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Organophosphate Insecticides Induced Histopathological and Biochemical Changes on Male Rats

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ABSTRACT

Background: Histopathological changes in some internal organs have been widely used as biomarkers for health evaluation. Histopathological lesions following pesticide chlorpyrifos (CPF) administration in some organs which has prompted us to undertake this study.

Methods: 30 mature male albino rats , weighing 120 – 150 gms, were randomly divided into 3 groups A, B& C. Oral administration of Chlorpyrifos was to groups B and C in dose of 5 and 10 mg/kg. B. Wt respectively. Group A kept as control . Body weight of each rat was recorded weekly. Ten animals from each group were sacrificed at end of the experiment to evaluate some biochemical alterations and the histopathological changes in some internal organs (kidney & spleen) and sexual organs(testis and epididymis).

Results: Chlorpyrifos, an organophosphate insecticide ,was orally administered to male rats at the doses of 5 and 10 mg/ kg.B.Wt for 30 successive days. Rats in Group C which exposed to high dose (10 mg/ kg.B.Wt) showed significantly reduced body weight and body weight gain. Weight of different vital organs were significant increases and decrease in the weight of testes (Group B & C) as compared to control. Different tissues of male rats administer CPF(5 mg/ kg.B.Wt) showed mild histopathological changes in liver ,testis & epididymis while administration of high dose (10mg/ kg.B.Wt) of chlorpyrifos to male rats for 30 days has produced significant pathological changes in liver , testis and epididymis. Chlorpyrifos increased serum transferase enzymes (AST and ALT) activity. Group A shows no pathological changes.

Conclusion: Our study has shown that 30 days administration of chlorpyrifos at dose of 10 mg/kg .B.Wt causes sever histopathological changes which leading to damages of some vital organs along with relative decrease in weights of almost all vital organs.

Keyword: Organophosphorus insecticides, chlorpyrifos, histopathology; liver, kidney, spleen, sexual organs& body weight

INTRODUCTION

In recent years , the use of pesticides has common for increasing criticism, with the aspects of public concern including both the possible accumulation of pesticides

residues in food and crops, therefore they could be a source of many biochemical and physiological disturbance in animals and humans **(Awasthi and Parkash ,2007 and Shalini et al 2006).**

Goel et al, 2007 mentioned that organophosphorus pesticides are large group of pesticides which are widely used for a variety of agricultural and public health applications and the exposure to these pesticides is known to produce variety of biochemical changes, some of which may be responsible for the adverse biological effects leaves residues on crops and also contaminates reported in man and animals. It could induce adverse effects on the immune system, pancreas, kidney and reproductive systems **(Bebe , Panemanogalore, 2003 Lin et al 2003, and Amer et al,2000)**

Chlorpyrifos is a non-systemic insecticide that effective by direct contact, ingestion and inhalation **(Yurumez et al., 2007 and Nolan et al., 1984)**. Toxicity chlorpyrifos (CPF) in mammalian & animals has increased in recent years. Histopathological lesions have been widely used as biomarkers for health evaluation (**Rekha et al ,2013**). Few reports regarding histomorphological changes in some vital organs following pesticide chlorpyrifos administration which has prompted us to undertake this study.

Relative organ weight can be the most sensitive indicator for toxicity of experimental compound, as significant differences in organ weight between treated and control animals might be used as a arkers to detect early biochemical effects of pesticides before adverse clinical health effects occur. (**Michael *et al*., 2007; Hernandez *et al*., 2006, Bailey *et al.*, 2004 , Wooley, 2003 and Trimbell, 1991) .** In fact, as a consequence the repeated doses of CPF and CPM, may be lead to significant disturbances in the biochemical parameters and functions of liver and kidney (**Mansour and Mossa, 2010 and Verma *et al*., 2007**). The biochemical changes such as total protein, glucose and total lipids contents in living organisms exposed to insecticides may explain their toxicities.

Due to the above facts, our study is designed to evaluate the effect of orally administered Chlorpyrifos at different doses for 30 successive days on kidney and some sexual organs (testis and epididymis) and some enzymatic activity of albino rats.

MATERIALS AND METHODS

Pesticide Details:

Chlorpyrifos are an organophosphorus insecticides which introduced by Giba-Geigy AG (Novartis). It was purchased from the market with Molecular formula C9 H11 Cl3 – NO3 PS

Animals:

Thirty male albino rats weighing 120-150 g. Rats were obtained from the National Institute of Ophthalmology, fed on basal diet and watered *ad-libitum*.

Experimental design:

Thirty mature male rats (120-150g) were divided into 3 groups. The first group was kept as a control, whereas the second and third groups were administered orally Chlorpyrifos in doses of 5 and 10 mg/kg.b.wt. respectively daily for 30 successive days and the sexual organs weight were recorded .

Body weight and body weight gain assay:

The body weight of each treated and untreated rats was recorded every week .The biological value of the treatments was assessed by the determination of its effect on body weight gain (BWG) at the end of each experimental period using the following formula **(Rezq and El-Khamisy, 2011**):

$$BWG = Final\ Body\ Weight - Initial\ Body\ Weight$$

Rats were weighed at the beginning of the study, on Day 0, and then body weight gains were calculated for 0-4 weeks

Relative organ weight assay:

At the end of experiment ,all rats sacrificed, the sexual organs(testis, epididymis, seminal vesicle and prostate)and some vital organs (liver, spleen and kidneys) from males were carefully dissected out according to (**Stanley *et al*., 2005)** as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Whole Body weight (g)}} \times 100$$

Blood samples :

It was obtained from each rat at the end of the experimental period, left to clot and the serum was separated for biochemical analysis. The activities of AST, ALT and AP were determined according to the method of **Reitman and Frankel (1957) and Roy (1970)** respectively.

Histopathological Examination

Sampling

At the end of the experiment, 10 rats of each group were sacrificed ,rats were decapitated and liver, testis, and epididymis were removed immediately and were fixed in 10% formalin and routinely processed for histopathological evaluation using conventional paraffin embedding technique. Paraffin section of approximately 4-5 µm were stained with hematoxylin and eosin (**Boncroft,2008**).

Statistical analysis:

The results were subsequently analyzed following the statistical methods established by **Snedecor (1982)**

RESULT AND DISCUSSION

Pesticides have been one of the most effective compounds discovered by man to protect agricultural products from the harm of Pests. Chlorpyrifos is an extensively used organophosphate pesticide and due to its wide-spread use it posses potential

harm to humans **(Kavitha,. and Venkateswara Rao,2009)** . The toxic effects can be attributed to the physiologic features of vital organs. In fact any drug or chemical in the systemic circulation will be delivered to these organs in relatively high amounts. **(Griffin et al., 1999 and Nolan et al., 1984).**

Body weight and body weight gains:

The results tabulated in(**Table 1)** indicate a significant decrease in body weight gains of male rats treated with tested insecticide(Group C) compared to control.

Our data are in agreement with the results reported by **(Mohamed ,2014 ,Jaiswal and Verma 2012, Ambali *et al*. 2011a** , **Bozkurt *et al*. 2010, Johnson *et al*. 2009 and Hancock *et al*. 2007),** who found that significant reduction of body weight gain in rat after administration of chlorpyrifos, and methyl-parathion with no effects on physical or reflex development

Relative organ weight:

The organ weight ratios in toxicological studies is an integral component in the assessment of pharmaceuticals, chemicals, and medical devices **(Sellers *et al*., 2007). Wilson *et al*., 2001 ,** alterations in body weight may lead to increases or decreases in some organ-to-body weight ratios**.** The results of **(Tables 2)** show that there were significant increases in relative weights of liver, spleen & kidneys in treated male rats(Group B & C) as compared to control; on the other hand, there were significant decreases in weight of the testes of male rats(Group C) compared to control.

Our results are coincided with (**Mossa and Abbassy 2012, Jacobsen *et al.*, 2004 , Kang *et al.* 2004 & Yoshida *et al.* 1985**) who found that administration of chlorpyrifos to male and female rats &mice significantly increase in relative liver and kidney weights in treated animals.

Biochemical and Histopathological Findings:

In the present study, rats administered CPF (Group C) showed significant increase of serum activities of AST, ALT and AP **(Table3**). The elevated transferees enzymes denoted the adverse effect of CPF on hepatic function. Our results are confirmed histopathologically as liver of male rats administer of chlorpyrifos (10 mg kg.B.wt)for 30 days showed focal degenerative & necrotic changes which replaced by mononuclear inflammatory cells. Most hepatocytes around necrotic foci have vascular degeneration & some of them have pyknotic nuclei **(Fig1),** vascular degeneration of hepatocytes as well as marked vasiculitis of portal blood vessel with swollen of its endothelial cell lining & degenerative changes of its muscular wall**(Fig2)&** in **(Fig3)** liver has marked mononuclear cell infiltration around portal area as well as most hepatocytes having vascular degeneration .Activation of Kupffer cells could be seen . Some hepatocytes showing pyknotic nucleiand portal area with marked mononuclear cell infiltration & bile duct proliferation. The portal blood vessels showing marked vasculitis **(Fig4)** . Previous work has reported dilatation of central vein, degradation, congestion, oedema, hyalinosis, fibrosis and necrosis in the liver of rats (**Mansour and Mossa, 2010a, 2011).** Also, hepatocellular degeneration and necrosis was recorded in rat treated with profenofos The hepatic function tests

confirmed by the histopathological lesions observed in the present study. Degeneration and necrosis in the hepatocytes, inflammatory cells infiltration, and Kupffer cells proliferation were frequently observed in CPF-treated group. These observations indicated marked changes in the overall histoarchitecture of liver in response to CPF. Our results are supported by other studies conducted on CPF and other OP insecticides **(Heikal et al., 2011, Mansur et al 2011**,**Mansur and Mossa 2010 and Tuzmen et al., 2008).**

Repeated exposure of high dose (10mg kg.B.Wt) of chlorpyrifos to male rats for 30 days showed significant patho-morphological changes in sexuall organs. The previous results were supported histopathologically as Seminiferous tubules some detachment of spermatogenic cells from its basement membrane & leyding cells have so showing me degenerative changes **(Fig 5)** as well as Seminiferous tubules showing marked degenerative changes of spermatogenic cells& sever congestion of interstitial blood vessels could be seen accompanied by edema .The interstitial leyding cells have degenerative changes and necrosis **(Fig 6).** The epididymis of male rats exposed to chlorpyrifos (10 mg kg. B. wt)for 30 days showing some spermatids inside the lumen of its tubules. Edema in the interstitial tissue between epididymal tubules could be seen accompanied by some degenerative changes of interstitial connective tissue**(Fig 7) &**epididymal tubules have degenerative and necrosis of its epithelial lining as well as detachment of its epithelial ling from basement membrane . Edema between epididymal tubules could be seen**(Fig 8) .** Organophosphorus pesticides have the ability to cross the blood-testis barrier inducing oxidative stress and lipid testes (**Okamura et al,2009, Nahid et al, 2009 Uzunhisarcileli et al,2007 and Pant and Srivastava, 2003**) This in turn may cause degeneration of spermatogenic and leydig cells, which disrupt spermatogenesis. The sperms themselves may also be damaged by oxidative effects of organophosphorus pesticides. Sexual organs of male rats exposed to lower doses (5 mg kg.B.Wt) of chlorpyrifos did not exhibit any significant pathological changes.

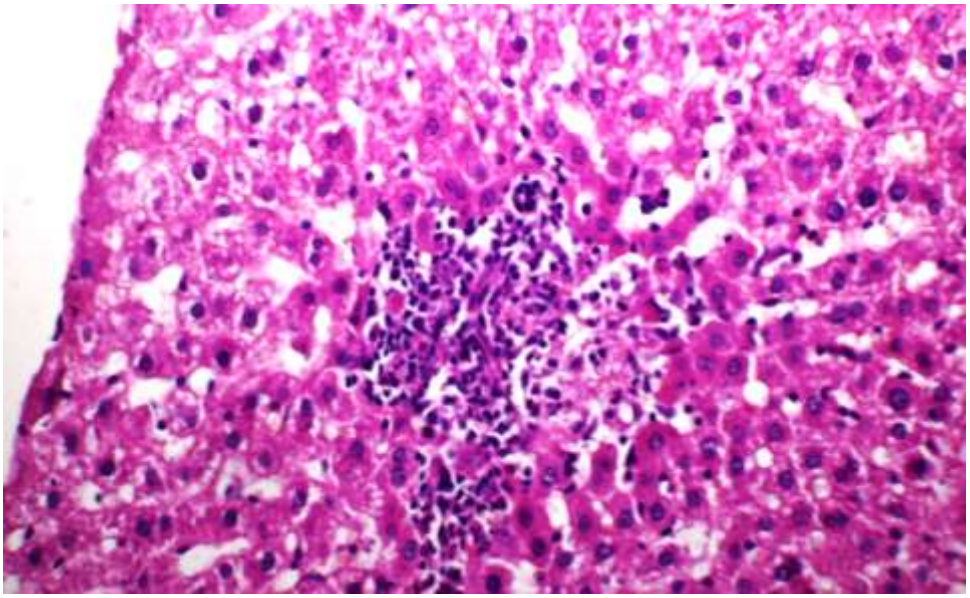

Fig 1: Liver of male rats exposed to chlorpyrifos (10 mg kg.B.wt)for 30 days showing focal degenerative & necrotic changes which replaced by mononuclear inflammatory cells. Most hepatocytes around necrotic foci have vascular degeneration & some of them have pyknotic nuclei (H&EX400)

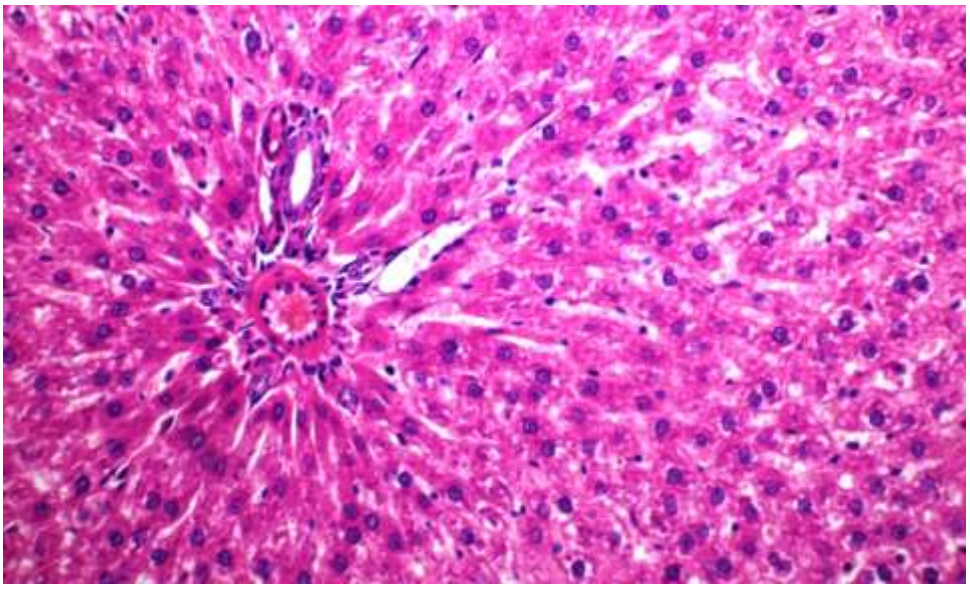

Fig 2: Liver of male rats exposed to chlorpyrifos (10 mg kg.B.wt)for 30 days showing vascular degeneration of hepatocytes as well as marked vasiculitis of portal blood vessel with swollen of its endothelial cell lining & degenerative changes of its muscular wall (H&EX400)

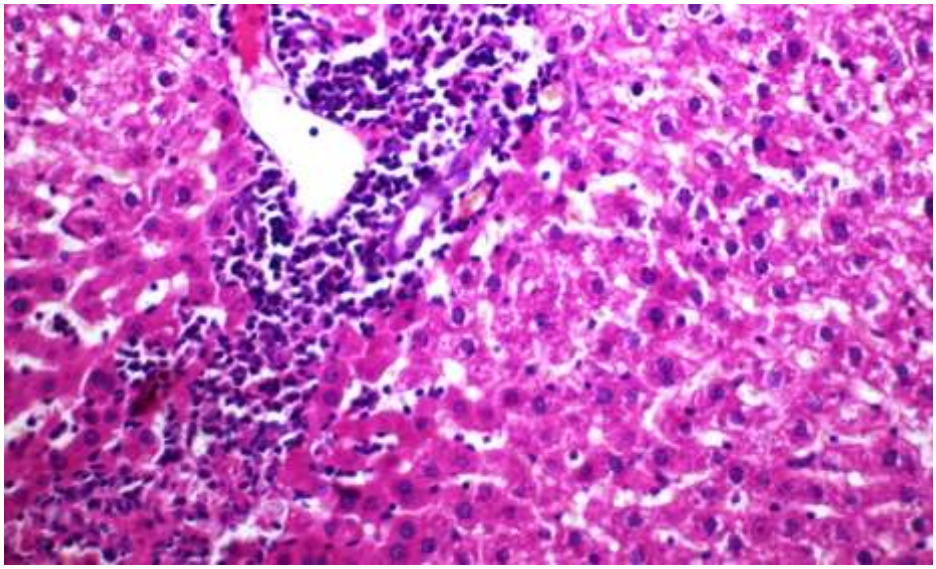

Fig3: Liver of male rats exposed to chlorpyrifos (10 mg kg.B.wt)for 30 days has marked mononuclear cell infiltration around portal area as well as most hepatocytes having vascular degeneration .Activation of Kupffer cells could be seen & some hepatocytes showing pyknotic nuclei(H&EX400)

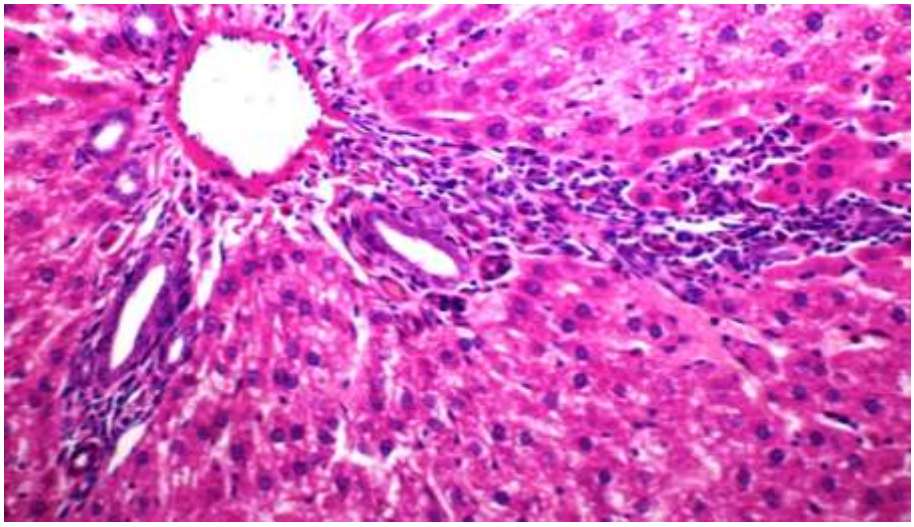

Fig4: Liver of male rats exposed to chlorpyrifos (10 mg kg.B.wt)for 30 days showing portal area with marked mononuclear cell infiltration & bile duct proliferation. The portal blood vessels showing marked vasculitis (H&EX400)

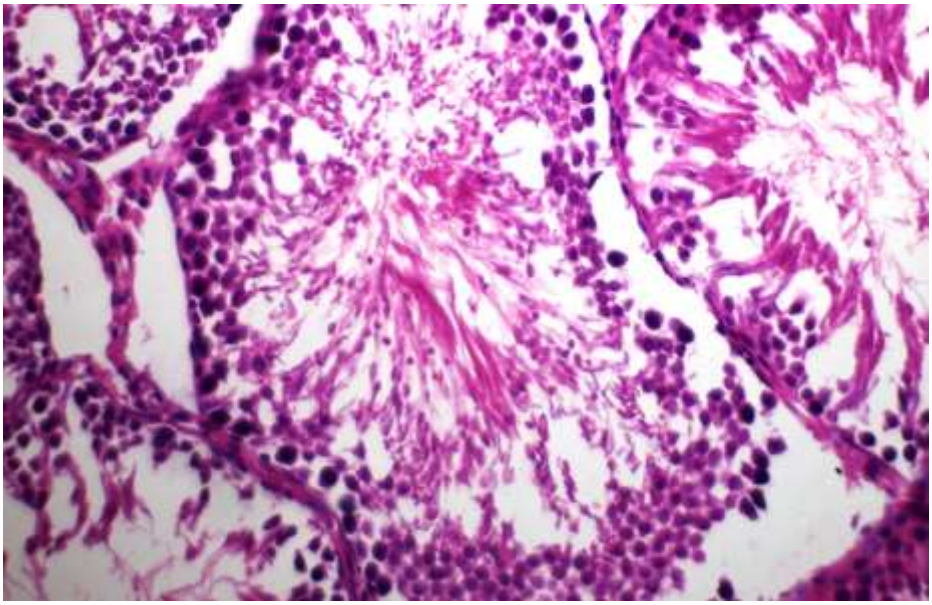

Fig 5 : Seminiferous tubules of male rats exposed to chlorpyrifos (10 mg kg.B.wt)for 30 days showing some detachment of spermatogenic cells from its basement membrane & leyding cells have some degenerative changes (H&E X 400)

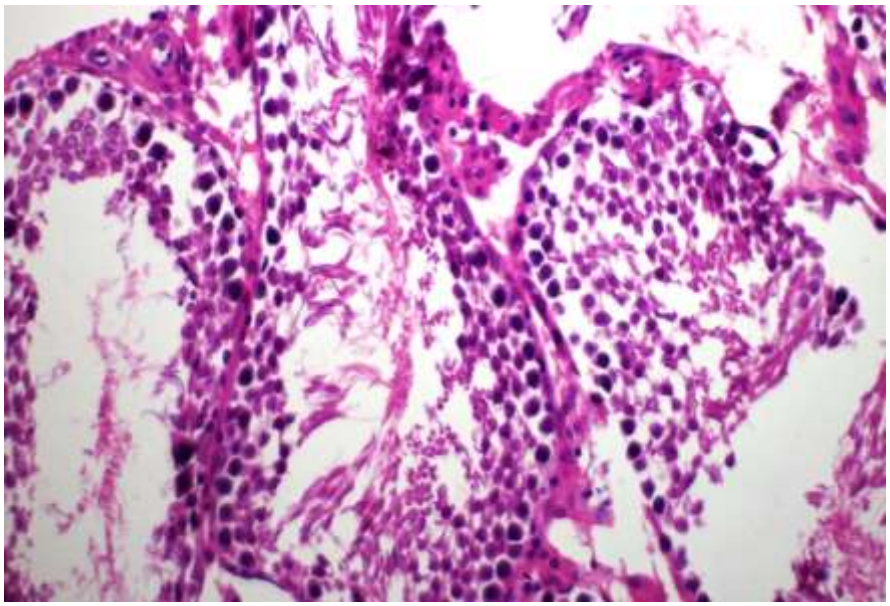

Fig 6 :Seminiferous tubules of male rats exposed to chlorpyrifos (10 mg kg.B.wt)for 30 days showing marked degenerative changes of spermatogenic cells& sever congestion of interstitial blood vessels could be seen accompanied by edema .The interstitial leyding cells have degenerative changes and necrosis(H&E X 400)

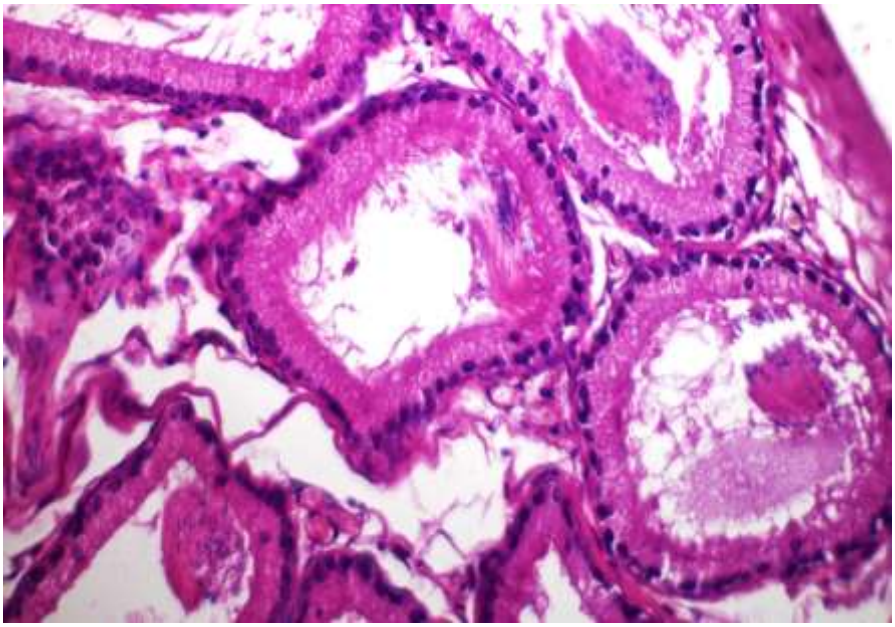

Fig 7 :Epididymis of male rats exposed to chlorpyrifos (10 mg kg.B.wt)for 30 days showing some spermatids inside the lumen of its tubules. Edema in the interstitial tissue between epididymal tubules could be seen accompanied by some degenerative changes of interstitial connective tissue (H&EX400)

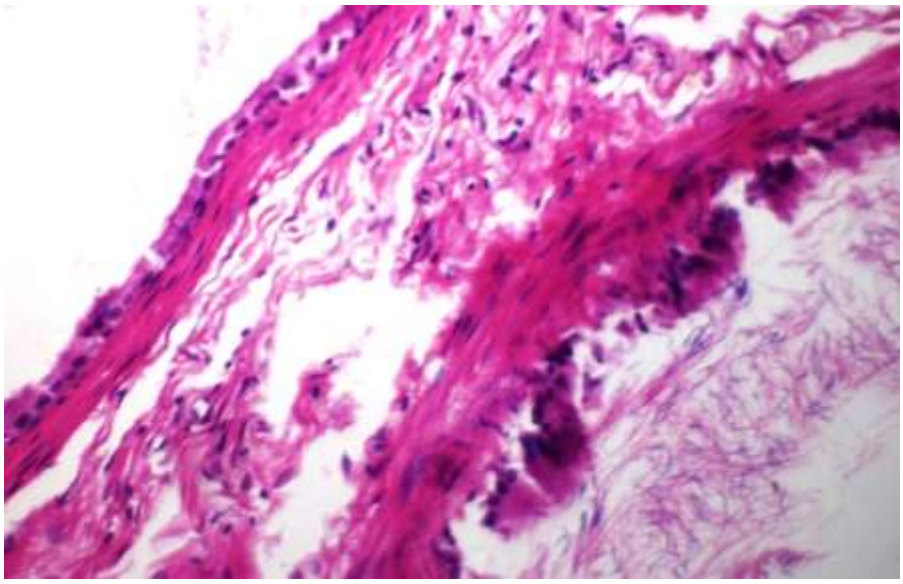

Fig 8 :Epididymis of male rats exposed to chlorpyrifos (10 mg kg.B.wt)for 30 days showing its tubules have degenerative and necrosis of its epithelial lining as well as detachment of its epithelial ling from basement membrane . Edema between epididymal tubules could be seen (H&EX400)

Table (1): Effect of oral administered to chlorpyrifos for 30 successive days on body weight and body weight gain of male rats (n=10)

Group	Dose mg/kg.B.Wt	Initial Body weight	Body weight after 30 days	Final weight gain%
C-ve	----	172.97 ± 7.356	193.5 ± 2.25	42
Chlorpyrifos	5	165.7 ± 6.89	188.7 ± 2.11	40
	10	170.03 ± 2.411	199.98 ± 4.58	35*

Value represents mean ± SE of 20 rats, * =p<0.05

Table (2): Effect of oral administration of chlorpyrifos for 30 successive days on sexual organs weight, and some internal organs of male rats.(mean±, n=10). Group

Group	Dose mg/kg.B.Wt	ALT U/L	AST U/L	AP U/L
Control	-----	37.6±3.5	44.6 ±1.32	79.35 ±1.22
Chlorpyrifos	5	80.02±4.2	79.0±0.77	79.98±0.45
	10	51.45**± 6.98	62.43**±2.99	96.99** ±0.6

significant at**, p<0.01

Table(3): Effect of oral administration of chlorpyrifos for 30 successive days on enzymatic activity in serum of male rats (mean±SE, n=10)

Group	Dose mg/kg.B. Wt	Weigh of sexual organs gm/100 gm b.wt				Weight of internal organs gm/100gm.b.wt		
C-ve		Testis	Seminal vesicle	Prostat	Epidid	liver	kidney	Spleen
		1.73±0.07	0.67±0.05	0.41 ±0.03	0.70 ±0.32	2.552 ±0.032	0.509 ±0.019	0.263 ±0.006
chlorpyrifos	5	1.526±0.08	0.5±0.02	0.2*** ±0.02	0.582 ±0.07	2.926** ±0.126	0.644* ±0.022	0.275* ±0.03
	10	1.04** *±0.04	0.158*** 0.014±	0.072*** ±0.01	0.38 ±0.02	2.976 ** ±0.074	0.646* ±0.049	0.289* ±0.014

Significant at***p<0.001 **p<0.01& *p<0.0

REFERENCE

Ambali, S.F., A.T. Abubakar, M.U. Kawu, C. Uchendu, M. Shittu and S.O. Salami,(2011a). Biochemical alterations induced by subchronic chlorpyrifos exposure in Wistar rats: ameliorative effect of zinc. The Journal of American Science, 7(9): 73-81.

Amer, H.A., W.M. Ahmed and S.I. Shalaby, (2000) Effect of chronic low dose oral administration of the organophosphorus selecron on some blood constituents and reproductive parameters in Baladi sheep. Egypt. J. Comp. Path. Clin. Path, 13(1): 81-88

Awasthi, M.D. and Prakash, N. B. (2007): Persistence of chlorpyrofos in soil under different moisture regimes. Pesti. Sci.

Bailey, Steven A.; Robert H. Zidell and Richard W. Perry, (2004). Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint? Toxicologic Pathology, 32: 448-466.

Bebe FN and Panemanogalore M (2003): Exposure of low doses of endosulfan and Chlorpyrifos modifies endogenous antioxidants in tissues of rats. J. Environ. Sci. Health.; 38(3): 349-363

Boncroft,M.Gamble(2008): Theory and Practical of histological techniques(6thed),Elsiver,Churchill Livingstone, China

Bozkurt, Ayhan; Yardan, Turker; Ciftcioglu, Engin; Baydin, Ahmet; Hakligor, Aylin; Bitigic, Medine and Bilge, Sirri, (2010). Time Course of Serum S100B Protein and Neuron-Specific Enolase Levels of a Single Dose of Chlorpyrifos in Rats. Basic & Clinical Pharmacology & Toxicology, 107(5): 893-898.

Chaudhary S and Sahal S. (1994) Lambda-cyhalothrin-induced changes in oxidative stress biomarkers in rabbit erythrocytes and alleviation effect of some antioxidants.Toxicology in Vitro.;21:392-397

Drury RA, Wallington EA. **(1980**).Technique. Fourth Edition Oxford University Press, New

E1-Deeb AEA, Abd E1-Aleem IM and Sherin S (2007): Harmful effect of some insecticides on vital parameters of albino rats. J. Egypt. soc. Toxicol; 36: 53-60.

EL-Hossary GG, EL-Gohary AA, Ahmed NS, Mohamed AS and Mansour SM (2009): Amelioration of Chlorpyrifos Induced Retinal and Renal toxicity by Vit D3. Australian Journal of Basic and Applied Sciences;3 (3): 2304-14.

Galakatu SS, Joshi DV, Patel BJ, Balani JK, Mohammad N and Kher AC (2012): Toxicopathological Studies on induced Chlorpyrifos toxicity in Wistar rats (Rattusnorvegicus). An international e Journal; 1(2).www.arkgroup.co.in.

Goel, A., V. Dani and D.K. Dhawan, (2007). Zinc mediates normalization of hepatic drug metabolizing enzymes in chlorpyrifos-induced toxicity. Toxicol. Lett., 169(1): 26-33.

Griffin P, Mason H, Heywood K and Cocker J (1999) Oral and dermal absorption of Chlorpyrifos: A human volunteer study. Occup. Environ. Med. ; 56 (1), 10-13.

Griffin P, Mason H, Heywood K and Cocker J (1999): Oral and dermal absorption of Chlorpyrifos: A human volunteer study. Occup. Environ. Med. ; 56 (1), 10-13

H. M. El-bendary*, M. H. Shaker, A. A. Saleh, S. E. Negm,M. E. Khadey and F. A. Hosam Eldeen(2014) Histopathological Changes Associated withExposure of Male Mice to Profenofos andChlorpyrifos. Annual Research & Review in Biology,4(5): 766-777

Hancock, S., M. Ehrich, J. Hinckley, T. Pung and B.S. Jortner,(2007). The effect of stress on the acute neurotoxicity of the organophosphate insecticide chlorpyrifos. (Special issue: Anticholinesterases). Toxicology and Applied Pharmacology, 219(2/3): 136-141.

Heikal TM, Ghanem HZ, Soliman MS.(2011): Protective effect of green tea extract aagganistt dimethoattee DNA damage and oxidant/antioxidant status in male ratsBiohealth. Sci. Bull 2011; 3(1): 1– 11.

Heikal TM, Mossa ATH, Marci GI and Rasoul MA (2012): Cyromazine and Chlorpyrifos induced Renal Toxicity in Rats: The Ameliorating affects of Green tea Extract. Environmental Analytical Toxicology; 2(5): 146.

Hernandez, Hernandez Antonio F., Gomez, Amparo M., Perez, Perez Vidal; Garcia-Lario, Jose V., Pena, Gloria, Gil, Fernando, Lopez, Lopez Olga, Rodrigo, Lourdes, Pino, Guadalupe and Pla, Antonio, (2006). Influence of exposure to pesticides on serum components and enzyme activities of cytotoxicity among intensive agriculture farmers. Environmental Research, 102(1): 70-76.

Issa AM, Gawish AM and Esmail GM (2011): Histological Hazards of Chlorpyrifos usage on gills and kidneys of Nile tilapia and the role of Vitamin E supplement in Egypt. Life Science Journal; 8(4): 113-123.

Jacobsen, H., G. Ostergaard, H.R. Lam, M.E. Poulsen, H. Frandsen, O. Ladefoged and O. Meyer,(2004). Repeated dose 28-day oral toxicity study in Wistar rats with a mixture of five pesticides often found as residues in food: alphacypermethrin, bromopropylate, carbendazim, chlorpyrifos and mancozeb. Food and Chemical Toxicology, 42(8): 1269-1277.

Jaiswal, C. and Y. Verma, (2012). Effect of alpha-tocopherol on haematological alteration induced by chlorpyrifos in albino rats. Haryana Veterinarian, 51: 108-110.

Johnson, Frank O., Chambers, Janice E., Nail, Carole A., Givaruangsawat, Sumalee and L. Carr, Russell, (2009). Developmental Chlorpyrifos and Methyl Parathion Exposure Alters Radial-Arm Maze Performance in Juvenile and Adult Rats. Toxicological Sciences, 109(1): 132-142.

Joshi, S.C., R. Mathur, A. Gajraj and T Sharma(,2003).Influence of methyl parathion on reproductive, parameters in male rats.Environ. Toxicol. Pharmacol. 14: 91-98.

Kammon AM, Brar R S. Banga H S and Sodhi S (2010): Patho-biochemical studies on hepatotoxicity and nephrotoxicity on exposure to Chlorpyrifos and Imidacloprid in layer chickens. VeterinarskiArchhiv; 80(5): 663-672.

Kang, Hwan Goo; Jeong, Sang Hee; Cho, Joon Hyoung; Kim, Dong Gyu; Park, JongMyung and Cho, Myung Haing, (2004). Chlorpyrifos-methyl shows anti-androgenic activity without estrogenic activity in rats. Toxicology, 199(2/3): 219-230.

Kavitha, P. and J. Venkateswara Rao,(2009): sub-lethal effects of profenofos on tissue-specific antioxidative responses in a euryhyaline fish, oreochrom Saf., 72(6): 1727-1733. is mossambicus. Ecotoxicol. and Environ

Lin, L., J. Liu, K. Zhang and Y. Chen, (2003). An experimental study of the effects of profenofos on antioxidase in rabbits. Wei. Sheng. Yan. Jiu., 32(5): 434-435.

Mansour SA and Mossa AH (2011): Adverse effects of exposure to low doses of Chlorpyrifos in lactating rats. ToxicolInd Health; 27 (3): p 213-214.

Mansour, S.A. and A.H. Mossa, (2010): Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. Pestic. Biochem. Physiol., 96: 14-23 .

Michael, B., B.L. Yano, R.S. Sellers, R. Perry, D. Morton, N. Roome, J.K. Johnson and K. Schafer, (2007). Evaluation of organ weights for rodent and non-rodent toxicity studies: A review of regulatory guidelines and a survey of current practices. Toxicol. Pathol., 35(5): 742-50.

Mohamed F. El-Tawil,**(2014).** Toxicological effects of short-term feeding with chlorpyrifos and chlorpyrifos-methyl insecticides on adult albino rats. Middle East Journal of Agriculture Research, 3(2): 208-220,

Mossa, A.T.H. and M.A. Abbassy, (2012). Adverse haematological and biochemical effects of certain formulated insecticides in male rats. Research Journal of Environmental Toxicology, 6(4): 160-168.

Nahid Akhtar, M.K. Srivastava and R.B. Raizada(2009): Assessment of chlorpyrifos toxicity on certain organs in rat, Rattus norvegicus. Journal of Environmental Biology, 30(6) 1047-1053

Nashwa, A. ; Abu Aita; Mahitab, A. H. and Amina,H. M. (2012): Clinicopathological and cytogenetic studies on the ameliorative effect of propolis against profenofos toxicity in rats. Glob. Vet. 9(6): 669-682.

Nolan RJ, Rick DL, Freshour NL and Saunders JH (1984): Chlorpyrifos: Pharmacokinetics in Human Volunteers. Toxicol. Appl. Pharmacol. ; 73: 8-15.

Okamura, A. ;Kamijima, M. ;Ohtani, M. K. ;Yamanoshita, O. ; Nakamura, D. ;Ito, Y. ;Miyata, M. and et al (2009): Broken sperm cytoplasmic droplets and reduced sperm motility are principle markers of decreased sperm quality due to organophosphorus pesticides in rats. J. Occup. Health, 51(6):478-487.

Oncu M, Gultekein F, Karaoz E, Altuntas I and Delibas N (2002): Nephrotoxicity in rats induced by Chlorpyrifos-ethyl and ameliorating effects of antioxidantsy. Hum ExpToxicol; 21 : p223-230.

Pal S, Kokushi E, Koyama Jb Uno S and Ghosha AR (2012): Histopathological alterations in gill, liver & kidney of common carp exposed to CPF. J environ Sci. Health B.; 4 7 (33): 180-195.

Reitman, S. and Frankel, S. (1957): Calorimetric method for the determination of serum transaminases activity of cholesterol. Clin. Chem., 19: 1350-1356.

Rekha, Sunanda Raina and Sajad Hamid (2013): Histopathological effects of pesticide-cholopyrifos on kidney in albino rats . *Int J Res Med Sci. Nov;1(4):465-475*

Rezq, Amr A. and El-Khamisy, E. Abeer, (2011). Hypolipideimic and Hypocholestermic Effect of Pine Nuts in Rats Fed High Fat, Cholesterol-Diet World Applied Sciences Journal, 15(12): 1667-1677.

Roy, A.V.(1970): A rapid method for alkaline phosphatase estimation. Clin. Chem.., 16:431.

Schellmann RG (1995): Toxic responses of the kidney. Casarett and Doull's Toxicology. The Basic Science of Poisons. Klaassen CD ed (7th) McGraw Hill Companies Inc, New York, NY:591-597.

Sellers, R.S., D. Morton, B. Michael, N. Roome, J.K. Johnson, B.L. Yano, R. Perry and K. Schafer, (2007). Society of Toxicologic Pathology position paper: organ weight recommendations for toxicology studies. Toxicol. Pathol., 35(5): 751-5.

-Shalini, R. ; Reena, K. ; Sharmila, R. and Kumar, R. (2006): Biodegeneration of fenitrothion in soil.Biochem.chromatog.,10:60-64.

Snedecor , G.W (1982): statistical methods . 6th ed . the iowa state Univ, Press , Ames , Iowa , USA.

Stanley, O. Aniagu, C. Florence, Nwinyi; D. David, Akumka; Gloria, A. Ajoku; Sunday, Dzarma; Kazeem, S. Izebe; Matthew Ditse; Patrick E.C. Nwaneri; Charles Wambebe and Karynius Gamaniel, (2005). Toxicity studies in rats fed nature cure bitters. African Journal of Biotechnology, 4(1): 72-78.

Trimbell, J.A., (1991). Principles of Biochemical Toxicology, pp: 144-147; Taylor and Francis; London; Second Edition.

Tripathi S and Srivastav AK (2010): Nephrotoxicity induced by long-term oral administration of different doses of Chlorpyrifos. ToxicolInd Health; 26 (7): 439-47.

Tuzmen N, Candan N, Kaya E, Demiryas N.(2008): Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidative defense mechanisms and lipid peroxidation in rat liver. Cell Biochem. Funct. 2008; 26: 119–124.

Uzunhisarcileli, M.; Kalender, Y. ; Dirican, K. ; Kalender, S.; Ogutcu, A. and Buyukkomurcu, F. (2007): Acute, sub-acute and subchronic administration of

methyl parathion-induced testicular damage in male rats and protective role of Vit. C & E. Pestic. Biochem. Physiol. ,87:115-122.

Verma, R.S., Mehta, Anugya and Srivastava, Nalini, (2007). In vivo chlorpyrifos induced oxidative stress: attenuation by antioxidant vitamins. Pesticide Biochemistry and Physiology, 88(2): 191-196.

Wilson, A.G.,(2001). Short term, subchronic and chronic toxicology studies. In: Principles and Methods of Toxicology. (W. Hayes, ed.), 4th Edition, pp 917–58, Taylor and Francis, Philadelphia, PA.

Wooley, A., (2003). Determination-General and reproductive toxicology. In: A Guide to Practical Toxicology Evaluation, Prediction and Risk, pp. 80–106. Taylor and Francis, New York.

Xing H, Li S, Wang Z, Gao X, Xu S and Wang X (2012): Histopathological changes and anti oxidant response in brain and kidney of common car p exposed to atarzine and CPF. Chemosphere; 88(4) 337-83.

York, Toronto;

Yoshida, A., T. Kosaka, T. Miyaoka, K. Maita, S. Goto and Y. Shirasu, (1985). Chlorpyrifos-methyl: 28-day oral toxicity study in mice. Unpublished Report No. GHF-R 80 from the Institute of Environmental Toxicology, Tokyo, Japan. Submitted to WHO by DowElanco, Indianapolis, USA.

Yurumez Y, Ikekeizceli I, Sozuer CM, Soyuer I and Yavuz Y(2007): Effect of interleukin–10 on Tissue Damage caused by organophosphate poisoning. Basic and clinical pharmacology and toxicology; 100: 323-327.