NEPHROPROTECTIVE EFFECT OF *Cuminum cymenum* ON CHLOROPYRIFOS INDUCED KIDNEY OF MICE

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**ABSTRACT**

Environmental pollution from pesticides is an important issue that attracts wide spread public concern. Among these organophosphate pesticides are routinely used in agriculture. Chloropyrifos toxicity has been largely associated with irreversible inhibition of acetyl-cholinesterase resulting in accumulation of acetylcholine in the cholinergic receptors. Organochlorine insecticides, caused degeneration in mouse kidney due to oxidative stress. *Cuminum cymenum* has shown significant antioxidant activity in several test methods. Cumin seeds have been reportedly used for traditional treatment of toothache, dyspepsia, diarrhea, epilepsy and jaundice. Thus the present study is designed to evaluate nephroprotective potential of *Cuminum cymenum* on chloropyrifos induced kidney of mice. Chlorpyrifos administration at 6 mg/kg b.wt for 4 weeks was followed by the administration of aqueous seed extract of *Cuminum cymenum* for 8 weeks at 75 mg/kg b.wt. Urea, uric acid and creatinine were increased evidently after chloropyrifos exposure. Degeneration was observed in glomerulus and bowmen’s capsule. PCT and DCT were also degenerated to greater extent in chloropyrifos administered group of mice. Effective restoration was observed in urea, uric acid and creatinine in *Cuminum cymenum* administered group of mice. Glomerulus, Proximal convoluted tubule, distal convoluted tubules and bowmens capsule was also restored effectively in *Cuminum cymenum* administered group of mice. It is evident from study that *Cuminum cymenum* has effective nephroprotective potential against chloropyrifos toxicity on kidney of mice.

**Key Words:** Acetylcholine; Cumin; Glomerulus; PCT; DCT

**INTRODUCTION**

Environmental pollution from pesticides is an important issue that attracts wide spread public concern. Among these, some organophosphate & organochlorine pesticides are routinely used in agriculture.

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Chlorpyrifos, O, O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothionate, is a broad-spectrum organophosphate insecticides used widely for the control of agricultural & domestic insect pests control. More importantly at low doses the chlorpyrifos oxon, a bioactivated metabolite of chlorpyrifos disrupts microtubule polymerization by forming adducts with specific tyrosine residues in tubulin.
However other putative mechanism have implicated in molecular mechanism of chlopyrifos toxicity. Among these, the induction of oxidative stress has received tremendous attention. Chlorpyrifos toxicity has been largely associated with irreversible inhibition of acetyl-cholinesterase (AChE) resulting in accumulation of acetylcholine in the cholinergic receptors. Organochlorine insecticides, caused degeneration in mouse kidney due to oxidative stress.

*Cuminum cyminum* is an aromatic plant and its fruit, known as cumin seed and is most widely used spices and medicinal plants in the world. The antibacterial activities of spices and essential oils have been known for a long time, and a number of researches on the antibacterial effect of spices, essential oils and their derivative have been reported.

*Cuminum cyminum* has shown significant antioxidant activity in several test methods. These effects are documented as their ability to prominently quench hydroxyl radicals, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and lipid peroxides. The other assays employed were ferric thiocyanate method in linoleic acid system, Fe2+ascorbate-induced rat liver microsomal lipid peroxidation (LPO), soybean lipoxygenase dependent lipid peroxidation and ferric reducing ability.

Thus the present study is designed to evaluate nephroprotective potential of *Cuminum cyminum* on chlopyrifos induced kidney of mice.

**MATERIALS AND METHODS**

**Pesticide**

Chlorpyrifos (Tₙ-Dursban) were used at an effective concentration, EC = 20% (w/v).

**Herbal Plant**

Seed of *Cuminum cyminum* was selected as a plant material for the study.

**Experimental model**

Swiss albino mice (*Mus musculus*) weighing 30±5gm were selected as an experimental model in the present study. The animals were housed at controlled environmental conditions 22±2°C, relative humidity 50±10%, and 12h dark-light cycle. All experimental procedures were conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

**METHODOLOGY**

**Chronic Toxicity Studies**

Selected pathogen-free mice were sorted and chlopyrifos was administered at 6 mg/kg b.wt dose for 4 weeks by Gavage method. Sacrifice was done on 2nd week and 4th week of chlopyrifos administration in each group.

**Herbal Administration**

Chlorpyrifos administration at 6 mg/kg b.wt for 4 weeks was followed by the
administration of aqueous seed extract of *Cuminum cyminum* for 8 weeks at 75 mg/kg b.wt. Animals were sacrificed on 4th and 8th weeks of herbal treatment.

**Sub-cellular Studies**

Mice were sacrificed from each group for histological analysis. Kidney is dissected out and washed three times in isotonic saline (0.85 w/v %) then fixed in 10% neutral formalin solution. Tissue was processed. Slides were stained with Hema-toxylene-Eosin (H & E) and examined morphometrical under Light Microscope.

**Biochemical Assessment**

Blood was collected by orbital puncture and centrifuged to separate the serum to carry out further biochemical analysis. With the separated serum biochemical analysis was performed to establish the effects of chloropyrifos induced toxicity and the remedial effect of seed of *Cuminum cyminum* on urea, uric acid and creatinine level through standard kit process (Hi Media) by spectrophotometer.

**RESULTS**

Urea level in control group of mice was 13.67 ± 1.20 mg/dl. In chloropyrifos 2 weeks and 4 weeks administered group of mice urea level was 41.33 ± 1.45 mg/dl and 52.67 ± 1.20 mg/dl. It was 30.67 ± 2.40 mg/dl and 18.00 ± 3.78 mg/dl after 4 weeks and 8 weeks administration of *Cuminum cyminum*. (Graph: 1).

Uric acid level in control group of mice was 3.067 ± 0.12 mg/dl. In chloropyrifos 2 weeks and 4 weeks administered group of mice uric acid level was 5.917 ± 0.05 mg/dl and 7.410 ± 0.13 mg/dl. It was 5.627 ± 0.15 mg/dl and 3.933 ± 0.14 mg/dl after 4 weeks and 8 weeks administration of *Cuminum cyminum*. (Graph: 2).

Creatinine level in control group of mice was 0.8400 ± 0.08 mg/dl. In chloropyrifos 2 weeks and 4 weeks administered group of mice creatinine level was 1.950 ± 0.05 mg/dl and 2.290 ± 0.09 mg/dl. It was 1.567 ± 0.06 mg/dl and 1.117 ± 0.06 mg/dl after 4 weeks and 8 weeks administration of *Cuminum cyminum*. (Graph: 3).
weeks administration of *Cuminum cyminum*. (Graph: 3).

**Graph - 3: Creatinine Level in serum of Mice**

Kidney of control mice shows normal glomerulus and bowmens capsule. PCT and DCT are also normal in structure (Figure: 1).

Kidney of four weeks chloropyrifos administrated mice shows frequent vacuolization in cortex region. Degenerated glomerulus and dilated Bowmen`s capsules were also observed (Figure: 2). Vacuolated space was observed in glomerulus. Dilated PCT & DCT were also observed (Figure: 3).

Kidney of four weeks chloropyrifos administered mice followed by eight weeks *Cuminum cyminum* administration show restoration in bowmens capsule. Restoration was also observed in renal cortex region. Little vacuolization was observed (Figure: 4). Podocyte was almost normal like. Bowmen`s capsule was also normal in structure. Restoration was observed in both cytoplasm and nuclear material (Figure: 5).
glomerulus were also observed. Vacuolated space observed in glomerulus. Dilated PCT & DCT were also observed.

Figure 4: show kidney of four weeks chloropyrifos administered mice followed by eight weeks Cuminum cyminum administration with restoration in bowmens capsule. Restoration was also observed in renal cortex region. Little vacuolization was observed.

Figure 5: show kidney of four weeks chloropyrifos administered mice followed by eight weeks Cuminum cyminum administration with restoration in glomerulus. Podocyte was almost normal like. Bowmen’s capsule was also normal in structure. Restoration was observed in both cytoplasm and nuclear material.

DISCUSSION
Chloropyrifos is thought to be primarily metabolized in the liver by multiple, specific cytochrome P450 enzymes through several reaction pathways chloropyrifos elicits a number of additional effects, including hepatic dysfunction, haematological and immunological abnormalities, embryotoxicity, genotoxicity and neurobehavioral changes. The toxic effect of profenofos and chlorpyrifos on hepatic lesion leading to congestion and hemorrhages of spleen. Also lymphocytes occurred, which many be affected on the immunity. These findings were confirmed with results of Chaudhary. Inhibition of cholinesterase by organophosphoric pesticides or their metabolites plays a key role in toxicity. However, inhibition of other enzymes, such as neuropathy target esterase or other beta esterase’s and the direct effects of organophosphates on tissues are also important. In present study urea, uric acid and creatinine were increased evidently after chloropyrifos exposure. Chronic exposure to chloropyrifos can alter the structural and functional integrity of the kidney, induce oxidative stress, and cause nephrotoxicity, which may lead to renal failure. The glomerular tubules of the kidney were vacuolated due to edema, with excessive toxicity concentration and destruction of the glomerular tubules occurred which may be due degenerative changes. Degeneration of renal tubules resulted from collection of albuminous material lining.
during its excretion in the urine\textsuperscript{16,17}. Necrosis of tubular epithelium, cloudy swelling of epithelial cells of renal tubules, narrowing of the tubular lumen, contraction of the glomerulus and expansion of space inside the Bowman’s capsule were observed in the kidney tissues of fish after exposure\textsuperscript{18}. In present study degeneration were observed in glomerulus and bowmen’s capsule. PCT and DCT were also degenerated to greater extent in chloropyrifos administered group of mice. In \textit{Cuminum cyminum} treated rats, the levels of cholesterol, cholesterol/phospholipids ratio & 3methyl glutacyl COA-reductors activity were reduced\textsuperscript{19}. Another study demonstrated a relative potent relaxant effect of aqueous macerated extracts. Therefore, the bronchodilators effect of extraction of \textit{Cuminum cyminum} may be responsible for its antitussive property\textsuperscript{20}. Effective restoration was observed in urea, uric acid and creatinine in \textit{Cuminum cyminum} administered group of mice. Glomerulus, Proximal convoluted tubule, distal convoluted tubules and bowmens capsule was also restored effectively in \textit{Cuminum cyminum} administered group of mice. Restoration was more with increased duration of \textit{Cuminum cyminum} exposure.

**CONCLUSION**

Thus it is concluded from study that \textit{Cuminum cyminum} acts effectively against chloropyrifos induced toxicity on both biochemical and histological parameters of kidney of mice. It restores these parameters to normal level. It is evident from study that \textit{Cuminum cyminum} has effective nephroprotective potential against chloropyrifos toxicity.

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