RECOVERY OF MALE REPRODUCTIVE HEALTH IN CYPERMETHRIN EXPOSED RATS BY TESTOSTERONE

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Abstract

The present study was designed to evaluate the anti androgenic activity of cypermethrin in adult male albino rats. Animals were randomly divided into three groups. Rats in group I served as control, Rats in group II were administered orally with 50 mg/Kg body weight of cypermethrin over a period of 60 days, whereas animals in group III were maintained on same experimental regimen as that of rats in group II but in addition they received intra-peritoneal injection of testosterone at a dose of 4.16 mg/kg body weight on days 1, 7 and 14. At 61st day, all the rats from control and experimental groups were sacrificed and analyzed for the reproductive endpoints such as serum hormone levels like testosterone, FSH and LH levels and testicular histological changes. A significant reduction in the serum testosterone levels associated with a significant increase in the FSH and LH levels in the serum were noted in cypermethrin exposed rats as compared to controls. The testicular architecture in cypermethrin exposed rats showed disrupted epithelial membrane with loss of seminiferous tubules and lumen with reduced spermatozoa in rats. Conversely, supplementation of testosterone reduced the circulatory levels of LH and FSH with a significant increase in the serum testosterone levels in testosterone plus cypermethrin exposed rats over cypermtehrin alone exposed rats. Testosterone supplementation restored the testicular architecture as evidenced by reduction in the degeneration of epithelial membrane with lumen occupied by spermatozoa. To conclude, anti androgenic activity of cypermerthin leads to disruption of testicular architecture and decreased the hormonal levels, whereas testosterone supplementation reverse cypermethrin-induced deterioration of male reproductive health in rats.

Keywords: Cypermethrin, testes, spermatogeensis, histology, testosterone, FSH, LH.

INTRODUCTION

Cypermethrin (CP) [(R,S)-a-cyano-3-phenoxybenzyl (1R,S)- cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane] is one of the synthetic (II) pyrethroid insecticides that resemble pyrethrums structurally (Adelsbach and Tjeerdema, 2003). Like other pyrethroids, CP is not target specific and its exposure causes several harmful effects in humans and wildlife including reproductive disorders (Rignell-Hydbom *et al*., 2004; De Jager et al., 2006; Carbone *et al*., 2007; Mocarelli *et al*., 2008). Animal studies indicated that exposure to CP leads to neurotoxicity, immunotoxicity and genotoxicity and reproductive toxicity (Mani *et al*., 2002; Wang *et al*., 2010; Noaishi *et al*., 2013). Earlier studies reported that exposure of rodents to CP negatively affect sperm quality and quantity and also disrupts testicular testosterone production (Solati *et al*., 2010; Luccio-Camelo and Prins, 2011; Fang *et al*., 2013; Hu *et al*., 2013; Sahaar *et al*., 2016). It has been shown that CP can able to modulate testicular pro- and anti-oxidant status thereby provokes oxidative stress in rats (Sharma *et al*., 2014). Thus, it seems apparent that CP induced male reproductive toxicity at least in part mediate inhibition of testosterone biosynthesis and oxidative stress.

Androgens are one of the key factors in the regulation of spermatogenesis and sperm maturation events in male fertility (Wang *et al*., 2009). Thus, chemicals which interfere with testosterone synthesis or its actions and/or both adversely affect the fertility efficacy in males. Synthetic pyrethroids can able to interact with the intrinsic endocrine system thereby acts as endocrine

disruptors and linked to impairment of reproductive and developmental aspects (Han *et al*., 2008). Previously, it has been shown that CP can able to interact with androgen receptor *in vitro* and thus possess anti-androgenic effects (Xu *et al.,* 2008). Testosterone synthesis (testicular Leydig cells) and spermatogenesis (testicular Sertoli cells) are regulated by pituitary gonadotropins, leutinizing hormone (LH) and follicle stimulating hormone (FSH), respectively. Moreover, the feed-back mechanisms induced by testosterone are believed to crucial for the regulation of pituitary gonadotropins. Thus, it is conceivable that CP may target pituitary gonadotropins thereby affect testosterone synthesis. However, studies related to the effect of CP on the changes in the circulatory levels of FSH, LH and testosterone is not clear.

In view of the above, the present study was aimed to investigate the effect of CP on circulatory FSH, LH and testosterone levels in rats. In addition we also determined whether the supplementation of testosterone ameliorates CP-induced changes in the selected hormones.

MATERIALS AND METHODS

Chemicals

Cypermethrin PESTANAL® (CAS-No: 67375-30-8, with 99% purity by HPLC) purchased from Sigma-Aldrich (St.Louis, Missouri, USA). The other reagents were used in the experiments of analytical grade and purchased from local standard commercial firms.

Maintenance of animals

Adult albino male rats of (70 days old, weighing150 ±10g) Wistar strain was purchased from an authorized vendor (M/S Sri Venkateswara Enterprises, Bengaluru, India). Rats were maintained (six/cage) in sterilized polypropylene cages with following sizes: 18" x 10" x 8". Paddy husk was used as the bedding material for rats. The animals were acclimatized to the laboratory conditions over a period of 10 days. The laboratory conditions were as follows: temperature: 25 ± 2^0 C; 12 h light/12 h dark cycle and the relative humidity 50 ± 5%. The rats were fed with standard rodent chow and provided water *ad libitum* during acclimatization and the experimental period. We strictly adhered to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India (CPCSEA, 2003) with respect to the experimental procedures. The experiments performed were also approved by the Institutional Animal Ethical Committee (Reg. No. 1677/ PO/Re/S/2012/ CPCSEA dt. 21.12.2015).

Experimental Design

Healthy rats were divided into three groups with six animals per group. Animals in group I was considered as control group. Animals in this group did not receive any treatment. Animals in groups II and III were considered as experimental groups. Animals in group II were exposed to cypermethrin at a dose of 50mg/Kg body weight. Whereas rats in group III were exposed to both cypermethrin at a dose of 50mg/Kg body weight and intra-peritoneal injection of testosterone at dose of 4.16 mg/kg body wt. on 1, 7 and 14 days.

Necropsy

The rats from control and experimental groups were fasted overnight, weighed and humanely euthanized via cervical dislocation. Blood was drawn immediately via cardiac puncture using a heparinized syringe and serum was separated by centrifugation at 2000g for 15 min after overnight storage at 4 ^oC. Serum was stored at - 20^0 C until further hormone analysis.

Measurement of Serum hormone levels

The levels of serum hormones such as FSH, LH and testosterone in both control and experimental rats were measured by using a commercial kit [Master CLIA (Chemi Luminiscent Immunosorbent assay) vast enabled kit]. Each sample was run in duplicate. The intra- and inter-assay coefficients of variation were performed and found to be less than 10% for these assays. The estimated sensitivity of these hormone assays in this method is about 100 pg/ml.

Histoarchitecture of Testis

Testis was fixed in the Bouin's solution for 24 h followed by dehydration step using ascending concentrations of alcohol. After clearing in xylol, the specimens were embedded in the paraffin wax. Thin sections were chopped off (5µm thickness) and stained with hematoxylin and eosin Y (Bancraft and Stevens, 1982). The histological changes of the testis were observed using Olympus microscope (Model No:CX41, Olympus; Japan) with scale bar = 25 µm.

Statistical analysis

In the present study, the values were represented as mean ± SD of six rats. The data was analyzed statistically [SPSS Software (version 16.0; Chicago, Illinois, USA)] using one-way analysis of variance (ANNOVA) followed by post-hoc test, Dunnett's multiple comparison test. The differences between the groups were considered significant at $p < 0.05$.

RESULTS

Serum hormone levels

The circulatory levels of testosterone was significantly ($p < 0.05$) decreased in CP administered rats as compared to control rats (Fig 1). Concomitantly, the levels of FSH and LH were significantly ($p < 0.05$) increased in CP treated rats over controls (Fig 2&3). Conversely, injection of testosterone significantly increased the levels of testosterone with a significant reduction in the circulatory levels of FSH and LH in CP and testosterone administered rats over controls.

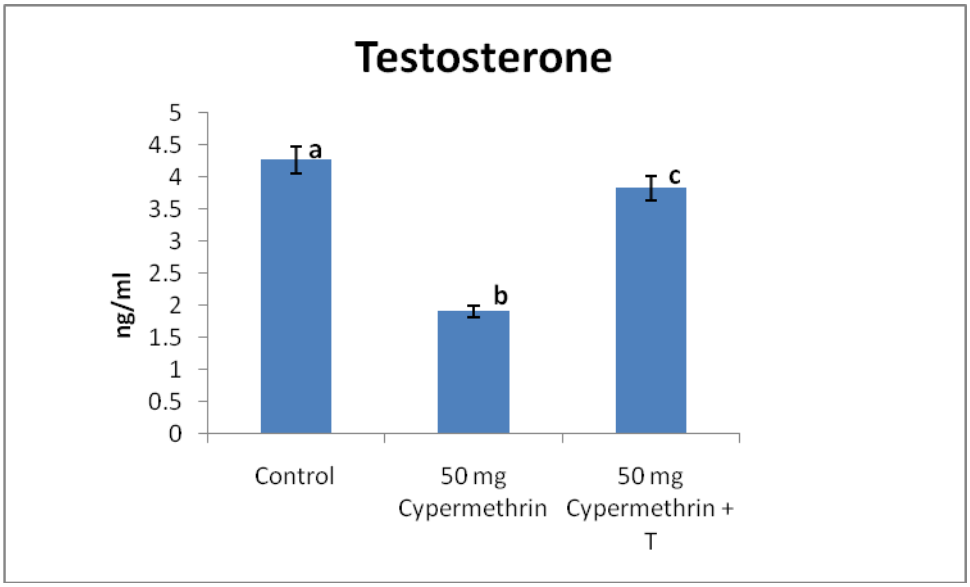


Fig. 1: The levels of serum testosterone in the testes of control and experimental rats. Bars represented as mean ± SD of 6 rats. Bars denoted with different letters are significantly different between different groups ($p < 0.05$).

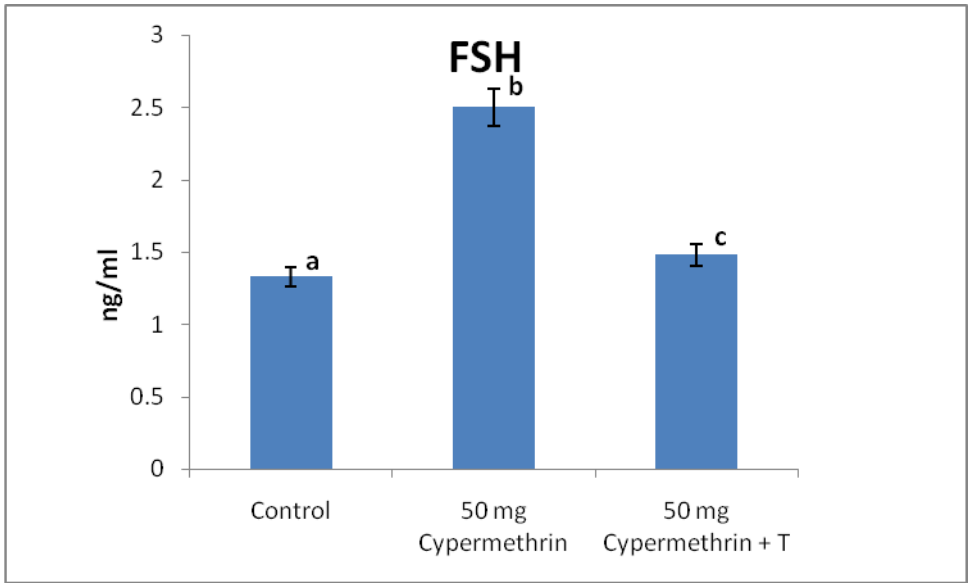


Fig. 2: The levels of serum FSH in the testes of control and experimental rats. Bars represented as mean ± SD of 6 rats. Bars denoted with different are significantly different between different groups ($p < 0.05$).

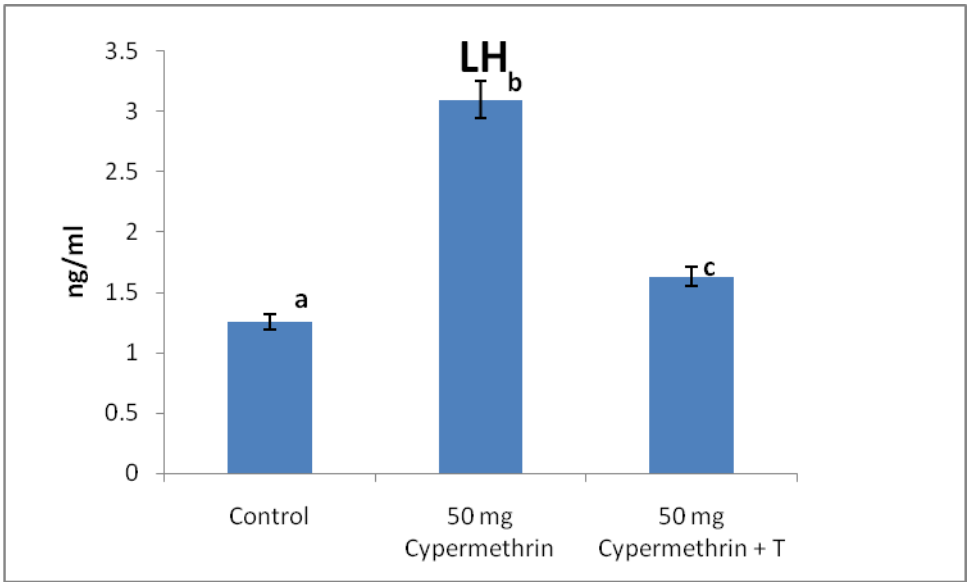
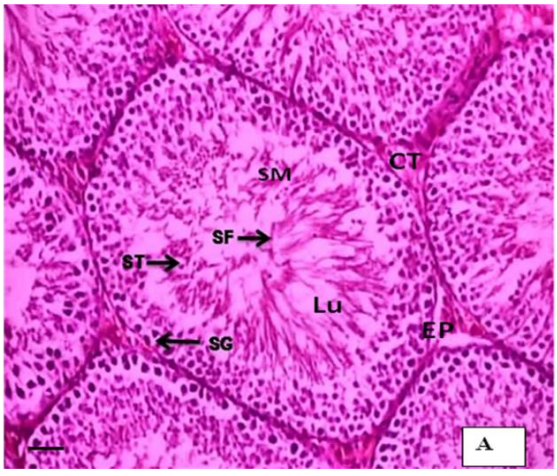
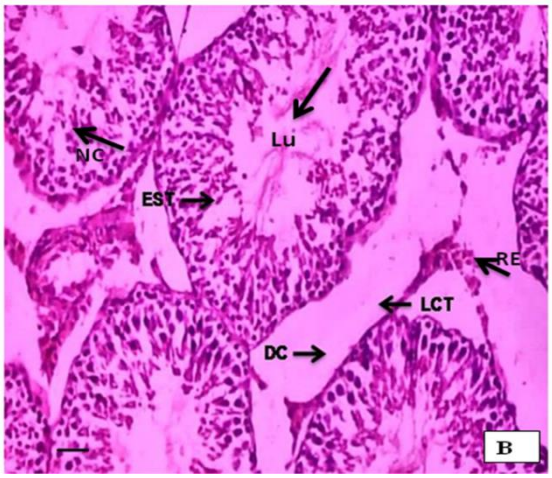


Fig. 3: The levels of serum LH in the testes of control and experimental rats. Bars represented as mean ± SD of 6 rats. Bars denoted with different letters are significantly different between different groups ($p < 0.05$).

Histological studies

Histological features of testis of control rats showed intact epithelial membrane with organized seminiferous tubules in uniform size and shape and lumen filled with spermatozoa (Fig. 4A). In contrast, significant reduction in the size of tubules associated with elongated seminiferous tubules, widening of lumen, ruptured epithelium with necrosis and reduced number of germ cells were noticed in Cypermethrin exposed rats (Fig. 4B). On the other hand, the transverse section of testis in Cypermethrin and testosterone injected rats showed restoration of ruptured epithelium and increased number of germ cells with organized seminiferous tubules (Fig. 4C).





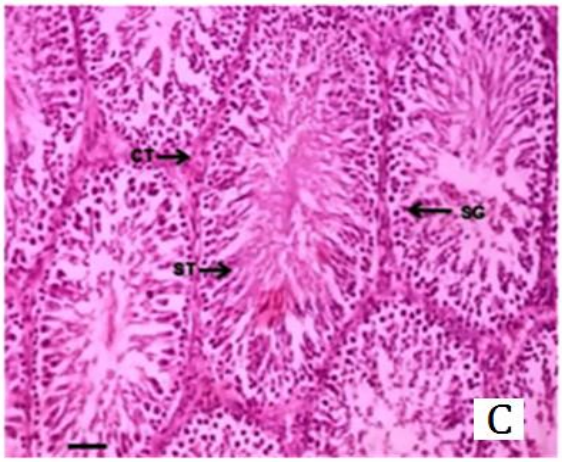


Fig.4: Photomicrographs of testicular architecture of control rats (A) and rats treated with cypermethrin 50mg/Kg.B.Wt (B) and cypermethrin + testosterone (C). Lu=lumen; EP=epithelium; SM=sperm.; SF = Sperm Flagella; SG=Spermatogonia; ST=Semeniferous Tubule; EST = Elongated Seminiferous Tubules; NC = Necrosis; LCT= Loss of Connective Tissue; DC = Degenarative changes; RE = Ruptured epithelium. Scale bar=25 μm.

DISCUSSION

It is well known that testosterone plays a key role in the regulation of structural and functional integrity of male reproductive organs. In the present study, the deterioration of structural integrity of testis in CP administered rats could be linked to the inadequate levels of androgen supply. We also found a significant decrease in the serum testosterone levels in CP administered rats. Previously, it has been shown that exposure of rats to CP deteriorates the testicular architecture associated with reduced spermatogenesis, and testicular steroidogenesis (Hu et al., 2013). In the present study, rats administered with CP caused seminiferous tubule damage associated with reduction and deformation of spermatogonia and spermatocytes. Similar structural deformities were noted by Hu et al., 2013 in male rats exposed to CP over a period of 15 days. Further, these authors demonstrated that CP induced structural changes include swollen mitochondria, widened endoplasmic reticulum and abnormal golgi complex morphology (Hu et al., 2013). Other studies also reported that disruption of testicular structural integrity in CP exposed rats (Li et al., 2013; Sharma et al., 2014; Fang et al., 2014). Androgens are critical for structural and functional aspects of male reproductive organs. In this study, decreased testosterone levels associated with structural deformity of testis in CP exposed rats may support this notion. Testosterone levels are also regulated by hypothalamo-pituitary hormones. In the present study, a significant decrease in the testosterone levels with a significant increase in the serum FSH and LH levels could be disturbances in the feed-back mechanism in CP administered rats. The present results are in agreement with previous studies (Hayes et al., 2001; Hu et al., 2013; Sahar et al., 2016).

One of the important findings of this study revealed that testosterone supplementation restored the structural integrity of testis and also increased the testosterone levels in CP administered rats. Steroidogenesis is a process through which the testosterone biosynthesis occurs in testicular Leydig cells. A significant reduction in the serum testosterone levels might reflect CP-induced Leydig cell toxicity in rats (Hu et al., 2013). On the other hand, supplementation of testosterone recovered the structural integrity of testis which in turn might be responsible for the restoration of serum testosterone levels in CP exposed rats. Accordingly, we noticed restoration of testicular architecture with lumen occupied by spermatozoa. Previously, administration often testosterone restored male reproductive functions including spermatogenesis and sperm maturation events in lead exposed rats (Reshma and Reddy, 2015). Further, a reduction in the levels of serum FSH and LH in testosterone and CP administered rats as compared to CP alone administered rats might be recovered the mechanism of pituitary axis by exogenous testosterone.

CONCLUSION

From the results we conclude that administration of CP to male rats deteriorates structural integrity of testis, disrupts pituitary-testicular axis thereby affects serum FSH, LH and testosterone levels. On the other hand, supplementation of testosterone ameliorates the testicular architecture and reduced the serum FSH and LH levels in testosterone and CP administered rats. Thus, we suggest that testosterone could be used as one of the therapeutic agents against CP-induced deterioration of male reproductive functions. Further, analysis of sperm quality and quantity and also assessing the male fertility variable in testosterone plus CP exposed rats is warranted.

Acknowledgements

We are thankful to Head, Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam, (Women's University), Tirupati for providing laboratory facilities. Authors also thankful to DST-CURIE, Central Instrumentation Facility, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati, for permitting to utilize Phase contrast microscope for taking photographs.

References

1. Adelsbach, T.L. and Tjeerdema, R.S. 2003.Chemistry and fate of fenvalerate and esfenvalerate. Reviews of Environmental Contamination and Toxicology, 176:137-154.

2. Rignell-Hydbom, A., Rylander, L., Giwercman, A., Jönsson, B.A., Nilsson-Ehle, P. and Hagmar, L. 2004. Exposure to CB-153 and p,p'-DDE and male reproductive function. Human Reproduction, 19:2066-75.

3. De Jager, C., Farias, P., Hernandez Avilla M., Barraza, A., Diaz Sanchez, V., Cerezo, G., Ayotte, P., Dewailly, É. and Bailey, J.L. 2006. Reduced Seminal Parameters Associated with Environmental DDT Exposure in Men from Chiapas, Mexico. Journal of Andrology, 27(1):16-27.

4. Carbone, P., Giordano, F., Nori, F., Mantovani, A., Taruscio, D., Lauria, L. and Figa-Talamanca, I. 2007. The possible role of endocrine disrupting chemicals in the aetiology of cryptorchidism and hypospadias: a population-based case-control study in rural Sicily. International Journal of. Andrology, 30(1): 3-13.

5. Mocarelli P, Gerthoux, P.M., Patterson, D.G., Milani, S., Limonta, G., Bertona, M., Signorini, S., Tramacere, P., Colombo, L., Crespi, C., Brambilla, P., Sarto, C., Carreri, V., Sampson, E., Turner, W. and Needham, L. 2008. Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. Environmental Health Perspective, 116:70–77.

6. Mani, C., Freeman, S., Nelson, D.O. et al. 1999. Species and strain comparisons in the macromolecular binding of extremely low doses of [14C] benzene in rodents, using accelerator mass spectrometry. Toxicology and Applied Pharmacology, 159 (2):83-90.

7. Wang, H., Wang, Q., Zhao, X.F., Liu, P., Meng, X.H., Yu, T., Ji, Y.L, Zhang, H., Zhang, C., Zhang, Y. and Xu, D.X. 2010. cypermethrin exposure during puberty disrupts testosterone synthesis via down regulation StAR in mouse testes. Archives of Toxicology, 84: 53-61.
8. Noaishi, M.A., Allah, A.A. and Afify, M.M. 2013. Oral and dermal exposure of chlorpyrifos and cypermethrin mixture induced cytogenetic, histopathological damage and oxidative stress in rats. Journal of American Science, 9:56–65.

9. Jalal Solati, Ramin Hajikhani, Roohollah Toodeh Zaeim. 2010. Effects of Cypermethrin on Sexual Behaviour and Plasma Concentrations of Pituitary-Gonadal Hormones. International Journal Of Fertility And Sterility, 4 (1): 23-28.

10. Luccio-Camelo, D.C. and Prins, G.S. 2011. Disruption of androgen receptor signaling in males by environmental chemicals. Journal of steroid Biochemistry and Molecular Biology, 127: 74–82.

11. Fang, L.Y. Chen, P.. HuJ-Jing, L.I. and Chun, X.L. 2013. Effects of Cypermethrin on Male Reproductive System in Adult Rats. Biomedical and Environmental Sciences, 26(3): 201-208.

12. Sahar, M. El-Sheshtawy, Lobna, S. El- Gebaly , Maha, R. B. Ebai, Nabila, I. El-Sheikh. 2016. Toxic Effects of Cypermethrin on Male Fertility and Some Hepatic Biochemical Parameters in Male Albino Rats. Egyptian Journal of Chemistry and Environmental Health, 2 (2):66-77.

13. Hu, J.X, Li, Y.F, Li, J., Pan, C., He, Z., Dong, H.Y.et al. 2013.Toxic effects of cypermethrin on the male reproductive system: With emphasis on the androgen receptor. Journal of Applied Toxicology, 33:576–85.

14. Sharma, P., Firdous, S. and Singh, R. 2014. Neurotixic effect of cypermethrin and protective role of resveratrol in wistar rats. International Journal of Nutrition, Pharmacology and Neurological Diseases, 4(2): 104-111.

15. Hua Wang, Su-Fang Wang, Huan Ning,Yan-Li Ji, Cheng Zhang et al., 2011. Maternal Cypermethrin Exposure During Lactation Impairs Testicular Development and Spermatogenesis in Male Mouse Offspring. Environmental Toxicology, 26(4):382-94.

16. Han, Y, Xia, Y., Han, J., Zhou, J., Wang, S., Zhu, P., Zhao, R., Jin, N., Song, L., Wang, X. 2008. The relationship of 3-PBA pyrethroids metabolite and male reproductive hormones among non-occupational exposure males. Chemosphere., 72: 785–790.

17. Xu, L.C., Liu, L., Ren, X.M. et al. 2008. Evaluation of androgen receptor transcriptional activities of some pesticides in vitro. Toxicology, 243: 5965.

18. CPCSEA, 2003. Committee for the Purpose of control and supervision on experiments on animals, CPCSEA guidelines for laboratory animal facility, Indian Journal of Pharmacology, 35: 257–274.

19. Bancraft, J.D. and Stevens, A. 1982.Theory and Practice of Histological Techniques, seconded. Churchill Livingstone, New York.

20. Li, Y.F., Pan, C., Hu, J.X., Li, J., Xu, L.C. 2013. Effects of cypermethrin on male reproductive system in adult rats. Biomedical and Environmental Sciences. 26: 201–8.

21. Hayes, F.J., DeCruz, S., Seminara, S.B. et al. 2001. Differential regulation of gonadotropin secretion by testosterone in the human male: absence of a negative feedback effect of testosterone on follicle stimulating hormone secretion. The Journal of Clinical Endocrinology and Metabolism , 86: 538.

22. Reshma Anjum, M., Sreenivasula Reddy, P. 2014. Recovery of lead-induced suppressed reproduction in male rats by testosterone. Andrologia., xx, 1–8.