

Protective Effect of Alpha Lipoic Acid and Royal Jelly Against the Side Effects of Cyclophosphamide in Testis of Male Albino Rats

Original
Article

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ABSTRACT

Introduction: Cyclophosphamide (CP) is a widely used anti-cancer drug which can induce serious male infertility.

Aim of the study: The present study aims at determining the protective role of alpha-lipoic acid and royal jelly in improving biochemical, histological and ultrastructural disorders in the testis of rats induced by CP.

Materials and Methods: 64 rats were separated into eight groups each of 8 rats. (G1) control group, (G2) alkaline solution treated group, (G3) DMSO treated group, (G4) Alpha-lipoic acid (LA) treated group, (G5) Royal jelly (RJ) treated group, (G6) CP treated group (5 mg/kg b.wt) three days per a week for 4 weeks, (G7) CP plus LA treated group (25 mg/kg b. wt) and (G8) CP plus RJ treated group (1g/kg b. wt). Animals were sacrificed after 4 weeks.

Results: CP leads to an increase in lipid peroxidation in form of malonaldehyde (MDA) and decline in superoxide dismutase (SOD) in comparison with control. It caused histopathological changes in testes of rats including vacuolation, exfoliation of germ cells in the lumina of the seminiferous tubules, and maturation arrest. Furthermore, oedema was also observed and congestion of the intertubular blood vessels. Ultrastructurally, the boundary tissue of some tubules displayed noticeable changes; it was thickened and folded. Sertoli cells displayed an increase in the number of lysosomes and their nuclei showed signs of pyknosis, cytoplasmic vacuolation. Administration of LA and RJ to CP-treated rats revealed marked improvement in the altered level of SOD and MDA activities when compared with the CP-treated group and showed improvement in histopathology and ultrastructure of the testis.

Conclusion: Treatment of the cyclophosphamide with the alpha-lipoic acid-induced great regaining of the damaged testicular tissues and royal jelly induced little regaining of the damaged testicular tissues, so LA was effective in treatment with CP than RJ with CP. This enhancement because of their antioxidant properties and its scavenging abilities against active free radicals.

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Key Words: Alpha lipoic acid, cyclophosphamide, histopathological and ultrastructural study, royal jelly, testis.

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INTRODUCTION

Anti-cancer drugs are compounds which are of high environmental significance because of their deficiency of specific mode of action and they can be particularly destructive to living organisms even at low concentrations^[1]. Anticancer drugs are cytotoxic, genotoxic, and also are released into the environment as a result of incomplete metabolism, they display high biological activity. Consequently, they pose a serious threat to the environment and human health due to their mutagenic, carcinogenic and/or reproductive toxicity properties^[1,2,3].

Cyclophosphamide (CP), was introduced in 1960, effective against a broad spectrum of human cancers, including benign diseases, chronic and acute leukemia, multiple myeloma, lymphomas, systemic lupus erythematosus, multiple sclerosis, rheumatic arthritis, some forms of leukemia, and some solid tumors and

widely used as anti-neoplastic drug^[4]. This alkylating agent considered the “strongest” medication and generally used by rheumatologists^[5].

CP toxicity in testis and spermatozoa is due to oxidative stress which induced biochemical and physiological damage^[6] and also leads to disturbances in gonadotropin secretion, testicular damage, and decreased plasma testosterone levels are found in patients undergoing treatment with CP^[7,8]. Additionally, mitochondria and abnormal morphology of spermatozoa plasma membranes produce reactive oxygen species (ROS) through nicotinamide adenine dinucleotide-dependent oxidoreductase systems, respectively and the nicotinamide adenine dinucleotide phosphate-dependent^[9].

CP-induced reproductive damage was predominantly due to the production of oxidative stress, lipid peroxidation, DNA damage and decreased glutathione levels^[10].

Alpha-lipoic acid (LA) directly quenches free radicals, inhibits reactive oxygen-generators and regenerates other antioxidants so it considered as an ideal antioxidant^[11].

LA has antioxidant properties as it scavenges hydroxyl radicals^[12]. LA can cross biological membranes easily and suppresses free radicals, owing to its small size and high lipophilicity^[13] and can improve mitochondrial function by stimulating sirtuin 1 and 3^[14]. LA is also well-known for its anti-inflammatory properties, which are attributable to the LA-dependent down-regulation of the pro-inflammatory NF- κ B pathway as studied elsewhere^[15]. LA contains two thiol-groups, which may be reduced rapidly in many tissues to its dithiol and dihydroliipoic acid (DHLA) form^[16,17].

Royal jelly (RJ) is a nutritive secretion formed by the worker bees, rich in carbohydrates, proteins, vitamins and minerals^[18]. RJ has remarkable antimicrobial properties so it has been used as traditional medicine^[19]. Young worker bees secrete RJ by its hypopharyngeal glands. RJ is used in pharmaceutical, food and cosmetics productions due to its exceptional biological properties. Several studies confirm that RJ has anti-inflammatory, antibacterial, anti-ageing, antimutagenic potentials, antioxidant, antitumor, anti-diabetic and anti-fatigue. These activities are principally due to bioactive components it contains^[20].

RJ has many pharmacological activities: neurotrophic, hypoglycemia, antioxidant, hypocholesterolemia and hepatoprotective, hypotensive and blood pressure regulatory, antitumor, antibiotic, anti-inflammatory, immunomodulatory and anti-allergic, general tonic, antiaging etc^[21] and also has antiviral, anti-ulcerous and antibacterial activities^[22,23].

The current study was performed to study the biochemical, histopathological and ultrastructure changes of CP effect on testis and the role of LA and RJ in ameliorating these changes.

MATERIALS AND METHODS

Materials

1- Cyclophosphamide (CP)

Cyclophosphamide (CP) was purchased from Baxter Oncology GmbH Kantstrasse 2 D-33790 Halle, Germany. CP dissolved in saline and was orally treated in a dose of 5 mg/kg b. wt.

2- Alpha Lipoic Acid (LA)

LA was purchased from Sigma Alderich Chemical Company Inc. Product of China store at: 2-80C, MW:206.33, mp:60-620 °C, assay: \geq 98.0% (HPLC). LA dissolved in 100 ml (25 ml Di Methyl Sulpho Oxide (DMSO) + 75 ml distilled water) and we used NaOH to neutralize the solution PH=7 and was given orally in a dose of (25 mg/kg b.wt) three times a week for 4 weeks.

3- Royal jelly (RJ)

RJ was purchased from Safe for Pharmaceutical Products for Pharco-Pharmaceuticals (Store at Temperature

not exceeding 300 °C) dissolved in 10 ml distilled water and was given orally in a dose of (1g/kg b.wt) three times a week for 4 weeks.

4- Animals

The experimental animals used in this work were random-bred 64 white male albino rats (*Rattus norvegicus*) weighing 140- 170g, The animals were kept in plastic cages with wired covers and under normal laboratory conditions for different periods used. The animals were supplied with classic rat chow ad libitum and water and they were kept under observation for a week before the start of the experiment. In this study, animal care was carried out following the European Community Directive (86/609/EEC) and national rules, this is in accordance with the NIH Guidelines for care and use of Laboratory Animals, 8th editions. This was administrated by the committee of the Zoology Department, Beni-Seuf University, Egypt.

Then the animals were separated into eight groups each of 8 rats.

Group 1: This group was served as a control group and received saline orally.

Group 2: This group was received alkaline solution orally.

Group 3: This group was given DMSO orally.

Group 4: This group received an oral dose of lipoic acid (25 mg/kg b.wt) three times a week for 4 weeks^[24].

Group 5: received an oral dose of royal jelly (1g/kg b.wt) three times a week for 4 weeks^[25].

Group 6: was orally treated with CP (5 mg/kg b.wt) three days per week for 4 weeks^[26].

Group 7: was orally treated with CP (5 mg/kg b.wt) then received an oral dose of lipoic acid (25 mg/kg b.wt) three times a week for 4 weeks.

Group 8: was orally treated with CP (5 mg/kg b.wt), then received an oral dose of royal jelly (1g/kg b.wt) three times a week for 4 weeks.

METHODS

Tissue Homogenate Preparation

Testes homogenate, obtained by grinding a small piece (1g) of freshly excised tissue in 10 volumes of 0.9% saline solution, was kept at -20 °C till use in the determination of superoxide dismutase (SOD) using the methods of Rest and Spitznagel^[27], and lipid peroxidation (LPO) according to Ohkawa *et al*^[28].

Histological Preparations

After 4 weeks, the animals from each group were sacrificed under mild diethyl ether anaesthesia. Small pieces of testis were fixed in 10 % neutral buffered formalin solution for 24 hours. Tissue specimens were embedded in paraffin using a conventional method. Paraffin sections of some testes were stained with haematoxylin and eosin^[29].

Ultrastructural Preparations

After 4 weeks of specimens from testes cut into small pieces measuring about 1 mm³ and directly fixed in fresh 3% glutaraldehyde-formaldehyde at 4°C for 18-24 hours. Then the specimens were washed in phosphate buffer (pH 7.4) and then post-fixed in isotonic 1% osmium tetroxide for one hour at 4°C^[30]. Serial dehydration in alcohol was carried out. The specimens were then passed through propylene oxide solution. Finally embedded in Epon epoxy resin. Semithin sections were cut from these blocks at 1.0 µm thickness by ultra-cute Reichert-Jung ultramicrotome with the aid of glass knives, stained with toluidine blue stain and examined by light microscope to detect the area of interest. Ultrathin sections (70-90 nm) were then prepared using the ultramicrotome glass knives, stained with uranyl acetate and lead citrate^[31] and examined with a Joel JEM-2100 transmission electron microscope worked at an accelerating voltage 80 kV.

Statistical Analysis

Analysis of data was performed using student ANOVA test followed by Duncan's multiple range tests post hoc analysis values. A value of $P < 0.05$ was considered statistically significant. Analysis of data was expressed as mean \pm SEM. Package for the Social Sciences (SPSS for WINDOWS, version 20.0; SPSS Inc, Chicago)^[32] was used for the statistical analysis of data.

RESULTS

Determination of Antioxidant Parameters

(Figures 1 and 2 and Table 1) indicated an increase in lipid peroxidation in form of malonaldehyde (MDA) and decrease in superoxide dismutase (SOD) in comparison with control, slightly alkaline solution, DMSO, CP+ LA and CP+ RJ groups. Although LA and RJ administration made an increase in SOD activity and decrease in MDA similar to control groups. Concerning one way ANOVA, markers of testicular tissue lipid peroxidation and superoxide dismutase of all groups are given in figures (1,2) respectively. CP administration resulted in a significant ($P < 0.001$) increase in MDA level (46.17 ± 2.65 nmol/g tissue) when compared to the control group (9.20 ± 0.38 nmol/g tissue). CP administration resulted in a significant decrease in SOD level (58.07 ± 1.90 U/g tissue) when compared to the control group (86.41 ± 1.43 U/g tissue). However, administration of LA and RJ to CP-treated rats revealed marked amelioration in the altered level of MDA and SOD activities when compared with the CP-treated group.

Histopathological Observations of the Testis

A) Testis of Control Rats

Histologically, the testes control, alpha-lipoic acid and royal jelly respectively showed normal structure. It consists of several seminiferous tubules. Each tubule is lined with spermatogenic cells which produce sperms and Sertoli cells which act as supporting cells. The

spermatogenic cells include spermatogonia, primary and secondary spermatocytes and spermatids. The tubules are lined with interstitial supporting tissue which composed of connective tissues, blood vessels and Leydig cells (Figures 3a,b and c).

B) Testis of Rats Treated with Cyclophosphamide

The present study indicated that the testes of cyclophosphamide treated rats showed degenerative alterations in the seminiferous tubules and interstitial tissue. These changes including thickening of the basement membrane and congestion of blood vessels (Figure 4a), hyperplasia of germ cells in which the spermatogenic cells increase in number and the tubules appeared as a solid mass (Figure 4b). Desquamation and exfoliation of the germ cells in the lumen of the seminiferous tubules were observed (Figure 4c), in addition to oedema and maturation arrest (Figure 4d). Moreover, atrophy of some tubules and vacuolation in the spermatocytes of other tubules were evident (Figures 4e and f).

C) Testis of Rats Treated with Cyclophosphamide Plus Alpha-lipoic Acid and Royal Jelly

In contrast, the tubules of the testis of rats treated with cyclophosphamide plus alpha-lipoic acid (Figure 5a), and cyclophosphamide treated rats plus royal jelly (Figure 5b) restored their regular shape, and the spermatogenic layers were comparatively well preserved.

Ultrastructural Observations of the Testis

A) Testis of Control Rats

The electron microscopic examinations of the control testis showed that each seminiferous tubule is surrounded by a boundary tissue which consists of an inner layer of loosely-arranged collagenous fibres. The seminiferous tubule is lined with spermatogenic cells and Sertoli cells (Figure 6a). Sertoli cells rest on the basal lamina. Their nuclei are large, infolded and possess a prominent nucleus. The nucleoplasm is homogenous and the cytoplasm contains mitochondria, lysosomes and rough endoplasmic reticulum (Figure 6a). The primary spermatocytes are large spherical cells, their nuclei appeared spherical with centrally clumps of chromatin substance their cytoplasm contains mitochondria and rough endoplasmic reticulum and contain large nucleoli (Figure 6a). The spermatids are rounded cells and contain large spherical nuclei which contain chromatin clumps in a lightly stained nucleoplasm and their cytoplasm contains free ribosomes, lysosomes and mitochondria (Figure 6b). Leydig cells are relatively large and their cytoplasm contains numerous lipid droplets and rough endoplasmic reticulum (Figure 6c). Each cell possesses a large nucleus containing a prominent nucleolus and a dense rim of heterochromatin adherent to the inner side of the nuclear envelope (Figure 6 d, e).

B) Testis of Rats Treated with Cyclophosphamide

The ultrastructural examination of the testis of the cyclophosphamide treated rats showed degenerative changes in the spermatogenic cells and their surrounding

tissues. The boundary tissue appeared thickened, folded and contained a large number of collagenous fibres, where Sertoli cells showed distended shrunken nucleus (Figure 7a). In some Sertoli cells, the cytoplasm contains large vacuoles, lipid droplets and degenerated mitochondria (Figure 7a). The cytoplasm of the spermatogonia contained many large vacuoles, lipid droplets and damaged mitochondria (Figure 7b). The primary spermatocytes seemed with the degenerated nucleus and fragmented chromatin (Figure 7b). The primary spermatocytes seemed with the degenerated nucleus and fragmented chromatin (Figure 7b). The spermatid appeared degenerated with lysis of cytoplasmic component and other cells containing some lysosomes, giant mitochondria and shrunken nuclei (Figure 7c). Leydig cells showed many vacuoles, shrunken nuclei and decreased number of lipid droplets (Figure 7d). Sperms showed a marked decrease in their number (Figure 7e).

C) Testis of Rats Treated with Cyclophosphamide Plus Alpha-Lipoic Acid and Royal Jelly

Supplementation of alpha-lipoic acid to the cyclophosphamide treated rats showed noticeable improvement in the microscopical structure of the testis. The basal lamina restored its normal structure. Sertoli cells and the spermatogonia retained their normal organelles with few vacuolated mitochondria (Figure 8a). The primary spermatocytes seemed normal with normal mitochondria (Figure 8b). The spermatids retained their normal organelles (Figure 8c). Leydig cells showed few vacuoles and marked an increase in the lipid droplets similar to the control ones (Figure 8d). Sperms showed marked amelioration in their number (Figure 8e). On the other hand, testes of rats treated with cyclophosphamide plus royal jelly showed great recovery spermatogonia, Sertoli, primary spermatocytes, spermatids, Leydig cells except for few vacuoles and a moderate improvement in several sperms (Figures 9a, b, c, d and e).

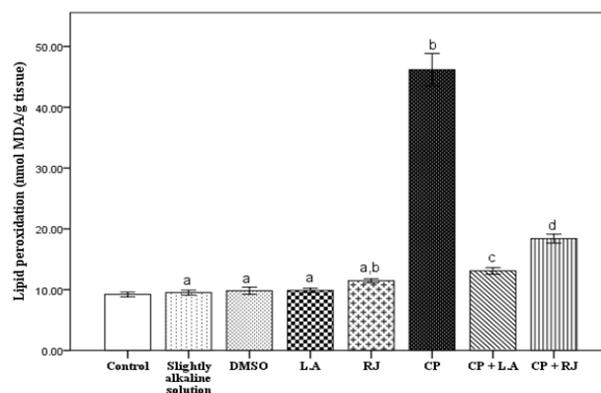


Fig. 1 : Lipid peroxidation (MDA) (nmol/g tissue) of control, slightly alkaline solution, Di Methyl Sulpho Oxide (DMSO), alpha lipoic acid (LA), royal jelly (RJ), cyclophosphamide (CP), cyclophosphamide Plus alpha lipoic (CP+ LA) acid and cyclophosphamide Plus royal jelly (CP+ RJ) for 30 days in testis homogenate

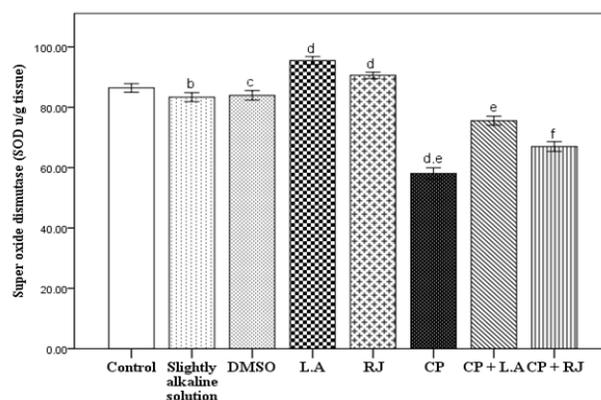
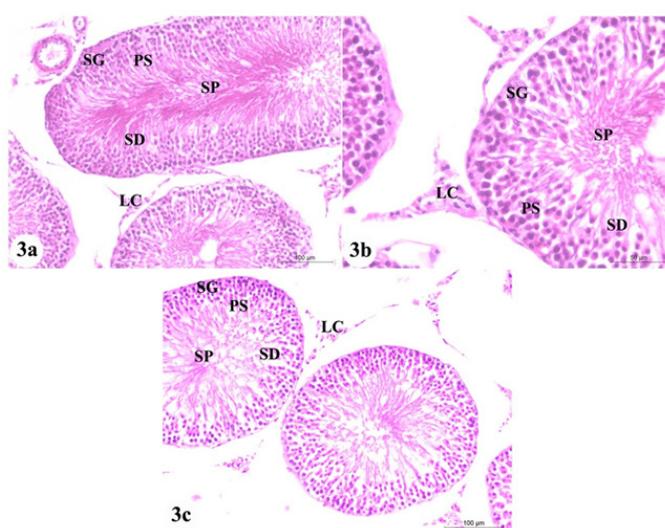
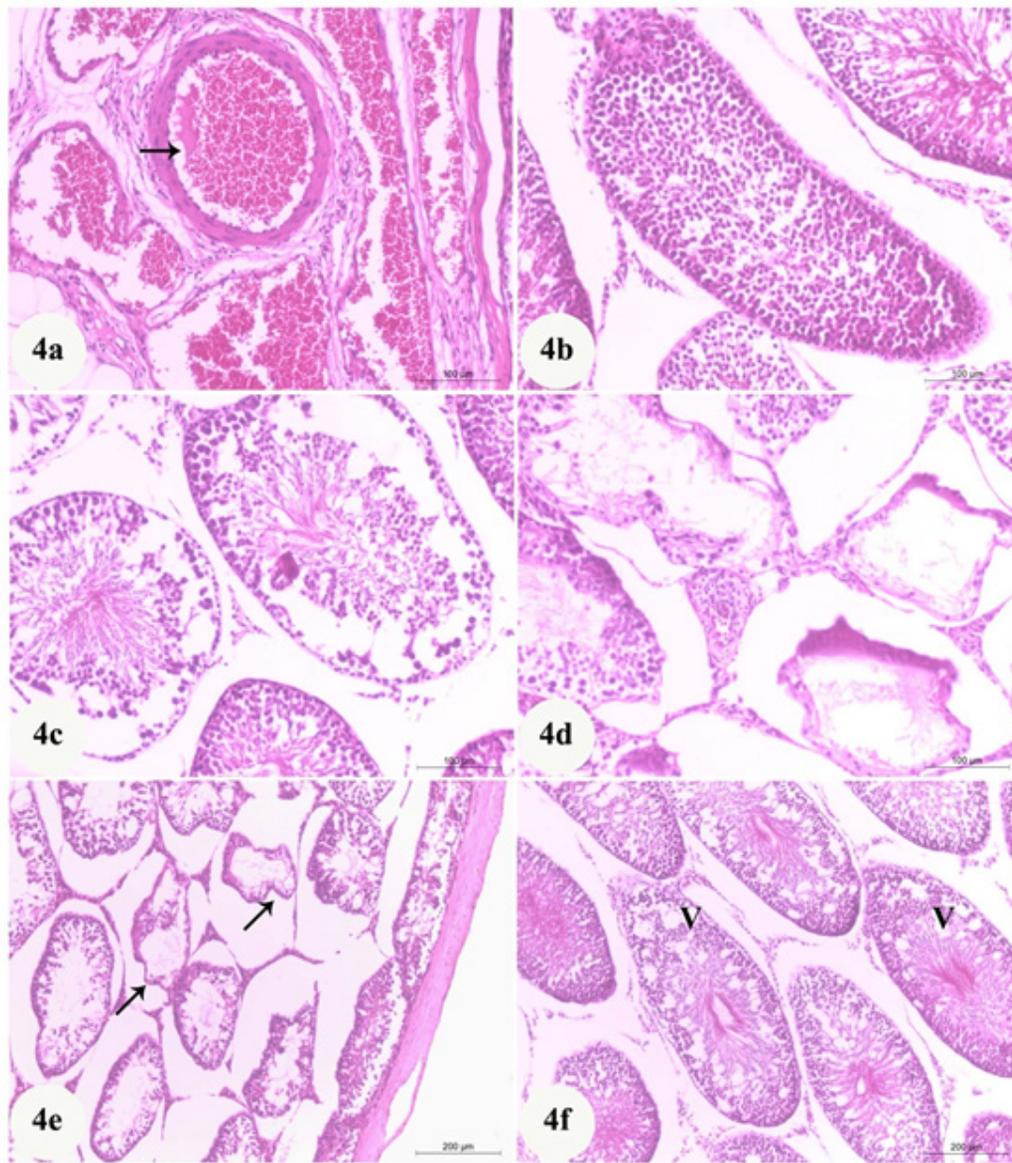


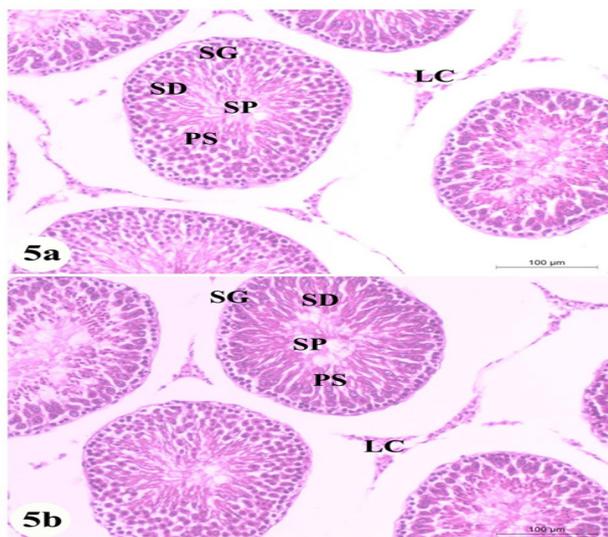
Fig. 2: Superoxide dismutase (SOD) (u/g tissue) of control, slightly alkaline solution, Di Methyl Sulpho Oxide (DMSO), alpha lipoic acid (LA), royal jelly (RJ), cyclophosphamide (CP), cyclophosphamide Plus alpha lipoic (CP+ LA) acid and cyclophosphamide Plus royal jelly (CP+ RJ) for 30 days in testis homogenate



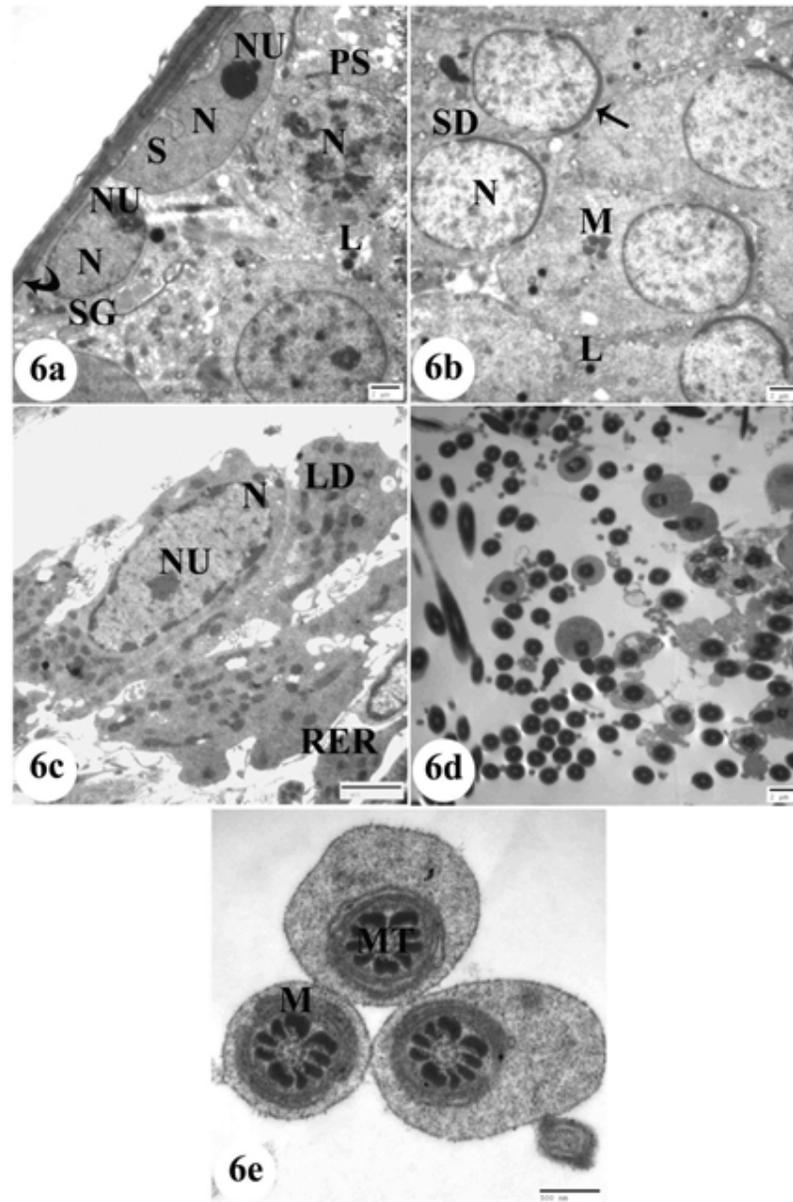
Figs. 3 (a, b&c): photomicrograph of testis sections of control rats, alpha-lipoic acid and royal jelly respectively stained with H&E showing seminiferous tubules, Leydig cells (LC), spermatogonia (SG) resting upon the basal lamina of the seminiferous tubules that are oval in shape, primary spermatocytes (PS), recognized by their large nuclei containing coarse clumps of chromatin, spermatids (SD) with rounded nuclei and sperms in the lumen of the tubules (SP). (H&E X = 200, 400, 200 respectively)



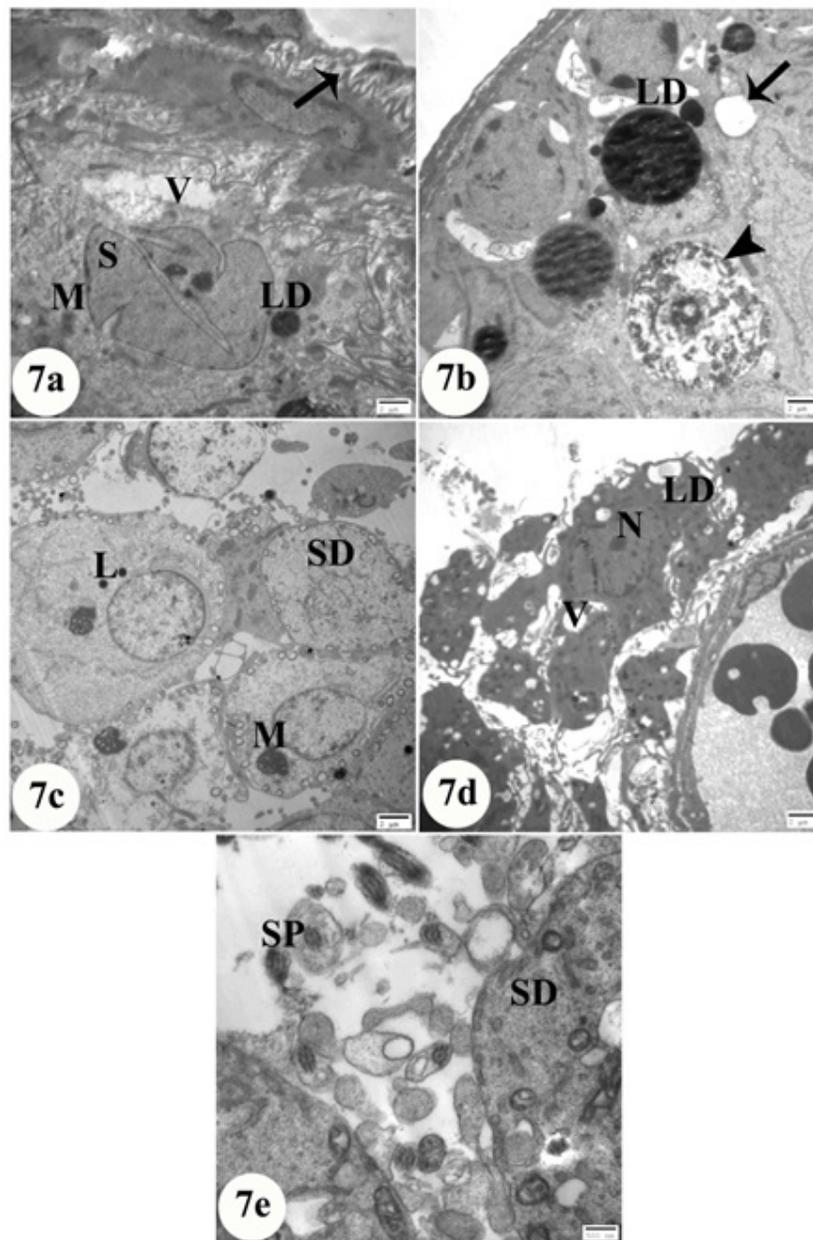
Figs. 4 (a-f): photomicrograph of testis sections of CP treated rats. a): showing thickening of basement membrane and congestion of blood vessels (arrow). b): showing hyperplasia of germ cells. c): showing desquamation and exfoliation of the germ cells in the lumen of the seminiferous tubules. d): showing oedema and maturation arrest. e&f): showing atrophy of some tubules (arrow) and vacuolation (V) in the spermatocytes. H&E X= 200, 200, 200, 200, 100, 100 respectively.



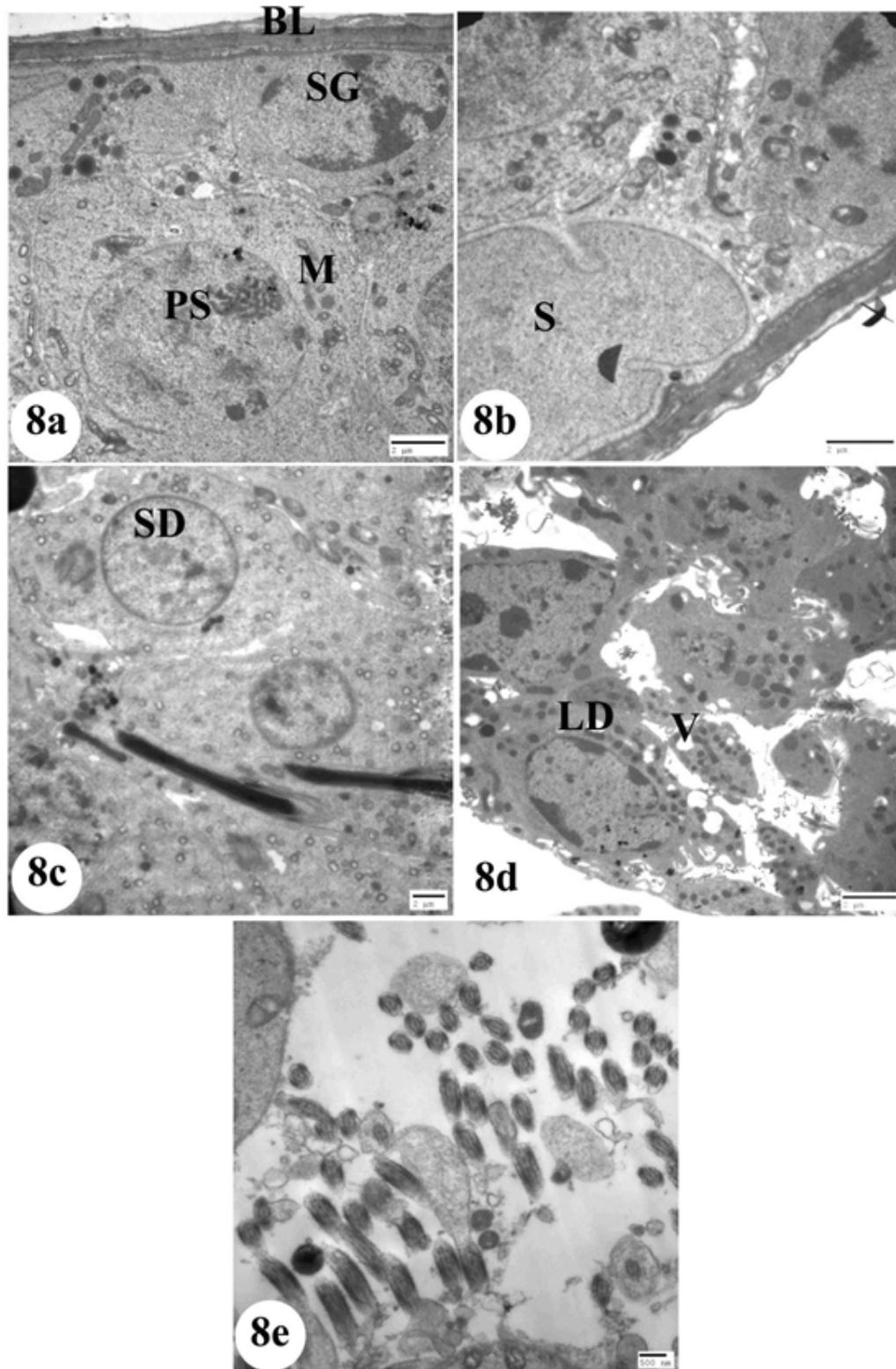
Figs. 5 (a&b): photomicrograph of testis sections of cyclophosphamide plus alpha-lipoic acid and cyclophosphamide plus royal jelly respectively showing almost normal structure of seminiferous tubules, Leydig cells (LC), spermatogonia (SG), primary spermatocytes (PS), spermatids (SD) and sperms (SP). H&E X= 100, 100 respectively.



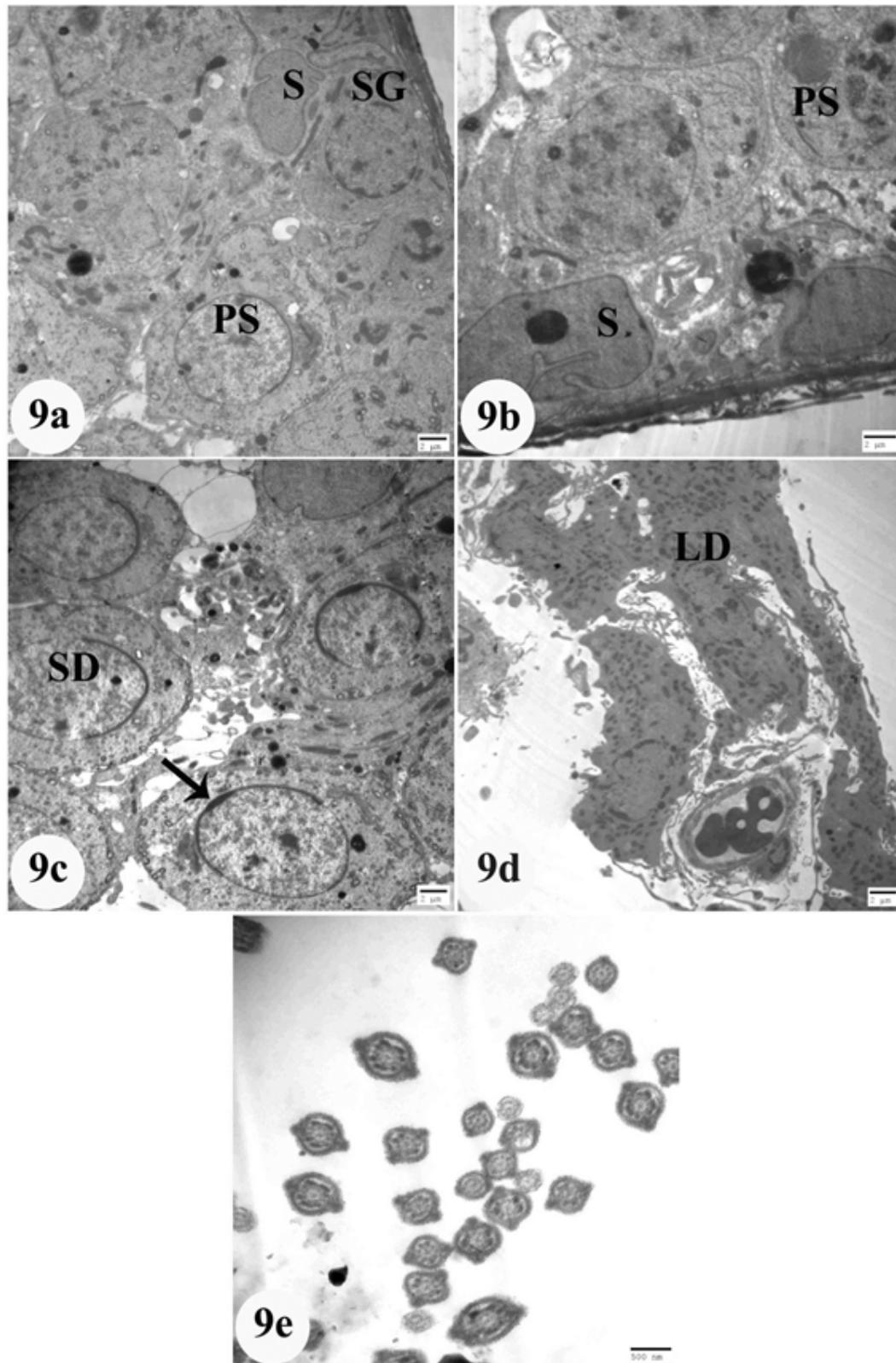
Figs. 6 (a-e): Electron micrographs of ultrathin sections of testis of control rats showing: a) Normal testis enveloped by the boundary layers (curved arrow), Sertoli cell (S) resting on basal lamina (BL) with large nucleus (N), nucleolus (NU), spermatogonia (SG) possess spherical nucleus (N), nucleolus (NU) and primary spermatocyte (PS) with large spherical nucleus (N), lysosomes (L). b) Spermatide (SD) with spherical nucleus (N), acrosomal membrane (arrow), mitochondria (M), lysosomes (L). c) Leydig cell with large nucleus (N), nucleolus (NU) and rough endoplasmic reticulum (RER) and lipid droplets (LD). d) showing cross-section of sperms at the middle piece. (LD). e) Higher magnification showing middle piece containing mitochondria (M) and microtubules (MT). TEM X= 1200, 1200, 2500, 1200, 10000 respectively.



Figs. 7 (a-e): Electron micrographs of ultrathin sections of testis of CP treated rats showing: a): Spermatogonial cells with thickened boundary tissue, folded and contained a large number of collagenous fibres (arrow), where Sertoli cells (S) showed distended shrunk nucleus, vacuolation (V), mitochondria (M) and lipid droplets (LD). b): Showing Spermatogonia with many large vacuoles (arrow), lipid droplets (LD), damaged mitochondria and degenerated nucleus of primary spermatocytes appear with fragmented chromatin. (arrowhead). c): Showing degenerated spermatid (SD) with lysis of cytoplasmic component and other cells containing some lysosomes (L), giant mitochondria (M) and shrunken nuclei. d): Showing vacuolation in Leydig cells (V) with irregular shrunken nucleus (N) and decreased number of lipid droplets (LD). e): showing degenerated spermatids (SD) and marked decrease in sperms (SP). TEM X= 1200, 1200, 1200, 1200, 5000 respectively.



Figs. 8 (a-e): Electron micrographs of ultrathin section of testis of CP and alpha-lipoic acid-treated rats showing marked improvement of basement membrane (BL), spermatogonia (SG), primary spermatocytes (PS) with normal mitochondria (M), Sertoli cell (S), spermatids (SD), Leydig cells (LD) with little vacuoles and marked amelioration in number of sperms. TEM X= 2000, 2500, 1200, 2000, 4000 respectively.



Figs. 9 (a- e): Electron micrographs of ultrathin section of testis of CP and royal jelly treated rats showing improvement of spermatogonia (SG), primary spermatocytes (PS), Sertoli cell (S), spermatids (SD) with normal acrosomal membrane (arrow), Leydig cells (LD) with little vacuoles and moderate improvement in number of sperms. TEM X= 1000, 1200, 1000, 1000, 6000 respectively.

Table 1: Protective effect of alpha lipoic acid and royal jelly against cyclophosphamide induced changes in lipid peroxidation (MDA) and Superoxide dismutase (SOD) in testis tissues of all experimental groups

Groups	Lipid peroxidation (n mol MDA/g tissue)	Super Oxide Dismutase (SOD) (U/g tissue)
G1 (-ve control)	9.20 ± 0.38	86.41 ± 1.43
G2 (slightly alkaline solution)	9.51 ± 0.40a	83.37 ± 1.49b
G3 (DMSO)	9.82 ± 0.57a	83.95 ± 1.56c
G4 (LA)	9.86 ± 0.35a	95.50 ± 1.32d
G5 (RJ)	11.45 ± 0.33a,b	90.58 ± 1.04d
G6 (CP)	46.17 ± 2.65b	58.07 ± 1.90d,e
G7 (CP + LA)	13.08 ± 0.54c	75.55 ± 1.49e
G8 (CP + RJ)	18.38 ± 0.73d	67.00 ± 1.63f
One way ANOVA (<i>P</i> value) F = 144.494, (<i>p</i> <0.001) F = 68.605, (<i>p</i> <0.001)		

- Data are expressed as mean ± standard error (SE)

- Means with the same superscript letter are non-significant at *P* > 0.05, whereas others are significant at *P* < 0.05 and highly significant at *P* < 0.01.

DISCUSSION

Cyclophosphamide (CP) is the most common anticancer drugs, but its clinical efficacy is also linked with testicular toxicity^[33]. In our study CP- induced an increase in lipid peroxidation in the form of malonaldehyde (MDA) and decrease in superoxide dismutase (SOD). Parallel results were reported by Ghosh *et al*^[34], and especially induce lipid peroxidation in the mitochondria of rat testis^[35] which may result in mitochondrial disintegration membrane ultrastructure. The generation of free radicals and other reactive oxygen species (ROS) as well as LPO have been informed to be the major mechanisms in CP-toxicity^[36,37]. Such oxidative stress generates biochemical and physiological disturbances^[38]. Administration of CP under different conditions and doses has been established to be an excellent model to produce syndromes of oxidative stress^[39]. Generally, CP administration in testis lead to increase in lipid peroxidation and this increase considered one of the toxic manifestations of CP. Spermatozoa are principally susceptible to ROS-induced injury and peroxidative damage because of their high concentrations of polyunsaturated fatty acids and low antioxidant capacity^[40].

Loss of fertility occurred due to the production of extreme amounts of ROS in semen can overcome the antioxidant-defence mechanisms of spermatozoa and seminal plasma stimulating DNA fragmentation and loss of sperm function, that is related to per-oxidative damage to the sperm plasma membrane^[41]. ROS commonly plays a critical role in the inhibition of steroidogenesis, spermatogenesis, and male fertility^[42]. It is a recognized fact that LA as a dithiol scavenger protects cells against CP-induced LPO^[43] and this is clear in our results as treatment

with LA lead to decrease in MDA and increase in SOD activity similar to control groups. The observed protective action of lipoic acid against CP induced ROS formation maybe because of its antioxidant property. Besides, LA supplementation restored the activities of testicular marker enzymes as well as the levels of thiols and ROS to near normalcy and the histopathological studies proved the cytoprotection rendered by LA^[44].

RJ administration improved oxidative stress^[45]. Similar results by El-Nekeety *et al*^[46] who stated that RJ supplementation improved antioxidant enzyme activities such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in fumonisin rat.

Another study demonstrated that CP treatment induced a significant increase in oxidative stress, which is severely impaired function and structure of testis^[47]. The present histopathological study showed that testes of CP treated rats revealed degenerative alterations in the seminiferous tubules and interstitial tissue. These changes including thickening of basement membrane and congestion of blood vessels, hyperplasia of germ cells. Desquamation and exfoliation of the germ cells, in addition to oedema and maturation arrest. Moreover, atrophy of some tubules and vacuolation in the spermatocytes of other tubules were evident and these results coincide with the findings of Hosseini *et al*^[48] who illustrated that CP increased the disintegration of epithelial cells in seminiferous tubule as shedding into the lumen, decomposition of Sertoli cells, widening of the interstitial space and vacuolization in interstitial tissues. Atrophy in the most of the tubules of the Leydig cells and dispersion with pyknotic nuclei were also observed.

Maturation arrest observed because of the testosterone inhibition as a result of the damage of the Leydig cell. CP caused a histopathological reduction in number and size of the seminiferous tubules, irregular seminiferous tubules, less number of germ cells and spermatocytes, significant maturation arrest, vacuolation and degeneration in spermatogonia, perivascular fibrosis, reduced seminiferous epithelial layers and hyalinization of intertubular tissue^[49,50].

Vacuoles appeared in the spermatocytes may be produced by the fluid within the ground cytoplasm of the germinal epithelial cells and this lead to an increased pressure pushing the surrounding cells in all directions giving rise to vacuoles formation between the adjacent cells^[51].

Moreover, congestion of blood vessels occurred to CP-treated rats may be because of increased breakage of blood capillaries that lead to further augmentation of interstitial oedema and consequently to disorganization of Leydig cells in the interstitial tissues of the testis^[52].

The current ultrastructural examination of the testis of the CP treated rats showed degenerative changes in the surrounding tissues and their spermatogenic cells. The boundary tissue appeared thickened and Sertoli cells

showed distended shrunken nucleus which confirmed by previous reports Sakr *et al*^[53] who illustrated that animals treated with CP for 4 weeks different displayed ultrastructural changes.

The current study also detected mitochondrial damage presented as vacuolization and loss of cristae. In some Sertoli cells, the cytoplasm contains large vacuoles, lipid droplets and degenerated mitochondria. The cytoplasm of the spermatogonia contained many large vacuoles, lipid droplets and damaged mitochondria. The primary spermatocytes looked with the degenerated nucleus. Some spermatids embodied giant mitochondria and shrunken nuclei. Leydig cells showed many vacuoles, shrunken nuclei and decreased number of lipid droplets. Similar findings were founded by Andriano *et al*^[54] who illustrated that the degeneration of Sertoli cells may be attributed to endocrine disrupted after treatment with chemicals. Furthermore, Risbridger *et al*^[55] revealed that the cytoplasmic vacuolization in Sertoli cells was accompanied by sloughing of the germ cells.

In the present study, rats treated with Alpha-lipoic acid (LA) showed an improvement in histological and ultrastructure of testes sections comparable with CP treated rats. The protection of LA because of its ability to scavenge the singlet oxygen, H₂O₂, hydroxyl radicals formed in many metabolic processes and during lipid peroxidation^[56].

LA is a dithiol scavenges hydroxyl radicals, hydrogen peroxide, the singlet oxygen and also chelates the ferrous ion involved in the production of hydroxyl radicals^[57] and treatment of free radical-related diseases. Fahmy *et al*^[58] reported that the use of honey mixture for 5 days resulted in a marked reduction of inflammatory cells infiltration, although the main blood vessels were still dilated and congested.

In the present study, LA treated animals showed minimal histologic abnormalities and the cells restored their regular shape, and the spermatogenic layers were comparatively well-preserved.

Collectively, the present results supported the idea that reproductive toxicity of CP is mediated through oxidative stress, while LA, as a strong and safe antioxidant, can protect the reproductive system from CP-induced damages that usually result in infertility. These results are in conchoids with many investigators who stated that CP treatment leads to testicular toxicity^[59].

Worker honey bees produced royal jelly (RJ) which consider special food for the queen honeybee^[60]. The protective effect of RJ because of its component vitamins, antioxidant vitamins A, E, C, vitamin D and vitamin B complex^[61]. These vitamins themselves had anticancer effect^[62,63,64,65]. RJ has a lot of therapeutic effects besides being an anti-oxidant and anti-cancer agent^[66].

RJ administration improved sperm viability, oxidative stress, testosterone concentration, sperm DNA damage induced by chemicals that affect male reproductive

organs^[67,68]. In our work, CP treated rats plus royal jelly restored their regular shape, and the spermatogenic layers were comparatively well preserved. These results are similar to results of Abdel-Hafez *et al*^[69] who explained that RJ leads to biochemical and histopathological improvement in CP induced prostatic tissue toxicity. These results revealed that this enhancement was related to a decrease in the tissue oxidative damage and apoptosis. Bayer^[70] found that RJ had clear protection against cellular damage.

Concerning the present ultrastructural observations of the testis after RJ administration showed recovery in spermatogonia, Sertoli, primary spermatocytes, spermatids, Leydig cells except few vacuoles and moderate improvement in number of sperms these results are conchoids with results of Raafat *et al*^[71] who illustrated that cisplatin affected on testes cells and RJ restore all changes as most seminiferous tubules were seen to be almost similar to those of the control group. Moreover, Leydig cells appeared almost like those seen in controls. RJ contains vitamins E and C^[72] and vitamin E increased sperm count in the testicular as well as epididymis lumina and reduced the occurrence of morphological changes in these organs^[73] and this is clear in our ultrastructure observations explained by an improvement in the number of sperms.

CONCLUSION

The present study recommended that alpha-lipoic acid and royal jelly protects against CP-induced testicular damage via their antioxidant effects. Moreover, this study suggested that alpha-lipoic acid and royal jelly may be used parallel with CP, to improve CP-induced injuries in histopathology and ultrastructure of testes and oxidative stress parameters. Lipoic acid-treated animals showed minimal histologic abnormalities than royal jelly.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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الملخص العربي

التأثير الوقائي لحمض الألفا ليبويك وغذاء ملكات النحل على الآثار الجانبية للسيكلوفوسفاميد في ذكور الجرذان البيضاء

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المقدمه: يعتبر السيكلوفوسفاميد من أكثر الأدوية المضادة للسرطان انتشاراً ويؤدى إلى العقم.

تهدف هذه الدراسة الى تقييم التأثير الوقائي لحمض الألفا ليبويك وغذاء ملكات النحل فى تحسين التغيرات البيوكيميائية، الهستوباثولوجية والتركييبية الدقيقة للخصى والناجمة عن السيكلوفوسفاميد.

المواد والطرق: تم تقسيم الجرذان إلى ثمانية مجموعات: المجموعة الأولى وتمثل المجموعة الضابطة، المجموعة الثانية أخذت محلول قلوئى، المجموعة الثالثة أخذت مادة داي ميثيل سلفوكسيد (DMSO)، المجموعة الرابعة أخذت حمض الألفا ليبويك، والمجموعة الخامسة أخذت غذاء ملكات النحل، والمجموعة السادسة عولجت بمادة السيكلوفوسفاميد (٥ ملجم / كجم من وزن الجسم) ثلاث مرات فى الإسبوع لمدة أربعة أسابيع، والمجموعة السابعة عولجت بمادة السيكلوفوسفاميد يليها حمض الالفيا ليبويك (٢٥ ملجم / كجم من وزن الجسم) بينما المجموعة الثامنة عولجت بمادة السيكلوفوسفاميد يليها جرعة من غذاء ملكات النحل (١ ملجم / كجم من وزن الجسم). وقد تم الذبح بعد أربعة أسابيع لتجميع عينات السيرم والأنسجة لعمل بعض القياسات البيوكيميائية، الهستوباثولوجية والتركييبية الدقيقة على التوالي.

النتائج: أوضحت الدراسة أن السيكلوفوسفاميد أدى إلى إرتفاع فوق أكسدة الدهون (LPO) ونقص فى إنزيم السوبر أكسيد ديسميتيز (SOD) بالمقارنة بالمجموعة الضابطة. كما أوضحت التغيرات الهستوباثولوجية للسيكلوفوسفاميد تغيرات واضحة فى الخصى والمتمثلة فى تحرك عدد من الخلايا إلى تجاويرف الأنبيبات المنوية، فجوات فى السيتوبلازم، تغير فى نضج الخلايا بالإضافة إلى إحتقان فى الأوعية الدموية وتورم النسيج.

وقد أظهر الفحص بالمجهر الإلكتروني زيادة فى سمك الغشاء القاعدى والتوائه، ظهور فجوات فى السيتوبلازم وكذلك فجوات فى ميتوكوندريا خلايا سرتولى.

الخلاصة: وقد أحدث معاملة الجرذان بحمض الالفيا ليبويك مع السيكلوفوسفاميد تأثيرات محسنة فى الخصى بالمقارنة بالعلاج بغذاء ملكات النحل حيث أدى إلى انخفاض فوق أكسدة الدهون وارتفاع إنزيم السوبر أكسيد ديسميتيز وكذلك تحسن التغيرات الهستوباثولوجية والتركييبية الدقيقة. وهذا التحسن ممكن أن يكون نتيجة الخواص المضادة للأكسدة والقدرة الفائقة على التخلص من الشوارد الحرة.