Int J Pharma Res Health Sci. 2016; 4 (6): 1489-1494
DOI:10.21276/ijprhs.2016. 06.11
S C Joshi *et al.*
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Effects of Tebuconazole (A fungicide) on Reproduction of Male Rat

S C Joshi *, Nandan Gulati , Bhawana Sharma, Priyanka Sharma

Reproductive Toxicology Unit, Department of Zoology, University of Rajasthan, Jaipur-302004, India.

ARTICLE INFO

Received: 24 Nov 2016
Accepted: 20 Dec 2016

ABSTRACT

Tebuconazole, a triazole fungicide, widely used in agriculture by farmers was evaluated to determine its effects on testes of rats. In the present study, Tebuconazole dissolved in olive oil was administered orally to albino male rats at dose level of 250 mg /kg b.wt/day for 15, 30 and 45 days. Reproductive toxicity of tebuconazole was evaluated on the basis of weight analysis of testes and accessory sex organs, fertility, sperm dynamics, hormonal analysis and histopathological studies. There was a decrease in the weight of testes, epididymis, ventral prostate and seminal vesicle. The result showed highly significant decline in sperm density and motility. Post fertility test showed 70%, 90%, and 100% negative results. A decrease in serum testosterone, FSH and LH levels were observed in all the treated groups. The histopathological observations also support the occurrence of toxicity being caused due to exposure of tebuconazole. The observations are thus indicative of the reproductive toxicity caused by tebuconazole at different dose durations in the testes of rats.

Keywords: Tebuconazole, testes, testosterone, LH, FSH, fertility

1. INTRODUCTION

The Pesticides are extensively being used to boost agriculture production. Apart from affecting target species, these toxic chemicals also influence physiology of numerous non-target species including man directly or through the food chain [1]. Environmental pollution from pesticides is an important issue that attracts widespread public concern in developing countries [2]. Unfortunately, the risk of acute exposure to these compounds is a constant threat in developing nations and these chemicals are responsible for numerous cases of poisoning annually in non-target wildlife and acute mammalian neurotoxicity and reprotoxicity [3]. These are mostly non-selective, widespread applied, possess toxic properties [4], and in some cases are very refractory.

Corresponding author *
Prof. S.C. Joshi
Centre for Advance Studies,
Dept. of Zoology,University of Rajasthan,
Jaipur- 302004, India
Mobile no. - 9414360717
E-mail: s_c_joshi2003@rediffmail.com

They include a great variety of substances like insecticides, acaricides, herbicides, fungicides and algaecides, different both in composition and properties with the purpose to kill, destroy or repel undesirable living organisms [5].

Triazole fungicides are used agriculturally to control rust and mildew on fruit, vegetables, cereals and seeds, residential and commercial turf, and in pharmaceutical applications for the treatment of local and systemic fungal infections [6]. Triazoles inhibit the biosynthesis of ergosterol, an essential component of fungal cell membranes, via. inhibition of cytochrome P450 dependent enzyme lanosterol 14α-demethylase [7]. Cyp51 is evolutionarily conserved between plants, fungi and animals and in animals is critical for cholesterol synthesis and therefore steroid biosynthesis [8]. Besides Cyp51, triazoles also modulate the gene expression and enzyme activity of multiple cytochrome P450 (CYP) and other metabolic enzymes in mammalian liver and other tissues [9].

Tebuconazole (TEB) ((RS)-1-p-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl) pentan-3-ol) is a systemic triazole fungicide used on crops such as barley, wheat, peanuts, and orchard fruits. Its mechanism of fungicidal activity is inhibition of a-lanosterol demethylase, which decreases ergosterol biosynthesis [10]. Tebuconazole is a potent xenobiotic to which exposure can cause metabolic alterations and death of different organisms. The tebuconazole fungicide had been classified by the US EPA as Group C-Possible Human Carcinogen [11]. TEB is persistent in soils and presents moderate mobility [12]. Studies with rats show that perinatal exposure to tebuconazole produced immunological neurobehavioral and neuropathological deficits [13]. However, there are no sufficient information was available concerning tebuconazole's effects on the reproductive systems [14]. Therefore, the present investigation was undertaken to elucidate the effect of tebuconazole on the reproductive system of adult albino rats.

2. MATERIALS AND METHODS

Animal model

Adult male albino rats (*Rattus norvegicus*) of an average body weight 180–200 g were used for experimentation. The animals were kept in clean polypropylene cages covered with chrome-plate grills and maintained in a room with controlled room temperature (20±5^{0}C) with 12:12 h light/dark cycle. The animals were fed with food pellets procured from Ashirwad Industries, Chandigarh, as well as germinated/sprouted gram and wheat seeds as an alternative feed. Tap water was supplied *ad libitum*.

Chemical and dose

Technical grade Tebuconazole [(RS)-1-p-Chlorophenyl-4,4-Dimethyl-3-(1H-1,2,4-Triazol-1-ylmethyl) Pentan-3-ol], obtained from Gupta chemical Pvt. Ltd., Jaipur, was used for experimentation. The insecticide was dissolved in olive oil and administered to animals through oral intubations at daily doses of 250 mg kg^{-1} b.wt. per day for 15, 30 and 45 days.

Experimental procedure

Animals were intended into four groups containing six animals each. Group I animals served as control and were administered olive only. The animals of group II, III, and IV were treated with tebuconazole of 250 mg kg^{-1} bwt per day for 15, 30 and 45 days, respectively. At the end of the experimentation, the rats were weighed, and sacrificed under light ether anesthesia. The male reproductive organs were removed, washed with distilled water, dried, weighed, and processed for biochemical and histopathological studies.

Fertility test

The mating exposure test of the animals was performed. Rats were cohabited with normal proestrous females in the ratio of 1:3. The vaginal plug and presence of sperms in the vaginal smear were checked for positive mating. Females were separated and resultant pregnancies were noted when dams gave birth.

Sperm dynamics

The sperm motility in cauda epididymis and sperm density of testicular and cauda epididymis were determined [15].

Biochemical parameters

The total protein [16], sialic acid [17], glycogen [18] and cholesterol levels were determined by Zlatkis, Zak, and Boyle 1953 [19].

Hormonal analysis

Testosterone, leutinizing hormone (LH), and follicle stimulating hormone (FSH) were estimated through chemiluminescence in fully automatic Advia Cemtaus Immuno Assay System.

Testicular histology

Testes of rats exposed to tebuconazole and control were fixed in Bouin's fixative for at least 48 h, processed by paraffin wax impregnation method, cut using a rotary microtome at 5 mm thickness, and stained with hematoxylin and eosin (HE) for light microscopic examination.

Statistical analysis

The data were analyzed statistically using Student's ''t'' test and the significance of differences was set at $p< 0.01$ and $p< 0.001$.

3. RESULTS

Testes and reproductive organ weight

Oral administration of tebuconazole caused a significant ($P \leq 0.01$) decrease in the weight of testes, epididymis and seminal vesicles at the duration of 15 days and highly significant ($P \leq 0.001$) decrease in the weight of testes, epididymis and seminal vesicles in 30 and 45 days at the dose of 250 mg/kg bwt/ day in the experimental rats. However, tebuconazole showed a non significant decrease in the weight of ventral prostate at the duration of 15 days wherever a significant ($P \leq 0.01$) and highly significant ($P \leq 0.001$) decrease was observed at the duration of 30 and 45 days (Table 1.1).

Sperm Dynamics, Motility and Fertility

The sperm density in testes and cauda epididymis decreased highly significantly (P ≤ 0.001) after tebuconazole administration in the all dose duration (Table 1.2) and a severe impairment of sperm motility in cauda epididymis was also observed in the experimental rats. Control rats showed 100% positive fertility in the mating exposure test while the rats exposed to 250 mg/kg b. wt/day dose level showed 70, 90 and 100% negative fertility in the duration of 15, 30 an 45 days respectively (Table 1.2).

Biochemical changes in testes after administration of tebuconazole

The glycogen and sialic acid contents in testes decreased significantly (p≤0.01 and p≤0.001) while testicular protein and cholesterol increased significantly (p≤0.01 and p≤0.001) in tebuconazole treated rats (Table 1.3). These changes were in dose duration dependent manner.

Serum concentration of total testosterone, FSH and LH

Rats administered with tebuconazole exhibited significant (p≤0.01 and p≤0.001) reduction in the level of serum testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in the 30 and 45 days at the dose of 250 mg/kg bwt/ day in comparison to control rats (Table 1.4). However, tebuconazole showed a non significant decrease in the level of serum testosterone, FSH and LH at the duration of 15 days.

Testicular histopathology

Histoarchitecture of control rat testes exhibits normal morphology of seminiferous tubule with all successive stages of spermatogenesis, lumen filled with spermatozoa and sertoli cells are present.

Testes of rats treated with tebuconazole (250 mg/kg^{-1} b.wt/day for 15 days) showed degenerated germinal epithelium and lumen with less sperms. Further, loosened tunica propria, few spermatocytes, and few numbers of Leydig and Sertoli cells with increased intertubular space and lumen with cellular debris were found in testes of rats treated with tebuconazole (250 mg/ kg^{-1} b.wt/day for 30 days). Spermatogenic elements changed into debris in the lumen causing complete arrest of spermatogenesis at high dose level of acephate. Ruptured germinal epithelium with disrupted interstitial cells with damaged Leydig cells was also seen in 45 days duration (Figure 1a-d).

4. DISCUSSION

Tebuconazole, is one of the triazole fungicide that produced neuropathological, neurobehavioral and immunological defects in different species, however disturbances in reproductive functions are less known [20]. The present study revealed that administration of tebuconazole at the dose level of 250 mg/kg/b.wt./day for 15, 30 and 45 days to male rats resulted in reproductive toxicity. Administration of tebuconazole showed a significant decrease in weight of testes and accessory sex organs. The weight of testes is largely dependent on the mass of differentiated spermatogenic cells and the reduction in the weight of testes may be due to reduced tubule size, decreased number of germ cells, elongated spermatids, spermatogenic arrest and inhibition of steroid synthesis by Leydig cells [21 22 23]. The reduction in weight of accessory sex organs may be due to low availability of androgens or antiandrogenic activity of tebuconazole [24]. Generally, maintenance of weights of accessory reproductive glands depends on testosterone level [25]. Reduction in sperm motility and density suggested an undersupply of testosterone to the testes and epididymis, thereby possibly causing impaired epididymal function, as it is known that the structure and function of the testes and epididymis is dependent on androgens [26 27]. Reduced sperm count in testes and epididymis may also be due to inhibition of meiotic division of spermatocytes or may be due to the suppressive effect of the tebuconazole on spermatogenesis [28 29]. The decrease in fertility potentials reported after the treatment of male rats has been attributed to impairment in sperm motility and viability [30].

Table 1.1: Testes and accessory sex organ weight analysis (Tebuconazole 250 mg/kg.b.wt./day)

Treatment	Testes	Epididymis	Seminal Vesicle	Ventral Prostate
	mg/100 g body wt.			
Group I Control (Vehicle only)	1212.42 ±53.61	510.26 ±6.76	412.62 ±12.21	344.27 ±17.24
Group II 15 Days	998.46* ±39.41	461.34* ±15.41	358.21* ±16.41	310.12^{ns} ±27.84
Group III 30 Days	886.32** ±63.21	412.34** ±15.41	349.26* ±18.26	281.36* ±19.21
Group IV 45 Days	836.24** ±43.36	397.41** ±17.26	332.61** ±19.34	242.81** ±27.23

Mean ± SEM of 6animals (Group II; III; IV compared with Group I)
ns = Non significant, * = P ≤ 0.01 (Significant), ** = P ≤ 0.001 (Highly significant)

Table 1.2: Sperm Dynamics and Fertility (Tebuconazole 250 mg/kg.b.wt./day)

Treatment	Sperm motility (%)	Sperm density (million/ml)		Fertility (%)
	Cauda Epididymides	Testes	Cauda Epididymides	
Group I Control (Vehicle only)	70.46 ±4.02	4.35 ±0.37	46.62 ±0.86	100 % (+)ve
Group II 15 Days	34.32** ±5.38	1.87* ±0.69	36.14** ±0.91	70 % (-)ve
Group III 30 Days	18.96** ±6.34	0.98** ±0.54	21.24** ±0.87	90 % (-)ve
Group IV 45 Days	11.07** ±7.65	0.76** ±0.65	16.01** ±1.10	100 % (-)ve

Mean ± SEM of 6animals (Group II; III; IV compared with Group I)
ns = Non significant, * = P ≤ 0.01 (Significant), ** = P ≤ 0.001 (Highly significant)

Table 1.3: Biochemical changes in testes (Tebuconazole 250 mg/kg.b.wt./day)

Treatment	Protein	Sialic acid	Cholesterol	Glycogen
	mg/g			
Group I Control (Vehicle only)	252.56 ±11.84	5.30 ±0.19	6.01 ±0.40	2.56 ±0.13
Group II 15 Days	289.10* ±10.61	4.74* ±0.21	7.41* ±0.37	1.85* ±0.17
Group III 30 Days	321.76** ±9.27	4.09** ±0.24	9.87** ±0.49	0.98** ±0.23
Group IV 45 Days	330.20** ±8.18	3.71** ±0.22	11.52** ±0.64	0.51** ±0.24

Mean ± SEM of 6animals (Group II; III; IV compared with Group I)
ns = Non significant, * = P ≤ 0.01 (Significant), ** = P ≤ 0.001 (Highly significant)

Table 1.4: Changes in serum hormonal level (Tebuconazole 250 mg/kg.b.wt./day)

Treatment	Testosterone ng/ml	Lutenising Hormone (LH) Miu/ml	Folicle Stimulating Hormone (FSH) Miu/ml
Group I Control (Vehicle only)	2.79 ±0.43	1.76 ±0.48	0.53 ±0.09
Group II 15 Days	2.24^{ns} ±0.20	1.12^{ns} ±0.37	0.43^{ns} ±0.10
Group III 30 Days	1.28** ±0.17	0.63** ±0.54	0.30** ±0.08
Group IV 45 Days	0.86** ±0.14	0.41** ±0.39	0.19** ±0.06

Mean ± SEM of 6animals (Group II; III; IV compared with Group I)
ns = Non significant, * = P ≤ 0.01 (Significant), ** = P ≤ 0.001 (Highly significant)

Administration of tebuconazole also changes the biochemical parameters of the reproductive tract. Protein biosynthesis is a key factor for testicular development and spermatogenesis. Elevation in the total Protein content in testes may be due to hepatic detoxification activity which results in the inhibitory effect on the activity of enzyme involved in the androgen biotransformation [31 32 33]. The structural integrity of the acrosomal membrane is dependent upon sialic acid; the reduced sialic acid content might alter the structural integrity of acrosomal membrane, ultimately affects the metabolism, motility and fertilizing capacity of spermatozoa [34]. A significant increase in cholesterol level was observed after tebuconazole treatment. This increased testicular cholesterol concentration may reflect reduced conversion of cholesterol to testosterone [35 36] which resulted in accumulation of cholesterol in the testes, and thus impaired spermatogenesis [37]. Tebuconazole treated rats showed a significant decline in glycogen content. Reduced glycogen reflects decrease in the number of postmeiotic germ cells, which are thought to be the sites of glucose metabolism [38 39]. A decrease in glycogen content could also be due to inhibited the glycogen synthesis which eventually decreases spermatogenesis [40].

Testosterone, an important androgen, plays a pivotal role in maturation, spermatogenesis and the maintenance of accessory sex organs [41]. Tebuconazole treatment caused a significant decrease in the testosterone level of treated rats. The significant reduction of testosterone level in blood indicates the reduction of androgen level in treated animal. The production of the sperm cells (spermatozoa) and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH) [42]. The reduction in the serum level of testosterone could probably be due to the decrease of serum levels of FSH, LH and prolactin which are essential for the gonadal development and steroidogenesis in rats [5]. The decrease in the serum concentrations of FSH, LH and testosterone in the treated animals clearly indicates the action of extract on the secretion of pituitary gonadotropins and in turn in the testosterone biosynthesis in the testis and reproductive organs [43]. Thus the present investigation suggests that fungicide tebuconazole is highly toxic to reproductive function and alter the fertility of animals.

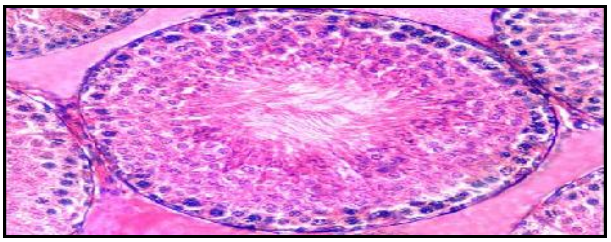
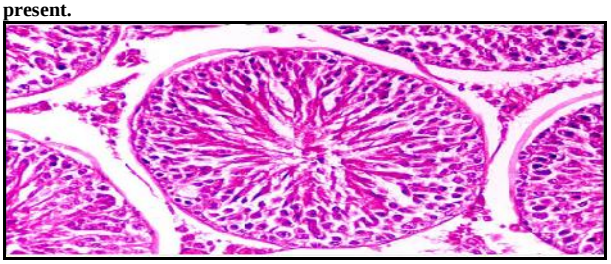
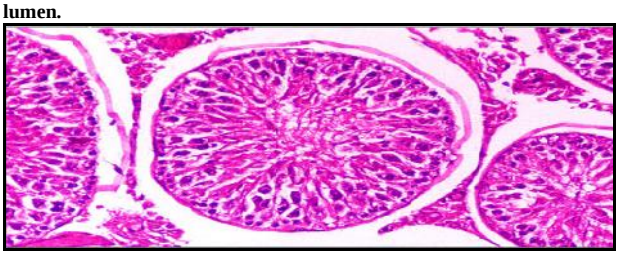


Fig 1.(a) Micro-photograph of control rat testes showing all the successive stages of spermatogenesis i.e. normal morphology of seminiferous tubules. Lumen is filled with sperm. Leydig cells are also present.

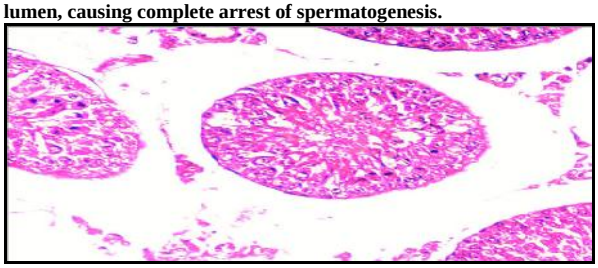


(b) Photograph of testes treated with Tebuconazole 250mg/kg b.wt. for 15 days showing vacuolated interstitial tissues with disrupted connective tissues. Tunica propria loosened at several sites. Inhibition of spermatogenesis seen as there is presence of celluar debris in the lumen.



(c) Photograph of testes treated with Tebuconazole 250mg/kg b.wt. for 30 days showing reduced size of seminiferous tubules with increased Intertubular spaces. Germinal epithelium loosened and even damaged

at some sites. Spermatogenic elements changed in to debris in the lumen, causing complete arrest of spermatogenesis.



(d) Photograph of testes treated with Tebuconazole 250mg/kg b.wt. for 45 days showing irregular tubule with highly reduced size having vacuolated epithelium and showing complete spermatogenic arrest. Ruptered germinal epithelium with disrupted interstitial cells having damaged leydig cells could be seen.

5. CONCLUSION

The present study revealed that oral administration of tebuconazole induced reproductive toxicity in male albino rats. Toxic effects of tebuconazole were pronounced at high dose level. The conclusion can be drawn that tebuconazole administration affect spermatogenesis leading to poor semen quality and reduced male fertility but these effects were dose and duration dependent. These observations suggested the limited use of such toxic insecticides to improve the quality of life for human welfare.

6. REFERENCE

1. Sharma RK and Goyal AK. Agro-pesticides and andrology. Int J Pharm Pharm Sci 2014; 6(10): 12-19.
2. Sudakin DL and Power LE. Organophosphate exposures in the United States: A longitudinal analysis of incidents reported to poison centers. J Toxicol Environ Health, Part A 2007; 70: 141-47.
3. Kaur S and Dhanju CK. Biochemical effects of some organophosphorus pesticides on the ovaries of albino rats. Ind J Physiol Pharmacol 2005; 49(2): 148-52.
4. Bhuvaneswari S, Aravind R, Kaviyarasan V, Kalaivanan K, Hariram SB. A comparative study on antibacterial activity of common weeds. Int J Pharm Biosci 2011; 2: 677-683.
5. Martina G, Katarina S, Beata H, Jan D, Martin M. Identification of chromosomes and aberrations after exposure to pesticide in bovine lymphocytes. Egypt Acad J biolog Sci 2010; 2(2): 5- 9.
6. Goetz AK, Ren H, Schmid J E, Blystone CR, Thillainadarajah I, Best DS, Nichols HP, Strader LF, Wolf DC, Narotsky MG, Rockett JC, Dix DJ. Disruption of testosterone homeostasis as a mode of action for the reproductive toxicity of triazole fungicides in the male rat. Toxicol Sci 2007; 95(1): 227–239.
7. Barton HA, Tang J, Sey YM, Stanko JP, Murrell RN, Rockett JC, Dix DJ. Metabolism of myclobutanil and triadimefon by human and rat cytochrome P450 enzymes and liver microsomes. Xenobiotica 2006; 36: 793-906.
8. Zarn JA, Bruschweiler BJ, Schlatter JR. Azole fungicides affect mammalian steroidgenesis by inhibiting sterol 14 alpha-demethylase and aromatase. Environ. Health perspect 2003; 111: 255-261.
9. Tully DB, Bao W, Goetz AK, Ren H, Schmid JE, Strader LF, Wood CR, Best DS, Narotsky MG, Wolf DC. Gene expression profiling in liver and testis of rats to characterize the toxicity of triazoles fungicides. Toxiciol Appl Pharmacol 2006; 215: 260-273.
10. Obanda DN, Shupe TF, Catallo WJ. Resistance of Trichoderma harzianum to the biocide tebuconazol – Proposed biodegradation pathways. Hulzforschung 2008; 62: 613-619.
11. U.S. EPA (Environmental Protection Agency). Chemicals Evaluated for Carcinogenic Potential. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC 2006.
12. EFSA. Conclusion on the Peer Review of Tebuconazole. EFSA Scientific Report 2008; 176: 1–109.
13. Moser VC, Barone S, Smialowicz RJ, Harris MW, Davis BJ, Overstreet D. The effects of perinatal tebuconazole on adult neurological, immunological and reproductive function in rats. Toxicol Sci 2001; 62: 339-52.
14. Taxvig C, Hass U, Axelstad M, Dalgaard M, Boberg J, Andeasen HR, Vinggaard AM. Endocrine-disrupting activities in vivo of the fungicides tebuconazole and epoxiconazole. Toxicol Sci 2007; 100(2): 464-73.
15. Prasad MRN, Chinoy NJ, Kadam KM. Changes in succinate dehydrogenas levels in the rat epididymis under normal and altered physiologic conditions. Fertil Steril 1972; 23: 186-90.
16. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol Reagent. J Biol Chem 1951; 193: 265-75.
17. Warren L. The thiobarbituric acid assay of Sialic acid. J Biol Chem 1959; 234: 1971-75.
18. Montgomery R. Determination of glycogen. Arch Biochem Biophys 1957; 67: 378-81.
19. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. J Lab Clin Med 1953; 41: 486-92.
20. Joshi SC, Gulati N. Effects of tebuconazole on reproductive function of male rat. J Cell and Tissue Res 2005; 5(2): 457-460.
21. Sujatha R, Chitra KC, Latchoumycandane C, Mathur PP. Effect of lindane on testicular antioxidant system and steroidogenic enzymes in adult rats. Asian J Androl 2001; 3: 135-138.
22. Sanhez LC, Reyes BE, Labez Carrill OL. Organophorous pesticides exposure alters sperm chromatin structure in mexican agricultural workers. Toxicol Appl Pharmacol 2004; 94: 108-13.

23. Ananthan.G, Kumaran.B. Effect of Mancozeb on the Specific activities of Testicular Phosphatases and Protective role of Vitamin C in Albino rats. Bull Env Pharmacol Life Sci 2013; 2(7): 56-61.
24. Bian Q, Qian J, Xu L, Chen J, Song L, Wang X. The toxic effects of 4-tert-octylphenol on the reproductive system of male rats. In Food and chemical toxicology. British Ind Biol Res Associa 2006; 44: 1355-1361.
25. Nour El-Hoda, AZ. Evaluation of the reproductive toxicity of chlorpyrifos methyl, Diazinon and Profenofos pesticides in male rats. Int J Pharmacol 2009; 5: 51-57.
26. Robaire B, Seenundun S, Hamzeh M, Lamour SA. Androgenic regulation of novel genes in the epididymis. Asian J Androl 2007; 9(4): 545-53.
27. Cornwall GA. New insights into epididymal biology and function. Hum Reprod Update 2009; 15(2): 213-227.
28. Akbarsha MA, Kadalmani B, Girija R, Faridha A, Hamid KS. Spermatotoxic effect of carbendazim. Indian J Exp Biol 2001; 39(9): 921-4.
29. Obianime AW, Aprioku JS. Comparative study of artesunate, ACTs and their combinants on the spermatic parameters of the male guinea pig. Niger J Physiol Sci 2009; 24(1): 1-6.
30. Nwanjo HU, Iroagba IN, Nnatuanya IN, Eze NA. Antifertility activity of dihydroarteminisinin in male albino rats. The Internet Journal of Endocrinology, 2007; 4(1): p3.
31. Walsh LP, Stocco DM. Effects of lindane on steroidogenesis and steroidogenic acute regulatory protein expression. Biol Reprod 2000; 63(4): 1024-1033.
32. Venkataramana GV, Sandhya Rani PN, Murthy PS. Impact of malathion on the biochemical parameters of gobiid fish Glossogobius giuris (Ham). J Environ Biol 2006; 27: 119-122.
33. Thejashwini MS, Krishna Ram H, Shivabasavaiah. Reversible antifertility effect of Cyamposis psoralioides in male swiss albino mice. International Journal of Advance Biological Research 2012; 2(4): 657-665.
34. Murono EP, Derk RC, Akgul Y. In vivo exposure of young male rats to methoxychlor reduces serum testosterone levels and ex vivo Leydig cell testosterone formation and cholesterol side-chain cleavage activity. Reprod Toxicol 2006; 21(2): 148–53.
35. Narayana K, Prashanthi N, Nayanatara A, Kumar HH, Abhilash K, Bairy KL. Neonatal methyl parathion exposure affects growth and functions of the male reproductive system in the adult rat. Folia Morphol (Warsz) 2006; 65(1): 26-33.
36. Ngoula F, Watcho P, Dongmo MC, Kenfac A, Kamtchouing P, Tchoumboue J. Effects of primphos-methyl (an organophosphate insecticide) on the fertility of adult male rats. Afr Health Sci 2007; 7(1): 3-9.
37. Ohi M, Dalsenter PR, Andrade AJM, Nascimonto AJ. Reproductive adverse effects of fipronil in wistar rats. Toxicol Lett 2004; 146(2): 121-127.
38. Bone W, Jone AR, Morin C, Nieschlag E, Cooper TG. Susceptibility of glycolytic enzyme activities and motility of spermatozoa from rat, mouse and human to inhibition by proven and putative chlorinated antifertility compounds in vitro. J Androl 2001; 22(3): 464-470.
39. Joshi SC, Mathur R, Gulati N. Testicular toxcity of chlorpyrifos (an organophosphate pesticide) in albino rat. Toxicol Ind Health 2007; 23: 439-444.
40. Sisodia R, Yadav RK, Sharma KV, Bhatia AL. Spinacia oleracea modulates radiationinduced biochemical changes in mice testis. Indian J Pharm Sci 2008; 70(3): 320–6.
41. Joshi SC, Tibrewal P, Sharma A and Sharma P. Evaluation of toxic effect of 2,4-D (2,4-dichlorophenoxyacetic acid) on fertility and biochemical parameters of male reproductive system of albino rats. Int J Pharm Sci 2012; 4: 338-342.
42. Maarke JE, Roelofsa B, Roberto Temminga A, Aldert H. Piersmab,a,Martin van den Berga, Majorie B.M. van Duursen. Conazole fungicides inhibit Leydig cell testosterone secretionand androgen receptor activation in vitro. Toxicology Reports 2014; 1: 271–283.
43. Ramesh V, Kumaran B. Spermatotoxic Effect of Hexaconazole in Adult Rats. IAAST 2013; 4 (2): 56-61.

Conflict of Interest: None

Source of Funding: Nil