# **Research Article**

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# Histopathological effects of pesticide-cholopyrifos on kidney in albino rats

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#### **ABSTRACT**

**Background:** 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 us to undertake this study.

**Methods:** The present study was conducted on 45 inbred adult Wistar albino rats of either sex, weighing 145 – 165 gms. These animals were randomly divided into 3 groups A, B, C. 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. 3 animals from each group were sacrificed after 1 week, 2<sup>nd</sup> week, 4<sup>th</sup> week, 6<sup>th</sup> week and 8<sup>th</sup> week of initiation of experiment to see the histological changes in the kidney architecture.

**Results:** Group A shows no histological alterations. Group B-No histological alterations in the kidney after 1 week. From 2 weeks-8 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. In Group C- the Kidneys of 1 week Chlorpyrifos treated rats exhibited shrunken glomeruli and hypertrophy of renal tubular epithelium. From  $2^{nd}$  week, the changes seen were more pronounced than Group B

**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. Hence this study brought into light the renal toxicity induced by chlorpyrifos which was found to be significant at high dose level.

Keywords: Pesticide, Kidney, Vacuolization, Fibrosis, interstitial edema

#### **INTRODUCTION**

Environmental Pollution, when considered in its broadest context, is a by-product of human activities and its 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 (Cope et al., 2004).<sup>1</sup>

Food and agricultural organization (FAO) has defined the term pesticide as: "Any substance or mixture of substances intended for preventing, destroying or

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controlling any pest including vectors of humans or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with production, processing storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feed stuffs or substances which may be administered to animals for control of insects, arachnids or other pests in or their bodies (Food and Agricultural organization of United Nations, 2002).<sup>2</sup>

Pesticides have played vital role in controlling agricultural, industrial, home and public health pest worldwide (Bjorling-Poulsen et al., 2008). 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 (Yang and Deng, 2007). Despite this alarming figure, there is currently no global system to track and stem poisoning or diseases associated with pesticide use. 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 Gbaruko et al., 2009.<sup>5</sup>

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. 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.

(Abdel and Saleh, 1999)<sup>6</sup>

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 (Tripathiand Srivastava, 2010).<sup>7</sup>

Chlorpyrifos [0, 0-diethyl-o(3, 5, 6-trichloro-2-pyridil) phosphor-dhioate] is a member of organophosphate class of pesticides that elicits broad spectrum insecticidal activity against a number of important arthropod pests (Racke, 1993). Chlorpyrifos kills insects upon contact by affecting normal function of nervous system.

Chlorpyrifos is a non-systemic insecticide designed to be effective by direct contact, ingestion and inhalation. Poisoning occurs as a result of agricultural use, accidental exposure, suicide and rarely homicide (Yurumez et al., 2007).

Chlorpyrifos is firstly activated to its active metabolite Chlorpyrifos – oxon by oxidative desulfuration which in turn is responsible for mammalian toxicity through inhibition of cholinesterase (Timchalk et al., 2002; Betancourt and Carr, 2004; Tongbai and Damrongphol, 2011). 10-12 Once Cholinesterase has been inactivated, acetylcholine accumulates throughout the nervous system (Latuszynska et al., 1999)<sup>13</sup> Toxicity of pesticide cause adverse effect on many organs like Kidney, Liver, Brain and Blood cell (Bebe and Panemanogalore, 2003).<sup>14</sup> Chlorpyrifos is readily absorbed from Gastrointestinal tract. In single dose oral study conducted on Human volunteers Chlorpyrifos was 70% absorbed from gastrointestinal tract (Nolan et al., 1984)<sup>15</sup> and in rats absorption of Chlorpyrifos through Gastrointestinal tract after single dose gavage study ranged from 84-90%. After entering the body of organism Chlorpyrifos is eliminated primarily through kidneys in urine. In rats, following oral intake of Chlorpyrifos, about 90% is removed in urine and 10% is excreted in faeces. Barr etal. in 2005<sup>16</sup> reported detectable 3,5,6 trichloropyridil (TCP) a metabolite of Chlorpyrifos in urine of 90% of the approximately 2000 samples collected from US residents (aged 2-59 yrs). The elimination half life for this metabolite (TCP) in humans following oral or dermal exposure was approximately 27 hrs (Nolan et al., 1984). 15

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 (Yurumez et al., 2007).

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.

The histological structure of the kidney of human beings and that of rats is very similar. So in the present study Albino rat were taken as experimental animal to study the effect of Chlorpyrifos in kidney. Also the unilobar kidney of the rat resembles each lobe of multilobar kidney of human beings. And unit of gross structure of kidney is 'lobe'. Moreover general microscopic structure of Nephron and its disposition within tubules in similar in both humans and rats (Ham and Cormach, 1979).<sup>17</sup>

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 us 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.

#### **METHODS**

In the present study, Albino rats served as experimental animals.

#### Collection of 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).

# Grouping of Animals

After two weeks of acclimatization, the rats were randomly divided into following groups. Identification number was given to rats of each group.

Groups	Number of Animals	Identification Number
Group A:	Control – 15 Animals	$A_1 - A_{15}$
Group B:	Experimental (15 Animals) Group I	$B_1 - B_{15}$
Group C:	Experimental (15 Animals) Group II	$C_1 - C_{15}$

The animals were group housed (12 hours light / dark cycle) in labeled cages in a room where temperature was maintained at  $25^{\circ} \pm 2^{\circ}$ C. The cages were made of solid

plastic sides and base and stainless steel grid top. Rice husk was used as bedding material. The animals were fed with standard laboratory feed and water ad-libitum throughout the experimental period. The animals were observed for abnormal physical or behavioral change throughout the experimental period. The study was done from December 2011 to February 2012.

#### Pesticide Details

Chemical used for the study was Chlorpyrifos, an Organophosphorus pesticide. It was purchased from the market with Molecular formula  $C_9 H_{11} Cl_3 - NO_3 PS$ 

# **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	Date of Adm. of 1 <sup>st</sup> Dose	Route of Adm.	Duration	Dose
$B_1 - B_3$	7/12/2011	Oral	1 Week	5mg/kg
$B_4 - B_6$	7/12/2011	Oral	2 Weeks	5mg/kg
$B_7 - B_9$	7/12/2011	Oral	4 Weeks	5mg/kg
$B_{10} - B_{12}$	7/12/2011	Oral	6 Weeks	5mg/kg
$B_{13} - B_{15}$	7/12/2011	Oral	8 Weeks	5mg/kg

# Group C

Experimental group	Date of Adm. of 1 <sup>st</sup> Dose	Route of Adm.	Duration	Dose
$C_1 - C_3$	7/12/2011	Oral	1 Week	10mg/kg
$C_4 - C_6$	7/12/2011	Oral	2 Weeks	10mg/kg
$C_7 - C_9$	7/12/2011	Oral	4 Weeks	10mg/kg
$C_{10} - C_{12}$	7/12/2011	Oral	6 Weeks	10mg/kg
$C_{13} - C_{15}$	7/12/2011	Oral	8 Weeks	10mg/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. A piece of cotton soaked in ether was placed in desiccators' jar and then the animals to be sacrificed were placed in

the jar and lid was closed. 3rats from each of 3 groups were sacrificed after last dose at the end of 1<sup>st</sup> week (14<sup>th</sup> of December 2011), 2<sup>nd</sup> week (21<sup>ST</sup> of December 2011), 4<sup>th</sup> week (4<sup>th</sup> of January 2012), 6<sup>th</sup> week (18<sup>th</sup> of January 2012) and 8<sup>th</sup> week (1<sup>st</sup> of February 2012) from the date of initiation of experiment. Sacrificing of animals was followed by dissection a mid-line incision through the skin extending from xiphisternum to pubic symphysis was given with a knife and was extended laterally at its lower end to achieve maximum exposure of abdominal cavity and the kidneys were exposed by displacing intestines. Later each kidney was removed in total and then washed with distilled water. After dissection and removal of kidney 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). A piece of paper carrying the serial number of specimen was kept along with the specimen.

#### **Fixation**

Kidney tissue was kept inside the tissue capsule and immediately fixed in 10% formation solution containing (Formalin - 100 ml, Sodium chloride - 8.5 grams & Distilled water - 900 ml) (Drury and Wallington, 1967). <sup>18</sup> The dissected specimen was kept in fixative for a period of 48 -72 hours.

# Preparation of Tissue for Section Cutting

This done by paraffin wax embedding method. Various steps used are:

- 1) Dehydration: After fixation the specimen was washed thoroughly with water and passed through ascending grades of ethyl alcohol. The specimen was given two changes of absolute alcohol for 2 -4 hours
- 2) *Clearing:* After dehydration, it is necessary to clear the specimen and to remove alcohol. This was done by adding xylene. Two changes were given in xylene
- 3) Paraffin wax impregnation: The tissue was impregnated with molten wax contained in electrically heated paraffin wax embedding water bath. The temperature is kept at 58°C (melting point of paraffin, 58°C 60°C) 2 or 3 changes after 2-4 hours were given.
- 4) Casting (Block making): This was done by taking Lockhart's L-shaped moulds of required sizes.
- 5) Sectioning: After trimming the paraffin wax block, sections was cut with rotary microtome. The sections of  $5-7 \mu$  thickness were cut.

- 6) Fixation of sections on the slides: The slides were smeared with a drop of Mayer's egg albumin. Slides smeared with the solution were dried and then dropped obliquely in water bath of lukewarm water so as to lift the section on the slides.
- 7) Staining: Staining was done by Harris Haemotoxylin and Eosin stain

# Steps of Staining the Section

- The slides were placed on hot plate with surface bearing tissue upwards for melting the wax.
- The slides were treated with xylene for 3-5 minutes for removal of paraffin wax. Two changes were given in xylene.
- Xylene is immiscible in water and weak alcohol. So xylene was removed by treating the slides with absolute alcohol for ½ 1 minute.
- The slides were treated with descending grades of alcohol of 90%, 70%, 50% respectively for 1-2 minutes in each for gradual hydration to avoid shrinkage.
- The slides were left under running tap water for few minutes.
- The slides were treated with Haemotoxylin solution for 5-7 minutes.
- The slides were rewashed under running tap water till colour changed to blue.
- The slides were treated with 1% acid alcohol solution (1% HCl in 70% alcohol) for differentiation. It was kept for about ½ to 1 minute and the colour changes from blue to red by the action of acid.
- Again slides were dipped in water and colour changed to blue.
- The slides were treated with 1% aqueous-eosin for 1-2 minutes.
- Excess of stain was washed with water.
- Dehydration was done by passing slides through ascending grades of alcohol for ½ to 1 minute in each.
- The slides were cleared with two changes in xylene.
- The slides were mounted with DPX (Distrene Plasticizer Xylene) solution.
- The slides were covered with cover slips.
- The slides were left overnight to dry completely so that coverslips were adherent to the slides.

# **RESULTS**

Group A (Control)

### Microscopic Observations

No histological changes observed

Group B (5 mg/kg body weight Chlorpyrifos)

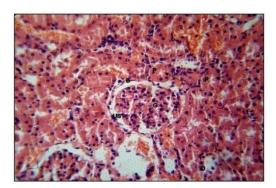


Figure 1: Photomicrograph of section of Kidney of group a (control) rat showing normal structure of renal glomeruli (G), bowman's capsule lined by squamous epithelium, distinct urinary space (us), the proximal tubule (P) are lined with cubiodal epithelium with brush border (arrows) and distal convoluted tubules (D) are lined with low cuboidal epithelium. H & E stain x 400.



Figure.2: Photomicrograph of section of kidney of group B of 1 week chlorpyrifos (5 mg/kg b wt) treated rat showing normal glomeruli (G), bowman's capsule (B) and distinct urinary space (US), proximal convoluted tubule (P) lined by cuboidal epithelium and distal convoluted tubule (D) lined by low cuboidal epithelium. H&E stain x 400.

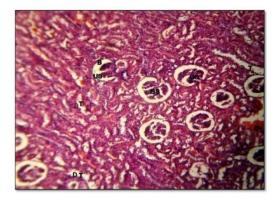


Figure.3: Photomicrograph of section of kidney group B of 2 week chlorpyrifos (5 mg/k b wt) treated rat showing shrunken glomerulus (GS), bowman's capsule (B) and increased urinary space (US), dilated blood vessel (BV), dilated tubule (DT). H&E stain x 100.

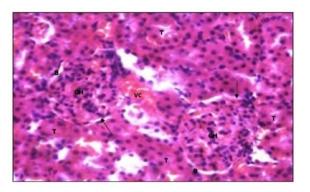


Figure 4: Photomicrograph of section of kidney of group B of 4 week chlorpyrifos (5 mg/kg b wt) treated rat showing increased cellularity in glomerulus (GH), obliterations of urinary space (arrows) between bowman's capsule (B) and glomerulus. Hypertrophy of tubular epithelium (T) almost obliterating the lumen and vascular congestion (VC). H&E stain x 400.

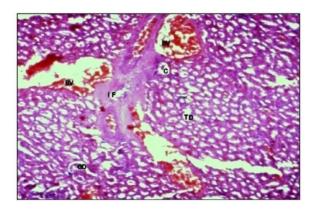


Figure 5: Photomicrograph of section of kidney of group B of 8 week chlorpyrifos (5 mg/kg b wt) treated rat showing, degenerating glomerulus (GD), degenerating tubules (TD), eosinophilic casts within the lumen of the tubules (C), dilated blood vessels (BV) and fibrosis (IF) in the interstitium. H&E stain x 100.

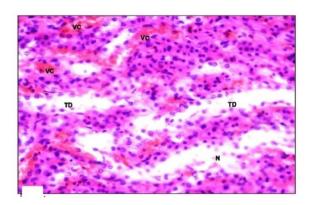


Figure 6: Photomicrograph of section of kidney of group c of 4 week chlorpyrifos (10 mg/kg b wt) treated rat showing degenerated tubules (TD), necrotic nuclei within the lumen (N) and vascular congestion (VC).

H&E stain x 400.

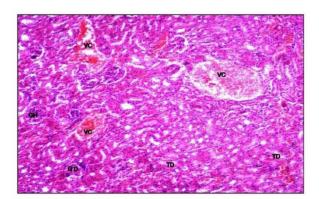


Figure 7: Photomicrograph of section of kidney of group c of 6 week chlorpyrifos (10 mg/kg b wt) treated rat showing hypercellularity in some glomeruli (GH) and some are showing degeneration (GD), tubular degeneration (TD) and vascular congestion (VC).

H&E stain x 100.

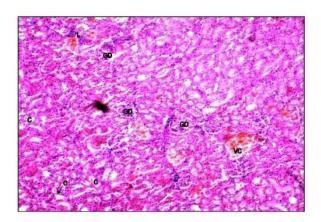


Figure 8: Photomicrograph of section of kidney of group c of 6 week chlorpyrifos (10 mg/kg b wt) treated rat showing degenerating glomeruli (G), eosinophilic casts within the tubules (c), vascular congestion (vc) and foci of lymphocytic infiltration (L). H&E stain x 100.

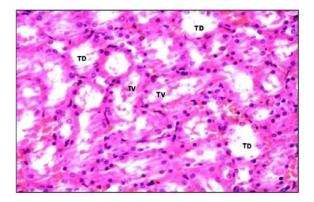


Figure 9: Photomicrograph of section of kidney of group c of 6 week chlorpyrifos (10 mg/kg b wt) treated rat showing degenerated tubules (TD) and vacuolization with the tubules (TV). H&E. stain x 400.

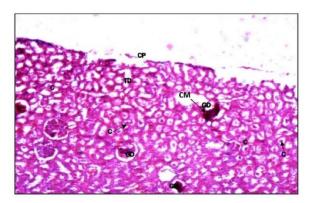


Figure 10: Photomicrograph of section of kidney of group c of 8 week chlorpyrifos (10mg/kg b wt) treated rat showing eroded capsule (CP) degenerated glomerulus (GD) seen as clumps of amorphous material (CM), many eosinophilic cast within the tubules (C) and degenerated tubules (TD). H&E stain x 100.

#### Microscopic Observations

Histological examination of Group B kidney (5 mg/kg/day Chlorpyrifos exposed) after one week of treatment showed insignificant changes. Following 2 week exposure, complete renal capsule was visualized. There were few shrunken glomeruli, thus more spaces appeared between bowman's capsule and glomeruli. The renal tubules showed dilatation and proximal convoluted tubule showed wide lumen with loss of brush border. There was mild hyperemia and few dilated renal vessels were also seen.

Chlorpyrifos treatment for 4 weeks caused increased Cellularity in glomeruli tightly fitting the Bowman's capsule, thus there was no space between the glomerulus and Bowman's capsule which was suggestive of proliferative glomerulonephritis and may be because of proliferation of endothelial cells, mesangial cells or infiltration with inflammatory cells. The proximal and distal tubules exhibited hypertrophy of epithelial cells (cloudy swelling) and obliteration of lumen. A focus of lymphocytic infiltration was seen in the interstitium. There was more vascular congestion after 4 weeks of treatment with Chlorpyrifos.

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 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)

#### Microscopic Observations

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

The kidney of 1 week Chlorpyrifos treated rats exhibited shrunken glomeruli and there was wide urinary space between glomerulus and bowman's capsule and congested tubules due to the hypertrophy of tubular epithelium and obliteration of lumen. Medulla of the kidney in the group C showed dilated blood vessels.

Following 2 week Chlorpyrifos treatment the cellularity of glomeruli was increased at several places leaving almost no space between the Bowman's capsule and glomerulus. The tubules were dilated. A few tubules displayed separation of epithelial cells from underlying basement membrane.

After 4 week Chlorpyrifos treatment, large numbers of glomeruli were seen to have hypercellularity and congestion, thus filling the Bowman's capsule and obliterating the urinary space. Tubules exhibited severe degeneration; there were necrotic nuclei in the lumina of the tubules. There were few foci of lymphocytic infiltration in the interstitium. Lymphocytic infiltration was considered as sign of toxicity and consequent action of defensive mechanism. At places vascular congestion was seen.

Following 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 undistinguished. 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. Interstitial 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.

#### 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 (Nolan R. J et al., 1984, Griffin et al., 1999). 15,19

#### Microscopic Changes

The present study revealed that Chlorpyrifos treatment to rats caused histological alterations in the kidneys. The changes observed at initial stage of treatment were shrinkage of cortical glomeruli with increased urinary spaces between Bowman's capsule and glomeruli after 1<sup>st</sup> week of treatment at higher dose (10 mg/kg) in Group-C rats where as these changes appeared a week later that is after 2<sup>nd</sup> week in rats treated with low doses (5 mg/kg) in Group-B. Shrinkage of glomeruli with wide urinary spaces derives its support from the findings of Srivastava et al. (1990), Eldeeb at al. (2007), El-Hossary et al., Tripathi and Srivastava (2010), Issa et al. (2011), Galakatu et al. (2012),

Xing et al. (2012).<sup>7,20-25</sup> Shrinkage of Glomeruli was probably due to renal vasoconstriction in response to nephrotoxicant (Schellman, 1995).<sup>26</sup>

In the present study there was dilatation of both proximal and distal convoluted tubules. The proximal convoluted tubules also showed loss of brush border after 2<sup>nd</sup> week of treatment with Chlorpyrifos doses of 5 mg/kg (Group B) body weight. Whereas there was even separation of tubular epithelium from basement membrane in some fields along with dilations of tubules after 2 weeks in Group-C (10 mg/kg). These findings are in agreement with the observations made by Srivastava et al. (1990), Tripathi and Srivastava (2010), Kumar (2012). 7,20,27

These changes may be in response to nephrotoxic drug as proximal convoluted tubules are the most common site of toxicant induced renal injury. The reason for this relate in part to the selective accumulation of xenobiotics into this segment of nephron. Maintenance of tubular integrity depends on cell to cell and cell to matrix adhesions, after a chemical insult adhesion of nonlethally damaged cells to basement membrane is compromised, leading to their detachment from basement membrane and later on it may lead to sloughing of cells and formation of intratubular casts. Loss of Brush border in Proximal convoluted tubules can result from toxicant induced alterations in membrane integrity, Cytoskeleton component and ATP depletion (Schellmann, 1995).<sup>26</sup>

The foregoing study showed glomerular hypercellularity and obliteration of urinary spaces after 4 weeks of treatment with Chlorpyrifos in doses of 5mg/kg and after 2<sup>nd</sup> and 4<sup>th</sup> weeks of treatment with Chlorpyrifos in doses of 10 mg/kg body weight. This is in agreement with reports made by Oncu et al. (2002) Triparthi and Srivastava (2010), Mansour and Mossa (2011), Kammon et al. (2011).<sup>7,28-30</sup> Hypercellularity in glomeruli was suggestive of proliferative glomerulornephritis which may be because of proliferation of endothelial cells, mesangial cells or infiltration with inflammatory cells (Kumar 2012).<sup>27</sup>

Tubular epithelial swelling in the present study was observed after 4<sup>th</sup> week of treatment with Chlorpyrifos in Group B & C. There was increase in tubular height thus obliterating the lumina of the tubules. This is in agreement with reports made by Mansour and Mossa (2010a), Tripathi and Srivastav (2010), Kammon et al. (2011), Issa et al. (2011), Heikel et al. (2012), Pal et al. (2012).<sup>7,23,29-32</sup> Tubular epithelial swelling may be attributed to disruption of cell volume and ion homeostasis by toxicants, thus increasing permeability and inhibiting energy production, resulting into ATP depletion. Following ATP depletion Na<sup>+</sup>, K<sup>+</sup>, ATP activity decreases resulting in K<sup>+</sup> efflux, Na and Ca<sup>-</sup> influx, cell swelling and ultimately cell membrane rupture. This influx may be a trigger for cell swelling and cell death (Schellmann, 1995).<sup>2</sup>

Tubular degeneration was noticed in foregoing study after 6<sup>th</sup> and 8<sup>th</sup> 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), Oncu et al (2002), Benjamin N et al. (2005), Eldeeb et al. (2007), Kammon AM (2010), Tripathi & Srivastava (2010), Kumar et al. (2011), Mansour and Mossa (2011) Ambali et al. (2011a), Pal et al. (2012), Bhandaniya et al. (2012) and Galakatu et al. (2012).

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 4<sup>th</sup> week of treatment whereas after 6<sup>th</sup> and 8<sup>th</sup> week of treatment there were many eosinophilic casts within the lumen of tubules. The present study is in agreement with Tripathi and Srivastava (2010), Bhandaniya et al. (2012).<sup>7,26</sup> Further, in the present study degeneration of glomeruli as well as deposition of amorphous eosin positive substance has been noticed after 6<sup>th</sup> and 8<sup>th</sup> week in Group-B and C in Chlorpyrifos exposed rats. Degeneration of glomeruli has earlier been reported by Srivastava et al. (1990), Tripathi and Srivastava (2010), Ambali et al. (2011a), Bhandaniya et al. (2012) and Pal et al. (2012).<sup>7,20,32-34</sup>

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 (6<sup>th</sup> 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 Latuszynska et al. (1999), Delibas (2002), Oncu et al. (2002) and Tripathi and Srivastava (2010).<sup>7,13,28,35</sup> This infiltration of lymphocytes was suggestive of interstitial nephritis and may be attributed to hypersensitivity after exposure to toxic drug.

Interstitial oedema observed after 6<sup>th</sup> week in Group-C and interstitial fibrosis in both Groups B and C after 8<sup>th</sup> week of treatment. The present study was in agreement with E1-Hossary et al. (2009).<sup>22</sup>

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)<sup>27</sup> 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 2<sup>nd</sup> week of treatment with 10 mg/kg body weight Chlorpyrifos (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 6<sup>th</sup> and 8<sup>th</sup>week of treatment. This reactive hyperemia could be an attempt to get rid of toxicant. Similar observations were reported by Oncu et al. 2002., EL-Hossary GG (2009), Cao et al. (2006), Kammon et al. (2010).

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.

#### **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 body weight Chlorpyrifos. 3 animals from each group were weighed at 2 weekly interval for 8 weeks and weight gain was evaluated in each group. Rats were sacrificed on 1st, 2nd, 4th, 6th and 8th week after initiation of the experiment. Kidneys were removed after dissection. 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 u thickness were cut and stained with Haematoxylin and Eosin stain and observations were made microscopically.

#### Microscopic changes

Group A: No histopathological changes seen

Group B: 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

Group C: Histological changes seen were more pronounced than Group B.

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.

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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