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Effect of Acephate on Testicular Functions of Albino Rats

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ABSTRACT

In the present study commonly used insecticide acephate dissolved in olive oil was administered orally to albino male rats at dose level of 25, 50 and 75mg /kg b.wt/day for 30 days. Reproductive toxicity of acephate 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 30%, 60%, and 80% 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 acephate. The observations are thus indicative of the reproductive toxicity caused by acephate at different dose levels in the testes of rats.

Keywords- Acephate, fertility, sperm dynamics, hormonal analysis.

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INTRODUCTION

Organophosphorus (OP) compounds constitute a heterogeneous category of chemicals specifically designed for the control of pests, weeds or plant diseases [1] and there have been increasing concerns about the reproductive toxicity of various organophosphate insecticides in humans and animals [2-4]. These include cholinergic and noncholinergic biological disturbances [5-9].

These compounds may affect semen quality in adult males either directly or indirectly via interference with endocrine status [10, 11]. By reducing acetylcholinesterase activity OPs block nerve impulses which leads to suppression of the brain's release of hormones that stimulate the gonadotrophic hormones (LH and FSH) [12]. Suppression of gonadotrophins might have caused decrease in sperm density in testes [13]. OPs can also cause reproductive toxicity by inhibiting DNA synthesis in the seminiferous epithelium and are cytotoxic for spermatogenic cells [14]. OPs elicit morphophysiological damage of sperm, with cytogenetic damage of male germ cells [14, 15].

Acephate is an important systemic organophosphorus insecticide with toxicity attributed to bioactivation on metabolic conversion to methamidophos which acts as an acetylcholinesterase (AChE) inhibitor [16, 17]. It is used for control of a wide range of biting and sucking insects, especially aphids, including resistant species, in fruit, vegetables, vine, and hop cultivation and in horticulture. It also controls leaf miners, lepidopterous larvae, sawflies and thrips in the previously stated crops as well as turf, mint and forestry [18]. Acephate and its primary metabolite, methamidophos, are toxic to various species. A number of studies showed the toxicity of acephate on different organisms which indicate it as a potent neurotoxic, mutagenic, carcinogenic and cytotoxic compound [19].

The present investigation has been conducted to evaluate the effects of acephate on sperm dynamics, hormonal parameters and testicular histopathology in male albino rats.

MATERIALS AND METHODS

Chemical

Acephate (Chemical name- O, S-dimethyl acetylphosphoramidothioate; Trade name- Orthene; Chemical family- Organophosphate) (KR exports pvt. Ltd., Jammu, India) dissolved in olive oil and administered orally via gavage.

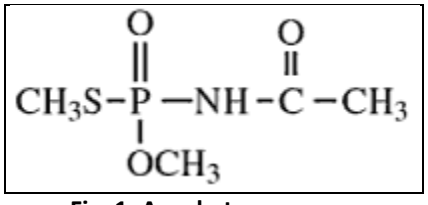

Fig. 1: Acephate

Animals

Male albino rats (*Rattus norvegicus*) weighing 150-200 gms were used and they were housed in separate polypropylene cages, under controlled conditions of temperature (22±3°C), humidity (50±5%) and light (12 light : 12 dark cycle), fed with standard pellet diet (Ashirwad Industrial Ltd., Punjab, India) and water *ad libitum*. The rats were divided into four groups containing 6 animals each. Acephate was dissolved in olive oil and administered to animals via gavage at daily doses of 25, 50 and 75 mg/ kg^{-1} b.wt for 30 days. Only olive oil was given to the control (GI) simultaneously with all the treated groups (GII, GIII and GIV). At the end of the experiment rats were euthanized under light ether anesthesia for testicular & accessory sex organ weight analysis, sperm dynamics, hormonal and histopathological studies.

Sperm Dynamics and Hormonal Measurements

Sperm Motility

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 counting chambers of haemocytometer. The results were finally expressed as percent motility [20].

Sperm Density

Total number of sperms were counted using haemocytometer after further diluting the sperm suspension from cauda epididymis and testes. The sperm density was calculated in million per ml as per dilution [20].

Fertility Test

The mating exposure test of all the animals was performed. They were cohabited with proestrus females in the ratio 1:3. The vaginal plug and presence of sperm in the vaginal smear was checked for positive mating. Females were separated and resultant pregnancies were noted, when dam gave birth. The number and size of litters delivered were recorded.

Hormonal analysis

Testosterone, Leutinizing Hormone (LH), and Follicle Stimulating Hormone (FSH) were estimated through chemiluminescence in fully automatic Advia Cemtaus Immuno Assay System.

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Testicular Histopathology

Testes of rats were fixed in Bouin's fixative for at least 48 hours, processed by the paraffin wax impregnation method and after using a rotary microtome; these were cut at 5µm thickness and stained with haematoxylin and eosin (H&E) for light microscopic examination.

Statistical Analysis

The data obtained from the above experiments were subjected to statistical analysis. Data were represented as mean ± S.E.M. The differences were compared for statistical significance by "t- test" by using SPSS software (16.0 version) and they were considered non significant at $P \leq 0.05$, significant at $P \leq 0.01$ and highly significant at $P \leq 0.001$. Graphical representation of data has been done using Microsoft Excel 2007.

RESULTS

The weight of the reproductive organs (Table 1.1)

Table 1.1: Testes and accessory sex organ weight analysis after administration of acephate for 30 days

Treatment	Sperm motility (%)	Sperm density (million/ml)		Fertility (%)
	Cauda Epididymides	Testes	Cauda Epididymides	
GI Control (Vehicle only)	68.92 ±2.10	4.22 ± 0.72	46.92 ±0.35	100%(+)ve
GII (Acephate 25mg/kg b.wt./day)	45.67[b] ±5.14	1.78[b] ±0.12	42.56[b] ±0.96	30%(-)ve
GIII (Acephate 50 mg/kg b.wt./day)	42.69[c] ±3.28	1.21[b] ±0.19	36.45[c] ±1.11	60%(-)ve
G IV (Acephate 75 mg/kg b.wt./day)	31.12[c] ±5.48	0.82[c] ±0.08	28.22[c] ±2.03	80%(-)ve

Mean ± of 6animals (GII; GIII and GIV compared with GI)
a = $P \leq 0.05$ (Non significant)
b = $P \leq 0.01$ (Significant)
c = $P \leq 0.001$ (Highly significant)

The mean relative weight of the testes, epididymis, seminal vesicle and ventral prostate were decreased after administration of acephate at different dose levels for 30 days when compared to control group. A significant decrease in the weight of testes and seminal vesicles was observed at medium dose level whereas highly significant decline was noticed in high dose treated rats. The weight of Vas Deferens was significantly decreased at all the dose levels as

compared to control. Further, the weight of epididymis was reduced significantly at low dose level whereas highly significantly at medium and high dose levels. Non significant reduction was observed in the weight of ventral prostate at low dose level.

Sperm Dynamics and Fertility Test (Table 1.2)

Table 1.2: Sperm dynamics and fertility test after administration of acephate for 30 days

Treatment	Testes	Epididymides	Vas Deferens	Seminal Vesicle	Ventral Prostate
	mg/100g body wt.				
GI Control (Vehicle only)	1214.01 ±45.23	502.82 ±7.32	145.32 ± 6.67	405.66 ± 20.19	356.12 ± 28.32
GII (Acephate 25mg/kg.b.wt./day)	1030.17[b] ±36.18	421.44[b] ± 20.96	118.56[b] ±8.21	321.11[b] ± 15.63	302.43[a] ± 13.71
GIII (Acephate 50 mg/kg b.wt./day)	975.11[b] ± 46.00	406.88[c] ±12.20	115.35[b] ±5.28	304.84[b] ±23.12	255.82[b] ± 12.33
GIV (Acephate 75mg/kg b.wt./day)	912.29[c] ±25.57	378.85[c] ±14.63	115.48[b] ±5.37	288.19[c] ±12.97	242.35[b] ± 18.50

Mean ± of 6animals (GII; GIII and GIV compared with GI)
a = P ≤ 0.05 (Non significant)
b = P ≤ 0.01 (Significant),
c = P ≤ 0.001 (Highly significant)

The sperm density in testes and cauda epididymis decreased significantly (P ≤ 0.001) after high dose of acephate administration. A severe impairment of sperm motility in cauda epididymis was also observed. Control rats showed 100% positive fertility in the mating exposure test while the rats exposed to 25 mg dose level showed 30% negative fertility, 50mg showed 60% negative fertility, and 75mg/kg b.wt./day showed 80% negative fertility after 30 days.

Effects of acephate on Serum testosterone, LH and FSH levels in rats (Fig. 2.1- 2.3)

The serum levels of testosterone, LH and FSH were significantly reduced at medium dose level while highly significant reduction was seen at high dose level as compared to control group.

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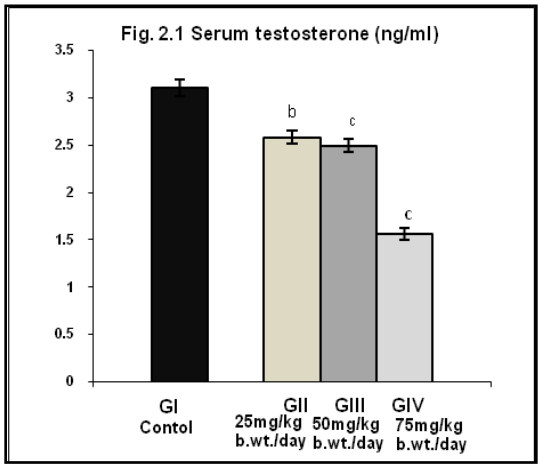
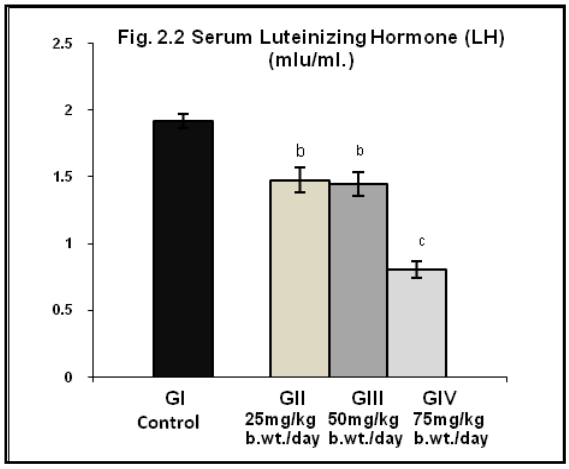
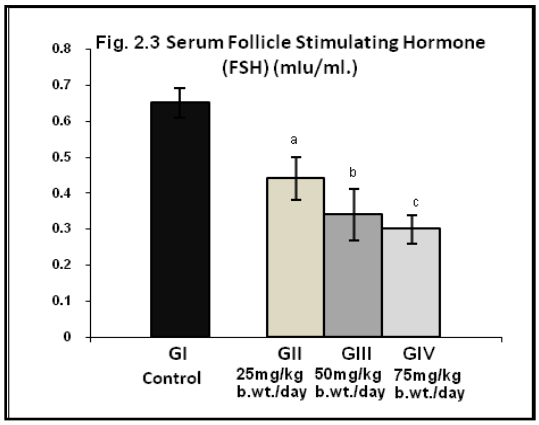
Fig. 2.1- 2.3: Effects of acephate on Serum testosterone, LH and FSH levels in rats







Mean ± SEM of 6 animals
a = P ≤ 0.05 (Non significant)
b = P ≤ 0.01 (Significant)
c = P ≤ 0.001 (Highly significant)
(GII; GIII and GIV compared with GI)

Testicular Histopathology (Fig: 3)

Histopathological examination of the testes revealed that the high dose of acephate induced severe degenerative changes as compared to low dose level. Microphotograph of control rat testes showed normal morphological architecture with all the successive stages of spermatogenesis. Lumen filled with spermatozoa and Sertoli cells are present. Testes of rats treated with acephate (25 mg/ kg^{-1} b.wt/day for 30 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 acephate (50 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 (75 mg/ kg^{-1} b. wt/day for 30 days).

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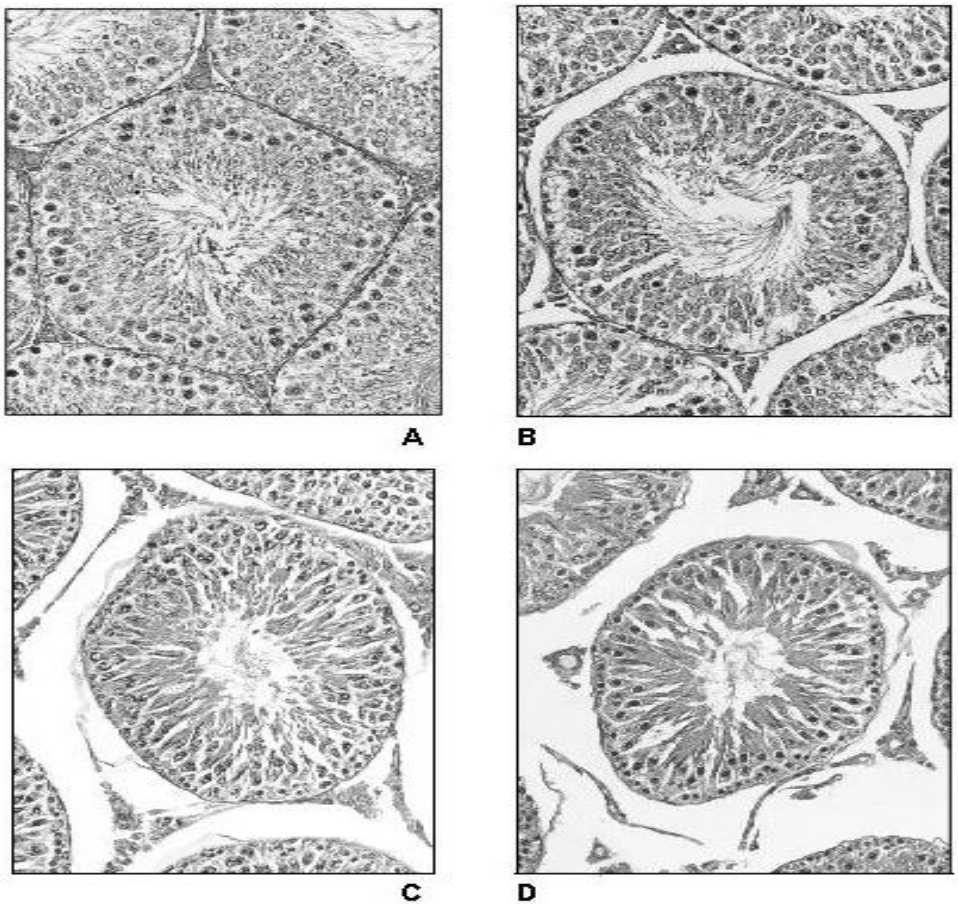

Fig. 3: Histopathology of testes after exposure with acephate in rats (A) Testes of control rat (B) Testes of rat treated for 30 days with acephate of daily doses 25mg/ kg^{-1}b.wt (C) 50 mg/ kg^{-1}b.wt and (D) 75 mg/ kg^{-1}b.wt
(H&E 200X for each microphotograph)

DISCUSSION

The present study revealed that administration of acephate produces significantly decline in the weights of testes and accessory sex organs in comparison to control rats at different dose levels for 30 days. The decrease in testicular weight in the treated rats may be due to reduced tubular size as confirmed by the histopathological findings of the testes which show degeneration and atrophy of non functioning seminiferous tubules [21]. Also, spermatogenic arrest and inhibition of steroid biosynthesis of Leydig cells, a site of steroid biosynthesis may contribute to the decline of testes weight [22]. The reduction in weight of accessory sex organs may be due to low availability of androgens or antiandrogenic activity of acephate [23].

Sperm motility is considered one of the most important parameters evaluating the sperm fertilizing ability [24, 25]. Choudhary and Joshi, [26] found a reduction in percent of mobile sperm in rats exposed to different doses of endosulfan. Other studies showed that

different chemical agents also compromised the mobile capacity of sperm and reduced fertility of animals [27, 28]. The decreased motility of sperm in cauda epididymis indicates less ability of sperm to interact with the oocyte plasma membrane [29].

Decreased sperm density in the cauda epididymides is an indicator of reduced spermatogenesis as a result of the toxicity of any agent [30]. Biologically active gonadotropins are essential for normal sperm production, growth, development and maturation of testes and cauda epididymis [31]. Reduction in sperm density may be due to alteration in androgen gonadotrophin [32-34). The observed negative fertility may be attributed to lack of forward progression, reduction in density of spermatozoa and altered biochemical milieu of cauda epididymis [35].

Testosterone is the principal androgen of the testes and it is essential for sperm production and maintenance [36]. There may be two mechanisms by which insecticides could reduce circulating levels of testosterone; first by enhancing its degradation, excretion or tissue uptake or second by depressing circulating LH levels and thereby reducing LH dependent testicular steroidogenesis [37]. It is well established that organophosphorous pesticides reduce acetylcholinesterase activity and block nerve impulses. This effect may alter the release of pituitary hormones, namely FSH and LH, leading to the reduction of sperm production in the testes [38].

The present investigation is supported by our earlier laboratory findings which revealed that administration of pesticides adversely affect the hormonal level, sperm dynamics and histoarchitecture of the testes [4, 21, 39]. Testicular histopathology is the most sensitive and reliable method for detecting effects of toxicant on sperm production [40]. Decreased LH, FSH, and testosterone hormone concentration could be effective in decreased spermatogenesis and the number of testicular germinal cells [41, 42].

CONCLUSIONS

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

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