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ADVERSE EFFECTS OF PHOSMET ON MALE FERTILITY IN RATS

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SUMMARY

Assessments of the reproductive toxicity of organophosphorus insecticides are important public health issues. The adverse reproductive effects of one of the commonly used organophosphorus members, namely; phosmet on male rats were investigated. The tested insecticide was given orally to male rats at five dosage levels (phosmet, 0.81, 1.62, 3.25, 6.5 and 13 mg kg^{-1} b.wt) for 60 consecutive days. Plasma testosterone levels, sex organs weights, semen quality as well as histopathological finding of testis were the criteria used to evaluate the reproductive efficiency of the treated rats. A dose-dependant decrease was observed in the weights of the most genital organs, sperm cell concentrations and sperm motility associated with a significant increase in the percentage of dead morphologically abnormal spermatozoa of treated rats. Plasma testosterone level was declined significantly in phosmet treated groups at 3.25, 6.5 and 13 mg kg^{-1} b.wt. Sperm cell concentrations was severely affected by phosmet at high dose level (3.25, 6.5 and 13 mg kg^{-1} b.wt,). Histopathological examination of the testes revealed a mild, moderate and severe histopathological changes in rats treated with phosmet and severity was dose dependent. It is concluded that, phosmet induced adverse effects on male rats fertility by impairing both testicular function and structure.

INTRODUCTION

Male infertility is related to many factors among those factors is the misuse of drugs and chemicals (Amann and Berndtson 1986, Burke, 1986). Organophosphorus insecticides and pesticides are a group of chemicals widely used throughout the world in agricultural and animal field and may contaminate the environment and induce

serious problems to man and animals. Prolonged exposure to these contaminants could cause chronic toxicity, teratogenicity and male reproductive failure (Nafstad et al., 1983, Schlegel et al., 1991, Fukushima, 1991; Kelce and Wilson, 1997, Lacorte et al 1997., Cavanna and Molinari, 1998; Kitamura et al., 2000)).

Some organophosphorus compounds such as dimethyl-methyl phosphate (DMMP), dimethoate, quinalphos, diazinion, deltamethrin, trimethyl phosphate (TMP), phoxim, carbofuran, chlorpyriphos, prograf (FK506), DDT, fenvalerate and dichlorvos are known to impair male fertility, suppress sexual developments, cause testicular degeneration and alter semen picture in male rats (Dunnick et al., 1984, Afifi et al., 1991, Ray et al., 1992, Abdel-Aziz et al., 1994, Cho and Park, 1994, Atef et al., 1995, Pant et al., 1995, Brweslin et al., 1996, Hisatomi et al., 1996, Benphouma et al., 2001, Xu et al., 2004 and Okamura et al., 2005).

Phosmet is an organophosphorus broad spectrum insecticide used extensively worldwide to protect fruit, vegetables and grain crops and also as ectoparasiticides for farm animals (Roberts and Hutson, 1999).Because of prolonged exposure of humans and animals to this insecticide and a lack of information on its reproductive effects on male rats. Therefore, the present study was established to evaluate the adverse effects of phosmet (0.81, 1.62, 3.25, 6.5 and 13 mg kg^{-1} b.wt) on male fertility in rats when given orally at five dosage levels for 60 consecutive days.

MATERIAL AND METHODS

Drugs:

I- Phosmet (Zoecon RF-43, Wellmark Int., IL, USA) was available as emulsifiable concentrate and dissolved in fresh corn oil just before administration and given once daily by gastric intubation. Phosmet was administered in doses of (0.81, 1.62, 3.25, 6.5 and 13 mg kg^{-1} b.wt) (1/160, 1/80, 1/40, 1/20 and 1/10 from LD_{50} in rats). LD_{50} of phosmet in rats was determined as 130 mg kg^{-1} b.wt .

Animals:

Sixty male wistar rats of 12-14 month old and 150-200 gram body weight were used. The animals were kept under hygienic conditions and fed on rat-dietary cubes and water ad libitum.

Experimental Design:

The male rats were divided into six equal groups of 10 rats each. One group was kept as a non-treated control and given orally corn oil (0.5 mL/rat/day) while the 1st, 2nd, the 3rd, the 4th and 5th groups were dosed orally with the tested drugs at doses of (0.81, 1.62, 3.25, 6.5 and 13 mg kg^{-1} b.wt in rats, respectively. The tested insecticide was administered once daily for 60 successive days

to cover a complete spermatogenic cycle which ranges from 56-60 days in rats (Hershberger et al., 1969).

Blood samples were collected from the orbital plexus of rats of each group just before and at 60 days after administration. Clear plasma was separated by centrifugation at 3000 r.p.m. for 15 minutes.

Testosterone estimation:

The obtained plasma was assayed for testosterone content by using radioimmunoassay technique described by Yen and Jaffe (1978).

Weight of the genital organs:

Following blood sampling, rats were weighed, sacrificed by decapitation (at 60 days of administration). The testes, prostate glands and seminal vesicles were dissected out and weighed. Weights of testes and accessory glands were calculated in relation to its body weight.

Sperm concentrations and progressive motility: the epididymal content of each rat was obtained by cutting the tail of the epididymis and squeezing it in a sterile clean watch glass. The epididymal content was diluted 10 times with isotonic sodium citrate solution (2.9%) and thoroughly mixed to estimate the sperm concentrations and progressive motility (Bearden and Fluquary, 1980).

The percentage of live and abnormal spermatozoa: Eosin-nigrosin stained smears were prepared to determine the percentage of live and morphologically abnormal spermatozoa (Miller and Rass, 1952).

Histopathological examination: Testes of rats (5 rats from each group) were taken at 60 days of administration and processed for histopathological examination using the method of Drury and Wallington (1980) and Harris (1988).

RESULTS

The effect of phosmet on male fertility in rats are recorded in table 1 and 2.

Successive oral administration of phosmet (0.81, 1.62, 3.25, 6.5 and 13 mg kg^{-1} b.wt) for 60 days to male rats revealed a significant decrease in sperm count, sperm motility and significant increase in abnormal spermatozoa at 60 days of administration as compared to that of control (Table 1). The obtained results revealed that plasma testosterone levels were significantly decreased at 60 days of drug administration as compared to that of control in rats treated with phosmet at 3.25, 6.5 and 13 mg kg^{-1} b.wt. (Table1). A significant decrease in the weights of testes and accessory genital organs observed only in high dose levels (6.5 and 13 mg kg^{-1} b.wt) at 60 days of the experimental period (Table 2).

Histopathological examination of testes of rats given phosmet (0.81 and 1.62 mg kg^{-1} b. wt)

for 60 days showed no pathological alterations as compared to that of control groups. The large doses of phosmet (3.25, 6.5 and 13 mg kg^{-1} b. wt) caused pathological alterations in the genital organs as compared to that of control group (Fig. 1) characterized by vacuolization in the primary and secondary spermatocytes,, proliferation of interstitial leydig cells, atrophy of the seminiferous tubules, incomplete spermatogenesis and slight decrease in the concentration of sperms in seminiferous tubules up to necrobiotic changes in spermatogonial cell. These changes ranged from mild (Fig. 2), moderate (Fig. 3) to severe degenerative changes (Fig. 4) depending on the administered dose, the higher the dose, the more the pathological alterations.

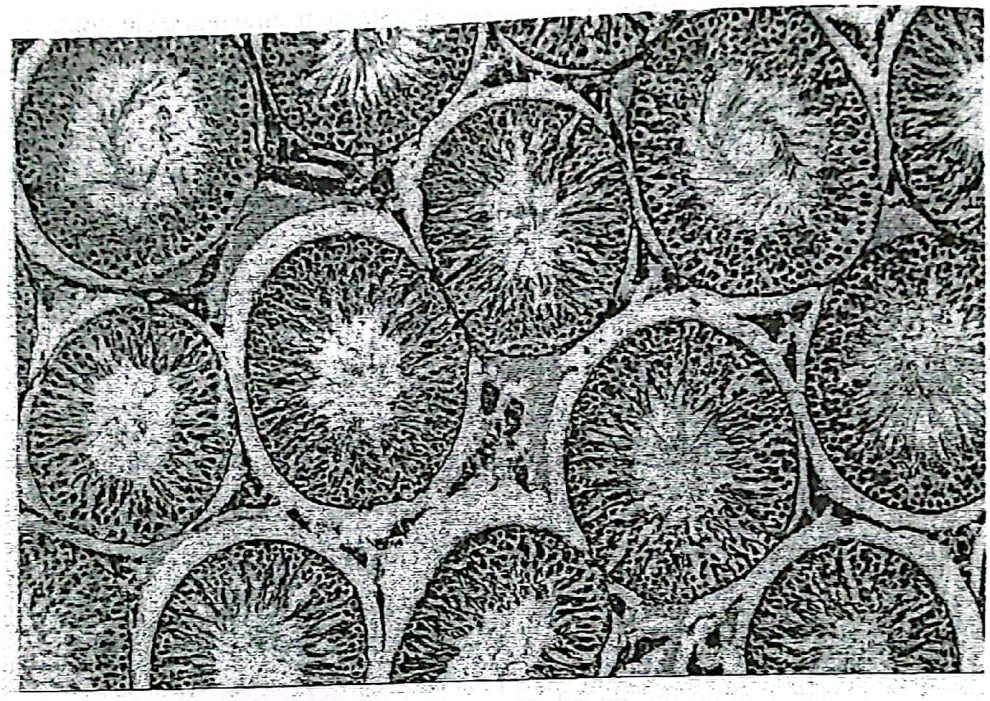

Figure (1): Normal testicular section of rats non treated showed normal appearance of spermatogonial cells, basement membrane, seminephrous tubules and interstitial tissues with excellent spermatogenesis (H&E x 200)

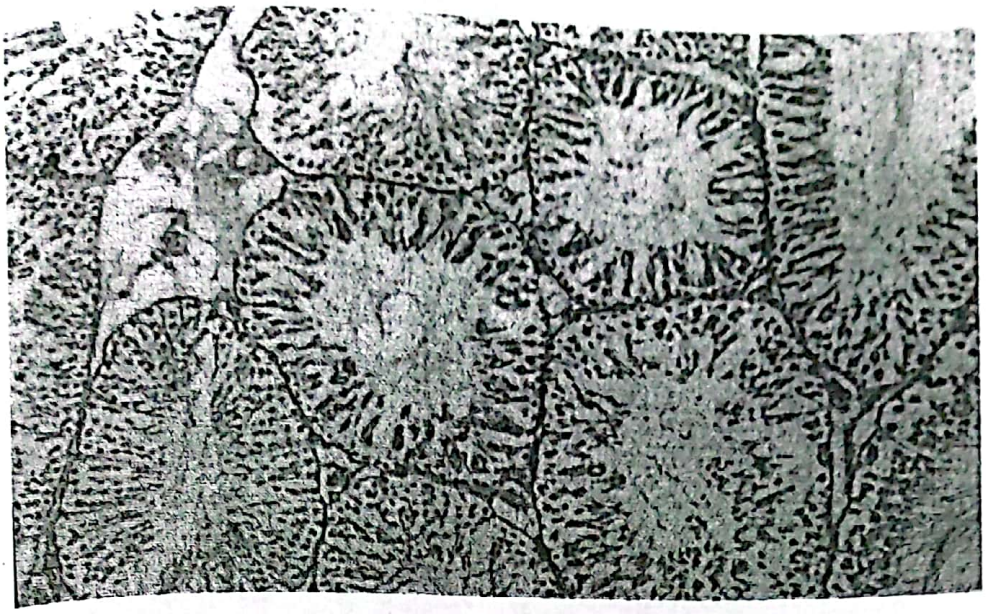

Figure (2): Testicular section of rats treated with 3.25 mg/kg b.wt. for 60 days showed esinophilic appearance of the necrotic spermatogonial cells with defective spermatogenesis (H&E x 200)

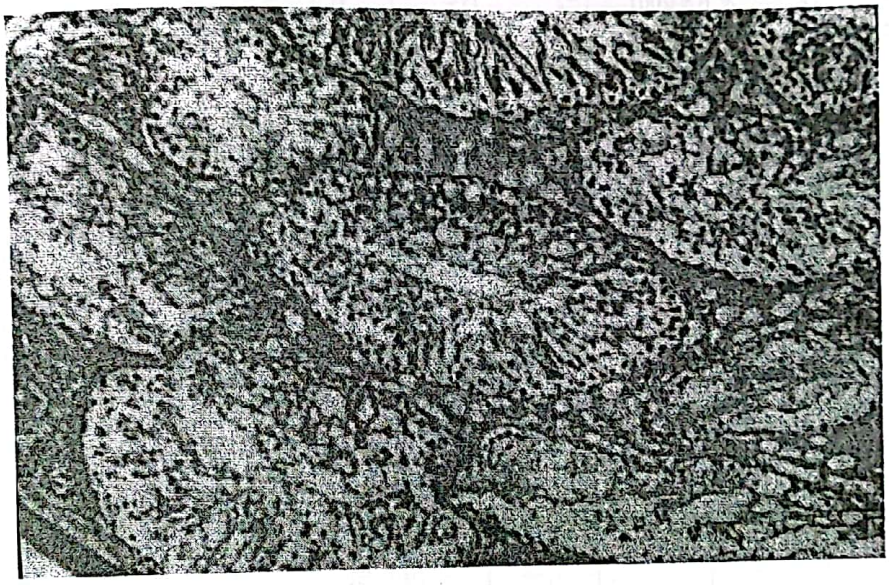

Figure (3): Testicular section of rats treated with 6.5 mg/kg b.wt. for 60 days showed marked interstitial oedema , loss of the spermatogonial epithelium with presence of only cell debris in the seminephrous tubules lumina (H & E x 200)

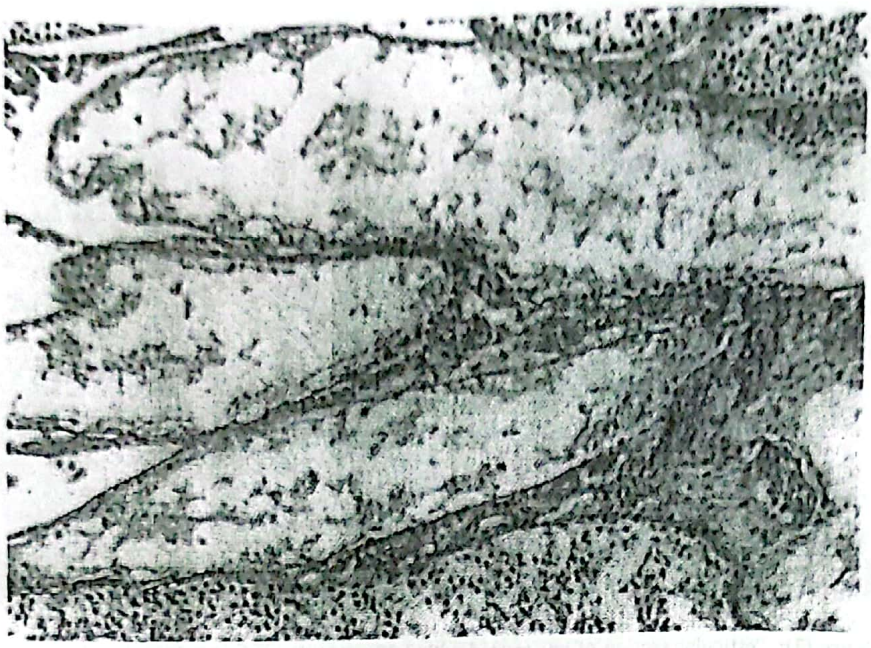

Figure (4): Testicular section of rats treated with 13 mg/kg b.wt. for 60 days showing atrophied seminephrous tubules and more prominent interstitial oedema (H & E x 200)

Table 1: Findings of sperm examination and plasma testosterone level in male rats (n = 10) treated with oral gavage of phosmet for 60 consecutive days.

Parameter	Dose (mg/kg)					
	0	0.81	1.61	3.25	6.5	13
Sperm count (x10^6/ ml)	1.82±0.37	1.74±0.3	1.46±0.3	1.1±0.29**	0.79±0.16***	0.64±0.11***
Sperm motility(%)	83.6±17.18	73±13.4	52.3±9.7**	43.7±7.29***	32.5±7.41***	22.2±4.9***
Sperm abnormalities (%)	2.4±0.5	2.9±0.4	3.1±0.81	3.4±0.67**	3.9±0.8***	5.6±1.2***
Plasma Testosterone level (ng/ml)	3.5±0.71	3.53±0.64	3.24±0.62	2.67±0.6*	2.11±0.3***	1.65±0.36***

Values are mean ± SD.*, **and*** indicate significant difference at $P < 0.05$, < 0.01, $P < 0.001$ levels respectively, when compared with the control group.

Table 2: Relative genital organ weight (g/100 g body weight) of male rats (n = 10) treated with oral gavage of phosmet for 60 consecutive days.

Relative organ weights	Dose (mg/kg)					
	0	0.81	1.61	3.25	6.5	13
Testes	1.46±0.3	1.43±0.38	1.41±0.3	1.35±0.25	1.2±0.24***	0.86±0.19***
Prostate	0.45±0.1	0.45±0.09	0.43±0.09	0.39±0.08	0.35±0.096	0.26±0.06***
Seminal vesicle	0.61±0.13	0.54±0.14	0.49±0.16	0.48±0.09	0.45±0.1*	0.31±0.05***
Epididymis	0.51±0.1	0.46±0.1	0.43±0.1	0.41±0.09	0.32±0.07***	0.23±0.06***

Values are mean ± SD. *and*** indicate significant difference at $P < 0.05$, $P < 0.001$ levels respectively, when compared with the control group.

DISCUSSION

Administration of chemicals and drugs specially organophosphorus insecticides is one of the main causes of male infertility (Burke, 1986, Pant et al., 1995, Benrhouma et al., 2001). The present study was performed to investigate the adverse effects of the commonly used organophosphorus insecticide, phosmet on male fertility in rats when orally administrated at five dosage levels (0.81, 1.62, 3.25, 6.5 and 13 mg kg^{-1} b.wt) for 60 consecutive days.

The obtained results revealed that oral administration of the tested insecticide had a dose-dependent adverse effects on male genital organs as evidenced by a marked decrease in weights of genital organs and significant alteration of semen picture. There was also marked reduction in the plasma testosterone levels. Moreover, significant increase in the percentage of dead and morphologically abnormal spermatozoa associated with testicular degeneration in the treated groups as compared to control rats. These findings are nearly similar with those previously reported for other insecticides in rats by Dunnick et al. (1984), Afifi et al. (1991), Ray et al. (1992), Abdel-Aziz et al. (1994), Atef et al. (1995), Breslin et al. (1996). Benrhouma et al. (2001) and Okamura et al. (2005).

The decreased plasma testosterone level may be attributed to the depressant effects on testosterone production by leydig cells (Rosen et al. 1988, Serova et al., 1994). Antiandrogenic activity of organophosphorus pesticides is one of the main causes of decreased plasma testosterone level. Tamura et al. (2003) reported that organophosphorus pesticides disrupt normal endocrine function by binding to steroid hormone receptors. Fenthion is an organophosphate insecticide has acted as a potent antagonist of the androgenic activity of dihydrotestosterone and could block androgen-dependent tissue growth (Kitamura et al., 2003).

The observed reduction in the weight of testes and accessory genital organs could be attributed to decreased plasma testosterone level recorded in the present study. These results were supported by Alexander (1978) who reported that the development and maintenance of accessory sex organs and their secretions depend on androgen level. The obtained results were similar with that previously reported by Atef et al. (1994) and Afifi et al. (1991) they reported that oral administration of dimethoate and phoxim to male rats for 65 and 60 consecutive days, respectively cause a dose-dependent decrease in the weight of sex organs and a decrease in the plasma testosterone level.

The reduction in the sperm concentration and motility in rats treated with the tested

insecticide may be attributed to the disturbance in spermatogenesis (Crotty et al., 1995) or due to the decreased testosterone level in plasma. Our results were consistent with that reported by Zhan et al. (2000) who mentioned that oral administration of phoxim to male rats at higher doses (24.5 and 73.5 $mgkg^{-1}$ b.wt) for 60 successive days showed significant reduction of daily sperm production and decreased sperm motility in a dose-dependent-manner. It has been reported that organophosphates as fenchlorphos and fenthion interfere with the energy production process required for sperm vitality and motility. Thomas et al. (1978) attributed the adverse reproductive effects of methyl-parathion in male rats to its ability to interfere with the metabolism and/ or uptake of steroid hormones at the cell receptor level. The antifertility action of trimethyl-phosphate in rats may be attributed to the presence of methyl group which had an antispermatogenic effect (Jackson and Jones, 1968). Similarly, the effect of phosmet and related compounds as antispermatogenic hazards in male rats could be attributed to the presence of such methyl groups.

Histopathological examination following repeated oral administration of phosmet for 60 days in male rats revealed mild, moderate and severe degenerative changes in testes. The observed results were agreed with those previously reported for phoxim in male rats (Atef et al., 1994).

From the aforementioned results, it could be concluded that, phosmet when given orally to male rats for 60 consecutive days caused adverse effects on male fertility as evidenced by marked reduction in the plasma testosterone level, significant decreased in weight of genital organs, decrease in the sperm count and progressive motility of spermatozoa associated with increased dead and abnormal spermatozoa and induced histopathological changes in testes in a dose-dependant manner. The authors advised to avoid prolonged exposure to these insecticides as they impair both testicular functions and structures in rats.

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دراسة الأثار الضارة لمبيد فوسميت علي الخصوبة في ذكور الفئران

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قسم الفسيولوجي والكيمياء الحيوية والأدوية, كلية الطب البيطري والثروة الحيوانية جامعة الملك فيصل, الأحساء

ان دراسة الأثار السلبية للتسمم بالمبيدات العضوية الفسفورية علي الوظائف التناسلية من اهم اهداف الصحة العامة لهذا تم بحث الأثار السلبية لمبيد الفوسميت الأكثر شيوعا في الأستخدام بالطب البيطري علي الخصوبة بالفئران. وقد اعطي هذا المبيد لذكور الفئران بخمس جرعات مختلفة وهي 0,81-1,62-3,25-6,5-13 مجم/كجم من وزن الحيوان لمدة قدرها 60 يوما متتالية. تم تقييم تأثير هذا المبيد علي الوظائف التناسلية في الفئران عن طريق قياس مستوي هرمون التستوسترون بالبلازما وقياس خصائص السائل المنوي ووزن الأعضاء التناسلية والفحص الهستوباثولوجي للخصية. وقد اظهرت النتائج وجود تأثير معنوي لهذا المبيد علي خصائص السائل المنوي وتتمثل بوجود نقص معنوي في عدد الحيوانات المنوية وسرعتها وارتفاع مستوي التشوهات بالحيوانات المنوية. كما لوحظ وجود نقص معنوي بمستوي هرمون التستوسترون . واثبتت الدراسة بان هناك تأثير معنوي سلبي لهذا المبيد علي وزن الأعضاء التناسلية. كما اوضحت الدراسة وجود العديد من التأثيرات الباثولوجية لهذا المبيد علي انسجة الخصية حيث وجد العديد من التغيرات الباثولوجية. مما سبق اثبتت هذه الدراسة بأن هذا المبيد يؤثر تأثيرا سلبيا علي الخصائص التناسلية بالفئران.