EFFECT OF DICHLORVOS PESTICIDE ON FERTILITY OF LABORATORY MALE MICE (*Mus musculus* L.)

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ABSTRACT

Dichlorvos is one of organophosphate insect pesticides which is widely distributed in environment. This study deals with the effect of this pesticide on sperms number , sperm abnormalities and spermatogenesis in laboratory male mice (*Mus musculus* L.) which were treated with (0.1 mg/day , 0.05 mg/day) of the pesticide for a period of 15 day.

intraperitoneal injection of Dichlorvos in laboratory mice resulted in a significant decrease in sperms number with both doses as compared with the control group. The result also showed a significant increase of sperm abnormalities with both doses. Moreover, pesticide injection caused a significant decrease in spermatogonia and primary spermatocyte with both doses whereas the statistical analysis did not show any significant difference in tubular diameter when compared with the control group.

INTRODUCTION

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is an organophosphate compound used to control of household and control ectoparasites in domestic animals [1,2] . It has been classified by WHO as highly hazardous and toxicity compound ; The lethal dose LD_{50} is 56 mg/kg [3].

Most human poisonings have resulted from the splashing of concentrated formulation onto the skin [4]. The mechanism of pesticide toxicity is mainly by blocking of acetyl cholinesterase – an enzyme which decomposes acetyl choline , immobilization of this enzyme result in accumulation of excessive amounts of acetyl choline in nervous tissue and muscular motor plates, as well as, symptoms of endogenic poisoning by this neuro hormone [5].

The increasing knowledge of the reproductive toxicity of environmental chemicals has raised public concern as to whether the current use of pesticides could adversely affect human. Among pesticides and their related chemicals , organophosphate insecticides such as quinalphos and aldrin that provoke massive germ cell degeneration in laboratory animals [6,7]. In addition [8] have observed that parathion (an organophosphate insecticide) caused tubular depletion and blockade in adult mice.

Interest in the reproductive effects of these organophosphate compounds has heightened in the recent years as a result of studies which show that several organophosphorous compounds including chloropyrifos, dimethyl methyl phosphate and malathion impair fertility, suppress libido, deteriorate semen quality and causes testicular degeneration in rodents following repeated exposure [9,10] . Moreover , the investigators [11,12] have shown that repeated exposure to dimethoate decreases serum testosterone levels, testicular weight, and sperm motility and increases the percentage of dead and abnormal sperm in rats and rabbits

MATERIALS AND METHODS

Dichlorvos Pesticide:

The pesticide diviGoz 50 obtained from the local markets in a shape of volumetric bottle (100 ml) with a concentrated 50%, produced by Indian company (BHARAT INSECTSCIDES Ltd) number of manufacture group 02.

Laboratory animals:

In this study laboratory male mice *Mus musculus* L. strain BALB/C had been used .These mice bred and housed in the animal house of the Biology Department / College of Education/ Basrah University. Animals were maintained in light- controlled room and at a temperature of (22±3c°) through the experiment . The food was prepared in the laboratory by mixing crude protein, ground soya bean, wheat flour, wheat bran, milk powder, mineral and vitamins.[13]

Preparation of animals:

In this study 24 intact male mice (7-8) weeks of age and (22-25)g body weight had been injected with Dichlorvos in the intraperitoneal region (I.P). These males were divided into three groups. Eight animals (n=8) in each group as follows:

1- The control group treated with distil water (0.1ml/animal)
2- The second group treated with low dose 0.1ml of Dichlorvos=(0.05 mg/day)
3- The third group treated with high dose 0.1ml of Dichlorvos =(0.1 mg/day).

The three groups were treated for 15 days .

Method of sperms count :

The method of [14] was used in this test as follows:

The right epididymis was cut into small parts and were put in test tubes with 2ml of formalin salt which consists of 5 grams of sodium pecarbonate and 100 ml formalin with temperature of 37-40°c. Then 0.1 ml of eosin stain (5%) was added to the solution, the samples were put in centrifuge (1500cycle/minute) for 5 minutes. After that a drop of the resulted solution was put in the middle groove of a heamocytometer slide, and then the sperms were counted in the five squares .

The total sperms = numbers of sperms in five squares × 10000

Percentage of normal and abnormal spermatozoa :

The Method of [15] was used in this study. The epididymis was put in a Petri dish containing 5 ml (0.9%) physiological saline then it was cut into six parts or more by using sharp razor and appointed tong , After that, drops of the final produced solution was spreading on the slide and dried. The slides were stained by 1% eosin for 5-10 minutes and then left to be dried. For each sample,100 sperms were counted on each slide (five slides) and then the normal and abnormal percentage of sperm was determined.

Histological study :

Histological sections of male mice testes were taken for both matrices according to [16] which included fixation, dehydration, clearing, embedding and staining. Eosin-hematoxilin stain was used to stain slide sample with 7µ thickness. The following parameter were determined by[17] .

1- The diameter of somniferous tubules.
2- Numbers of spermatogonia. 3- Numbers of primary spermatocyte.

Statistical analysis:

Analysis of variance was used to assess the data by using SP_{SS} program version 10 . [18]

RESULTS

Effect of Dichlorvos on sperm numbers and sperm abnormalities :

Effects of Dichlorvos pesticide on sperm count and sperm abnormalities of the male mice are presented in table (1). The results showed a significant decrease ($p<0.01$) in sperm numbers and a significant increase ($p<0.01$) in abnormal sperms of the males treated with (0.1, 0.05 mg/day) compared with the control group. The majority of abnormalities included the changes of head and tail shape as show in pictures(2,3,4,5,6,7 and 8) compared with control group (picture 1).

Effect of Dichlorvos on spermatogenesis:

Table(2) represented the results of Dichlorvos effects on spermatogenesis (pictures 10,11) . The results showed a significant decrease ($p<0.01$) in the spermatogonia and primary spermatocyte in the mice treated with (0.1,0.05 mg/day) , where as, there is no significant effect in the tubular diameter in the males treated with both doses compared with the control group (picture 9).

Table (1). Effect of Dichlorvos on the abnormalities and numbers of the mice sperms (N=8)(Mean ± standard error)

Treatment	Sperm numbers ($mm^3 \times 10^3$)	Normal sperm %	Abnormal sperms (%)	
			Ab. head	Ab. tail
Control group distil water	710.0 ± 1.89	80.25 ± 1.79	11.90 ± 0.81	7.95 ±0.45
Dichlorvos 0.05 mg/day	*480.0 ± 2.00	*57.92 ± 2.56	*27.06 ± 2.03	*14.78 ± 0.87
Dichlorvos 0.1 mg/day	*390.12 ± 2.06	*52.77 ± 2.38	*32.70 ± 1.23	*15.45 ± 0.94

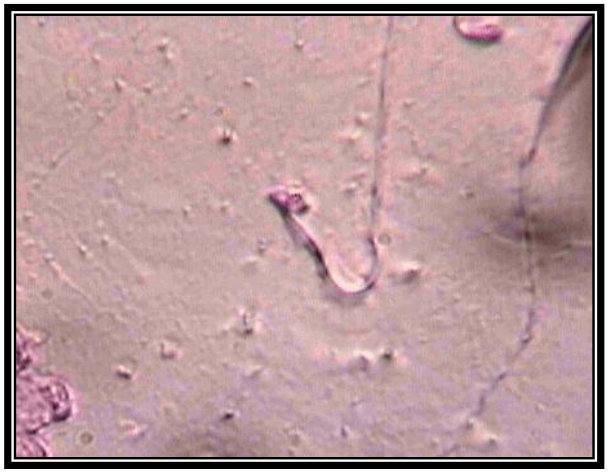
* There is a significant difference($p<0.01$) compared with the control.

Table (2). Effect of Dichlorvos on spermatogenesis of the male (N=8)(Mean ± stander error)

Treatment	Spermatogonia numbers	Primary spermatocyte numbers	Tubular diameter(µm)
Control group distil water	35.25 ± 0.79	29.62 ± 0.80	143.62 ± 2.05
Dichlorvos 0.05 mg/day	*22.32 ± 1.14	*18.76 ± 1.04	141.86 ± 2.18
Dichlorvos 0.1 mg/day	*17.18 ± 1.02	*15.72 ± 1.04	140.73 ± 2.64

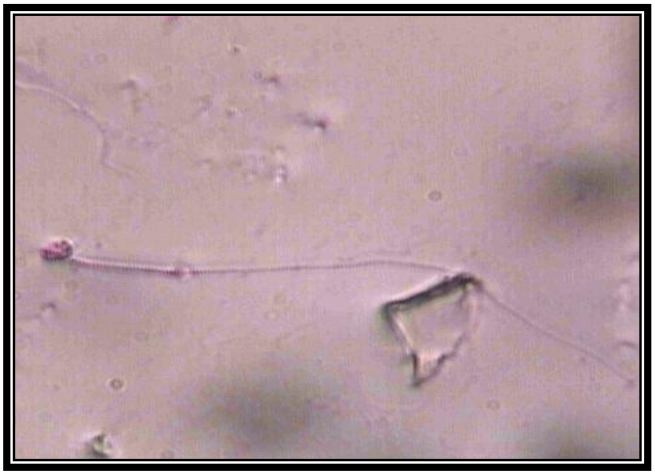
*There is a significant difference($p<0.01$) compared with control.

(1) Normal sperm 400X E.H)

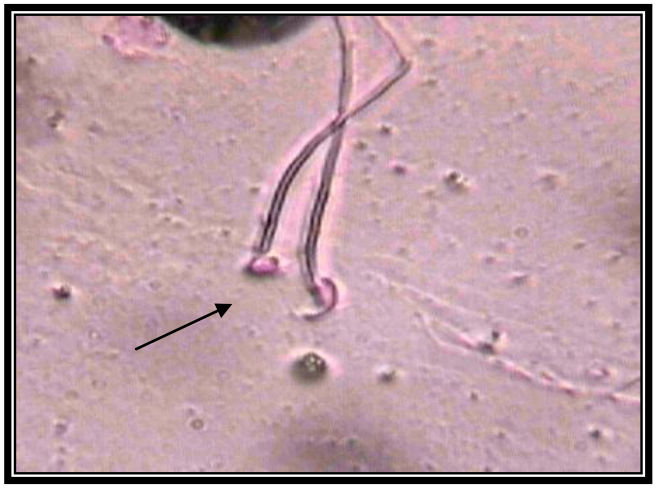


(2) Abnormal sperm (lacking of hook) 400X

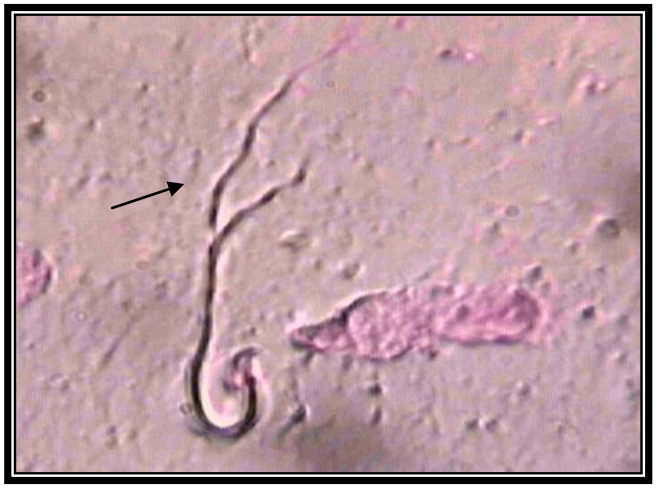
(3) Abnormal sperm (amorphous head) 400X



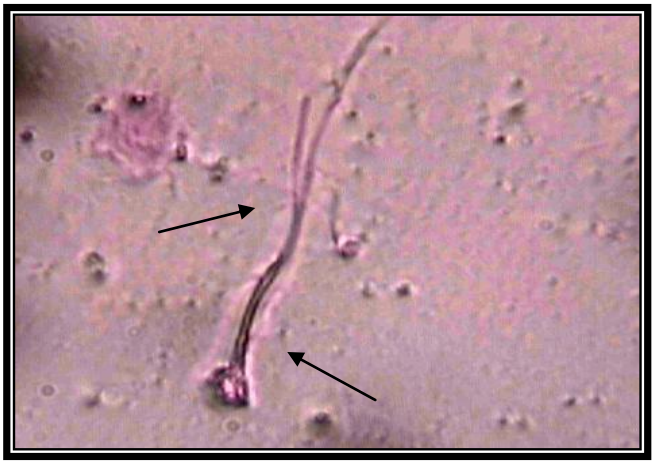
(4) Abnormal sperm (sickle head) 400X



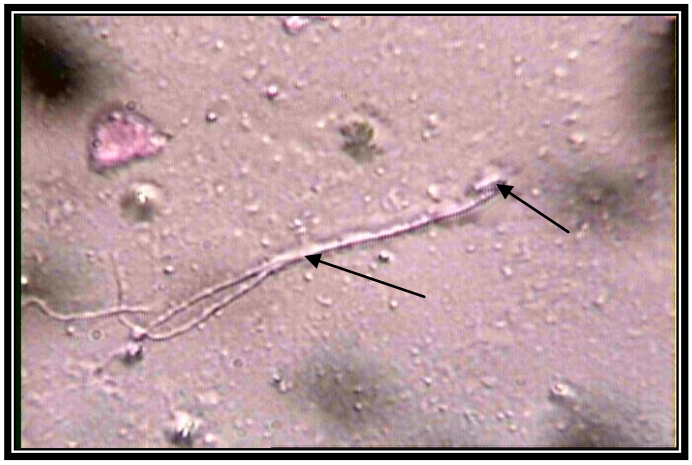
(5) Abnormal sperm (global head) 400X



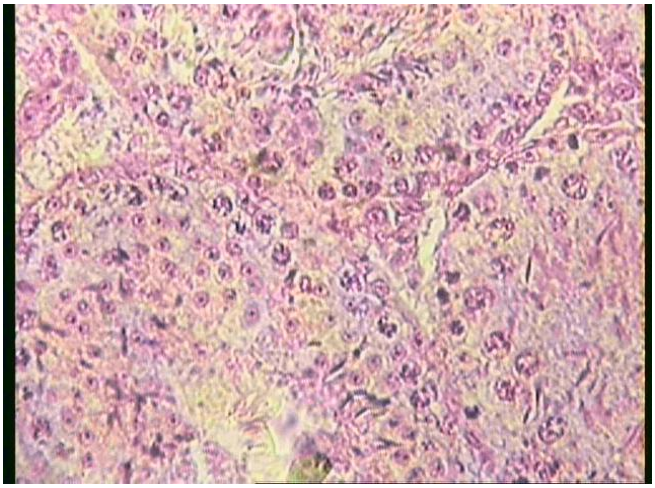
(6) Abnormal sperm (schizoid tail) 400X



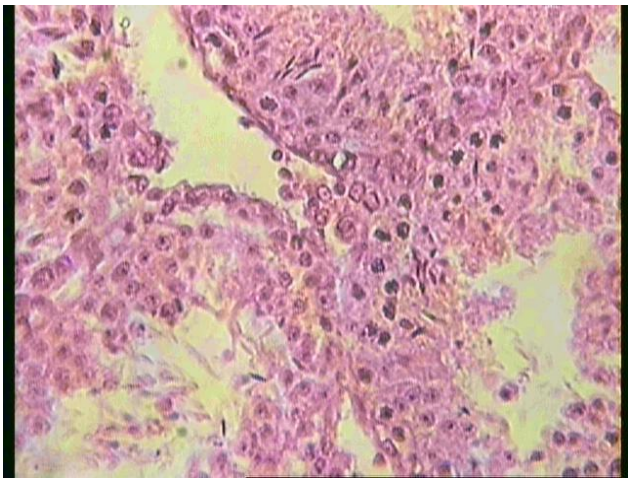
(7) Abnormal sperm
(lacking hook and schizoid tail) 400X



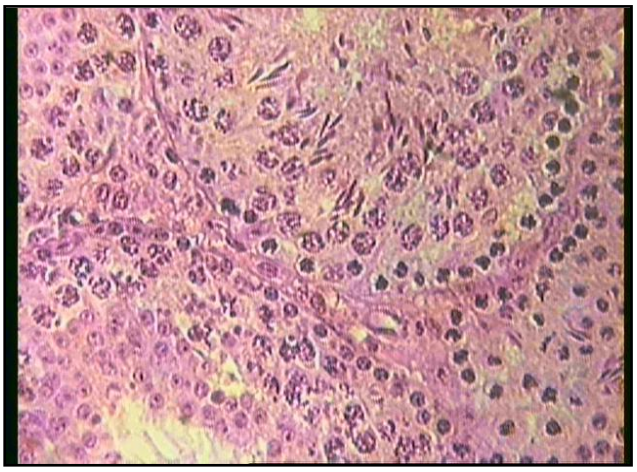
(8) Abnormal sperm
(sickle head and schizoid tail) 400X

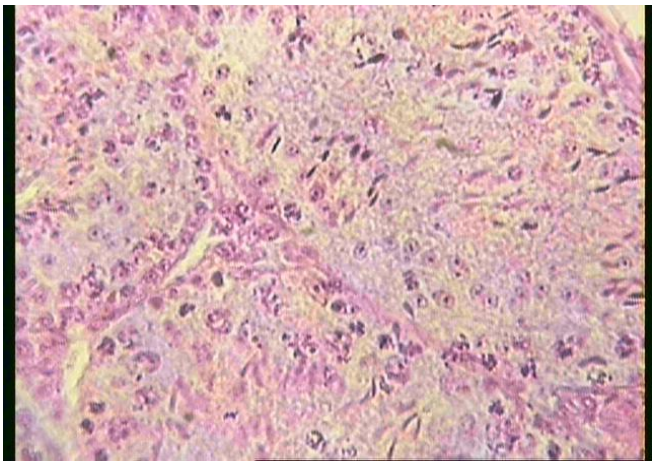


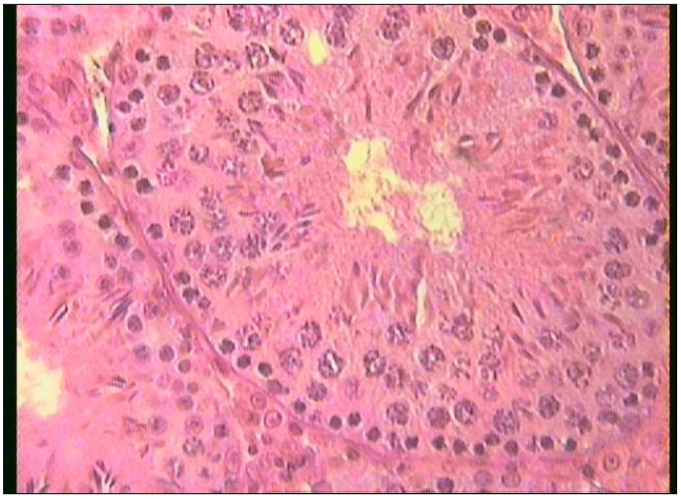
(9) Testicular section of mice (control group)
S=spermatogonia ,PS=primary spermatocyte



(10) Testicular section of mice
(low dose 0.05 mg/day of Dichlorvos)







(11) Testicular section of mice
(high dose 0.1 mg/day of Dichlorvos)

DISCUSSION

Effect of Dichlorvos Pesticide on sperm abnormalities:

The present study was showed the significant abnormalities that occurred in the sperm shapes. [19] found that sperm DNA is sensitive to organophosphate pesticide exposure and it seemed to play an important role in the genesis of sperm chromatin alterations. Moreover, the chromatin structure of abnormal sperm reflecting a variety of anomalies during spermatogenesis after exposure to chemicals [20,21] .

[22] reported that workers exposure to organophosphate pesticides in Chinese pesticide factory had increased the prevalence of sperm aneuploidy. A positive association between organophosphate pesticide metabolites levels and sex null and total aneuploidy frequencies had been reported in agricultural workers [23].

The present results from animal experiments indicated that chemical-induced alkylation of sperm protamines causing a significant stress on chromatin structure, by blocking normal disulfide-bond formation. Furthermore, some protamine alkylation bind to DNA only in a very small fraction of the total binding to the sperm nucleus, indicating that nuclear proteins are alternative molecular targets within the germ cell [24].

Effect of Dichlorvos Pesticide on sperm count and spermatogenesis:

The result of the present study showed that Dichlorvos caused significant depression in sperm numbers and spermatogenesis which may occur due to the effect Dichlorvos on the levels of sex hormones especially testosterone hormone which played an important role in the spermatogenesis [25]. Moreover, [26] found that Dichlorvos diminished the number of sertoli and leydig cells which have a role in providing the nutrients and incubation to spermatides [27]. In addition, the germinal cell sloughing may reflect a functional damage of sertoli cells which their function may be affected by the Dichlorvos pesticide [28].

[29] found that parathion is a more potent cytotoxic compound for mice resulting in decrease of the body and testicular weight. Also [30] found that exposure the rats to Dichlorvos caused a necrosis in the germinal epithelium. The testicular toxicant effects of organophosphate pesticide goes beyond genotoxic damage to compromise testosterone production by altering leydig cell steroidogenesis [31] .

The effect of organophosphate pesticides on reproductive function are suspected by reducing brain acetyl cholinesterase activity and monoamine levels, thus impairing hypothalamic and / or pituitary endocrine function and gonadal processes , indicating that FSH and LH are the hormones most effected which have an important role in the spermatogenesis [32]. [33] reported that malathion elicits a decrease in the number of renewing spermatogonia in immature rats. Also, [34] postulated that organophosphate pesticides inhibit the spermatogonial mitosis which produced by spermatogenic differentiated cells.

Conclusion :

Dichlorvos elicits a toxic effect on germinal cell of the testis , being toxic both to spermatogonia ,primary spermatocyte and sperm . sperm abnormalities may result from a variety of mechanisms , mainly affecting the DNA structure and function .

تأثير مبيد الدايكلورفوس على خصوبة ذكور الفئران المختبرية.*Mus musculus* L

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الخلاصة

الدايكلورفوس من المبيدات العضوية الفسفورية الحشرية الواسعة الانتشار في البيئة ،اذ تناولت هذه الدراسة تأثير هذا المبيد على عدد النطف وتشوهاتها وعلى عملية نشأة النطف لذكور الفئران المختبرية *Mus musculus* L. والمعاملة بالجرعتين (0.1 ملغم/يوم و 0.05 ملغم/يوم) ، ولمده 15 يوماً .

أظهرنتائج حقن مبيد الدايكلورفوس في منطقة الخلب .I.P للفئران المختبرية أنخفاض معنوي في عدد النطف وبالجرعتين عند مقارنته مع مجموعة السيطرة. كما بينت النتائج وجود أرتفاع معنوي في تشوهات النطف وبالجرعتين، بالاضافة الى ذالك ، ان حقن المبيد سبب انخفاض معنوي في عدد خلايا سليفات النطف وعدد الخلايا النطفية الابتدائية وبالجرعتين ، بينما لم يظهر التحليل الإحصائي وجود أي فارق معنوي لقطر النبيب المنوي عند مقارنته مع مجموعة السيطرة.

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