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ORIGINAL ARTICLE

Alpha-tocopherol and ginger are protective on Cyclophosphamide-induced gonadal toxicity in adult male albino rats

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ABSTRACT

Background and aim: The ameliorative effect of alpha-tocopherol (vitamin E) and Zingiber officinale (ginger) on cyclophosphamide-induced gonadal toxicity are studied in adult male rats. **Methods:** Forty-four adult male albino rats were used in this study in four groups. Group I served as normal control, groups II–IV received cyclophosphamide intraperitoneally at a daily dose of 20 mg per kg body weight for 7 days but group III and IV received supplementary vitamin E and ginger respectively for two weeks starting one week before the start of the cyclophosphamide. **Results:** The sperm counts of the normal (I), cyclophosphamide treated group alone (II), cyclophosphamide with vitamin E (III) and with ginger (IV) showed 23.5, 8.5, 14.2, 18.1 ($\times 10^6$ per cubic milimeter) and abnormal morphology was most frequently found in group II (16.2%) followed by groups III (6.3%), IV (5.9%) and I (2.2%). Testosterone level, malondialdehyde enzyme activity, and red cell catalase and superoxide dismutase activities also correlated well with the morphological parameters. Incidence of apoptosis and the number of TUNEL-stained cells as well as pathological findings in reproductive organs also correlated with these parameters. **Conclusion:** Both vitamin E and ginger have protective effect from the cyclophosphamide-induced gonadal toxicity. The mechanism is largely unknown but empirical supplementation of vitamin E and ginger would be recommended before and during cyclophosphamide chemotherapy.

INTRODUCTION

Cyclophosphamide is an anticancer and immunosuppressive agent with a very narrow therapeutic index that undergoes a complicated process of metabolic activation and inactivation,[1,2] biotransformed principally in the liver to active alkylating metabolites by a mixed function microsomal oxidase system. These metabolites interfere with the growth of susceptible rapidly proliferating malignant cells by cross-linking with the DNA of tumor cells.

A range of adverse effects of cyclophosphamide have been reported, including reproductive toxicity in human and animals. Adult male patients treated with cyclophosphamide have demonstrated diminished sperm counts and an absence of spermatogenic cycles in their testicular tissue.[3] Long-term treatment with cyclophosphamide injures progeny, decreases the weight of the reproductive organs, and impairs fertility.[4] Thus long-term side effects like gonadal toxicity have become an important issue in both men and women treated with cyclophosphamide.[5,6]

Vitamin E is a fat-soluble vitamin that exists in eight different forms. Each form has its own biological activity, which is the measure of potency or functional use in the body. Alpha-tocopherol (α-tocopherol) is the name of the most active form of vitamin E, as it has the highest bioavailability, with the body preferentially absorbing and using this form.[7] Vitamin E acts as an antioxidant to protect the cells from the damage by free radicals, which often produced as by-products of energy metabolism.[8] Vitamin E has also been shown to play a role in immune function, DNA repair, other metabolic processes[8] and prevention of sperm loss.[9]

Ginger (*Zingiber officinale*) is a member of the family of plants that includes cardamom and turmeric. The strong aroma of ginger is the result of pungent ketones including gingerol, the extract that primarily has been used in research studies. It is categorized as a food additive by the US Food and Drug Administration.[10] However, it is a commonly used medicinal herb throughout the world dating back 2500 years. It is used to aid digestion and treat stomach upset, diarrhea, and nausea.[11] No specific dosing studies have been performed for ginger; however, most clinical researches have used between 250 mg and 1 g of the powdered root in capsular form taken one to four times daily.[12]

In the last years, concerns have been raised about the possible mutagenic effect of chemotherapy on sperm.[13] While gonadal toxicity of cyclophosphamide has been well demonstrated, little and conflicting data were reported about the protective effect of some natural plants and its comparison with well known synthetic agents on testicular function.

The present work was undertaken as an attempt to minimize male germ cell damage during treatment with chemotherapy in rats by demonstrating the ameliorative influence of each of

alpha-tocopherol (vitamin E) and ginger on the toxicity of cyclophosphamide regarding, semen quality, testosterone level and histopathology. Along with understanding the possible underlying mechanism of toxicity through estimation of rate of apoptosis induction, oxidative stress markers such as catalase (CATA) and superoxide desmuatase (SOD) enzymes and lipid peroxidation markers (malondialdehyde enzyme).

MATERIALS AND METHODS

Animals

The present study was carried out on forty-four mature male albino rats weighted (200–250 g), obtained from animal breeding house of Faculty of Veterinary Medicine, Zagazig University. Animals were accommodated to the laboratory conditions for 2 weeks before starting the experiment.

Materials

Cyclophosphamide as a white water soluble crystalline powder (ASTA Medica AG Pharmaceutical, Frankfurt, Germany), vitamin E (EPICO, Cairo, Egypt) and ginger as commercial oil (Bader Company, Cairo, Egypt).

Experimental protocol

Rats were divided into four groups of 11 rats each. Group I served as a control group. Group II rats received intraperitoneal cyclophosphamide at a daily dose of 20 mg per kg body weight for 7 days.[14] Groups III and IV rats received vitamin E at a daily dose of 36 mg per kg body weight[15] and ginger at a daily dose of 200 mg per kg body weight[16] respectively by gavage for 1 week before the use of cyclophosphamide at a same dose with group II. The dose of vitamin E was converted into equivalent rat dose according to a previous study.[17] Both vitamin E and ginger were gavaged to rats one and half hour before cyclophosphamide treatment.

At the end of the experimental period (14 days), blood samples were collected from each rat (3 mL in plain tubes and 4 mL in heparinized tubes). The former blood samples were used for serum separation to estimate testosterone hormone and malondialdehyde enzyme. While, heparinized blood samples were used to estimate antioxidant enzymes activities; red cell catalase and superoxide dismutase.

Then all studied animals were sacrificed and weights (g) of testis, epididymis and seminal vesicles were recorded using electronic balance (Scaltec, Göttingen, Germany). Epididymis was utilized immediately to evaluate sperm quality parameters.

Evaluation of sperm quality

Epididymal content of each rat was obtained immediately by cutting the tail of epididymis and squeezing it gently to obtain the fresh undiluted semen in a clean Petri dish. Collection was done as described by Prasad *et al.*[18] to precede the following examinations; sperm cell count, sperm motility,[19] sperm viability and abnormalities.

Estimation of serum testosterone level

Testosterone was assayed in serum samples that were stored at −20 °C by the use of Roche Elecsys testosterone reagent kit (Roche Diagnostics, Indianapolis, IN, USA) and modular analyzer the minimum detectable limit was 0.02 ng/mL.[20]

Estimation of serum malondialdehyde UMol/L

Malondialdehyde (MDA) was measured in serum using the spectrophotometer at a wave length of 535 nm.[21]

Estimation of red cell CATA and SOD activities

The activity of red cell CATA enzyme was measured using spectrophotometer at a wave length of 240 nm.[22] While red cell SOD was estimated using the spectrophotometer at a wave length of 560 nm.

Histopathological examination

Specimens from testes, epididymis, seminal vesicles and prostate were taken immediately from each rat for histopathological examination. The specimens were then fixed in Bouin's fluid for 48 h. Later, they were dehydrated in graded levels of ethanol, cleared in xylene, and embedded in paraffin wax for sectioning. The 5-μm thick sections were cut, mounted on glass slides, and stained with hematoxylin and eosin for light microscopy.

In situ detection of apoptosis in testicular germ cells

The testicular germ cells apoptotic detection was performed on paraformaldehyde-fixed 3um paraffin sections using The DEdEnd Colorimetric TUNEL System (G7131, G7132; Promega, Madison, WI, USA) according to the manufacturer's instructions. This system depends on end-labeling the fragmented DNA of apoptotic cells using a modified TUNEL assay. Biotinylated nucleotide is incorporated at the 3′-OH DNA ends using the enzyme terminal deoxynucloetidyl transferase (TdT). Horseradish- peroxidase-labeled streptavidin (Streptavidin HRP) is then bound to these biotinylated nucleotides, which are detected using the peroxidase substrate, hydrogen peroxide, and the stable chromogen, diaminobenzidine (DAB).

Statistical analysis

The obtained data were analyzed by ANOVA and LSD (Least Significant Difference) to determine the significance of differences among means. Then data were expressed as mean ± standard deviation.

RESULT

The results of the present work revealed that exposure of adult male rats to cyclophosphamide had deleterious effects on both testicular histology and all male fertility parameters investigated in our work. On the other hand, co-administration of either vitamin E or ginger had a corrective influence on the previously mentioned deleterious effects.

Weight of sexual organs

The obtained data from treated groups clearly revealed significant decrease in weights of the testes, epididymis and seminal vesicles of cyclophosphamide treated group in comparison to the control and other treated groups. However, pre and co-treatments with vitamin E and ginger significantly restored this decrement as shown in Table 1. This restoration was more obvious in Group IV.

Epididymal spermatozoa examination

The mean of the sperm cell count and the percentage of live and motile sperm showed statistical significant decrease in

Table 1 Comparison between mean values of control and treatment groups regarding weight of sexual organs and epididymal sperm character

Groups	Weight of sexual Organs (g) $\bar{X} \pm$ SD Testis **	Epididymal sperm characters Epididymis **	Seminal vesicle **	Motility **	Sperm cell count ($\times 10^6$/mm^3) $\bar{X} \pm$ SD **	Abnormality (%) $\bar{X} \pm$ SD **	Life (%) $\bar{X} \pm$ SD **
Control	1.94 ± 0.07 a	1.50 ± 0.50 a	1.38 ± 0.03 a	91.80 ± 2.10 a	23.50 ± 2.00 a	2.16 ± 0.15 c	94.50 ± 3.20 a
CP	1.23 ± 0.14 c	0.50 ± 0.30 c	0.63 ± 0.07 c	37.30 ± 6.90 c	8.52 ± 1.00 d	16.23 ± 1.70 a	30.00 ± 8.90 c
Vit.E + CP	1.57 ± 0.10 b	0.90 ± 0.43 ab	0.81 ± 0.10 b	60.80 ± 7.40 b	14.16 ± 1.50 c	6.30 ± 0.73 b	61.60 ± 7.50 b
Ginger + CP	1.71 ± 0.15 ab	1.10 ± 0.20 ab	0.91 ± 0.06 b	70.30 ± 6.50 b	18.13 ± 1.90 b	5.90 ± 0.87 b	72.00 ± 7.30 b

*P < 0.05; **P < 0.001.

a, b,c Means within the same column having different letters significantly different.

CP, cyclophosphamide; Vit. E, vitamin E.

cyclophosphamide treated groups, while, there were significant increase in the number in groups with supplementation with vitamin E or ginger.

Investigation of sperm morphology in the present study revealed marked head and tail abnormalities in cyclophosphamide treated group compared to normal sperm morphology of the control (Fig. 1a). Head abnormalities were in the form of double head (Fig. 1b), abnormal hook shape (Fig. 1c) and detached head (Fig. 1d). While, tail abnormalities were represented only by coiled tail (Fig. 1e). Other recorded abnormalities like coiled mid piece as well as broken mid piece which considered as minor abnormalities were observed. The prevalence of these abnormalities was 16.23%, 6.3% and 5.9% in groups II, III and IV respectively as presented in Table 1. The last prevalence revealed that; pre and co-treatments with vitamin E and ginger with cyclophosphamide resulted in significant decrease in the percent of sperm cell abnormalities especially in ginger co-treated group.

Serum testosterone level

A significant decrease in the serum testosterone level was observed in cyclophosphamide treated group compared to the control (see Table 2). However, a significant increase in serum testosterone level was observed in Group III (vitamin E and cyclophosphamide) and Group IV (ginger and cyclophosphamide).

Lipid peroxidation marker level

A significant increase in MDA level was noticed in cyclophosphamide treated group compared to control and other treated groups (see Table 2). While, pre and co-administration of vitamin E and ginger decreased MDA levels in Groups III and IV. However significant restoration in MDA level by decrement was noticed in ginger and cyclophosphamide treated group when compared with vitamin E and cyclophosphamide treated group.

Oxidative stress markers levels

CATA and SOD enzymes activities showed significant decrease in their levels in cyclophosphamide treated group when compared with control and other treated groups. Pre and co-administration of either vitamin E or ginger improved oxidative stress and increased the level of both CATA and SOD enzymes activities compared to cyclophosphamide treated group. However this increment was significant ($P < 0.001$) in ginger and cyclophosphamide treated group when compared with vitamin E and cyclophosphamide treated group.

Histopathological examination

Histopathological examination of tissue sections from testis, epididymis, prostate gland and seminal vesicles of cyclophosphamide treated rats revealed marked tissue alterations which were restored to a significant degree by pre and co-treatments of vitamin E and ginger oil.

Concerning Group II (cyclophosphamide-treated group), testicular tissue showed marked interruption of spermatogenesis compared to normal control (Fig. 1f). The former represented by different degenerative changes and necrosis together with disorganization of spermatogonial cell layers (Fig. 1g). Spermatogonial cell death was apparent in most of the seminiferous tubules as pyknosis, karyorrhexis, karyolysis and nuclear fragments with variation in the staining intensity (Fig. 1h).

Degeneration and loss of the germinal epithelium within the seminiferous tubules were apparent. The later appeared as if lined by Sertoli cells with few or no spermatogenic cells particularly mature spermatid. Early atrophic changes were apparent as some tubules appeared atrophied with complete absence of the spermatogonial cell layers (Fig. 2a).

Inhibited spermiation was obvious (Fig. 2b) characterized by maturation arrest at primary and secondary spermatid with presence of elongated spermatid in the same tubule together with younger spermatid at the periphery of the seminiferous tubules with presence of radial orientation (Fig. 2c). Multinucleated spermatid giant cells were more conspicuous in large number of the seminiferous tubules (Fig. 2d and e). Interstitial edema was apparent (Fig. 2f).

It was pronounced that in some animals the entire testis was affected while, in other animals scattered tubules were affected.

The aforementioned histological alterations were restored to a good degree in those animals pre and co-treated with either vitamin E (Group III) or ginger (Group IV). But the most pronounced restoration was seen in group IV. Those restorative effects presented by normal testicular morphology and spermatogenesis especially in group IV with slight degeneration of spermatid and spermatozoa in some tubules (Fig. 2g and h). A large number of the seminiferous tubules showed beginning of active spermatogenesis represented by appearance of marked division of the spermatogonial cells (Fig. 3a) and normal spermiation, appearance of normal

Table 2 Comparison between mean values of control and treated groups regarding testosterone level, malondialdehyde (MDA) and oxidative enzymes highest bioavailability (HB)

Groups.	Testosterone level (ng/mL) $\bar{X} \pm$ SD **	MDA (UMOL/L) $\bar{X} \pm$ SD **	CATA (u/g) HB $\bar{X} \pm$ SD **	SOD (u/g) HB $\bar{X} \pm$ SD **
Control	2.02 ± 0.14 a	1.17 ± 0.17 c	61.6 ± 8.60 a*	1749.5 ± 80.50 a
CP	0.6618 ± 0.13 c	30.63 ± 3.14 a	18.01 ± 3.60 c	898.55 ± 64.30 d
Vita E + CP	1.355 ± 0.12 b	16.51 ± 2.25 b	27.5 ± 5.20 c	1105 ± 126.30 c
Ginger + CP	1.625 ± 0.20 b	12.66 ± 1.70 b	41.82 ± 3.10 b	1277.73 ± 52.40 b

*P < 0.05; **P < 0.001.

a, b,c Means within the same column having different letters significantly different.

CATA, catalase ; CP, cyclophosphamide; SOD, superoxide dismutase ; Vit. E, vitamin E.

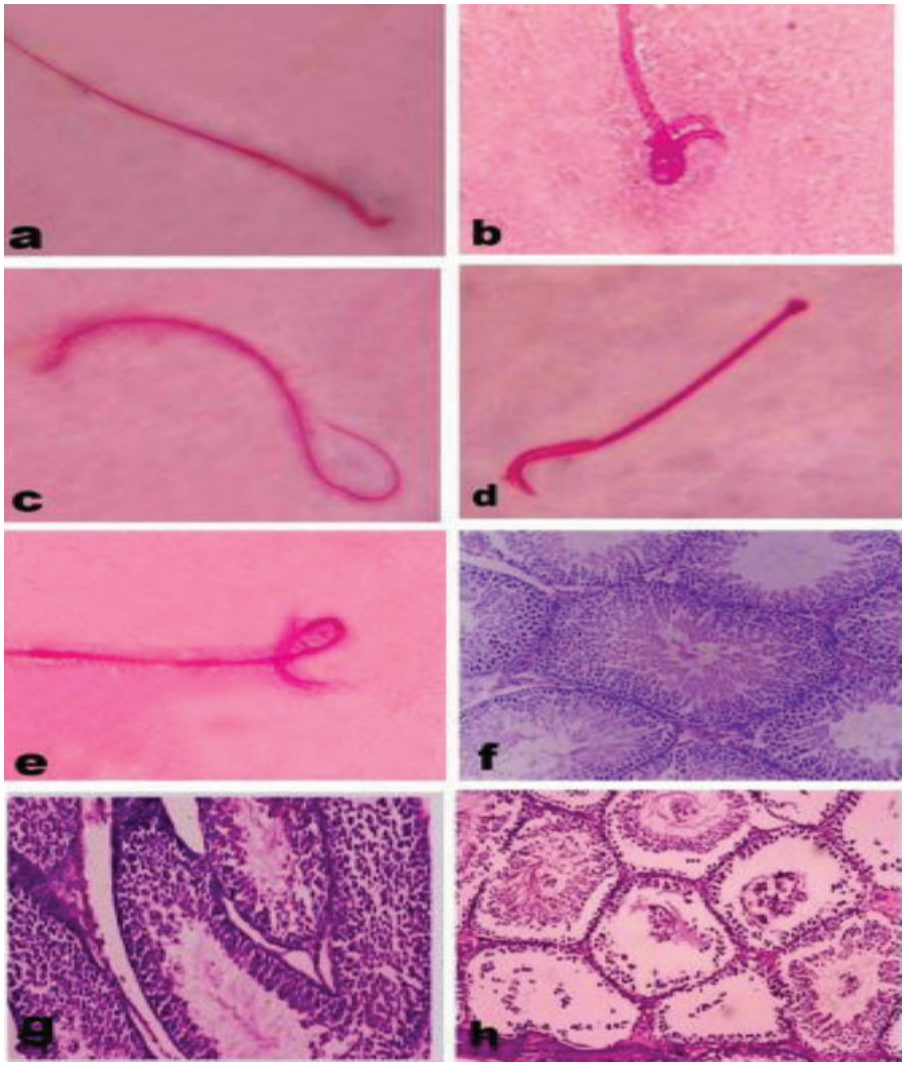

Figure 1 (a) the normal morphology of rat epididymal spermatozoa from control group. (b, c, d and e): rat epididymal spermatozoa from cyclophosphamide treated group showing (b) Double head and coiled mid piece (second abnormality). (c) Abnormal hook shape. (d) Detached head. (e) Broken mid piece (eosin and negrosin stain ×400). (f) Normal testis of control group, notice the normal spermatogonial cells layers, normal spermatogenesis and multiple sperms in the lumen of the seminiferous tubules. (hematoxylin and eosin×200). Testis of a cyclophosphamide-treated rat showing (g) disorganization of the spermatogonial cell layers and (h) various degenerative and necrotic changes of those cells (hematoxylin and eosin ×200).

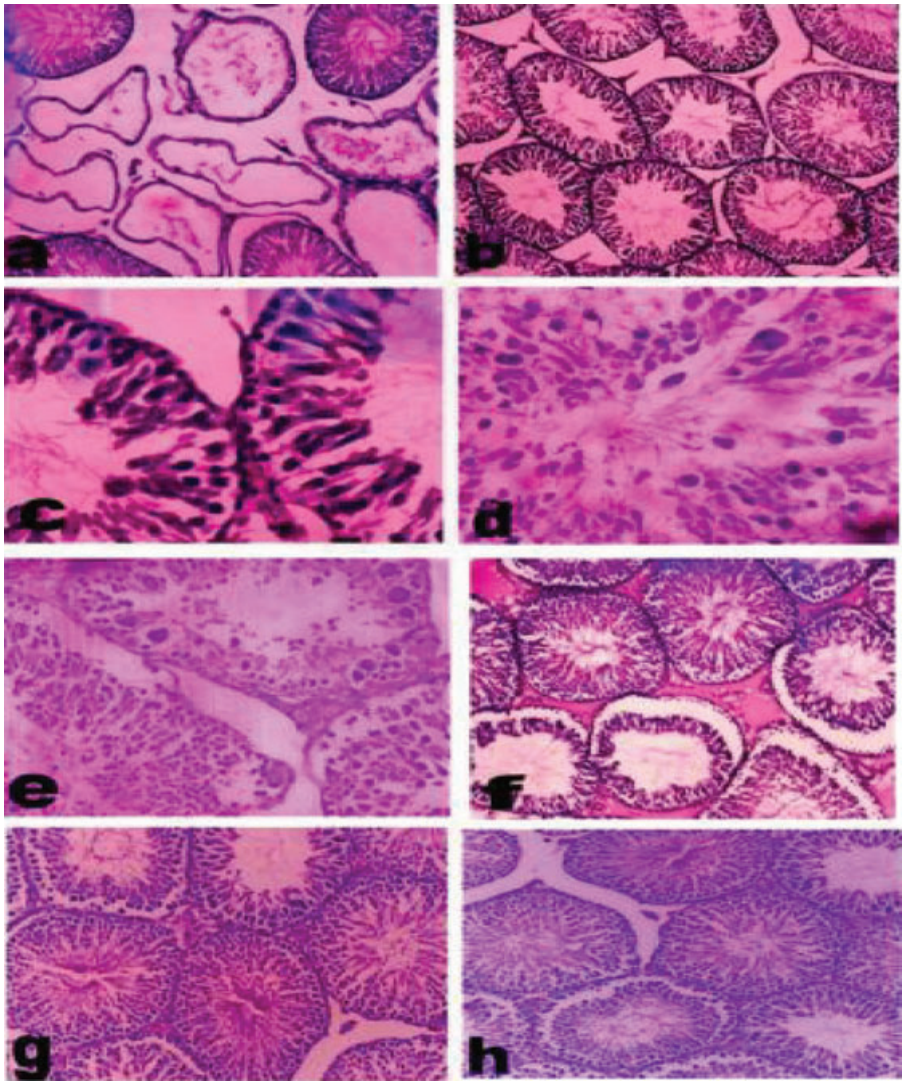

Figure 2 Testis of cyclophosphamide treated rat showing (a) Atrophic changes of large number of seminiferous tubules with complete absence of spermatogonial cells. (b) Inhibited spermiation. (c) Abnormal spermatid forms. (d) Necrotic spermatogonial cells and beginning of spermatid giant cell formation. (e) Multiple multinucleated spermatid giant cells in seminiferous tubules. (f) Marked interstitial oedema. (g) Testis of ginger and cyclophosphamide-treated rat showing active spermatogenesis in large number of the seminiferous tubules. (h) Testis of vitamin E and cyclophosphamide-treated rat showing active spermatogenesis with appearance of active sperms in the lumen of some tubules (hematoxylin and eosin ×200, ×400).

spermatid and an obvious active sperm in the lumen of these tubules (Fig. 3b).

Regarding the examined prostate gland of the cyclophosphamide treated group, it revealed degeneration and desquamation of the prostatic acinar cells lining. Some other acini showed cystic dilatation which appeared lined with flattened epithelium (Fig. 3c). While large number of the acinar cells showed marked hyperplasia with formation of polyps penetrating the acinar lumen (Fig. 3d).

Some rats showed marked metaplastic changes of the prostatic acinar epithelium. The later appeared lined with stratified epithelium. Dysplastic changes were also evident in some scattered tubular acini.

Seminal vesicles of cyclophosphamide treated animals revealed; marked vacuolar degeneration (Fig. 3e) as well as necrosis of the lining epithelium, with marked nuclear degeneration as pyknosis and karyorrhexis. Large numbers of detached epithelial cells were observed in the lumen.

Increased activity of the lining epithelium was evident in some animals together with marked hyperplastic changes (Fig. 3f). The nuclei of those epithelial cells were vesicular with marked pleomorphism.

Pre and co-treatments of vitamin E and ginger protected both the prostatic and seminal vesicle epithelium against the effect of cyclophosphamide. That protective effect represented by appearance of normal epithelium with restoration of most of the degenerative and necrotic changes.

In situ detection of apoptosis

Microscopic examination of testicular sections stained by TUNEL method showed a low incidence of apoptosis among the germ cells in the seminiferous tubules of control groups (Fig. 4a and b). In cyclophosphamide-exposed rats, the incidence of apoptosis increased significantly in all stages of the testicular germ cells as the

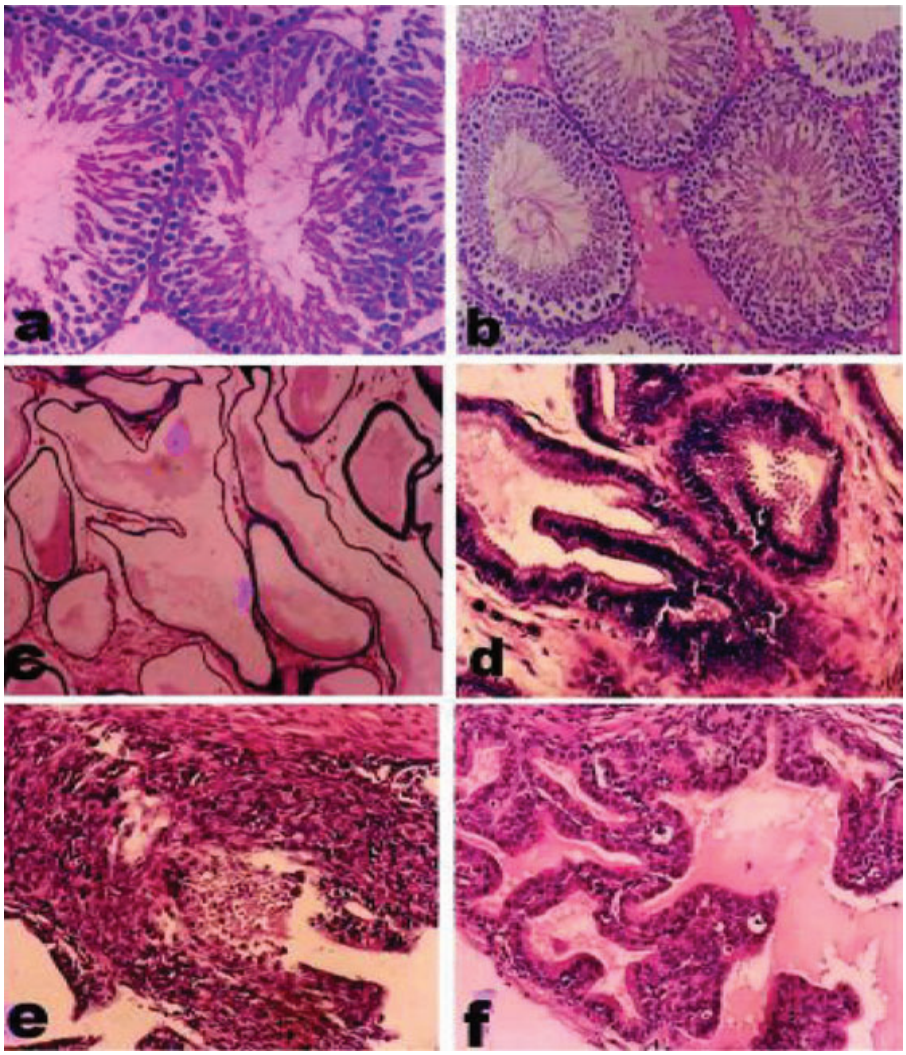

Figure 3 (a) Testis of a vitamin E and cyclophosphamide-treated rat showing division of the spermatogonial cells with beginning of active spermatogenesis. (b) Testis of ginger and cyclophosphamide-treated rat showing normal spermatid forms and active sperm in the lumen of seminiferous tubules. (c and d) Prostate gland of cyclophosphamide -treated rat revealing (c) Cystic dilatation and flattened epithelium of the prostatic acini. (d) Marked hyperplasia with formation of polyps penetrating the acinar lumen. Seminal vesicles of cyclophosphamide-treated rat showing (e) marked vacuolar degeneration of the lining epitheliumand (f) Hyperplasia as well as increased activity of the lining epithelium (hematoxylin and eosin ×200, ×400).

number of TUNEL-stained cells were high (Fig. 4c and d). While, examination of testicular sections from rats pre and co-treated with either vitamin E or ginger oil, revealed low incidence of apoptosis among the germ cells in comparison with the cyclophosphamide treated group. Ginger oil ameliorated cyclophosphamide induced apoptosis more than vitamin E.

DISCUSSION

Despite the continuous improvement of cancer treatment protocols, altered testicular function and infertility frequently represent major adverse effects of oncologic treatments. Thus, strong efforts are needed to avoid or at least to reduce these complications that are particularly relevant in young men without children.[13]

Our present study revealed marked protective role of both vitamin E and ginger oil on cyclophosphamide-induced male gonadal dysfunction. The later represented by altered male gonadal weight, disturbed sperm quality parameters, decreased testosterone level, disturbed oxidative stress and lipid peroxidation markers in addition to altered spermatogenesis, testicular histology and increased incidence of apoptosis among germ cells,. The findings of the present study pass in accordance with similar studies which previously reported that cyclophosphamide induced testicular androgenic and gametogenic dysfunction.[23,24]

Other clinical trials found patients who had received cyclophosphamide showed a severe gonadal failure characterized by reduced testicular size, very low sperm count and some degree of Leydig cell impairment.[5,13] Many experimental studies proved that, the reproductive system was damaged by cyclophosphamide administration, as they noticed reduction in weight of testis and epididymis and weight of the body,[25] histological changes of the testis and the penis observed by microscope and terminal deoxynucleotidyl transferase biotin-dUTP-X nick end labeling[14] and significant decrease in sperm count and motility with an increase in dead and abnormal sperm,[25] in addition to significant decrease in blood testosterone level.[14,23,25] The decrease in testosterone level served as a proof

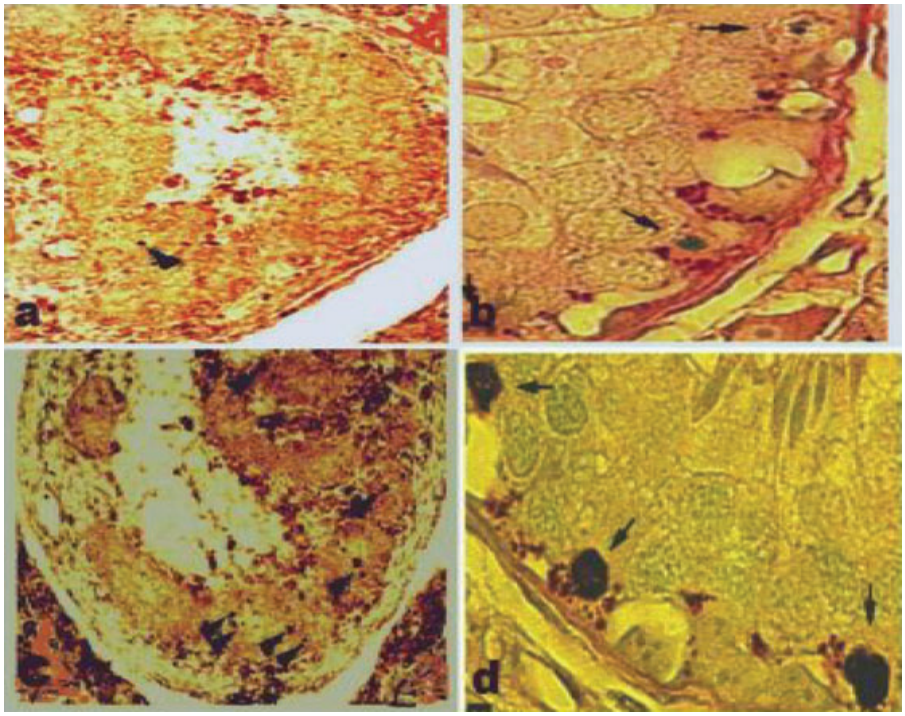

Figure 4 TUNEL-positive cells (arrows) were stained in sections from testis of (a and b) control and (c and d) of a cyclophosphamide-treated rat.

for the damage of testis.[14] Those changes could be attributed to cyclophosphamide-induced oxidative stress due to significant increase in testicular reactive oxygen species (ROS) level, along with a significant decrease in cellular thiol levels.[25] The oxidative stress is believed to be involved in the etiology of toxicities of many xenobiotics.[26]

The result of the present work showed that cyclophosphamide decreased the level of both CATA and SOD activities and increased the level of MDA activities. That disturbed activities suggested that free radical generation and lipid peroxidation could be from the mechanisms by which cyclophosphamide and its derivatives exert their toxic effects in different tissues. This suggestion passes parallel with other studies which reported that cyclophosphamide administration has been associated with free radical mediated oxidative stress.[27,28]

Histological examination of testicular tissue of cyclophosphamide treated group revealed disturbed spermatogenesis, severe diffuse damage of seminiferous tubules that reached to early atrophic changes with complete loss of the spermatogonial cells together with degeneration and necrosis of Leydig cells. In addition, various degenerative and necrotic changes were observed among the epithelial cells lining prostatic acini and seminal vesicles. Both testicular and secondary organs damage could be the cause of sperm abnormalities observed in our study. The former pathological alterations were more or less similar to a previous observation[29] who attributed those alterations to the oxidative stress induced by increasing lipid peroxidation production by cyclophosphamide as well as to the ability of the activated metabolites of cyclophosphamide (which are alkylating agents) to cause cross-linking of DNA strands, interfering with normal cell division in all rapidly proliferating tissues. In addition, cyclophosphamide can disrupt the redox balance of tissues suggesting that biochemical and physiological disturbances may result from oxidative stress caused by the generation of free radicals and ROS[30] and due to their high polyunsaturated fatty acid content, spermatozoa plasma membranes are highly sensitive to ROS-induced damage and lipid peroxidation.[31]

The TUNEL assay in the present study revealed an increased number of TUNEL positive cells in cyclophosphamide treated group compared to control group as well as vitamin E and ginger pre and co-treated groups. Similar results were mentioned that cyclophosphamide induces apoptosis in male germ cells.[31] That increased incidence of apoptosis may be attributed to cyclophosphamide could increases single- and double-strand breaks in DNA of the testicular germ cells (which were TUNEL positively stained in our study) and cross-links in spermatozoa.[4,32] On the other hand, Nandi *et al.*[33] related the increased germ cells apoptosis in testicular tissue to testosterone withdrawal (a result which was obtained in our study), suggesting that testosterone may function as a cell survival factor, in some way protecting germ cells from apoptotic death. They also added that the molecular mechanism by which testosterone does so, however, has not yet been elucidated.

In the present study we administrated both vitamin E and ginger before and during cyclophosphamide treatment to strengthen the antioxidant system, eliminate oxidative reaction and counteract or ameliorate the toxicity induced by cyclophosphamide. It is well known that antioxidants form an important part of a cells defense against free radical damage. The present work showed that both of vitamin E and ginger improved all studied parameters with the superiority of ginger. The possible mechanism of vitamin E protection is attributed to its well-known antioxidant effects on the testicular injury and scavenging free radical as well as it may have some stimulatory effects on gonadotrophin releasing of pituitary anterior lobe.[29,34] It has been claimed that α-tocopherol is the most important lipid-soluble antioxidant, and that it protects cell membranes from oxidation by reacting with lipid radicals produced in

the lipid peroxidation chain reaction. The oxidized α-tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol.[9]

Recently, much attention has been focused on the protective effects of antioxidants and naturally occurring substances against substances induced toxicities. However, little is known about herbal plants as protective agents against cyclophosphamide-induced testicular toxicity. In our work, we chose ginger as one of the available medicinal plants in Egypt that has antioxidative reputation and compared its ameliorative effect on cyclophosphamide gonadal toxicity with that of the potent antioxidant, vitamin E, as in 21st century, there is a long tradition of the use of medicinal plants for health recovery[35] and direction for complementary and alternative medicine where herbal medicinal treatment shows a promising effect.

This high restorative activity was explained by potent androgenic and antioxidant activities in male rats, increased testosterone level and testicular weight produced by *Z. officinale* extracts and its major active phenolic ingredients.[36,37] Moreover, *Z.officinal* treatment increased activities of testicular antioxidant enzymes; superoxide dismutase, glutathione and catalse and reduced level of malondialdhyde.[38] Some pungent constituents present in ginger and other zingiberaceous plants have potent antioxidant and anti-inflammatory activities, and some of them exhibit cancer preventive activity in experimental carcinogenesis.[36] The anticancer properties of ginger are attributed to the presence of certain pungent vallinoids; gingerol and paradol, as well as some other constituents like shogaols, zingerone etc. A number of mechanisms that may be involved in the chemopreventive effects of ginger and its components have been reported from the laboratory studies in a wide range of experimental models.[36,39] The present work therefore suggested that the co-administration of ginger with cyclophosphamide therapy not only ameliorates gonadal toxicity through its antioxidant and androgenic activities but also it potentiates the chemo preventive effects of cyclophosphamide.

Finally, the findings of the present study proved that the protective effect of ginger is superior to that of vitamin E in reproductive toxicity induced by cyclophosphamide as was reported that the protective effect of Z. officinale was found to be better than that of vitamin E in cisplatin-induced nephrotoxicity.[26]

CONCLUSION

Experimental data of our work suggested that cyclophosphamide treatment is associated with antigonadal activities as well as induction of oxidative stress in gonad that can be ameliorated significantly by vitamin E and ginger oil co-administration. The later proved a positive role for medicinal herbs in combination with conventional chemotherapy.

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