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The ameliorative effects of DMSA and some vitamins against toxicity induced by lead in the testes of albino rats. II

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Abstract Lead is a poison that affects virtually every system in the body. The current study was planned to examine the toxic effects of lead acetate on the histological picture of testes, and the protective roles of DMSA, combined vitamins C and E, and DMSA combined with vitamin C plus vitamin E against the histopathological changes in the testes of albino rats induced by lead acetate. Oral administration of lead acetate caused necrosis of spermatogenic cells in the seminiferous tubules, congestion of interstitial blood vessels, severe interstitial edema and complete necrosis in the seminiferous tubules. Co-administration of DMSA with lead acetate minimized the histopathological changes exhibited by lead acetate in the affected organ compared with lead acetate-intoxicated rats. Lead acetate combined with vitamin C plus vitamin E supplemented rats showed mild congestion of the interstitial blood vessels and the seminiferous tubules with its components appeared normal compared to DMSA treated rats. Treatment with DMSA combined with vitamin C plus vitamin E showed more or less normal histological appearance of the testes in lead acetate induced histopathological changes in the affected organ. These results show that DMSA, as a chelating agent for lead, and the combination of vitamins C and E as antioxidants reduced the toxic effects of lead on the histological structure of testes in albino rats but did not provide complete protection. Whereas, the supplementation of DMSA combined with both vitamins C and E provide complete protection against toxicity induced by lead in the testes of albino rats.

Introduction

Lead is known to induce a broad range of physiological, biochemical, histological and behavioral dysfunctions in laboratory animals and humans including nervous system (Flora et al., 2006), kidneys (Rastogi, 2008), liver (Kasten-Jolly

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et al., 2010), male and female reproductive systems (Flora et al., 2011). Its negative impact on reproduction is well known (Alexander et al., 1996; Gandley et al., 1999; Bonde et al., 2002). However, the mechanism of its activity in male reproductive system is not fully understood. Experimental studies showed that lead caused suppression of spermatogenesis in rats depending on the dose-quantity (Sokol, 1989, 1990) and sperm production rate and fertility decreased(Johansson and Wide, 1986). It has been reported that treatment of rats with 10 mg/kg lead acetate once a week for 6 and 9 weeks caused a decrease of sperm counts and of absolute concentration of a motile sperm (Hsu et al., 1997). It has been found that mice receiving 0.25% and 50% lead acetate showed a reduced sperm count and a reduced percentage of motile sperm in the epididymis (Wadi and Ahmad, 1999). Wang et al. (2013) found spermatogenesis distribution including reduced layers of germ cells, distributed germ cell alignment, a decreased number of spermatozoa in the testis of mice exposed to 1.0 or 1.5 g/L of lead acetate. Lead poisoning caused a reduction in epididymal sperm counts and sperm motility in rats treated with 1000 ppm of lead acetate for 28 days (Asadpour et al., 2013).

Makhlouf et al. (2008) reported that after oral administration of lead acetate for 3 months, degenerative changes in seminiferous tubules with irregularity in their basement membranes and lined with one or two layers of small acidophilic cells in rat testis were observed. It has been reported that oral administration of 20 mg/kg b.w. of lead acetate for 42 days caused atrophication of seminiferous tubules, reduction in the number of leydig cells and destruction in the parts of sertoli and leydig cells with pyknotic nucleus in mice testis (Shan et al., 2009). Oral administration of 16 mg/kg b.w. of lead acetate for 21 days changed the arrangement and shape of spermatogonial cells and reduced the number of sertoli cells in mice testis (Garu et al., 2011). It was found that after oral administration of 25 mg/kg b.w. lead acetate for 3 months, loss of normal architecture of the testicular tissue in the form of thin walled seminiferous tubules with wide lumen and vacuolation in the spermatogenic epithelium mostly separating primary spermatocytes from spermatogonia and surrounding nuclei of sertoli cells was noticed. Also apoptotic bodies were found among the basal part of the spermatogenic epithelium (El-Shafai et al., 2011). Oral administration of 15 mg/kg b.w., 20 mg/kg b.w. and 30 mg/kg b.w. lead acetate for 3 months led to degenerative changes of spermatogonia and spermatocytes to advanced degeneration and vacuolation with pyknosis and necrosis of spermatogonia and sertoli cells in rabbit's testis (Ahmed et al., 2012). Oral administration of 1000 ppm of lead acetate for 28 days caused degeneration of seminiferous tubules, germ cells necrosis and vacuolar degeneration especially in secondary spermatocytes in rat testis (Asadpour et al., 2013). Also, oral administration of 6 mg/kg b.w. of lead acetate led to degeneration of interstitial spaces and narrowing of lumen and morphological alternation of sperm cells in rat testis (Isaac et al., 2013).

It has been shown that lead impairs sperm shape and reduces sperm count (Acharya et al., 2003; Ait Hamadouche et al., 2009). However, the mechanism by which lead toxicity leads to impaired sperm counts and morphology is still unclear. It has been reported that lead, an example of heavy metals, is capable of inducing oxidative stress (Tomascik-Cheeseman et al., 2004). Lead, in particular, can accumulate in the reproductive system and has been implicated in the development of oxidative stress via reactive oxygen species (ROS) and lipid peroxidation (Vaziri and Sica, 2004; Marchlewicz et al., 2004; Mishra and Acharya, 2004). In view of this, many investigators have used a variety of antioxidants and chelating agents for lead, including vitamin C (Hsu et al., 1998a; Saad and El-Sayed, 2014), vitamin E (Patra et al., 2011) and DMSA (Flora et al., 1997; Malvezzi et al., 2001) to prevent the occurrence and subdue oxidative stress in tissue. It has been reported that supplementation of vitamin E and/or C reduced sperm ROS generation, prevented loss of sperm motility and oocyte penetration capacity in lead-exposed rats (Hsu et al., 1998b; El-Neweshy and El-Sayed, 2011). The interaction between vitamin C and E jointly might have a more efficient protective action against lead toxicity by protection of lipid structure against peroxidation (Buettner, 1993). Therefore, vitamin C and vitamin E function together to protect membrane lipids from damage (Frei, 1991). Animal studies suggested that DMSA is an effective chelator of lead in soft tissues but it is unable to chelate lead from bone (Flora et al., 1997, 2008). Vitamin C and/or vitamin E alone, or both in combination with conventional chelator was found to decrease lipid peroxide levels of soft organs (Patra et al., 2001).

The aim of the present work, therefore, was to investigate the toxic effect of exposure to lead on the histological picture of albino rat testis and the possible protective effects of DMSA, vitamin C combined with vitamin E and the combination of DMSA with both vitamins C and E on lead induced toxicity in this organ.

Material and methods

A total of 30 male albino rats weighting about 160–180 g, obtained from the nation experimental house in Helwan, Egypt were housed in individual stainless cages at room temperature and exposed to 12 h light/dark cycle. They had access to standard rodent laboratory diet and drinking water *ad libitum* throughout the whole experimental period.

After 2 weeks of long acclimatization period, they were randomly divided into 5 equal groups (containing each 6 animals) according to dietary treatments applied for 6 weeks: rats from group 1 (G1) served as untreated control and were fed with the standard diet and normal drinking water; rats from the other 4 groups were orally treated with lead acetate (100 ppm in drinking water) and animals in groups 3, 4 and 5 were additionally treated with 50 mg/kg b.w. DMSA (G3), 160 mg/kg of vitamin C combined with 50 mg/kg b.w. of vitamin E (G4), and 160 mg/kg b.w. of vitamin C combined with 50 mg/kg b.w. vitamin E and 50 mg/kg b.w. of DMSA (G5) two times/week, respectively. The animals were observed daily for signs of toxicity. At the end of experimental period (on the week 6), animals were given rest overnight and then on the next day, they were sacrificed under light ether anesthesia.

Histopathological studies

Fresh specimens were taken from the testis of each animal and dissected into two pieces and fixed in neutral buffered 10% formalin. The specimens were prepared for histological studies and light microscopic studies using haematoxylin and eosin stains (H&E) (Druy and Wallington, 1980).

Results

Light microscopic examination using H&E

Group I (G1) (control rats), sections of testes from control rats showed the normal structure of the testis. The seminiferous tubules appeared rounded or oval in their outlines, and each surrounded by a thin basement membrane. The spermatogenic cells were seen in regularly arranged rows with different stages of spermatogenesis. They were arranged from the basal compartment to the lumen of the tubule starting from spermatogonia, primary spermatocytes, secondary spermatocytes, rounded and elongated spermatids till mature spermatozoa in the lumen. The seminiferous tubules were surrounded by a thin connective tissue layer with myoid cells (Fig. 1A).

Group II (G2) (lead-intoxicated rats): examination of sections obtained from testes of lead intoxicated rats revealed seminiferous tubules with necrosis of spermatogenic cells and congestion of the blood vessels (Fig. 1B). Severe interstitial edema as well as congestion of interstitial blood vessels were noticed (Fig. 1C) and complete necrosis of seminiferous tubules were seen in some cases (Fig. 1D).

Group III (G3) (lead combined with DMSA treated rats): animals that treated with lead together with DMSA showed mild necrosis of some seminiferous tubules as well as congestion of the interstitial blood vessels (Fig. 2A) and mild interstitial edema as well as congestion of blood vessels (Fig. 2B).

Group IV (G4) (lead combined with both vitamins C and E treated rats): animals that received lead together with vitamins C and E revealed mild congestion of the interstitial blood vessels and the seminiferous tubules appeared approximately normal (Fig. 2C).

Group V (G5) (lead combined with vitamins C, E and DMSA treated rats): animals of this group which were treated with lead together with both vitamins C and E in addition to DMSA showed more or less normal histological appearance of testis. It means that DMSA combined with vitamins C and E has recovered the testis cells from the lead acetate toxicity (Fig. 2D).

Discussion

It is well known that lead exposure induces male reproductive toxicity (Winder, 1989; Batra et al., 2001; Asadpour et al., 2013; Fahim et al., 2013). It has been reported that the accumulation of lead in testis is a substantial basis for resulting in the spermatogenesis and sperm development to be suppressed (Wang et al., 2013). The primary mechanism of the toxic action of lead appears to be a disruption of the hypothalamic control of pituitary hormone secretion and in turn,

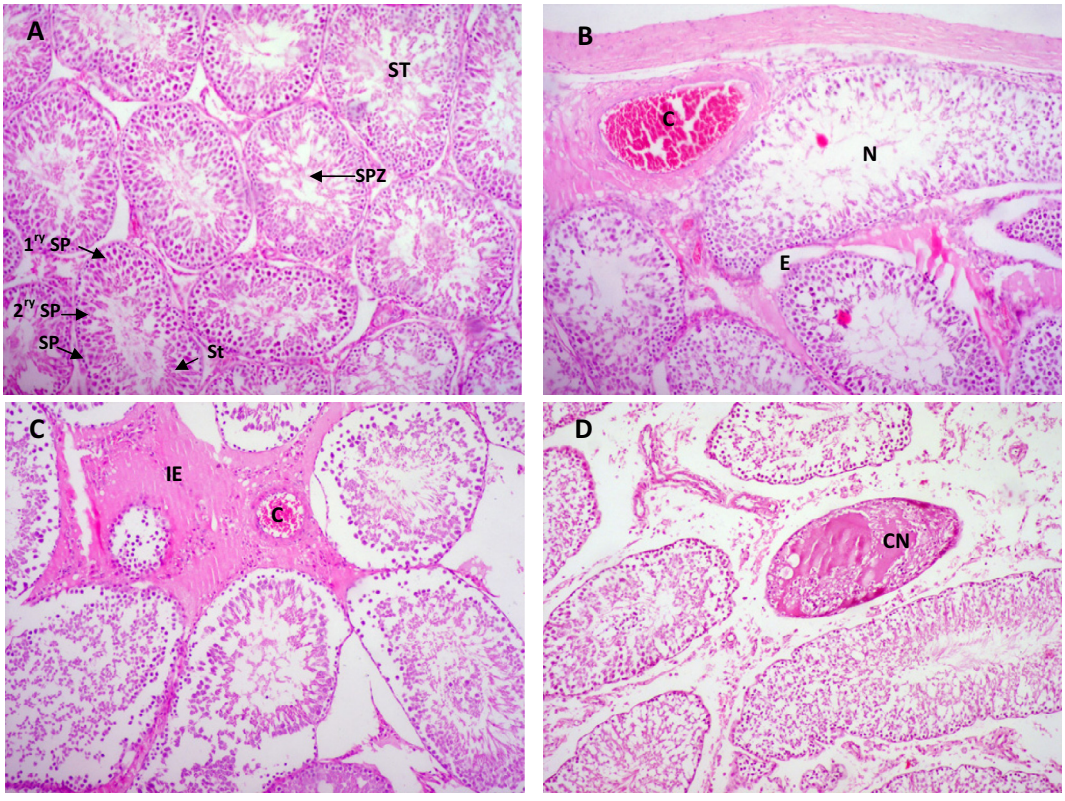

Figure 1 Photomicrograph of the normal control testis (G1) showing (A) seminiferous tubules (ST) arranged as rounded or oval structures, spermatogonia (SP_1), primary spermatocytes (SP_2), secondary spermatocytes (SP_3), spermatids (SP_4) and spermatozoa (SP_5), (B) lead-intoxicated group (G2) necrosis of spermatogenic cells (N) in the seminiferous tubules and congestion of interstitial blood vessels (C) in addition to edema in the interstitial spaces between the seminiferous tubules (E), (C) severe interstitial edema (IE) as well as congestion of interstitial blood vessels (C) and (D) complete necrosis (CN) in the seminiferous tubules. (H&E) (10×).

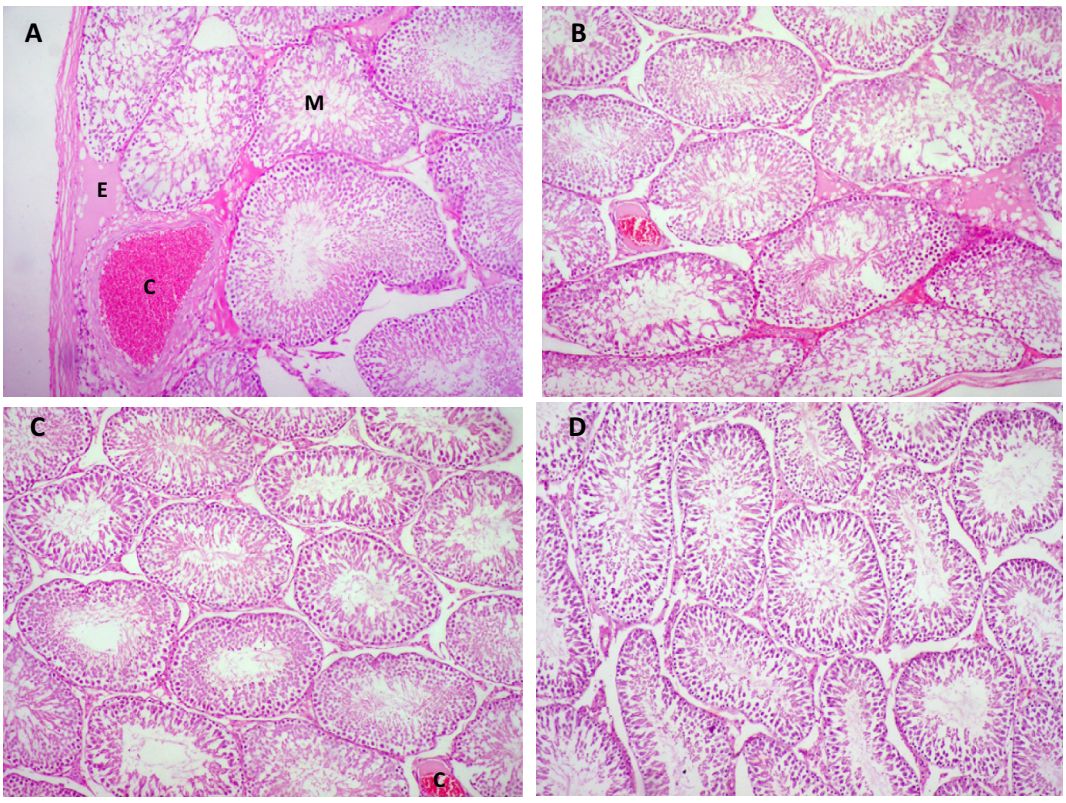

Figure 2 Photomicrograph of the rat testis of group 3 (DMSA treated group) (G3) showing (A) mild necrosis of some tubules (MN) as well as congestion of the interstitial blood vessels (C) associated with edema (E), (B) mild interstitial edema (E) as well as congestion of the blood vessels (C). (C) (lead acetate combined with vitamins C and E) (G4) showing mild congestion of the interstitial blood vessels (C) and (D) (lead acetate combined with DMSA and vitamin C plus vitamin E treated group) (G5) showing more or less normal histological appearance. (H&E) (10×).

spermatogenesis (Sokol, 1987). Since males do not possess accessory reproductive organs, reproductive potential relates to three factors; sperm availability, quality and quantity (Tsuji and Karagatzides, 2001). It was found that disorganization and disruption of spermatogenesis in lumen of seminiferous tubules were noticed in the testis of rats exposed to lead (Batra et al., 2001). Also, it has been reported that cells of seminiferous show signs of degeneration including heterochromic nuclei, irregular basal lamina and vacuolization in albino rats receiving 25 mg/kg b.w. of lead acetate (El-Shafai et al., 2011). It was also reported that exposure to lead acetate reduced sperm density and sperm activity, and increased sperm malformation in mice testis (Wang et al., 2013).

In this study, we evaluated the effect of 100 ppm of lead acetate exposure on male reproductive system in rats. We found that exposure to lead acetate resulted in necrosis of spermatogenic cells in the seminiferous tubules, congestion of blood vessels, severe interstitial edema as well as congestion of interstitial blood vessels and complete necrosis in the seminiferous tubules in some cases. These results are consistent with the above reports and indicated that lead exposure induced toxicity to male reproductive system, especially to spermatogenesis, sperm development and sperm maturation.

It has been shown that lead can cause oxidative stress (Acharya et al., 2003), resulting in increased lipid peroxidation and decreased antioxidant defense mechanism (Bokara et al., 2008; Adegbesan and Adenuga, 2007). In view of the facts that lead acetate exposure has been shown to induce oxidative stress in the testis of rodents (Ercal et al., 1996; Acharya et al., 2003). So, it is valuable to examine whether combined vitamin C with E, as antioxidants, would prevent oxidative stress-induced histological changes in the testicular cells of rat subjected to lead exposure.

Several studies have examined the role of antioxidant on lead-induced oxidative stress. It has been shown that oral administration of vitamin E has partly alleviated lead-induced histological changes in rat testis (Asadpour et al., 2013). Other studies examining the effect of vitamin C on chronic lead toxicity have shown the beneficial effect of vitamin C, antioxidant, on lead-induced changes in the testis of wistar rats (El-Neweshy and El-Sayed, 2011). Also, the supplementation of vitamin E was found to reduce the lead-induced morphological changes in mice testis (Fahim et al., 2013). In lead-exposure rats, supplementation of vitamin E and/or vitamin C reduced sperm ROS generation, prevented loss of sperm motility (Hsu et al., 1998b). However, it has been reported that the interaction between vitamin E and other antioxidant may have a more efficient protective action against lead toxicity. Vitamin E and C jointly protect lipid structures against lipid peroxidation (Buettner, 1993).

The results of this study showed that vitamin C combined with vitamin E is capable of reducing the deleterious impact of lead-induced histopathological changes in the rat testis, in accordance with literature reports. Mishra and Acharya

(2004) reported that the supplementation of vitamin C combined with vitamin E reduced the abnormal sperm population in swiss mice induced by lead. Thus, it can be speculated that vitamin E alone or in combination with vitamin C, as in this study, may decrease the lead-induced lipid peroxidation in the testis of rat.

It has been reported that DMSA is a potent thiol-chelating agent in reducing lead concentration in soft tissues and blood (Flora et al., 2008). However, the available literature does not provide any information on the role of DMSA in lead-induced testicular injury in experimental animals. But, for example it has been stated that vitamin C and/or E alone or in combination with conventional chelator, $CaNa_2$ EDTA, was found to decrease the lead induced lipid peroxide levels of liver and brain in rat (Patra et al., 2001). The observation of this study showed that DMSA alone does not completely remove the toxic effect of lead on the rat testis but leads to mild necrosis of some seminiferous tubules, mild congestion of interstitial blood vessels and mild interstitial edema, whereas, the interaction between DMSA and vitamin C and E jointly completely removed the toxic effect of lead on the testis of rat.

In conclusion, lead in its acetate form caused degenerative changes in spermatogenesis, seminiferous tubules and the interstitial blood vessels. These morphological alternations may explain on the basis that lead exposure enhances reactive oxygen species (ROS) and lipid peroxidation which may lead to tissue damage (Vaziri and Sica, 2004). Our results, also show that the combination between DMSA, as a chelating agent, and both vitamin E and C, as antioxidants, has more beneficial effect in removing the toxic effect of lead on the testis in rats than that of DMSA alone and of interaction between vitamin C and E. So, it can be concluded that the combination between DMSA and both vitamins C and E can remove the impact of lead toxicity in male genital organs.

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