In the present study, the effects of lead acetate were studied on male reproductive health in rats at two time selected points viz., rats at pubertal age and rats that were exposed to lead during perinatal period (embryonic development and lactation).

Exposure to lead acetate during perinatal/pubertal period did not affect the body weights. The weights of liver, kidneys and brain were also not significantly changed in rats exposed to lead acetate during perinatal and pubertal period when compared to their respective control rats indicating that the metabolic activity in these organs is not affected. No significant differences in the weights of accessory sex organs such as seminal vesicles, prostate gland and vas deferens were observed in rats exposed to lead acetate during perinatal/pubertal period, suggesting that these reproductive organs are not the targets of lead acetate at doses 0.05 and 0.15%. It is well known that lead is a neurotoxic chemical, and exposure to lead causes many behavioral abnormalities like wall rearings, aggressiveness, and crossings in animal models (Khaled Kahloula et al., 2009). However, in the present study, no such abnormal behavioral changes were observed in the experimental rats indicating that the selected doses of lead acetate (0.05 or 0.15%) may not be effective to impair brain functions.

Rats exposed to lead acetate either at perinatal and/or pubertal period showed a decrease in the weights of the testis and epididymis, when compared to their corresponding group of control animals. Studies of Wang et al. (2008) and Smith et al. (2008) also report that exposure to lead acetate decreases the weights of testes and epididymis in male rats. Whereas administration of testosterone into rats exposed to lead during perinatal/pubertal period increases the weights of testis and epididymis when compared to lead exposed respective control rats. The weight of the testis is largely dependent on the mass of differentiated spermatogenic cells and it has been used as a measure of spermatogenesis. Since, a strong correlation exists between weight of the testis and number of germ cells (Sinha Hikkim et al., 1989), the reduction in the testicular weight indicates possibility of germinal loss in the lead exposed rats. From the data it is evident that testis and epididymis are the vulnerable targets of lead toxicity, irrespective of the time points selected and the changes are temporary, since administration of testosterone restores the weights of both testis and epididymis.
In the present study, sperm count, sperm motility, sperm viability and sperm functional test were performed to assess the quality and functional status of sperm, which play an important role in male fertility determination. Significant decrease in sperm count, sperm motility, sperm viability and sperm membrane integrity (HOS test) was observed in rats exposed to lead acetate during perinatal/pubertal period when compared to the control rats. Whereas administration of testosterone partially restored the sperm parameters in rats exposed to lead acetate during perinatal/pubertal period when compared to its corresponding control animals. The decreased testosterone levels might be responsible for the observed sperm reduction in experimental animals.

The activities of 3β-HSD and 17β-HSD decreased significantly in the testis of rats exposed to lead during perinatal and/or pubertal period, when compared with their corresponding controls. The decreased steroidogenic enzyme activity indicates decreased steroidogenesis. The decreased steroidogenesis is known to affect spermatogenesis and reproductive activities in rats. Consistent with our results, Biswas and Ghosh (2004) demonstrated decreased activity levels of 3β- and 17β-hydroxysteroid dehydrogenases, in the testis of rats exposed to lead.

Testosterone administration has significantly improved the sperm quality and density and also a significant increase in the steroidogenic enzyme activities in lead intoxicated rats. One plausible mechanism might be due to restoration of testicular architecture in testosterone supplemented rats, which in turn maintains the germ cell numbers and also sustains the Leydig functions, since it is well known that male hormone plays a key role in maintaining the structural and functional integrity of testis (Bain, 2010).

Oxidative stress plays a key role in many pathophysiological disorders, including disorders of male reproductive health. A significant increase in lipid peroxidation in the testis of rats exposed to lead acetate during perinatal/pubertal period has been observed when compared to the control rats. It has been reported that lead affects the testicular functions like steroidogenesis and spermatogenesis by inducing oxidative stress (Ait Hamadouche et al., 2009). A significant decrease in the levels of catalase and SOD in the testis is observed in rats exposed to lead acetate during perinatal/pubertal period when compared to the control rats.
Testosterone plays a major role in pro-and antioxidant balance in the reproductive tissues (Alvarez et al., 2007). Earlier, it has also reported that testosterone administration increases the catalase activity in the testis (Peltola et al., 1996) signifying the role of testosterone in antioxidant defense mechanism. Testosterone supplementation in lead exposed rats showed a significant increase in the activity levels of catalase and SOD in the testis. The lipid peroxidation levels in the testosterone injected lead exposed rats are significantly decreased over their respective control animals.

The deposition of lead in the testis is consistent with the sensitivity of the reproductive system to lead toxicity. Earlier it has been reported that exposure to lead during adulthood (Rader et al., 1981) results in accumulation of lead in the blood and soft tissues. The results of the present study also demonstrates that exposure to lead acetate at 0.05 or 0.15 % levels during perinatal and pubertal period results in significant accumulation of lead in the testis of rats. The bioaccumulation of lead in testis of testosterone injected lead exposed rats did not show any significant change from that of lead exposed rats indicating that testosterone does not chelate the lead levels in the testis of rats.

Histological observations of the transverse section of testis of the control rats have showed that seminiferous tubules contain all stages of spermatogenesis and interstitial cells. The transverse section of testis of lead exposed rats have showed a decrease in the number of spermatocytes, spermatids and sperms in the lumen of seminiferous tubules and the interstitial tissue contains clusters of leydig cells. Administration of testosterone restores the structural integrity of testes in rats exposed to lead during perinatal/pubertal period.

Fertility tests are one set of reproductive endpoints which give valuable information regarding reproductive efficiency of male/female rats. Perinatal and pubertal lead exposed rats have showed a reduction in reproductive performance to sire offspring in a fixed time period. Number of implantations and number of live fetuses are significantly reduced, whereas the number of copulation trials, pre-implantation loss and post-implantation loss are increased in rats cohabited with lead exposed rats. The reduced reproductive performance in the lead-exposed groups may be due to reduced testosterone levels. In addition, the increase in non-motile sperm and decrease in epididymal sperm numbers and viable sperm noticed in lead acetate
exposed rats might be the reason for the decrease in fertility. Decreased sperm motility, viability and altered sperm membrane integrity in rats exposed to lead acetate during perinatal and pubertal period might be responsible for the decreased male fertility.

Decrease in number of mating trials and copulation trails by testosterone injected lead exposed rats when compared with its corresponding controls indicates significant improvement in reproductive activity after testosterone administration. Pre-implantation loss and post-implantation loss are decreased in rats mated with testosterone treated lead exposed rats indicating testosterone ameliorates the lead-induced suppressed fertility in rats.

From the results, it is evident that exposure to lead during perinatal/pubertal period reduced male reproductive performance in rats. This might be due to decreased steroidogenesis and spermatogenesis. The decreased serum testosterone levels might be responsible for the decreased sperm density and poor sperm quality in rats. The inferior sperm quality and low sperm density might be responsible for suppressed reproductive performance in the male rats. Furthermore, the findings of the present study also provide evidence that testosterone treatment ameliorates most of the male reproductive abnormalities caused by the lead exposure; however, it was partial when compared to control rats.
Conclusions

1. Exposure to lead acetate during pubertal and perinatal period decreases the weights of testis, indicating that testis is the prime target for lead toxicity. In addition, the weights of epididymis also decreased in rats exposed to lead acetate during pubertal period suggesting that epididymis is also the target for lead toxicity. Supplementation of testosterone increases the weights of reproductive organs in lead acetate exposed rats. This suggests that lead induced decrease in testis and epididymis weights can be reversible upon testosterone treatment.

2. Exposure to lead acetate during pubertal and perinatal period decreases the selected sperm parameters like sperm count, motility, viability and HOS tail coiled sperms, indicating spermatotoxic effects of lead. Testosterone supplementation showed partial restoration of sperm count and sperm quality in pubertal rats. Whereas in perinatal rats, administration of testosterone though showed partial restoration, at 0.15% lead acetate, the restoration of sperm parameters was marginal when compared to control rats. This also suggests that the programming of the Sertoli cells might be started shaping early during fetal period.

3. Lead acetate exposure during pubertal and perinatal period decreases the activity levels of testicular steriodogenic enzymes like 3β-HSD and 17β-HSD confirming the possible derangements in testicular steriodogenesis, whereas, administration of testosterone increases the activity levels of testicular steriodogenic enzymes like 3β-HSD and 17β-HSD.

4. Lead acetate exposure during pubertal and perinatal period increases the lipid peroxidation levels with a significant decrease in the antioxidant enzymes activities in the testes, indicating that the testis is under oxidative stress, whereas, administration of testosterone decreases the lipid peroxidation levels in the testes of lead acetate exposed rats.

5. The number of mating trails increased with a decrease in mating index, number of implantations and live pups, increases the pre and post implantation loss in rats cohabited with lead exposed rats. On the other hand, administration of testosterone into lead exposed rats partially restored all these parameters.

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6. Many histological alterations are observed in the testis of rats exposed to lead acetate during pubertal and/or perinatal period, which includes decrease in the number of spermatocytes, spermatids and empty lumen devoid of sperms, whereas, administration of testosterone into lead acetate exposed rats partially restores the architecture of testis.

7. It can be concluded that supplementation of testosterone to rats exposed to lead acetate (0.05% and 0.15%), partially restored almost all reproductive variables assessed. Hence testosterone can be used to ameliorate lead-induced reproductive toxicity in rats.