



## Influence of lipoic acid on testicular toxicity induced by bi-n-butyl phthalate in rats



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### ARTICLE INFO

#### Article history:

Received 24 March 2014

Accepted 27 May 2014

Available online 6 June 2014

#### Keywords:

Bi-n-butyl phthalate

Lipoic acid

Antioxidant

Testes

Oxidative stress

### ABSTRACT

Bi-n-butyl phthalate (BNBP) is an environmental pollutant. The aim of this study was to evaluate the protective effect of lipoic acid (LA) against testicular dysfunction associated with the intake of BNBP-intoxicated rats. Adult male Wistar rats were divided into 4 groups of 6 animals each, and received medication orally for 14 days. Group I rats received 0.5 ml corn oil. Group II rats received LA (20 mg/kg B.W./day). Group III rats received BNBP (250 mg/kg B.W./day). Group IV rats received LA 24 h prior to BNBP intake. Testes weight, cauda sperm count and sperm motility were decreased significantly by 18.15%, 13.83% and 13.5%, respectively, after BNBP treatment. Significant increase by 12.1%, 10.20% and 11.51%, respectively, was observed in LA–BNBP rats. Significant increase by 1.53%, 1.5% and 1.8%, for serum follicle stimulating hormone, testosterone and total antioxidant status, respectively, were observed in LA–BNBP rats. Testicular lipid peroxides and lactate dehydrogenase enzyme were significantly decreased by 1.5 and 1.6 folds, respectively, in LA–BNBP rats were decreased after BNBP treatment. Testicular superoxide dismutase, catalase and glutathione reductase enzymes were significantly increased in LA–BNBP rats. LA–BNBP rats, decreased the damage to seminiferous tubules produced by BNBP intake. In conclusion, LA mitigated BNBP-induced testicular toxicity through antioxidant mechanism and by direct free radical scavenging activity.

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### 1. Introduction

Bi-n-butyl phthalate (BNBP) is an environmental pollutant, extensively used as a plasticizer in many products (ATSDR, 2001). As a result of the ubiquitous use of BNBP in personal care and consumer products, human exposure is widespread. Exposure through ingestion, inhalation and dermal contact are considered important routes of exposure for the general population (Rudel et al., 2003). Upon exposure, BNBP is rapidly metabolized and excreted in urine and feces (ATSDR, 2002; Silva et al., 2004). Upon exposure, phthalates are rapidly metabolized in humans to their respective monoesters, which can be further metabolized to oxidative products of their lipophilic aliphatic side chain. Monoesters and

the oxidative metabolites of phthalates may be free or conjugated as glucuronides excreted in urine and feces (Latini et al., 2005). The acute toxicity of BNBP is relatively low with oral LD<sub>50</sub> values of 8–20 gm/kg body weight in rats and 5–16 gm/kg body weight in mice (IPCS, 1997), however, long-term exposure to low doses of DBP in utero has been shown to induce serious impairment of the early reproductive (Gray et al., 1982; Wine et al., 1997; Foster et al., 2001) and neural development (Shiota et al., 1980; Shiota and Nishimura, 1982) in animals.

Although phthalate testicular toxicity has been recognized since 1945, the primary cellular targets of the phthalates remains elusive and the mechanisms responsible for the loss of germ cells after their exposure are still understood. Some studies have shown that, BNBP has potential testicular toxicity (Zhou et al., 2010). However, the mechanism of action of BNBP on male reproduction is not fully understood. Male rat fetus exposed to BNBP develops a number of reproductive tract abnormalities, including underdeveloped or

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absent reproductive organs, cryptorchidism, retained nipples and decreased sperm production (Barlow et al., 2004). The fetal testes of BNBP-exposed males are characterized by abnormal, poorly formed seminiferous cords containing multinucleated gonocytes (Creasy et al., 1983).

$\alpha$ -Lipoic acid (LA; 1,2-dithiolane-3-pentanoic acid), which plays an essential role in mitochondrial dehydrogenase reactions, is present in all kinds of prokaryotic and eukaryotic cells and has gained considerable attention as an antioxidant (Biewenga et al., 1997). Free LA has not been detected in human beings because it is bound to proteins. LA has used as therapeutic agent against atherosclerosis, rheumatism, AIDS, hepatic injury and diabetic polyneuritis (Packer et al., 1995; El-Beshbishy, 2007). LA protected against inflammation associated with tumor and ulcer (Packer et al., 1995; Ho et al., 2007). After therapeutic applications, free LA can be found in the circulation (Teichert and Preiss, 1995). LA characterized by high reactivity toward free radicals and its capability of increasing tissue levels of reduced glutathione and reducing the oxidative stress by decreasing significantly the formation of lipid peroxides (LPOs) and restoring the normal antioxidant enzymes profile (El-Beshbishy et al., 2011).

This study was conducted in order to investigate testicular toxicity and oxidative stress state associated with the intake of LA to BNBP-testicular intoxicated rats. The measured parameters included absolute testes weight, cauda sperm count, sperm motility, serum follicle stimulating hormone (FSH), serum testosterone and serum total antioxidant status (TAS). Also, testicular lactate dehydrogenase (LDH) enzyme, lipid peroxides (LPOs), antioxidant enzymes such as glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) were estimated.

## 2. Materials and methods

### 2.1. Chemicals and kits

BNBP (oily liquid) and LA (white powder) were purchased from Sigma–Aldrich chemical company (St. Louis, MO, USA). The testosterone and FSH enzyme immunoassay kits were purchased from ALPCO Diagnostics (NH, USA) and BioVendor–Laboratori medicina a.s. (Karasek, Czech Republic), respectively. The total antioxidant kit was purchased from Randox Laboratories Ltd. (Co. Antrim, UK). All other chemicals used in this study were of the highest available grades.

### 2.2. Animals

Adult male Wistar rats weighing  $170 \pm 10$  g were housed in clean polypropylene cages and maintained on a 12 h light/dark cycle and a temperature of 20–25 °C with ad libitum access to food and water. For 7 days before the experiment, rats were handled daily for 5 min to acclimate them to human contact and minimize their physiological responses to handling for subsequent protocols (Ma and Lightman, 1998). Animal care and procedures were in accordance with the European Communities Council Directive (86/609/ECC), R.D. 223/1988, and were approved by the local Medical Ethics Committee – Faculty of Applied Medical Sciences, Taibah University, Madinah, Saudi Arabia (protocol number AMS-296/1/14).

### 2.3. Experimental protocol

The animals were randomly divided into 4 groups consisting of 6 animals each.

- Group I rats served as normal control received 0.5 ml corn oil orally for 14 successive days.
- Group II rats served as drug control group and the dose of LA used in this study was 20 mg/kg body weight/day in 0.5 ml corn oil, orally for 14 successive days (El-Beshbishy, 2007; Bist and Bhatt, 2010).
- Group III rats treated with BNBP diluted with corn oil (250 mg/kg body weight/day) orally for 14 successive days (Chen et al., 2011).
- Group IV rats received LA 24 h prior to the administration of BNBP, orally for 14 successive days.

After termination of treatment, animals were killed after deeply anesthetized with 20% urethane. Blood was *via* the femoral artery and serum was obtained after centrifugation at 10,000 rpm for 8 min and stored at  $-80$  °C till the time of TAS, FSH and testosterone analysis. The testes and epididymis were quickly removed and weighed. The right testis were used for histopathological sectioning. The left epidid-

ymides were separated for sperm evaluation and left testes were blotted and dried after washing with ice-cold physiological saline and stored at  $-80$  °C till homogenate preparation.

### 2.4. Cauda sperm count and sperm motility

Cauda epididymides were dissected out, immediately minced in 5 ml of physiological saline and then incubated at 37 °C for 30 min to allow spermatozoa to leave. The percentage of motile sperm was recorded using a phase contrast microscope at 400 $\times$  magnification power. Total sperm count was determined by using a Neubauer<sup>®</sup> hemocytometer as previously described (Yokoi et al., 2003).

### 2.5. Preparation of testicular homogenate

Each sample was ice-thawed and homogenized (10% w/v) using the BulletBlender<sup>®</sup> homogenizer (Next Advance Inc., NY, USA) in 25 ml saline–merthiolate–triton (SMT) buffer and centrifuged at 20,000 rpm for 2 min at 4 °C to obtain the testicular homogenate supernatant (Blazak et al., 1993).

### 2.6. Protein measurement

Protein content of the testicular homogenate was measured using crystalline bovine serum albumin as standard (Bradford, 1976).

### 2.7. Assay of biochemical parameters

#### 2.7.1. Determination of serum TAS

20  $\mu$ l of serum was incubated in a cuvette with 1 ml of chromogen composed of 610 mmol/L of 2,2'-azino-di-(ethylbenzthiazoline sulphonate) and 6.1 mmol/L of metmyoglobin. The reactants were mixed well and the initial absorbance ( $A_1$ ) was recorded. Then, the substrate was added (200  $\mu$ l of mmol/L  $H_2O_2$ ). The contents were mixed well and the absorbance was recorded after 3 min ( $A_2$ ). The  $\Delta A$  was calculated for each of the sample and blank (Miller et al., 1993). The TAS was calculated using the following equation:

$$\text{TAS (mmol/L)} = \text{factor} \times (\Delta A_{\text{blank}} - \Delta A_{\text{sample}})$$

$$\text{Factor} = \text{concentration of standard} / (\Delta A_{\text{blank}} - \Delta A_{\text{standard}})$$

#### 2.7.2. Determination of serum FSH and testosterone hormones

Determination of serum FSH (ng/ml) and testosterone (ng/ml) was carried out using enzyme immunoassay diagnostic kits.

#### 2.7.3. Determination of testicular lactate dehydrogenase (LDH) enzyme

The testicular homogenate was used to estimate LDH enzyme (U/mg protein) spectrophotometrically using diagnostic kit.

#### 2.7.4. Determination of testicular lipid peroxides (LPOs)

A 1 ml 1%  $H_3PO_4$  and 1 ml 6.0% MDA in 0.25 M HCl were added to 0.5 ml of testicular homogenate. The assay mixture was shaken and heated on a boiling water bath for 45 min. After cooling, 4 ml of *n*-butanol was added to the mixture with shaking. After separation the *n*-butanol layer by centrifugation at 6000 rpm for 15 min, the difference between two optical densities (535 and 520 nm) was recorded. The LPOs was expressed as  $\mu$ mol MDA equivalent formed/mg protein (Buege and Aust, 1978).

#### 2.7.5. Determination of testicular glutathione reductase (GR) enzyme

The testicular GR activity was measured by mixing 0.1 ml testicular homogenate with 1 ml of 0.2 M sodium phosphate buffer, 1 mM GSSG and 0.1 mM nicotinamide adenine dinucleotide phosphate (NADPH). The absorbance was recorded at 340 nm. The enzyme activity was calculated as nmol NADPH oxidized/min/mg protein (McFarland et al., 1999).

#### 2.7.6. Determination of testicular catalase (CAT) enzyme

CAT activity was determined using reaction mixture (2 ml) consisting of 1.95 ml of 10 mM  $H_2O_2$  in 60 mM phosphate buffer (pH 7.0). The reaction was started by adding 0.5 ml of testicular homogenate and the absorbance was recorded at 240 nm after 3 min. One CAT unit is defined as the amount of  $H_2O_2$  converted into  $H_2O$  and  $\frac{1}{2}O_2$  in 1 min under standard conditions and the specific activity is reported as  $\mu$ mol  $H_2O_2$  consumed/min/mg protein (Aebi, 1984).

#### 2.7.7. Determination of testicular superoxide dismutase (SOD) enzyme

The xanthine oxidase method was used to measure the testicular homogenate SOD activity using purified bovine erythrocyte SOD (5000 U/mg solid) as a standard. The reaction between 50 mM xanthine, 50 mM xanthine oxidase 1000 U and 0.1 mM EDTA was used to generate superoxide radicals and uric acid at pH 7.8. The superoxide radicals produced reacted with 50 mM NBT to produce a red formazan dye that was measured spectrophotometrically at 250 nm. The SOD present in

the sample (0.1 ml testicular homogenate) competes with the NBT for superoxide radicals and so inhibits the production of formazan dye. The SOD activity was expressed as U/mg of protein (Oynagui, 1984).

### 2.8. Histopathological studies

For the histopathological observations at light microscopic level, fresh testes were immersed and fixed in 10% formalin saline. Following an overnight fixation, the specimens were dehydrated in ascending grades of alcohol, cleared in benzene and embedded in paraffin wax. The tissues were then cutted into 4  $\mu\text{m}$  thick sections, stained with hematoxylin/eosin (H&E) stain and examined by light microscopy using  $\times 40$  magnification power (Banchraft et al., 1996).

### 2.9. Statistical analysis

Results were reported as mean  $\pm$  SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA). All statistical analyses were performed using GraphPad InStat 3 (GraphPad Software, Inc. La Jolla, CA, USA) software. Graphs were sketched using GraphPad Prism version 4 software (GraphPad Software, Inc. La Jolla, CA, USA). In all cases,  $P < 0.05$  was considered significant.

## 3. Results

### 3.1. Effect on testes weight, cauda sperm count, sperm motility percentage

The weight of testes (gm) decreased significantly by  $-18.15\%$  ( $p < 0.05$ ) after BNPB treatment, compared to normal control rats. In contrary, a significant increase by  $+12.1\%$  ( $p < 0.05$ ) in the testes weight was observed in rats administered LA prior to BNPB, compared to BNPB-intoxicated rats (Fig. 1A). The cauda sperm count ( $\times 10^6/\text{rat}$ ) decreased significantly by  $-13.83\%$  ( $p < 0.05$ ) after BNPB treatment, compared to normal control rats. In contrary, a significant increase by  $+10.20\%$  ( $p < 0.05$ ) in cauda sperm count was observed in rats administered LA prior to BNPB, compared to BNPB-intoxicated rats (Fig. 1B). The sperm motility percentage

decreased significantly by  $-13.50\%$  ( $p < 0.05$ ) after BNPB treatment, compared to normal control rats. In contrary, a significant increase by  $+11.51\%$  ( $p < 0.05$ ) in sperm motility percentage was observed in rats administered LA prior to BNPB, compared to BNPB-intoxicated rats (Fig. 1C).

### 3.2. Effect on serum FSH and testosterone hormones

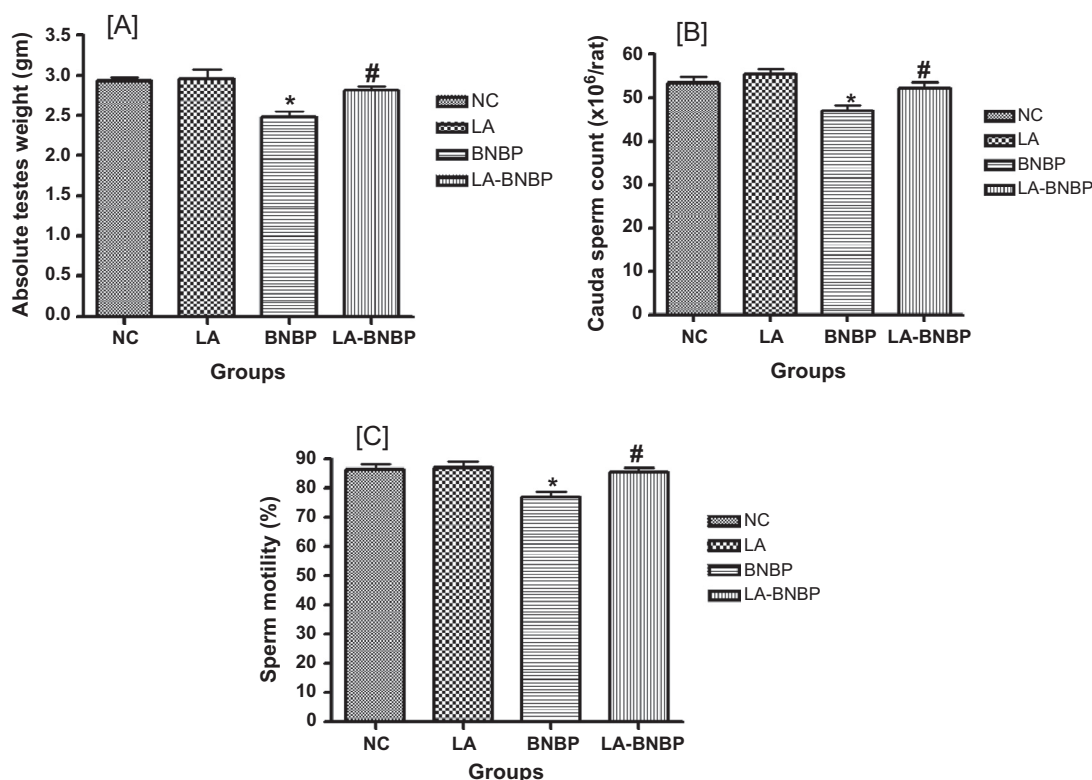
The serum FSH (ng/ml) decreased significantly by 2.6 folds ( $p < 0.05$ ) after BNPB treatment, compared to normal control rats. In contrary, a significant increase by  $+65.20\%$  ( $p < 0.05$ ) in serum FSH was observed in rats administered LA prior to BNPB, compared to BNPB-intoxicated rats (Fig. 2A). The serum testosterone (ng/ml) decreased significantly by  $-66.90\%$  ( $p < 0.05$ ) after BNPB treatment, compared to normal control rats. In contrary, a significant increase by 1.4 folds ( $p < 0.05$ ) in serum testosterone was observed in rats administered LA prior to BNPB, compared to BNPB-intoxicated rats (Fig. 2B).

### 3.3. Effect on serum TAS

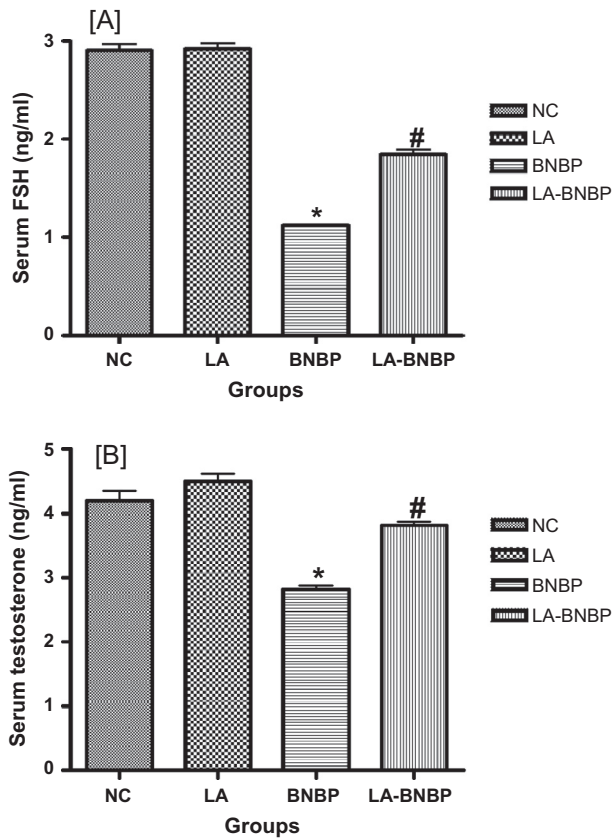
The serum TAS (mmol/L) decreased significantly by 2.5 folds ( $p < 0.05$ ) after BNPB treatment, compared to normal control rats. In contrary, a significant increase by 1.8 folds ( $p < 0.05$ ) in serum TAS was observed in rats administered LA prior to BNPB, compared to BNPB-intoxicated rats (Fig. 3A).

### 3.4. Effect on testicular LPOs and LDH enzyme

The testicular LPOs ( $\mu\text{mol}$  MDA equivalent formed/mg protein) increased significantly by 2 folds ( $p < 0.05$ ) after BNPB treatment, compared to normal control rats. In contrary, a significant decrease by 1.5 folds ( $p < 0.05$ ) in testicular LPOs was observed in rats



**Fig. 1.** Influence of co-administration of LA and BNPB on (A) absolute testes weight (gm), (B) cauda sperm count ( $\times 10^6/\text{rat}$ ) and (C) sperm motility (%). Values represent the mean  $\pm$  SEM ( $n = 6$ ). \*Significantly different from the NC group at  $p < 0.05$ . #Significantly different from BNPB-treated group at  $p < 0.05$ . LA:  $\alpha$ -lipoic acid; BNPB: bi-n-butyl phthalate; NC: normal control.



**Fig. 2.** Influence of co-administration of LA and BNP on serum (A) FSH (ng/ml) and (B) testosterone (ng/ml). Values represent the mean  $\pm$  SEM ( $n = 6$ ). \*Significantly different from the NC group at  $p < 0.05$ . #Significantly different from BNP-treated group at  $p < 0.05$ . FSH: follicle stimulating hormone; LA:  $\alpha$ -lipoic acid; BNP: bi-n-butyl phthalate; NC: normal control.

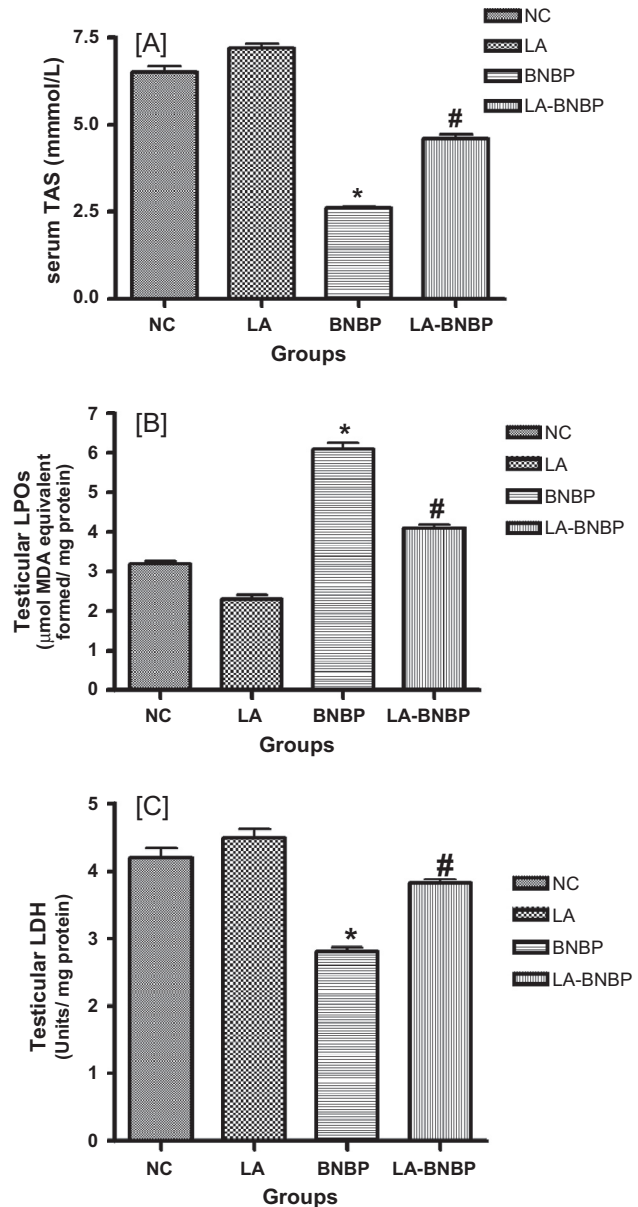
administered LA prior to BNP, compared to BNP-intoxicated rats (Fig. 3B). The testicular LDH (U/mg protein) increased significantly by 3.4 folds ( $p < 0.05$ ) after BNP treatment, compared to normal control rats. In contrary, a significant decrease by 1.6 folds ( $p < 0.05$ ) in testicular LDH was observed in rats administered LA prior to BNP, compared to BNP-intoxicated rats (Fig. 3C).

### 3.5. Effect on testicular SOD, CAT and GR enzymes

The testicular SOD (U/mg protein) decreased significantly by 1.6 folds ( $p < 0.05$ ) after BNP treatment, compared to normal control rats. In contrary, a significant increase by 1.4 folds ( $p < 0.05$ ) in testicular SOD was observed in rats administered LA prior to BNP, compared to BNP-intoxicated rats (Fig. 3A). The testicular CAT ( $\mu\text{mol H}_2\text{O}_2$  consumed/min/mg protein) decreased significantly by 2 folds ( $p < 0.05$ ) after BNP treatment, compared to normal control rats. In contrary, a significant increase by 1.8 folds ( $p < 0.05$ ) in testicular CAT was observed in rats administered LA prior to BNP, compared to BNP-intoxicated rats (Fig. 4B). The testicular GR ( $\mu\text{mol NADPH}$  oxidized/min/mg protein) decreased significantly by 1.6 folds ( $p < 0.05$ ) after BNP treatment, compared to normal control rats. In contrary, a significant increase by 1.4 folds ( $p < 0.05$ ) in testicular GR was observed in rats administered LA prior to BNP, compared to BNP-intoxicated rats (Fig. 4C).

### 3.6. Histopathological examination

The histopathological examination of the testicular sections from rats showed no histopathological changes in case of LA-treated rats, however, the BNP-treated rats, showed degeneration and

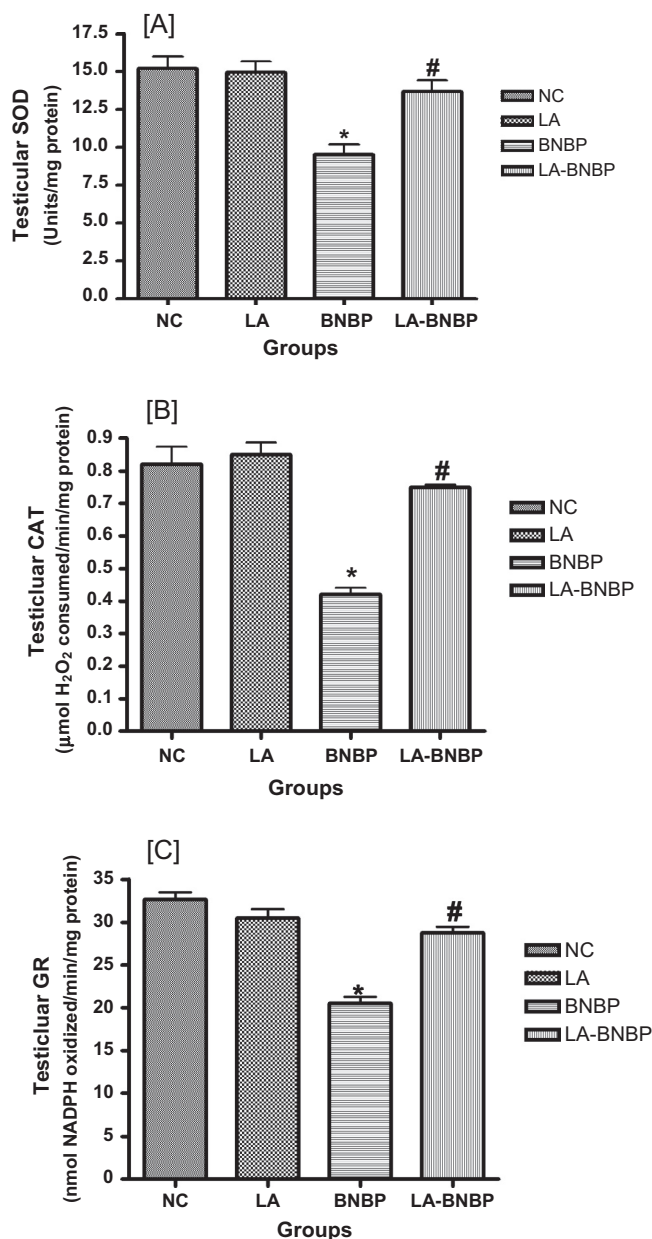


**Fig. 3.** Influence of co-administration of LA and BNP on (A) serum TAS (mmol/L), (B) testicular LPOs ( $\mu\text{mol MDA}$  equivalent formed/mg protein) and (C) testicular LDH (U/mg protein). Values represent the mean  $\pm$  SEM ( $n = 6$ ). \*Significantly different from the NC group at  $p < 0.05$ . #Significantly different from BNP-treated group at  $p < 0.05$ . TAS: total antioxidant status; LPOs: lipid peroxides; LDH: lactate dehydrogenase enzyme; LA:  $\alpha$ -lipoic acid; BNP: bi-n-butyl phthalate; NC: normal control.

atrophy were observed in some of the seminiferous tubules with complete absence of the spermatogenic series and sperms (Fig. 5C). The LA-BNP rats, showed slight damage to the seminiferous tubules (Fig. 5D). Stained rat testes sections were scored for the testicular injury through examining under the light microscope (50X magnification), with testicular histopathology score (0–4). Normal control rats (group I) and LA rats (group II) elicited score (0). BNP-intoxicated rats (group III) elicited the highest score (4) among all groups. However, LA-BNP rats (group IV) elicited score (2).

## 4. Discussion

The BNP has been utilized commercially as plasticizers, and become persistent environmental pollutant (Moffit et al., 2007).



**Fig. 4.** Influence of co-administration of LA and BNP on testicular (A) SOD (U/gm protein), (B) CAT ( $\mu\text{mol H}_2\text{O}_2$  consumed/min/mg protein) and (C) GR ( $\mu\text{mol NADPH}$  oxidized/min/mg protein). Values represent the mean  $\pm$  SEM ( $n = 6$ ). \*Significantly different from the NC group at  $p < 0.05$ . #Significantly different from BNP-treated group at  $p < 0.05$ . SOD: superoxide dismutase enzyme; CAT: catalase enzyme; GR: glutathione reductase enzyme; LA:  $\alpha$ -lipoic acid; BNP: bi-n-butyl phthalate; NC: normal control.

In recent years, more attention has been paid to whether exposure to environmental BNP affects human development and reproduction. It was reported that, the intake of BNP to the male rats showed decreased testosterone concentration (Kai et al., 2005; Gray et al., 2006), and an inverse correlation with sperm concentration and quality (Murature et al., 1987) and with sperm morphology but not correlated with ejaculate volume (Rozati et al., 2002). The results achieved from the present study, indicated that, the BNP intake to male rats resulted in decline in testes weight, sperm count and sperm motility. These findings were in accordance with another study showed that, mature male rats exposed to BNP had smaller testes, degeneration of seminiferous

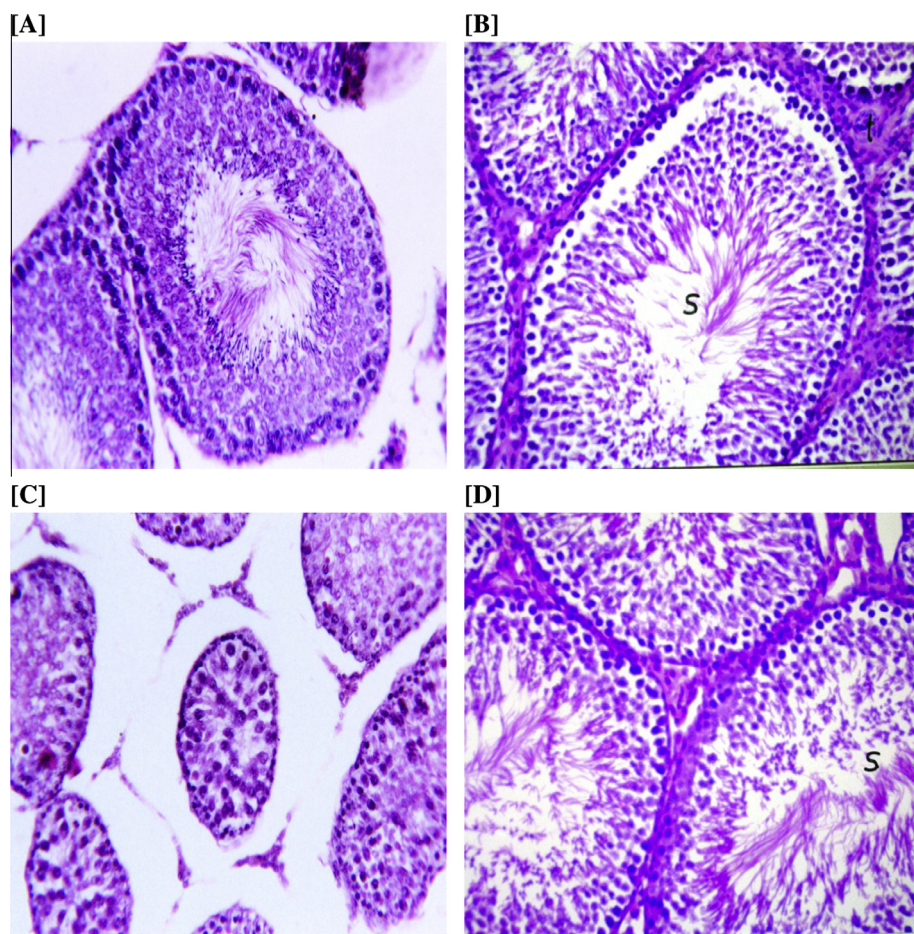
epithelium, decreased spermatogenesis (Barlow and Foster, 2003) and histopathological and biochemical alterations in sertoli cells (Creasy et al., 1983).

The development of the male reproductive tract is a dynamic process, requiring the interaction of many factors and hormones. Activation of specific pathways is required, involving the production and action of FSH and testosterone hormones, that are considered as a major essential factors for the development of the male internal and external male reproductive tract (Wilson, 1978). Our data revealed that, the intake of BNP is a potent testicular toxicant as the serum concentration of both FSH and testosterone hormones were declined. Antiandrogenic activities of BNP was detected in recombinant yeast assays (Jobling et al., 1995) as well as in mammalian and human cell systems (Terouanne et al., 2000; Körner et al., 2004). It was reported that, treatment of male rats with BNP produced significant decreases in testicular cytochrome P-450, cytochrome P-450 dependent microsomal steroidogenic enzymes (17 $\alpha$ -hydroxylase, 17-20 lyase) and in the maximal binding of a natural substrate (progesterone) to testis microsomes. This effect on the enzymes responsible for androgen production may be important as a mechanism of action involved in the development of BNP-induced testicular damage (Foster et al., 1983). Also, it was reported that, the exposure to BNP alters IL-1 $\beta$  secretion and subset expression of testicular macrophages, resulting in decreased testosterone production in rats (Zheng et al., 2010). Testosterone can both enhance or protect against toxicity, depending on the experimental model used (Nishino et al., 1998; Ahlbom et al., 2001). The protective property of testosterone against oxidative stress as well as on cell damage biomarkers such as lactate dehydrogenase enzyme and caspase-3 was demonstrated (Tunez et al., 2007).

These biochemical abnormalities were in parallel with the histopathological findings that showed severe damage and atrophy to the seminiferous tubules, existence of vacuoles in sertoli cells and loss of spermatogenesis in case of BNP-treated rats. The LA-BNBP rats, showed slight damage to the seminiferous tubules. BNP is considered an important class of sertoli cell toxicants. Sertoli cells are the primary supportive cells of the testis, facilitating the physical, hormonal and nutritive development of germ cells. Disruption of sertoli cell function results in impaired spermatogenesis and subsequent germ cell loss (Moffitt et al., 2007).

Antioxidants and other cell redox state modulating enzyme systems such as SOD, CAT and GR enzymes act as the first-line defense against reactive oxygen species in all cellular compartments and also extracellularly (Karihtala and Soini, 2007). The BNP-intoxicated rats exhibited significant depletion of testicular CAT, SOD and GR enzymes that was concomitant with significant elevation in LPOs and significant reduction of serum TAS and testicular LDH enzyme. These findings may be attributed to the fact that, BNP inhibits some forms of cytochrome P-450 and P-19, especially those responsible for hydroxylation of substrates (Foster et al., 1983; Ryu et al., 2007) and the generation of reactive oxygen species during BNP intoxication.

All the aforementioned biochemical abnormalities were ameliorated after the co-administration of LA and BNP to rats. The oral administration of LA to the BNP-intoxicated rats resulted in significant elevation of testicular CAT, SOD and GR enzymes, concomitant with significant reduction in testicular LDH enzyme and LPOs. Also, the absolute testes weight, cauda sperm count and sperm motility were improved after the intake of LA to the BNP-intoxicated rats. These results may elaborate the role of LA as free radical scavenger and antioxidant that may act as testicular toxicity protector. It was proved that, LA increased the TAS level, reduced LPOs and increased GSH and antioxidant enzymes levels, indicating its antiperoxidative and antioxidative effects (Chidlow et al., 2002;



**Fig. 5.** Histopathological examination of the testicular sections from rats (A) NC rats, showed no histopathological changes (testicular histopathology score-0), (B) LA-treated rats, showed no histopathological changes (testicular histopathology score- 0), (C) BNBP-treated rats, showed degeneration and atrophy were observed in some of the seminiferous tubules with complete absence of the spermatogenic series and sperms (testicular histopathology score- 4) and (D) LA–BNBP treated rats, showed slight damage to the seminiferous tubules (testicular histopathology score- 2). LA:  $\alpha$ -lipoic acid; BNBP: bi-n-butyl phthalate; NC: normal control.

Sivaprasad et al., 2004; El-Beshbishy et al., 2011). Also, it was reported that, LA works both inside the cell and at the membrane level, thereby giving dual protection to create a robust shield on the cell membrane, along with the liquid that surrounds the cell to tolerate higher volumes of free radical attack (Cronan et al., 2005). Besides, LA is well recognized as a natural cofactor of the pyruvate dehydrogenase and  $\alpha$ -dehydrogenase complexes. In addition, enantiomers of LA and their reduced forms (dihydrolipoic acid) act as extra- and intracellular redox couplers and powerful lipophilic free radical scavengers (Suzuki et al., 1991). Our results showed that, LA acts as a potent antioxidant capable of alleviating BNBP-induced oxidative stress and perturbation of antioxidant enzymes. LA has also been reported to assist with the Krebs cycle, which in turn will increase the level of reduced GSH, ATP, Krebs cycle enzymes and electron transport chain complex activities (Henriksen, 2006) and eventually lead to a reduced incidence of testicular dysfunction, thus ensuring sufficient ATP availability (Ibrahim et al., 2008).

Based on the findings of the present study, it may be concluded that, BNBP caused testicular toxicity through negatively affecting the activity of testosterone and FSH hormones and antioxidant enzymes. BNBP treatment also enhanced the LPOs level, demonstrating vulnerability of testicular membrane to BNBP exposure. LA which has emerged as a potential antioxidant and cell protecting compound mitigated testicular oxidative stress, testicular injury and LPOs produced by BNBP toxicity.

### Conflict of Interest

The authors declare that there are no conflicts of interest.

### Transparency Document

The [Transparency document](#) associated with this article can be found in the online version.

### Acknowledgements

This study was funded by Grant number 777/433 from the Deanship of Scientific Research, Taibah University, Al-madinah Al-munawarah, Saudi Arabia.

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