Lipoic Acid and *Calligonum Comosumon* Attenuate Aroclor 1260-Induced Testicular Toxicity in Adult Rats

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ABSTRACT: Aroclor 1260 is one of the more representative polychlorinated biphenyls found in biota. This study was designed to delineate the testicular toxicity of Aroclor 1260 and to elucidate the potential protective role of *Calligonum comosum (C. comosum)* and lipoic acid in adult rats. Aroclor 1260 was dissolved in corn oil and given to rats by gavage at doses 0, 20, 40, or 60 mg/kg/day for 15 consecutive days (Groups I, II, III, and IV, respectively). Groups V and VI were pretreated with *C. comosum* (200 mg/kg/day) and lipoic acid (35 mg/kg/day) respectively 24 h before Aroclor 1260 (40 mg/kg/day) treatment for 15 consecutive days. Aroclor 1260 (20, 40 or 60 mg/kg/day) treatment significantly decreased testes weight, sperm count and motility and daily sperm production. Serum testosterone was significantly decreased in response to treatment with 40 and 60 mg/kg/day of Aroclor 1260. LDH-X activity was significantly decreased at the three dose levels. Hydrogen peroxide (H_2O_2) production (in a dose-related manner) and lipid peroxidation were significantly increased in response to Aroclor 1260 (20, 40, or 60 mg/kg/day) treatment. Aroclor 1260 at the three dose levels decreased the activities of the antioxidant enzymes

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SOD, CAT, GPx, and GR and the non-enzymatic antioxidant GSH level. CAT, GPx and GSH showed a dose-response effect. These abnormalities were effectively attenuated by pretreatment with *C. comosum* (200 mg/kg/day) or lipoic acid (35 mg/kg/day). Histopathological examination showed a dose-related increase in morphological abnormalities of the testis in response to Aroclor 1260 treatment. In conclusion, Aroclor 1260 induced testicular toxicity at least, in part, by induction of oxidative stress. By reversal of biochemical and morphological changes towards normalcy, the cytoprotective role of *C. comosum* and lipoic acid is illuminated. In comparison, lipoic acid was more protective than *C. comosum* extract against testicular toxicity induced by Aroclor 1260. © 2016 Wiley Periodicals, Inc. Environ Toxicol 00: 000–000, 2016.

Keywords: aroclor 1260; testis; spermatogenesis; oxidative stress

INTRODUCTION

In the past few years, there has been increased interest in assessing the relationship between impaired male fertility and environmental factors (Jorgensen et al., 2001; Pflieger-Bruss et al., 2004). Human male fertility is a complex process and therefore a great variety of sites may be affected by exogenous noxae. Lifestyle factors as well as various environmental and occupational agents may impair male fertility (Brevini et al., 2005). Even though the commercial production of polychlorinated biphenyls (PCBs) was banned at the end of the 1970s, they continue to be a health problem due to their lipophilic nature, persistence in the environment and demonstrated toxic effects (Hsu et al., 2007). PCBs are a group of widely dispersed environmental contaminants that disrupt normal endocrine functions in human and mammals (Kimbrough, 1995). PCBs are used in transformers and capacitors, in pesticides and additive in paints, copying paper, adhesives, sealants and plastics (Safe, 1994; Erickson, 1997). PCBs are lipophilic and poorly catabolized and thus, they remain in tissues such as testes, ovary, adrenals, liver, adipose tissue, skin, and other organs as well as in plasma membranes (Kimbrough, 1995).

The effects of PCBs on male reproduction in vivo comprise impaired fertility in postnatally exposed rats, reduced matings, decreased concentration of testicular spermatozoa, lowered weight of the testis and accessory sex organs such as epididymis, ventral prostate, and seminal vesicle (Sridhar et al., 2004; Murugesan et al., 2005a).

Aroclors are commercially used PCB mixtures and usually given a four-digit number, of which the first two digits refer to the number of carbon atoms attached to the biphenyl ring and the latter two indicate the percentage (by weight) of chlorine (Andric et al., 2000a). Aroclor 1248 inhibited testicular 3β-hydroxysteroid dehydrogenase (3β-HSD) activity following 24 h *in vivo* exposure or 2 h exposure of intact interstitial cells (Andric et al., 2000a). Further studies also demonstrated that adult rats exposed to PCB (Aroclor 1254) significantly decreased the activity of steroidogenic enzymes such as cytochrome P450 side chain cleavage, 3β-and 17β-HSDs and antioxidant enzyme activities in Leydig cells (Murugesan et al., 2005b). Moreover, Aly et al. (2009) reported that Arochlor 1254 impaired spermatogenesis as evidenced, at least partly, by induction of oxidative stress in testicular mitochondria. Andric et al. (2000b) reported that the Aroclor 1260 based-transformer fluid Askarel inhibits rat testicular steroidogenesis (3βHSD activity) after in vivo and in vitro applications.

Lipoic acid (LA) is a naturally occurring nutraceutical, whose therapeutic effect has been related to its antioxidant activity and its ability to repair oxidative damage (El-Beshbishy et al., 2013). It is readily distributed and accumulates in several tissues where it is rapidly converted to its more potent antioxidant form dihydrolipoic acid (Packer et al., 1997). Because of its small size and high lipophilicity, it crosses biological membranes easily and quenches free radicals in both lipid and aqueous environments (Suzuki et al., 1991). Furthermore, LA may also act indirectly to maintain cellular antioxidant defense by enhancing the levels of other natural antioxidants, such as glutathione (GSH), tocopherol, and ascorbic acid (Sefah et al., 2010).

The local Saudi flora is very rich in a variety of plants of quite known folkloric or traditional medicinal uses. *Calligonum comosum (C. comosum)*, is a Saudi plant, belongs to family Polygonaceae, distributed throughout Saudi Arabia in tropical and subtropical regions and growing in sandy deserts, and known to have antioxidant activities (Ashour et al., 2012). Recently, a standardized chemical product from the aerial part of *C. comosum* was pharmacologically prepared containing major functional constituents, sterols and/or terpenoids, polyphenolic compounds such as flavonoids (Badria et al., 2007). This standardized extract is used to protect against haloperidol-induced oxidative stress in rat brain (Abdel-Sattar et al., 2014). Furthermore, this extract has been shown to have cardioprotective activity against doxorubicin toxicity (Ashour et al., 2012). Several antioxidative flavonoids have been isolated from *C. comosum*, including (+)-catechin, dehydrodicatechin A, kaempferol-3-*O*-rhamnopyranoside, quercetin (quercetin-3-*O*-rhamnopyranoside), isoquercitrin (quercetin-3-*O*-glucopyranoside), kaempferol-3-*O*-glucuronide, and mequilianin (quercetin-3-*O*-glucuronide) (Badria et al., 2007).

The idea that free radical scavengers are protective against pollutant induced toxicity (Korkmaz et al., 2010) led the authors to investigate the potential protective effect of *C. comosum* and LA against Aroclor 1260-induced testicular toxicity. To the best of our knowledge, the role of Aroclor 1260 in male infertility has not been fully investigated.

Therefore, the current study was designed to delineate the potential testicular toxicity of Aroclor 1260 and to elucidate the potential protective role of *C. comosum* and LA in adult rats.

MATERIALS AND METHODS

Chemicals

Aroclor 1260, pyrogallol, sodium azide, glutathione reductase, GSH, 5,50-dithiobis(2-nitrobenzoic acid), 2,4-dinitrophenylhydrazine were purchased from Sigma–Aldrich Chemical Company, St. Louis, MO. All other chemicals are of analytical grade.

Animals and Treatment

Forty-eight adult male Wistar rats (90 days) were obtained from our animal facility (Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia) and housed in clean polypropylene cages (6/cage/group) and maintained on a 12 h light:dark cycle and a temperature of 20–25°C. The animals were fed standard rat chow and water ad libitum. For 7 days before the experiment, rats were handled daily for 5 min to acclimatize them to human contact and minimize their physiological responses to handling for subsequent protocols (Ma and lightman, 1998**;** Vaithinathan et al., 2010). Aroclor 1260 was dissolved in corn oil and given to rats by gavage at doses 0, 20, 40, or 60 mg/kg/day for 15 consecutive days (Groups I, II, III, or IV respectively). Groups V and VI were pretreated with aqueous extract of *C. comosum* (200 mg/kg/day) and lipoic acid (35 mg/kg/day, dissolved in saline at alkaline pH, 7.8) respectively, 24 h before Aroclor 1260 (40 mg/kg/day) treatment for 15 consecutive days. Two additional groups of animals (Group VII and Group VII) were also included and treated with *C. comosum* (200 mg/kg/day) and lipoic acid (35 mg/kg/day) respectively for 15 consecutive days. Gavage volume was adjusted according to the weight of each rat. The doses and duration of treatment were selected as per previous publications (Andric et al., 2000b**;** Andric et al., 2003**;** Selvakumar et al., 2006**;** Liu et al., 2001). The control group of animals was maintained and gavaged corn oil vehicle alone. This study was carried out in accordance with the NIH guidelines. All animal treatment described was conducted according to Research Ethics Committee of King Abdulaziz University, Jeddah, Saudi Arabia (REC-KAU).

Necropsy

Twenty-four hours after the last dose, blood samples were collected from the retro-orbital sinus, under ether anesthesia. Samples were centrifuged and supernatant serum was separated from the clot as soon as possible and stored at −80°C until testosterone assay. The animals were euthanized under anesthetic ether. Testes and cauda epididymides were removed, cleaned from adhering fat and connective tissues and weighed in g. The cauda epididymides were used for sperm count and motility. One testis from each rat (the right) was homogenized in ice-cold phosphate buffer (pH 7.0) using a glass-teflon homogenizer. The homogenate was centrifuged at 10,000 × *g* for 30 min at 4°C and the supernatant was stored at - 80°C till used for biochemical and enzymes estimation as enzyme source. The left one was processed for evaluation of daily sperm production (DSP). Protein concentrations were determined using a BCA kit (Pierce, Rockford, USA) that employed bovine serum albumin as a standard.

Sperm Count and Motility

Epididymides were dissected out, weighed, immediately minced in 5 mL of physiological saline and then incubated at 37°C for 30 min to allow spermatozoa to leave the epididymal tubules. The percentage of motile sperm was recorded using a phase contrast microscope at a magnification of 400x. Total sperm number was determined by using a Neubauer hemocytometer as previously described (Yokoi et al., 2003). To determine sperm motility, 100 sperms each were observed in three different fields, and classified into motile and non-motile sperms, and the motility was expressed as percentage incidence.

Daily Sperm Production (DSP)

Daily sperm production was determined in adult rats as previously described (Blazak et al., 1993). The left testis was decapsulated and homogenized in 50 mL of ice-cold 0.9% sodium chloride solution containing 0.01% Triton X-100 using a Polytron homogenizer (Sharpe et al., 1995). The homogenate was allowed to settle for 1 min and then was gently mixed, and a 10 ml aliquot was transferred to a glass vial and stored on ice. After thorough mixing of each sample, the number of sperm heads (step 19 spermatid head) in four chambers of Neubauer type hemocytometer was counted under a light microscope with 40x objective. To calculate DSP, the number of spermatids at stage 19 was divided by 6.1, which is the number of days of the seminiferous cycle in which these spermatids are present in the seminiferous epithelium.

Serum Testosterone

Testosterone was measured by use of the Pathozyme Testosterone ELISA kit. Briefly, standards, specimens and controls were dispensed into appropriate wells, followed by testosterone HRP reagent and anti-testosterone reagent, before mixing thoroughly and incubating at 37°C for 90 min. Wells were then rinsed with de-ionized water and substrate solution was dispensed into each well, gently mixed and incubated for 20 min. The reaction was stopped with the ‘stop reagent’ and the absorbance recorded at 450 nm (Chen et al., 1991).

Testicular Lactate dehydrogenase-X (LDH-X)

Lactate dehydrogenase-X (LDH-X) activity was measured using α-ketovaleric acid as the substrate (Meistrich et al., 1977). The activity of LDH-X is expressed as μmol of NADH oxidized/min/mg protein.

Oxidative Stress Markers

Hydrogen Peroxide (H_2O_2) Generation

Hydrogen peroxide (H_2O_2) generation was assayed by the method of Pick and Keisari (1981). Briefly, the incubation mixture contained 1.641 mL phosphate buffer (50 mM, pH 7.6), 54 μL horseradish peroxidase (8.5 units/mL), 30 μL of 0.28 nM phenol red, 165 μL of 5.5 nM dextrose, and 600 μL of enzyme source, incubated at 35°C for 30 min. The reaction was terminated by the addition of 60 μL of 10 N sodium hydroxide. The absorbance was read at 610 nm against a reagent blank on a spectrophotometer. For standard curve, known amounts of hydrogen peroxide and all the above reagents except enzyme source were incubated for 30 min at 35°C, followed by addition of 60 μl of 10 N sodium hydroxide, and reading of optical density at 610 nm. The quantity of H_2O_2 produced was expressed as nmol of H_2O_2 generated/min/mg protein at 35°C.

Lipid Peroxidation (LPO)

Malondialdehyde (MDA), formed as an endproduct of the peroxidation of lipids, served as an index of the intensity of oxidative stress. MDA reacts with thiobarbituric acid to generate a coloured product that can be measured optically at 532 nm. A break down product of LPO, thiobarbituric acid reactive substance was measured by the method of Buege and Aust (1976). Briefly, the stock solution contained equal volumes of trichloroacetic acid 15% (w/v) in 0.25 N HCl and 2-thiobarbituricacid 0.37% (w/v) in 0.25 N HCl. One volume of the test sample (sperm suspension) and two volumes of stock reagent were mixed in a screw-capped centrifuge tube, vortexed and heated for 15 min on a boiling water bath. After cooling on ice the precipitate was removed by centrifugation at $1000 \times g$ for 15 min and absorbance of the supernatant was measured at 532 nm against blank containing all the reagents except test sample. The value is expressed as μmol of MDA equivalent formed/min/mg protein.

Assessment of Enzymic Antioxidants

Superoxide dismutase (SOD) was assayed by the method of Marklund and Marklund (1974). Briefly, the assay mixture contained 2.4 mL of 50 mM Tris–HCl buffer containing 1 mM EDTA (pH 7.6), 300 μl of 0.2 mM pyrogallol and 300 μL enzyme source. The decrease in absorbance was measured immediately at 420 nm against blank at 10 s intervals for 3 min on a spectrophotometer. The activity of enzyme was expressed in nmol of pyrogallol oxidized/min/mg protein. Catalase (CAT) was assayed as previously mentioned (Claiborne, 1985). Briefly, the assay mixture contained 2.40 mL of phosphate buffer (50 mM, pH 7.0), 10 μL of 19 mM hydrogen peroxide and 50 μL enzyme source (sperm suspension). The decrease in absorbance was measured immediately at 240 nm against blank at 10 s intervals for 3 min on a spectrophotometer. The activity of enzyme was expressed in μmol of hydrogen peroxide consumed/min/mg protein. Glutathione peroxidase (GPx) was assayed by the method of Paglia and Valentine (1967). Briefly, the assay mixture contained 1.59 ml of phosphate buffer (100 mM, pH 7.6), 100 μL of 10 mM EDTA, 100 μL of sodium azide, 50 μL of glutathione reductase, 100 μL of reduced glutathione, 100 μL of 200 mM NADPH, 10 μL of hydrogen peroxide and 10 μL enzyme source. The oxidation of NADPH was measured immediately at 340 nm against blank at 10 s intervals for 3 min on a spectrophotometer. The activity of enzyme was expressed in nmol of NADPH oxidized/min/mg protein. The activity of glutathione reductase was assayed by the method of Carlberg and Mannervik (1975). Briefly, the assay mixture contained 1.75 mL of phosphate buffer (100 mM, pH 7.6), 100 μL of 200 mM NADPH, 100 μL of 10 mM EDTA, 50 μL of 20 mM oxidized glutathione and 50 μL enzyme source. The oxidation of NADPH was measured immediately at 340 nm against blank at 10 s intervals for 3 min on a spectrophotometer. The activity of enzyme was expressed in nmol of NADPH oxidized/min/mg protein.

Reduced Glutathione (GSH)

The level of reduced glutathione (GSH) was determined as previously described (Moron et al., 1979) based on the reaction with Ellman's reagent (19.8 mg DNTB in 100 ml 0.1% sodium citrate). The absorbance was recorded at 412 nm using a spectrophotometer. The GSH content was expressed as μg/mg protein.

Histopathological Examination of the Testes

Autopsy samples were taken from the testes of sacrificed rats and fixed in Bouin's solution. Serial gradient alcohol dilutions were used for dehydration of the tissue samples. Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 5-micron thickness. The obtained tissue sections were stained by hematoxylin and eosin (Banchroft et al., 1996) for histopathological examination by the light microscope.

Statistical Analysis

Differences between obtained values (mean ± SD, $n = 6$) were compared by one way analysis of variance (ANOVA, GraphPad Instat) followed by the Tukey-Kramer multiple comparison test. A p value less than 0.05 was taken as a criterion for a statistically significant difference.

TABLE I. Effect of Aroclor 1260 and *C. Comosum* or lipoic acid on testes weights and sperm parameters

	Doses of Aroclor 1260 (mg/kg b.wt./day)					
Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
Absolute testes weight (g)	2.67 ± 0.22	2.25 ± 0.19^{a}**	2.22 ± 0.16^{a}***	2.05 ± 0.19 ^{a}***	2.55 ± 0.14^{b}*	2.6 ± 0.18^{b}*
Absolute epididymides weight (g)	0.84 ± 0.1	0.69 ± 0.09*	0.62 ± 0.12**	0.48 ± 0.08***	0.78 ± 0.05*	0.8 ± 0.04*
Cauda sperm count (x10^6/rat)	52.33 ± 4.59	44.5 ± 3.51^{a}*	41.5 ± 4.04^{a}**	36.33 ± 3.61^{a}***	48.83 ± 4.07^{b}*	50.67 ± 4.8^{b}**
Sperm motility %	89.11 ± 5.2	80.5 ± 3.67^{a}*	76.17 ± 3.82^{a}***	72.17 ± 5.12^{a}***	85.17 ± 4.22^{b}*	86.17 ± 3.82^{b}**
Daily sperm production (x10^6/testis/day)	28.83 ± 3.19	24.17 ± 2.14^{a}*	22 ± 2.28^{a}***	19.83 ± 2.32^{a}***	26.83 ± 2.64^{b}*	27.83 ± 2.48^{b}**

Group I: Control (vehicle), Group II: Aroclor 1260 (20 mg/kg/day), Group III: Aroclor 1260 (40 mg/kg/day), Group IV: Aroclor 1260 (60 mg/kg/day), Group V: Aqueous extract of *C. comosum (*200 mg/kg/day) + Aroclor 1260 (40 mg/kg/day), Group VI: Lipoic acid (35 mg/kg/day) + Aroclor 1260 (40 mg/kg/day). Data are expressed as mean ± S.D. ($n = 6$). Statistical analysis (ANOVA) for differences from corresponding controls: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. a: Significant difference of all treated Groups from Group I (control), b: Significant difference of Groups V and VI from Group III.

RESULTS

Testes and Epididymides Weights and Sperm Parameters

Table I shows the changes in testes and epididymides weights and sperm parameters. Treatment of male rats (Group II, Group III or Group IV) with Aroclor 1260 (20, 40, or 60 mg/kg) caused significant decrease in absolute testes ($p < 0.01$, $p < 0.001$, and $p < 0.001$, respectively) and epididymides ($p < 0.05$, $p < 0.01$, and $p < 0.001$ respectively) weights as compared to the corresponding control. Pretreatment with *C. comosum* extract or lipoic acid (Group V and VI respectively) reverted these values to normalcy ($p < 0.05$ and $p < 0.05$, respectively) for both testes and epididymides weights.

Sperm count was significantly decreased ($p < 0.05$, $p < 0.01$, and $p < 0.001$) in response to Aroclor 1260 (20, 40, or 60 mg/kg) treatment (Group II, III, and IV respectively) in a dose related pattern as compared to the corresponding control. Moreover, Aroclor 1260 treatment (Groups II, III, and IV) in a dose of 20, 40, or 60 mg/kg/day significantly decreased ($p < 0.05$, $p < 0.001$, and $p < 0.001$, respectively) both sperm motility and daily sperm production as compared to the related control. Pretreatment with *C. comosum* extract or lipoic acid (Group V and VI) significantly suppressed ($p < 0.05$ and $p < 0.01$, respectively) the

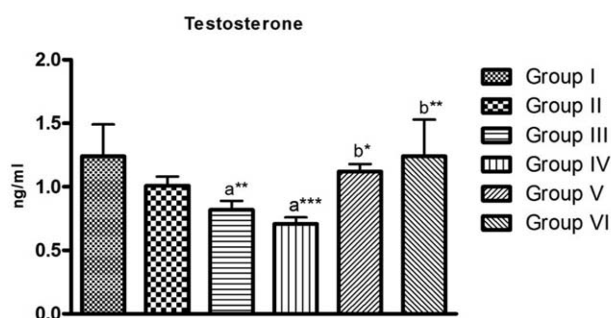

Fig. 1. Effect of Aroclor 1260 and *C. comosum* or lipoic acid on serum testosterone. Group I: Control (vehicle), Group II: Aroclor 1260 (20 mg/kg/day), Group III: Aroclor 1260 (40 mg/kg/day), Group IV: Aroclor 1260 (60 mg/kg/day), Group V: Aqueous extract of *C. comosum (*200 mg/kg/day) + Aroclor 1260 (40 mg/kg/day), Group VI: Lipoic acid (35 mg/kg/day)-+Aroclor 1260 (40 mg/kg/day). Data are expressed as mean ± S.D. ($n = 6$). Statistical analysis (ANOVA) for differences from corresponding controls: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a: Significant difference of all treated Groups from Group I (control), b: Significant difference of Groups V and VI from Group III.

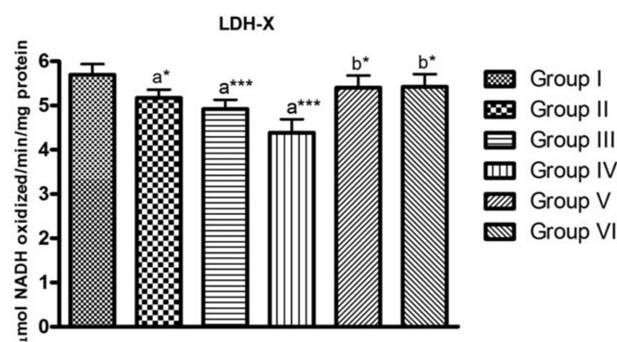

Fig. 2. Effect of Aroclor 1260 and *C. comosum* or lipoic acid on testicular LDH-X activity. Group I: Control (vehicle), Group II: Aroclor 1260 (20 mg/kg/day), Group III: Aroclor 1260 (40 mg/kg/day), Group IV: Aroclor 1260 (60 mg/kg/day), Group V: Aqueous extract of *C. comosum (*200 mg/kg/day) + Aroclor 1260 (40 mg/kg/day), Group VI: Lipoic acid (35 mg/kg/day) + Aroclor 1260 (40 mg/kg/day). Data are expressed as mean ± S.D. ($n = 6$). Statistical analysis (ANOVA) for differences from corresponding control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a: Significant difference of all treated Groups from Group I (control), b: Significant difference of Groups V and VI from Group III.

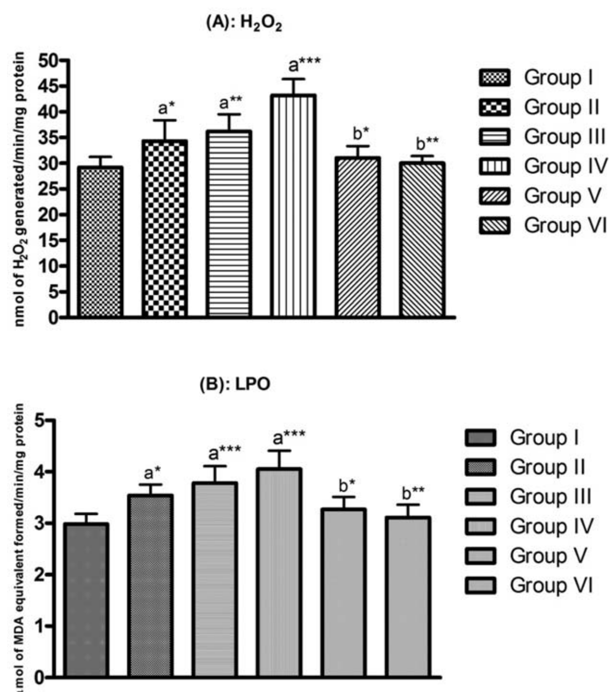

Fig. 3. Effect of Aroclor 1260 and *C. comosum* or lipoic acid on H_2O_2 production and LPO. Group I: Control (vehicle), Group II: Aroclor 1260 (20 mg/kg/day), Group III: Aroclor 1260 (40 mg/kg/day), Group IV: Aroclor 1260 (60 mg/kg/day), Group V: Aqueous extract of *C. comosum (*200 mg/kg/day) + Aroclor 1260 (40 mg/kg/day), Group VI: Lipoic acid (35 mg/kg/day) + Aroclor 1260 (40 mg/kg/day). H_2O_2: Hydrogen peroxide, LPO: Lipid peroxidation. Data are expressed as mean ± S.D. ($n = 6$). Statistical analysis (ANOVA) for differences from corresponding control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a: Significant difference of all treated Groups from Group I (control), b: Significant difference of Groups V and VI from Group III.

changes in sperm count and motility and daily sperm production induced by Aroclor 1260 (40 mg/kg/day) treatment.

Serum Testosterone and Testicular Lactate dehydrogenase-X (LDH-X)

Figure 1 represents a significant decrease ($p < 0.01$ and $p < 0.001$) in serum testosterone in response to Aroclor 1260 treatment (Group III and IV) at doses of 40 and 60 mg/kg respectively. Aroclor 1260 at a dose of 20 mg/kg/day did not cause any significant change in serum testosterone as compared to the corresponding control. Pretreatment with *C. comosum* extract or lipoic acid (Group V and VI) restored serum testosterone level ($p < 0.05$, $p < 0.01$ respectively) toward normalcy.

LDH-X was significantly decreased ($p < 0.05$, $p < 0.001$, and $p < 0.001$) in response to Aroclor 1260 treatment (Group II, III, and IV) at a dose of 20, 40, or 60 mg/kg respectively as compared to the related control (Fig. 2). Pretreatment with *C. comosum* extract or lipoic acid (Group V and VI respectively) significantly ($p < 0.05$) reverted the change in LDH-X activity toward normalcy.

Oxidative Stress Parameters

The change in H_2O_2 production in response to Aroclor 1260 (20, 40, or 60 mg/kg/day) treatment (Group II, III, and IV respectively) is presented in Fig. 3(A). H_2O_2 production was significantly increased ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) in a dose response pattern. Pretreatment with *C. comosum* extract (Group V) or lipoic acid (Group VI) significantly reverted ($p < 0.05$ and $p < 0.01$ respectively) the H_2O_2 production near to normalcy.

Figure 3(B) presents the change in lipid peroxidation (LPO). Animals treated with Aroclor 1260 (20, 40 or 60 mg/kg/day) showed significant increase ($p < 0.05$, $p < 0.001$ and $p < 0.001$ respectively) in LPO (Group II, III and IV respectively) as compared to the corresponding control. Pretreatment with *C. comosum* extract or lipoic acid (Group V and

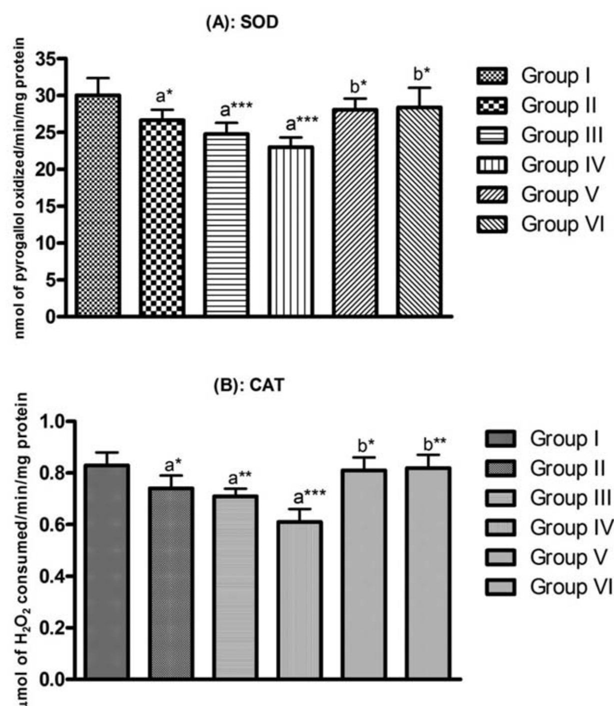

Fig. 4. Effect of Aroclor 1260 and *C. comosum* or lipoic acid on SOD and CAT activities. Group I: Control (vehicle), Group II: Aroclor 1260 (20 mg/kg/day), Group III: Aroclor 1260 (40 mg/kg/day), Group IV: Aroclor 1260 (60 mg/kg/day), Group V: Aqueous extract of *C. comosum (*200 mg/kg/day) + Aroclor 1260 (40 mg/kg/day), Group VI: Lipoic acid (35 mg/kg/day) + Aroclor 1260 (40 mg/kg/day). SOD: Superoxide dismutase, CAT: Catalase. Data are expressed as mean ± S.D. ($n = 6$). Statistical analysis (ANOVA) for differences from corresponding control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a: Significant difference of all treated Groups from Group I (control), b: Significant difference of Groups V and VI from Group III.

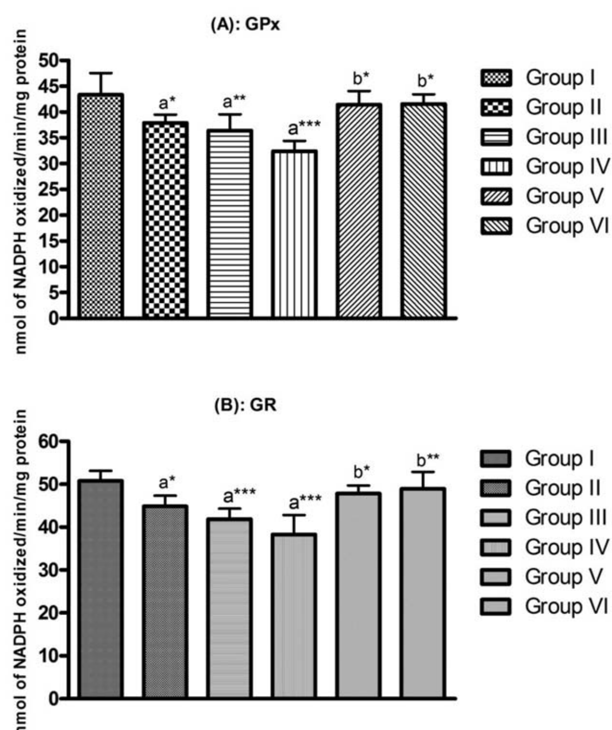

Fig. 5. Effect of Aroclor 1260 and *C. comosum* or lipoic acid on GPx and GR activities. Group I: Control (vehicle), Group II: Aroclor 1260 (20 mg/kg/day), Group III: Aroclor 1260 (40 mg/kg/day), Group IV: Aroclor 1260 (60 mg/kg/day), Group V: Aqueous extract of *C. comosum (*200 mg/kg/day) + Aroclor 1260 (40 mg/kg/day), Group VI: Lipoic acid (35 mg/kg/day) + Aroclor 1260 (40 mg/kg/day). GPx: Gltathione peroxidase, GR: Glutathione reductase. Data are expressed as mean ± S.D. ($n = 6$). Statistical analysis (ANOVA) for differences from corresponding control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a: Significant difference of all treated Groups from Group I (control), b: Significant difference of Groups V and VI from Group III.

VI respectively) significantly suppressed LPO ($p < 0.05$ and $p < 0.01$ respectively) induced by Aroclor 1260 (40 mg/kg/day) treatment.

Figure 4(A) presents the significant decrease ($p < 0.05$, $p < 0.001$ and $p < 0.001$) in SOD in response to Aroclor 1260 (20, 40 or 60 mg/kg/day respectively) treatment (Group II, III and IV) as compared to the related control. Pretreatment with *C. comosum* extract or lipoic acid (Group V and VI respectively) restored SOD activity ($p < 0.05$) toward normalcy.

Animals treated with Aroclor 1260 (20, 40 or 60 mg/kg/day) (Group II, III and IV respectively) showed significant decreased ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively) in CAT activity in a dose related pattern as compared to the corresponding control [Fig. 4(B)]. Pretreatment with *C. comosum* extract or lipoic acid (Group V and VI respectively) prevented these alterations ($p < 0.05$, $p < 0.01$ respectively) induced by Aroclor 1260 (40 mg/kg/day) treatment.

The activity of GPx was significantly decreased ($p < 0.05$, $p < 0.01$ and $p < 0.001$) in a dose response manner in response to Aroclor 1260 (20, 40 or 60 mg/kg/day respectively) treatment (Group II, III and IV respectively) as compared to the related control [Fig. 5(A)]. Pretreatment with *C. comosum* extract or lipoic acid (Group V and VI respectively) restored GPx activity ($p < 0.05$) toward normalcy.

Figure 5(B) displays the changes in GR activity induced by Aroclor 1260 treatment. GR activity displayed a significant decreased ($p < 0.05$, $p < 0.001$ and $p < 0.001$) (group II, III and IV respectively) in response to Aroclor 1260 (20, 40 or 60 mg/kg/day respectively) treatment as compared to the corresponding control. Pretreatment with *C. comosum* extract or lipoic acid (Group V and VI respectively) maintained GR activity ($p < 0.05$, $p < 0.01$ respectively) near normal activity.

Total reduced GSH content was significantly decreased ($p < 0.05$, $p < 0.01$ and $p < 0.001$) in a dose response manner in response to Aroclor 1260 (20, 40 or 60 mg/kg/day respectively) treatment (Group II, III and IV respectively) as compared to the related control (Fig. 6). Pretreatment with *C. comosum* extract and lipoic acid (Group V and VI respectively) restored GSH content ($p < 0.05$) toward normalcy.

Histopathological Examination

Normal testicular architecture with an orderly arrangement of germinal cells and Sertoli cells were observed in testes from control group [Group I, Fig. 7(A)]. Animals treated with *C. comosum* extract or lipoic acid (Groups VII and VIII) showed normal testicular histology and normal spermatogenesis (photos not shown). Aroclor 1260 treatment at doses 20, 40 or 60 mg/kg (Group II, III and IV respectively) resulted in

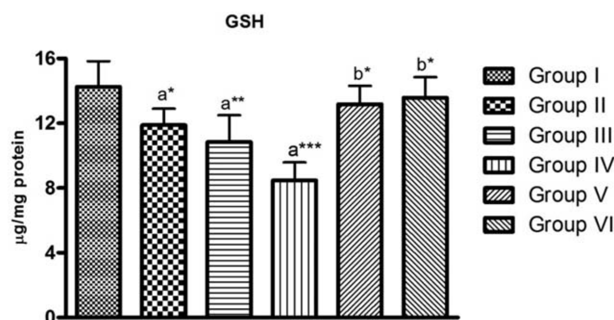

Fig. 6. Effect of Aroclor 1260 and *C. comosum* or lipoic acid on GSH level. Group I: Control (vehicle), Group II: Aroclor 1260 (20 mg/kg/day), Group III: Aroclor 1260 (40 mg/kg/day), Group IV: Aroclor 1260 (60 mg/kg/day), Group V: Aqueous extract of *C. comosum (*200 mg/kg/day) + Aroclor 1260 (40 mg/kg/day), Group VI: Lipoic acid (35 mg/kg/day)-+ Aroclor 1260 (40 mg/kg/day). GSH: Reduced glutathione. Data are expressed as mean ± S.D. ($n = 6$). Statistical analysis (ANOVA) for differences from corresponding control: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a: Significant difference of all treated Groups from Group I (control), b: Significant difference of Groups V and VI from Group III.

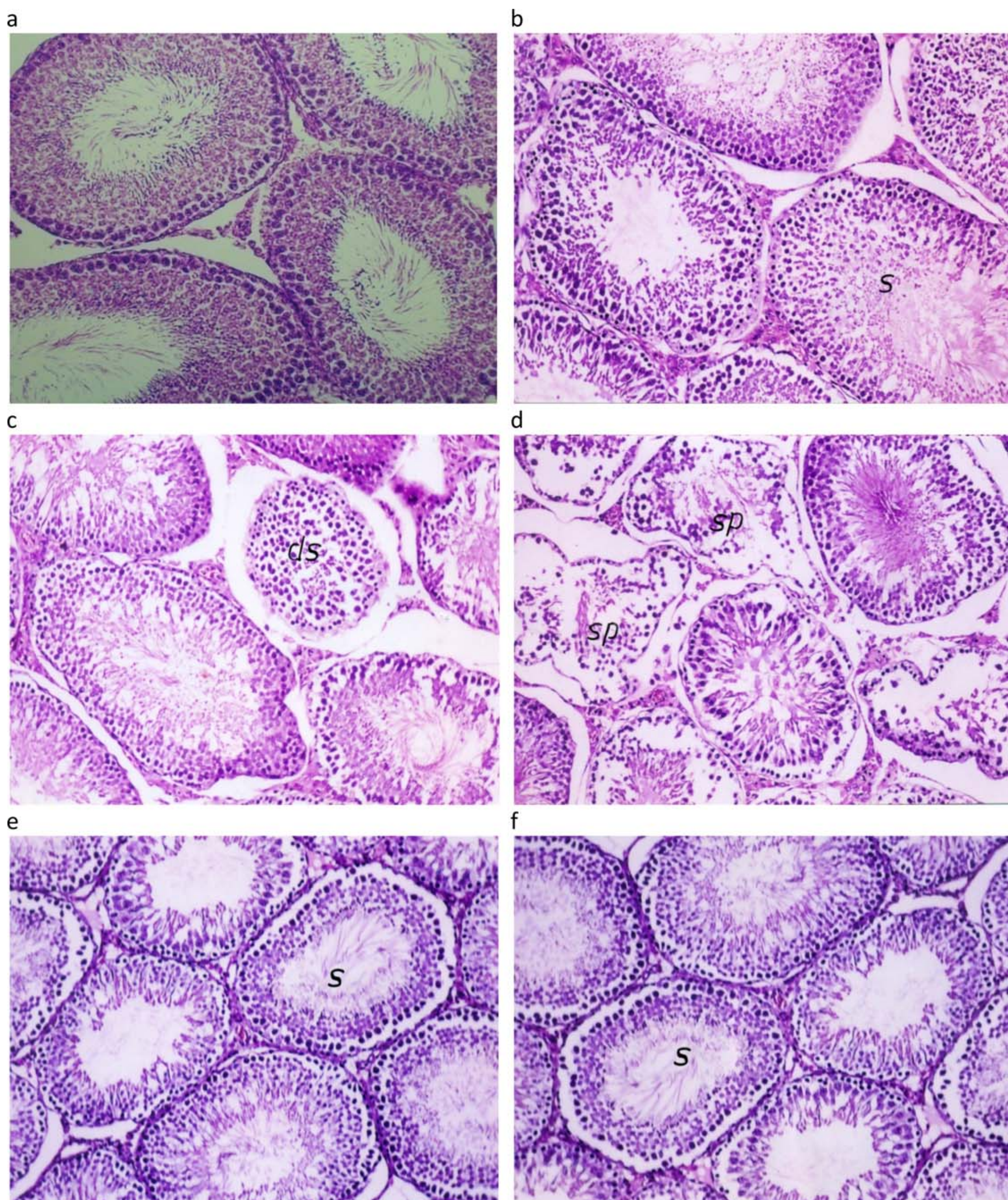

Fig. 7. Representative illustrations of histological morphology of rat testes. Testes from control (a), *C. comosumc* extract and lipoic acid (photos not shown) groups of rats exhibit normal testicular histology and normal spermatogenesis. Testes of rats treated with Aroclor 1260 display abnormal histological morphology. Aroclor 1260 at 20 mg/kg/day, (b) showing mild thickening of the basement membrane of some seminiferous tubules, focal hydropic degeneration and mild congestion of the interstitial tissue, 40 mg/kg/day, (c) showing decreasing in the number of spermatogonia as well as disorganization of spermatogenic cells with central sloughing in some seminiferous tubules, 60 mg/kg/day, (d) showing atrophy, shrinkage of seminiferous membrane and stoppage of all stages of spermatogenesis. Aroclor 1260 + *C. comosum* extract (e) and Aroclor 1260 + lipoic acid (f) group testes showing focal disorganization of spermatogenic cell with central sloughing in some seminiferous tubules. However, in spite of this focal changes, there was improvement in histopathologiacl morphology of testes of animals pretreated with *C. comosum* extract or lipoic acid. Cross sections of testes were stained with hematoxylin and eosin (H and E). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

histopathological changes in a dose-related manner. A dose level of 20 mg/kg showed mild thickening of the basement membrane of some seminiferous tubules, focal hydropic degeneration and mild congestion of the interstitial tissue [Fig. 7(B)]. Rats treated with 40 mg/kg of Aroclor 1260 showed decreasing in the number of spermatogonia as well as disorganization of spermatogenic cells with central sloughing in some seminiferous tubules [Fig. 7(C)]. A dose level of 60 mg/kg showed atrophy and shrinkage of seminiferous tubular membrane and stoppage of all stages of spermatogenesis [Fig. 7(D)]. Pretreatment of animals with *C. comosum* extract [Group V, Fig. 7(E)] or lipoic acid [Group VI, Fig. 7(F)] showed focal disorganization of spermatogenic cell with central sloughing in some seminiferous tubules. However, in spite of these focal changes, there was improvement in histopathological morphology of testes of animals pretreated with *C. comosum* extract [Group V, Fig. 10(E)] or lipoic acid [Group VI, Fig. 7(f)].

DISCUSSION

In this study, the testicular toxicity induced by Aroclor1260 was shown to be associated with reduction in both testes and epididymides weights as well as with reduction in the quality of sperm (count and motility). The weight of testes is largely dependent on the mass of the differentiated spermatogenic cells, and weight of the epididymides is dependent on the mass of spermatozoa (Aly and Khafagy, 2014). The observed reduction in the weight of testes and epididymides are due to the decreased number of germ cells and elongated spermatids in the testes and spermatozoa in the epididymides (Chapin et al., 1997). In the current study, administration of Aroclor 1260 decreased epididymal sperm count and motility which may be due to increased lipid peroxidation, reactive oxygen species (ROS) production and/or direct cytotoxicty (Andric et al., 2003). The reduction in sperm count in the epididymis was probably responsible for the decrease of the epididymal weight. Impaired sperm motility may result in infertility due to the failure of sperm to reach the site of fertilization as well as their ability to penetrate zona pellucida (Aly et al., 2009). In addition to decrease in sperm count and motility, the cytotoxicity of Aroclor 1260 was further justified by decrease in daily sperm production (DSP) manifested as decrease in step 19 spermatids/stage VII (Robb et al., 1978). Plasma membranes of the sperms have a high content of polyunsaturated fatty acid; so, they are highly sensitive to oxidative stress and lipid peroxidation (Lenzi et al., 2000). Lipid peroxidation has been shown to be associated with reduction in sperm mobility, viability and count (Kao et al., 2008). In this study, the decreased sperm count and motility and DSP may be due to lipid peroxidation. The reduction in DSP may be related to morphological abnormalities of testis in the Aroclor 1260 treated rats.

Testosterone is important for normal sperm production. In the absence of testosterone, germ cells differentiation is unable to progress and result in failure of spermatogenesis and infertility (Walker, 2010). Significant decrease in serum testosterone may be indicative of reduced steroidogenesis. This suggests that Aroclor 1260 induced oxidative stress might affect Leydig cells. This in turn decreased synthesis of testosterone thereby affects the sperm production (Sainath et al., 2011). LDH-X is the specific isozyme of LDH in the testes and it is often used as a marker of germ cell maturation (Meistrich, 1982). Changes in testicular levels of LDH-X activity have been previously associated with the presence of cytotoxicity induced by many toxicants (Chapin et al., 1985). The decreased testicular LDH-X activity, in this study, could be one of the contributory factors leading to reduced sperm concentration and motility.

In the present study, administration of Aroclor 1260 increased the levels of H_2O_2 and lipid peroxidation and concomitantly decreased the activities of CAT, SOD, GPx, and GR as well as GSH level in the rat testis. SOD is considered the first line of defense against deleterious effects of oxyradicals in the cell by catalyzing the dismutation of superoxide radicals to H_2O_2 and molecular oxygen. The reduction in the activity of CAT may reflect an inability to eliminate H_2O_2 produced by Aroclor 1260 due to enzyme inactivation caused by excess ROS production in the rat testis (Pigeolet et al., 1990). The reduced activity of GPx can be explained on the basis of the observed lack of the substrate GSH. Treatment with Aroclor 1260 also reduced GR activity. GR is involved in the supplementation of GSH to spermatogenic cells (Kaneko et al., 2002). The reduction in the activities of antioxidant enzymes and the increase in H_2O_2 and lipid peroxidation could reflect an adverse effect of Aroclor 1260 on the antioxidant system in the testis (Latchoumycandane et al., 2002). ROS such as hydrogen peroxide cause damage to sperm and other cytoplasmic organelle membrane structures through peroxidation of phospholipids, proteins, and nucleotides, thereby altering sperm motility (Ichikawa et al., 1999; Aly and Khafagy, 2011).

GSH plays an important role in the cellular antioxidant defenses and most importantly to remove H_2O_2 and organic peroxides (Powers and Lennon, 1999). The decreased GSH content may be attributed to the direct conjugation of Aroclor 1260 with glutathione or due to decreased GR activity (Aly, 2013). Pretreatment with *C. comosum* extract or lipoic acid protected the rat testis against lipid peroxidation induced by Aroclor 1260 by increasing antioxidant enzymes and decreasing ROS generation. This finding may be due to the high antioxidant activity of *C. comosum* due to its content of polyphenolic compounds (Badria et al., 2007). Lipoic acid, a dithiol, scavenges the singlet oxygen, H_2O_2, hydroxyl radicals and also chelates the ferrous ions involved in the production of hydroxyl radicals (Packer et al., 1997). Lipoic acid has the ability to correct deficient thiol status in the cell by increasing the de novo synthesis of GSH (Han et al., 1997).

The consequences of the recorded testicular toxicity was confirmed by histological examinations which indicated the ability of Aroclor 1260 (20, 40 or 60 mg/kg/day for 15 consecutive days) to induce pathological changes in a dose-related manner. These included focal hydropic degeneration, decrease in the number of spermatogonia, reduction in spermatogenesis as well as disorganization of spermatogenic cells. These findings are in line with our previously observed decreased daily sperm production and sperm count and motility. However, pretreatment with *C. comosum* extract or lipoic acid showed minimal histologic abnormalities, thereby highlighting its protective role in countering the testicular injury induced by Aroclor 1260.

In conclusion, Aroclor 1260 administration causes oxidative stress in the testis which is associated with abnormal histologic findings and concomitantly impairs spermatogenesis. *C. comosum* extract and lipoic acid have a protective role against Aroclor 1260-induced alteration in the adult rat spermatogenesis. The histopathological studies confirmed the cytoprotection rendered by *C. comosum* extract and lipoic acid. In comparison, lipoic acid was more protective than *C. comosum* extract against testicular toxicity induced by Aroclor 1260.

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