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Impact of Chronic Exposure to Pesticide Chlorpyrifos on Kidney of Albino Rats

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ABSTRACT

AIM:The organophosphorus pesticides are among the most widely use insecticides globally and they are readily available commercially for domestic and industrial purposes But as a consequence of their widespread use in agriculture and public health, these insecticides ultimately reach the environment and affect the life there in. There are few reports regarding histomorphological changes in kidney following pesticide Chlorpyrifos exposure which has prompted me to undertake this study.

MATERIAL AND METHODOLOGY: In the present study, Albino rats served as experimental animals. Healthy Wistar Albino rats, forty five in number of either sex weighing between 145 – 165 mg were taken for the study. The rats were procured from the Central Animal House of Government Medical College, Jammu. The investigation was conducted upon getting clearance from Institutional Animal Ethics Committee **(IAEC).**After two weeks of acclimatization; the rats were randomly divided into divided into 3 groups A, B and C with 15 animals each. Group A served as control group, Group B were daily administered Chlorpyrifos at a dose of 5 mg/kg b wt and animals in Group C received daily an oral dose of 10 mg/kg b wt Chlorpyrifos

Rats were sacrificed on 6^{th} and 8^{th} week after initiation of the experiment. Kidneys were removed after dissection, examined grossly and after making coronal section. These specimens were then subjected to standard histological proceedings by paraffin embedding method.

RESULTS: Grossly the kidneys of rats in Group B showed congestion, thickening and adherence of capsule at some places .With increasing dose and duration of treatment with Chlorpyrifos i.e after 8^{th} week the kidneys were small in size, shrunken and had irregular contour.In Group C the kidneys were grossly congested and friable to touch and the capsule was thickened. After 8 weeks of exposure the kidneys were small in size, shrunken and had irregular contour with thickened outer capsule.**Microscopic changes: Group** B –After 6 weeks Shows degeneration of glomeruli at some places and at some locations there was hypercellularity in the glomeruli. Renal tubules were dilated due to atrophy of epithelial lining. Degenerating tubules showed tubular vacuolization and ragged appearance. These were suggestive of acute tubular necrosis. There were eosin positive casts in lumen of the tubules. There was increased vascularity and dilated blood vessels. Foci of lymphocytic infiltration were seen in the interstium. Following 8 weeks of chlorphyrifos exposure all these histomorphological changes were increased in severity and also a band of fibrosis was seen in the interstium.

Group C –There were few foci of lymphocytic infiltration and vascular congestion. 6 week after Chlorpyrifos treatment revealed degeneration in some of glomeruli and a few showed hypercellularity. Several degenerating tubules were seen and their lumina were filled with eosinophilic casts. There was increased vascularity in the form of dilated blood vessels and interstitial oedema was seen. There was a band of fibrosis within interstitium.

CONCLUSION: The present study showed that significant histomorphological changes were caused in the kidneys of rats administered with Chlorpyrifos. These changes were markedly different from the control rats. There was shrinkage of glomerulus at initial stages of treatment, tubular dilation, glomerular hypercellularity, hypertrophy of tubular epithelium, degeneration of renal tubules, deposition of eosin positive substance in the glomerulus and renal tubules. There were infiltration of lymphocytes in the interstitium and increased vascularity in the form of dilated vessels fibrosis and interstitial oedema.

All these changes were suggestive of glomerulonephritis, acute tubular necrosis and interstitial nephritis leading to acute renal failure progressing to Chronic renal failure with increasing duration. Hence this study brought into light the renal toxicity induced by Chlorpyrifos which was found to be significant at high dose level..

Keywords: Chlorpyrifos, Pesticide, Nephroticity

INTRODUCTION

Environmental Pollution , when considered in its broadest context, is a by-product of human activities and is significance is in what ways it affects directly or indirectly the living population . One of the ways of environmental pollution is by chemical pesticides.

The term pesticide covers a wide range of compounds including insecticides, fungicide, Herbicide, rodenticide, plant growth regulators and others **(1).**

Pesticides have played vital role in controlling agricultural, industrial, home and public health pest worldwide **(2).** However, their use poses animal and human health concerns because of their toxicity, widespread use and release into the environment**.** According to the World Health Organization, 3 million cases of pesticide poisoning occur every year, resulting in more than 250,000 deaths **(3).** Despite this alarming figure, there is currently no global system to track and stem poisoning or diseases associated with pesticide use**.** Exposure to pesticides normally occurs through lower level single or repeated exposure (for example, as residues in food products) **(4).** The high rate of poisoning may be attributed to a number of reasons, including farmers' poor knowledge about pesticides and pesticide use, less protection against exposures, little formal education of agricultural workers , minimal understanding of the health risks and, most importantly, inadequate safety warnings on the packages by the manufacturers **(5)**. Farmers and farm workers may be exposed by mixing, loading and applying pesticides or while performing duties not associated with pesticide application, for example, weeding or harvesting **(6).**

In India the first report of poisoning due to pesticide was from Kerala in 1958, where 100 people died after consuming wheat flour contaminated with pesticide. **(7).**

If the credits of pesticides include enhanced economic potential in terms of increased production of food, fiber and amelioration of vector borne diseases, then their debits have resulted in serious health implications to man and his environment.

In spite of undesirable and unwanted effects of pesticides on man, there is sequential rise in production and consumption of pesticides in India during last three decades. Currently there are 165 pesticides registered for use in India. The domestic demand in India accounts for 76% of the total pesticides used in the country as against 44% globally (**7**).

Many chemical formulations were synthesized, introduced and widely used in pest control programs. These synthetic chemical pesticides can be structurally classified into the following groups:-

a. Organochlorine pesticides.
b. Organophosphate pesticides.
c. Carbamates.
d. Synthetic pyrethroids. **(8)**

The organophosphate insecticides have superseded the organochlorines owing to their rapid biodegradability and shorter persistence in the environment. The organophosphorus pesticides are among the most widely use insecticides globally and they are readily available commercially for domestic and industrial purposes. They account for 50% of all insecticides applied worldwide. But as a consequence of their widespread use in agriculture and public health, these insecticides ultimately reach the environment and affect the life there in. Organophosphorus compounds exist in liquid and solid forms and are – Phosphorothioates, Phorodithioates and Phosphates **(9).**

Chlorpyrifos is a member of organophosphate class of pesticides that elicits broad spectrum insecticidal activity & kills insects upon contact by affecting normal function of nervous system.

Chlorpyrifos is responsible for mammalian toxicity through inhibition of cholinesterase (**10).**Once Cholinesterase has been inactivated, acetylcholine accumulates throughout the nervous system **(11)**. Toxicity of pesticide cause adverse effect on many organs like Kidney, Liver, Brain and Blood cell **(12).**In humans and experimental animals, the accumulation of acetylcholine results in cholinergic responses in the peripheral (muscarinic and nicotinic) and central nervous system and neuromuscular junctions. (13**).**

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The mortality rate of organophosphorus poisoning is high and the fatal issue is often related to delay in diagnosis or improper management. Acute treatment includes rapid administration of Atropine, which blocks the muscarinic effects and that of Pralidoxime which reactivates acetylcholine inhibited by organophosphates **(**14**) .**

Administration of sublethal doses of Chlorpyrifos resulted in renal damage, reproductive disorders and altered enzyme activities in liver and brain of experimental animals **(15)**.

Chlorpyrifos also has a role in increased lipid peroxidation and decreased antioxidant potential in increasing oxidative stress and all these factors attribute to nephrotoxicity **(16).**

Ojha et al. in 2011(17) studied genotoxic effect of Chlorpyrifos following acute and chronic exposure and observed significantly marked DNA damage in rat tissues namely the Liver, Brain, Kidney and Spleen.

The toxicity of organophosphate (CPF) in mammalian animals has received much attention in the recent years . Kidney , the major detoxification organ for many xenobiotics is frequently susceptible to nephrotoxic effects. Nephrotoxicity is one of the toxic manifestations of Chlorpyrifos after its long term as well as acute exposure .

As a consequence of renal heterogeneity, mechanism of chemically induced injury cannot be explored easily, but can be evaluated by studying histomorphological changes in different parts constituting renal tissue. Histopathological lesions have been widely used as biomarkers for health evaluation of organism exposed to pollutants and can be used as warning symptoms for organism health.

There are few reports regarding histomorphological changes in kidney following pesticide Chlorpyrifos exposure which has prompted me to undertake this study.

Keeping in view the above facts, present study is designed to evaluate the effect of orally administered Chlorpyrifos at different doses for 2 months on kidney architecture of Albino Rat.

MATERIAL AND METHODOLOGY

In the present study, Albino rats served as experimental animals. Healthy Wistar Albino rats, forty five in number of either sex weighing between 145 – 165 mg were taken for the study . The rats were procured from the Central Animal House of Government Medical College, Jammu. The investigation was conducted upon getting clearance from Institutional Animal Ethics Committee **(IAEC).**After two weeks of acclimatization, the rats were randomly divided into following groups.

The animals were group housed (12 hours light / dark cycle) in labeled cages in a room where temperature was maintained at $25^0 \pm 2^0$C. The animals were fed with standard laboratory feed and water ad-libitum throughout the experimental period.

EXPERIMENT PROTOCOL:

Experimental animals were given oral Chlorpyrifos using a cannula

Group A rats served as control and were left as such.

Drug regime in Group A and Group B were as follows:-

GROUP B

EXPERIMENTAL GROUP	DURATION OF DOSE	DOSE STRENGTH
Group B	**6 Weeks**	**5 mg/kg**
Group B	**8 Weeks**	**5 mg/kg**

GROUP C

EXPERIMENTAL GROUP	DURATION OF DOSE	DOSE STRENGTH

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Group C	**6 Weeks**	**10 mg/kg**
Group C	**8 Weeks**	**10 mg/kg**

Chlorpyrifos was diluted in distilled water to obtain desired concentration. Fresh dosing solution was made every time.

Parameters Studied:

Animal were anaesthetized by using Diethyl ether and then sacrificed**.** Rats from each group were sacrificed at the end of 6th week and 8th week from the date of initiation of experiment. Sacrifice was followed by Dissection.

After dissection and removal of kidney following parameters were studied

Macroscopic Changes-

Kidneys were examined first grossly and later on section of kidney was made in coronal plane.

Microscopic changes-

After proper tissue processing light microscopic study was done and histomorphological changes were documented as photomicrographs.

PREPARATION OF TISSUE FOR MICROSCOPY

After obtaining the kidney, it was cut into smaller pieces (5 mm) which were kept inside a special metallic container (tissue capsule) for Fixation .The fixation was followed by dehydration,clearing, paraffin wax impregnation, casting (block making), sectioning, fixation of sections on the slides & finally staining: Staining was done by:-Harris Haemotoxylin and Eosin stain

RESULTS

The present study was conducted on 45 inbred adult Wistar albino rats of either sex, weighting 145 – 165 gms. Oral Chlorpyrifos was given to the experimental groups B and C in dose of 5 mg/kg body weight and 10 mg/kg body weight respectively. Group A served as control and was left as such.

The animals were randomly divided into 3 groups (A, B, C) as follows and following observations were made.

On dissection of the rats of all groups (A,B & C), the organ of present study, Was the kidney .

Group A (Control)

III.Macroscopic Observations

No change was seen in the Kidney of Group A (Control) on Gross & cut-section.

Microscopic Observations

Histologically,no change seen in control group(Group A)

Group B (5 mg /kg body weight Chlorpyrifos)

III.Macroscopic Observations

Grossly, after 6th week of treatment with Chlorpyrifos, the kidney showed congestion and there was thickness of capsule and adhesions at places. 8 weeks after treatment with Chlorpyrifos, the kidney was small in size, shrunken and had irregular contour and the outer capsule was thickened.

Coronal section of kidney when viewed with naked eye in Group B, observed congestion at corticomedullary junction and even the base of the medulla was affected by the congestion . **After 8 weeks** of treatment whole of the medulla was seen to be congested due to which corticomedullary junction was obscured. Cortex was congested especially along the upper poles

IV Microscopic Observations

Histological examination of Group B kidney (5 mg/kg/day Chlorpyrifos exposed) after **6 weeks** Chlorpyrifos exposure, degenerating glomeruli were noticed at some places .The renal tubules also exhibited degenerative changes and appeared more dilated because of atrophy of epithelial lining and in few tubules vacuolization was seen in epithelium and some of the tubules showed ragged appearance. There were eosin positive casts within the lumen of the tubules. Some of tubules showed separated epithelial lining from the underlying basement membrane. There were foci of lymphocytic infiltration in the interstitium. .There was hyperemia in the medulla due to vascular congestion and dilated blood vessels. Following **8 weeks** Chlorpyrifos exposure, Histomorphological changes were increased in severity. There were many degenerating glomeruli , most of the renal tubules were damaged

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and lost their characteristic appearance. Most of the tubules showed vacuolization, their lumens were filled with eosin positive casts. There were dilated blood vessels. There was erosion of the outer capsule. A band of fibrosis was seen in the interstitium . These features were suggestive of acute renal failure proceeding towards chronicity.

Group C (10 mg/kg body weight)

III. Macroscopic Observations

Grossly in group C after 8 weeks treatment the kidneys of the exposed rats were small in size, shrunken and had irregular contour with thickened outer capsule.**Coronal section of kidney when viewed with naked eye After 6** weeks of treatment with Chlorpyrifos, red-brown coloured outer cortex was seen and visible haemorrhage was seen in whole of the medulla .**8 weeks** after treatment with Chlorpyrifos the cut section of the kidney showed no demarcation between cortex and medulla and whole of it was congested .

IV. Microscopic observations

Histological examination of Group C (10 mg/kg) Chlorpyrifos were as follows:-

The kidney after **6 week** Chlorpyrifos exposure, glomeruli were degenerated at certain places and contained amorphous eosin-positive material. Some of the glomeruli showed increased cellularity, thus filling the Bowman's capsule. Several renal tubular cells appeared foamy due to vacuolization in epithelium and displayed ragged appearance . Many tubules showed eosin positive casts within their lumen .There were foci of Chronic inflammatory cells. Vascular congestion was more marked and concentric onion skin thickening of tunica media was seen. After **8 weeks** treatment with Chlorpyrifos, all the histomorphological changes were more pronounced. Capsule was eroded at places . Many glomeruli showed degeneration and were seen as clumps of amorphous material within the Bowman's capsule . Several tubules possessed amorphous material and degenerated nuclei in their lumina. The lining of epithelial cells became indistinguished. Large deposits of eosin positive material appeared in between the tubules. There were many foci of chronic inflammatory cells around the tubules as well as around the blood vessels suggestive of interstitial nephritis. Increased vascularity was seen in the form of dilated and congested blood vessels engorged with blood. Intestitial tissue oedema was seen and a band of fibrosis was seen within the interstitium. These features were suggestive of acute renal failure leading to chronic renal failure.

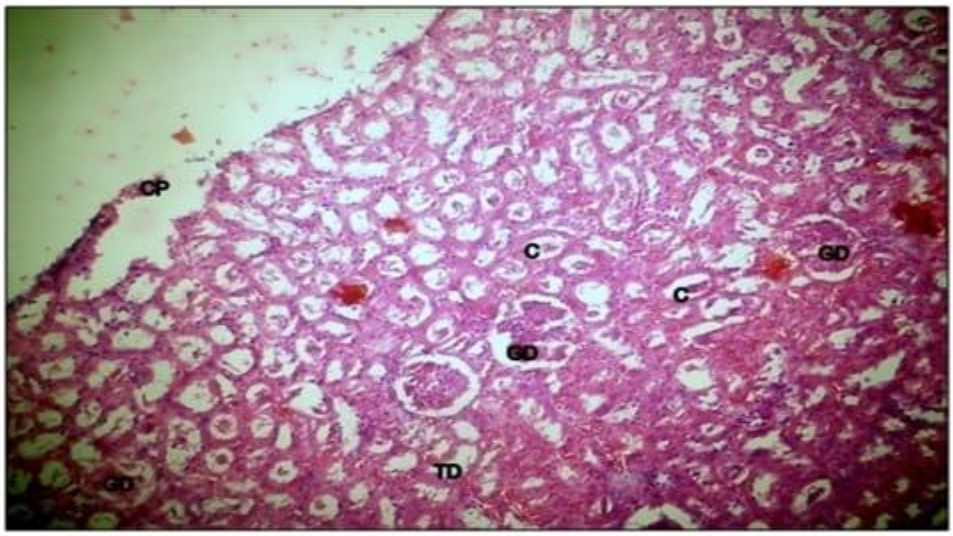

FIG. 48a PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP B OF 8 WEEK CHLORPYRIFOS (5 mg/kg b wt) TREATED RAT SHOWING ERODED CAPSULE (C), DEGENERATING GLOMERULI (GD), DEGENERATING TUBULES (TD) AND EOSINPHILIC CASTS WITHIN THE LUMEN OF TUBULES (C). H&E. STAIN X 100

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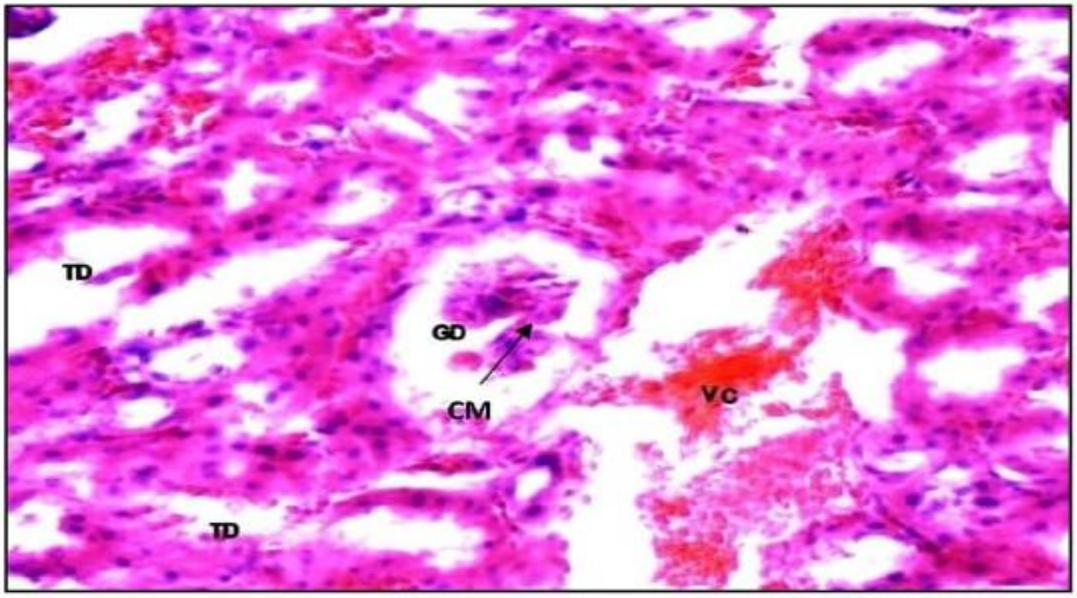

FIG. 60b PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP C OF 8 WEEK CHLORPYRIFOS (10 mg/kg b wt) TREATED RAT SHOWING DEGENERATED GLOMERULUS (GD) SEEN AS CLUMPS OF AMORPHOUS MATERIAL(CM), DEGENERATED TUBULES (TD) AND VASCULAR CONGESTION (VC). H&E STAIN X 400

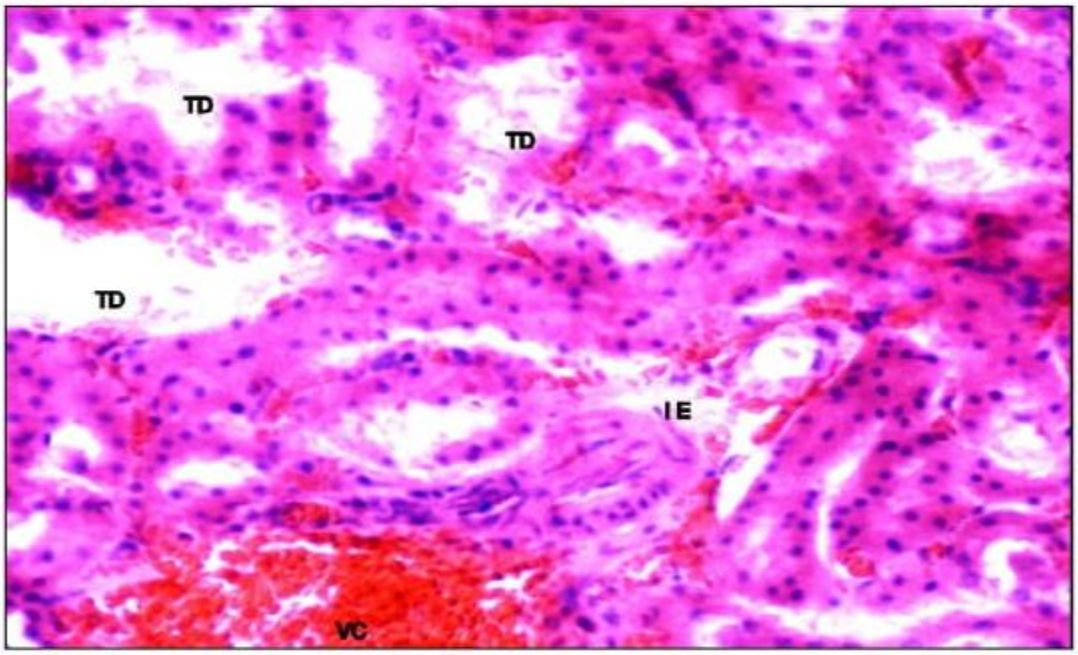

FIG. 60c PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP C OF 8 WEEK CHLORPYRIFOS (10 mg/kg b wt) TREATED RAT SHOWING SEVERE DEGENERATION OF TUBULES (T) INTERSTITIAL OEDEMA (IE) AND MASSIVE CONGESTION (VC). H&E. STAIN X 400

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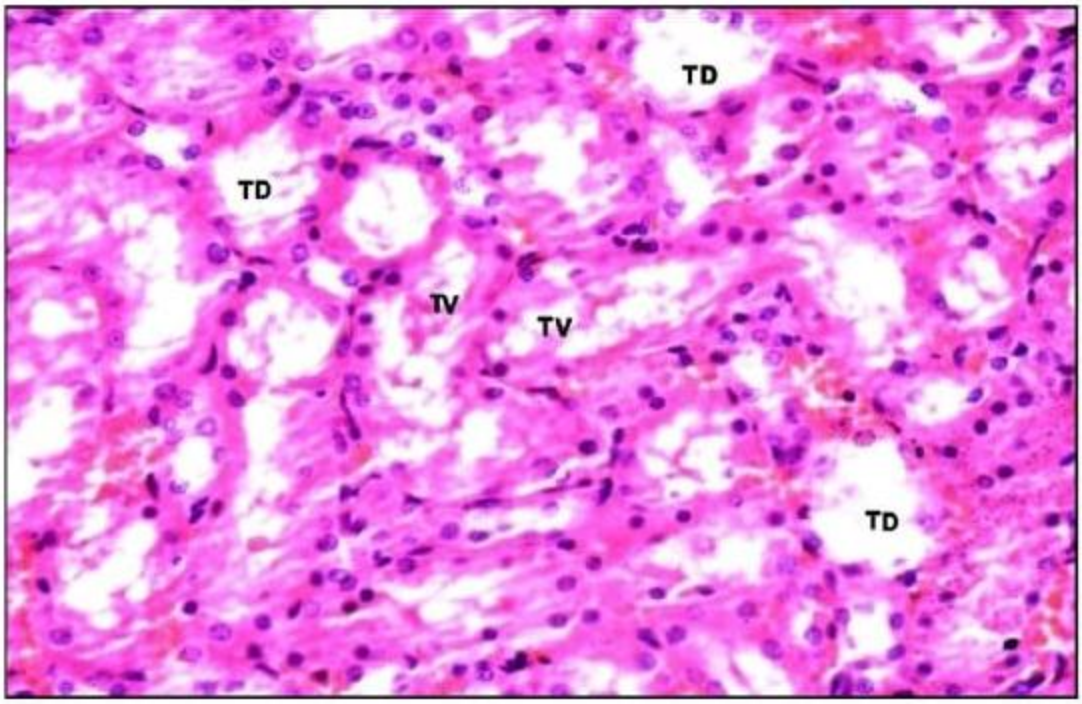

FIG. 59 PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP C OF 6 WEEK CHLORPYRIFOS (10 mg/kg b wt) TREATED RAT SHOWING DEGENERATED TUBULES (TD) AND VACUOLIZATION WITH THE TUBULES (TV). H&E STAIN X 400

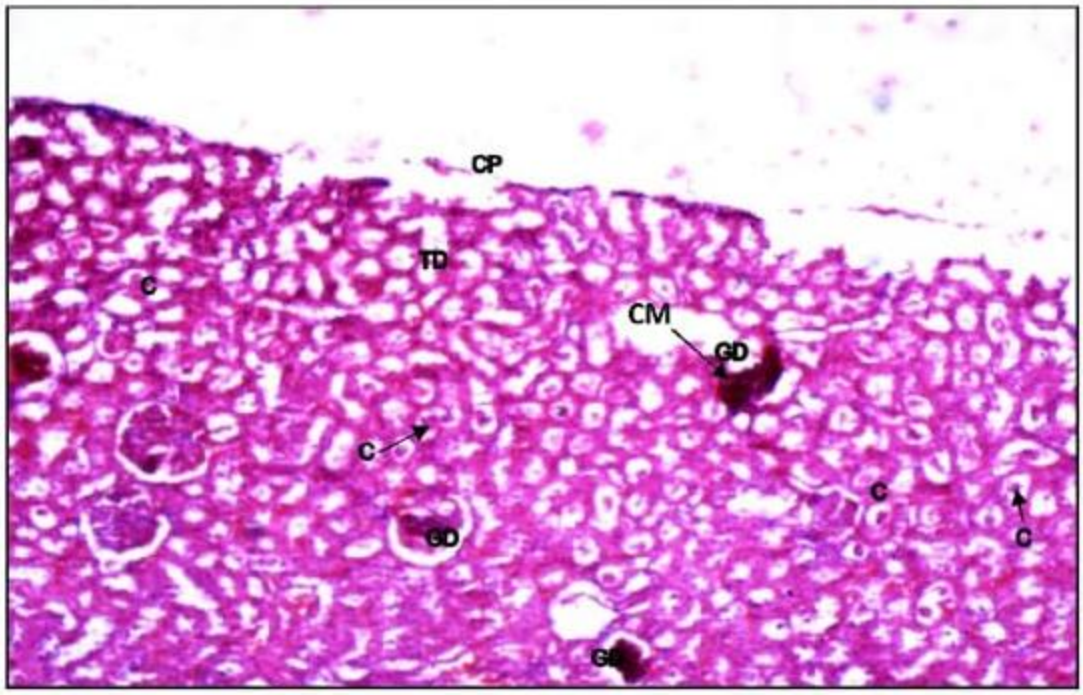

FIG. 60a PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP C OF 8 WEEK CHLORPYRIFOS (10 mg/kg b wt) TREATED RAT SHOWING ERODED CAPSULE (CP) DEGENERATED GLOMERULUS (GD)SEEN AS CLUMPS OF AMORPHOUS MATERIAL(CM), MANY EOSINOPHILIC CAST WITHIN THE TUBULES (C) AND DEGENERATED TUBULES (TD). H&E STAIN X 100

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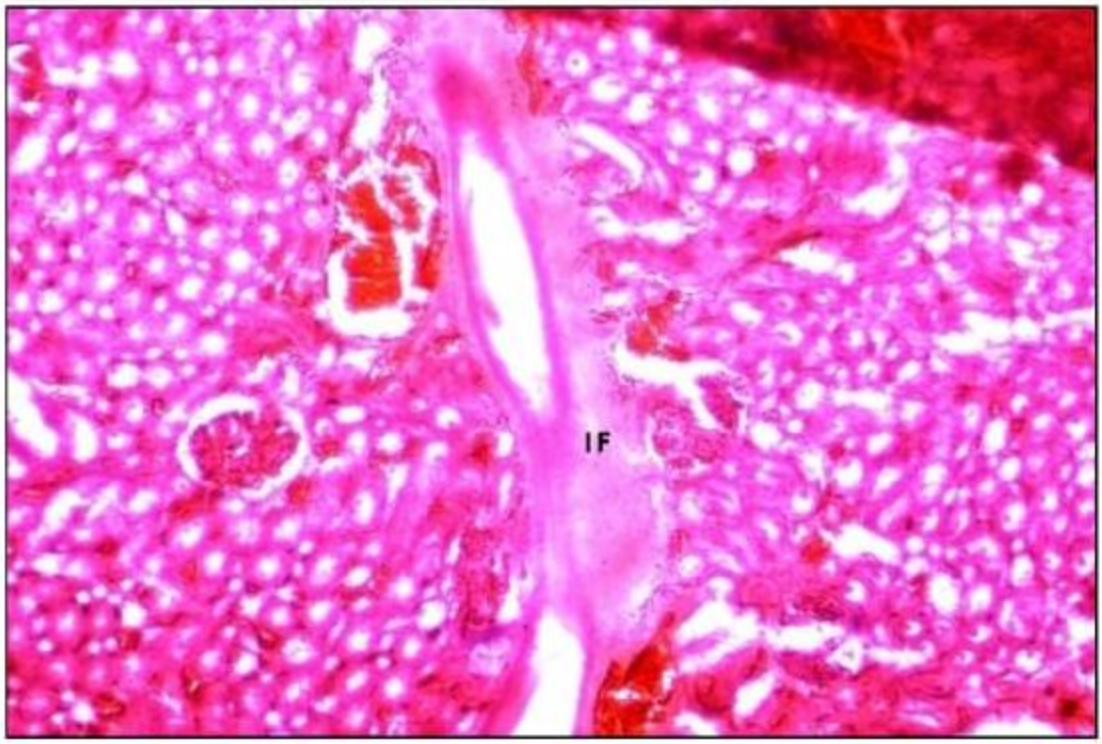

FIG. 63a PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP C OF 8 WEEK CHLORPYRIFOS (10 mg/kg b wt) TREATED RAT SHOWING INTERSTITIAL FIBROSIS (IF). H&E. STAIN X 100

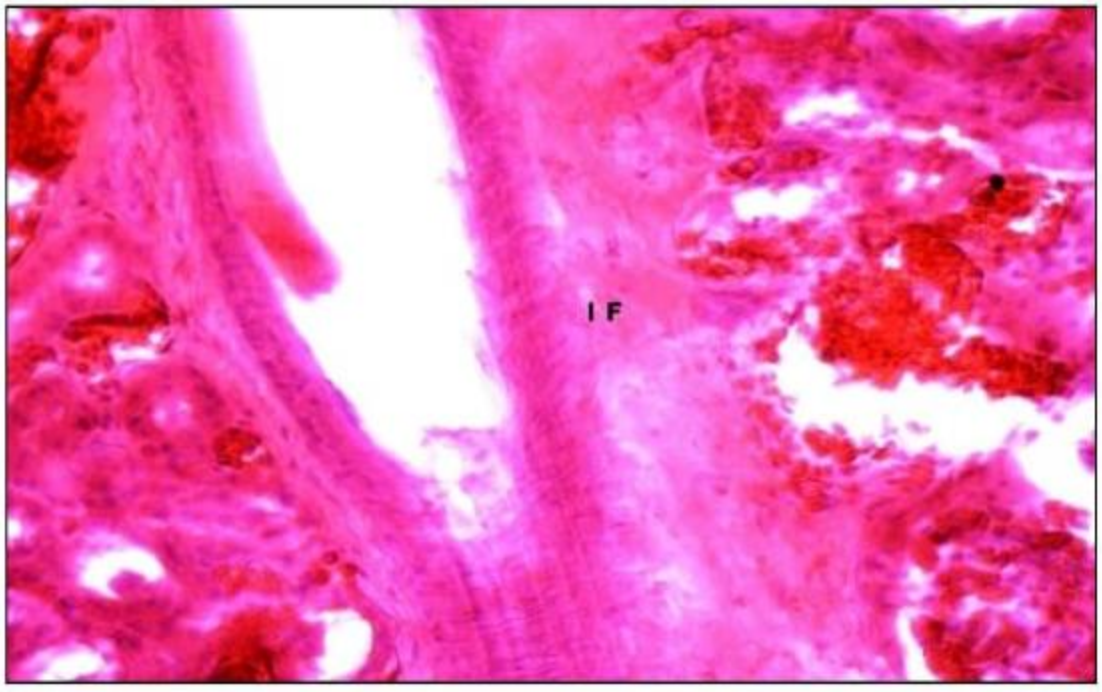

FIG. 63b PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP C OF 8 WEEK CHLORPYRIFOS (10 mg/kg b wt) TREATED RAT SHOWING INTERSTITIAL FIBROSIS (IF). H&E. STAIN X 400

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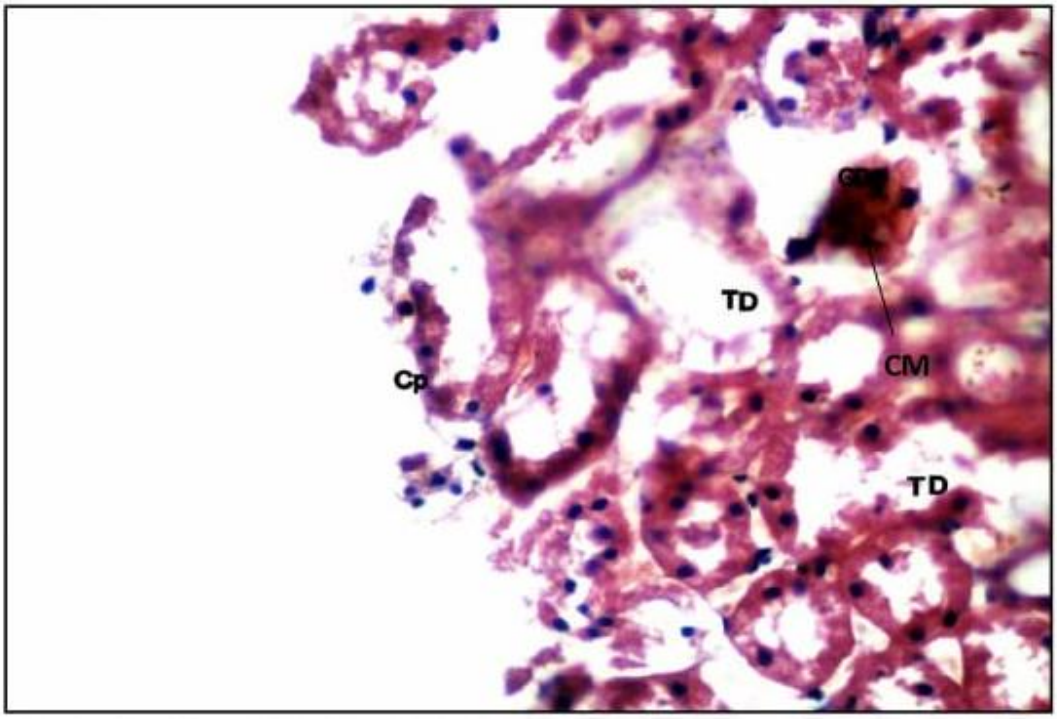

FIG. 60d PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP C OF 8 WEEK CHLORPYRIFOS (10 mg/kg b wt) TREATED RAT SHOWING DEGENERATED GLOMERULUS (GD) SEEN AS CLUMPS OF AMORPHOUS MATERIAL(CM), DEGENERATED TUBULES (TD) AND ERODED CAPSULE (CP)). H&E STAIN X 400

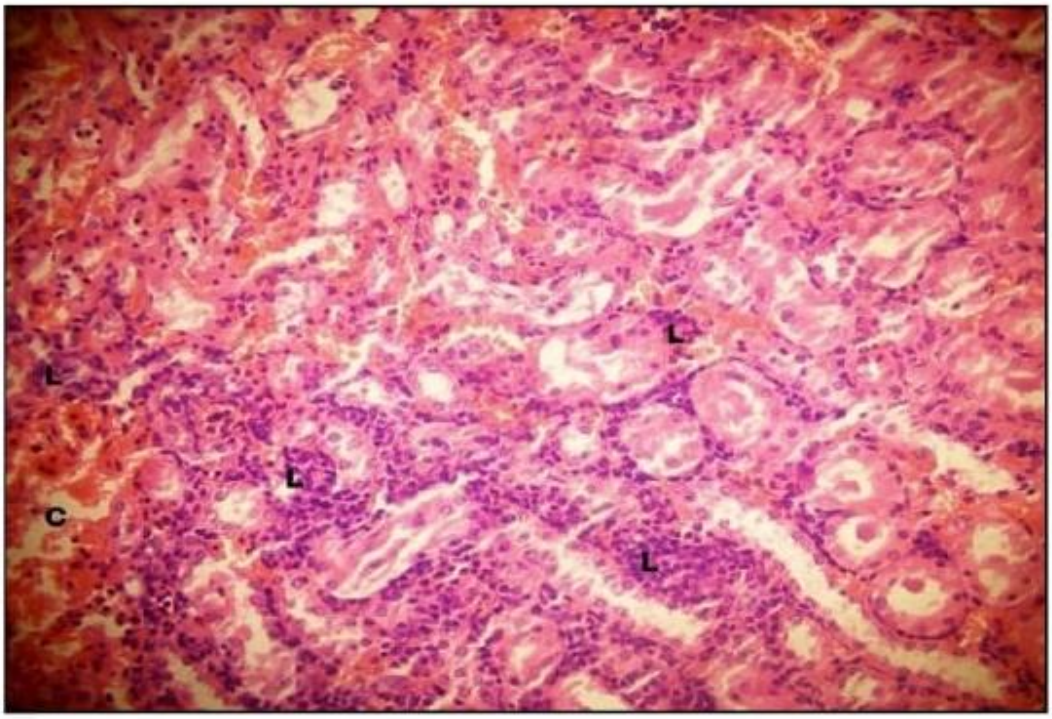

FIG. 61 PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP C OF 8 WEEK CHLORPYRIFOS (10 mg/kg b wt) TREATED RAT SHOWING MASSIVE INFILTRATION OF LYMPHOCYTES IN THE INTERSTITIUM (L) AND CONGESTION (C). H&E STAIN X 100

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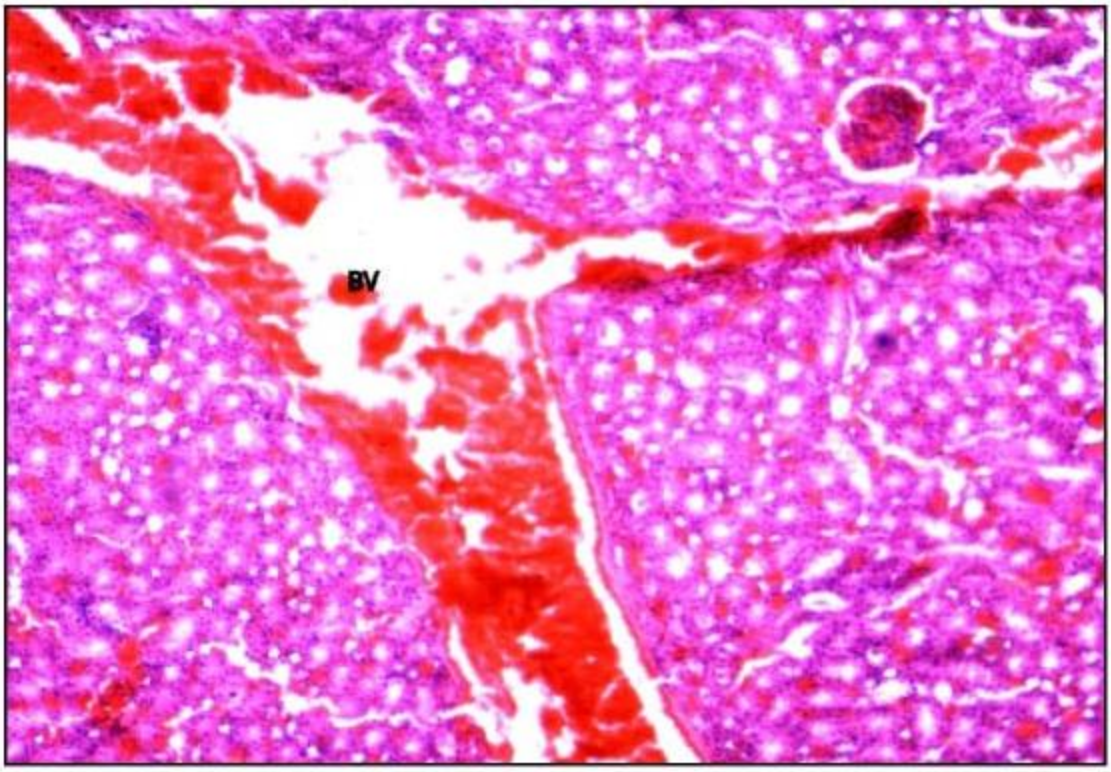

FIG. 62a PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP C OF 8 WEEK CHLORPYRIFOS (10 mg/kg b wt) TREATED RAT SHOWING DILATED BLOOD VESSEL (BV). H&E STAIN X 100

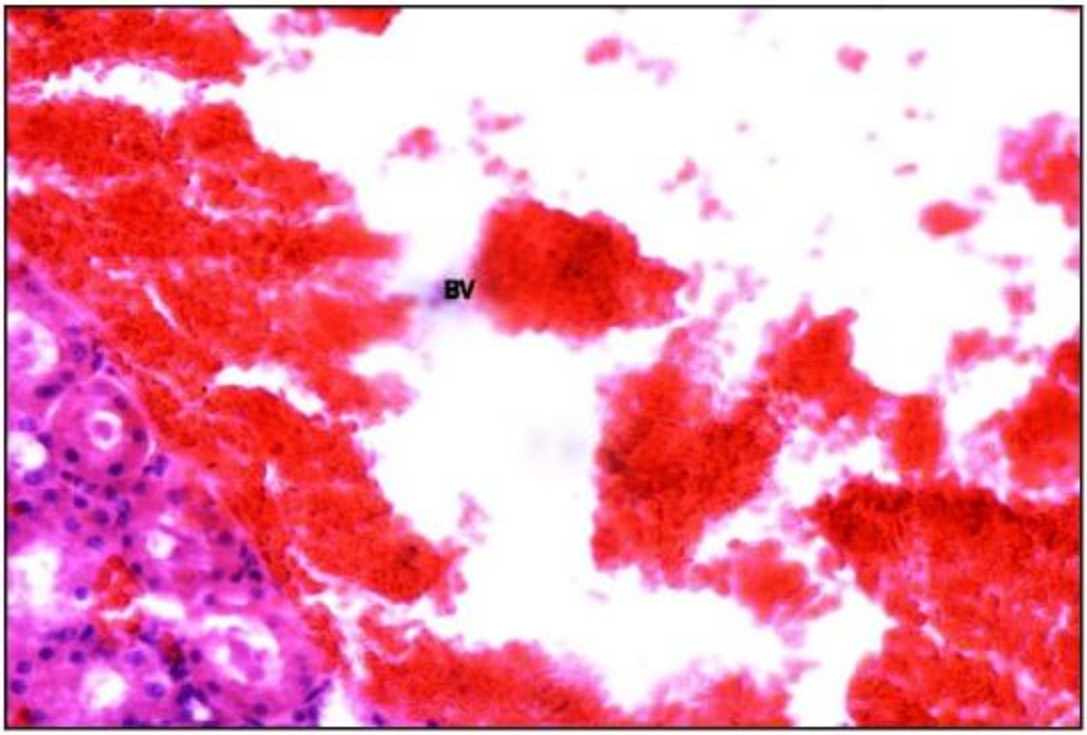

FIG.62b PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP C OF 8 WEEK CHLORPYRIFOS (10 mg/kg b wt) TREATED RAT SHOWING DILATED BIOOD VESSEL (BV). H&E. STAIN X 400

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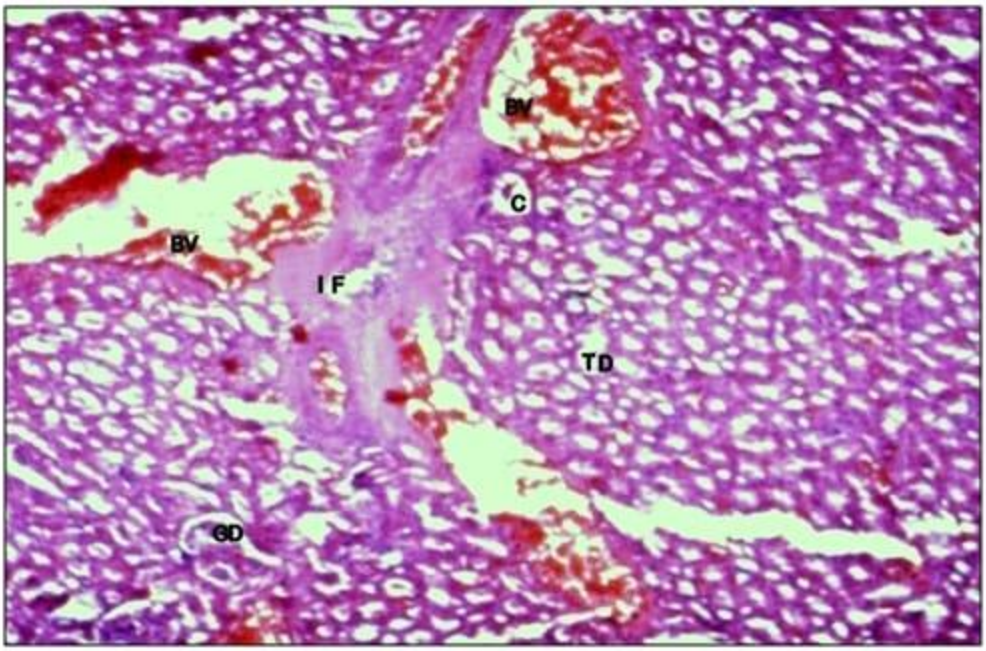

FIG. 48b PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP B OF 8 WEEK CHLORPYRIFOS (5 mg/kg b wt) TREATED RAT SHOWING, DEGENERATING GLOMERULUS (GD), DEGENERATING TUBULES (TD)., EOSINOPHILIC CASTS WITHIN THE LUMEN OF THE TUBULES (C), DILATED BLOOD VESSELS (BV) AND FIBROSIS (IF) IN THE INTERSTITUM H&E. STAIN X 100

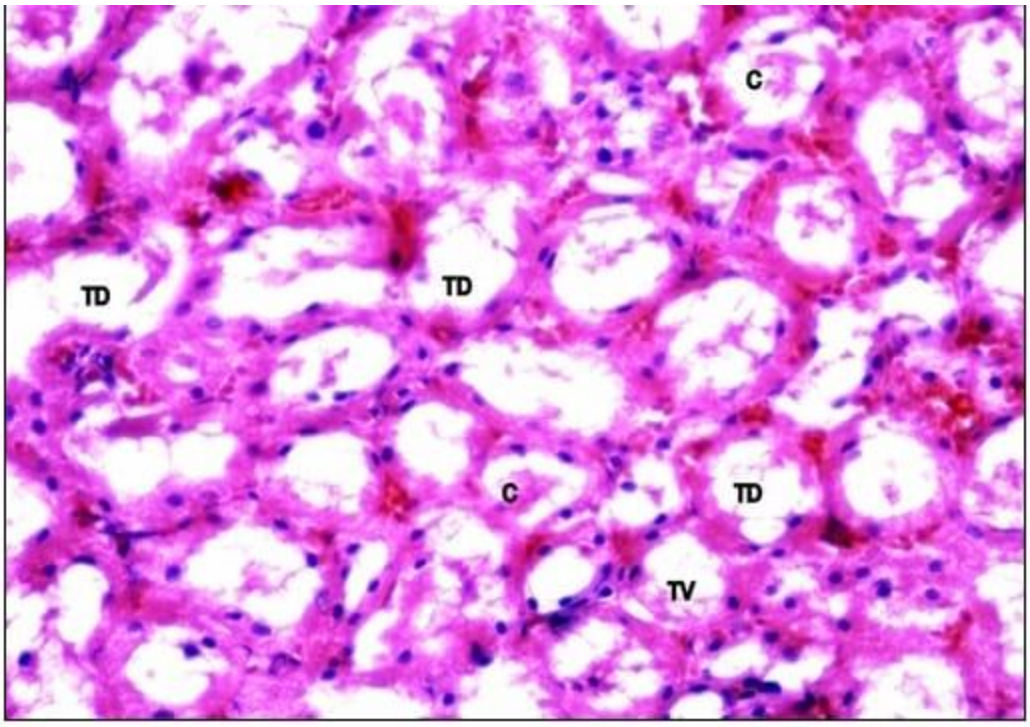

FIG. 48c PHOTOMICROGRAPH OF SECTION OF KIDNEY OF GROUP B OF 8 WEEK CHLORPYRIFOS (5 mg/kg b wt) TREATED RAT SHOWING SEVERE TUBULAR DEGENERATION (TD), EOSINOPHILIC CASTS WITHIN THE LUMEN OF THE TUBULES (C) AND TUBULAR VACUOLIZATION (TV). H&E. STAIN X 400

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DISCUSSION

Pesticides have been one of the most effective weapons discovered by man to protect agricultural products from the attack of Pests. Among these Chlorpyrifos is an extensively used organophosphate pesticide. Due to its wide-spread use it poses potential harm to nontarget organisms including humans. Chlorpyrifos exposure leads to extensive structural damage to the kidney. The unusual susceptibility of mammalian kidney to the toxic effects of noxious chemical can be attributed in part to the unique anatomic and physiologic features of this organ. Although the kidneys constitute only 0.5% of total body mass they receive about 20-25% of the resting cardiac output. Consequently any drug or chemical in the systemic circulation will be delivered to these organs in relatively high amounts. The process involved in forming concentrated urine also serves to concentrate potential toxicants into tubular cells. Therefore, a non-toxic concentration of chemical in the plasma may reach toxic concentration in the kidney. Progressive concentration of toxicants along the nephron may result in intraluminal precipitation of relatively insoluble compounds, causing acute renal failure secondary to tubular obstruction. Finally, renal transport, accumulation and metabolism of xenobiotics contribute significantly to the susceptibility of the kidney to toxic injury.

Several studies have supported the fact that major route of excretion of Chlorpyrifos is through kidneys in urine **(18).**

Macroscopic Changes

(a) Grossly, the kidneys in Chlorpyrifos treated rats in the present work were seen to be congested with increasing dose and duration of treatment. Also there was thickening and adherence of capsule at some places after 6th week 8 weeks after treatment with Chlorpyrifos the kidneys were small in size, shrunken and with irregular contour similar facts have been reported by (19) in glomerulosclerosis.
The present study is in agreement with(20) who observed congestion and patches of ecchymotic haemorrhages in kidney of layer chickens on gross examination when chickens were treated with single oral dose of 55 mg/kg body weight Chlorpyrifos. Bhandaniya et al. (2012)(21) also observed congestion in kidney and liver after exposing rats with Acephate (an organophosphorus pesticide) in higher doses for 28 days. However Corley et al. (1989)(22) observed no gross changes in the kidneys after treating rats with low doses of Chlorpyrifos.

(b) Coronal section of kidney:As the duration of treatment advanced variable degrees of corticomedullary haemorrhage was observed ranging from focal to complete obscuration of corticomedullary junction. In Group-C, after 8 weeks of treatment with Chlorpyrifos there was no demarcation between cortex and medulla and whole of the section was congested (giving dark red-brown appearance). Similar facts have been given by Kumar et al. (2012)(20) in cut section of kidney due to hypertension in which there were pin-point petechial haemorrhages on cortical surface giving it appearance of flea bitten kidney.

Microscopic Changes:-

The present study revealed that Chlorpyrifos treatment to rats caused histological alterations in the kidneys.

Tubular degeneration was noticed in foregoing study after 6th and 8th week of treatment with Chlorpyrifos in Group-B and Group-C. Tubular degeneration was in the form of tubular vacuolization, ragged appearance of epithelial lining, atrophy of epithelium and necrosis. Consistent results were documented by Srivastava et al. (1990)(23) in fresh water Cat fish after 96 hours of treatment with 2 mg/lt of Chlorpyrifos. Vacuolization due to hydropic degeneration was observed by Oncu et al (2002)(24) in rats exposed to 100 mg and 200 mg of intramuscular Chlorpyrifos for 6 days. Severe renal tubular desquamation and necrosis was reported by BenjaminN et al. (2005) (25) in diabetic and malnourished rats after 60 days of oral exposure of organophosphate pesticide in doses of 0.044 mg/day. The present study is in agreement with observations made by Eldeeb et al. (2007)(26) in broiler chicken, Mansour and Mossa (2010) (27) observed tubular degeneration in Chlorpyrifos exposed suckling rats through mothers milk and these mothers were exposed to Chlorpyrifos orally .Consistent results were observed by Tripathi & Srivastava (2010)(9) after 6th and 8th weeks of treatment with Chlorpyrifos in male rats in dose of 5 mg/kg and 10 mg/kg body

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weight. Kammon et al (2011)(20) observed tubular degeneration in broiler chicks exposed to Chlorpyrifos (0.8 mg/kg) after 24 days. Similarly Kumar et al. (2011)(19), Mansour and Mossa (2011)(28) in lactating rats, Ambali et al. (2011)(29) observed tubular degeneration.

Tubular vacuolization, necrosis and dilation due to atrophy of tubular epithelium noticed in kidney after exposure to toxicant might be a result of failure of ion pump transport of tubular cells. These alterations suggest incapability of renal cells to cope with functional disturbances provoked by toxicants.

In the rats Chlorpyrifos caused deposition of eosinophilic casts within the lumen of tubules which were few upto 4th week of treatment whereas after 6th and 8th week of treatment there were many eosinophilic casts within the lumen of tubules. The present study is in agreement with Tripathi and Srivastava (2010)(9) who observed eosinophilic casts within the lumen of tubules after 6th and 8th week of treatment with Chlorpyrifos in rats. Similar casts have been reported by Bhandaniya et al. (2012)(21) within lumen of renal tubules due to 1/20th LD_{50} of Acephate in rats in Group – II.

Further, in the present study degeneration of glomeruli as well as deposition of amorphous eosin positive substance has been noticed after 6th and 8th week in Group-B and C in Chlorpyrifos exposed rats. Degeneration of glomeruli has earlier been reported by Srivastava et al. (1990)(23) who have also documented glomerular degeneration in fresh water Cat fish treated with Chlorpyrifos (2 mg/lt) after 96 hrs. Degeneration of glomeruli has also been reported by Tripathi and Srivastava (2010)(9) after 6 and 8 weeks of treatment with Chlorpyrifos in male rats in doses of 5 mg/kg and 10 mg/kg. Similar findings were revealed by Ambali et al. (2011)(29) after Chlorpyrifos (21.3 mg/kg) exposure in mice; Bhandaniya et al. (2012) (21)

The presence of amorphous substances in glomerulus and tubules might be an indication of glomerulonephritis and/or incapability of renal tubules to counter the accumulated residues resulting from metabolic and structural alterations caused by Chlorpyrifos.

In the present study infiltration of chronic inflammatory cells i.e lymphocytes were observed in the interstitium of kidney of rats (6th week) ranging from few foci of infiltration with lymphocytes to many foci of lymphocytes in interstitium with increasing duration in both groups B and C. Consistent results were reported by earlier studies. (11), (25) and (9). This infiltration of lymphocytes was suggestive of interstitial nephritis and may be attributed to hypersensitivity after exposure to toxic drug.

Interstitial oedema observed after 6th week in Group-C and interstitial fibrosis in both Groups B and C after 8th week of treatment. The present study was in agreement with E1-Hossary et al. (2009)(30) who observed interstitial oedema after 15 days of treatment with Chlorpyrifos in rats. Periglomerular fibrosis in the cortex was observed by Mansour and Mossa (2010) (28)in rats exposed to 6.75 mg/kg for 28 days.

In the present study after 6 weeks of treatment with Chlorpyrifos (10 mg/kg body weight), changes in vessel wall were observed which were in the form of onion skin, concentric laminated thickening of tunica media of small renal vessels. This was typical of acute or severe elevation in blood pressure as described by Kumar (2012)(19) due to nephrosclerosis secondary to hypertension.

Generalized renal tissue hyperemia both in cortex and medulla was observed in the present study. Few vessels were dilated after 2nd week of treatment with 10 mg/kg body weight Chlorphyrifos (Group – C). Moderate renal congestion was observed after 4 weeks in both Group B & C rats. Massive dilatation of blood vessels was seen in rats of Group B & C after 6th and 8th week of treatment. This reactive hyperemia could be an attempt to get rid of toxicant. Similar observations were reported by Oncu et al. 2002.(25) Who treated rats with Chlorphyrifos in doses of 100 mg and 200 mg by intramuscular route for 6 days. Similarly EL-Hossary GG (2009)(30) observed congestion in the cortex of kidney in rats after Chlorpyrifos treatment. Renal congestion was also reported by Cao et al. (2006)(31) when he used intraperitoneal route of administration of Chlorpyrifos in doses of 2.5 mg/kg in rabbits. Kammon et al. (2010)(20) observed haemorrhages in kidney of Layer Chickens after treatment with Chlorpyrifos in doses of 55 mg/kg body weight orally. Kammon et al. (2010)(20) observed chronic

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oral administration of Chlorpyrifos in doses of 0.8 mg/kg body weight produced mild congestion & haemorrhage on day 24 and 45 in broiler chicken.

The present study showed variable intensities of changes, depending upon dose and duration of treatment and these changes were significantly different from those of control rats.

SUMMARY AND CONCLUSION

The present study is based upon the observations made on 45 albino Wistar rats weighing 145-165 gms to determine the effect of Chlorpyrifos on histomorphology of kidneys of these animals. The animals were group housed (12 hr light/dark cycle) with ad-libitum access to food and water. They were divided into 3 groups A, B and C with 15 animals each. Group A served as control group, Group B were daily administered Chlorpyrifos at a dose of 5 mg/kg b wt and animals in Group C received daily an oral dose of 10 mg/kg b wt Chlorpyrifos. Rats were sacrificed on 6th and 8th week after initiation of the experiment. Kidneys were removed after dissection, examined grossly and aiso after making coronal section. Then kidneys were cut into smaller pieces (5 mm size) and fixed in 10% formalin. These specimens were then subjected to standard histological proceedings by paraffin embedding method. Sections of 5-7 µ thickness were cut and stained with Haemotoxylin and Eosin stain and observations were made microscopically.

Macroscopic Changes

Grossly the kidneys of rats in control group were reddish brown in colour, soft, smooth in texture, the capsule was thin and was easily stripped off.

The kidneys of rats in Group B showed congestion, thickening and adherence of capsule at some places .With increasing dose and duration of treatment with Chlorpyrifos i.e after 8th week the kidneys were small in size, shrunken and had irregular contour.

In Group C the kidneys were grossly congested and friable to touch and the capsule was thickened. After 8 weeks of exposure the kidneys were small in size, shrunken and had irregular contour with thickened outer capsule.

Naked eye observations of coronal section of kidney

Group A (control) showed clear demarcation of outer cortex and inner medulla.

Group B (5 mg/kg b wt Chlorpyrifos) showed visible haemorrhage at corticomedullary junction ranging from focal to completely obscuring of corticomedullary junction. After 8th week there was no demarcation between cortex and medulla and whole of medulla was congested.

Group C (10 mg/kg b wt) After 6 weeks of treatment whole of the medulla was congested and after 8weeks there was no demarcation between cortex and medulla, whole of the coronal section of the kidneys showed dark reddish brown colour due to haemorrhage.

Microscopic changes

Group B –After 6 weeks of Chlorpyrifos exposure there was degeneration of glomeruli at some places and at some locations there was hypercellularity in the glomeruli. Renal tubules were dilated due to atrophy of epithelial lining. Degenerating tubules showed tubular vacuolization and ragged appearance. These were suggestive of acute tubular necrosis. There were eosin positive casts in lumen of the tubules. There was increased vascularity and dilated blood vessels. Foci of lymphocytic infiltration were seen in the interstium. Following 8 weeks of chlorphyrifos exposure all these histomorphological changes were increased in severity and also a band of fibrosis was seen in the interstium.

Group C –There were few foci of lymphocytic infiltration and vascular congestion. 6 week after Chlorpyrifos treatment revealed degeneration in some of glomeruli and a few showed hypercellularity. There were eosin positive casts within the lumen of many tubules. There were features of acute tubular necrosis in the form of vacuolization in the epithelium, ragged appearance of tubules and necrotic nuclei in the lumen of tubules. There were features of tubulointerstitial nephritis in the form of degeneration of tubules and infiltration of lymphocytes in the interstitium. Renal vessels showed concentric thickening of vessel wall. Following 8 weeks of treatment with Chlorpyrifos, outer capsule was seen to be eroded, there were many degenerating glomeruli with clumps of amorphous material within the Bowman's capsule. Several degenerating tubules were seen and their lumina were

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filled with eosinophilic casts. There was increased vascularity in the form of dilated blood vessels and interstitial oedema was seen. There was a band of fibrosis within interstitium.

The present study showed that significant histomorphological changes were caused in the kidneys of rats administered with Chlorpyrifos. These changes were markedly different from the control rats. There was shrinkage of glomerulus at initial stages of treatment, tubular dilation, glomerular hypercellularity, hypertrophy of tubular epithelium, degeneration of renal tubules, deposition of eosin positive substance in the glomerulus and renal tubules. There were infiltration of lymphocytes in the interstitium and increased vascularity in the form of dilated vessels fibrosis and interstitial oedema.

All these changes were suggestive of glomerulonephritis, acute tubular necrosis and interstitial nephritis leading to acute renal failure progressing to Chronic renal failure with increasing duration. Hence this study brought into light the renal toxicity induced by Chlorpyrifos which was found to be significant at high dose level.

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