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REPRODUCTIVE TOXICITY INDUCED BY CHLORPYRIFOS IN MALE RATS

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ABSTRACT

Chlorpyrifos (CPF) is one of the most organophosphate pesticides commonly used. Although many studies have been conducted on the insecticide chlorpyrifos but few of them had intended to study its toxic effect on the reproductive capacity. Therefore, the present study aimed at assessing the reproductive efficiency of male rats after exposure to a single dose of chlorpyrifos and estimation the hormone testosterone in serum as a marker to assess male fertility potential after treatment with pesticide. As well as study the rate of sperm production and counts as well as the motility, morphology and viability. Also shed light on the potential effects of chlorpyrifos toxicity on the activities of some enzymes (ALT, AST, ALP, and LDH) in addition to the lipid profiles in the serum of male rats. In addition, estimate mating and fertility indices by mating treated males with normal females. To clarify the histopathological changes occurred in the testicular tissue. The male rats were divided into two groups (25 mice per group); the first group was given solution of saline orally and considered as a control. The second group was received a dose of 9 mg chlorpyrifos/kg body weight/day (1/25 of LD_{50}) for 70 days.

Samples were taken from the blood and the separation of serum for use in physiological study. As well as the collection of samples from the testes of the histological study. Results showed that giving male rats this dose resulted in the emergence of many of the symptoms of toxicity such as diarrhea, redness of the eye and a decrease of body weight and genital organs weights. Histological findings of the testicular tissue showed cellular debris of the germ cells within seminiferous tubules as well as the presence of vacuolations in the germ cells to rupture and distort the full walls of seminiferous tubules with a decrease in the thickness of the layers of germinal epithelium. Histological changes have been linked to the previous significant decrease in the hormone testosterone, which has been associated with inhibition of mating and fertility indices. There is a clear lack in numbers of sperm as well as the mobility. As it turned out significant increase in abnormal forms (distorted), this appeared in the head and tail with the presence of cytoplasmic droplets on the tail. The results also showed an increase in the activity of enzymes that associated with increase in lipid profiles. These results can be concluded that exposure male rats, to this dose level caused a detrimental effect on reproductive tissues and thus on the fertility of males to reproduce.

Keywords: Chlorpyrifos; Reproductive toxicity; Testosterone; Fertility; Spermatozoa

INTRODUCTION

Organophosphates are among the most widely used synthetic insect pesticides. The widespread use of organophosphates has stimulated research into the possible existence of effects related with their

reproductive toxic activity (Joshi *et al.*, 2007). Occupational exposures to pesticides could diminish or destroy the fertility of workers sparked a concern about the effects of hazardous substances on male reproductive health. The issue of testicular toxicity is of growing concern as a large number of organophosphates viz., diazinon (ATSDR, 1994), and methyl parathion (Joshi *et al.*, 2003). Adversely affect the testicular functions in experimental animals. Owing to the extensive use of organophosphate pesticides in agriculture, there is a high risk of human exposure to these chemicals (Sarkar *et al.*, 2000).

Chlorpyrifos (CPF), (O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate) is a conventional organophosphorous insecticide, that was first registered as a broad spectrum insecticide in 1965. It was used widely to control a variety of pests in agriculture and animal farm (EPA, 1986). CPF interferes with the acetyl cholinesterase enzyme, which is necessary for normal nerve transmission (NRA, 2000). It has been reported that it was linked to male and female genital deformities (Sherman, 1995; ENDS, 1999). Additionally, the exposure of laboratory animals to CPF elicits a number of effects including hepatic (El-Shenawy and Al-Eisa, 2010; Mansour and Mossa 2010) and testicular damage (Joshi *et al.*, 2007).

Also, acute toxic effects of chlorpyriphos were studied on goats 24 hours after the exposure. The pesticide had moderate to marked potential to exert pathophysiological effects on the body (Kaur *et al.*, 1998; Seth *et al.*, 2000). In the context of chlorpyrifos toxicology, recent study reported that OP pesticides (e.g. CPF) can be accumulated and cause significant damage

with increased plasma levels of aminotransferases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) and lactate dehydrogenase (LDH) in a dose-dependent manner (Mansour and Moussa,2011).The present study was therefore, undertaken to compare and evaluate the possible toxic effects of CPF in producing the biochemical and histopathological alterations in the testes of male rats, which may have implications in managing humans with accidental exposures to such compounds.

MATERIAL AND METHODS

Chemicals

CPF technical grade (98%) was obtained from El-Helb Company for pesticides and chemicals, Egypt.

Animals

Healthy adult male albino rats of the Wistar strain *(Rattus norvegicus)* with proven fertility, 4-5 months of age and weighing 150 ± 5 g, were supplied from the Animal Breeding House of the Medical Research Institute (MRI), Alexandria University, Alexandria, Egypt. Animals were maintained at the animal care facility in the Zoology Department, Faculty of Science, in plastic cages under controlled temperature (23 ± 2°C), 12-h light/dark cycle and 50 ± 5% relative humidity. Water and food were available *ad libitum*. Rats were acclimatized to the laboratory environment for two weeks prior to the start of experiments.

Experimental design

After the period of acclimation, animals were divided into two groups with twenty five animals in each. The first group was used as control. The animals of control group were orally given saline (4 ml/kg). The second group was orally administered with CPF (9 mg/kg b.w.); about 1/25 LD_{50}. The selected dose of the CPF was based on studies of McCollister *et al.* (1974). The duration of the oral administration during the experiments lasts for 70 days for completion of the spermatogenic cycle and maturation of sperms in epididymis (Sarkar *et al.*, 2003).

Mating and Fertility indices

After the end of the administration course, males of control and experimental groups of rats (n=25/group), were mated 1:1 with untreated proven fertile, with regular estrus cycle, females for 5 days (complete one estrous cycle) (Fox and Laird, 1970). Mating was confirmed by the presence of vaginal plugs or deposition of spermatozoa at the vaginal orifice upon vaginal examination. The day of a vaginal plug that found was considered day 0 of gestation. Then mating and fertility indices were estimated and recorded.

Sperm quantity and quality

The left cauda epididymis was used for sperm motility and right cauda epididymis was used for sperm counts and morphology. Left cauda epididymis was first weighed, placed in a petri dish. An appropriate dilution (1:20) was made with physiological saline (0.9% NaCl). Cauda epididymis was nicked in few sites with a scalpel blade and kept at 37 °C to release the spermatozoa from the tubules. The sperm suspension was examined within 5 minute after their isolation from epididymis. The

counting of both motile and immotile sperms was done at 40x magnification. The calculated results were finally expressed as percent motility (Bearden and Fluquary, 1980; Freund and Carol, 1993). Right cauda epididymis was weighed, diluted in 1:20 with physiological saline (0.9% NaCl) solution in a petri dish and minced with a scalpel blade in the mid-to-distal region. Suspension was kept at 37 °C for 5 minutes for the dispersion of sperm into medium. Sperm suspension was pipette very gently 20 times and placed in a hemocytometer, and total number of the sperm heads was counted (Freund and Carol, 1993) at 40x magnification. Each sample was counted twice and means value was taken for calculation.

Body and genital organ weight

Initial and final body weights of male rats were recorded and subsequently weight changes were calculated. After fertility study, the rats were sacrificed by cervical dislocation. The testes and accessory sex organs (seminal vesicles, prostates and epididymis) were dissected out, trimmed off the attached tissues and weighed individually. Then, the organ/body weight ratio was calculated. Specimens of the testes were fixed immediately in Bouin's fluid for histological study.

Biochemical assays

At the end of the 70th day of the treatment course, blood samples were collected from anaesthetized males of all groups by puncturing the retro-orbital venous plexus with a fine sterilized glass capillary tube into dry tube. The gathered blood left for 20 min at room temperature, then centrifuged at 3000 rpm (600 g) for 10 min for the separation of serum. The serum was kept in a deep freezer (-20 °C) until analyses of certain

biochemical parameters. The biochemical measurements were performed according to the details given in the kit's instructions.

Enzymes activity determination

Activities of serum lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were measured by spectrophotometry using commercial kits (Boehringer Mannheim, GmbH, Mannheim, Germany).

Testosterone determination

Serum samples of the control and treated male rats were used for estimating testosterone concentration using radioimmunoassay (RIA) method. After incubation, the liquid contents in the tubes were withdrawn and the bound radioactivity was determined using gamma counter according to method described by Wilke and Utley (1987).

Determination of serum lipids profile

Serum concentrations of total lipids (TL) were assayed by the method of Knight *et al.* (1972) and that of cholesterol and triglycerides (TG) were determined by the method of Carr *et al.* (1993). High density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) were determined according to the methods of Warnick *et al.* (1983) and Bergmenyer (1985), respectively. Very low-density lipoprotein (VLDL) was calculated mathematically by dividing the values of TG by a factor of 5 according to Friedewald *et al.* (1972).

Histopathological examination

Testes of the control and treated rats were taken and fixed in Bouin's solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene,

embedded in paraffin, sectioned at 4–6 µm thickness and stained with Hematoxyline and Eosin (H&E) according to Lillie (1965) then examined microscopically for histological alterations.

Statistical analysis

Data were expressed as mean values ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess significant differences among treatment groups. For each significant effect of treatment, the post hoc Tukey's test was used for comparisons. The criterion for statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS statistical version 8 software package (SPSS® Inc., USA).

RESULTS

Morbidity and mortality

Male rats administered with CPF (9 mg/kg bw/day) for 70 days have shown signs of toxicity such as salivation, diarrhea, nose and eye bleeding and tremor. No death was recorded throughout the experimental group.

Body and organs weights

Body and organs weights of experimental male rats were presented in Table 1. Significant decrease in the body weight change was observed in the rats group that treated with CPF as compared to control rats. Absolute and relative reproductive organ weights (testes, epididymides, prostate and seminal vesicles) were reduced dramatically in the group treated with CPF as compared to the control.

Sperm characteristics

Data of sperm characteristics (Table 2) showed that the treatment of male rats with CPF significantly ($P < 0.01$) decreased sperm count and progressive motility (%), while increased immotile and morphologically abnormal sperms as compared to control group (Table 2).

Mating and fertility indices

Mating and fertility indices of the male rats given CPF at doses of 9 mg/kg/b.w. for 70 consecutive days were 52% and 54%, respectively compared to 100% in the control normal group as shown in Table 3.

Biochemical changes

Serum AST, ALT, ALP and LDH activities

The results of enzyme activities of male rats were shown in Table 4. Male rats exposed to CPF (9 mg/kg/day) showed an increase in the serum enzyme activities of AST, ALT, ALP and LDH levels compared to control.

Serum lipids profile

The lipids profile in sera of male rats were assayed showing significant ($P < 0.05$) increase in total lipids, triglycerides, total cholesterol, very low density lipoprotein cholesterol (VLDL-c) and low density lipoprotein cholesterol (LDL-c) in CPF treated group compared to the control group (Table 5), while the content of high density lipoprotein cholesterol (HDL-c) showed a significant ($P < 0.05$) decrease in CPF treated group compared to the control group (Table 5).

Serum testosterone

Data presented in Figure 1 showed significant decrease in serum testosterone concentrations ($P < 0.05$) in rats treated with CPF compared to control.

Histopathology

Figures 2A–2E showed photomicrographs of testes from the control and treated group. Histopathological examination of the testes of normal rats revealed active mature functioning seminiferous tubules associated with complete spermatogenial cell series (Fig. 2A). The histological architecture of the testicular pathology of CPF treated rats showed depression of spermatogenesis, Sertoli cell toxicity and degeneration of seminiferous tubules (Fig. 2B). The general architecture of some seminiferous tubules was disorganized (Fig. 2E). They were characterized by the accumulation of exfoliated germ cells (cellular debris) within the affected tubules (Fig. 2C, D) and appearance of cytoplasmic vacuolation (Fig. 2B, D).

DISCUSSION

The administration of chlorpyrifos in the present work brought a marked reduction in the testicular weight reflects regressive changes in seminiferous tubules. Similar reduction in the weight of testes was also observed by Joshi *et al.* (2007) with different doses and exposure time. Decrease in testicular weight was accompanied by necrotic changes (Udoh and Kehinde, 1999). The change in testicular weight has also corresponded to the presence or absence of postmeiotic germ cells (Nelson and Patenelli, 1965). Reduction in the number of spermatogenic elements and spermatozoa leads to reduction in the weight of testes (Takihara *et al.*, 1987).

Sperm motility is an important functional measurement to predict sperm fertilizing capacity. Any negative impact on motility would

seriously affect fertilizing ability. In the present study, marked inhibition of sperm motility in CPF treated rats may be because of low level of ATP content (Bai and Shi, 2002). Sperm motility may be affected by altered enzymatic activities of oxidative phosphorolytic process. Oxidative phosphorolytic process is required for ATP production, a source of energy for the forward movement of spermatozoa (Bett *et al.,* 1996). Full ATP pool is crucial for normal spermatozoa movement and a slight deprivation of ATP leads to reduction in motility, which may cause infertility. Sperm count is considered to be one of the important factor that affect fertility (Bett *et al.,* 1996). Suppression of gonadotrophins might have caused decrease in sperm density in testes (Joshi *et al.,* 2007). Also, toxicants have direct effect on Sertoli cell function, which appears to be involved in the control of spermiation, and when disturbed caused epithelial disorganization and subsequent tubular atrophy (Bedwal *et al.*, 1994). The negative fertility test may be attributed to lack of forward progression and reduction in the density of spermatozoa and altered the biochemical milieu of cauda epididymis.

In the present findings, increased level of cholesterol is attributed to decreased androgen concentration, which resulted in impaired spermatogenesis (Bedwal *et al.,* 1994). Similar effects by other insecticides have also been reported (Choudhary and Joshi, 2003; Joshi *et al.*, 2003, 2007). Reduction in the serum testosterone, clearly demonstrated the inhibitory effect of insecticides on the secretion of pituitary gonadotrophins (FSH and LH) and in turn on the testosterone biosynthesis in the testes of rat (Singh and Pandey, 1990). Marked

reduction in testosterone content in association with highly reduced circulating levels of this hormone confirmed alteration in the reproductive physiology of rats. The decrease in testosterone production may be induced by the stimulation of P450 aromatase (P450arom), which catalyzes estrogen production from androgen; thereby decreasing androgen levels (Saitoh *et al.*, 2001). These results suggested that chlorpyrifos exerts suppressive effects on testicular function and leads to infertility in rats.

The decrease in plasma testosterone levels, body weight, relative testes and epididymis weights observed in the present results confirmed earlier results of Grote *et al.* (2004) in rats and Sarpa *et al.* (2007) in mice. Sarpa *et al.* (2007) found that treatment with triphenyltin chloride during gestation days 6–17 of mice caused a decrease in weight gain and food intake. The decrease in relative testes and epididymis weights of rats treated with CPF in the present study agreed with results obtained by Yousef *et al.* (2010), who found that treatment with triphenyltin chloride caused a decrease in relative weight of testis and epididymides of rabbits. The present study declared that CPF caused a decrease in epididymal sperm count and sperm viability of rat. These results could be suggested that CPF impair male reproduction in rat by decreasing circulatory testosterone. The decline in ejaculate volume, sperm concentration, and total sperm output can be partly attributed to the CPF-induced reduction in testosterone. The observed decrease in sperm motility could be attributed in part to the concomitant abnormality of the sperms, decrease their viability (Table 2) and the decrease in body weight (Table 1). Moreover, CPF has been reported that it was able to generate reactive oxygen species

(ROS) in different tissue organs (Verma *et al.*, 2007; Mansour and Mossa, 2010) which coincides with the elevation in lipid peroxidation induced in seminal plasma. Overproduction of ROS can be detrimental to sperm as it is may be associated with male infertility (Akiyama, 1999). Thus, the spermatoxic effect of triphenyltin chloride might be due to induced free radicals.

The transaminases and phosphatases in semen play an important role in transamination and phosphorylation processes in sperm metabolism (El-Kashoury and Tag El-Din, 2010). The present results revealed a significant ($P < 0.05$) increase in the activities of serum AST, ALT, LDH and ALP of rats treated with CPF. El-Kashoury and Tag El-Din (2010) reported that CPF in different local manufactures (chlorozan, pestpan and pyriban) at different doses of 23.43, 21.40 and 17.43 mg/kg b.w., respectively significant decrease the activities of ALP and LDH. This fact is a conventional indicator of liver injury due to CPF- treatment (El-Banna *et al.*, 2009).

Recent literature demonstrated that CPF may affect liver metabolism and the leakage of certain intracellular enzymes, suggesting damage in hepatocytes. The increment of the activities of AST, ALT and LDH in plasma could be attributed to the leakage of these enzymes from the liver cytosol into the blood stream (Mansour and Moussa, 2011) which indicated liver damage and disruption of normal liver function (Caglar and Kolankaya, 2007).

In the present work, the serum content of lipid profile showed significant increases in TL, TG, TC and LDL-cholesterol were shown in CPF-treated rats compared to the control ones.

In the current study, it was evaluated the reproductive toxicity and the histological changes resulting from the administration of CPF to rats. Many environmental, physiological and genetic factors have been implicated in defective sperm function, the most common cause of infertility. Free radical-induced oxidative damage to spermatozoa is one such condition which has recently gained a considerable attention for its role in inducing poor sperm function and infertility (Russo *et al.*, 2006). Factors that can offer spermatozoa protection are, therefore, of great importance. In conclusion, the present results showed that exposure to CPF caused significant decrease the enzyme activities in serum, deterioration in sperm characteristics and male infertility of rats.

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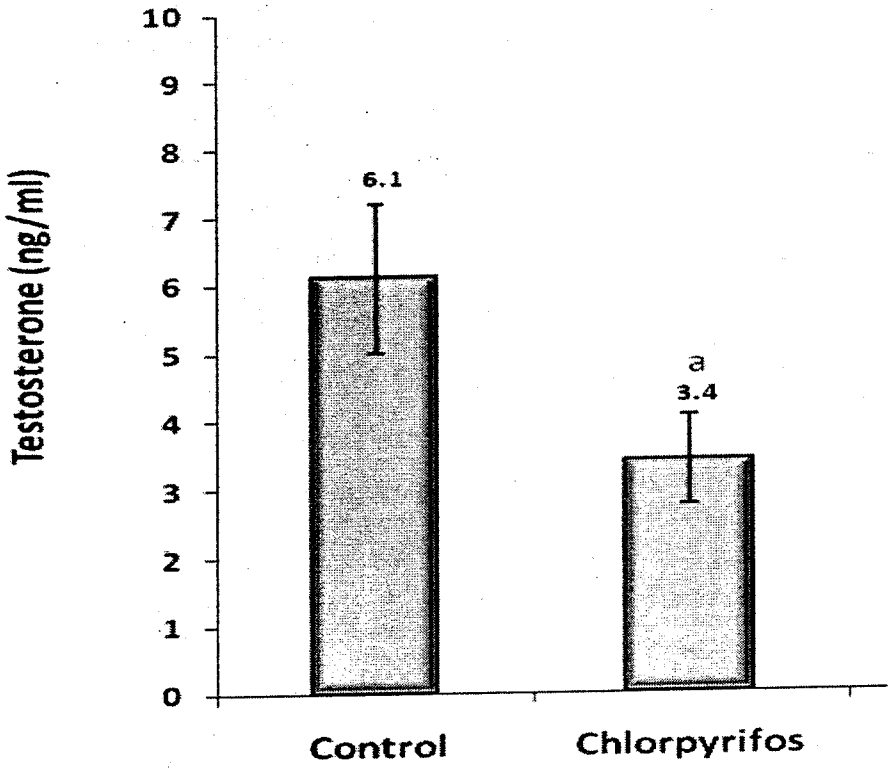
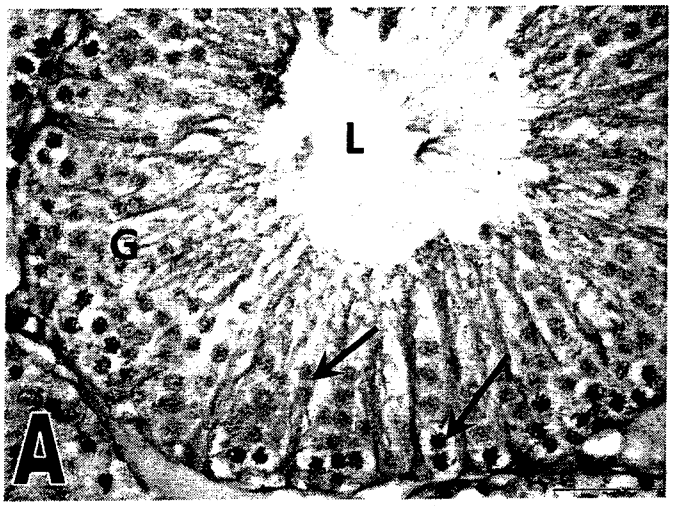


Fig. 1: Serum testosterone of male rats after oral administered with chlorpyrifos and/or propolis for 70 days. The data are presented as mean ± S.D, (n = 10). [a]Significant difference as compared with control group ($P \leq 0.05$). [b]Significant difference as compared with chlorpyrifos group ($P \leq 0.05$).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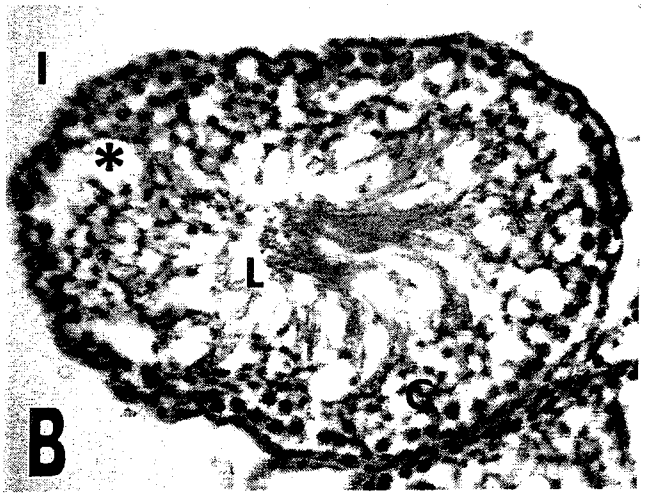
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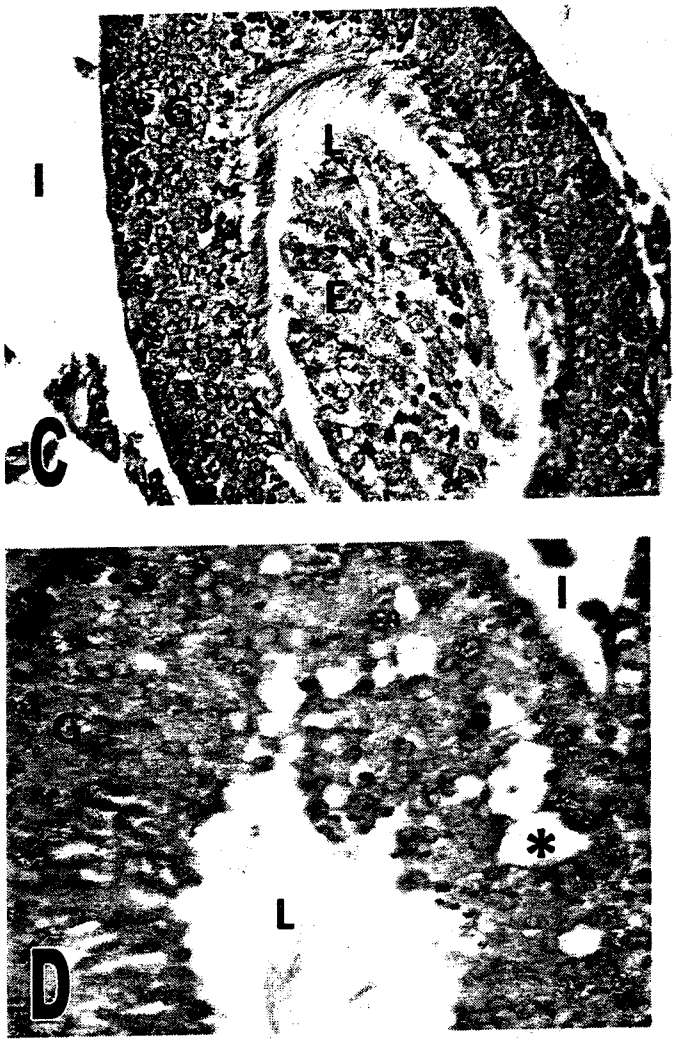
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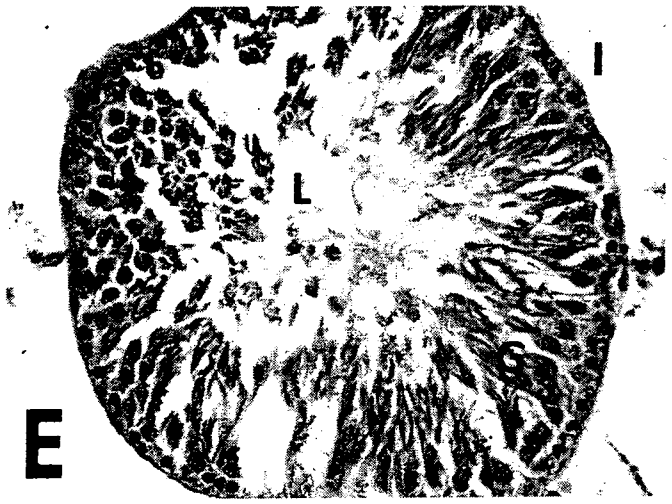

Fig. 2: Photomicrograph of testicular histology of the (A) control rats showing normal structure of germinal epithelium with primary spermatocytes, secondary spermatocytes, spermatids are seen. (B-E) Testes of rats treated with 9 mg/kg bw CPF, showing vacuolation and deterioration of abnormal germinal cells with destruction in components of the tubules. (E): exfoliation of debris of germ cells in the lumen, (arrow): germinal cells, (G): germinal epithelium, (*): vacuolation, (I): intercellular tubular spaces, (L): Lumen, H&E, original magnification 200X.

Table 1: Effect of oral administration of chlorpyrifos and / or propolis for 70 days on the body and sexual relative organs weight of male rats

	Control	CPF	Value of P
Body weights (g)			
Initial	172.7 ± 9.85	174.6±10.98	> 0.05
Final	219.5 ± 16.25	189.3 ± 13.6 [a]	0.021
Body weight change (%)	27.1 ± 2.36	8.4 ± 1.08 [a]	0.001*
Organs weights (g)			
Testes	2.68 ± 0.21	1.85 ± 0.36	> 0.05
Epididymides	0.83 ± 0.036	0.66 ± 0.105	> 0.05
Prostate	0.56 ± 0.098	0.36 ± 0.096 [a]	0.045
Seminal vesicles	1.82 ± 0.41	0.98 ± 0.11 [a]	0.021
Relative organ weights			
Testes	1.22 ± 0.10	0.98 ± 0.11	> 0.05
Epididymides	0.38 ± 0.025	0.35 ± 0.03	> 0.05
Prostate	0.26 ± 0.031	0.19 ± 0.02 [a]	0.05
Seminal vesicles	0.83 ± 0.08	0.52 ± 0.031 [a]	0.038

The data are presented as mean ± S.D, n = 25.

[a] Significant difference as compared with control group ($P \leq 0.05$).

[b] Significant difference as compared with chlorpyrifos group ($P \leq 0.05$).

Table 2: Sperm characteristics in male rats after oral administration of chlorpyrifos and / or propolis for 70 days

Sperm Parameters	Control	CPF	Value of P
Count/epididymis ($\times 10^6$)	91.2 ± 9.85	56.1 ± 4.65 [a]	0.012
Motility (%)			
Progressive	59.4 ± 4.68	25.1 ± 2.06 [a]	0.021
Non-Progressive	15.8 ± 2.10	29.3 ± 2.65 [a]	0.033
Immotile	24.7 ± 2.65	45.5 ± 3.98 [a]	0.041
Morphology (%)			
Normal	86.4 ± 7.98	62.4 ± 6.07 [a]	0.045
Abnormal head	5.2 ± 0.25	19.0 ± 1.06 [a]	0.022
Abnormal tail	7.6 ± 0.62	17.1 ± 1.20 [a]	0.035
Other abnormalities	0.7 ± 0.02	1.5 ± 0.106	0.05
Total abnormalities	13.5 ± 2.33	37.6 ± 2.96 [a]	0.013
Viability (%)	88.5 ± 7.90	56.5 ± 6.21 [a]	0.022

The data are presented as mean ± S.D, n = 25.

[a] Significant difference as compared with control group ($P \leq 0.05$).

[b] Significant difference as compared with chlorpyrifos group ($P \leq 0.05$).

Table 3: Functional fertility parameters of male rats after oral administration of chlorpyrifos and / or propolis for 70 days

	Control	CPF	Value of P
Number of males that used for mating	25	25	
Mating index (%)	25/25 (100)	13/25 [a] (52)	0.012*
Fertility index (%)	25/25 (100)	7/13 [a] (54)	0.025*

The data are presented as mean ± S.D, n = 25.

[a] Significant difference as compared with control group ($P \leq 0.05$).

[b] Significant difference as compared with chlorpyrifos group ($P \leq 0.05$).

Mating index (%) = Number of males inseminated females /total number of males cohabited with females ×100.

Fertility index (%) = Number of cohabited females becoming pregnant/number of non pregnant with evidence of vaginal plug×100.

Table 4: Effect of chlorpyrifos alone and its combination with propolis on serum ALT, AST, ALP and LDH activities of male rats after 70 days of oral administration

Enzymes activities (U/L)	Control	CPF	Value of P
ALT	88.51 ±8.62	121.36 ± 14.25 [a]	0.025
AST	156.82 ±16.25	188.41 ± 22.9 [a]	0.036
ALP	139.06 ±19.65	185.92 ±24.6 [a]	0.044
LDH	1259.25 ±108.6	2394.36 ± 110.3 [a]	0.027

The data are presented as mean ± S.D, (n = 10).

[a] Significant difference as compared with control group ($P \leq 0.05$).

[b] Significant difference as compared with chlorpyrifos group ($P \leq 0.05$).

AST: aspartate transaminase, ALT: alanin transaminase, AlP: alkline phosphatase, LDH: lactate dehydrogenase.

Table 5: Biochemical changes of serum lipids profile after oral administration of male rats with chlorpyrifos and / or propolis for 70 days

Parameters (mg/dl)	Control	CPF	Value of P
TL	289.98 ± 22.6	336.01 ± 27.5 [a]	0.05
TG	124.60 ± 15.6	136.71 ± 16.8	0.05
TC	102.67 ± 9.85	135.50 ± 10.2[a]	0.041
HDL-c	43.31 ± 3.99	32.43 ± 2.99 [a]	0.05
LDL-c	34.44 ± 2.85	75.75 ± 7.98 [a]	0.025
VLDL	24.92 ± 2.41	27.34 ± 2.88 [a]	0.05

The data are presented as mean ± S.D, (n = 10).

[a] Significant difference as compared with control group ($P \leq 0.05$).

[b] Significant difference as compared with chlorpyrifos group ($P \leq 0.05$).

TL; total lipid, TG; triglycerides, TC; total cholesterol, HDL-c; high density lipoprotein-cholesterol, LDL-c; low density lipoprotein-cholesterol and VLDL; volatile low density lipoprotein.

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السمية الإنجابية المستحثة بالكلوربيريفوس في ذكور الجرذان

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تستهدف الدراسة الحالية تقدير الكفاءة الإنجابية لذكور الجرذان بعد التعرض لجرعة واحدة من الكلوربيريفوس وتقدير هرمون التيستوستيرون فى سيرم الذكور كدلالة لتقييم الخصوبة المحتملة بعد المعاملة بالمبيد. وكذلك دراسة معدل إنتاج الحيوانات المنوية وعددها وكذلك حركتها ومظهرها وحيوتها. وأيضا إلقاء الضوء على الآثار المحتملة لسمية الكلوربيريفوس على أنشطة بعض الأنزيمات (الترانس أمينيز واللاكتيت ديهيدروجينيز والألكالاين فوسفاتيز) بالأضافة الى صورة الدهون في مصل ذكور الجرذان. علاوة على تقدير معامل التزاوج والخصوبة للذكور عند تزاوجها مع إناث طبيعية. وتوضيح التغيرات الهيستولوجية المرضية فى نسيج الخصية. وقد تم تقسيم ذكور الجرذان الى مجموعتين (٢٥ جرذ لكل مجموعة). المجموعة الأولى وهى الضابطة أعطيت محلول الملح. المجموعة الثانية أعطيت الكلوربيريفوس بجرعة ٩ مجم/كجم من وزن الجسم/يوم (١/٢٥ من الجرعة المميتة) لمدة سبعين يوم. وتم أخذ عينات من الدم وفصل السيرم لإستخدامها فى الدراسة الفسيولوجية. وكذلك جمع عينات من الخصى للدراسة الهيستولوجية. أوضحت النتائج أن إعطاء ذكور الجرذان هذه الجرعة أدت الى ظهور العديد من أعراض السمية مثل الإسهال وإحمرار العين ونقص فى وزن الجسم والأعضاء التناسلية. وقد أظهرت نتائج الدراسة الهيستولوجية على أنسجة الخصى فى الجرذان التى تم أعطاؤه كلوربيريفوس تغيرات واضحة عبارة عن بقايا من الخلايا الجرثومية داخل الأنيبيات المنوية للخصية وكذلك وجود تجاويف وفراغات فى الخلايا الجرثومية مع تمزق وتشوه كامل لجدر الأنابيب المنوية مع نقص فى سمك الطبقات الجرثومية الطلانية. وقد أرتبطت التغيرات الهيستولوجية السابقة بنقص معنوى فى هرمون التيستوستيرون والذى إرتبط مع تثبيط معامل التزاوج والخصوبة. هناك نقص واضح فى أعداد الحيوانات المنوية وكذلك بطء حركتها. كما إتضح زيادة معنوية فى الأشكال الغير طبيعية لها (المشوه) والتى ظهرت فى الرأس والذيل مع وجود قطيرات سيتوبلازمية على الذيل. كما أظهرت النتائج زيادة فى نشاط الإنزيمات والمصاحبة بزيادة فى الدهون. من هذه النتائج يمكن القول أن تعرض ذكور الجرذان لمستوى هذه الجرعة تسببت فى تأثير ضار وسام على الأنسجة التناسلية وبالتالى على قدرة الذكور على الأنجاب.