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OXICOLOGICAL AND PATHOLOGICAL STUDIES ON THE ADVERSE EFFECTS OF INSECTICIDE TRICHLORFON (METRIFONATE) IN ALBINO RATS WITH SPECIAL REFERENCE TO MALE FERTILITY

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ABSTRACT

The present investigation was carried out to study the adverse effects of organophosphorus insecticide trichlorfon by both therapeutic and toxic doses on albino rats with special reference to male fertility through recording the changes in serum biochemical parameters as well as the histopathological alterations.

In this study 21 mature male albino rats were divided randomly into three equal groups as follows: the first group was served as a control while the second and the third groups were orally administered trichlorfon by a stomach tube once daily for 65 days at a dose of 8 mg/kg.b.wt(therapeutic dose)&80 mg/kg.b.wt.(toxic dose)respectively. The experimental period extended to 65 days from the 1st day of administration of trichlorfon. At end of experimental period, rats were scarified. Blood samples were collected for separation of the serum for estimating serum levels of testosterone hormone, Leutinizing hormone (L.H), aspartate amino transferase (AST), alanine amino

transferase (ALT), total acid phosphatase and prostatic acid phosphatase. Testes, seminal vesicles and prostate glands were dissected and weighed. The cauda epididymis were extracted and prepared for semen analysis. Moreover, tissue specimens were collected from testes, seminal vesicles, prostate glands, liver and spleen for histopathological investigation.

Rats treated with either therapeutic or toxic dose of trichlorfon showed significant decrease in testicular weights, sperm motility (%), sperm cell concentration and total acid phosphatase levels and significant increase in sperm cell abnormalities (%), LH, AST and ALT levels compared with the control. Rats administered toxic dose of trichlorfon displayed a significant decrease in the seminal vesicle and prostate gland weights and testosterone hormone level compared with the control.

Histopathological findings in testes of rats treated with the therapeutic dose of trichlorfon revealed interstitial edema, congestion and hemorrhage in the testicular tissue. Moreover scanty secretions in some tubulo-alveolar glands with papillary projections of the lining epithelium of the seminal gland together with cystic dilated prostatic acini with scanty secretion. Degenerative changes in the liver and mild lymphoid depletion with few hemosiderosis in the spleen were seen. The fore mentioned lesion became abundant and intense in rats received toxic dose of the compound.

It could be concluded that, trichlorfon elicited a marked reduction in male fertility by using biochemical parameters, semen analysis as well as the histopathological picture.

INTRODUCTION

Organophosphate compounds (OPCs) are an important insecticide class, which are widely used in agriculture and domestic purposes to control insect pests ***Rodrigues et al. (2001)***. Trichlorfon is an organophosphate insecticide used to control the parasites of fish in aquatic environments ***Washington (1984)*** and for control of internal parasites in domestic animals ***Hayes and Lows (1990)***. The desirable effect during a treatment with organophosphates is a selective killing of the target parasite without any damage to the host ***Salte et al. (1987).*** However, this goal is not always achieved and damage to hosts may occur at variable extents ***Chandrasekara and Pathiratne (2005), Yoshimura and Endoh (2005)***. Under the name of metriphonate, trichlorfon made its way into the veterinary and human medicine e.g. for treatment of Schistosomiasis in man ***Lebrun and Cerf (1960)***, Cysticercosis in animals and man ***Malagon (1989)*** and in the experimental treatment of Alzheimer's disease in man ***Hallak and Giacobini (1989) and Tariot et al. (1997)***.

The intensive and widespread use of trichlorfon is considered a potential source of exposure for workers and animals with great hazard to livestock and human beings. The recurrent and prolonged exposure to this organophosphate insecticide even at low concentrations may lead to toxicity, reproductive failure and immunosuppression ***Nafstand et al. (1983).***

Trichlorfon was suspected of having negative reproductive effects ***Hayes and Lows (1990)***. Fetal abnormalities were produced in rats, hamsters and mice, when doses approaching the LD50 level when administrated during pregnancy ***Nordgren et al. (1978)***. In addition, an increased number of embryonic deaths, a decreased number of live fetuses and an increased number of fetal abnormalities were observed in

rats given a single oral dose of 80 mg/kg body weight, by stomach tube, on the 13th day of pregnancy ***Bearden and Flyquary (1980)***. Moreover, exposures to trichlorfon result in adverse effects on the other body organs. Edema of the brain, congestion and hepatic damage, pneumonia, and heart muscle changes were observed in rats given daily oral doses of 300 mg/kg body weight for 5 days. Brain disturbances and changes in the liver, kidneys, spleen, lungs and testicles were also seen in bulls given oral doses of 1, 2, or 5 mg/kg formulated trichlorfon (chlorophos) daily, or 5 mg/kg in a weekly intervals for 6 months ***Bearden and Flyquary (1980)*.**

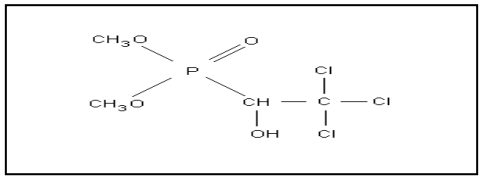
The present study was carried out to determine the adverse effect of trichlorfon on internal organs of rat particularly male genitalia through the possible effect of trichlorfon on male fertility, serum hormonal and enzymatic changes as well as the pathological alterations to the male genital organs, liver and spleen.

MATERIALS AND METHODS

Chemical compound:

Trichlorfon (Metriphonate)®, organophosphate insecticide was obtained from the Egyptian Company for Chemicals and Pharmaceuticals (ADWIA)

- **Chemical structure**: O,O-Dimethyl 2, 2, 2 Trichloro -1-hydroxy-ethyl-phosphonate ester.
- **Chemical formula**: $C_4H_8CL_3O_4P$



López-Arrieta and Schneider (2008)

Experimental Design:

The present study was carried out on 21 mature male albino rats weighing 150-180 gm body weight. These rats were divided randomly into three equal groups, the first group was served as a control where rats orally administered distilled water daily till end of experiment. The second group, were orally administrated trichlorfon dilution in distilled water by stomach tube once daily at a dose of 8 mg/kg.b.wt (therapeutic dose) ***Rodionov and Voronina (1973)*** for 65 days; to cover the entire spermatogenetic cycle ***Hershberger et al. (1969)*** While the third group were orally administered trichlorfon dilution in distilled water by stomach tube once daily at a dose of 80 mg/kg.b.wt.(toxic dose) ***Scali et al. (2002)*** for 65 days.

All rats used in this experiment were kept in metal cages under hygienic conditions, fed on a well-balanced ration and provided with water and food *ad-libitum* through the experimental period. The animals were scarified at the end of the experimental period.

Sampling and analysis:

- Hormonal estimation

Blood samples were collected at the end of the experiment for separation of the serum which kept at -20 °C till used. Serum testosterone and LH were estimated using the electrochmiluminescence immunoassay ***Wheeler (1995)*** and ***Tietz (1995)***.

- Biochemical and immunological analysis

Serum levels of some biochemical parameters as AST &ALT ***Bergmeyer et al. (1978)*** total acid phosphatase and prostatic acid phosphatase ***Young (2001)*** were estimated.

- Semen analysis

Rats were sacrificed at the end of the experiment, both seminal vesicles and prostate glands were dissected and weighed. The cauda epididymis was minced in normal saline and a drop of this epididymal suspension was picked up for semen analysis and recording the epididymal spermatozoal characters ***Bearden and Flyquary (1980).***

- Histopathological examination

Tissue specimens were collected from testes, seminal vesicles, prostate glands, liver and spleen and rapidly fixed in 10 % neutral buffered formalin. After proper fixation, thin paraffin sections about 5 microns were routinely prepared and stained with H& E stain for microscopic investigation ***Bancroft and Stevens (1996)***.

- Statistical analysis:

The obtained data were analyzed statistically using ANOVA test ***SAS Institute(1996).***

RESULTS

I. <u>Effect of trichlorfon on the male fertility</u>:

A- Male reproductive organs weights

Effect of trichlorfon orally given once daily for 65 days at a dose level of 8 mg/kg.b.wt (therapeutic dose) & 80 mg/kg.b.wt. (toxic dose) on the male reproductive organs weights of male albino rats (testes, seminal vesicles and prostate glands) was illustrated in **Table 1.**

As shown in table 1, it was cleared that, rats treated with the therapeutic and toxic doses of trichlorfon showed significant decreases in the weight of their testes ($p<0.05$).

Regarding to the effect of trichlorfon on both seminal vesicles and prostate glands, non-significant decreases in their weights were recorded in rats administered the therapeutic dose of trichlorfon while rats given the toxic dose of trichlorfon showed significant decreases ($p<0.05$) in the seminal vesicles and prostate glands weights.

B. Epididymal spermatozoal characters

The effect of trichlorfon on the characters of epididymal spermatozoa was detected **Table 2**. Significant decreases ($p<0.05$) in the sperm motility (%)and sperm cell concentration (x 10^6/ml) were found in rats after oral administration of trichlorfon once daily for 65 days at dose levels of 8 mg/kg.b.wt (therapeutic dose) and 80 mg/kg.b.wt. (toxic dose). Moreover, comparing with the control rats significant increases in the sperm cell abnormalities (%) were also observed in rats after oral administration of therapeutic and toxic doses **Figs. (1-3).**

C. Testosterone and leutinizing hormones

Table 2 revealed that, rats treated with the therapeutic dose of trichlorfon showed significant decrease ($p<0.05$) in leutinizing hormone while the testosterone hormone revealed non-significant decrease compared with the control. While rats administered toxic dose of trichlorfon showed significant decreases in the levels of both testosterone and leutinizing hormones compared with the control group.

2- <u>Effect of trichlorfon on some biochemical parameters</u>:

Comparing with those of control group, oral administration of therapeutic and toxic doses of trichlorfon in rats was associated with significant increases ($P<0.05$) in the levels of AST and ALT and significant decreases ($P<0.05$) in the total acid phosphatase levels. Moreover, non-significant decrease in the levels of prostatic phosphatase were recorded in these rats **Table 3.**

Table (1): Effect of orally administration of trichlorfon once daily for 65 days at a dose of 8 & 80 mg/kg.b.wt. on the male reproductive organs weights (g.) of albino rats.

(n=7) **(Mean ± SE)**

Group	Dose mg/kg b.wt.	Testes	Seminal vesicles	Prostate glands
G1	0	1.37±0.03 [a]	0.94±0.07 [a]	0.40±0.02 [a]
G2	8	0.86±0.02 [b]	0.79±0.02 [a]	0.34±0.01 [a]
G3	80	0.65±0.04 [c]	0.62±0.03 [b]	0.28±0.01 [b]

Means within the same column carrying different superscripts are significantly different at ($p<0.05$).

Table (2): Effect of orally administration of trichlorfon once daily for 65 days at a dose of 8 & 80 mg/kg.b.wt. on epididymal spermatozoal characters and testosterone & Leutinizing hormones levels in male albino rats.

(n = 7) **(Mean ± SE)**

Group	Dose mg/kg b.wt.	Sperm Progressive motility (%)	Sperm cell concentration ($x10^6$/ml)	Sperm abnormalities (%)	Testosterone hormone (ng/ml)	Leutinizing hormone (IU/ml)
G1	0	85.0±0.02[a]	31.66±0.01[a]	2.29±0.03[a]	3.30±0.01[a]	0.37±0.03[a]
G2	8	72.80±0.04[b]	15.36±0.04[b]	6.39±0.03[b]	2.71±0.02[a]	0.31±0.01[b]
G3	80	41.10±0.01[c]	9.00±0.05[c]	12.36±0.01[c]	1.86±0.02[b]	0.20±0.03[c]

Means within the same column carrying different superscripts are significantly different at ($p<0.05$).

Table (3): Effect of orally administration of trichlorfon once daily for 65 days at a dose of 8 & 80 mg/kg.b.wt. on some biochemical parameters in male albino rats.

(n = 7) **(Mean ± SE)**

Group	Dose mg/kg b.wt.	AST (U/L)	ALT (U/L)	Total acid phosphatase (U/L)	Prostatic phosphatase(U/L)
G1	0	95.06±0.02[a]	50.86±0.01[a]	13.53±0.04[a]	5.26±0.09[a]
G2	8	118.26±0.03[b]	65.88±0.03[b]	11.99±0.02[b]	4.80±0.02[a]
G3	80	141.49±0.03[c]	80.77±0.09[c]	11.32±0.03[b]	4.67±0.02[a]

Means within the same column carrying different superscripts are significantly different at ($p<0.05$).

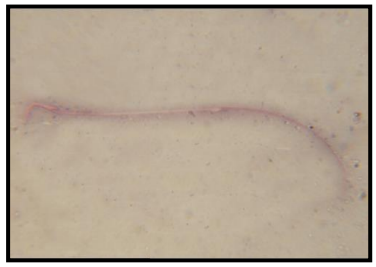
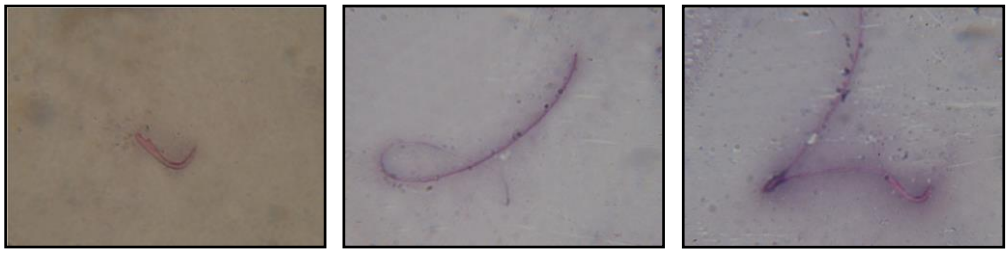


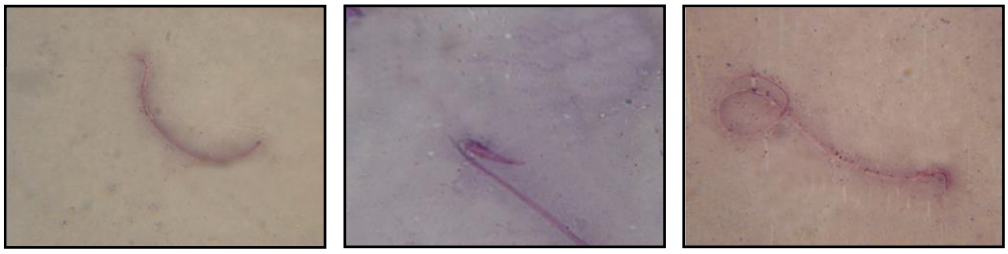
Fig. (1): Spermatozoa from the control male rats showing normal sperm.



(A) **(B)** **(C)**

Fig. (2): Spermatozoa from male rats orally administered trichlorfon once daily at a dose of 8 mg/kg. b.wt. for 65 days showing the following secondary abnormalities:

A- Detached head sperm **B- Detached tail sperm** **C- Bent tail sperm**



(A) **(B)** **(C)**

Fig.(3): Spermatozoa from male rats orally recieved trichlorfon once daily at a dose of 80 mg/kg.b.wt.for 65 days showing the following abnormalities:

A- Short "stunted"sperm (primary) **B- Abnormal hook-shape sperm (primary)** **C- Coiled-tail sperm (secondary)**

3- Histopathological findings:

- Testes:

Macroscopically, the testes of rats in both treated groups (therapeutic and toxic dose) were slightly decreased in size and slightly congested compared with control.

Microscopically, the histopathological examination of testes of rats treated with the therapeutic dose of trichlorfon revealed congestion, interstitial edema and hemorrhages. In addition, no spermatogenesis was detected in most of semineferous tubules **Fig. (4).** Concerning to the examined testes of rats administrated the toxic dose of trichlorfon showed absence of spermatozoa with vacuolation of the lining epithelium of semineferous tubules and interstitial edema **Fig. (5).** Severe hemorrhages in the interstitium and edematous thickening of tunica albugina were also observed. Moreover, the microscopic examination of the testes of rats in control group showed normal histological structure **Fig. (6).**

- Seminal vesicular gland:

Macroscopically, the seminal vesicular glands in both treated groups were apparently normal.

Microscopically, the seminal vesicles of **r**ats treated with the therapeutic dose of trichlorfon showed papillary projections of their lining epithelium with accumulation of little secretion in lumens of some tubulo-alveolar glands together with interstitial edema **Fig. (7).**

In rats given the toxic dose of trichlorfon, the examined seminal vesicles revealed extensive papillary projections together with the presence of excessive secretion in lumens **Fig. (8).** Moreover, no abnormal microscopical changes were detected in the seminal vesicles of rats in the control group **Fig. (9)**.

- Prostate glands:

Macroscopically, no clear obvious changes were detected on the prostate glands of both treated groups.

Microscopically: the acini of the prostate gland in rats treated with the therapeutic dose of trichlorfon revealed cystic dilatation and filled with little secretion causing pressure atrophy and flatting of their epithelium **Fig. (10).** In addition abundant stroma was also observed

The histopathological findings of the examined prostate glands of rats given the toxic dose of the trichlorfon were characterized by inflammatory cellular infiltration mainly neutrophils within the lumina and interstitial tissues. In some examined prostates, chronic inflammatory cells aggregation and fibrous connective tissue proliferation were seen in the interacinar tissues **Figs. (11 & 12).** In control group, the examined prostate glands showed no characteristic abnormal microscopical changes **Fig. (13).**

- Liver:

Macroscopically, the liver of rats treated with the therapeutic dose was apparently normal. Mean while the liver of rats treated with the toxic dose was decreased in size, dark red in color and slightly friable.

Microscopically, the examined liver of rats treated with therapeutic dose of trichlorfon revealed mild and focal degeneration of hepatocytes mainly of hydropic type **Fig. (14).** The examined portal areas showed round cells infiltration with endotheliosis and hyalinization of the muscular walls of some portal blood vessels.

The examined liver of rats administrated the toxic dose of trichlorfon showed severe and diffuse hydropic degeneration of hepatocytes. Multiple areas of hepatic necrosis manifested by pyknotic nuclei and cytoplasmolysis with fibrous tissue proliferation in portal areas were also detected **Fig. (15)**. Congestion of the portal blood vessels with bile ductular hyperplasia and mononuclear inflammatory cellular infiltration of the portal tract were also noticed. Moreover, the examined liver of rats in control group revealed normal hepatic cords arranged radially around central veins **Fig. (16)**.

- Spleen:

Macroscopically, the spleen of rats in both treated groups were dark red in color.

Microscopically, the spleen of rats treated with the therapeutic dose of trichlorfon showed lymphoid depletion **o**f white pulps **Fig. (17).** Aggregation of dark-brown granules of hemosiderin pigment scattered in the splenic parenchyma was detected. The examined spleen of rats given the toxic dose of the drug displayed severe edema and lymphoid depletion of the majority of white pulps **Fig.(18).** Extensive splenic hemosiderosis was also seen in some examined spleen. Moreover, no abnormal microscopic changes were observed in the spleen of rats in control group **Fig. (19**).

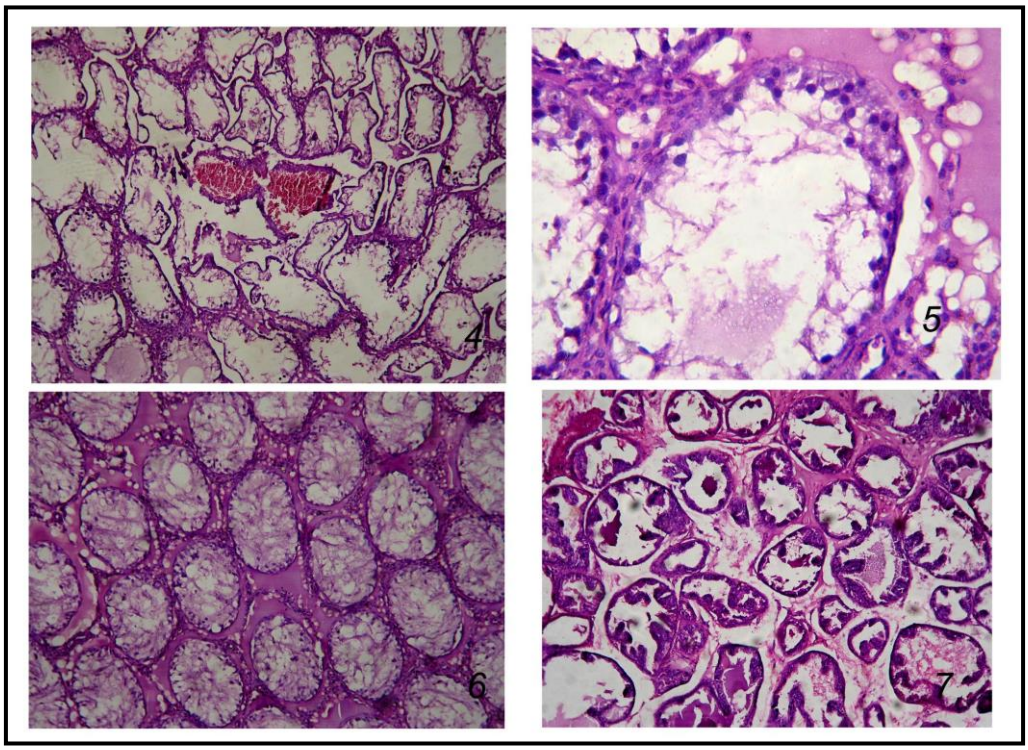


Fig. (4): Testicular section from rats treated with the therapeutic dose of trichlorfon showing semineferous tubules without spermatogenesis (a)and congested blood vessels(b). (H&E X120).

Fig. (5): Testicular section from rats treated with the toxic dose of trichlorfon showing interstitial edema(a) with vacuolated epithelium of semineferous tubules(b). (H&E X300).

Fig.(6): Testicular section from control rats showing normal testicular tissue. (H&E X120).

Fig. (7): Vesicular gland section from rats treated with the therapeutic dose of trichlorfon showing little secretion inside the luminae(a) with papillary projections of some acini (b). (H&E X120).

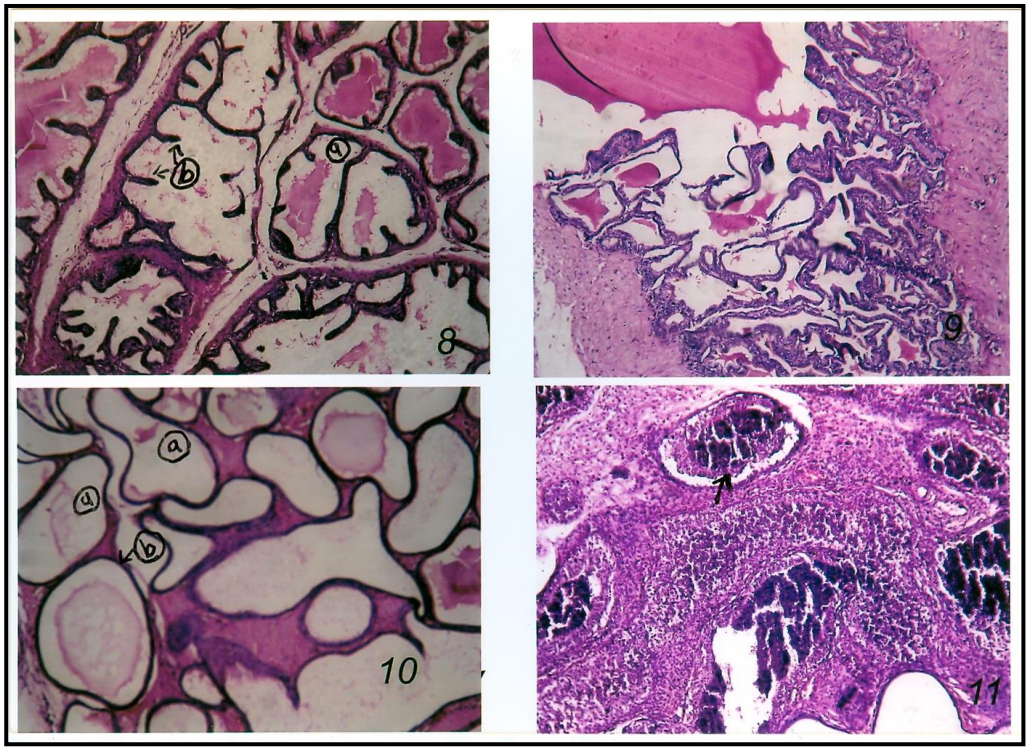


Fig. (8): Vesicular gland section from rats treated with the toxic dose of trichlorfon displaying excess secretion(a) and papillary projections in the tubulo-alveolar glands(b). (H&E X120).

Fig. (9): Vesicular gland section from control rats showing normal structure. (H&E X150).

Fig. (10): Prostate gland section from rats treated with the therapeutic dose of trichlorfon showing dilated acini(a) with the pressure atrophy of their lining epithelium(b). (H&E X120).

Fig. (11): Prostate gland section from rats treated with the toxic dose of trichlorfon showing chronic suppurative prostatitis.(H&E X120).

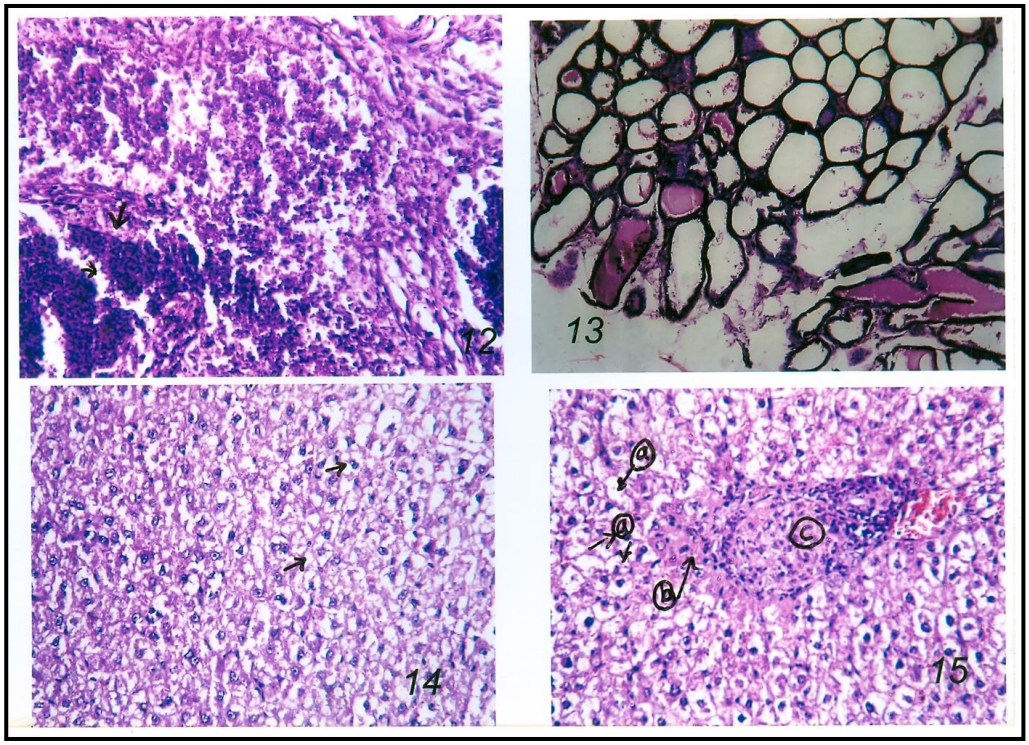


Fig. (12): High power of the previous figure showing intense neutrophils and fibrous tissue.(H&E X300).

Fig. (13): Prostate gland section from control rats displaying normal structure. (H&E X150).

Fig. (14): Liver of rats treated with the therapeutic dose of trichlorfon showing mild and focal hydropic degeneration in the hepatic cell.(H&E X300).

Fig. (15): Liver of rats treated with the toxic dose of trichlorfon demonstrating severe and diffuse hydropic degeneration(a) and necrosis(b) with portal fibrosis(c). (H&E X300).

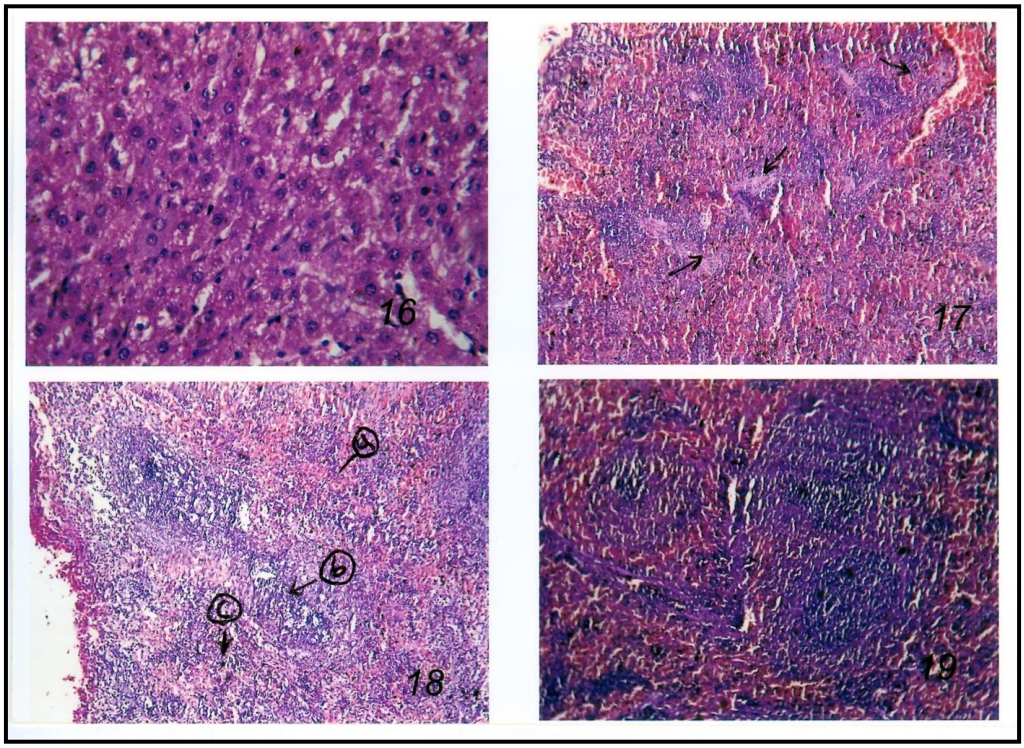


Fig. (16): liver section from the control rats showing normal hepatic parenchyma. (H&E X300).

Fig. (17): Spleen section from rats treated with the therapeutic dose of trichlorfon demonstrating lymphoid deplation. (H&E X120).

Fig. (18): Spleen section from rats treated with the toxic dose of trichlorfon showing edema(a), Lymphoid depletion(b) and hemosiderosis(c). (H&E X120).

Fig. (19): Spleen section from the control rats showing normal splenic tissue. (H&E X300).

DISCUSSION

Although the use of many pesticides is heavily regulated in some parts of the world, studies to assess toxicity of organophosphate insecticides used in Egypt are required to establish safety levels, as some of these are still licensed and more authoritarian control is needed. Trichlorfon is one of agrotoxic products, still discharged by agricultural production systems and frequently used products against parasites in farmed animals. Trichlorfon often applied in larger amounts by farmers. The repeated and indiscriminate use of this product in high concentrations and without specialized technical orientation may cause intoxication. Some of these effects on the non-target organism are expected, including tissue alterations ***Sturm et al. (1999b).***

Regarding to the reproductive toxicity in rats after oral exposure to trichlorfon in toxic dose, significant decreases in the weights of testes, seminal vesicles and prostate glands were recorded. Meanwhile exposure to therapeutic dose of trichlorfon was followed by significant reduction in the weight of testes with non significant changes in the weights of the accessory organs. These results are consistent to those recorded by ***Kuwar et al. (2006),*** who found that, treatment of albino rats with the organophosphorus(OP) insecticide phosphamidon at doses of 35 and 50 p.p.m in drinking water for one month elicited an obvious reduction in the weight of testes. On the other hand our results are disagreed with ***Okamura et al.(2005)*** who mentioned that, non significant difference in the reproductive organs weights of 10 weeks old Wister male rats subcutaneously injected with the organophosphorus(OP) dichlorvos at doses of 1,2,4 mg/kg .b. wt., 6 days/week for 9 weeks. These variations from our results may be attributed to the difference in species, dose and

duration of the insecticide administration. A possible explanation for our findings may be attributed to disturbance in the hormonal levels recorded in the present study. This suggestion was supported by ***Ray et al. (1992)*** who reported that, the development and maintenance of accessory sex organs and their secretions depend upon the androgen level where they found that, following treatment of Wister rats with quinalphos organophosphorus (OP) at dose level of 250 µg/kg.b.wt. i.p. for 13, 26 days resulted in a suppressive influence on gonadotrophin release and testosterone concentrations beside its detrimental action on the testes.

Moreover, another possible scenario for the decreases in the accessory organs weights through the recorded degenerative and destructive changes in the histopathological examination of these organs in our work. This opinion was supported by ***Kuwar et al. (2006)*** who mentioned that the reduction in the weight of testes could be due to the destruction, reduction and distortion of the seminiferous tubules.

The microscopic examination of male genital organs of rats received the therapeutic and toxic doses of trichlorfon revealed absence of spermatozoa with vacuolation of the lining epithelium of semineferous tubules in the examined testes with papillary projections of the lining epithelium of seminal vesicles and inflammation of prostate glands.

These histopathological changes were reflected on epididymal spermatozoal characters where decreases in the sperm motility and concentration with increases in sperm abnormalities postorganophosphorus administration were recorded. These results were previously recorded by several investigators ***Dunnick et al. (1984) and Pina et al. (2005).*** This effect of (OP) compounds may be due to changes in germ cell diameter, which is the essential first step in spermatogenesis,

resulting in hindering the production of viable spermatozoa endangering the population dynamic[30], alter sperm chromatin by phosphorylation of nuclear protamine leading to DNA damage during spermatid differentiation ***Pina et al. (2005)*** and inhibition of mitotic activity ***El-Nahas et al. (1989)*** or meiotic index of testicular cells ***El-Ashmawy et al. (1993)***.

On contrary, our findings were disagreed with ***Okamura et al. (2005)*** who recorded no effect of trichlorfon on sperm picture.

In addition administration of trichlorfon at toxic dose in rats was associated with vacuolation of the lining epithelium of semineferous tubules with interstitial edema and hemorrhages. These findings were supported by the changes in the levels of testosterone and leutinizing hormones in these rats where significant decreases in their levels were recorded. These findings were in a harmony with those of ***Ray et al. (1991 and 1992)*** who mentioned that, administration of (OP) quinalphos in rats resulted in suppression of plasma gonadotrophins and testicular testosterone production which may adversely interferes with the activity of the testicular steroidogenic enzymes and inhibits the pituitary gonadotrophins release.

Another possible explanation for the reduction of the testosterone hormone was clarified by ***Hong et al. (2007)*** who reported that, *in vitro* treatment of mouse leydig tumor cells with various concentrations of trichlorfon for 24 hrs resulted in suppression in the expression of steroidogenic acute regulatory protein (STAR), mRNA and protein and profoundly inhibited the activity of P450 side chain cleavage enzyme (P450SCC). The suppression in the expression of P450SCC mRNA and protein further accounted for the inhibitory action of trichlorfon on steroidogenesis.

The pathological picture of the liver was explained by ***Snow and Watson (1973),*** who mentioned that, liver damage was occurred in a greyhound dog treated once with 11 mg/kg dichlorvos in capsules. On the same line cytoplasmic vacuolization of liver cells was noted in male Fischer 344 rats receiving 4 or 8 mg/kg/day dichlorvos by gavage for 5 days per week for 2 years ***NTP (1995).*** The authors stated that these changes were minor in extent but have been associated with lipid accumulation in cells. The majority of pesticides are biotransformed in metabolites by the mixed function oxigenases in liver and, in some cases, the metabolites are more toxic than the original product. Also the acetyl choline inhibition was probably associated with the presence of dichlorvos, the main metabolite of trichlorfon ***Garcia-Repetto et al. (1995).***

In the present investigation, it was cleared that, exposure of rats to trichlorfon showed even at the therapeutic dose severe pathological alterations in the hepatic tissues. These alterations manifested by mild and focal hepatic degeneration with round cells infiltration of portal areas in rats administrated with therapeutic dose of trichlorfon. While in toxic doses, these degenerative changes became diffuse and accompanied by presence of multiple areas of hepatic necrosis with bile ductular hyperplasia, fibrous tissue proliferation and mononuclear inflammatory cellular infiltration of the portal tract.

These hepatic degeneration and necrosis were reflected on the results of serum parameters of liver function tests where significant increases in the levels of AST and ALT in both therapeutic and toxic dose treated groups were predominant. Moreover this hepatotoxicity of trichlorfon may be related to its per oxidative property in the microsoms or to the main specific inhibitory effects on cholinesterase ***Kravtsova (1983).***

Immunosuppression after oral exposure to trichlorfon was cleared in the present investigation. The microscopic examination of the spleen of rats treated with the therapeutic dose of trichlorfon showed lymphoid depletion of white pulps. This splenic microscopic picture became more severe in rats given the toxic dose where lymphoid depletion of the majority of white pulps with extensive splenic hemosiderosis were prevalent. These findings were in accordance with those mentioned by ***Desl et al. (1978)*** in rabbits administrated 0.3-2.5 mg/kg dichlorvos 5 days a week for 6 weeks and in male C57B mice administrated single oral dose of 120 mg/kg dichlorvos ***Casale et al. (1983).***

Finely according to the obtained results it concluded that,

- Trichlorfon elicited a marked harmful effect on male fertility of rats which was evidenced by degenerative changes in the testis and the abnormalities of epididymal spermatozoa.
- Trichlorfon had an immunsuppressive effect represented by depletion of the splenic lymphoid depletion.
- The liver is sensitive organ for trichlorfon where hepatic degenerative changes were recorded even at low concentration.

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دراسات باثولوجيه و سميه على التأثيرات الضارة للمبيد الحشرى ترايكلوروفون (ميتريفونات) مع دلألة تأثيره على الخصوبة فى ذكر الجرزان

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استهدف هذا البحث دراسة تأثير المبيد الحشري الفوسفورى العضوي "ترابكلورفون" على الخصوبة فى ذكور الجرذان البيضاء وكذلك رصد التغيرات البيوكيميائية و النسيجية التى يمكن أن تصاحب استخدامه فى تلك الحيوانات.

تم استخدام 21 من ذكور الجرذان البيضاء البالغه، قسموا بالتساوى الى ثلاث مجموعات هى المجموعة الضابطة (المجموعة الأولى)، الجرذان التى تجرعت بالفم الجرعة العلاجية من الترابكلورفون (8 مجم/كجم من وزن الجسم) يوميا لمدة 65 يوم"لكى يتم تغطية كل فترة تكوين و تطور الحيوانات المنوية فى الجرذان والتى تمتد من60–65 يوم" (المجموعة الثانية)، الجرذان التى تجرعت بالفم الجرعة السامة من الترابكلورفون (80مجم/كجم من وزن الجسم) يوميا لمدة 65 يوم (المجموعة الثالثة).

بعد انتهاء فترة التجربة (65 يوم) تم ذبح الجرذان و تم أخذ الخصى و غدتا البروستاتا والحوصلة المنوية وتم تسجيل أوزانهم ثم أخذت عينات من البربخ بعد معاملتها لفحص السائل المنوي كذلك تم أخذ عينات من الدم لفصل المصل و ذلك لعمل الاختبارات البيوكيميائيه. كما تم أخذ عينات من الكبد،الطحال،الخصية،البروستاتا، الحوصلة المنوية وتم معاملتها وتجهيزها للفحص الهستوباثولوجى.

ولقد أسفرت النتائج عن أن كلا من الجرعتين المستخدمتين من هذا المبيد الحشري قد أحدثت نقصا معنويا فى وزن الخصية و فى معدل حركة و تركيز الحيوانات المنوية و كذلك فى مستوى انزيم

الأسيد فوسفاتيز كما أحدثتا زيادة معنوية فى معدل تشوهات الحيوانات المنويه ومستوى (الـ انتش) هرمون وانريمات (اسبارتيت ترانس امينيز) & (الانين ترانس امينيز) مقارنة بالمجموعه الضابطة بينما أوضحت النتائج أن فقط المجموعة التى تجرعت الجرعة السامة من المبيد الحشري أظهرت نقصا معنويا فى أوزان البروستاتا و الحوصلة المنوية و كذلك مستوى هرمون التيستورون مقارنة بالمجموعة الضابطة. كما أظهر الفحص الباثولوجى وجود تغيرات بسيطة فى أنسجة الكبد ، الطحال, الخصية, البروستاتا و الحوصلة المنوية فى الجرذان التى تجرعت الجرعة العلاجية من الترابكلورفون ولكنها كانت أكثر شدة و انتشار– مع ملاحظة وجود نفص فىالخلايا المنوية فى مراحل تكوينها المختلفة– فى الجرذان التى تجرعت الجرعة السامة من المبيد الحشري و قد تمثلت التغيرات الباثولوجيه فى وجود تغيرات تنكسيه فجوية موضعية فى الخلايا الكبدية، اضمحلال فى عدد الخلايا الليمفاوية مع وجود مادة الهيموسيدرين فى الطحال بالاضافه الى ملاحظة وجود وزمه خلاليه و احتقان و بقع نزفيه فى الأنسجة الخصويه و كذلك وجود نتوءات خملية فى النسيج الطلائى المبطن للحوصلة المنوية مع ملاحظة و جود أسناخ بروستاتيه متسعة ومتحوصلة مع نقص فى افرازات غدة البروستاتا.

وقد خلصت الدراسة الى وجود تأثير سلبي وهدام للمبيد الحشري (التراى كلورفون) على الخصوبة فى ذكور الجرذان البيضاء وكذلك على بعض القياسات البيوكيميائية و الباثولوجية فى تلك الحيوانات والدراسة توصى بعدم الاستخدام المفرط بجرعات أعلى من العلاجية من هذا المبيد الحشري و ذلك للتأثيرات القوية السامة و الضارة التى أحدثها عند اعطاء الجرذان البيضاء جرعتة السامة.