



EFFECT OF AN ORGANOPHOSPHOROUS INSECTISIDE DICHLOROVOS ON THE REPRODUCTIVE PERFORMANCE IN MALE RAT (*RATTUS NORVEGICUS*)

Bhawana Sharma, Priyanka Sharma and S. C. Joshi*

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

***Corresponding Author: Prof. S. C. Joshi**

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

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ABSTRACT

Dichlorovos is widely used as a potent pesticide in many countries and has been shown to produce some adverse health effects. The present investigation was designed to clarify whether dichlorovos causes detectable adverse effects on rat testes. Dichlorovos was administered to rats orally at dose level of 20 mgkg⁻¹bwt.⁻¹day⁻¹ for 45 days. Study showed significant (P<0.05) decline in the body weight and reproductive organs weight, and significant (P<0.05) reduction in sperm counts with 80% infertility in dichlorovos treated animals. A significant reduction in testicular glycogen (P<0.05) and sialic acid (P<0.01) was also noticed. Contrary to these, protein (P<0.01) and cholesterol (P<0.05) contents of testes were significantly increased. In addition, serum acid phosphatase activity significantly (P<0.05) increased, while serum alkaline phosphatase (P<0.05) and serum testosterone, LH and FSH levels (P<0.01) were diminished. Thus, the obtained results indicated that dichlorovos shows reproductive toxicity on biochemical and hormonal level in male albino rats.

KEY WORDS: Dichlorovos, Epididymis, Fertility, Testosterone.

INTRODUCTION

Pesticides have a serious impact on the environment and unexpected threat to man's well being by their exponential increase in the production, use and deposition. It is calculated that male-related disorders are probably present up to 40% to 50% in childless couples, alone or in combination with female factors.^[1]

Organophosphorus (OP) compounds have largely been implicated in reproductive deficit^[2] and have an adverse effect on male fertility.^[3] Organophosphate (OP) induces toxicity through inhibition of acetyl cholinesterase (AChE) in addition to multiple other mechanisms and it can also induce oxidative stress by generating free radicals and altering antioxidants level of the free radical scavenging enzyme activity.^[4]

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate), also known as DDVP, is an organophosphate pesticide that is widely used to treat domestic animals and livestock for internal and external parasites, and to protect crops from insect infestation.^[5] Its annual world production reaches four thousand tonnes.^[6] DDVP (traded as Ota-piapia) is indiscriminately used because of its cheap production, efficacy, accessibility and affordability. It is most commonly used organophosphate pesticides in developing countries and classified by the WHO as a Class IB, 'highly hazardous' chemical.^[7] Like other pesticides, dichlorvos is absorbed through the skin,

ingested or inhaled.^[8] Dichlorvos is one of the environmental pollutant which showed a broad spectrum toxicological effects and biochemical dysfunctions constituting serious hazards to health.^[9] Dichlorvos is reported to cause toxicity to pancreas^[10], kidney^[11], brain^[12], immune system^[13] and reproductive system.^[14]

The aim of the present study was to investigate the reproductive performance of male rats exposed to an organophosphorous insecticide dichlorovos using organ weight, biochemical and hormonal analysis and fertility parameters.

MATERIALS AND METHODS

Animal and housing

The present study was carried out on inbreeds proven fertile male albino rat (*Rattus norvegicus*) of Wistar strain weighing 150-200g. The animals were kept in clean polypropylene cages covered with chrome plate grills and maintained in airy room with controlled room temperature (20±5°C) with 12:12 hrs light and dark cycle. The animals were fed with food pellet procured from Ashirwad Industries, Chandigarh (India), as well as germinated/sprouted gram and wheat seeds as an alternative feed. Fresh water was supplied *ad libitum* throughout the study. The guidelines for care and use of animals for scientific research^[15] were strictly followed throughout the course of investigation. The Experimental

protocol has approval of the Institutional Animal Ethics Committee (IAEC).

Before starting the experiment the male rat were kept with normal oestrous female for normal mating.

Test compound and dose

Technical grade dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) obtained from Sudarshan Crop, Ltd., Jaipur (Raj.) (India) was used for experimentation. The insecticide was dissolved in olive oil and administered to animals through oral intubations at the daily dose of $20 \text{ mgkg}^{-1}\text{b.wt}^{-1}\text{day}^{-1}$ for 45 days. The dose was decided after determination of LD_{50} values of dichlorvos in laboratory.

Treatment Protocol

Animals were divided into four groups containing 6 animals each

Group I

This group served as control was not given any treatment.

Group II

This group served as vehicle treated control was given olive oil (0.5 ml) for 45 days.

Group III

Animals of this group were administered with dichlorvos at oral dose of $20 \text{ mgkg}^{-1}\text{b.wt}^{-1}\text{day}^{-1}$ in 0.5 ml olive oil for 45 days.

Group IV

This group served as recovery group was administered with dichlorvos at oral dose of $20 \text{ mgkg}^{-1}\text{b.wt}^{-1}\text{day}^{-1}$ in 0.5 ml olive oil for 45 days and then kept without any treatment for next 45 days.

The dose level selected was five times less than LD_{50} value as suggested in number of papers and our preliminary experiments and duration was selected keeping in mind spermatogenetic cycle of rat.

Autopsy schedule

At the end of the experiment, the rats were weighed and sacrificed under light ether anesthesia. The male reproductive organs were dissect out, washed with distilled water, dried, weighed and processed for sperm dynamics and biochemical studies.

Fertility test

The mating exposure test of all the animals was performed. They were cohabited with normal pro oestrous females in 1:3 ratios. The vaginal plug and presence of sperms in the vaginal smear was checked for positive mating. Females were separated and resultant pregnancies were noted when dams gave birth.^[16]

Body and organ weight measurements

Initial body weight was taken on the first day of treatment and final body weight of each animal was recorded after the last day of treatment. Treated animal were sacrificed along with control animals. Blood (2ml) was collected in sterile tubes by cardiac puncture for hematological studies and allowed to clot. Further, clotted blood was centrifuged to isolate serum for serological studies. At autopsy, the reproductive organs (Testes, Epididymis, Seminal Vesicle, Prostate and Vas deferens) were excised from the surrounding tissue and blotted free of blood for weighing.

Sperm dynamics

The sperm motility in cauda epididymis and sperm density of testicular and cauda epididymis was determined.^[17] The epididymis was removed immediately after anesthesia. Epididymal fluid with spermatozoa was diluted with physiological saline (0.9% NaCl) and placed on a neubauer's chamber slide. The sperm suspension was examined within 5 min after their isolation from epididymis. The results were determined by counting both motile and immotile sperms in at least ten separate and randomly selected fields. The results were finally expressed as percent motility. The sperm density was calculated in testes and epididymis in million/ml as per dilution.

Biochemical parameters

The total protein^[18], sialic acid^[19], glycogen^[20], and cholesterol^[21] were assessed in testes by standard methods. The acid and alkaline phosphatase levels in serum were determined by following the method of King and Jagathesan.^[22]

Hormonal analysis

Serum levels of testosterone, FSH and LH of control and treated groups were estimated by radioimmunoassay (RIA) technique by LKB gamma counter.^[23]

Statistical analysis

The data were analyzed statistically by using ANOVA and the significance of differences was set at $P \leq 0.05$ (significant) and $P \leq 0.01$ (highly significant) levels.

RESULT AND DISCUSSION

The study showed significant decrease ($p \leq 0.05$) in body weight after the administration of dichlorvos at dose level of $20 \text{ mgkg}^{-1}\text{b.wt}^{-1}\text{day}^{-1}$ as compared to control group (Table 1). Reduction the body weight can be attributed to the hormonal imbalance caused due to pesticide toxicity. It has been reported that the reduced levels of testosterone propionate causes decrease in the body mass index (BMI).^[24]

Similarly, the weight of testes, epididymis, prostate and seminal vesicles in treated group were also significantly ($p \leq 0.05$) decreased, whereas the recovery group showed a non significant increase in the weight of reproductive organs (Table 1). A significant ($p \leq 0.01$) decrease was

observed in the weight of testes might be attributed to loss of germ cell and elongated spermatids.^[25] Dichlorovos reduced spermatogenic potential by reducing their number of sertoli cells, as weight of testes is largely dependent on the mass of differentiated spermatogenic cells^[26] or might be due to decreased production of seminiferous tubular fluid, which contributes to weight of testis.^[27] The decreased testes weight in the dichlorovos treated rats clearly indicated that dichlorovos caused structural and functional alteration in the reproductive organs which could be due to imbalance of reproductive hormones. Similarly the reduction in weight of accessory sex organs might be due to low availability of androgens or antiandrogenic activity of dichlorovos.^[28]

A severe impairment of sperm motility in cauda epididymis was observed and the sperm density in testes and cauda epididymis decreased significantly ($p \leq 0.05$) after dichlorovos administration, while recovery group revealed non-significant decrease as compared to control (Table 2). A significant reduction ($p \leq 0.05$) in cauda epididymal sperm motility and density suggested an undersupply of testosterone to the epididymis, thereby possibly causing impaired epididymal function, as it is known that the structure and function of the epididymis is dependent on androgens.^[29] Organophosphate insecticides block nerve impulses by reducing acetylcholinesterase activity which leads to suppression of gonadotrophic hormones (LH and FSH). Suppression of gonadotrophins might be decreased sperm density in testes.^[30]

Control rats showed 100% fertility rate by mating exposure test. The rats administered with dichlorovos at $20 \text{ mg kg}^{-1} \text{ b.wt}^{-1} \text{ day}^{-1}$ dose level showed 20% fertility rate whereas recovery group exhibited 30% fertility rate as compared to control group (Table 2). A decrease in fertility potentials in treated rats has been attributed to impairment in sperm motility and viability. The 80% infertility in rats might be attributed to lack of forward progression and reduction in density of spermatozoa and altered biochemical milieu of cauda epididymis.^[31]

In biochemical parameters, testicular protein and cholesterol increased significantly ($p \leq 0.05$) after the administration of dichlorovos in rats, as well as recovery group. Glycogen and sialic acid contents in testes decreased significantly ($p \leq 0.01$) in dichlorovos treated rats and recovery group rats (Table 3). Protein biosynthesis is a key factor for testicular development and spermatogenesis. A high level of total protein content usually indicates of hepatic detoxification, which

results in inhibitory effects on the activities of enzymes involved in androgen biotransformation.^[32] These effects might be responsible for inhibition of spermatogenesis. Increased level of cholesterol may be due to decreased androgen production, which resulted in accumulation of cholesterol in testes and thus, impaired spermatogenesis.^[33]

A fall in glycogen level reflects a decrease in the number of post meiotic germ cells which are thought to be the sites of glucose metabolism.^[11] Inhibition of glycogen synthesis may affect the maturational process of spermatozoa and eventually decreases spermatogenesis process.^[34] The structural integrity of 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.^[35]

The study revealed a significant increase ($P < 0.05$) in acid phosphatase and decrease in alkaline phosphatase activity in serum, where as a vice versa was observed in recovery group that is acid phosphatase decreased and alkaline phosphatase increased (Table 4). The increase in acid phosphates activity might be the result of enhancement of cell membrane permeability with disturbance in the transphosphorylation process as a result of cellular degeneration or might be due to results of labalization of lysosomal system.^[36] A significant ($P < 0.05$) decrease in the alkaline phosphatase activity is considered as an indicator of decreased steroidogenesis and inhibition of testicular functions.^[37]

Testosterone is the principal androgen of the testes and is essential for sperm production and maintenance of accessory sex organs.^[38, 39] The level of testosterone, FSH and LH decreased significantly ($P < 0.01$) after treatment with dichlorovos (Table 4). The recovery group also showed significant ($P < 0.05$) ($P < 0.01$) decrease in comparison to control. A reduced serum testosterone demonstrated the inhibitory effects of dichlorovos on the secretion of pituitary gonadotrophins (FSH and LH) and in turn on testosterone biosynthesis. A low level of testosterone arrests spermatogenesis.^[40] The hormonal changes produced by dichlorovos favour direct toxic effect of insecticide or possibly through a change in the neuroendocrine environment resulting into acetylcholinesterase inhibition.^[41] Androgen deprivation not only suppresses spermatogenesis, leading to low sperm concentration, but alters the epididymal milieu also, which renders it hostile for physiological maturation and survival of spermatozoa.^[42]

Table 1: Values of body weight and reproductive organ weight of male rats orally administered dichlorovos for 45 days

Treatment	Body weight (gm)		Reproductive organ weight/ Index (mg / 100 gm body weight)			
	Initial	Final	Testes	Epididymis	Prostate	Seminal vesicle
Group I Control	182.56 ±2.58	184.98 ±3.24	1170.84 ±5.29 [632.95]	525.91 ±5.01 [284.30]	412.36 ±3.54 [222.92]	361.25 ±7.36 [195.29]
Group II (vehicle treated control)	175.37 ±1.05	180.50 ^{ns} ±1.56	1164.94 ^{ns} ±4.45 [630.68]	521.34 ^{ns} ±5.61 [282.24]	422.54 ^{ns} ±3.79 [228.75]	355.79 ^{ns} ±9.07 [192.62]
Group III 20mg/kg.b.wt/day for 45 days	178.25 ±0.82	149.95* ±1.25	959.84** ±8.01 [519.64]	441.24** ±13.65 [238.88]	309.12* ±3.54 [167.35]	266.83* ±5.75 [144.45]
Group IV 20mg/kg.bwt/ day for 45 days + then kept without any treatment for next 45 days (Recovery)	179.75 ±1.00	180.04 ^{ns} ±1.18	963.29** ±10.22 [521.51]	458.18* ±9.62 [248.05]	330.35 ^{ns} ±4.32 [178.84]	284.41 ^{ns} ±4.03 [153.97]

(Mean ± SEM of 6 Animals)

ns = non-significant,

* = significant (P<0.05),

** = highly significant (P<0.01) Group II, III Compared with group I

Table 2: Values of sperm dynamics and fertility tests of control and orally administered dichlorovos in rats for 45 days

Treatment	Sperm density (million/ml)		Sperm motility (%)	Fertility rate
	Testis	Cauda epididymis	Cauda epididymis	
Group I Control	5.31 ±0.22	49.02 ±0.41	66.59 ±2.14	100%
Group II (vehicle treated Control) % Change	5.18 ^{ns} ±0.47 [-0.56]	47.55 ^{ns} ±0.34 [-0.95]	64.34 ^{ns} ±3.96 [-0.37]	100%
Group III 20mg/kg.b.wt/day for 45 days % Change	0.88* ±0.17 [-83.61]	18.43** ±0.5 [-62.03]	29.12* ±3.29 [-56.10]	20%
Group IV 20mg/kg.bwt/ day for 45 days + then kept without any treatment for next 45 days (Recovery) % Change	1.18 ^{ns} ±0.29 [-77.22]	21.29 ^{ns} ±0.47 [-56.14]	33.65 ^{ns} ±3.90 [-49.27]	30%

(Mean ± SEM of 6 Animals)

ns = non-significant,

* = significant (P<0.05),

** = highly significant (P<0.01) Group II, III & IV Compared with group I

Table 3: Values of biochemical parameters in testes of control and orally administered dichlorovos in rats for 45 days

Treatment	Protein	Sialic acid	Cholesterol	Glycogen
	mg/g			
Group I Control	253.12 ±3.25	5.29 ±0.21	5.33 ±0.19	2.72 ±0.17
Group II (vehicle treated Control) % Change	245.36 ^{ns} ±4.61 [-0.30]	5.11 ^{ns} ±0.15 [-1.13]	5.27 ^{ns} ±0.23 [-1.12]	2.75 ^{ns} ±0.09 [+1.10]
Group III	284.95**	4.49**	7.52*	0.91*

20mg/kg.b.wt/day for 45 days % Change	± 7.64 [+12.91]	± 0.28 [-14.14]	± 0.05 [+42.69]	± 0.13 [-65.13]
Group IV 20mg/kg.bwt/ day for 45 days + then kept without any treatment for next 45 days (Recovery) % Change	279.90* ± 6.56 [+10.91]	4.64* ± 0.25 [-11.28]	7.14* ± 0.02 [+35.48]	1.37* ± 0.31 [-47.50]

(Mean $\pm$ SEM of 6 Animals)

ns = non-significant,

* = significant ($P < 0.05$),** = highly significant ($P < 0.01$) Group II, III & IV Compared with group I**Table 4: Values of control and orally administered dichlorovos for 45 days in serum of male albino rats**

Treatment	Acid Phosphatase KA units	Alkaline Phosphatase KA units	Testosterone ng/ml	Lutenising Hormone (LH) ng/ml	Folicle Stimulating Hormone (FSH) ng/ml
Group I Control	4.89 ± 0.23	64.08 ± 3.54	5.10 ± 0.31	4.88 ± 0.52	0.76 ± 0.05
Group II (vehicle treated Control) % Change	4.71 ^{ns} ± 0.47 [-1.63]	62.42 ^{ns} ± 2.21 [-1.02]	5.01 ^{ns} ± 0.43 [-1.78]	4.92 ^{ns} ± 0.48 [+0.81]	0.79 ^{ns} ± 0.09 [-2.63]
Group III 20mg/kg.b.wt/day for 45 days % Change	6.50* ± 0.57 [+35.13]	53.51* ± 2.30 [-15.62]	1.43** ± 0.20 [-71.45]	2.46** ± 0.37 [-50.00]	0.39** ± 0.04 [-50.00]
Group IV 20mg/kg.bwt/ day for 45 days + then kept without any treatment for next 45 days (Recovery) % Change	6.17 ^{ns} ± 0.42 [+28.27]	56.72 ^{ns} ± 1.38 [-10.56]	1.74** ± 0.17 [-65.26]	2.64* ± 0.54 [-46.34]	0.42** ± 0.08 [-46.15]

(Mean $\pm$ SEM of 6 Animals)

ns = non-significant,

* = significant ($P < 0.05$),** = highly significant ($P < 0.01$) Group II, III & IV Compared with group I**CONCLUSION**

Dichlorovos exposure not only altered the hormonal and biochemical structure but also reduced male fertility in wistar rats. Dichlorovos treatment also caused a significant decrease in the testosterone, LH and FSH levels of treated rats and thereby, suggesting the dwindling of androgen status or provides an indirect evidence for antiandrogenic action of pesticide. Hence, the use of dichlorovos must be limited due to its hazardous effects.

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REFERENCES

- Bungum M. Sperm DNA integrity assessment: A new tool in diagnosis and treatment of fertility. *Obstet Gynecol Int*, 2012.
- Volk M, Jaklic H, Zorn B, Peterlin B. Association between male infertility and genetic variability at the PON1/2 and GSTM1/T1 gene loci. *Reprod Bio Med Online*, 2011; 23(1): 105-10.
- Joshi SC, Bansal B. Reproductive toxicity of monocrotophos in male rats. *Int. J. Toxicol and Appl Pharm*, 2012; 2(1): 6-11.
- Sharma Y, Bashir S, Irshad M, Gupta SD, Dogra TD. Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. *Toxicol*, 2005; 206: 49-57.
- Yarsan E, Cakir O. Effects of dichlorvos on lipid peroxidation in mice on subacute and subchronic periods. *Pestic Biochem Phys*, 2006; 86: 106-9.
- Guvenc D, Aksoy A, Das YK, Atmaca E, Yavuz O. 3-nitrotyrosine levels in dichlorvos-induced neurotoxicity. *Arh Hig Rada Toksikol*, 2014; 65: 109-12.
- Das, S. A Review of Dichlorvos Toxicity in Fish. *Curr World Environ*, 2013; 8(1): 143-9.
- D'Souza UJA, Narayana K. Induction of seminiferous tubular atrophy by single dose of 5-fluorouracil (5-FU) in Wistar rats. *Indian J Physiol Pharmacol*, 2001; 45: 87-94.

8. Edem VF, Kosoko A, Akinyoola SB, Owoeye O, Rahamon SK, Arinola OG. Plasma antioxidant enzymes, lipid peroxidation and hydrogen peroxide in wistar rats exposed to Dichlorvos insecticide. *Arch Appl Sci Res*, 2012; 4(4): 1778-81.
9. Ojo AO, Oyinloye BE, Ajiboye BO, Ojo AB, Akintayo CO, Okezie B. Dichlorvos induced nephrotoxicity in rat kidney: protective effects of alstonia boonei stem bark extract. *IJP*, 2014; 1(7): 429-37.
10. Hagar HH, Fahmy AH. A biochemical, histochemical, and ultrastructural evaluation of the effect of dimethoate intoxication on rat pancreas. *Toxicol Lett*, 2002; 133: 161-70.
11. Verma RS, Srivastva N. Effect of chlorpyrifos on thiobarbituric acid reactive substances scavenging enzyme and glutathione in Rat tissues. *Indian J Biochem Biophysics*, 2003; 40: 423-28.
12. Diwivedi N, Bhutia YD, V. Kumar, Yadav P, Kushwaha P, Swarnkar H. Effects of combined exposure to dichlorvos and monocrotophos on blood and brain biochemical variables in rats. *Hum Exp Toxicol*, 2010; 29: 121-9.
13. Neishabouri EZ, Hassan ZM, Azizi E, Ostad SN. Evaluation of immunotoxicity induced by diazinon in C57bl/6 mice. *Toxicol*, 2004; 196: 173-79.
14. Oral B, Guney M, Demirin H, Ozguner M, Giray SG, Take G. Endometrial damage and apoptosis in rats induced by dichlorvos and ameliorating effect of antioxidant vitamins E and C. *Reprod Toxicol*, 2006; 22: 783-90.
15. Indian National Science Academy (INSA). Guidelines for care and use of animals in scientific research reported by Indian National Science Academy, New Delhi, 2000.
16. Ratnasooriya WD, Dharmasiri MG. Effects of Terminalia catappa seeds on sexual behaviour and fertility of male rats. *Asian J Androl*, 2000; 2: 213-19.
17. Prasad MRN, Chinoy NJ, Kadam KM. Changes in succinate dehydrogenase levels in the rat epididymis under normal and altered physiologic conditions. *Fertil Steril*, 1972; 23: 186-90.
18. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin Phenol Reagent. *J Biol Chem*, 1951; 193: 265-75.
19. Warren L. The thiobarbituric acid assay of Sialic acid. *J Biol Chem*, 1959; 234: 1971-75.
20. Montgomery R. Determination of glycogen. *Arch Biochem Biophys*, 1957; 67: 378-81.
21. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. *J Lab Clin Med*, 1953; 41: 486-92.
22. King EJ, Jagathesan KN. Method for estimation of acid and alkaline phosphatase. *J Clin Pathol*, 1959; 12: 85.
23. Belanger A, Caron S, Picard V. Simultaneous radioimmunoassay of progestins, androgens and estrogens in rat testis. *J Steroid Biochem*, 1980; 13: 185-90.
24. McLachlan RI, O'Donnell L, Meachem SJ, Stanton PG, De Kretser DM, Pratis K, Robertson DM. Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. *Recent Prog Horm Res*, 2002; 57: 149-79.
25. D'souza UJA, Narayana K. Induction of seminiferous tubular atrophy by single dose of 5-fluorouracil (5-fu) in wistar rats. *Indian J Physiol Pharmacol*, 2001; 45(1): 87-94.
26. Anway MD, Leathers C, Skinner MK. Endocrine disruptor vinclozolin induced epigenetic transgenerational adult onset disease. *Endocrinol*, 2006; 147(12): 5515-23.
27. 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: 135-38.
28. Jana K, Jana S, Samanta PK. Effect of chronic exposure to sodium arsenate on hypothalamic-pituitary-testicular activities in adult rats: possible an estrogenic mode of action. *Reproductive Biol & Endocrinol*, 2006; 4: 4-9.
29. Robaire B, Seenundun S, Hamzeh M, Lamour SA. Androgenic regulation of novel genes in the epididymis. *Asian J Androl*, 2007; 9(4): 545-53.
30. Choudhary N, Joshi SC, Goyal R. Effect of malathion on testicular cell population dynamics and fertility on male rat. *National J Life Sci*, 2005; 2(1-2): 17-21.
31. Nwanjo HU, Iroagba IN, Nnatuanya IN, Eze NA. Antifertility activity of dihydroartemisinin in male albino rats. *Int J Endocrinol*, 2007; 4(1): 3.
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: 119-22.
33. 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.
34. Joshi SC, Bansal B, Jasuja ND. Evaluation of reproductive and developmental toxicity of cypermethrin in male albino rats. *Toxicol & Environ Chem*, 2011; 93(3): 593-602.
35. Muroso 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.
36. Johal MS, Sharma ML, Ravneet. Impact of low dose of organophosphate, monocrotophos on the epithelial cells of gills of *Cyprinus carpio communis* Linn- SEM study. *J Environ Biol*, 2006; 27: 663-67.
37. Latchoumycandane C, Gupta SK, Mathur PP. Inhibitory effects of hypothyroidism on the testicular functions of postnatal rats. *Biomed Lett*, 1997; 56: 171-77.

38. Mac Donald AA, Herbison GP, Showell M, Farquhar CM. The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. *Hum Reprod*, 2010; 16(3): 293-311.
39. Joshi SC, Tibrewal P, Sharma A, 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: 338-42.
40. Kavlock R, Cumming A. Mode of action: inhibition of androgen receptor function vinclozolin, induced malformation in reproductive development. *Crit Rev Toxicol*, 2005; 35(8-9): 721-26.
41. Pareek TK, Joshi AR, Sanyal A, Dighe RR. Insights into male germ cell apoptosis due to depletion of gonadotropins caused by GnRH antagonists. *Apoptosis*, 2007; 12(6): 1085-1100.
42. Thejashwini MS, Krishna Ram H, Shivabasavaiah. Reversible antifertility effect of Cyamopsis psoraloides in male swiss albino mice. *Int J Adv Biol Res*, 2012; 2(4): 657-65.