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Effect of malathion on reproductive system of male rats

Nisha Choudhary*, Rekha Goyal and S.C. Joshi

Reproductive Toxicology Unit, Department of Zoology, University of Rajasthan, Jaipur-302 004, India

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Abstract: *The pesticides are one of the most potentially harmful chemicals liberated in the environment in an unplanned manner. Malathion is widely used as a potent pesticide in many countries and has been shown to produce some adverse health effects. A study was conducted to asses the effects of malathion on the male reproductive system of wistar rats. The pesticide was administered to rats orally at dose levels of 50, 150 and 250 mg/kg/body wt./day for 60 days. In comparison to the control rats, there was a significant reduction in the weight of testes, epididymis, seminal vesicle and ventral prostate. Testicular and epididymal sperm density were decreased in the animals treated with malathion. Pre and post fertility test showed 80% negative results after treatment. Biochemical profile of the testis revealed a significant decline in the contents of sialic acid and glycogen. Whereas a significant increase in the protein content of testis and testicular cholesterol was observed. The activity of testicular enzyme acid phosphatase increased significantly, while decreased alkaline phosphatase activity was found. Malathion also suppressed the level of testosterone significantly. Results of the present study clearly suggest that malathion induce toxic effects on the male reproductive system of rats.*

Key words : *Malathion, Testis, Spermdynamics, Testosterone*
PDF of full length paper is available with author ***(*nisha_choudhary@yahoo.com)***

Introduction

These days pollution of the environment by pesticides is a problem of great importance and is of every body's concern. There is a great concern that like other chemicals, these pesticides may modify the normal functioning of human and wild life endocrine system. Malathion an organophosphate pesticide has been reported for human birth defects (Lindouout, 1987). Exposure to malathion showed decreased testes weight and activity of testicular enzymes (Balasubramaniam, 1987). It is also reported to cause multiple system organ failure (Betrosian and Ballami, 1996; Johal *et al.,* 2007) and effects antioxidant defense system in human fetus (Gupta *et al.,* 1992). Malathion when given orally to mice caused degenerative effects on testes leading to their infertility (Kumar and Nath, 1997). In present study a sufficient number of well validated end points were investigated in order to provide comprehensive risk assessment of the entire reproductive system. We examined the toxic influence of malathion on fertility, testicular biochemistry and serum testosterone level of male rats.

Materials and Methods

Test animal : Twenty four healthy adult male wistar rats (100 days old) weighing 150-200 g were maintained at a room temperature (20° ± 5°C) and uniform light dark cycle (14 : 10 : : L : D) with relative humidity 55 ± 5%. They were fed on standard commercial pelleted feed (Ashirwad Food Industries Ltd., Chandigarh, India) and fresh water *ad-libitum* throughout the study. The weight of animals were measured weekly to see any change in the body weight. Males were cohabited with proestrus females in the ratio of 1 : 3, only fertile males were used for the study. The study was approved by the ethical committee of the Department of Zoology, University of Rajasthan Jaipur - 302004 (India). The "guidelines for the care and use of animals for scientific research" was strictly followed (INSA, 2000).

Testing dose: Technical grade malathion (Diethyl [dimethoxy thiophosphorylthio] Succinate S-1, 2 bis (ethoxy carbonyl) ethyl o, o- dimethyl phosphodithioate) obtained from Hoechst Bombay, India was used for experimentation. The pesticide was administered to male rats orally at dose levels of 50, 150 and 250 mg/kg b.wt./ day for 60 days.

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 male reproductive organs were removed, weighed on electronic balance and processed for detailed spermdynamical, biochemical and hormonal 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 16th of pregnancy.

Sperm motility: Sperm motility was assayed by the method of Prasad (1972). The epididymis was removed immediately after anaesthesia 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.

Table - 1: Biochemical changes in the testes of rat after malathion treatment

Parameters	Control (G-I)	50 mg/kg.b.wt./day (G-II)	150 mg/kg.b.wt./day (G-III)	250 mg/kg.b.wt./day (G-IV)
Glycogen (mg/gm)	2.70 (± 0.13)	1.36 (±0.06)*	0.45 (±0.01)*	0.28 (±0.01)**
Sialic acid (mg/gm)	5.10 (±0.19)	4.48 (±0.10)	4.54 (±0.07)*	4.09 (±0.05)**
Protein (mg/gm)	255.30 (±17.20)	338.62 (±3.55)*	373.74 (±8.45)*	373.99 (±12.81)**
Cholesterol (mg/gm)	5.92 (±0.41)	7.06 (±0.85)	9.00 (±0.28)*	9.50 (±1.19)**
Acid phosphatases (KA units)	4.59 (±2.28)	11.80 (±1.27)*	11.78 (±0.90)*	12.48 (±2.66)*
Alkaline phosphatases (KA units)	65.14 (±2.28)	41.73 (±0.40)	42.38 (±1.74)*	42.40 (±2.00)*

Values given are mean of results obtained from 6 animals
* Significant ($p \leq 0.001$) Group II, III and IV compared with Group I
** Significant ($p \leq 0.001$) Group IV compared with Group II

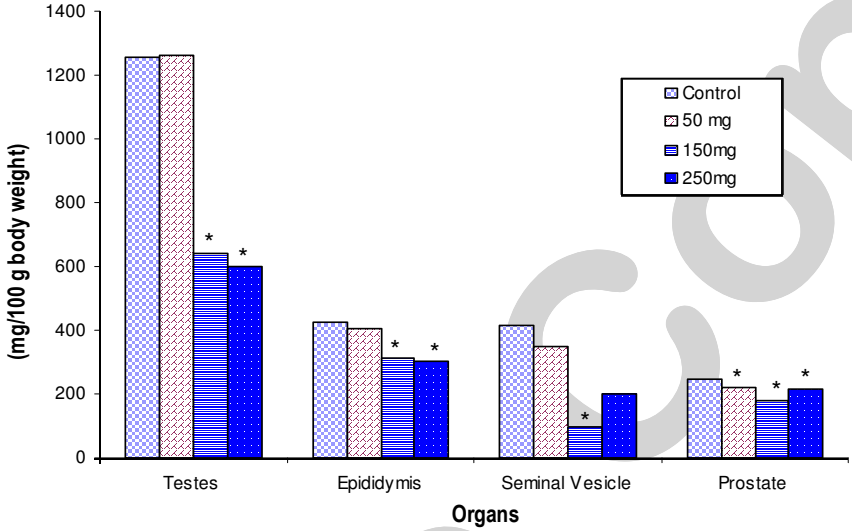

Fig. 1: Changes in organ weights after malathion treatment (values given are of 6 determination, * $p \leq 0.001$)

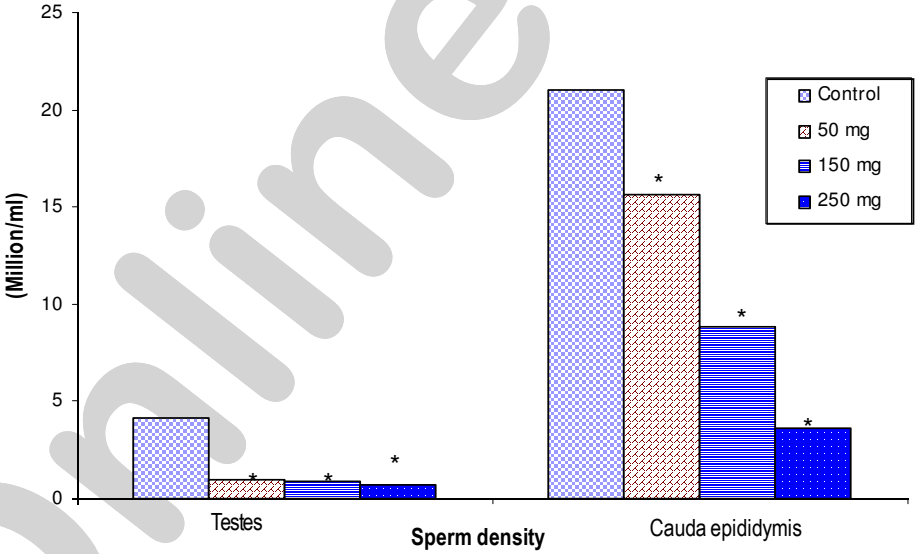

Fig. 2: Sperm density in testes and cauda epididymis after malathion treatment (values given are of 6 determination, * $p \leq 0.001$)

Sperm density: Sperm density was assayed by the method of Prasad (1972). Briefly total number of sperms were counted using haematocytometer after further diluting the sperm suspension of cauda epididymis and testes. The sperm density was calculated in million / ml as per the dilution.

Biochemical parameters: The total protein (Lowry *et al.*, 1951), Sialic acid (Warren, 1959), glycogen (Montgomery, 1957) and cholesterol (Zlatkis *et al.,* 1953) were assayed. Acid and alkaline phosphatase enzymatic activity were also determined by King and Jagthessan (1959) method.

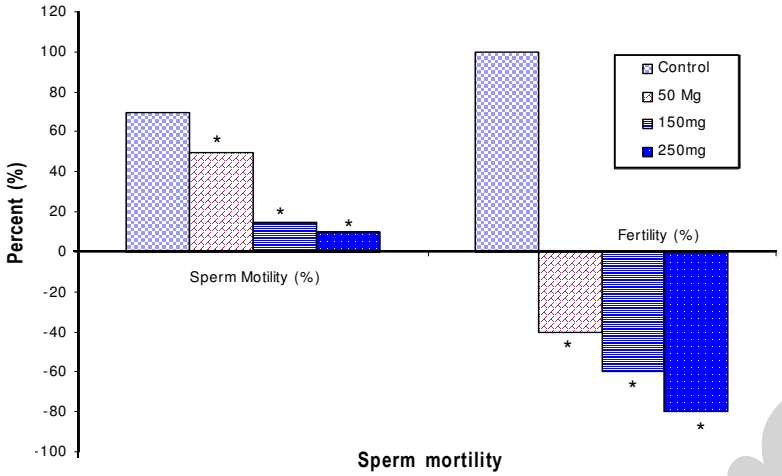

Fig. 3: Sperm motility in cauda epididymis and fertility test after malathion treatment (values given are of 6 determination * $p \leq 0.001$)

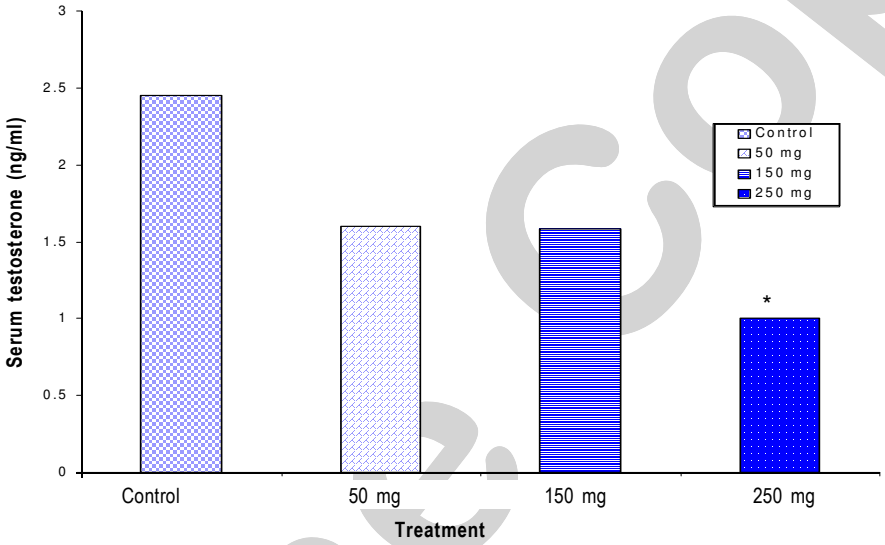

Fig. 4: Altered serum testosterone after malathion treatment (values given are of 6 determination * $p \leq 0.001$)

Hormonal analysis: Radio immunoassay of testosterone level was also performed (Belanger *et al.*, 1980).

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 ($p \leq 0.001$).

Results and Discussion

The exponential increase in the production use and disposition of pesticides have a profound impact on the environment and creates unforeseen hazards to man's well being (Chia, 2000; Karallieda *et al.*, 2003 and Karanthi *et al.*, 2004). The present observations obtained after oral administration of malathion at various dose levels are shown in the Table 1 and Figs. 1-4.

The study revealed that administration of malathion to male rats resulted in reproductive toxicity. 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 and elongated spermatids (Sanhez *et al.,* 2004). The observed reduction in weight of accessory sex organs may be due to reduced bio-availability of estrogenic and/or antiandrogenic activities of malathion (Linder *et al.,* 1988). 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 (Bedford, 1983). 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 (Vawda and Davies, 1986). A positive correlation between testosterone and motility or fertilizing capacity of the spermatozoa has been reported. Compounds of seminal vesicle secretion also acts as energy source for sperm motility (Eliasson, 1985). Low caudal epididymal sperm density may be due to alteration in androgen metabolism. The physiological and biochemical integrity of epididymis are dependent on androgens (Mukherjee *et al.,* 1992). 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 (Joshi *et al*., 2003).

Treatment with malathion also changes the biochemical parameters of the reproductive tract like other pesticides (Bhatnagar *et al*., 1996). 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 testes. Similarly reduction in testicular sialic acid content may be due to absence o spermatozoa or reduced androgen production (Levinsky *et al.,* 1983). 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 (Dikshith and Datta, 1972; Venkataramana *et al*., 2006). Increased concentration of cholesterol in testes suggests that impairment of spermatogenesis is due to decreased androgen concentration (Bedwal *et al*., 1994). A significant reduction in the alkaline phosphatases activity may be attributed to the decreased osteoblastic activity of bone, since it is formed and present in the osteoblasts (Naqvi and Vaishnavi, 1993). The increase in acid phosphates activity may be the result of labialization of lysosomal system (Johal *et al.,* 2003). The reduction in serum testosterone demonstrated the inhibitory effects of malathion on the secretion of pituitary gonadotrophins (FSH and LH) (Prakash and Venkatesh, 1996) and in turn on the testosterone biosynthesis like other pesticides (Avbel *et al.,* 1981 ; Singh and Pandey, 1990). Hence, from these results it can be concluded that malathion exerts testicular toxicity in *wistar rats.*

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