ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Cauda Epididymal Lesion in Male Rats after Subchronic Exposure of Malathion

Nisha Jain[*], Arti Sharma[1] and SC Joshi[2]

[*] Department of Zoology, Government P.G. College, Dausa, Jaipur, Rajasthan, India
[1] Department of Zoology, Raj Rishi Government College, Alwar, Rajasthan, India
[2] Reproductive Toxicology Unit, Department of Zoology, University of Rajasthan, Jaipur, India

ABSTRACT

The present study sought to investigate the toxic influence of malathion, an organophosphate pesticide on epididymal function. Wistar male rats were administered malathion at daily dose of 50, 150 and 250 mg/kg/b.wt per day for 60 days. Caput, corpus and cauda epididymis were subjected to biochemical and histopathological analysis. A decrease in the weight of epididymis was observed. Also significant decrease in the sperm motility and sperm density in cauda epididymis was observed. Statistically a significant increase in protein content and decrease in sialic acid, glycogen content was observed at various dose levels of malathion treated rats. Histoarchitecture of caput, corpus and cauda epididymis showed various degenerative changes. In caput and corpus epididymis, the inter tubular epithelium is fused, diffused stereocilia, lumen with few spermatozoa and more giant cells. Whereas the cauda epididymis showed fusion of tubular epithelium with reduced inter tubular stroma, cell without stereocilia and lumen contains cellular debris with scanty immature spermatozoa. From the above mentioned findings it has been concluded that the epididymal function and structure is seriously affected by toxic effect of malathion.

Corresponding author
Email: nisha_choudhary@yahoo.com

ISSN: 0975-8585

INTRODUCTION

Pesticides comprise one of the major groups of chemicals introduced in the environment by man himself. Man is constantly exposed to these chemicals either through the food and water that he consumes or through the air he breathes. Due to extensive use of these pesticides it is almost impossible for anyone to avoid daily exposure to low level of several different pesticide residues. In recent years there is great concern that likes other chemicals these pesticides may modify the normal functioning of human and wild life endocrine system, including developmental, behavioral and reproductive hazards [1].

Malathion an organophosphate pesticide is used to kill insects on agricultural crops, stored products, on golf courses, in home and gardens. It is also used to kill mosquitoes medflies, fleas or pests and to treat head lice on human [2,3]. The organophosphate pesticide like methylparathion have been found to cause arrested sperm production and increased proportion of abnormal sperm [4].The Chloropyrifos causes atrophied leydig cells and decreased activity of testicular enzyme [5,6]. Malathion has also been reported for human birth defects [7], and multiple system organ failure [8,9]. Exposure to Malathion showed decreased testes weight and activity of testicular enzymes [10]. Degeneration of Seminiferous tubule and various sperm abnormalities has also been observed [11]. It is apparent from the literature that organophosphate causes toxicity still the thorough studies and new finding on reproductive toxicity, specifically on epididymis are lacking which needs attention in greater details. It is there for the present study was designed to provide comprehensive risk assessment of malathion on epididymal function and structure.

MATERIALS AND METHODS

Animal Model: Healthy Albino rats *(Wistar)* weighing 150-200 gms, 100 days old were used for experimentation. The animals were housed in polypropyline cages, measuring 430 x 270 x 150 mm. The animals were fed on pelleted standard rat chow supplemented with soaked grams and wheat. Water was provided *ad-libitum*. They were acclimatized for 7 days to the laboratory conditions at 22-24 ^{0}C with provision 12 h light: 12h dark cycle. The "guidelines for the care and use of animals for scientific research" was strictly followed (12).

Test Material: The Technical Grade Malathion (Diethyl[dimethoxy thiophosphorylthio] Succinate S-1,2bis [ethoxy carbonyl] ethyl o, o- dimethyl phosphodithioate) an organophosphate pesticide was obtained from excel India Ltd., Mumbai.

Median lethal dose (LD50) of Malathion: The LD_{50} is statistically derived single dose of a substance that can be expected to cause death in 50% of the animals. In this investigation various calculated doses (mg/kg. b. wt) of insecticide was given orally. Six animals were tested for each dose level. Poisoning symptoms and mortality was observed daily for three days following the treatment. Results of the toxicity were analyzed statistically (13) for the determination of LD_{50} of the malathion. Acute oral LD_{50} value was 1375-2800mg/ kg.b.wt for rats.

Experimental procedure: Animals were divided into four groups of six animals each. Group I animals were kept as control and were administered olive oil only, whereas animals of Group II, III and IV were treated with 50, 150, 250 mg/ kg b.wt./day of test compound. At the end of the experimentation, the rats were weighed, sacrificed under light ether anesthesia. The caput, corpus, cauda and other male reproductive organs were removed, weighed and processed for detailed sperm dynamical, biochemical, hormonal and histopathological studies.

Fertility Test: The mating exposure test of all the animals was performed. They were cohabited with proestrous females in the ratio of 1: 3.The vaginal plug and presence of sperms in the vaginal smear was checked for positive mating. The mated females were separated to note the implantation sites on day 16^{th} of pregnancy.

Sperm motility: Sperm motility was assayed by the method of Prasad (14). The epididymis was removed immediately after anesthesia and known weight of cauda epididymis was gently teased in a specific volume of physiological saline (0.9% NaCl) to release the spermatozoa from the tubules. The sperm suspension was examined within five minutes 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.

Sperm density: Sperm density was assayed [14]. Briefly total number of sperms were counted using haematocytometer after further diluted the sperm suspension from cauda epididymis and testes. The sperm density was calculated in million/ ml as per the dilution.

Biochemical Parameters: The total protein [15], Sialic acid [16] and glycogen [17] were assayed.

Hormonal Analysis: Radio immunoassay of testosterone level was also performed [18].

Histology: Cauda epididymis was fixed in Bouin's fixative and cut into pieces and processed through ethanol-xylene series. It was then embedded in paraffin and bee wax (3:1) (M.P. 55-62 oc). Sections were cut at 5 um thick and stained with Harris haematoxylene and eosin (H & E).

Statistical Analysis: Data were tested for normal distribution and then analyzed by analysis of variance (ANOVA) and the significance of difference was set up at ($P< 0.001$).

RESULTS AND DISCUSSION

Sperm are man's immediate and personal connection to the future of our species and the disappearance of half of this connection is hard to ignore. Sperm content in healthy men around the world have fallen about fifty percent in the last fifty years and exposure to pesticide is an important cause of this decline [19]. The present observation obtained after oral administration of malathion at various dose levels are shown in the legends and figure 1-8. A

significant reduction in the weight of epididymis was observed. The spermatozoa motility and density (Table1, Figure. 2, 3) in cauda epididymis was significantly decreased in a dose dependent manner [20]. A short decline in fertility in malathion treated rat was also observed. The epididymal biochemistry showed depletion of glycogen, sialic acid and elevation of epididymal protein (Figure 5-7). There was reduction in serum testosterone (Table 2, figure 8). The study also revealed that administration of malathion to male rats resulted in reproductive toxicity. The weight of epididymis is largely dependent on the mass of differentiated spermatogenic cells and the reduction in the weight of epididymis may be due to reduced tubule size, decreased number of germ cells and elongated spermatids [21]. Sperm motility is affected by altered enzymatic activities of oxidative phosphorylation. Full ATP pool in crucial for normal spermatozoal movement and a slight derivation of ATP leads to reduction in motility, which may cause infertility [22]. Another factor, which caused decrease in sperm motility, may be androgen deprivation effect of the pesticides. The epididymal spermatozoa are highly dependent on testosterone and epididymal protein for their final maturation and development of progressive motility and fertilizing capacity [23].

Table 1: Weight and sperm dynamic changes of rat Epididymis after Malathion treatment.

Parameters	Control	50mg/Kg.b.wt/day	150mg/Kg.b.wt/day	250mg/Kg.b.wt/day
Epididymis (wt in mg)	425.68±19.57	406.54 ± 19.20 ^{ns}	404.67 ±11.24*	395.15 ±28.61*
Sperm motility (%)	69.61±3.58	49.01 ±2.43*	19.72 ±1.31*	9.99 ±1.02*
Sperm density (million/ ml)	21.00±0.37	16.59 ±1.65*	10.85 ±1.08*	7.98 ±0.87*
Fertility (%)	100%	20% -ve	40%-ve	80% -ve

Table 2: Biochemical Changes in the Epididymis of rat after Malathion treatment.

Parameters	Control	50mg/Kg.b.wt/day	150mg/Kg.b.wt/day	*250mg/Kg.b.wt/day*
Glycogen (mg/gm)	1.69 ±0.13	0.99 ±0.25*	0.49 ±0.23*	0.23 ±0.02*
Sialic Acid (mg/gm)	4.53 ±0.08	4.17 ±0.05*	3.27 ±0.01*	3.20 ±0.01*
Protein (mg/gm)	199.21±4.94	215.93 ±9.87*	218.87 ±1.80*	255.10 ±13.86*
Serum Testosterone (ng/ml)	2.40 ±0.25	2.30 ±0.13*	2.00 ±0.02*	1.50 ±0.20*

Values given are Mean± SE of results obtained from 6 animals.
* = Significant (P≤0.001)
ns = Non significant

A positive correlation between testosterone and motility or fertilizing capacity of the spermatozoa has been reported. Low caudal epididymal sperm density may be due to alteration in androgen metabolism. The physiological and biochemical integrity of epididymis

ISSN: 0975-8585

are dependent on androgens [24]. The 80% negative fertility test may be attributed to lack of forward progression and reduction in density of spermatozoa and altered biochemical milieu of cauda epididymis [4].

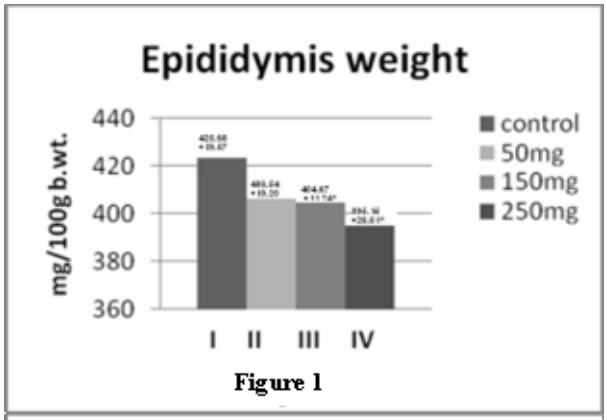


Figure 1

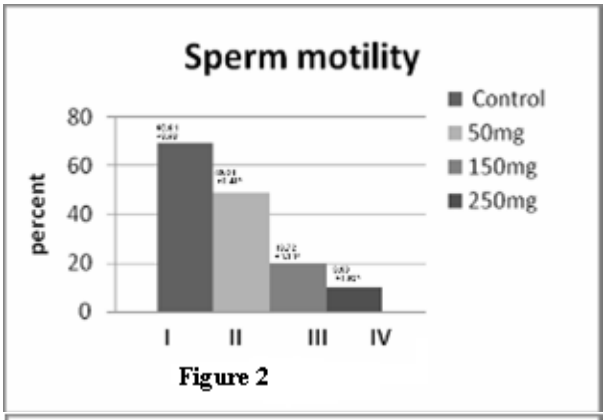


Figure 2

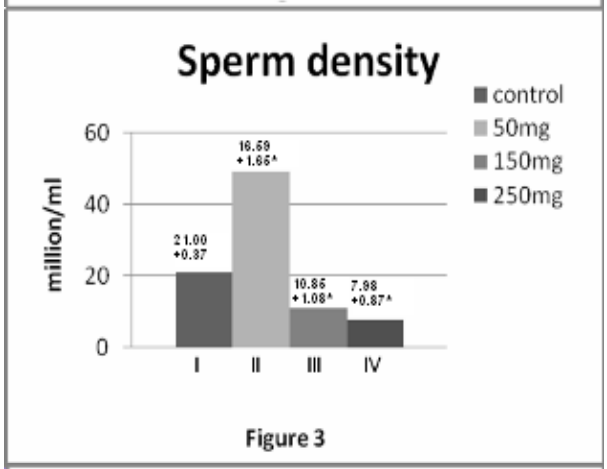


Figure 3

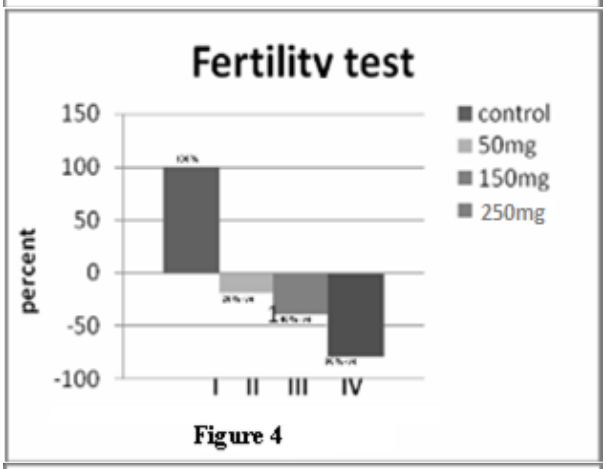


Figure 4

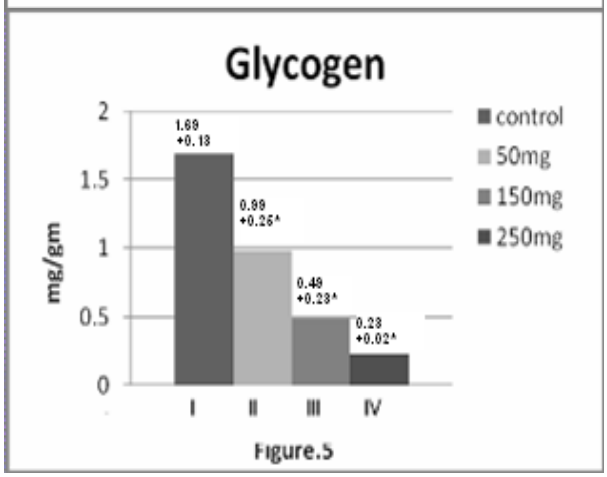


Figure.5

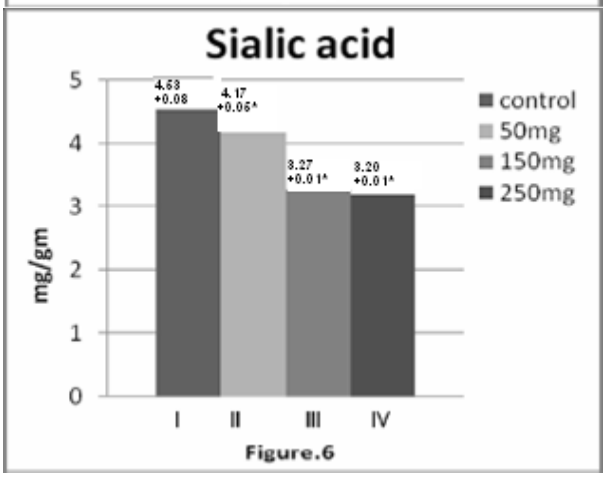


Figure.6

ISSN: 0975-8585

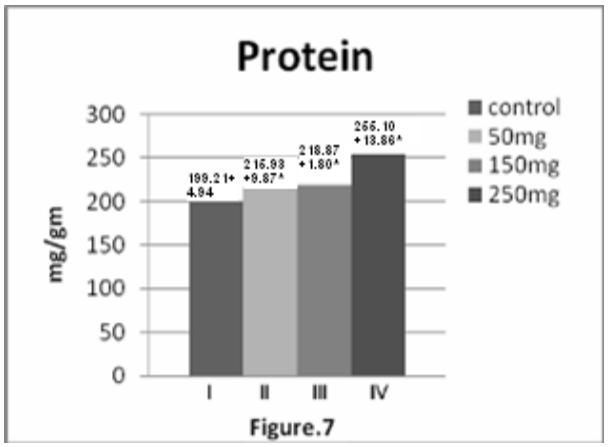


Figure.7

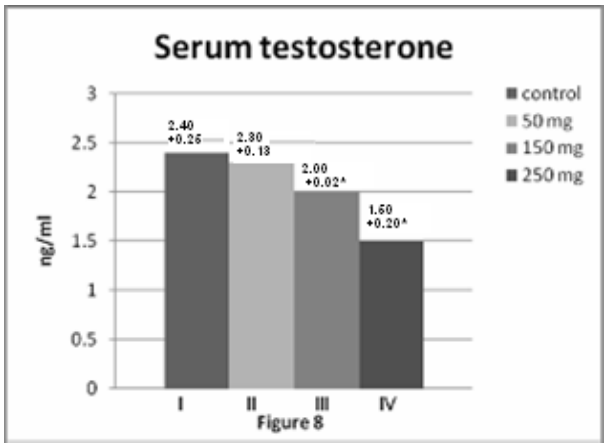
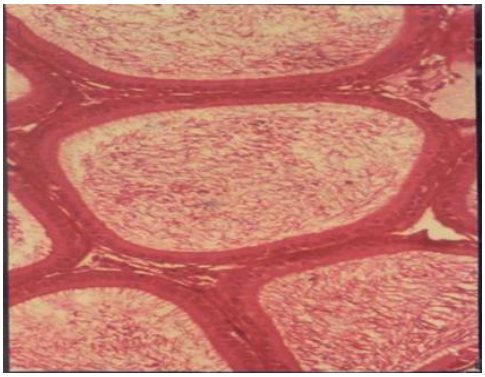
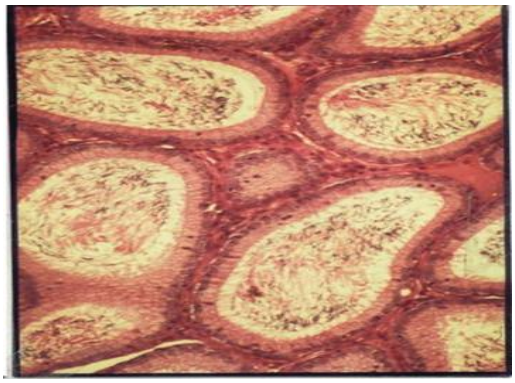


Figure 8

Administration of malathion also changes the biochemical parameters of the reproductive tract like other pesticides [25]. A fall in glycogen level may be due to interference in glycogenolysis. Since glycogen is an energy source for general metabolism and constant supply of glucose is essential for proper functioning of epididymis [26] similarly reduction in sialic acid content may be due to absence of spermatozoa or reduced androgen production [27]. Elevation in total protein content may be due to the hepatic detoxification, which results in the inhibitory effect on the activities of enzymes involved in the androgen biotransformation [28]. The reduction in serum testosterone demonstrated the inhibitory effects of malathion on the secretion of pituitary gonadotrophins (FSH and LH) [29,30] and in turn on the testosterone biosynthesis like other pesticides [31, 32].

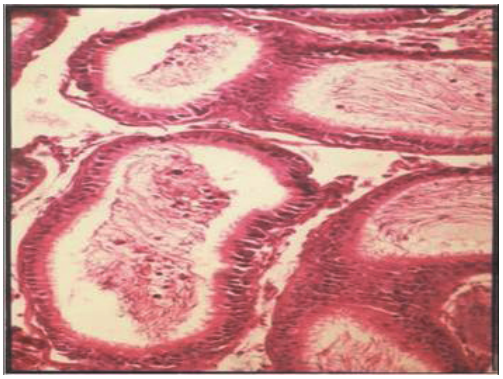


Microphotograph I
Microphotograph of cauda epididymis of control rats showing large tubules, lined with psuedo stratified columnar epithelial cells with long prominent stereocilia.

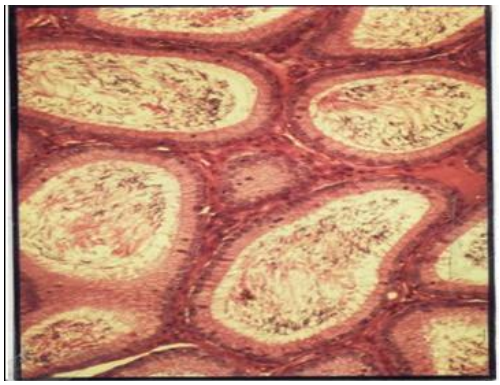


Microphotograph II
Microphotograph of 50 mg dose of cauda epididymis showing degenerative changes, the inter tubular epithelium is fused, diffused stereocilia, lumen contains spermatozoa with giant cells.

ISSN: 0975-8585



Microphotograph III
Microphotograph of cauda epididymis of 150 mg dose exhibits tubules with short stereocilia. Cluster of spermatozoa are present in the lumen. Inter tubular stroma is disrupted.



Microphotograph IV
Microphotograph of 250 mg dose of cauda epididymis showing reduced tubular size but increased epithelial height. Lumen is devoid of spermatozoa.

Microphotograph (Fig.9-I) showed the cauda epididymis of control rat with normal histological features like large tubules lined with pseudostratified columnar epithelial cells with long prominent stereocilia. Inter tubular stroma contains connective tissues and blood vessels. The lumen is filled with mature spermatozoa, whereas marked histological changes were observed in the epididymis of malathion treated rats (Fig.9 II-IV) like other pesticides [33, 34]. Epididymis plays an integral role in male reproduction by providing a favorable fluid micro environment for sperm maturation and storage and fluid secreted by epididymis is controlled by neurotransmitter substances that is allocrine and paracrine hormones [35]. The physiological and biochemical integrity of epididymis depend upon androgen deficiency of androgens caused a marked reduction in tubular diameters regression of epididymal epithelium, decline in spermatozoal number in cauda epididymis and change in composition of epididymal plasma [36, 37] .In conclusion the result of the present study showed the toxic effect of malathion on epididymal structure and function of male rats.

REFERENCES

[1] USEPA 1997.US, Environmental protection agency. Special report on Environmental endocrine disruption. An effect assessment and analysis 1997, EPA / 630/R-96/012.
[2] Hazarika A, Sarkar SN, Hazare S, Kataria M, Malik JK. Toxicology 2003; 185(2): 1-8.
[3] Agency for Toxic Substances and disease Registry (ATSDR) 2003; 121:75-85.
[4] Joshi SC, Mathur R, Gajraj A, Sharma T. Env Toxicol Phamacol 2003; 14: 91-98.
[5] Bhangalew C, Norton SP, Gupta S, Bhatnagar U, Gupta A. J Environ Zoo 1992; 6(1): 17-19.

[6] Karanthi S, Lia J, Olivier K, Pope, JC. Toxicol Appl Pharmacol 2004; 196: 183-190.
[7] Lindouout DR. Teratology 1987; 36:7-9.
[8] Betrosian A, Ballami A. J Toxicol Clin Toxicol 1996; 34(2).
[9] Abdelgadirand EH, Adam SEI. J Pharmacol Toxicol 2011; 6(1): 69-75.
[10] Balasubramaniam K. Med Sci Res 1997; 15: 219-30.
[11] Kumar S, Nath A. Ind J Mendel 1997; 14: 27-29.
[12] INSA (Indian National Science Academy).. Guidelines for care and use of Animals In Scientific Research Indian National Science Academy, New Delhi, 2000.
[13] Finney,DJ. Probit analysis (3rd) Cambridge University Press.Cambridge1971; : 33.
[14] Prasad MRN. In: Pharmacology and the future of man Proc 5th Int Cong Pharmacology Sanfransisco, 1972
[15] Lowry OH, Oser Rought, MJ, Randoll RJ. J Biol Chem 1951; 193(3):257-265.
[16] Warren L. J Biol. Chem. 1959; 234:1971-1975.
[17] Montgomery R. Arch Biochem Bio phys 1957; 67:378-89.
[18] Belanger A, Caron S, Picard V. J Steriod Biochem 1980; 13:185-190.
[19] Sharpe RM. J Endocrinol 1993; 56:357-360.
[20] Cevdet U, Omer V,Cansu A, Yuksel A. Repr Toxicol 2009; 28(4):542-549
[21] Sanhez Penal LC, Reyes, BE, Labez carrill OL. Toxicol Appl Pharm 2004; 94 (1):108- 13.
[22] Bedford JM. Biol Report 1983; 28:108-120.
[23] Vawda A I, Davies AG. Acta Endocrinol. 1986; 1130:436-441.
[24] Mukherjee M, Chatopadhyay S, Mathur PP. Andrologia 1992; (24):113-116.
[25] Bhatnagar VK, Malviya AN. Ind J Biochem Biophys 1986; 15: 23-25.
[26] Levinsky HR, Singer M, Barnet M, Sangive M, Allaloaf D. Arch Androl Toxicol 1983; 74:270-275.
[27] Gupta G, Shrivastva K, Setty BS. J Exp Biol 1993; 31:350-355.
[28] Grover JK, Vyas V, Shrinivas M, Das SN, Gupta DR, Mitra DK. Indian J Exp Biol 2001, 39:1160-1162
[29] Prakash N, Venkatesh N. Indian J Pharm 1996; 28:257-260.
[30] Latchoumycandane C, Chitra KC, Mathur PP. Repr Toxicol 2002; 16:161-172.
[31] Avbel AF, Plymate SR, Word, GS, Garrison MJ, Farris BL. Fert Ster 1981; 36: 427- 428.
[32] Chia SE. Ind J Androl 2000; 23:45-46.
[33] Akbarsha MA, Sivaswamy P. Ind J Exp Biol 1998; 36: 34 -38.
[34] Gangadharan B, Murugan M, Arun , Mathur PP. Asia Androl 2001; l 3: 285-288.
[35] Brooks DE. In: The spermatozoa. Fawcett, D.E. and Bedford JM (eds), Urban and Schwargenber Baltimore 1979; 23-24.
[36] Brooks DE. Physical Rev 1981; 61:515-555.
[37] Kasturi A, Manivannan B, Ahmed RN, Shaikh PD, Pathan KN. Indian J Exp Biol 1995; 33 (10):725-729.