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Monocrotophos induced inhibition of the activities of testis and accessory reproductive organs in male mice

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SUMMARY

Monocrotophos was administered orally to adult male albino mice at dose level of 3.0 mg/kg body weight/day/mice for 50 days. The treatment has found to affect spermatogenesis as well as the endocrine functions of the testis as indicated by gravimetric, histopathological and biochemical changes. The treatment has caused degenerative changes in the seminiferous tubules and Leydig cells of the testis and regression of the epididymis, seminal vesicle, vas deferens, prostate, Cowper's gland and levator ani. Similarly, cauda epididymal sperm count and sperm motility have shown significant reduction. There was a significant reduction in the protein, glycogen, sialic acid, acid and alkaline phosphatase and increase in cholesterol in the testis of monocrotophos treated mice compared with the control. The causative factors for these changes due to monocrotophos administration were discussed.

Key words: Monocrotophos; Mice; Testes; Sperm count; Sperm motility

INTRODUCTION

Monocrotophos (MCP) is an organophosphorus insecticide is widely used for agricultural pest control in India, because of its effectiveness at low dosages (Ray *et al.,* 1985). MCP has high oral toxicity at cellular level (Skripsky and Loosi, 1985). The presence of MCP in the aquatic environment would adversely affect many non-target species like fish and these species die because of malfunctioning of cells (Mount and Putnick, 1963). Laboratory studies indicate that exposure of fish to sublethal concentration of MCP leads to many histopathological lesions in the brain like necrosis of neurofibrillar region, vascular dilation, nuclear pyknosis, vacuolation etc. (Santhakumar, 2000). Since MCP is used for the control of common crop pests in this area, nontarget organisms are also likely to be exposed to the same always. Recently, Ratnasooriya *et al.* (1996) reported Monocrotophos inhibited fertility in female rats (in terms of uterine implants and implantation index) and in male rats, reduced sperm count and motility of cauda epididymis. The present study has been designed to show the effect of MCP on biochemical and histological transformations in the testis and accessory reproductive organs of adult mice.

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MATERIALS AND METHODS

Monocrotophos

Monocrotophos (phosphoric acid dimethyl) (1-

methyl) (methyl amino)–3-oxo-n-propnyl) ester) was purchased from United Phosphorus Ltd., Bombay at 36% concentration.

Animal model

Healthy, adult, virgin inbred male albino mice *(Mus musculus)* of Swiss strain weighing 30 - 35 g and 80 - 90 days old were maintained at room temperature of 28 ± 2ºC with lighting schedule of 12 h light and 12 h darkness. They were fed with a balanced diet as prescribed by Central Food and Technological Research Institute (CFTRI, Mysore) formula and water *ad libitum.*

Dose and duration of treatment

MCP was dissolved in 0.9% saline to make up the desired volume. The first group of animals were maintained on a standard diet and served control. The second group of animals received MCP orally by means of an intragastric catheter at dose level of 3.0 mg/kg body weight for 50 days.

Autopsy schedule

After 24 h of last treatment of respective duration, the animals were weighed and sacrificed by cervical dislocation.

Data collection

Testes, epididymis, vas deferens, seminal vesicle, ventral prostate, Cowper's gland and levator ani were dissected out, blotted free of blood and carefully made free from surrounding fat and connective tissues then weighed up to the nearest milligram on electronic balance. From the epididymis, caput and cauda regions were separated out. The organs from one side of each animal were fixed in bouin's fluid for histological studies. The tissues were embedded in paraffin, sectioned at 5 μm, stained with Haematoxylin-eosin (Gurr, 1962). The organs from the other side were processed for biochemical estimations like protein (Lowry *et al.,* 1951), cholesterol (Peters and Vanslyke, 1946), glycogen (Carrol *et al.,* 1956), sialic acid (Jourdian *et al.,* 1971), acid and alkaline phosphatase (Bessey *et al.,* 1946). The cauda epididymal sperm suspension was prepared in normal saline and count and motility of cauda epididymal spermatozoa of control and treated mice were determined by the method described by Kempinas and Lamano Carvalho (Kempinas and Lamano-Carvalho, 1987).

Statistical analysis

The data were statistically analyzed by using the student's '*t*' test. '*P*' values less than 0.05 were considered significant.

RESULTS

Effect on body weight

The body weight ($P < 0.05$) was significantly decreased in the MCP treated mice compared to that of control mice (Table 1).

Changes in the testis

Gravimetric and histometric changes

MCP administered orally at a dose 3.0 mg/kg body weight for 50 days has significantly reduced the weight of testis ($P < 0.001$). Histometric data revealed significant reduction ($P < 0.001$) in the diameter of testis and seminiferous tubules (Tables 1 and 2).

Table 1. Changes in the body and reproductive organ weights due to treatment of MCP

Treatment	Body weight (g)	Testes	Epididymis	Vas deferens	Seminal vesicle	Ventral prostate	Cowper's gland	Levator ani muscle
Control	32.61 ± 0.24	384.84 ± 6.07	109.69 ± 1.13	133.93 ± 1.13	350.29 ± 2.27	61.00 ± 0.44	81.00 ± 0.54	107.00 ± 0.44
Monocrotophos	31.60 ± 0.25*	343.63 ± 2.81***	94.54 ± 2.23**	101.20 ± 4.01***	331.51 ± 2.27**	49.00 ± 0.54	51.4 ± 0.24***	73.67 ± 0.13***

Organ weights: mg/100 g body weight; Dose: 3.0 mg/kg body weight; Duration: 50 days. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, when compared control.

Table 2. Changes in the testis due to the treatment of MCP

Treatment	Weight of testis (mg/ 100 g b.w.)	diameter of testis (μm)	Diameter of seminiferous tubule (μm)	Acid phos-photase (μM/mg/30 mts)	Alkaline phosphatase (μM/mg/30 mts)	Protein (mg/ 100 mg)	Choles-terol (μg/ 100 mg)	Glycogen (μg/100 mg)	Sialic acid (mg/g)
Control	384.84 ± 6.07	286.4 ± 2.73	91.8 ± 2.27	7.138 ± 0.08	2.903 ± 0.00	3.6 ± 0.24	2.36 ± 0.09	1.309 ± 0.17	5.9 ± 0.31
Monocrotophos	343.63 ± 2.31***	207.6 ± 2.31***	70.6 ± 2.81***	4.594 ± 0.09***	2.859 ± 0.01*	2.166 ± 0.11***	4.00 ± 0.24***	0.641 ± 0.01*	4.02 ± 0.25

Dose: 3.0 mg/kg body weight; Duration; 50 days. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, when compared to control.

Biochemical changes

A significant decrease in the acid phosphatase ($P < 0.001$) activity, protein ($P < 0.001$) and glycogen ($P < 0.01$) contents were observed in the MCP treated animals. Whereas, cholesterol ($P < 0.001$) content and alkaline phosphatase ($P < 0.05$) activity was increased significantly (Table 2).

Histological changes

In the histological sections of the testis, a significant reduction in the number of spermatogonia ($P < 0.001$), spermatocytes ($P < 0.001$) and spermatids ($P < 0.001$) was observed. Necroses in tubular epithelium, shrinkage of Sertoli cells were recorded. Pyknosis in the primary and secondary spermatocytes was observed. No spermatozoa were observed in the lumen of seminiferous tubules in the MCP treated group. The Leydig cells in the MCP received groups were degenerated (Table 3 and Fig. 1).

Changes in accessory reproductive organs

Significant decrease in the weight of epididymis ($P < 0.001$), vas deferens ($P < 0.001$), seminal vesicle ($P < 0.001$), ventral prostate ($P < 0.001$), Cowper's gland ($P < 0.001$) and Levator ani ($P < 0.001$) was observed due to treatment of MCP. In cauda and caput epididymis, the protein ($P < 0.01$) content

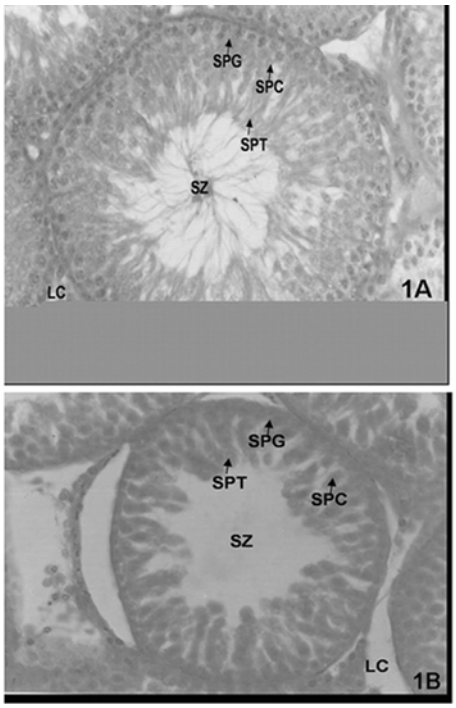

Fig. 1. (**A**) T. S. of control mice showing normal seminiferous tubules with all types of spermatogenic elements and spermatozoa in the lumen. Note the healthy Leydig cells × 400. (**B**) T. S. of testis of MCP treated mice showing shrinkage of seminiferous tubules, decreased interstitium and Leydig cells. Note the significant decrease in the spermatogonia, spermatocytes and spermatids and absence of spermatozoa × 400. Leydig cells : LC, Spermatozoa : SZ, Spaermatocyte : SPC, Spermatogonia : SPG, Spermatid : SPT.

Table 3. Changes in spermatogenic elements due to treatment of MCP in mice

Treatment	Spermatogonia	Spermatocytes	Spermatids	Spermatozoa	Sperm count in cauda epididymis (millions/ml)	Sperm motility (%) in cauda epididymis
Control	85.4 ± 1.12	85.2 ± 0.37	96.0 ± 0.54	Numerous	35.6 ± 0.60	78.4 ± 2.46
Monocrotophos	46.6 ± 0.51***	65.4 ± 0.75***	13.2 ± 0.58***	Nil	6.2 ± 0.73***	8.6 ± 0.06***

Dose: 3.0 mg/kg body weight; Duration: 50 days. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, when compared to control.

Table 4. Changes in the biochemical contents of epididymis and vas deferens due to MCP treatment in albino mice

Treatment	Cauda epididymis		Caput epididymis		Vas deferens	
	Protein (mg/100 mg)	Cholesterol (μg/100 mg)	Protein (mg/100 mg)	Cholesterol (μg/100 mg)	Protein (mg/100 mg)	Glycogen (μg/100 mg)
Control	1.14 ± 0.068	0.312 ± 0.012	4.6 ± 0.245	0.24 ± 0.007	3.16 ± 0.098	1.137 ± 0.013
Monocrotophos	0.68 ± 0.091**	0.432 ± 0.012***	3.6 ± 0.245*	0.388 ± 0.004***	2.76 ± 0.075*	0.844 ± 0.029***

Dose: 3.0 mg/kg body weight; Duration: 50 days. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, when compared to control.

was decreased and cholesterol ($P < 0.001$) content was increased significantly in all the animals treated with MCP. In vas deferens, the protein ($P < 0.05$) and glycogen ($P < 0.001$) content decreased significantly when compared to that of control (Tables 1 and 4).

Sperm count and motility

Though sperms are available in the cauda epididymal preparation, their number and motility was significantly reduced ($P < 0.001$) when compared to that of control (Table 3).

DISCUSSION

The pesticides that are used to control the pests are slow poison to human system. Reports are available on the degenerative changes in seminiferous tubules and haemorrage in intertubular areas of testis due to repeatedly exposed pesticides in rats (Dutta and Dikshith, 1973). Degenerative changes in seminiferous epithelium, spermatogenic cells, shrinkage and hyalinization of seminiferous tubules, increase in intertubular space were observed after exposure of BHC to mice (Nigam *et al.*, 1979). Degenerative changes in Sertoli cells and decreased number of spermatogenic elements were observed after exposure of rats Carbofuran (Pant *et al.*, 1997). Dimethoate is an organophosphorus insecticide caused testicular damage, damage to sperm production and reduction in testosterone level when fed to adult male rats (Afifi *et al.*, 1991).

In the present study the reduction in the weight of testis may be due to the decreased production of seminiferous tubular fluid, which contributes to the weight of testis (Ghosh *et al.*, 1992). The reduced protein content may also be another reason, as the growth rate of any organ is proportional to its protein content. It is evident pituitary FSH stimulates the development of spermatogonia to spermatocytes and also maintains the spermatogenic process (Connel and Eik-Nes, 1968; Johnson and Ewing, 1971; Holt *et al.*, 1973; Dorrington and Armstrong, 1975). Both FSH and LH/ICSH are necessary for meiosis and development of spermatids (Lostroch, 1963). The androgens induce meiosis, formation and development of spermatids in response to FSH (Chemes *et al.*, 1979; Heneji and Srivastava, 1984; Russel *et al.*, 1987; Hall, 1994). The observed reduction in the number of spermatogonia, spermatocytes and spermatids may indicate lowered availability of FSH and LH/ICSH, which are essential for initiation and maintenance of spermatogenesis. It is known that sperm production cannot proceed optimally to completion without continuous androgen supply (Mohri *et al.*, 1978). However, the incidences of low sperm count imply MCP induced infertility through the consequence of an array of factors in biochemical events in tissues due to imbalance in hormonal availability. Low level of protein in the testis after the treatment of MCP may impede the arrest of spermatogenesis and cause inhibition of gonadotrophin and androgen output. Further atrophy in androgen target organs, rarefaction in stage specific spermatogenic cells and decrease of protein contents point out that MCP exhibited its antiandrogenic effects in mice.

The glycogen content in the cell indicates energy storage. Sertoli cells and spermatogonia often contain glycogen and secrete substrates from the blood and provide source of reserve carbohydrates

for seminiferous tubular cells, and the glycogen level is found to be directly proportional to the steroid hormones synthesis (Gregoire *et al.*, 1967). Therefore, the decreased glycogen content of the testis after the administration of MCP may be due to reduced number of spermatogonia and this decreased glycogen content may provide less energy for spermatogenic activity, which might have resulted decrease in spermatogenic number.

Acid and alkaline phosphatase, which was associated with lysosomes, have an important role in the metabolism of carbohydrates, phospholipids and nucleotides (Monicalilly, 1996). Further these enzymes are also associated with membrane ion transport mechanisms, motility and viability of spermatozoa and these are indicators of androgen levels (Eliasson and Lindholmer, 1976; Sidharthan *et al.*, 1993). They also regulate the aecretory activity of the testis and are widely distributed in the testis and are important in the physiology of sperm (Mann, 1964). In the present study the activity of acid phosphatase is decreased while the alkaline phosphatase is increased in the testis due to MCP treatment. The decrease in the acid phosphatase activity indicates the failure in the acidification mechanism, which might have resulted in the poor sperm development and decline in endogenous androgen production (Breton *et al.*, 1996). The increased cholesterol content of testis after the administration of MCP also indicates reduced conversion of cholesterol to androgen, which is dependent on the availability of LH/ICSH (Catt *et al.*, 1974; Rommerts *et al.*, 1974).

Epididymis is responsible for many important functions such as the secretion and synthesis of proteins involved in the metabolism and physiological maturation of spermatozoa (Sarkar, 1996). The reduction in the protein content of the cauda epididymis is attributed to non-availability of androgens, which are responsible for protein synthesis in the epididymis (Cameo and Blaquire, 1976). Several investigators have reported that the conversion of cholesterol to pregnanalone in the leydig cell depends upon the availability of LH (Catt *et al.*, 1974). The increased level of cholesterol in the cauda epididymis shows hampered steroidogenesis, which has lead to reduced conversion of cholesterol to androgens, which may be due to inhibition in the release of pituitary LH. Sialic acid is a sialomucoprotein essential for the maintenance of the structural integrity of the sperm membrane and sperm maturation (Chinoy and Sequeira 1989). Therefore a reduction in the sialic acid concentration in the testis could be responsible for the morphological abnormalities observed in spermatozoa. Antiandrogenic action of the MCP is reflected in the regression and disintegration of Leydig cells, caput and cauda epididymis, vas deferens, seminal vesicle, ventral prostate, Cowper's gland, Levator ani.

Therefore it may be concluded that long-term exposure of MCP brings the degenerative changes in the testis and accessory reproductive organs by imbalancing the availability of the hormones. Further the action of MCP whether mediated through hypothalmo-hypophyseal axis or direct through both is to be determined.

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