2 immunostaining (deep brown color) in testicular tissue of both control group (Fig. 4A) and GIN-treated group (Fig. 4B). While, sections from the DEX-treated rats exhibited negative Bcl-2 immunostaining (Fig. 4C). However, rats treated with GIN and DEX simultaneously showed an increase in Bcl-2 positive cells, as compared to the DEX group (Fig. 4D). Regarding Bax examination, either control (Fig. 5A) or GIN-treated group (Fig. 5B) showed a negative immunoreactivity for Bax in the testicular tissues. While, intense positive Bax immunostaining (deep brown color) was found in spermatogenic cells and Leydig cells of the DEX group (Fig. 5C). Combined treatment with DEX and GIN, however, showed weak immunoreactivity for Bax, when compared with the result of DEX group (Fig. 5D). Obtained results of Bcl-2 and Bax provided evidence that GIN has anti-apoptotic effect because it inhibited DEX-induced apoptosis in the testis.

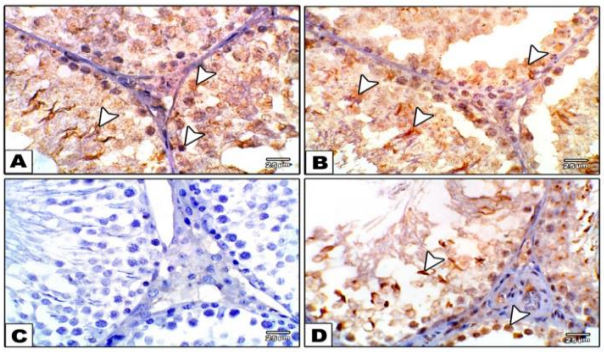

Fig. 4. Photomicrographs of rat testis sections in the different experimental groups

immunostained with Bcl-2. (A): Section from testis of control group. (B): Section from testis of GIN group. Both (A) and (B) sections reveal strong positive immunostaining for Bcl-2 (arrow head). (C): Section from testis of DEX group show negative immunoreactivity for Bcl-2. (D): Section from testis of group treated with DEX plus GIN simultaneously show positive immunoreactivity for Bcl-2 (arrow head).

4. Discussion

Treatment with DEX is frequently associated with cellular toxicity to various target tissues. Testis has been found to be one of the target organs of DEX, so it can adversely affect this organ causing pathophysiological alterations and testicular dysfunction [23,24]. The pathophysiology of testis induced by glucocorticoids was attributed to production of oxidative stress and tissue injury [23,25]. Currently, attempts have been made to determine the best therapeutic regimen that can alleviate oxidative stress for prevention of testicular disorder induced by glucocorticoids. The present study was, therefore, designed to assess the protective effects of GIN against DEX-induced testicular injury in adult male rats.

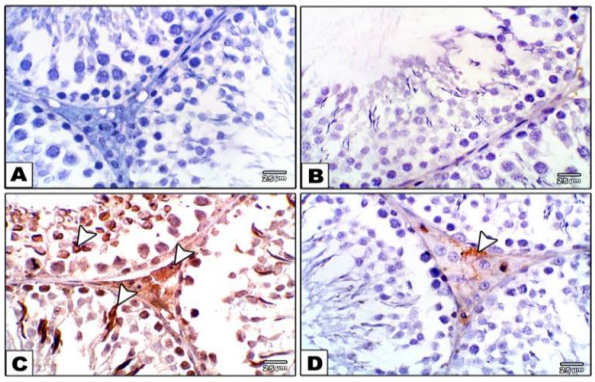

Fig. 5. Photomicrographs of rat testis sections in the different experimental groups immunostained with Bax. (A): Section from control group. (B): Section from GIN group. Sections of (A) and (B) display negative immunostaining for Bax. (C): Section from DEX group exhibits positive Bax immunostaining (deep brown) in the spermatogenic cells and Leydig cells (arrow head). D: Section from group treated with DEX & GIN reveals weak positive immunostaining for Bax (arrow head).

Obtained results of the present study showed that, DEX administration in male adult rats produced a significant decline in serum total testosterone levels, suggesting Leydig cells injury and testicular dysfunction. Similar findings of DEX-induced disturbances in hormonal secretion of testis were recorded in animal studies (23,26,27]. DEX-induced deprivation of testosterone may be due to the effect of the drug on hypothalamus and pituitary gland which led to inhibition of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion. In addition, ability of DEX to induce damage to Leydig cell and to decrease the sensitivity of it to gonadotropins resulted in reduction in testosterone biosynthesis and secretion [5,6,28].

It has been reported by several previous studies that the herbal medicines were effectively used in the treatment of various diseases and protection against chemicals-induced toxicity, when these herbal medicines are used in combination with conventional therapeutics [29]. In the current study, we examined the protective impact of ginseng (GIN) against DEX-induced testicular toxicity in adult male rats. Obtained results showed that, administration of GIN in male rats treated with DEX, concurrently, resulted in a significant increase in serum total testosterone concentrations, as compared to the result of DEX group. This finding suggested amelioration of disorders of both hypothalamus–pituitary–testicular axis and Leydig cells function, due to administration of GIN [30,31]. Present result is in accordance with results published by several studies on the effect of GIN on serum testosterone level. Omar and Abdalhafid [32] reported that GIN administration in rabbits led to increases in sperm counts and testosterone levels, and they suggested that GIN possesses pro-fertility properties which might be a product of both its potent androgenic activities and antioxidant properties. Ok *et al*. [33] found that Wild GIN administration prevents the decrease in testosterone levels induced by bisphenol A. In another study, Khodir *et al*. [34] demonstrated that GIN attenuates the lowering effect of doxorubicin on serum testosterone levels in male rats.

Testicular dysfunction in DEX-treated animals might be secondary to oxidative tissue damaging effect of this drug [6]. Supporting

this, current study provided evidence that DEX has the ability to induce oxidative stress and hence testicular tissue damage, since obtained results showed significant elevation in the testicular content of MDA accompanied by decreased testicular antioxidant parameters including GSH, SOD and CAT in DEX-treated adult male rats. The present findings are consistent with several published studies. Mukherjee *et al*. [1] observed an increase in MDA content accompanied by significant decreases in activities of antioxidant enzymes SOD, CAT and glutathione peroxidase (GPx) in testis of golden hamsters after treatment with DEX. Similar results have been reported in male offspring of Wistar rats following maternal treatment with DEX during lactation [6]. It seems clearly that, the ability of DEX to induce oxidative stress in the testicular tissue is implicated in the mechanisms by which DEX-lowered serum level of testosterone, because oxidative stress might cause either degeneration of Leydig cells or inhibition of gonadotropin secretion [35].

Malondialdehyde (MDA) is a product of lipid peroxidation, so it used as a marker of oxidative stress, which reflects a disturbance in the balance of redox state of cells and production of free radicals [36,37]. Both GSH (a non-enzymatic antioxidant) and antioxidant enzymes can stabilize the redox state of the cells, consequently they can play a big role in the defense mechanism against free radicals and reactive molecules-induced cell injury. GSH functions in the first line of the defense mechanism against free radicals-induced cell injury. It can act as a reducing agent for oxidant molecules (such as, H_2O_2 and hydroperoxides) as it donates hydrogen, and this process is catalyzed mainly by glutathione peroxidase (GPx) enzyme. Also, GSH is used in detoxification of xenobiotics through conjugation reactions which are catalyzed by glutathione-S-transferase (GST) enzyme. On the other hand, both SOD and CAT can also exert an effective role in the first line used for cellular protection against free radicals. SOD can catalyze the dismutation of superoxide radicals ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2) [38]. While, CAT catalyzes the breakdown of H_2O_2 into H_2O and O_2 [39]. Therefore, decline in both cellular GSH content and antioxidant enzymes activity (such as SOD and CAT) in the testicular tissue of rats treated with DEX can lead to an imbalance between redox state of the cells and production of free radicals leading ultimately to oxidative stress and lipid peroxidation (increased production of MDA).

In the current study, oral administration of GIN suppressed testicular lipid peroxidation and oxidative stress as reflected by markedly decreased the concentration of MDA associated with a significant increase in the activities of antioxidant enzymes CAT and SOD as well as GSH content in the testis of DEX-treated rats. This result could explain the antioxidative impact of GIN against free radical and oxidative stress produced by the action of DEX. The present finding is in line with the finding of Elgharabawy and Emara [40] who observed that GIN protect the testis of rats against oxidative damage induced by chromium picolinate through normalizing the levels of testicular antioxidant parameters including GSH, SOD, and CAT. In another study, treatment with GIN effectively protected against lipid peroxidation induced by cadmium in testis, as indicated by decreased testicular content of MDA [41]. Also, GIN significantly increased plasma levels of total antioxidant capacity and decreased levels of total oxidative species in cisplatin-induced testicular damage in rats [42]. Moreover, Oh *et al*. [43] and Yokozawa *et al* [44] reported that administration of ginsenoside Rb2 or ginsenoside-Rd to mice led to increase in the enzymatic and non-enzymatic antioxidant parameters, and decrease in lipid peroxidation. The antioxidant properties and free radical scavenging activity of GIN could be attributed to its phenolic, flavonoid and ginsenosides contents which can inhibit oxidative stress and possibly increase the antioxidant enzymatic activity [8,45].

Regarding histopathological examinations, H&E sections exhibited that administration of DEX in male rats induced testicular tissue damage, as manifested by degenerative changes including loss of spermatogenic cells, appearance of necrotic areas inside seminiferous tubules, detachment of spermatogenic cells from the basement membrane of the seminiferous tubule and degeneration of interstitial tissue, which contain

Leydig cells. Obtained histopathological changes in the testis which showed marked signs of tissue defects could explain a part of the reasons beyond the reduction of serum testosterone level, and seems to be secondary to the induction of oxidative stress and lipid peroxidation following treatment with DEX in adult male rats.

Present histopathological findings are in harmony with the results of several previous studies. Hanafy and Hassan [46] reported that DEX caused several histopathological alterations in testicular tissue of Japanese quail leading to testicular dysfunction. Also, Dare *et al*. [47] reported that DEX produced severe testicular degeneration, seminiferous tubule damage and Leydig cells atrophy. In addition, DEX was found to induce spermatogenesis defects including epithelial vacuolization, sloughing of germ cells, reduction of seminiferous tubule diameter and reduction in the number of sperm heads [48].

Histopathological studies using Masson's trichrome stain in the present study showed marked increase in collagen fibers deposition in the testicular tissue in DEX-exposed rats, when compared to the control. This finding suggested that DEX-induced oxidative stress can cause testicular tissue fibrosis and dysfunction. Similar results were revealed by El-Wassimy *et al*. [49] who reported that DEX increases formation and accumulation of collagen fibers between seminiferous tubules of male rats.

Treatment of DEX group with GIN simultaneously led to amelioration of the histological defects induced by DEX in testicular tissue. The results exhibited that GIN declined the formation and deposition of collagen fibers between seminiferous tubules of rats. However, this beneficial effect of GIN could be attributed to its bioactive ingredients that have the ability to suppress oxidative stress and tissue injury induced by DEX [8,9]. Interestingly, this attribution is supported by obtained results of the current study which demonstrated marked improvement in the testicular antioxidant defense markers by GIN in rats treated with DEX. The present findings are in line with several published studies. Of these, Mahmoud *et al*. [50] reported that GIN has the ability to repair the testicular injury caused by mercury chloride through decreasing fibrosis and collagen fibers accumulation between seminiferous tubules of rats. Also, Kamel *et al.* [51] found that GIN could restore normal histology and spermatogenesis in rats after testicular injury by methotrexate drug. In similar study, Aslan *et al*. [42] stated that GIN improved testicular damage induced by cisplatin in rats.

Apoptosis is a normal biological process which is essential for controlling cell numbers and reducing defective germ cells during spermatogenesis in testicular tissues, so disturbances in apoptosis can result in reproductive dysfunction [52]. It is known that, abnormally accelerated apoptosis of germ cells may lead to an imbalance of cell proliferation and cell death, resulting in spermatogenic impairment [53]. Increased production of free radicals is thought to play a role in testicular cell apoptosis via activation of the mitochondrial pathway, which includes both pro-apoptotic protein (Bax) and anti-apoptotic protein (Bcl-2) [54]. The present immunohistochemical results showed that DEX induced testicular apoptosis as indicated by observed decrease in Bcl-2 and increase in Bax immunoreactivity in the testis of rats.

The present finding of DEX-induced apoptosis is supported by several previous studies. Khorsandi *et al*. [48] and Nassan *et al*. [52] reported that DEX treatment downregulates the anti-apoptotic marker Bcl-2 in the seminiferous tubules in the testis of rats. Mukherjee *et al*. [1] recorded increase in the number of apoptotic cells in the testis of rats, as evidenced by increased Bax/Bcl-2 ratio and caspase-3 expression. Adding to this, Mahmoud *et al*. [55] demonstrated that DEX increased Bax expression in testicular tissue of mice, which led to induction of apoptosis. According to the current finding together with the previous one, the apoptotic effect of DEX could be attributed to its ability to induce oxidative stress and production of free radicals [1,23].

In the current study, treatment of DEX group with GIN successfully resulted in inhibition of DEX-induced testicular apoptosis, as indicated by decreased Bax and increased Bcl-2 in the tissue of the testis. In this line, Liu *et al*. [56] demonstrated that GIN and ginsenoside Rg3

protected mice against testicular damage and reduced spermatogenic cells apoptosis induced by heat stress, as they increased the Bcl-2 immunoreactivity while decreased Bax immunoreactivity in the testis. Moreover, Jin *et al*. [57] reported that GIN has the ability to inhibit apoptosis by increasing endogenous antioxidant status and inhibiting ROS production.

In conclusion, the current findings added support that exposure to DEX in adult male rats induces oxidative stress, apoptosis and histopathological alterations in the testis, accompanied by decline in serum level of testosterone. GIN, as a medicinal plant that has a strong antioxidative activity, can effectively protect against all adverse effects induced by DEX; including oxidative stress, apoptosis and histopathological alterations; in the testis of adult male rats.

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