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Pentoxifylline Efficiency in Protecting Testes Against Cadmium Toxicity

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Abstract: This study was performed to evaluate the protective role of pentoxifylline in preventing the detrimental effect of cadmium on the rat fertility. Thirty rats were assigned into five groups. Control group was daily administered saline for 60 days. Cadmium group was administered daily with cadmium chloride for 30 days then with saline only for 30 days. Pentoxifylline plus cadmium group was administered daily with saline for 30 days then with $CdCl_2$ plus pentoxifylline for 30 days. Pentoxifylline pre-cadmium group was administered daily with pentoxifylline for 30 days then with $CdCl_2$ for 30 days. Pentoxifylline post-cadmium group was administered daily with $CdCl_2$ for 30 days then with pentoxifylline for 30 days. Cadmium caused a significant weight reduction in the body and reproductive organs; serum and testicular testosterone, zinc; antioxidant enzymes and semen characteristics while it caused significant increase in testicular cholesterol and Malodialdehyde (MDA). Histopathologically cadmium caused impairment in the testes. Pentoxifylline treatment improved the estimated parameters. It could be concluded that the pentoxifylline treatment improved the evaluated parameters and cloud be used for protection against cadmium toxicity.

Key words: Cadmium, pentoxifylline, oxidative stress, rat, testes

INTRODUCTION

Cadmium (Cd) is one of major occupational and environmental toxicants ranked eighth in the top 20 hazardous substances priority list (ATSDR, 1999), occurring in the environment naturally and as a pollutant emanating from industrial and agricultural sources (Jarup and Akesson, 2009). Exposure to cadmium occurs as a result of atmospheric emission during Cd production and processing from combustion of fossil energy sources, waste and sludge, phosphate fertilizers and deposition of waste and slag at disposal sites (Toman *et al*., 2009). The general population is exposed to Cd via contaminants found in drinking water and food (ATSDR, 2008).

Cadmium is a highly reactive metal, adversely affecting many mammalian organ systems, including kidney, liver, lung, pancreas, testis, prostate, ovary and placenta (Bridges and Zalups, 2005; Thompson and Bannigan, 2008; Zhang *et al*., 2008; Liu *et al*., 2010). Moreover, cadmium is a recognized reproductive toxicant and has been reported to reduce male fertility and altered sexual behavior in both humans and rodents (Thomas and Brogan, 1983). Most animals with scrotal testes are susceptible to cadmium-induced testicular toxicity (King *et al*., 1999). Testes are included among the most target organs for cadmium intoxication (Stajn *et al*., 1997; Siu *et al*., 2009). Rodent testes are more susceptible to cadmium toxicity than liver as manifested in testicular damage without pathological changes to other organs (Adaikpoh and Obi, 2009; Siu *et al*., 2009). Exposure to cadmium can negatively affect the male reproductive system via degenerative changes in testes, epididymis and seminal vesicles (Corton *et al*., 1999). Acute Cd exposure results in complete testicular hemorrhagic necrosis in Cd-sensitive strains. Cadmium appears to cross the endothelial cell layer of the blood-testis barrier and to drastically increase the vascular permeability of testis. This increased permeability causes fluid to rush into the testis which overwhelms the draining function of the lymphatic system, resulting in hemorrhage, ischemia and finally, necrosis (Wong *et al*., 2004).

Although, cadmium is a well-known testicular toxicant, its mechanism of toxicity on this organ has not been completely elucidated. Among the proposed mechanisms for its toxicity on the testes are: circulatory failure due to vascular damage (Gunn and Gould, 1970, 1969; Johnson, 1977; Prozialeck *et al*., 2008). The injurious effects of cadmium which consist initially of interstitial

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edema, followed by hemorrhage, necrosis and finally testicular atrophy (Gunn and Could, 1975) are considered to be selective for the vascular bed of the testes since they occur at a dose that is not obviously toxic to vascular beds in other organs (Gunn and Gould, 1970, 1975). Also cadmium acts as an inducer of oxidative stress (Thompson and Bannigan, 2008; Tremellen, 2008; Turner and Lysiak, 2008) and lipid peroxidation (Ardais *et al*., 2008; Acharya *et al*., 2008; Ola-Mudathir *et al*., 2008; Ognjanovic *et al*., 2010). Moreover, it is well established that Cd significantly alters the circulating levels of several hormones, e.g., testosterone; Luteinizing Hormone (LH); Follicule Stimulating Hormone (FSH); Adrenocorticotrophic Hormone (ACTH) and Prolactin (PRL) (Lafuente *et al*., 2003, 2004).

Pentoxifyline (PTX) is a xanthine derivative which has inhibitory effects on xanthine oxidase (Hammerman *et al*., 1999). Xanthine oxidases considered as a candidate for oxygen free radical formation in cell. Recently, PTX has gained considerable interest as a ROS scavenger. The potential antioxidant effects of PTX have confirmed in several *in vitro* studies (Freitas *et al*., 1994; Bhat and Madyastha, 2001; Horvath *et al*., 2002). Pentoxifylline down regulates Tumor Necros is Factor alpha (TNFα) production, this cytokines provokes a rise in hydrogen peroxide from mitochondria (Bilsborough *et al*., 2002; Jackson *et al*., 2002). Pentoxifylline is a competitive non-selective phosphodiestrase inhibitors (Samardzic *et al*., 2000; Schermuly *et al*., 2001) and it may have some effects on production of nitric oxide and epidermal growth factors (Abdollahi and Simaiee, 2003). Pentoxifylline has the ability to improve perfusion in the impaired microcirculation of peripheral and cerebral vascular disorders as it has been shown to improve tissue oxygenation and endothelial function (Okumura *et al*., 2009) because it has rheologic and vasodilating properties (Salam *et al*., 2005) through decreasing blood viscosity; increasing erythrocyte flexibility (Ward and Clissold, 1987), reducing platelets aggregation and fibrinogen levels (Weithmann, 1981). Also, it has a potent stimulatory effect of prostacycline in vascular beds (Weithmann, 1981) and it is an adenosine receptor antagonist (Churchill and Bidani, 1982) and has the ability to increase the neutrophils motility (Crocket *et al*., 1987) and modulate the macrophage functions (Dominguez-Jimenez, 2002), so it has anti-inflammatory effect.

As in acute Cd toxicity, testis has been shown to be also a target organ following chronic Cd exposure (Saygi *et al*., 1991; Swiergosz *et al*., 1998). However, in comparison to acute Cd-induced testicular damage, the changes in testicular structure following repeated Cd exposure has not been fully explored, therefore, the present investigation was not only designed to study the effect of chronic cadmium administration on the testes and the male fertility but also to evaluate the effect of pentoxifylline in alleviating the detrimental effect of cadmium toxicity.

MATERIALS AND METHODS

Chemicals: Pentoxifylline and cadmium were purchased from (Sigma Chemical Company. Saint Louis, Missouri, United State of America).

Experimental animals and protocol: A total of 30 adult male Wister rats were used in the current study, weighing 195-210 g which were obtained from the Experimental Veterinary and Agriculture Station belonging to King Faisal University, at AL Asha, Saudi Arabia, housed in the Physiology Laboratory in plastic cages. The rats were acclimatized in controlled environment (temperature: 20-23°C and 12 light/12 h dark schedule). The rats were fed on standard food pellet and water *ad libitum*. The practices during experimentation were consistent to the principles of the Ethical Committee for Research at King Faisal University. Following 1 week acclimation, the animals were assigned into the following groups with 6 rats each: group 1(Cadmium treated group) were intubated daily with 0.5 mL saline only for 30 days then with 1/20 Lethal dose, 50% (LD_{50}) $CdCl_2$ (2.5 mg kg^{-1} BW) in 0.5 mL saline (Mohamed, 2009) for 30 days. Group 2 (Pentoxifylline plus cadmium treated group) was intubated daily with saline for 30 days then with $CdCl_2$ pluspentoxifylline (100 mg kg^{-1} BW (Taye *et al*., 2009)) for 30 days. Group 3 (Pentoxifylline pre-cadmium treated group) were intubated daily with pentoxifylline for 30 days then $CdCl_2$ for 30 days. Group 4 (Pentoxifylline post-cadmium treated group) were intubated daily with $CdCl_2$ for 30 days then with pentoxifylline for 30 days. Group 5 (control group) were received daily 0.5 mL saline for 60 days which representing complete spermatogenic cycle (Hershberger *et al*., 1969).

Body weight: The body weight of individual rat was recorded twice, at the beginning and at the end of the experimental period.

Reproductive organ weights: All rats were euthanized by decapitation at the end of the experiment. Blood samples were collected in clean dry test tubes. Immediately after blood collection. The testes; epididymis and accessory sex glands (seminal vesicles and prostate) were removed, blot dry; grossly examined and weighed. The Index Weight (IW) of each organ was calculated according to Matousek (1969).

Sperm count: Epididymal spermatozoa were counted by a modified method by Yokoi *et al.* (2003). Briefly, epididymides were detached from the testicles. The epididymis was minced in 5 mL phosphate buffer saline (pH 7.2), set in a rocker for 10 min and incubated at room temperature for 2 min. The supernatant fluid was diluted 1:100 with a solution containing 5 g sodium bicarbonate ($NaHCO_3$), 1 mL formalin (35%) and 25 mg eosin per 100 mL distilled water. An aliquot of this solution was placedin each counting chamber of the haemocytometer and allowed to stand for 5 min to be counted under a light microscope at x200 magnification.

Epididymal alive sperm percent: According to the method by Bearden and Fuquary (1980), a drop of epididymal contents of each rat was mingled with an equal drop of eosin-nigrosin stain and thin film was made on a clean slide. Two hundred sperms were examined per slide with 400 magnification. The average was determined for determining the viability percent.

Sperm motility: The progressive motility of the caudal epididymal sperm was evaluated microscopically as described by Sonmez *et al.* (2005). The content of cauda epididymis was obtained with a pipette and diluted to 2 mL with tris buffer solution. Motility estimates were performed from the three different fields in each sample. The average was used as the final motility score. The percentage of motility was evaluated at x400 magnification.

Sperm abnormalities: Using a light microscope at x400 magnification, a total of 300 sperm was counted on each slide and the percentages of morphologically abnormal spermatozoa were recorded according to Evans and Maxwell (1987).

Testosterone assay: The obtained blood samples were centrifuged for 15 min at 3000 rpm. The sera were separated and were stored at -80°C until testosterone level was measured according to method described by Orr and Mann (1992) using Radio-immunoassay (RIA) kits (Diagnostic Products Cooperation, Los Angeles, California) with a sensitivity of 0.2 ng mL^{-1} and intra-assay coefficient of variation of 12.8%.

Antioxidant enzyme activities and oxidative stress assays: One testis was kept at -70°C then homogenized in cold potassium phosphate buffer (pH 7.4). The testicular homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for determination of Malodialdehyde (MDA) (Ohkawa *et al.*, 1979), Glutathione (GSH) (Sedlak and Lindasy, 1968), Catalase (CAT) (Aebi, 1984) and Superoxide Dismutase (SOD) (Nishikimi *et al.*, 1972) activities using commercial available Colorimetric Assay kits (Bio diagnostic, Egypt) according to the manufacturer guides.

Testicular zinc, cholesterol and testosterone content: Testicular cholesterol and zinc content was determined in testicular homogenate colorimetrically using available commercial kits obtained from (Diamond Diagnostic, Egypt) and (Quimica Clinica Alpicada, SA, Spain) according to Richmond (1973) and Johansen and Eliasson (1987), respectively. Also, testicular testosterone content was assayed by RIA kits as described above.

Light microscopic examination: The other testes was fixed in 10% formalin solution, dehydrated in ascending grades of alcohol and embedded in paraffin (Culling,1983). Sections of 4 μm thickness were taken, stained with Hematoxylin and Eosin (H&E) and examined by light microscopy.

Statistical analysis: The obtained data were expressed as means±standard errors . The significance of differences was calculated by one-way analysis of variance followed by Duncan's multiple range test (SAS, 2001). The difference between means was considered significant when $p<0.05$.

RESULTS AND DISCUSSION

Body and reproductive organs weight: The final body weight and the index weight of the testis, accessory sex glands and the epididymis decreased significantly ($p\leq0.05$) in groups treated with cadmium, cadmium plus pentoxifylline, pentoxifylline pre-cadmium and pentoxifylline post-cadmium treated groups compared to control group. However, this reduction was less declared in the groups treated with cadmium plus pentoxifylline and pentoxifylline post-cadmium (Table 1).

Epididymal sperm characteristics: Epididymal sperm count; alive sperm and motility (%) decreased significantly ($p\leq0.05$) in groups treated with cadmium, cadmium plus pentoxifylline, pentoxifylline pre-cadmium and pentoxifylline post-cadmium treated groups compared to control group , this reduction was less conspicuous in the groups treated with cadmium plus pentoxifylline and pentoxifylline post-cadmium (Table 2). Furthermore, there was a significant increase ($p\leq0.05$) in sperm abnormalities (%) in groups treated with cadmium, cadmium pluspentoxifylline, pentoxifylline pre-cadmium and pentoxifylline post-cadmium treated groups compared to control one. This increase was less marked in pentoxifylline post-cadmium treated group (Table 2).

Table 1: Effect of cadmium and pentoxifylline (PTX) on the body and reproductive organs weight of male rats

	Parameters*				
Groups	Initial body weight (g)	Final body weight (g)	Testes (IW)	Epididymis (IW)	Accessory sex glands (IW)
Control	199.00±0.97^{a}	244.20±2.30^{a}	1.61±0.01^{a}	0.76±0.01^{a}	0.89±0.01^{a}
Cd	199.70±1.54^{a}	199.30±3.02^{b}	1.09±0.01^{b}	0.57±0.02^{b}	0.62±0.01^{b}
PTX+Cd	201.00±1.57^{a}	222.50±2.14^{c}	1.36±0.02^{c}	0.63±0.01^{cd}	0.69±0.01^{c}
PTX pre-Cd	199.80±2.85^{a}	214.50±1.61^{d}	1.12±0.02^{b}	0.62±0.01^{c}	0.63±0.01^{b}
PTX post-Cd	199.80±2.24^{a}	224.20±1.54^{c}	1.31±0.04^{c}	0.66±0.01^{d}	0.74±0.00^{d}

N = 6; *Mean±SE; $^{a-e}$Means in the same column carrying different superscript letters are significantly different ($p \leq 0.05$); IW = Index Weight; Cd = Cadmium; PTX = Pentoxifylline; Control: Rats were intubated daily 0.5 mL saline for for 60 days. Cd: Rats were intubated with saline for 30 days then with 2.5 mg kg^{-1} of $CdCl_2$ BW in 0.5 mL saline for 30 days. PTX+Cd: Rats were intubated with saline for 30 days then with $CdCl_2$ plus pentoxifylline at a dose100 mg kg^{-1} BW 30 days. PTX pre-Cd: rats were intubated daily with pentoxifylline for 30 days then with $CdCl_2$ for 30 days. PTX post-Cd: Rats were intubated with $CdCl_2$ for 30 days then with pentoxifylline for 30 days

Table 2: Effect of cadmium and pentoxifylline (PTX) on the epididymal sperm characteristics in male rats

	Parameters*			
Groups	Sperm count (×10^{6})	Sperm motility (%)	Alive sperm (%)	Sperm abnormalities (%)
Control	336.70±1.89^{a}	93.83±1.08^{a}	91.67±0.88^{a}	6.17±0.48^{a}
Cd	222.50±1.26^{b}	45.50±1.54^{b}	71.17±0.79^{b}	19.83±0.48^{b}
PTX+Cd	269.80±1.58^{c}	80.00±0.73^{c}	78.00±0.52^{c}	11.17±0.60^{c}
PTX pre-Cd	231.00±1.61^{d}	72.67±1.17^{d}	73.50±0.67^{b}	13.00±0.37^{d}
PTX post-Cd	288.00±2.96^{e}	84.50±1.28^{e}	83.67±0.92^{e}	9.67±0.49^{e}

N = 6; *Mean±SE; $^{a-e}$Means in the same column carrying different superscript letters are significantly different ($p \leq 0.05$); IW = Index Weight; Cd = Cadmium; PTX = Pentoxifylline; Control: Rats were intubated daily 0.5 mL saline for for 60 days. Cd: Rats were intubated with saline for 30 days then with 2.5 mg kg^{-1} of $CdCl_2$ BW in 0.5 mL saline for 30 days. PTX+Cd: Rats were intubated with saline for 30 days then with $CdCl_2$plus pentoxifylline at a dose100 mg kg^{-1} BW 30 days. PTX pre-Cd: Rats were intubated daily with pentoxifylline for 30 days then with $CdCl_2$ for 30 days. PTX post-Cd: Rats were intubated with $CdCl_2$ for 30 days then with pentoxifylline for 30 days

Table 3: Effect of cadmium and pentoxifylline (PTX) on the oxidative stress markers in male rats

	Parameters*			
Groups	MDA (μmol g^{-1})	GSH (mg g^{-1})	SOD (μ g^{-1})	CAT (μ g^{-1})
Control	6.06±0.27^{a}	18.25±0.33^{a}	1.24±0.06^{a}	35.99±0.82^{a}
Cd	23.85±0.37^{b}	8.34±0.33^{b}	0.76±0.03^{b}	13.7±0.500^{b}
PTX+Cd	8.45±0.34^{c}	13.93±0.25^{c}	1.05±0.040^{c}	21.50±0.51^{c}
PTX pre-Cd	10.46±0.27^{d}	11.38±0.26^{d}	0.96±0.040^{c}	15.81±0.32^{d}
PTX post-Cd	7.35±0.25^{e}	14.45±0.40^{c}	1.21±0.050^{a}	29.01±0.36^{e}

$^{a-e}$Means in the same column carrying different superscript letters are significantly different ($p \leq 0.05$). N = 6; *Mean±SE; Cd = Cadmium; PTX = Pentoxifylline; Control: Rats were intubated daily 0.5 mL saline for for 60 days. Cd: Rats were intubated with saline for 30 days then with 2.5 mg kg^{-1} of $CdCl_2$ BW in 0.5 mL saline for 30 days. PTX+Cd: Rats were intubated with saline for 30 days then with Cd Cl_2 plus pentoxifylline at a dose100 mg kg^{-1} BW 30 days. PTX pre-Cd: Rats were intubated daily with pentoxifylline for 30 days then with $CdCl_2$ for 30 days. PTX post Cd: Rats were intubated with $CdCl_2$ for 30 days then with pentoxifylline for 30 days. MDA = Malondialdehyde; GSH = Glutathione; CAT = Catalase; SOD = Superoxide Dismutase

Oxidative stress markers: Table 3 shows that MDA increased significantly ($p \leq 0.05$) in cadmium, cadmium plus pentoxifylline, pentoxifylline pre-cadmium and pentoxifylline post-cadmium treated groups compared to control one. The greatest increase was in cadmium treated group. Moreover, there was a significant decrease in GSH, SOD and CAT in cadmium, cadmium plus pentoxifylline, pentoxifylline pre-cadmium and pentoxifylline post-cadmium treated groups compared to control one. The greatest reduction was in cadmium treated group.

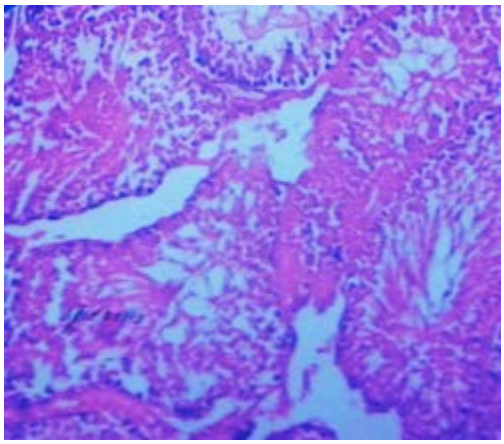

Fig. 1: Testis of control rats showing various developing stages of spermatogenia lining the seminiferous tubules with the presence of interstitial cells. (X200, H&E)

Serum testosterone and testicular testosterone; zinc and cholesterol: Table 4 shows that testicular cholesterol content increased significantly ($p \leq 0.05$) in cadmium, cadmium plus pentoxifylline, pentoxifylline pre-cadmium and pentoxifylline post-cadmium treated groups compared to control one. The greatest increase was in cadmium treated group. Moreover, there was a significant decrease in both of serum testosterone level and testicular zinc and testosterone content in cadmium, cadmium plus

Table 4: Effect of cadmium and pentoxifylline (PTX) on the serum testosterone and testicular zinc; cholesterol and testosterone content in male rats

	Parameters*			
Groups	Serum testosterone (ng mL^{-1})	Zinc (µg mL^{-1})	Cholesterol (mg g^{-1})	Testicular testosterone (ng g^{-1})
Control	2.22±0.13^a	18.5±0.380^a	16.05±0.26^a	9135.70±44.300^a
Cd	0.88±0.03^b	8.78±0.46^b	46.84±1.03^b	705.70±8.3000^b
PTX+Cd	1.24±0.10^c	12.65±0.48^c	19.09±0.39^c	8174.60±80.700^c
PTX pre-Cd	0.98±0.03^b	9.68±0.20^b	36.56±0.62^d	6242.10±206.00^d
PTX post-Cd	1.84±0.04^d	16.42±0.37^d	21.85±0.42^e	8503.50±100.00^e

N = 6; *Mean±SE; $^{a-e}$Means in the same column carrying different superscript letters are significantly different ($p \leq 0.05$); Cd = Cadmium; PTX = Pentoxifylline; Control: Rats were intubated daily 0.5 mL saline for for 60 days. Cd: Rats were intubated with saline for 30 days then with 2.5 mg kg^{-1} of $CdCl_2$ BW in 0.5 mL saline for 30 days. PTX+Cd: Rats were intubated with saline for 30 days then with $CdCl_2$ plus pentoxifylline at a dose100 mg kg^{-1} BW 30 days. PTX pre-Cd: Rats were intubated daily with pentoxifylline for 30 days then with $CdCl_2$ for 30 days. PTX post-Cd: Rats were intubated with $CdCl_2$ for 30 days then with pentoxifylline for 30 days

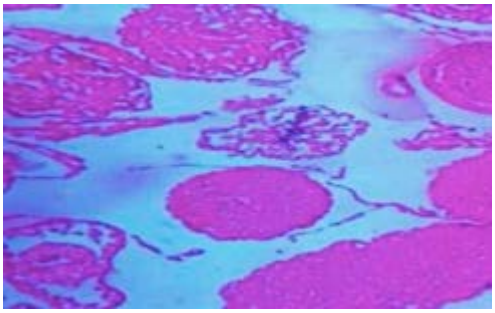

Fig. 2: Testis of cadmium treated rats showing some seminiferous tubules appeared as homogenous and esinophilic mass. Other tubules appeared atrophied and corrugated with separation of the tubules (X200, H&E)

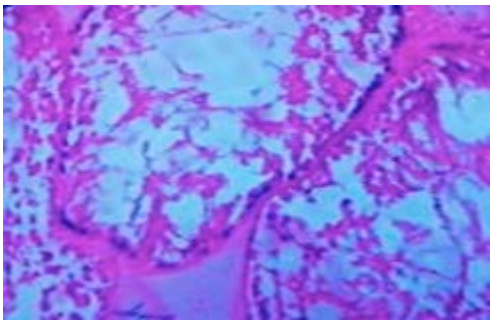

Fig. 3: Testis of cadmium treated rats showing interstitial edema and necrosis of interstitial cells, the seminiferous tubules display degeneration in the lining spermatogenic cells with few mature spermatozoa (X400, H&E)

pentoxifylline, pentoxifylline pre-cadmium and pentoxifylline post-cadmium treated groups compared to control one. The greatest reduction was in cadmium treated group.

Pathological finding: Microscopic examination of the testes of control rats showed well organized seminiferous tubules with various developing stages of spermatogenesis with the presence of intact interstitial cells (Fig. 1). Testicular tissue of rats treated with

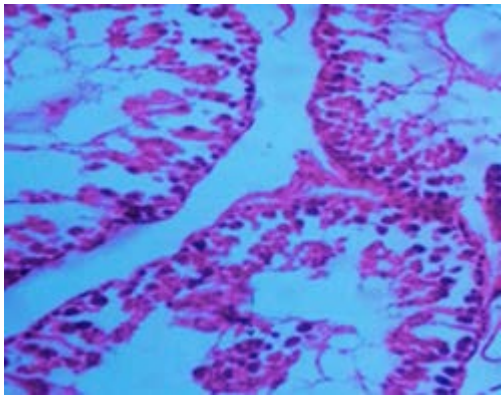

Fig. 4: Testis of cadmium treated rats showing some tubules contained intraluminal collection of degenerated and desquamated spermatogenic cells (X400, H&E)

cadmium revealed that some seminiferous tubules appeared as homogenous and esinophilic mass; the tubules appeared atrophied and corrugated with separation of the tubules (Fig. 2). Other tubules were showing interstitial edema and necrosis of interstitial cells, the seminiferous tubules display degeneration in the lining spermatogenic cells with few mature spermatozoa (Fig. 3). Some tubules contained intraluminal collection of degenerated and desquamated spermatogenic cells (Fig. 4). Cadmium plus pentoxifyllinetreated rat testes showed mild destruction in some areas of the interstitial Connective Tissue (CT) and the lining spermatogenic cells. Other showed improvement in the number of mature sperm (Fig. 5 and 6). Meanwhile, testicular tissue of pentoxifylline pre-cadmium treated rats showed destruction in the interstitial connective tissue associated with separation of the tubules (Fig. 7). Testes of pentoxifylline post-cadmium treated rats showed that there was moderate degenerative change in the interstitial CT with very minimal degenerative change in the lining spermatogenic cells associated with marked presence of mature sperms inside the tubular lumen (Fig. 8).

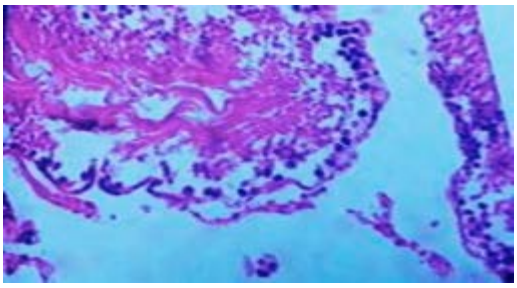

Fig. 5: Testis of cadmium plus pentoxifylline treated rat showing mild destruction in some areas of the interstitial connective tissue and the lining spermatogenic cells. Other showed improvement in the number of mature sperm (X400, H&E)

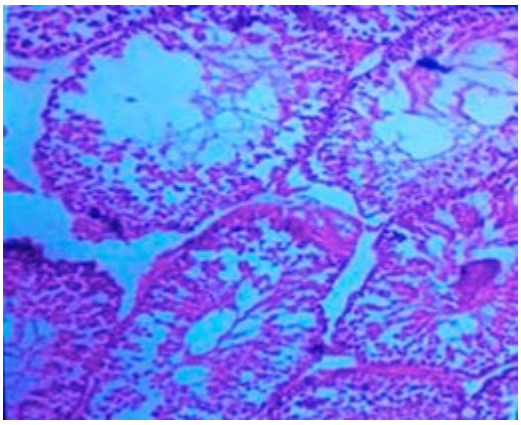

Fig. 6: Testis of cadmium plus pentoxifylline treated rat showing mild to moderate pathological lesions (X200, H&E)

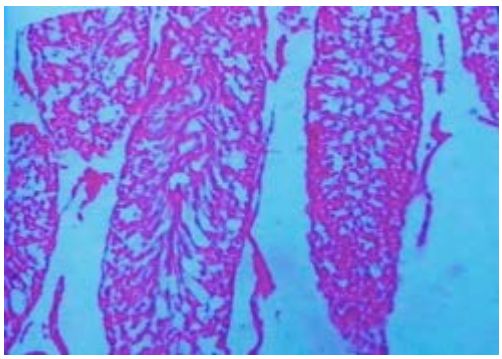

Fig. 7: Testis of pentoxifylline pre cadmium treated rat showing destruction in the interstitial connective tissue associated with separation of the tubules (X200, H&E)

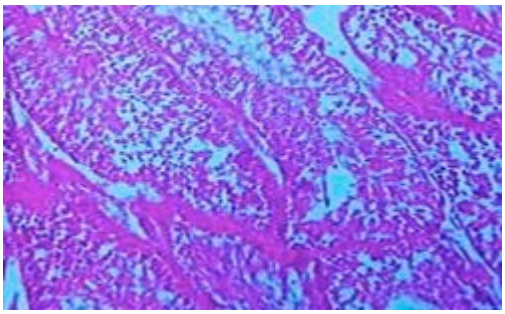

Fig. 8: Testis of pentoxifylline post cadmium treated rat showing very minimal degenerative change in the lining spermatogenic cells associated with marked presence of mature sperms inside the tubular lumen (X200, H&E)

In the present study, the relative weights of the testes, epididymis and accessory glands decreased significantly in the $CdCl_2$ treated rats. Our results agree with the results of El-Demerdash *et al*. (2004), Aziz *et al*. (2007), Zielinska-Psuja *et al*. (1979) and Kamel *et al*. (2011). The reduction in the reproductive organ weights could be attributed to the reduction of the body weight or to decrease in testosterone level. Losing even 10% of body weight is sufficient to cause decrease in the reproductive organs weight (Chapin *et al*., 1993a, b; Ji *et al*., 2010). The reduction in the body w eight can result in both decrease in testosterone production and accessory sex gland weight (Rehm *et al*., 2008). The testes weight is ultimately depending on the cluster of the differentiated spermatogenic cells; the reduction in testicular weight may be attributed to decreased steroidogenic enzyme activity, number of germ cells and inhibition of spermatogenesis (Takahashi and Oishi, 2001). The present investigation showed that cadmium treated rat had inferior sperm quality. Since, the cadmium significantly reduced the alive sperm and abnormalities percent and sperm count, this reduction in the sperm count may be attributed to the adverse effect of cadmium on spermatogenic process. The adverse effect of cadmium on spermatogenesis might be attributed to either the decrease in serum testosterone level or cadmium induced LPO. One of the indicators of the chemical toxicity of the male reproductive system is the reduction of testosterone level (Yoshida *et al*., 2002). Testosterone is a prerequisite for normal spermatogenesis (Holdcraft and Braun, 2004). The reduction in testosterone level in the $CdCl_2$ treated rats may be due to reduction of utilization of cholesterol by the Leydig cells. In the present study, the cholesterol level in $CdCl_2$ treated group increased significantly in comparison to the control group. The high level of cholesterol in the testes of the $CdCl_2$ treated rat might be an indication of decreased production of testosterone by the leydig cells. The stimulated Leydig cells function are

impaired by the high cholesterol level (Shimamoto and Sofikitis, 1998). Many enzymes in the leydig cells are included in the testosterone synthesis from cholesterol e.g., cytochrome P450 17α-hydroxysteroid dehydrogenase ($P450_{17\alpha}$), 17α hydroxysteroid dehydrogenase (17β-HSD) and cytochrome P450 cholesterol side-chain cleavage (P450sec). Cholesterol converted to pregnenolone by P450scc, pregnenolone is catalyzed by $P450_{17\alpha}$ to produce 17-hydroxyprogesterone and androstenedione then androstenedione being converted by 17β-HSD to testosterone (Payne and Youngblood, 1995). Cadmium administration to rats caused significant decrease in testicular 17βHSD activities (Neill *et al*., 2001; Gupta *et al*., 2004a, b; Manna *et al*., 2008). In agreement with the results by Santos *et al*. (2004), Blanco *et al*. (2007) and Ji *et al*. (2010). The results displayed obvious histopathological changes in the form of multifocal areas of ischemic necrosis; edema; widening in the interstitial space by diffused esinophilic fluid infilteration; complete necrosis and sloughing of all layers of seminiferous tubules and there were marked atrophy with complete absence of sperms in cadmium exposed group. Moreover, cadmium administration caused significant decrease in both sperm count and the percentage of live sperm along with significant increase in abnormal sperm percentage when compared with the control. The changes observed are harmonious with the decrease of testosterone levels.

In the present study, there was a significant increase in MDA level in $CdCl_2$ treated rats with a significant decrease in antioxidant components (HSG; SOD and CAT), our results run in line with the results reported that the Lipid Peroxidation (LPO) levels in testes homogenates were significantly increased in Cd treated group than that of control group (Gupta *et al*., 2004a; El-Shahat *et al*., 2009). Moreover, Stajn *et al*. (1997), Patra *et al*. (1999) and Kini *et al*. (2011) found that GSH and SOD were depleted due to cadmium administration while LOP increased in different organs including male sex organs and gave rise to changes in the antioxidant defense system. One of the mechanisms by which Cd induced toxicity is turmoil the balance of the antioixidant and prooxidant ratio through generation of Reactive Oxygen Species (ROS) (Kara *et al*., 2005). Lipid Peroxidation (LPO) level in the tissues is an indicator of oxidative stress (Tandon *et al*., 2003; Alvarez *et al*., 2004). Both acute and chronic cadmium administration is accompanied with high level of LPO in several organs including male sex organs (Gupta *et al*., 2004b; Kara *et al*., 2005). Administration of single dose of cadmium increased LPO while it decreased the GSH in liver (Muller, 1986). Glutathione (GSH) reduction leads to elevation of LPO (Bagchi *et al*., 1996; El-Maraghy *et al*., 2001). Both sperm and testicular cells are liable to oxidative damage by ROS or free radical as the polyunsaturated fatty acids contents in their cellular membrane are very abundant (Alvarez and Storey, 1995), the dysfunction and the structural damage of the cell may be resulted from lipid peroxidation of the cellular membrane. Subsequently, the observed abnormalities in the sperms and in the testicular structures in our study may be refer to the peroxidation of polyunsaturated fatty acids in their plasma membranes by cadmium. Seminal oxidative stress may be involved in sperm Deoxyribonucleic Acid (DNA) damage which in turn may affect the sperm quality (Smith *et al*., 2006). Pentoxifylline can attenuate oxidative metabolism and preserve antioxidant enzyme (Wong *et al*., 2002) co-treatment, pre or post-treatment with PTX was efficient in the prevention of oxidative damage produced by cadmium which resulted in significantly decrease LPO and restored testicular activities of GSH; SOD and CAT; increase testicular weight, increased both sperm count and the percentage of live sperm along with significant decrease in both abnormal and dead sperms percentage and the pathological lesions in the testicular tissues were less severe and with different stages of spermatogenesis when compared with the cadmium treated rats. Pentoxifylline has been shown to enhance sperm motility *in vivo* (Okada *et al*., 1997) and *in vitro* (Yovich, 1993; Kovacic *et al*., 2006; Mirshokraei *et al*., 2001). Pentoxifylline has successively been used as a pretreatment to activate testicular and epididymal sperm motility for Intra Cytoplasmic Sperm Injection (ICSI) (Terriou *et al*., 2000) and to raise bovine fertilization capacity *in vitro* fertilization (Numabe *et al*., 2001). Pentoxifylline inhibits cyclic Adenosine Monophosphate (cAMP) phosphodiesterase thus increasing intracellular cAMP. Cyclic adenosine monophosphate has a role in sperm kinematics. So, treatments that increase intracellular cAMP concentration cause an increase in kinematics and sperm motility (Henkel and Schill, 2003). The differentiation promoting effect of FSH was connected to protection against germ cell apoptosis and that both effects could be mimicked by the intracellular cAMP elevating drug PTX. Their data showed that the *in vitro* effects of supra physiological concentration of FSH on human spermatogenesis were mediated by the classical FSH signal transduction pathway involving cAMP as second messenger. Pentoxifylline may thus be useful as an alternative means for intracellular cAMP elevation in men with high circulating FSH concentrations leading to desensitization of the FSH receptor. The protection rendered by PTX may be due to its antioxidant effects. The anti-inflammatory properties of PTX such as a reduction of LPO associated with the intracellular cAMP

(Bhat and Madyastha, 2000; Chen *et al*., 2002; Maxwell *et al*., 2002) may reduce the severity of pathological lesions induced by cadmium. In the present study, there were significant increase in serum testosterone inpentoxifylline plus cadmium and PTX post-cadmium treatment when compared to cadmium treatment, it was possible that the PTX triggered a mechanism that block the cadmium damage to interstitial cells as evident by histopathological examination.

CONCLUSION

From the present study, it can be concluded that cadmium administration induces biochemical and histopathological changes in the rat testicular tissues. The elevated oxidative stress produced by cadmium intoxication might be responsible, at least in part for histopathological changes. Pentoxifylline had protective influence against Cd toxicity evident by lowering of LPO level and least testicular histopathological alteration in the rat treated with PTX plus cadmium or PTX post-cadmium treatment but not PTX pre-cadmium treatment.

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