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Full Length Research Paper

A comprehensive effect of acephate on cauda epididymis and accessory sex organs of male rats

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In the present study, thirty six (36) adult male Wistar rats were divided into six groups each containing 6 Wistar rats (n = 6). Group I served as untreated control group while group II served as the positive control and group III served as the negative control. Group IV, V and VI were considered as the experimental groups. Group II received tetracyclin (28.6 mg/kg/b.wt/day), while group III received vehicle (olive oil 0.5 ml/100 g body weight/day). Group IV, V and VI were administered "acephate" and dissolved in olive oil at dose level of 25, 50 and 75mg/kg b.wt/day for 30 days, respectively. Reproductive toxicity of acephate was evaluated on the basis of weight analysis of cauda epididymis and accessory sex organs, fertility, sperm dynamics, protein content, sialic acid content and histopathological studies. There was a decrease in the weight of epididymis, ventral prostate, vas deferens and seminal vesicle. The results showed highly significant decline in sperm density and motility. Post fertility test showed 30, 60, and 80% negative results. A statistically significant increase was noticed in protein content whereas sialic acid content was decreased in the cauda epididymis and accessory sex organs. 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 male rats.

Key words: Acephate, fertility, sperm density, sialic acid, toxicity.

INTRODUCTION

The important impact of men's reproductive health on a couple's fertility is often overlooked. Several studies have suggested that human semen quality and fecundity is declining (Aitken et al., 2004; Jørgensen et al., 2006). Environmental pollutants, occupational exposures and lifestyle have been explored as possible contributors to those changes (Homan et al., 2007). Volatile organic compounds (Wagner et al., 1990), heavy metals (Benoff et al., 2000) or xenoestrogens like some polychlorinated biphenyls (Rozati et al., 2002), phthalate esters (Duty et al., 2003) and pesticides (Carreño et al., 2007; Joshi et al., 2011) may compromise reproductive male function. Pesticides alter the male reproductive function by altering sperm count and sperm shape, alter sexual behavior or increase infertility in animals and human beings (Figà-Talamanca et al., 2001; Sweeney, 2002; Sheiner et al., 2003; Chang et al., 2004; Presibella et al., 2005; Jensen et al., 2006; Joshi and Sharma, 2011). There have

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been increasing concerns about the effects of various organophosphate insecticides in humans and animals. In mammals, the primary site of action of organophosphate pesticides is the central and peripheral nervous system as they inhibit acetylcholinesterase (AChE), the enzyme that hydrolyses the neuro-transmitter acetylcholine (Slotkin et al., 2006; Bajgar et al., 2009). These include cholinergic and non-cholinergic biological disturbances (Bomser et al., 2002; Gordon Mack, 2003). The widespread use of organophosphates has stimulated research into the possible existence of effects related with their reproductive toxic activity (Joshi et al., 2011; Joshi and Sharma, 2011).

Acephate is an important systemic organophosphorus (OPs) insecticide with toxicity attributed to bioactivation on metabolic conversion to methamidophos which acts as an acetylcholinesterase (AChE) inhibitor (Thapar et al., 2002; Trevizan et al., 2005). 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. 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 (Singh and Jiang, 2002).

Little information is available about the effects of acephate on cauda epididymis and accessory sex organs of male rats. Hence, the present study is aimed to find out the effects of acephate on epididymis and accessory sex organ weight response, sperm dynamics, fertility, protein content, sialic acid content and histopathology.

MATERIALS AND METHODS

Chemical

Acephate (Chemical name- O, S-dimethyl acetylphosphoramidothioate; Trade name- Orthene; Chemical family- Organophosphate) (KR exports pvt. Ltd., Jammu, India) was dissolved in olive oil (0.5 ml/100 g body weight/day) and administered orally via gavage (Figure 1).

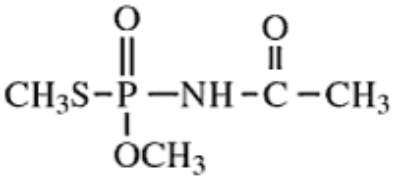

Figure 1. Acephate.

Animal model

The present study was carried out on inbreeds proven fertile male albino rats (*Rattus norvegicus*) of Wistar strain weighing 150 to 200 g (Visweswaran and Krishnamoorthy, 2012). The animals were housed in a hygienic, well-ventilated room with natural light and dark cycles (12 h dark, 12 h light) with relative humidity 55 ± 5%. They were individually housed in clean polypropylene cages (12” × 10” × 8”) with sawdust bed and covered with stainless steel wire lids. They were fed on standard commercial pellet feed procured from Ashirwad Food Industries Ltd., Chandigarh (Punjab) and fresh water was provided *ad libitum* throughout the study.

Experimental design

The rats were divided into following groups:

Group I: Untreated control.
Group II: Positive control (tetracycline 28.6 mg/kg b.wt/day)
Group III: Negative control received vehicle (olive oil 0.5 ml/100 g body weight/day) only.
Group IV: Acephate 25 mg/kg b.wt/day for 30 days.
Group V: Acephate 50 mg/kg b.wt./day for 30 days.
Group VI: Acephate 75 mg/kg b.wt/day for 30 days.

Parameters studied

Epididymis and accessory sex organs were excised blotted free of blood and weighed. The various parameters were performed by following methods:

Organ weight: Weight of epididymides, seminal vesicle, ventral prostate and vas deferens were recorded.

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 (Prasad et al., 1972).

Sperm density: Total number of sperms were counted using haemocytometer after further diluting the sperm suspension from cauda epididymis. The sperm density was calculated in million per ml as per dilution (Prasad et al., 1972).

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.

Biochemical analysis

Biochemical analysis of tissues were done by following standard methods: Total protein (Lowry et al., 1951) and Sialic acid (Warren, 1959)

Histopathology

Cauda epididymis and accessory sex organs of rats were fixed in

Table 1. Sperm dynamics and fertility (Acephate for 30 days).

Treatment	Sperm motility (%)	Sperm density (million/ml)	Fertility (%)
	Cauda epididymides		
Group I (untreated control group)	68.79±2.14	46.89 ±0.41	100 (+)ve
Group II (positive control) (Tetracycline)	26.59±3.28	24.12±1.98	90 (-)ve
Group III negative control (olive oil)	68.92±2.10	46.92±0.35	100 (+)ve
Group IV (25 mg)	45.67*±5.14	42.56*±0.96	30 (-)ve
Group V (50 mg)	42.69**±3.28	36.45**±1.11	60 (-)ve
Group VI (75 mg)	31.12**±5.48	28.22**±2.03	80 (-)ve

Mean ± of 6 animals. * = P ≤ 0.01 (significant), ** = P ≤ 0.001 (highly significant).

Table 2. Tissue biochemistry (Acephate for 30 days).

Treatment	Protein (mg/g)				Sialic acid (mg/g)			
	Cauda epididymis	Seminal vesicle	Ventral prostate	Vas deferens	Cauda epididymis	Seminal vesicle	Ventral prostate	Vas deferens
Group I (untreated control group)	203.51±5.42	235.23±7.81	229.10±3.51	258.53±6.24	5.12±0.12	5.31±0.32	5.21±0.21	5.32±0.33
Group II (positive control (Tetracycline)	247.21±2.85	275.12±8.57	262.84±8.81	286.74±5.55	3.79±1.02	2.61±0.12	3.73±0.15	2.87±0.30
Group III (negative control (Olive Oil)	203.71±7.83	235.24±8.26	228.20±4.01	258.23±6.50	5.00±0.02	5.29±0.25	5.08±0.15	5.75±0.23
Group IV (25 mg)	234.72*±5.44	265.98*±5.02	238.16^{ns}±6.20	271.58^{ns} ±5.33	4.21*±0.21	3.72*±0.35	4.56*±0.92	4.46*± 0.30
Group V (50 mg)	242.26*±9.02	269.12*±6.70	250.94^{ns}±6.21	270.07^{ns}±8.25	4.11*±0.26	3.46*±0.50	3.53*±0.44	3.42*±0.54
Group VI (75 mg)	246.42**±3.13	274.71*±9.03	262.34*±9.05	286.38*±5.20	3.80*±0.34	2.63**±0.02	3.85**±0.12	2.90**±0.50

Mean ± of 6 animals. * = P ≤ 0.01 (Significant). ** = P ≤ 0.001 (highly significant).

Bouin's fixative for at least 48 h, processed by the paraffin wax impregnation method and after using a rotary microtome, these were cut at 5 µm 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 ± SEM. The differences were compared for statistical significance by "t- test" by using statistical package for social sciences (SPSS) software (16.0 versions) and they were considered significant at P ≤ 0.01 and highly significant at P ≤ 0.001. Graphical representation of data has been done using Microsoft Excel 2007.

RESULTS

Acephate brought mark alterations in cauda epididymal weight, function and histology. 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). Table 1 shows the sperm motility, sperm density and fertility (%) in untreated control, positive control, negative control and various dose level treated groups. Group IV demonstrated significant decrease in sperm motility as well as sperm density as compared to untreated control group. Group V and Group VI showed marked reduction in both the parameters as well as fertility percentage in comparison to Group I. A gradient increase was observed in toxicity in treated group. Table 2 shows the protein and sialic acid concentration in cauda epididymis, seminal vesicle, ventral prostate and vas deferens. Group IV and V demonstrated elevation in protein level as compared to Group I in cauda epididymis and seminal vesicle. Protein concentration remained

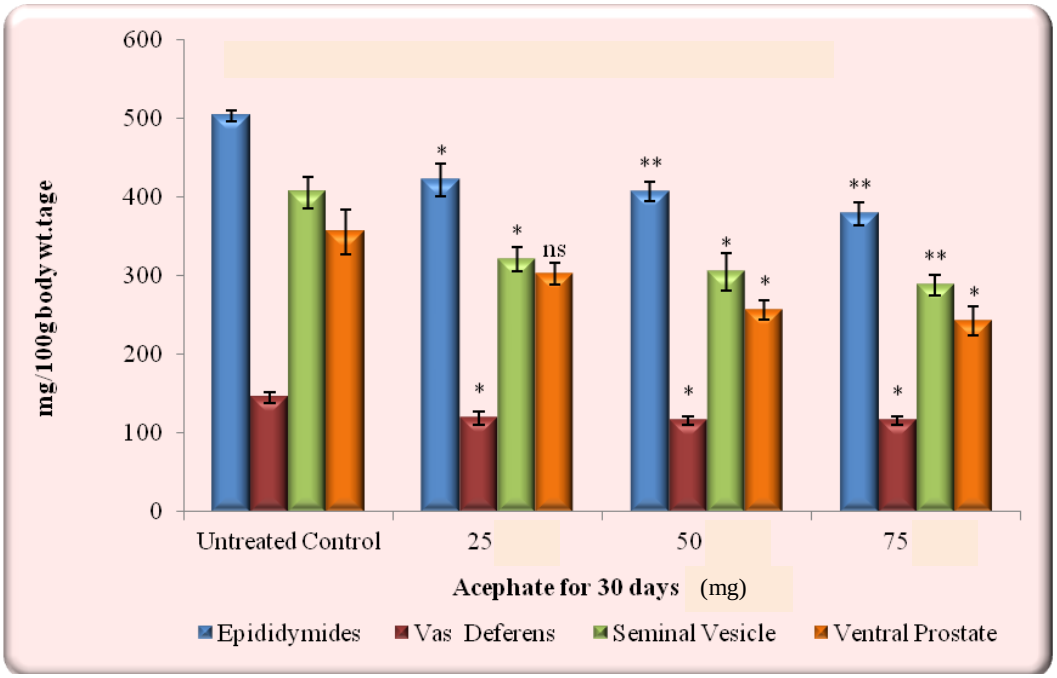

Figure 2. Epididymis and accessory sex organ weight (acephate for 30 days). Mean ± of 6 animals. * = P ≤ 0.01 (Significant), ** = P ≤ 0.001 (highly significant).

unaltered in ventral prostate and vas deferens. A marked reduction in sialic acid concentration has also been observed from all the four tissues. Group VI showed highly significant increase in protein concentration in cauda epididymis as compared to untreated group while marked decrease in silaic acid concentration has been reported in seminal vesicle, ventral prostate and vas deferens. In the present study, elevation in protein (Table 2) may be due to hepatic detoxification activity which results in the inhibitory effect on the activity of enzyme or production of enzymes lost involved in the androgen biotransformation (Venkataramana et al., 2006). Another reason for elevation in protein content may be stimulation of growth proteins and RNA synthesis. Elevation in protein content caused by other insecticides has also been reported (Joshi et al., 2003; Ngoula et al., 2007).

Sialic acid acts as a lubricant to facilitate the downward movement of sperm and to reduce friction among spermatozoa (Gupta, 2001). A significant decrease in the sialic acid concentration was noticed which may be due to the anti spermatogenic activity or reduced androgen production. The reduced sialic acid content of seminal vesicles caused deteriorating effects on the structural integrity of sperm cells (Bone et al., 2001).

Administration of acephate showed adverse effect on histoarchitecutre of seminal vesicle with disruptive changes of muscles and connective tissue along with highly reduced secretion in the lumen (Figure 4a to d). The sex differentiation and growth of seminal vesicles are highly dependent on androgens (Curry and Atherton, 1990), thus reduced androgen level have adverse effects on histo-architecture of seminal vesicles. Reduced epithelial folding of seminal vesicle was observed in methyl parathion treated rats compared to control (Prashanthi et al., 2006). These studies suggest that the OP pesticides may influence the semen quality by affecting the seminal vesicle functions in albino rats. Thus, obtained results collectively indicate that acephate caused toxic effects on male reproductive functions. Present study indicates limited use of such toxic insecticides to improve the quality of life for human welfare.

DISCUSSION

Several pesticides have reduced the organ weight by affecting either hypothalamus or pituitary or both (Joshi et al., 2011; Okazaki et al., 2001). In our study, the reduction in weight of accessory sex organs (Figure 2) may be due to low availability of androgens or antiandrogenic activity of acephate (Latchoumycandane et al., 2002b). Sperm parameters such as count, motility and morphology are the key indices of male fertility (Table 1), as these are the prime markers in testicular spermatogenesis and epididymal maturation. Decline in human sperm counts and motility over the recent decades may be attributed to increased exposure to

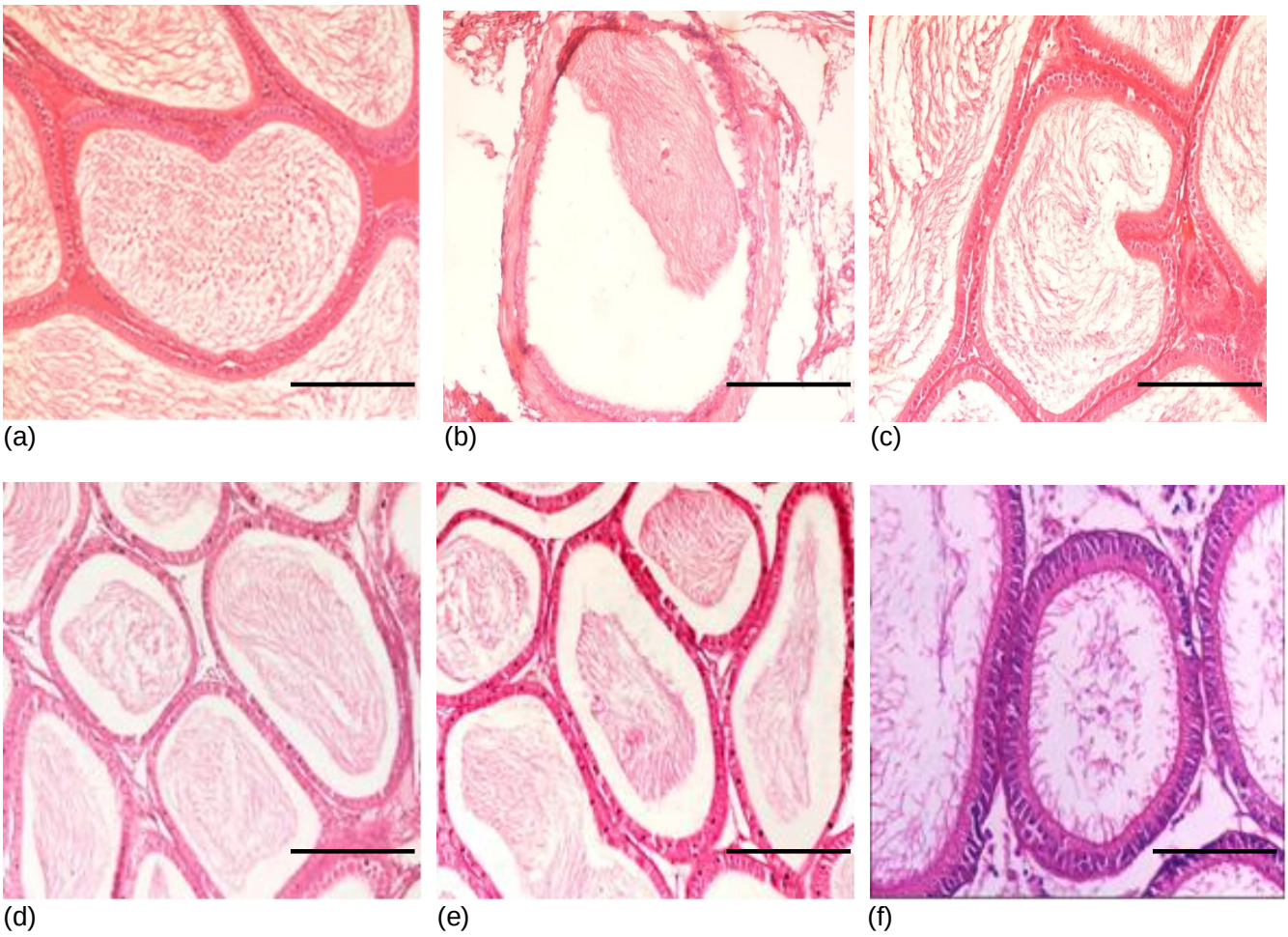

Figure 3. (a-f) Photomicrographs of cauda after exposure with acephate in rats. a. Untreated control b. Positive control (Tetracycline 28.6 mg./kg b.wt./day),c. Negative control (olive oil), d. Acephate 25 mg./kg b.wt./day, e. Acephate 50 mg./kg b.wt./day, f. Acephate 75 mg./kg b.wt./day. H&E. stain, Bar = 100 µm

environmental endocrine disruptors like OP compounds (Yuan et al., 2010).

Epididymides are the site of differentiation, maturation and storage of spermatozoa (Yuan et al., 2010). The physiological and biochemical integrity of epididymis are dependent on androgens. Low caudal epididymal sperm density in our study may be due to alteration in androgen metabolism (Duty et al., 2003). The loss of sperm motility in the treated rats may be due to change in sperm membrane properties. Similar effect on sperm motility is also reported with other pesticides and this negative impact affects fertilizing ability of the sperm. Low fructose concentration in seminal vesicle may be another cause of low sperm motility (Bharshankar and Bharshankar, 2000). Bai and shi (2002) reported that low levels of adenosine triphosphate (ATP) content may affect sperm motility. Reduction in sperm motility may be androgen deprivation effect of acephate (Uzumcu et al., 2004).

Proteins are the most important and abundant macro-molecules playing a vital role in the architecture and physiology of the cell and in cellular metabolism (Mommsen and Walsh, 1992). Sialic acid acts as a lubricant to facilitate the downward movement of sperm and to reduce friction among spermatozoa (Gupta, 2001). A significant decrease in the sialic acid concentration was noticed, which may be due to the anti spermatogenic activity or reduced androgen production. The reduced sialic acid content of seminal vesicles caused deteriorating effects on the structural integrity of sperm cells (Bone et al., 2001). The epididymis plays an essential role in male fertility, and disruption of epididymal function can lead to obstructive azoospermia.

Formation and function of the epididymis is androgen-dependent. Acephate at various dose levels produced many degenerative changes in the cauda epididymis and accessory sex organs along with various pathological changes. Deficiency of androgens caused a marked reduction in tubular diameters (Figure 3a to d), regression

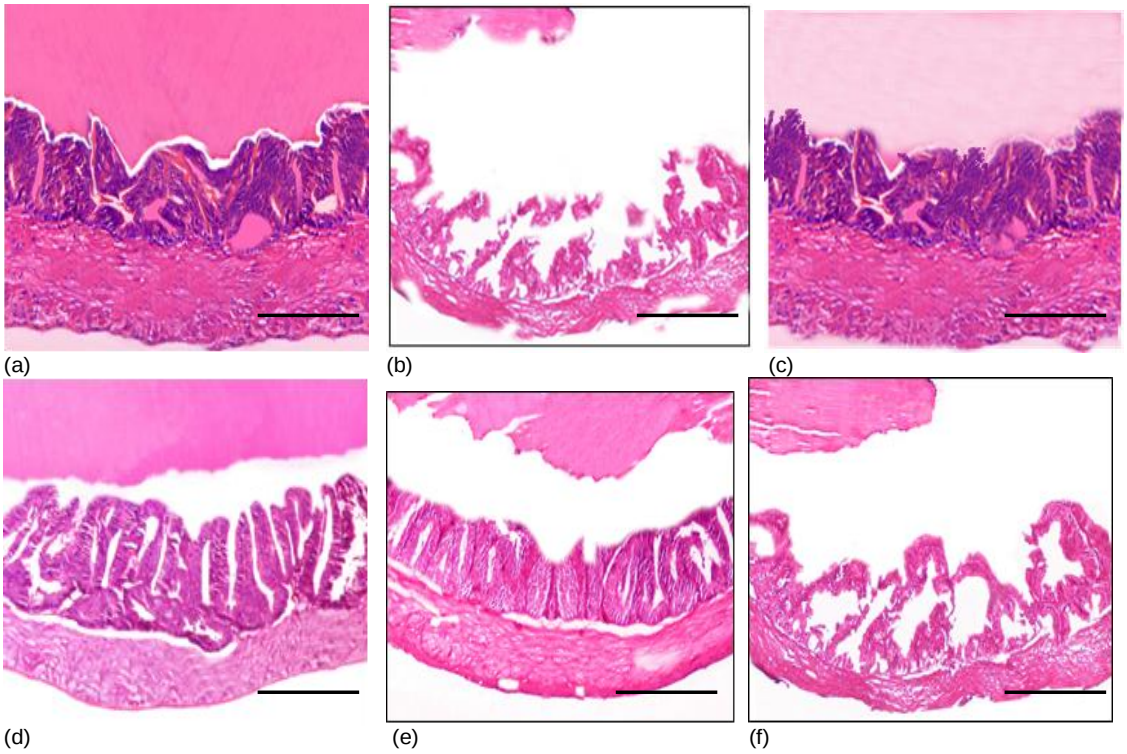

Figure 4. (a-f). Histopathology of seminal vesicle after exposure with acephate in rats. a. Untreated control, b. Positive control (Tetracycline 28.6 mg/kg b.wt/day),c. Negative control (olive oil), d. Acephate 25 mg/kg b.wt/day, e. Acephate 50 mg/kg b.wt/day, f. Acephate 75 mg/kg b.wt/day. H&E. stain, Bar = 100 µm.

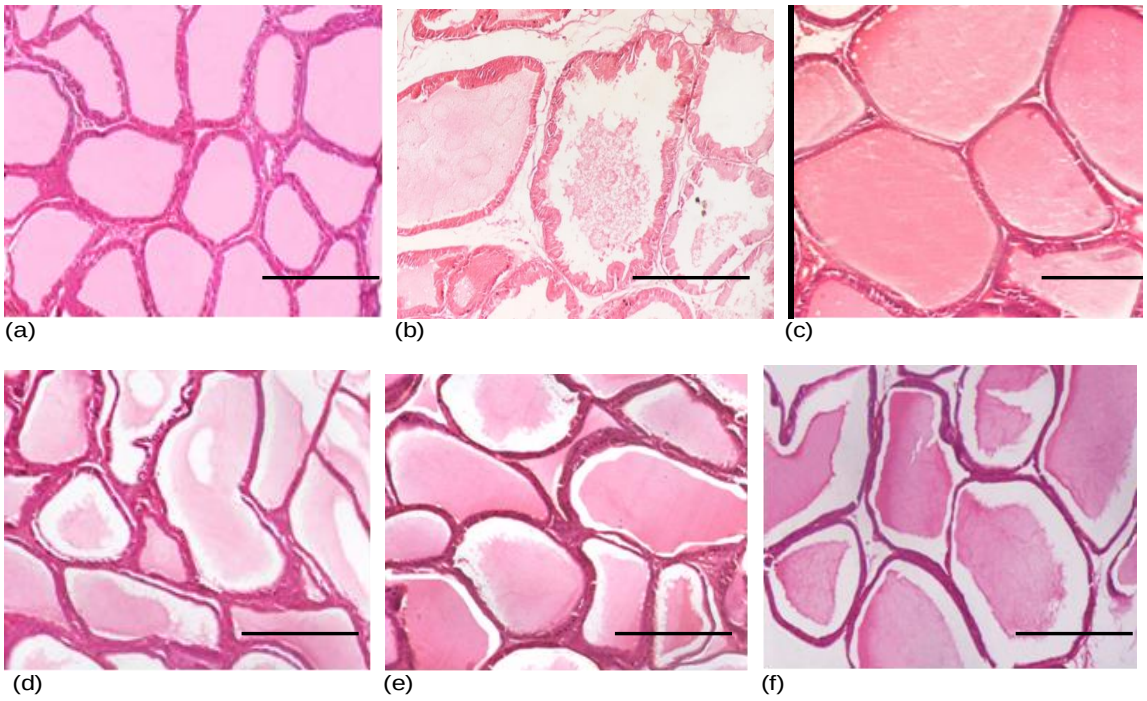

Figure 5. (a-f) Histopathology of prostate after exposure with acephate in rats. a. Untreated control, b. Positive control (Tetracycline 28.6 mg/kg b.wt/day),c. Negative control (olive oil), d. Acephate 25 mg/kg b.wt/day, e. Acephate 50 mg/kg b.wt/day, f. Acephate 75 mg/kg b.wt/day. H&E. stain, Bar = 100 µm.

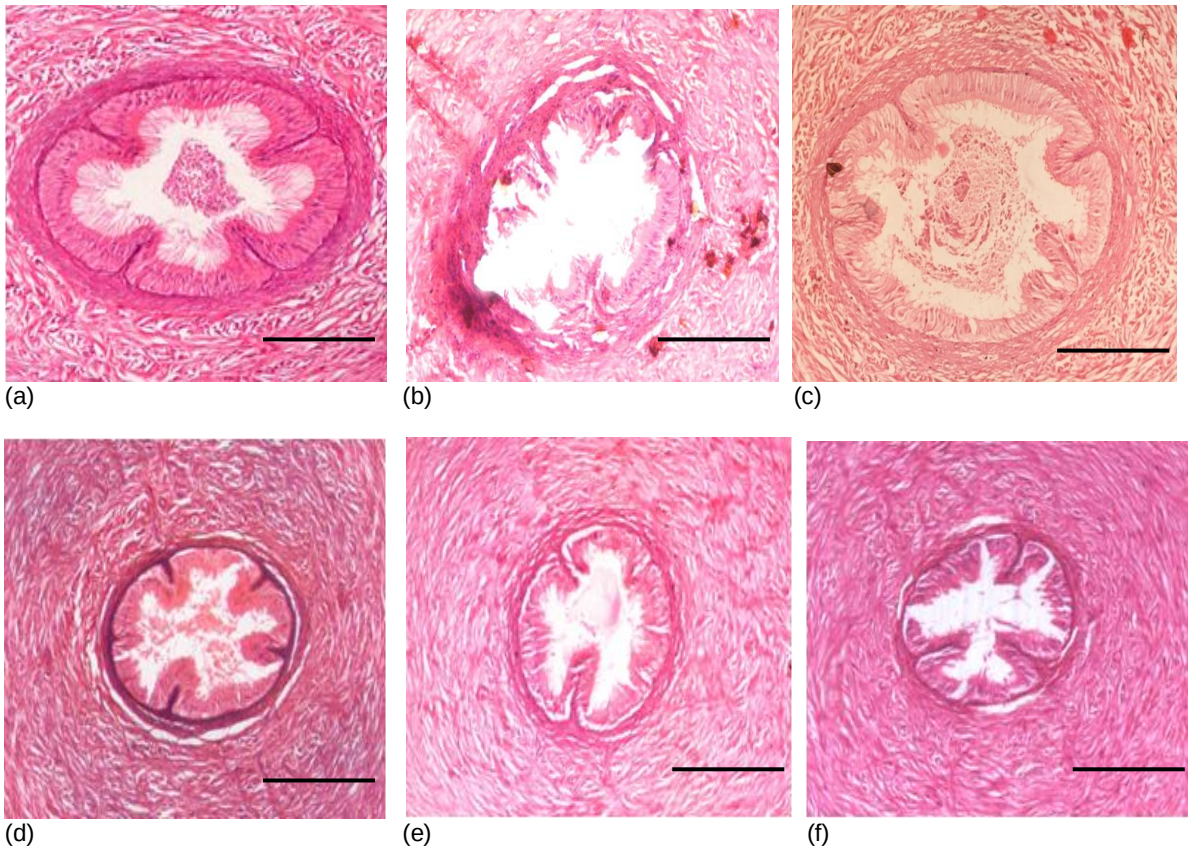

Figure 6. (a-f) Histopathology of vas deferens after exposure with acephate in rats. a. Untreated control, b. Positive control (Tetracycline 28.6 mg/kg b.wt/day),c. Negative control (olive oil), d. Acephate 25 mg/kg b.wt/day, e. Acephate 50 mg/kg b.wt/day, f. Acephate 75 mg/kg b.wt/day. H&E. stain, Bar = 100 µm.

of epididymal epithelium, severe decline in spermatozoa number in cauda and changes in the composition of epididymal plasma. OPs significantly increased cytoplasmic vacuolation and nuclear shrinkage in the epithelial cells of the rat's ductus epididymis (Okamura et al., 2009; O'Hara et al., 2011).

Prostate plays key role in male reproduction and its secretion is essential for the normal function of spermatozoa. Decrease in weight of ventral prostate was observed after treatment with many pesticides (Joshi et al., 2011; Bian et al., 2006; Kim et al., 2005). After treatment with acephate, ventral prostate showed reduced alveoli (Figure 5a to d), with very thin and disorganized cuboidal epithelial cells lining, and lumen was filled with very little secretion. Little work is done for the effects of OPs on the histoarchitecture of ventral prostate.

Vas deferens is a thick-walled muscular tube. Mucosal lining is described to have longitudinal folds resulting into irregular outline of its lumen. These folds are believed to allow for expansion during ejaculation. Its pseudostratified epithelium may possess tuft of microvilli (stereocilia) similar to that of epididymis to absorb the excess fluid produced by the testes. Administration of acephate caused degenerative changes in the vas deferens epithelium along with the absence of spermatozoa in the lumen (Figure 6a to d) that may be due to reduced androgen level. Structural damage to mammalian vas deferens may lead into infertility and carcinogenicity (Aziz et al., 2008).

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