International Journal of Pharmacology

ISSN 1811-7775

science alert

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OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2016.340.350

Research Article

Impact of *Ginkgo biloba* Extract on Reproductive Toxicity Induced by Single or Repeated Injection of Cisplatin in Adult Male Rats

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Abstract

This study was conducted to evaluate the possible protective effect of *Ginkgo biloba* (GB) against testicular damage and oxidative stress induced by acute or subchronic intoxication with cisplatin (CIS) in rats. Sixty male albino rats were classified into 6 equal groups (n = 10). Rats were treated orally with 150 mg kg b.wt. day^{-1} GB extract for 5 successive days before and 25 successive days after induction of toxicity by i.p. injection of CIS. Rats received CIS either acutely as a single injection of CIS (24 mg kg^{-1} b.wt.) or sub chronically with four equal doses of CIS (6 mg kg^{-1} b.wt.) once weekly. After 72 h from the last CIS injection, pathological and oxidative testicular toxicity as well as serum testosterone level and sperm indices were evaluated. Rats exposed to subchronic CIS treatment (alone or with prior GB co-treatment) displayed a significant decrease ($p<0.05$) in reproductive organs weight, caudal sperm count and motility, serum testosterone level and testicular GSH-PX and significant increase ($p<0.05$) in sperm abnormalities and testicular MDA. This reproductive toxicity was accompanied with necrotic and degenerated lesions in the seminiferous tubules. By contrast, in rats exposed to acute CIS treatment, prior treatment with GB partially ameliorated the subsequent harmful effects of CIS. In conclusion, pretreatment with GB mildly attenuated the reproductive damage induced by single CIS injection, while it failed to improve the damage induced by subchronic treatment.

Key words: *Ginkgo biloba*, cisplatin, sperm characters, reproductive toxicity, male rat

Received: December 21, 2015 **Accepted:** February 25, 2016 **Published:** April 15, 2016

Citation: Asmaa F. Khafaga and Aida E. Bayad, 2016. Impact of *Ginkgo biloba* extract on reproductive toxicity induced by single or repeated injection of cisplatin in adult male rats. Int. J. Pharmacol., 12: 340-350.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chemotherapy is the most effective and optimal mode for treatment of wide variety of malignancy. Cisplatin (cis-diammine dichloro platinum-II, CIS) has emerged as one of the most efficient and commonly used antineoplastic drugs, which still used successfully in the treatment of various types of malignancy including testicular cancer (Ahmed *et al*., 2011). Testicular cancer was reported as the most common type of cancer in men between 15 and 35 years old, the overall cure rate in these patients exceeded 95% after using of CIS-based treatment. Unfortunately, lasting infertility and azoospermia were common side effects of CIS treatment in some male patients (Chaudhary and Haldas, 2003). However, recovery of spermatogenesis takes place after the stoppage of chemotherapy, in about 50 and 80% of patients by 2 and 5 years, respectively (Howell and Shalet, 2005). This variation in the spermatogenic recovery duration relies mainly on the dose and duration of the chemotherapy regimes (Petersen *et al*., 1994). In the previous studies, acute exposure to a single dose of CIS can resulted in dose-dependent disturbance of spermatogenesis with prolonged azoospermia (Chaudhary and Haldas, 2003; Cherry *et al*., 2004), while; chronic and subchronic treatments with very small dose of CIS induce reduction in spermatogenic function in rats but failed to completely abrogate spermatogenesis (Marcon *et al*., 2008). However, there is no sufficient information about the deleterious effect of CIS on male fertility when use similar doses of CIS by two different regimes of exposure (acute exposure with a single injection or subchronic exposure to four divided doses) under the same experimental condition. The molecular mechanism by which CIS induce reproductive toxicity still unclear. However, it was generally ascribed to oxidative stress mediated by increased free radical generation and depletion of antioxidants (Amin *et al*., 2008). Consequently, antioxidants have been used to protect nonmalignant cells and organs against damage mediated by CIS (Amin *et al*., 2008). Over the past decade, *Ginkgo biloba* (GB) has been used as a popular traditional Chinese medicine and it is one of the widely used herbal drugs that acts through several mechanisms including antioxidant effects, alterations in signal transduction, inhibition of platelet activating factor and decrease adhesion of blood cells to endothelium (Zhou *et al*., 2004). The alleged bene cial effects of GB leave extract might be obtained through a combination of one or more of the basic mechanisms of action resulted in antiasthmatic, antidiabetic, cardioprotective and potent neuroprotective activities, including memory enhancement, mental alertness and decrease in mental fatigue (Naik *et al*., 2006; Naik and Panda, 2007). Additionally, it is used in treatment of some neurological diseases like Alzheimer's, dementia and other cognitive dysfunctions (Kwon *et al*., 2004) and posses protective effect of the cell membrane against oxidative damage induced by free radicals (Ahlemeyer and Krieglstein, 2003). Therefore, this study was designed to evaluate the detrimental effect of acute and subchronic treatment with CIS on reproductive organs of adult male albino rats and to assess the ameliorative role of GB on CIS induced reproductive toxicity in two different administration regimes.

MATERIALS AND METHODS

The protocol of this study was approved by the Institutional Animal Care and Use Committee of Alexandria University, Egypt. The experiment was executed by Pathology Department and Veterinary Services Center, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Animals: Sixty adult male albino rats (5 months old and 150-200 g b.wt.) were obtained from Animal Facility, Alexandria University, Egypt. Animals were housed in standard galvanized metal cages under a natural light cycle of 12/12 light/dark and room temperature of 25-27°C. Rats were supplied with a standard rodent chow diet with *ad libitum* access to water. Animals were allowed 2 weeks acclimatization period before use in experiment to ensure normal growth and behavior.

Chemicals and plant: Cisplatin (CIS) was purchased from Oncotic Pharma Production GmbH, Am Pharmapark, Germany. *Ginkgo biloba* (GB) extract was obtained from Nature's Bounty, Bohemia, USA. The extract is standardized to contain 24% Ginkgo flavono glycosides, 14.4 mg and terpene lactones (6%) which represent the major active contents of GB. Kits for determination of serum testosterone hormone was obtained from Diagnostic Product Co., Los Angles, USA. Kits for determination of testicular glutathione peroxidase and malondialdehyde were obtained from Biodiagnostic Co., Giza, Egypt.

Experimental protocol: Rats were randomly allocated into six equal groups (n = 10): Group 1 (negative control group) received intra-gastric distilled water using stomach tube (5 mL kg^{-1} b.wt., vehicle of GB) daily, concurrently with intraperitoneal (i.p.) injection of saline (5 mL kg^{-1} b.wt. vehicle of CIS) once weekly. Group 2 (positive control group) received intra-gastric GB water extract (150 mg kg^{-1} b.wt., at the same dose volume) daily; dose of GB were determined

according to the previously reported pharmacological properties of this herbal plant (Gong *et al*., 2006). Group 3 (acute toxicity with CIS) received single i.p., injection of CIS (24 mg kg^{-1} b.wt.) 72 h before euthanization of rats. This dose of CIS was previously used by De Freitas *et al*. (2009) to induce toxicity in rats. Group 4 (subchronic toxicity with CIS) received i.p., injection of CIS once weekly (6 mg kg^{-1} b.wt., with cumulative dose 24 mg kg^{-1} b.wt.). Group 5 (GB+acute CIS toxicity) received single i.p., injection of CIS (24 mg kg^{-1} b.wt.) in concomitant with daily intra-gastric dosage of GB (150 mg kg^{-1} b.wt.) started five days before the first injection of CIS and continued up to the end of the experiment. Group 6 (GB+subchronic CIS toxicity) received i.p., injection of CIS once weekly (6 mg kg^{-1} b.wt., with cumulative dose 24 mg kg^{-1} b.wt.) concurrently with daily intra-gastric dosage of GB (150 mg kg^{-1} b.wt.) started five days before the first injection of CIS and continued up to the end of the experiment. In groups 5 and 6, the treatments with GB and CIS were separated by 60 min to overcome the possible drug interaction. The experiment was conducted for 30 consecutive days. After 72 h from the last CIS injection, rats were euthanized. Blood, reproductive organs (testes, epididymis and prostate gland) and the epididymal content were obtained from treated and control rats. All experimental rats were weighed at specific killing time.

Blood sampling: At the end of the experiment, rats from each group were anesthetized with light ether, blood samples were drawn from their retro-orbital plexus before scarification by decapitation. The blood samples were collected without anticoagulant for obtaining serum.

Preparation of serum: Blood samples were left in slope position to clot at room temperature, centrifuged at 3000 rpm for 15 min and the clear non-hemolysed supernatant serum was collected then kept frozen at -20°C until used for determination of testosterone level.

Examination of epididymal sperm: After scarification of rats, the epididymal content of each control and treated rats were taken immediately by sharp cutting of the tail of epididymis and squeezed gently on sterile glass slide to estimate the progressive motility, sperm cell count and sperm abnormalities according to the method described by Bearden and Fuquay (1980).

Progressive sperm motility: A drop of sperm suspension was placed over a warm clean glass slide then put a cover slip. The progressively motile sperm percentage was microscopically estimated at 400x magnification.

Sperm cell count: For counting epididymal sperm, a hemocytometer and a pipette of RBCs counting were used. A drop of undiluted caudal epididymal content was withdrawn up to the mark 1.0 and the pipette was filled up to the mark 101 by sodium bicarbonate solution 5%. The number of sperm in five squares was multiplied by 10,000. The sperm cell count in millimeter cube was estimated.

Sperm abnormalities: A drop of the epididymal content of each rat was mixed with an equal drop of eosin-nigrosin stain and then the film was spread on a clear slide. Two hundreds sperm were randomly observed per slide under light microscope at 400x magnification and percentages of morphologically abnormal sperm (detached head and coiled tail) were recorded.

Weight of the reproductive organs: After scarification of rats and epididymal sperm examination, the testes, epididymis and prostate gland were dissected out, grossly examined and weighed.

The Index Weight (IW) of each organ was calculated as described by Matousek (1969):

$$\text{Index Weight (IW)} = 100 \times \frac{\text{Organ weight}}{\text{Body weight}}$$

Determination of serum testosterone levels: Assessment of the total serum testosterone according to Demetrious (1987) using solid-phase radioimmunoassay (RIA) kits. The protocol was based on presence of testosterone-specific antibody immobilized to the wall of the polypropylene tube.

Determination of antioxidant enzyme activity and oxidative stress assays in testicular tissues: One testis of each rat was kept frozen at -70°C for assessment of glutathione peroxidase (GSH-px) activity and lipid peroxidation (LPO) content, the testis washed with ice-cold tris-HCl buffer (0.05 M, pH 7.4) containing 0.25 M sucrose, dried, weighed and then homogenized separately in the ice-cold buffer with twelve strokes in a tight-fitting Potter-Elvehjem homogenizer. The w/v ratio of the tissue to the homogenization buffer was (1:10 w/v). Supernatants were collected for determination of LPO (quanti ed as malondialdehyde, MDA) and glutathione peroxidase (GSH-px) activity in testicular tissues. The MDA measured spectrophotometrically after its reaction with thiobarbituric acid (TBA) and formation of a pink complex

Table 1: Effect of cisplatin and/or its combination with *Ginkgo biloba* extract on index weight of the reproductive organs in adult male albino rats

	Groups					
Parameters	1	2	3	4	5	6
Testis (g)	1.62±0.08^{a}	1.53±0.13^{a}	0.51±0.04^{bc}	0.38±0.05^{c}	0.74±0.04^{b}	0.47±0.07^{c}
Epididymis (g)	0.68±0.04^{a}	0.70±0.06^{a}	0.17±0.03^{bc}	0.10±0.01^{c}	0.26±0.00^{b}	0.14±0.01^{bc}
Prostate gland (g)	0.52±0.02^{a}	0.51±0.02^{a}	0.16±0.01^{c}	0.07±0.02^{d}	0.36±0.01^{b}	0.08±0.02^{d}

Means bearing different letters within the same row are significant at (p<0.05), each value represents Mean±SD, N = 10 for each group

Table 2: Effect of cisplatin and/or its combination with *Ginkgo biloba* extract on sperm characters in adult male albino rats

	Groups					
Parameters	1	2	3	4	5	6
Sperm count (10^6 mL^{-1})	120.00±12.91^{a}	123.33±18.56^{a}	103.33±20.28^{a}	15.00±2.89^{b}	110.00±12.91^{a}	20.00±5.77^{b}
Sperm motility (%)	86.50±3.43^{a}	84.00±3.19^{a}	60.83±8.46^{b}	12.67±1.45^{c}	70.00±2.89^{b}	15.00±2.04^{c}
Sperm abnormalities (%)	13.00±2.86^{c}	14.75±3.30^{c}	59.50±10.32^{b}	76.67±1.76^{a}	50.33±4.10^{b}	53.67±0.88^{b}

Means bearing different letters within the same row are significant at (p<0.05), each value represents Mean±SD, N = 10 for each group

Table 3: Effect of cisplatin and/or its combination with *Ginkgo biloba* extract on serum testosterone levels, testicular GSH and testicular MDA in adult male albino rats

	Groups					
Parameters	1	2	3	4	5	6
MDA (nmol g^{-1} tissue)	3.83±0.28^{d}	3.99±0.26^{d}	8.99±0.12^{b}	11.95±0.36^{a}	7.23±0.15^{c}	10.88±0.45^{a}
GSH-Px (IU g^{-1} protein)	33.00±1.47^{a}	34.75±1.38^{a}	22.27±0.43^{b}	18.30±0.45^{c}	23.20±0.42^{b}	17.50±0.65^{c}
Testosterone (ng mL^{-1})	2.90±0.32^{a}	3.01±0.82^{a}	0.21±0.04^{b}	0.05±0.01^{b}	0.54±0.15^{b}	0.06±0.01^{b}

Means bearing different letters within the same row are significant at (p<0.05), each value represents Mean±SD, N = 10 for each group

(Placer *et al*., 1966) and GSH-px activity determined according to the method of Paglia and Valentine (1967).

Histopathological examination: One testes, epididymis and the prostate gland were collected from each rat and immediately fixed in 10% neutral phosphate-buffered formalin for at least 24 h then processed through the conventional paraffin embedding technique (Culling, 1983). Sections of five micro thicknesses were stained with Mayer's hematoxylin and eosins (HE) according to the method described by Bancroft *et al*. (2013) for the light microscope examination to obtain a representative photomicrograph for the tissues.

Statistical analysis: The obtained results were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (SAS., 2004). Data are presented as means plus or minus the standard error. The minimum level of significance was set at p<0.05.

RESULTS

Reproductive organs weight: The index weights of testes, epididymis and prostate gland in rats treated with acute and subchronic CIS with or without co-treatment with GB was significantly decreased (p<0.05) compared to the control groups. While, the reduction was less pronounced in group received acute single dose of CIS with GB prior treatment (Table 1).

Sperm characters

Sperm count: After subchronic treatment with CIS (either alone or co-treated with GB), the caudal sperm count decreased significantly (p<0.05) compared with the control group. While, acute intoxication with CIS (either alone or pretreated with GB) caused non-significant reduction in epididymal sperm count compared with the control group (Table 2).

Sperm motility: The obtained results showed that there was significant decrease (p<0.05) in the progressive sperm motility percentage in all treated groups of the experiment as compared with control groups. The degree of decrease in sperm motility percentage was more pronounced in rats treated subchronically (group 4). While, in groups 5, prior treatment with GB in acute CIS toxicity enhanced the sperm motility (Table 2).

Total sperm abnormalities (detached head and coiled tail): The obtained results displayed significant increase (p<0.05) in the percent of sperm abnormalities in all treated groups, but it was more pronounced in group 4. The treatment with GB in acute CIS (group 5) decreased the sperm abnormalities (Table 2).

Effect on serum testosterone levels, testicular GSH and testicular MDA: In Table 3, rats intoxicated acutely or subchronically with CIS and co-treated or not with GB showed

significant decrease ($p<0.05$) in serum testosterone levels compared to the control group. While, there was a significant increase ($p<0.05$) in levels of testicular MDA in all treated groups, the degree of increasing in testicular MDA was more pronounced in group 4 while, the lowest increase was in group 5. There was a significant decrease ($p<0.05$) in GSH-Px activities in all treated groups compared to the control group (Table 3).

Histopathological finding

Testes: Testicular tissue of control and GB treated groups showed normal arrangement of spermatogenic and sertoli cells in various stages of maturation without histopathological lesions (Fig. 1a and 2a). While, the testicular tissues of groups 4 and 6 treated subchronically with CIS and co-treated or not with GB, showed similar patterns of lesions without any noticeable improvement associated with prior treatment with GB. The noticed lesions were moderate to severe atrophy and loss of the normal histoarchitecture of the majority of seminiferous tubules, the seminiferous tubules, depletion of most germinal epithelium, buckled and corrugated basement membrane, focal separation of germ cells from basal lamina and aggregation of multinucleated giant cells. Moreover, completely necrotic tubules were filled with extensive acidophilic foamy materials with exfoliation of their necrotic germ cells into lumen. Regarding to the interstitium, there were congestion and thickening of the intertubular blood vessels with edema that was represented by faint eosinophilic material (Fig. 1b-d and 2b). On the other hand, groups treated acutely with single dose of CIS (with or without GB co-treatment) showed similar pattern of lesions but with low incidence for group pretreated with GB reflecting mild protective effect for GB co-treatment, the detected lesions included different degrees of tubular necrosis with desquamation of necrotic tissues into the lumen, depletion and destruction of tubular germ cells, corrugated basement membrane, focal separation of basement membrane and presence of acidophilic foamy materials in some seminiferous tubules, the interstitium showed moderate edematous changes with congested interlobular blood vessels (Fig. 1e, f and 2c).

Epididymis: Epididymis of the negative and positive control rats showed normal histological structure with normal sperm density (Fig. 3a, b). While, rats treated subchronically with CIS (with or without GB pretreatment) exhibited histopathological alterations composed of sloughing of lining epithelial cells within the lumen of both caput and cauda epididymis, the majority of the epididymal ducts appeared devoid of mature spermatozoa within their lumina and vacuolization of few germinal epithelium of some cauda epididymal ductules were evident (Fig. 3c, d). Rats received acute treatment with CIS (co-treated with GB or not) showed relative decrease in sperm density of some epididymal tubules and interstitial congestion (Fig. 3e).

Prostate gland: Prostate gland of the control and GB treated rats had normal histologic structure (Fig. 4a). Rats received subchronic CIS treatment with or without GB exhibited picture of histopathological alterations composed of interstitial congestion and edema with desquamated epithelium admixed with leukocytes-mainly neutrophils and lymphocytes-within the lumen of some glandular acini. Moreover, the majority of the acini observed with no luminal secretions. (Fig. 4b, c). Prostate of rats received single dose of CIS displayed moderate dilatation of some acini with no luminal secretions (Fig. 4d). While, rats received single dose of CIS in commitment with GB showed nearly normal histoarchitecture of prostate with sufficient amount of glandular secretions.

DISCUSSION

In the current study, a significant reduction in the reproductive organs weight was recorded in all groups intoxicated with CIS which may contributed to the decrease in serum testosterone levels. These results are in agreement with the findings of Seethalakshmi *et al*. (1992) and Amin *et al*. (2012). Moreover, it's known that the weight of the testis reflect the mass of the differentiated spermatogenic cells, therefore, the decline in the testicular weight may be due to decreased number of germ cells and inhibition of spermatogenesis (Takahashi and Oishi, 2001). In current study, rats intoxicated subchronically with CIS had significantly lowered sperm count and sperm motility. While, it has significant increase in sperm abnormalities. On the other hand, rats intoxicated with single dose of CIS had significant decrease in sperm motility and significant increase in sperm abnormalities with non-significant decrease in sperm count, the same results were recorded by Seethalakshmi *et al*. (1992) and Amin *et al*. (2012). The reduction in sperm count may be due to the deleterious effect of CIS on spermatogenesis which could be ascribed to either induction of apoptosis in target cells or CIS induced lipid peroxidation (LPO). The molecular weight of CIS is 300 Da and the size is a few angstroms. Substances with such low molecular weight and size are

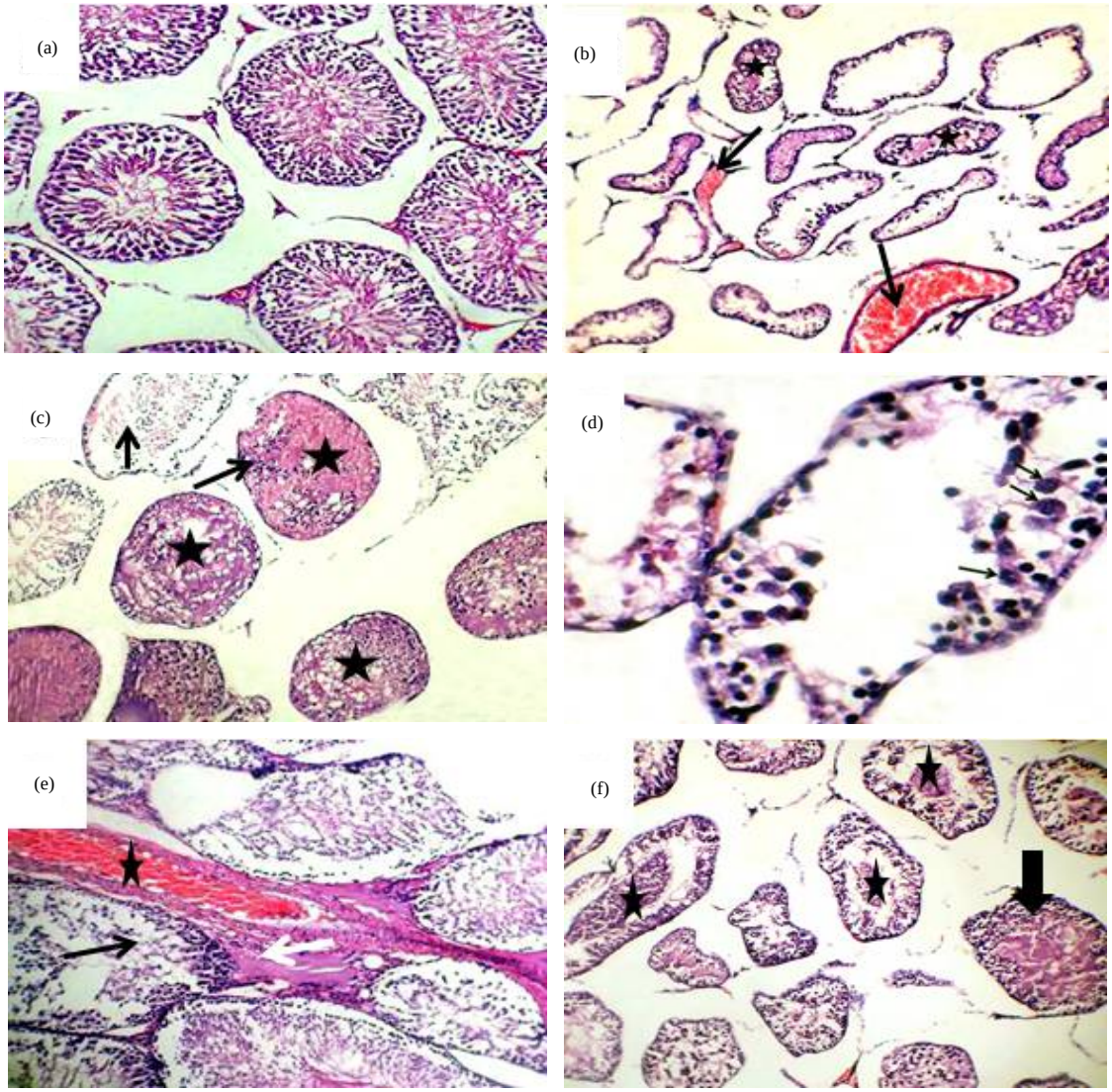

Fig. 1(a-f): Representative photomicrograph of a cross-sections of the testes of (a) Control group, (b-d) Group received 4 repeated doses of CIS and (e and f) group received single dose of CIS, H.E: (a) Normal arrangement of spermatogenic cells in various stage of maturation (250x), (b) Completely atrophied seminiferous tubules with loss of normal architectures, depletion of most of their germ cell, irregular basal lamina, congested and thickened intertubular blood vessel (arrows) and foamy acidophilic material within the lumen of some tubules (stars) (250x), (c) Completely degenerated tubule filled with an acidophilic foamy hyaline materials (stars) with exfoliation of germ cells into lumen of other tubules (arrows) (250x), (d) Drastic reduction in germ cells and aggregation of multinucleated giant cells (arrows) (400x), (e) Depletion and destruction of tubular germ cells (black arrow) and dilatation of interstitial tissues with acidophilic foamy materials (white arrow) with congested thickened interlobular blood vessel (star) (250x) and (f) Necrosis of intratubular elements and desquamation of necrotic tissues into lumen of some tubules (stars) with foamy acidophilic material within the lumen of other tubule (arrow) (250x)

known to pass the permeability barrier easily (Okumura *et al*., 1975). Therefore, CIS could cross the basal and luminal compartments resulting in direct effect on different germ cells, which expressed as cell death and/or creating unsuitable tubular environment impairing the maturation process of the germ cells. Normal testosterone level reflects the healthy condition of the reproductive system; hence, the decreased level of testosterone considers as one of the indicators of the chemical toxicity on reproductive system (Yoshida *et al*., 2002). Furthermore, testosterone is essential to maintain the

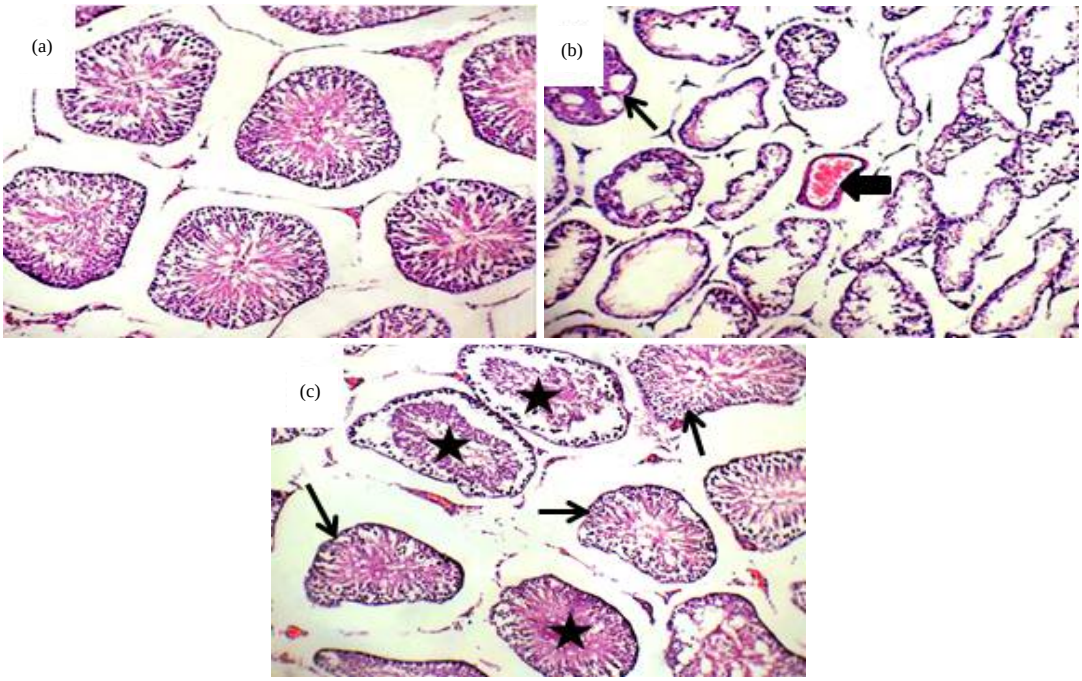

Fig. 2(a-c): Representative photomicrograph of cross-sections of the testes of (a) Group received GB, (b) Group received GB+4 repeated doses of CIS, (c) Group received GB+single dose of CIS, stained with H.E, (250x): (a) Normal arrangement of spermatogenic cells in various stage of maturation, (b) Completely atrophied seminiferous tubules with loss of normal architectures, depletion of most of their germ cell, irregular basal lamina, congestion and thickening of intertubular blood vessel (thick arrow) and foamy acidophilic material in lumen of some tubules (thin arrow) and (c) Necrosis and desquamation of germinal epithelium into lumen of some tubules (stars) with corrugated basement membrane which focally separated from germ cells while other tubules appeared with nearly normal histoarchitecture

normal structure and function of the male accessory sex glands and its lack might disrupt spermatogenesis (Boockfor, and Blake, 1997). In the current investigation, CIS treated groups showed significant reduction in serum testosterone level; this finding was in agreement with that described by Seethalakshmi *et al.* (1992). It is well known that the biosynthesis of testosterone is controlled by LH hormone, the previous study reported that administration of CIS (7-9 mg kg^{-1}) depressed testosterone synthesis by reducing the number of LH receptors, the activities of cholesterol side chain cleavage enzyme and 17-alpha hydroxylase (Maines *et al.*, 1989). The LPO act as an indicator of the oxidative stress, which plays a major role in the toxicity of many drugs including CIS. The MDA is a known stable end product of LPO which used to measure the cumulative LPO indirectly. Plasma membrane of sperm contains abundance of unsaturated fatty acids and a very low concentration of cytoplasmic antioxidants (Aitken *et al.*, 1993). Which led the sperm to be highly susceptible to LPO therefore, the increased LPO can lead to oxidative damage to sperm DNA, alter membrane functions and impair motility and the development of spermatozoa (Aitken *et al.*, 1989). In our study, rats intoxicated with CIS had significant increase in MDA level in the testicular tissues indicating the generation of LPO which in turn leads loss of membrane structure and function. These results were in agreement with those of Amin *et al.* (2012). It is worth noting that testes have very high levels of glutathione than other organs, which protecting the spermatogenic cells from ROS damage beside the important role it plays in the proliferation and differentiation of these cells (Teaf *et al.*, 1985). In CIS intoxicated rats, GSH level was significantly decreased, this result was supported by the finding of Amin *et al.* (2012). Regarding the histopathological examination, CIS provoked some histopathological alteration in the testis such as shrinkage, degeneration and/or complete atrophy of the majority of the seminiferous tubules with loss of its normal histoarchitecture. Moreover, there were incomplete spermatogenesis, irregular corrugated basement membrane, vacuolization and focal separation of germ cell from basal lamina with aggregation of multinucleated giant cells. Moreover, coagulative necrosis of the germinal epithelium which followed by hyalinization of the luminal

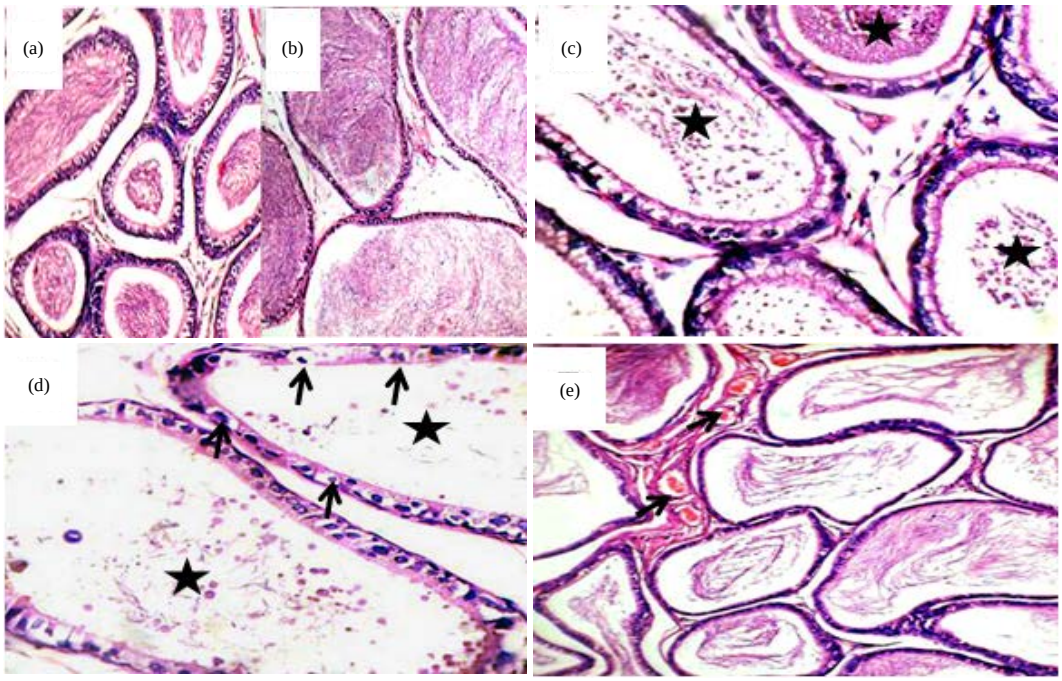

Fig. 3(a-e): Representative photomicrograph of cross-sections of the epididymis of (a) Control group (caput), (b) Group received GB (cauda), (c) Group received 4 repeated doses of CIS (caput), (d) Group received GB+4 repeated dose of CIS (cauda) and (e) Group received single dose of CIS, H.E: (400x): (a and b) Normal histological structure with normal sperm density, (c and d) Epididymal ductules were nearly devoid from mature spermatozoa contained sloughed germ cells (stars) and vacuolization of few germinal epithelial cells of some cauda epididymal ductules (arrows) and (e) Moderate decrease in sperm density of some tubules with interstitial congestion (arrows)

content were evident. Also, CIS caused interstitial congestion and edema. Vacuolization of the germinal epithelium and Sertoli cells may be attributed to the smooth endoplasmic reticulum dilatation represent the changes in cellular permeability (Creasy and Foster, 2002). These changes may be explained by the ability of CIS to induced LPO and reduction in testosterone hormone which is required for the attachment of different generations of germ cells within the seminiferous tubules. Therefore, low level of intratesticular testosterone may consequently lead to detachment of germ cells from seminiferous epithelium which may initiate germ cell apoptosis (Blanco-Rodriguez and Martinez-Garcia, 1998). These results are in harmony with those reported by (Seethalakshmi *et al*., 1992; Amin *et al*., 2008; Amin *et al*., 2012). The majority of the epididymal ducts of CIS intoxicated rats had no or low numbers of spermatozoa in their lumina. This nding was parallel to the significant reduction in epididymal sperm count. Picture of germinal epithelium sloughing within the lumen of some epididymal ducts was evident indicating testicular dysfunction (Narayana *et al*., 2006). As a consequence of CIS administration, prostates showed necrosis and desquamation of some glandular epithelial cells, decreased glandular secretions and interstitial congestion and edema, these findings could be attributed to either the decreased level of serum testosterone or the CIS-induced LPO. Furthermore, prostatitis in rats treated with CIS could be ascribed to immunosuppressive effect of CIS (Karalliedde *et al*., 2010). Co-administration of GB causes mild improvement in levels of some evaluated parameters in rats intoxicated with single dose of CIS albeit, it was not identical to control levels. In this group, pretreatment with GB ameliorated the reduction in the reproductive organs weight and CIS-induced testicular oxidative damage beside the improvement in incidence and occurrence of some histopathological lesions of testes, epididymis and prostate. The alleged beneficial effect of GB leave extract might be mostly obtained through its antioxidant properties. The antioxidative properties of GB may contribute to its ability to reduce the MDA content in the testicular tissues by scavenging free radicals (Naik *et al*., 2006; Naik and Panda, 2007) which are the main toxic byproducts of many metabolic processes in biological membranes (Akiyama, 1999). That could be attributed to its active components, namely, avonoglycoside and terpene lactones. Moreover, the

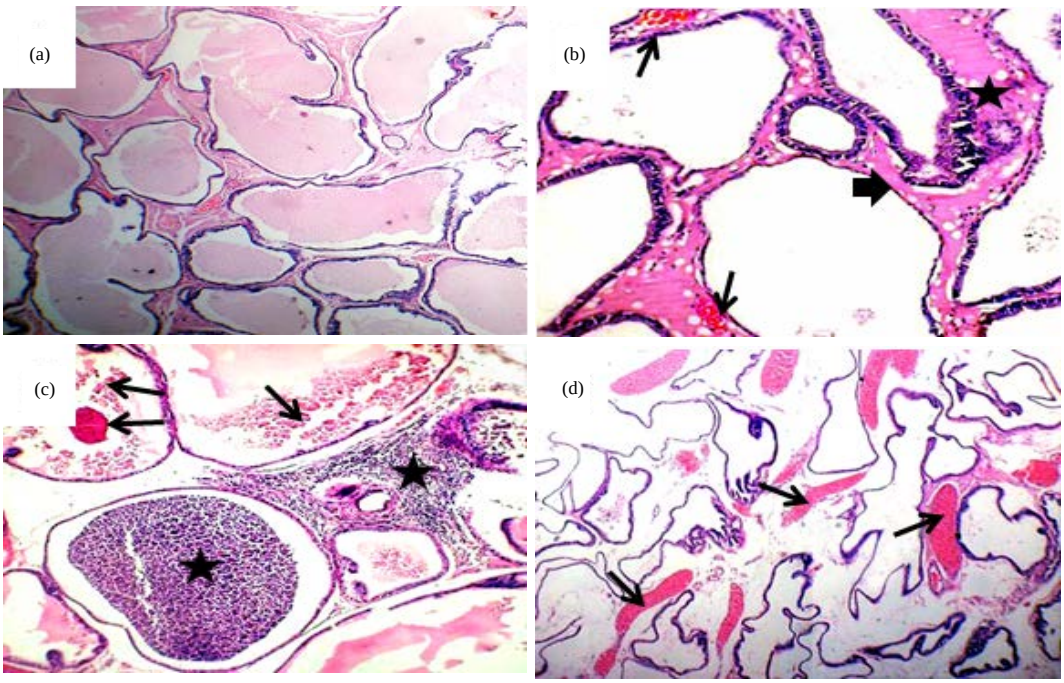

Fig. 4(a-d): Representative photomicrograph of cross-sections of the prostate gland of (a) Control group, (b and c) Group received 4 repeated doses of CIS, (d) Group received single dose of CIS, H.E: (a) Prostate with normal histological structure. (250x), (b) Interstitial congestion (thin arrow) and edema (star) with moderate dilatation and thinning of lining epithelium of some acini (thick arrow) with no luminal secretions (400x), (c) Desquamation of glandular epithelium (arrows) with infiltration of leukocytes in the interstitium and the lumen of acini (stars) (400x) and (d) Congestion of the interluminal B.Vs (arrows) with moderate dilatation of some acini and no luminal secretions (250x)

improvement in the histological structure of testes, epididymis and prostate gland was correlated to the potential role of GB in scavenging ROS generated by CIS which consequently followed by improvement in the reproductive organs weight. Similar results were declared by (Amin *et al*., 2008, 2012) whose concluded that GB treatment effectively attenuated the oxidative stress induced by CIS in testes. While, uncompleted preventive role of GB on other evaluated parameters might be explained by the difference in CIS dose used to intoxicate rats in the current study. On the contrary, GB failed to protect rats intoxicated with CIS in subchronic regimes, since all evaluated parameters in rats pretreated with GB have non-significant difference with those treated subchronically with CIS alone. Other interesting features of the current investigation were the presence of significant difference in some evaluated parameters (oxidative parameters and sperm characters) between the two groups treated with CIS alone in two different regimes. In addition to increase the incidence and severity of the recorded histopathological lesions in subchronically treated rats. The CIS in its free form have a very short half-life and is rapidly excreted in the urine (Rosenberg, 1979). However, it is known that CIS reacts with proteins to form stable covalent complexes and so, the elimination of bound CIS depend on the turnover of proteins it is bound to (Vermorken *et al*., 1986). In addition, CIS has low molecular weight and size which allow it to cross the permeability barrier easily and directly affect the germ cell. These facts can be interpreted the severe adverse impact of subchronic administration of CIS on fertility parameters which may attributed to the cumulative effect of bound CIS, without ignoring the length of treatment period which allow CIS to adversely affect the different stages of spermatogenesis; while, the single administration of CIS mainly affect the mature spermatozoa with less marked effect on different stages of spermatogenesis.

CONCLUSION

This study provides evidence that CIS adversely affect male reproductive organ tissues and sperm characters through increasing the oxidative damage with reference to that the harmful effect of subchronic treatment exceeded that induced by acute single treatment. Furthermore, pretreatment with GB mildly attenuated this damage induced

by single CIS treatment. But on the contrary, it couldn't ever improve the situation when the subchronic treatment was used.

ACKNOWLEDGMENTS

The authors thank Dr. Ayman El Sayed Taha Ass. Professor of Poultry Breeding and Production, Department of Animal Husbandry and Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Egypt for performing the statistical analysis.

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