

## Protective Effect of Quercetin and or Zinc against Lead Toxicity on Rat Testes

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**Abstract:** The present study was conducted to evaluate the protective effect of quercetin and/or zinc chloride on lead nitrate-induced toxicity in rat testes. Fifty adult male albino rats were divided into 5 groups and treated daily for successive 21 days as follows: Group 1 received 1 ml of 0.9% saline intraperitoneally. Group 2 received lead nitrate (20 mg/kg b.wt./day) by gastric intubation. Group 3 pre-treated with zinc chloride (2 mg of /kg b.wt./day) intraperitoneally then lead nitrate after half an hour. Group 4 received quercetin orally (50 mg/kg b.wt./ day) then lead nitrate after half an hour. Group 5 pre-treated with quercetin and zinc chloride (an hour and half an hour, respectively) before lead nitrate administration by the same manner and dosage. On the day 21, the animals were sacrificed and testes were removed for histological and ultrastructure studies. Sections of testes of group 2 showed shrinkage of seminiferous tubules with degeneration and marked widening of interstitial spaces. Arrangement of germinal epithelium was disturbed where most of the cells appeared degenerated. Ultrastructure changes were revealed in the form of vacuolation within Sertoli cell cytoplasm with the increase in the number and size of lysosomes. These toxic effects were improved in sections of testes of groups 3, 4 and 5. The obtained results showed that quercetin and/or zinc chloride improved toxic effects of lead nitrate on rat testes.

**Key words:** Lead Nitrate • Zinc • Quercetin • Rat • Testes

### INTRODUCTION

Spermatogenesis can be considered to be one of the markers of proper health. The increase in the number of and the exposure to, physical and chemical agents induces significant problems for human fertility. Exposure to heavy metal impairs reproductive capacity by causing severe testicular degeneration, seminiferous tubule damage with necrosis in rats [1, 2].

The toxicity of lead is mainly connected with its influence on the enzymatic systems of cells, which leads to many biochemical disorders. Sub-chronic oral lead intoxication may affect body organs and testes for several years even in absence of continued exposure [3]. Many studies have shown that reproductive toxicity is an important feature of lead toxicity [4, 5]. During lead exposure, it accumulates toxically inducing a significant increase in apoptotic cell death in the seminiferous tubules of young growing rats[4]. It is also associated

with disruption of spermatogenesis and histoarchitecture with lowered enzyme activities in testis [6].

Flavonoids are plant phenolic compounds with strong antioxidant properties found in many dietary sources such as tea, onion, broccoli, apple and green beans [7]. Flavonoids can prevent oxidative damage as a result of their ability to scavenge reactive oxygen species such as hydroxyl radical and superoxide anion [8] and metal chelating [9]. It has also been shown that flavonoids inhibit oxidative stress and lipid peroxidation induced by pesticides in experimental animals [10-12]. Quercetin is a member of the flavonoid family [10]. It has been reported that the biological effects of quercetin are as follow: Anti-cancerogenic, antiviral, anti-ischemic, anti-inflammatory and antiallergenic as well as preventive influence in atherosclerosis and coronary heart disease [13, 14]. Furthermore, quercetin protected human hepatocytes from ethanol-induced cellular damage [15] and exhibited neuroprotective effects and anti-inflammatory activity in rats [16-18].

On the other hand, Zinc (Zn) is an anti-oxidant trace element that is present in all tissues and fluids of the body. It is required for cell proliferation, differentiation, normal growth, immune functions and wound healing. Also, Zn is an essential mineral for spermatogenesis [19].

Lead interacts with trace metals especially zinc at the stage of their intestinal absorption and distribution in tissues as well as with their biological functions. Lead can substitute ions of other metals in many metallo-enzymes, leading to their inhibition. It has been shown that increased dietary intake of zinc reduces the accumulation and toxicity of lead, probably by decreasing its intestinal absorption [6]. Moreover, the beneficial role of zinc against cadmium-induced testicular damage has also been reported, although its anti-oxidant mechanism is unclear [2, 19, 20]. Nevertheless, several mechanisms have been proposed for zinc. One mechanism is that zinc's protection of the testes is mediated by the induction of metallothionein (MT) against heavy-metal toxicity [2, 21]. Alternatively, zinc may directly antagonize the toxic effects of heavy metal and/or stabilize cell membranes and protect lipid peroxidation by free radicals [1, 19, 22]. Lead (Pb) on the other hand is a toxic non-essential trace element. Divalent zinc and lead potentially have similar chemical characteristics [23].

Therefore, this study was conducted to demonstrate protective effect of quercetin and or zinc chloride on lead nitrate-induced toxic effects on the testes.

## MATERIALS AND METHODS

**Chemicals:** Lead nitrate, quercetin and zinc chloride were obtained from Sigma Chemicals Co., St. Louis, Mo., USA. Lead nitrate was dissolved in distilled water and given orally by gastric intubation at dose 20 mg/kg b. wt. according to Moldowan and Behr [24]. Zinc chloride was dissolved in 0.9% NaCl and injected intraperitoneally (i.p.) at dose 2 mg of /kg b. wt. according to Goulart *et al.* [25]. Quercetin was dissolved in distilled water and given orally by gastric intubation at dose 50 mg/kg b. wt. [26].

**Animals and Experimental Design:** Fifty adult male albino rats weighing about 120-160 g were used in the present study. Rats were obtained from the Central Animal House of the Farm Animal of the Egyptian Organization for Vaccine and Biological Preparation at Helwan, Egypt. Standard chow and water were given ad libitum. Rats were

housed 5 per cage, acclimated for 1 week prior to treatments. Rats were divided into 5 groups, 10 rats each. Group1 received i.p. injection of normal saline at 1 ml. Group 2 received lead nitrate orally. Group 3 received i.p. injection of zinc chloride half an hour before oral administration of lead nitrate. Group 4 received quercetin orally half an hour before lead nitrate. Group 5 pre treated with both quercetin and zinc chloride (An hour and half an hour, respectively) before lead nitrate administration. All treatments were received by the same manner and dosage daily for 21 successive days. On the day 21 of completion of treatment the animals were sacrificed under ether anesthesia and testes were removed and fixed in the suitable fixative for histological and ultrastructural studies.

**Histological Studies:** Testes sections from all groups were fixed in formalin and embedded in paraffin. Sections of 5 um thickness were stained with hematoxylin and eosin using standard procedures [27].

**Ultrastructure Studies:** Testicular tissues were further processed for ultrastructure evaluation by transmission electron microscopy (TEM); the testis samples were cut into small pieces of about 1 mm<sup>3</sup> and fixed in 2.5% glutaraldehyde for 24-48hr. The specimens were then washed in 0.1M phosphate buffer (pH7.4) 3-4 times for 20 min. every time and post-fixed in a buffered solution of 1% osmium tetroxide at 4°C for 2 hr. After dehydration in graded concentrations of ethyl alcohol, the tissue specimens were cleared in two changes of propylene oxide and embedd in Epon resin. Semithin sections (~1 um thick) were stained with 1% toluidine blue and examined by using a light microscope. Areas of interest were selected and the blocks were trimmed accordingly. Ultrathin sections (60-70 nm) were cut with a diamond knife using an ultra microtome (MT6000-X L RMC, Inc.), mounted on copper grids and double -stained with uranyl acetate and lead citrate [28]. Grids were viewed and photographed using a transmission electron microscope (JEOL JEM-1200 EX 11, Japan) operated at 60-70 kV (Faculty of Science, Ain Shams University).

## RESULTS

**Histological Studies:** In the control group, there was regular and compact arrangement of seminiferous tubules with intact interstitium (Fig. 1).

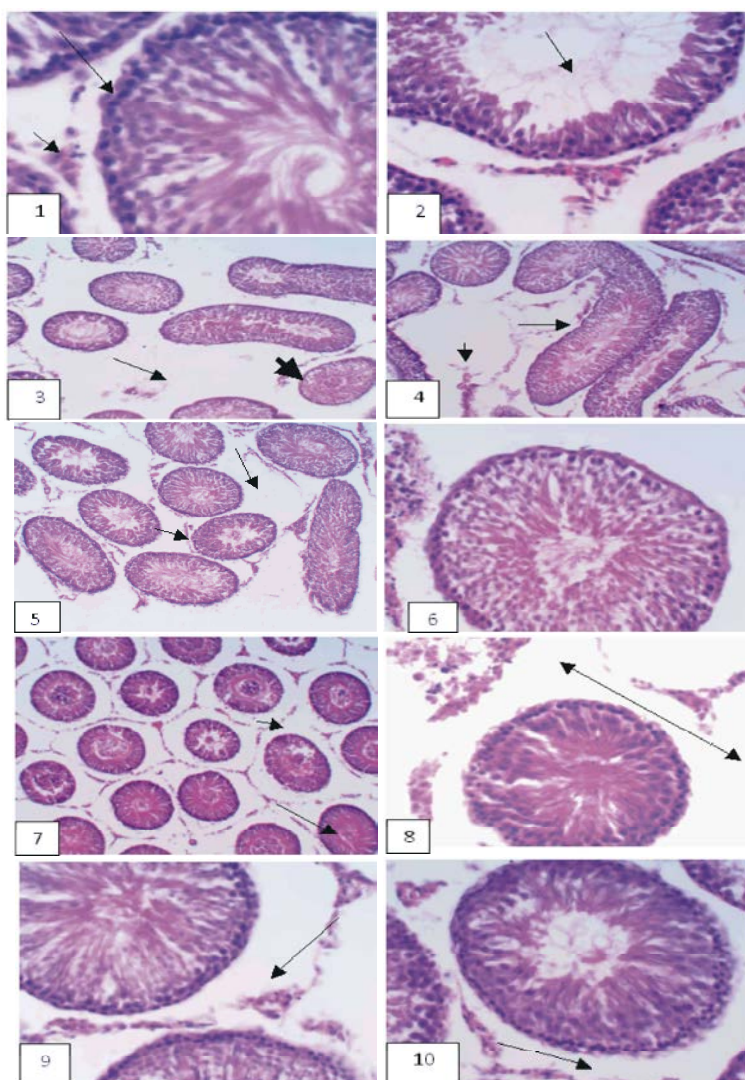


Fig. 1: Photomicrograph of testis section of control rat showing normal structures of the seminiferous tubules (Long arrows) and interstitial tissue (Short arrow). (H&E; X 400)

Fig. 2: Showing necrosis of almost all the spermatogenic cells and disappearance of sperms (Long arrow). (H&E; X 400)

Fig. 3: Showing shrunken seminiferous tubules (Short arrow) with mark widening of interstitial space (Long arrow). (H&E; X 100)

Fig. 4: Showing bizarre shapes of the seminiferous tubules (Long arrow) with widening of interstitial space (Short arrow). (H&E; X 100)

Fig. 5: Showing slight improvement in the testicular structures; widening of connective tissue spaces (Long arrow) and shrinkage in the seminiferous tubules (Short arrow). (H&E; X 100)

Fig. 6: Showing improvement in the seminiferous tubules. (H&E; X 400)

Fig. 7: Showing large number of spermatozoa in the lumen of tubules (Long arrow); separated tubules from each other by wide spaces (Short arrow). (H&E; X 100)

Fig. 8: Showing partial recovery of the germinal epithelium with marked widening of intersitail tissue (Double head) (H&E; X 400)

Figs. 9, 10: Photomicrographs of testis section of rats treated with quercetin, zinc chloride and lead nitrate showing restoration of spermatogenesis in most of the seminiferous tubules and expansion of intertubular space (Arrow) (H&E; X 400)

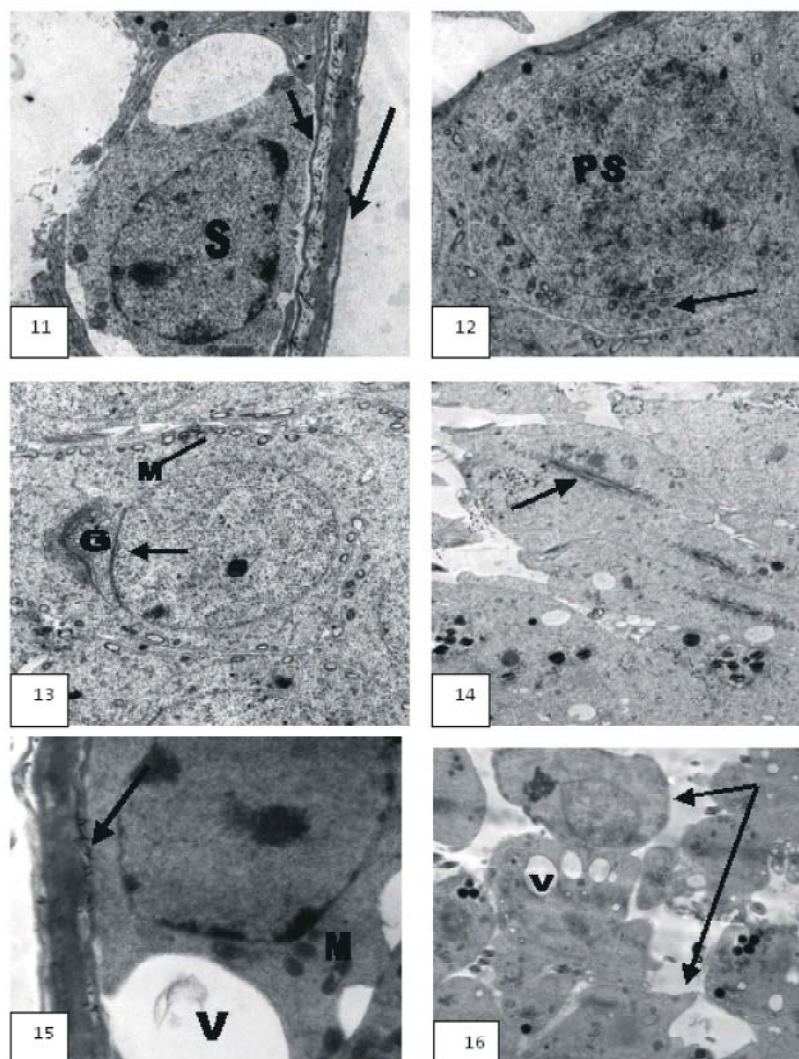


Fig. 11: Showing normal seminiferous tubule surrounded by thin basal lamina (Short arrow) followed by the boundary tissue (Long arrow), Sertoli cell (S) resting on the basal lamina and having nucleus with prominent nucleolus. (X 8000)

Fig. 12: Showing primary spermatocyte (PS) with spherical nucleus containing clumps of heterochromatin. The cytoplasm contains numerous mitochondria (Arrow). (X 10000)

Fig. 13: Showing early spermatid with acrosomal formation (Arrow), peripheral arrangement of mitochondria (M) and well developed Golgi apparatus (G). (X 10000)

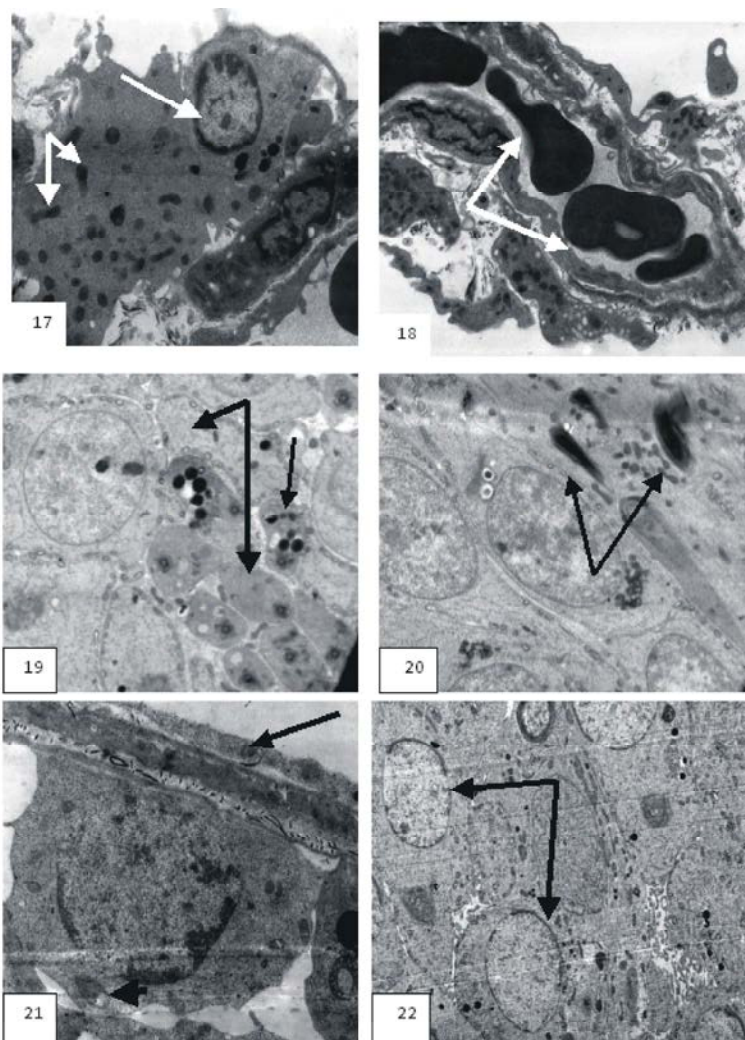
Fig. 14: Showing late spermatids with elongated nuclei (Arrow). (X 10000)

Fig. 15: Showing thickened boundary tissue (Arrow), disorganized spermatogenic cells with altered cytoplasmic organelles, vacuoles of various sizes (V) and swelling mitochondria (M). (X 15000)

Fig. 16: Showing deformed spermatids with abnormal nuclei (double arrows), Notice the presence of vacuoles in the cytoplasm (V). (X 5000)

Sections of testis of rats injected with lead nitrate showed variable degrees of degenerative changes in the seminiferous tubules up to complete cellular destruction. These were represented by necrotic features of almost all

spermatogenic cells and disappearance of sperms (Fig. 2), shrunken seminiferous tubules with mark widening of interstitial space (Fig. 3) and appearance of bizarre shapes of the seminiferous tubules as revealed in Figure 4.



- Fig. 17: Showing degenerative changes in Leydig cells where they exhibited ill defined cell membrane, irregular nuclear envelope (arrow) and mitochondrial disruption with loss of cristae (Double arrows). (X 10000)
- Fig. 18: Showing red blood cells (RBCs) scattered in the interstitial space (Double arrows). (X 8000)
- Fig. 19: Showing abnormal spermatids (Double arrows) and necrotic debris in the luminae of the seminiferous tubules. Notice the presence of numerous lysosomes (Arrow). (X 6000)
- Fig. 20: Showing abnormal sperms with decreasing number (Arrow). (X 6000)
- Fig. 21: Showing irregular boundary tissue and swelling mitochondria (Arrow). (X 12000)
- Fig. 22: Showing almost normal spermatogenic cells (Double arrows). (X 4000)

The testis tissue of rats treated with zinc chloride prior to lead nitrate showed slight improvement in the testicular structures; widening of connective tissue spaces and shrinkage in the seminiferous tubules (Fig. 5 & 6). The testis tissue of rats treated with quercetin and lead nitrate showed large numbers of spermatozoa in the lumen of tubules but the tubules themselves were still detached from each other by wide spaces (Fig. 7). In addition, partial recovery was manifested in the germinal epithelium (Fig. 8).

The testis tissue of rats treated with quercetin, zinc chloride and lead nitrate showed restoration of spermatogenesis in most of the seminiferous tubules whereas expansion of intertubular spaces were still observed (Figs. 9 & 10).

**Examination of the Ultrastructure:** The ultrastructure examination of rat testes from control group showed normal seminiferous tubule surrounded by thin basal lamina followed by the boundary tissue, Sertoli cell (S)

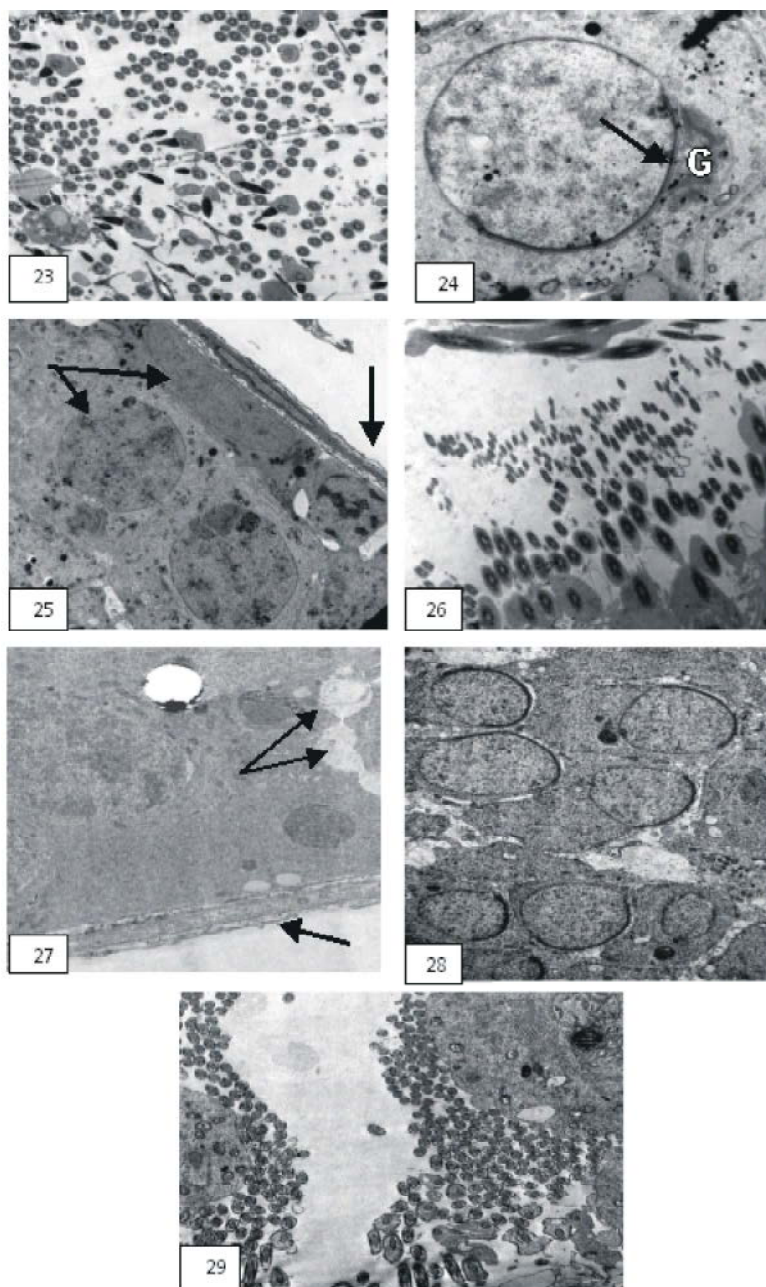


Fig. 23: Showing an increase in the number of sperms. (X 4000)

Fig. 24: Showing nearly normal early spermatid with acrosomal formation (Arrow), well developed Golgi apparatus (G) and peripheral arrangement of mitochondria. (X 12000)

Fig. 25: Showing almost normal structure of Sertoli cell and spermatogenic cells (double arrows). Notice nearly normal boundary tissue (Arrow). (X 4000)

Fig. 26: Showing an increase in the number of sperms. (X 4000)

Figs. 27-29: Electron micrographs of testis section of rats treated with quercetin and lead nitrate.

Fig. 27: Showing nearly normal boundary tissue (Arrow). Notice the presence of vacuoles of various sizes (Double arrows). (X 8000)

Fig. 28: Showing almost normal spermatids. (X 4000)

Fig. 29: Showing an increase in the number of sperms. (X 8000)

resting on the basal lamina and having nucleus with prominent nucleolus (Fig. 11), primary spermatocyte (PS) with spherical nucleus containing clumps of heterochromatin and cytoplasm containing numerous mitochondria (Fig. 12). Figure 13 showed early spermatid with acrosomal formation, peripheral arrangement of mitochondria (M) and well developed Golgi apparatus. Late spermatids with elongated nuclei were observed (Fig. 14).

Testes sections of rats injected with lead nitrate showed thickened boundary tissue, disorganized spermatogenic cells with altered cytoplasmic organelles, vacuoles of various sizes and swelling mitochondria (Fig. 15). Also, noticed was the presence of vacuoles in the cytoplasm and deformed spermatids with abnormal nuclei (Fig. 16).

Degenerative changes in Leydig cells were evidenced where they exhibited ill defined cell membrane, irregular nuclear envelope and mitochondrial disruption with loss of cristae (Fig. 17). Red blood cells (RBCs) were scattered in the interstitial space (Fig. 18).

Appearance of abnormal spermatids, necrotic debris in the lumina of the seminiferous tubules and numerous lysosomes were additionally noticed (Fig. 19). Abnormal sperms with decreasing number were main features in this group (Fig. 20).

Irregular boundary tissue and swelling mitochondria were still present (Fig. 21) in the testis tissues of rats orally given zinc chloride and injected with lead nitrate. Some improvement in the testicular structure as observed by almost normal spermatogenic cells (Fig. 22) and increase in the number of sperms (Fig. 23).

The testis tissue of rats orally given quercetin and i.p. injected with lead nitrate showed more improvement as observed by nearly normal early spermatid with acrosomal formation, well developed Golgi apparatus and peripheral arrangement of mitochondria (Fig. 24), almost normal structure of Sertoli cell and spermatogenic cells and nearly normal boundary tissue (Fig. 25). An increase in the number of sperms was observed (Fig. 26). The testis tissue of rats orally given quercetin, lead nitrate and injected (i.p.) with zinc chloride showed obvious improvement in most of the seminiferous tubules.

Most of the seminiferous tubules were more similar to those of the control group (Figs. 27-29) while other tubules showed vacuoles of various size (Fig. 27).

## DISCUSSION

The present study indicated that, the administration of the lead nitrate for 21 days induced many

histopathological changes in the testicular tissue of albino rats. These changes include necrosis of almost all the spermatogenic cells, disappearance of sperms, shrunken seminiferous tubules with marked widening of interstitial spaces and bizarre shapes of the seminiferous tubules. Similar observations were detected in reproductive system of rat by Makhoul *et al.* [29].

In the present work, there was gradual increase in the thickness of the boundary tissue. This thickening might result from increase in the amount of collagenous fibers that could result from either over production of collagen fibers by fibroblasts or decreased rate of collagen phagocytosis [30, 31].

In the current study, disorganized spermatogenic cells with altered cytoplasmic organelles, vacuoles of various sizes and abnormal sperms with decreasing number were observed. These findings might be due to loss of those populations via apoptosis or differentiation failure. Similar explanation was reported in mutant mice [32]. These changes might be explained by the reduced expression of Sertoli cell growth factor [Glial cell line-derived neurotrophic factor (GDNF)] as well as retraction of the Sertoli cells cytoplasmic processes that are normally supporting germ cells, that might depress the spermatogonial differentiations [33, 34].

Light microscopic analyses revealed that lead nitrate-induced numerous histopathological changes in testis tissues of diabetic and non-diabetic rats [35].

Makhoul *et al.* [29] found that lead induced apoptotic changes in most of the germ cells. These cells might be the most affected cells owing to their proliferating character so they might be the target for toxic effect of lead [36]. In accordance to these observations, it has been found that lead affect mitotic spindle in lead treated rats [37]. These changes might be due to the induction of the oxidative mechanisms by lead which induced both apoptotic and degenerative effects in the germ cells or secondary to Sertoli cell injury. Similar observations were reported in the germ cell of lead treated rat testis by using TUNEL technique [4]. However, reactive oxygen species (ROS) induced cell death might occur through apoptosis or necrosis [38]. Also, they might be implicated in the defect in spermatogenesis and residual body-like changes, including multiple lipid droplets in lead treated mice [39].

The present investigation reported abnormal spermatids, necrotic debris in the luminae of the seminiferous tubules and the presence of numerous lysosomes. Same explanation was reported by some authors in rat treated with nitrofurazone, who reported that it might be a sign of germ cell degeneration [40].

Lead caused degenerative changes in the spermatids of the seminiferous tubules of mice testis. It also caused cellular abnormalities in testosterone-producing Leydig cells. These morphological alterations may explain why lead induces infertility in male subjects [41].

Reduced width of germinal epithelium that was seen in this study seems to be due to damage of germinal cells as it was reported previously by other researcher [42]. Lead intoxication mainly affected spermatids [42]. Lead induced apoptosis of the germinal cells which was reported by Adhikari *et al.* [3] is a possible mechanism for loss of germinal epithelium [43]. Batra *et al.* [4] observed significant reduction in type A spermatogonia after lead toxicity associated with decrease of other germ cell spermatogonia (6).

The animals with lead and zinc treatments showed very little disarrangement of germinal epithelium [4].

In the present work, degenerative changes in Leydig cells were designated where they exhibited ill defined cell membrane, irregular nuclear envelope and mitochondrial disruption with loss of cristae. These findings might reflect degeneration of these cells and decrease in testosterone level which simultaneously arrested spermatogenesis. Consistent with present findings, degenerative changes were observed after diazepam treatment in rat testis [44].

Reactive oxygen species (ROS) and its metabolites is the subject of intense research because of their active role in cellular physiology and pathogenesis of number of diseases [45]. Makhlof *et al.* [29] suggested that oxidative damage is a major cause of lead-induced testicular damage.

The present investigation delineated an improvement in the histological picture of the testes, including almost normal spermatogenic cells and an increase in the number of sperms, in case of zinc chloride against lead toxicity. These results are in agreement with those of Rafique *et al.* [42] who postulated that the toxic effects of Pb on male reproductive system could be ameliorated by Zn supplementation. As evidence has shown that Zn exists in spermatozoa within the seminiferous tubules and helps spermatogenesis, Pb may result in disruption of the metabolic functions of enzymes containing Zn, inducing testicular damage. Batra *et al.* [43] reported that there was a 30% reduction in Pb deposition in the testes when Zn was co administered. The protective effect of Zn on reproductive toxicity of Pb may be attributed to competition between Pb and Zn, or reduction of available Pb-binding sites in the testicular tissue. Kulikowska-karpinska and Moniuszko-jakoniuk [46] reported that as the dietary Zn increased, severity of Pb toxicity

decreased, including decline of Pb concentration in the blood, liver and kidneys. Piao *et al.* [47] demonstrated that Zn administration together with Pb decreased hepatic and renal uptake of Pb. Testicular degeneration was observed in the rats exposed to high doses of Pb and that, with concomitant administration of Pb and Zn, both testes and epididymis presented nearly normal pictures [5].

Zinc reduces the toxic effects of lead with an anti-oxidant mechanism [20]. Several mechanisms have been proposed for the protection provided by zinc. One mechanism is that zinc may stabilize lipid membrane and protects lipid peroxidation by free radicals, thereby protecting tissues [2, 19].

The present study, showed more improvement as observed by nearly normal early spermatid with acrosome formation, well developed Golgi apparatus and peripheral arrangement of mitochondria, almost normal structure of Sertoli cell and spermatogenic cells, nearly normal boundary tissue and an increase in the number of sperms, in case of quercetin against lead toxicity. These results are in agreement with those of Anjaneyulu and Chopra [7] who stated that quercetin, a potent anti-oxidant defense pathway in vivo and vitro. Kalender *et al.* [48] also indicated that a low dose of chlorpyrifos causes testicular toxicity in male rats and antioxidants catechin and quercetin can ameliorate this toxicity. These protective effects may be due to antioxidant effects of catechin and quercetin.

Flavonoids can prevent oxidative damage as a result of their ability to scavenge reactive oxygen species such as hydroxyl radical and superoxide anion [8]. The antioxidant properties of flavonoids depend on both metal-chelating properties and free radical scavenging of reactive oxygen species [9]. Antioxidants are exogenous or endogenous compounds acting in several ways, including scavenging reactive oxygen species or their precursors, inhibiting ROS formation and binding metal ions needed for the catalysis of ROS generation [49].

Flavonoids are known to improve erectile function by their antioxidant and anti-inflammatory properties [50, 51]. Farombi *et al.* [52] suggested that consumption of dietary foods rich in quercetin may be beneficial against sperm toxicity, testicular damage and endocrine pathology by cadmium and thus indicating that quercetin could be clinically useful.

The use of quercetin in combination with 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) minimized its toxicity as revealed from decreasing histological changes in tissue and abnormal sperm rate and increasing sperm motility and sperm concentration [53].



Using quercetin as a polyphenol compound should be reconsidered and it is the time to stop the slow poisoning of letrozole (Aromatase inhibitor) that can promote spermatogenesis in male patients [54].

Quercetin supplementation to Streptozotocin - induced diabetic rats for five consecutive weeks is a potentially beneficial agent to reduce testicular damage in adult diabetic rats, probably by decreasing oxidative stress [55].

Therefore, it can be concluded quercetin or zinc chloride may play a crucial protective role against lead nitrate toxicity in rat testes. Nevertheless, using both together increases the potency of such protective role.

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