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Phytoremedial Potential of *Azolla filiculoides* against Profenofos Induced Nephrotoxicity on Swiss Albino Mice

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ABSTRACT

In present study the organophosphate was administered orally at the dose of 15 mg kg^{-1} of body weight to Swiss albino mice for 45 days to observe the effect on kidney of mice. The mice were then administered orally with *Azolla filiculoides* at the dose of 10 mg kg^{-1} body weight per day for 30 days. The Kidney Function Test (KFT), lipid peroxidation and histopathology of kidney tissue were evaluated in respective treated groups. The study reveals that profenofos causes deleterious effect on biochemical parameters, as there was immense increase in the kidney function test levels. The histopathological observations also revealed high degree of degeneration in renal cells. But, after *Azolla filiculoides* administration for 30 days there was amelioration in the kidney function test levels as well as restoration in the nephrocytes reveals nephro-protective effect. The findings suggest that the *Azolla filiculoides* is the suitable antidote against arsenic induced toxicity and possesses nephroprotective activity.

Key words: Profenofos, nephrotoxicity, *Azolla filiculoides*, antidote, antioxidant

INTRODUCTION

Currently used pesticides, however, are broad-spectrum biocides that are poisonous not only to target arthropods but also to vertebrates and mammals. Profenofos [O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] is an organophosphorus insecticide and acricide extensively used for the control of insects in agriculture and house-hold purposes (Das *et al*., 2006). Profenofos is reported to be highly toxic to human, animals and aquatic organisms (Moustafa *et al*., 2008; Kavitha and Rao, 2009). Experimental histopathological studies revealed that exposure to profenofos, induced structural changes in liver and kidney cells of rats, confirming its potent hepatotoxicity and renotoxicity (Farrag and Shalby, 2007). The primary effect of profenofos in studies of acute toxicity, short and long-term studies of toxicity was inhibition of acetyl cholinesterase activity and this was linked with signs of neurotoxicity at high levels of inhibition. High dose of the profenofos induced tissue caused vacuolization, haemorrhage and hyperplasia of Kupffer cells in the liver. In addition, swelling in Bowman's capsules and tubular degeneration in the kidney were well reported (Amer *et al*., 2007). In the present scenario profenofos is widely used by the farmers for the better yield of crops in many states of country. But, this pesticide has caused lots of health related problems in farmers. So, antidote search against profenofos is the prime objective of the study.

Azolla filiculoides (Water fern) is a species of *Azolla*, with occurrences in South Africa (McConnachie *et al*., 2003, 2004) Asia (Kitoh *et al*., 1993), Australia, South, Central and North America (Svenson, 1944) and South, West, Central and North Europe (Hussner and Losch, 2005). *Azolla* has become progressively significant in waste water treatments to eliminate different metals, to remove nitrogenous compounds from the water and useful in the detoxification of industrial effluents (Shiny *et al*., 2004; Gardea-Torresdey *et al*., 2005; Stepniewska *et al*., 2005; Sela *et al*., 1989; Ahmady-Asbchin *et al*., 2012; Sood *et al*., 2012). The chronic effect of profenofos on mammals can be eliminated by giving suitable antidote to the affected one. Therefore, the present study deals to evaluate the antidote effect of *Azolla filiculoides* against profenofos induced nephrotoxicity.

MATERIALS AND METHODS

Animals: Thirty female Swiss albino mice (30±2 g) were obtained from animal house of Mahavir Cancer Institute and Research Centre, Patna, India (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008). The study was approved by the Institutional Animal Ethics Committee (IAEC) with No. IAEC/2010/08/05. Food and water to mice were provided *ad libitum* (prepared mixed formulated feed by the laboratory itself). Animals were maintained in colony rooms with 12 h light/dark cycle at 22±2°C.

Chemicals: Commercially available profenofos, [O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] (50% E.C, specific gravity 1.34, trade name: "Carina", PI Industries Ltd.) was purchased from the local pesticide market of Patna, Bihar, India.

Plant material: *Azolla filiculoides* is a heterosporous, upto 2.5 cm large floating fern with polygonal or triangular in shape (Lumpkin and Plucknett, 1980). It consists of two-lobed leaves and rhizomes. In the present study, fresh leaves of *A. filiculoides* were collected from the local pond of Patna, Bihar. The identity of the leaves of *A. filiculoides* was confirmed by Dr. Ramakant Pandey (Botanist), Department of Biochemistry, Patna University, Patna, Bihar, India. The collected leaves of *A. filiculoides* were shade dried and were grinded to fine powder. The powder was then soaked in 70% ethanol for 48 h and finally extracted with 5% absolute ethanol using soxhlet apparatus for 6-8 h and the residue was concentrated and dried at 37°C. The dose was finally made to 10 mg kg^{-1} body weight for oral administration.

Treatment protocol: The animals were assigned into three groups, control, profenofos treated and *A. filiculoides* treated. The profenofos treated group was administered profenofos at the dose of 15 mg kg^{-1} body weight orally daily for 45 days to observe the nephrotoxicity effect. Upon profenofos treated group, ethanolic extract of *A. filiculoides* at the dose of 10 mg kg^{-1} body weight was administered orally daily for 30 days to observe the antidote effect.

Biochemical analysis: After the entire treatment protocol the experimental animals were anaesthesized and sacrificed. Blood sample were collected by orbital sinus puncture method (Van Herck *et al*., 1998) and their serum were extracted. The Kidney Function Test (KFT), Urea,

Uric acid and Creatinine were performed from blood serum through their respective methods (Berthelot, 1859; Fawcett and Scott, 1960; Bonsnes and Taussky, 1945; Toro and Ackermann, 1975).

Lipid peroxidation (LPO): Thiobarbituric Acid Reactive Substances (TBARS), as a marker for LPO, were determined by the double heating method (Draper and Hadley, 1990). The theory of the method was a spectrophotometric measurement of the colour produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this intention, 2.5 mL of 100 g L^{-1} trichloroacetic acid (TCA) solution was added to 0.5 mL serum in a centrifuge tube and incubated for 15 min at 90°C. After cooling at Room Temperature (RT), the mixture was centrifuged at 3000 g for 10 min and 2 mL of the supernatant was added to 1 mL of 6.7 g L^{-1} TBA solution in a test tube and again incubated for 15 min at 90°C. The solution was then cooled at RT and its absorbance was measured using Thermo Scientific UV-10 (UV-Vis) spectrophotometer (USA) at 532 nm.

Statistical analysis: Results are presented as Mean±SD and total variation present in a set of data was analysed through one-way analysis of variance (ANOVA). Difference among means has been analysed by applying Dunnett's test at 99.9% ($p<0.05$) confidence level. Calculations were performed with the GraphPad Prism Program (GraphPad Software, Inc., San Diego, USA).

RESULTS

Morbidity and mortality: The mice after profenofos exposure (15 mg kg^{-1} body weight per day) for 45 days have shown signs of toxicity such as sluggishness in the animal especially drowsiness, nausea and giddiness. Lack of co-ordination was the prominent observation. Although, no mortality was observed during exposure of profenofos.

Biochemical changes: The biochemical assessment shows the nephroprotective activity of *A. filiculoides*. In comparison to control mice group serum urea, uric acid, creatinine and lipid peroxidation activity were significant increase ($p<0.05$) in profenofos treated group. But, these are significantly lowered ($p<0.05$) in *A. filiculoides* treated group (Fig. 1-4).

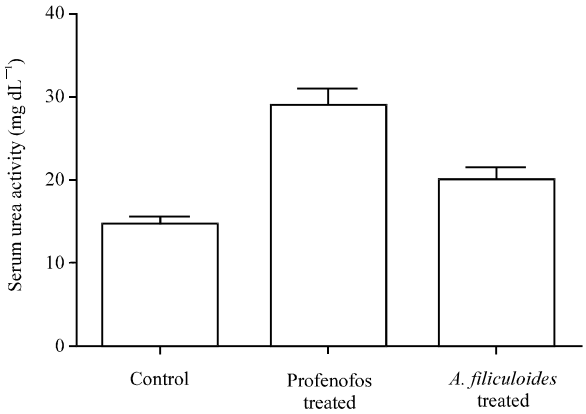

Fig. 1: Effect of *Azolla filiculoides* on profenofos induced toxicity showing serum urea activity (n = 6, values are Mean±SD)

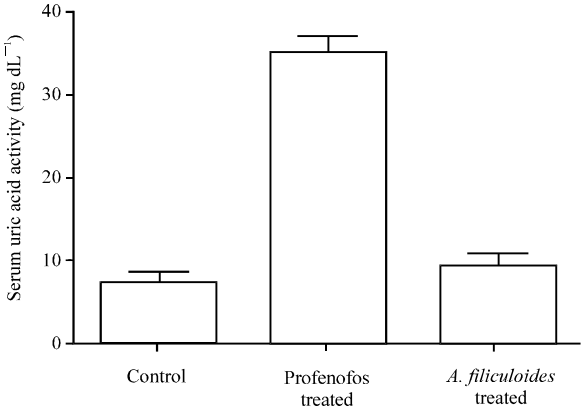

Fig. 2: Effect of *Azolla filiculoides* on profenofos induced toxicity showing serum uric acid activity (n = 6, values are Mean±SD)

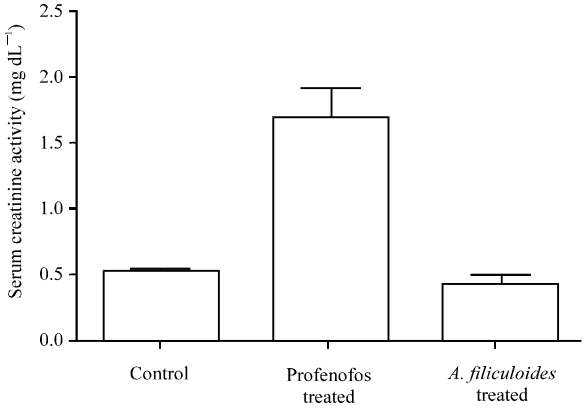

Fig. 3: Effect of *Azolla filiculoides* on profenofos induced toxicity showing serum creatinine activity (n = 6, values are Mean±SD)

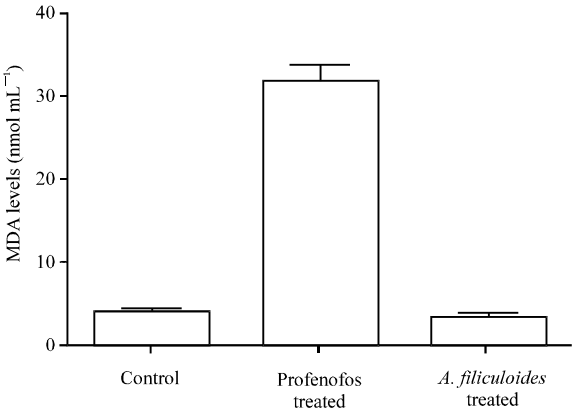

Fig. 4: Effect of *Azolla filiculoides* on profenofos induced toxicity showing serum lipid peroxidation activity (n = 6, values are Mean±SD)

Histopathological changes: In the histopathological study of kidney, degenerated convoluted and distal tubules and glomeruli as well as Bowman's capsule were observed in 45 days of profenofos treated mice. The epithelial cells of these tubules have lost their integrity as the agglutination of the epithelial cells nucleus is very prominent at many places. The oral administration of extract of *A. filiculoides* for 30 days to the 45 days profenofos toxicated mice, the restoration in the glomeruli with Bowman's capsule and architecture of convoluted tubules and distal tubules has been observed (Fig. 5-7).

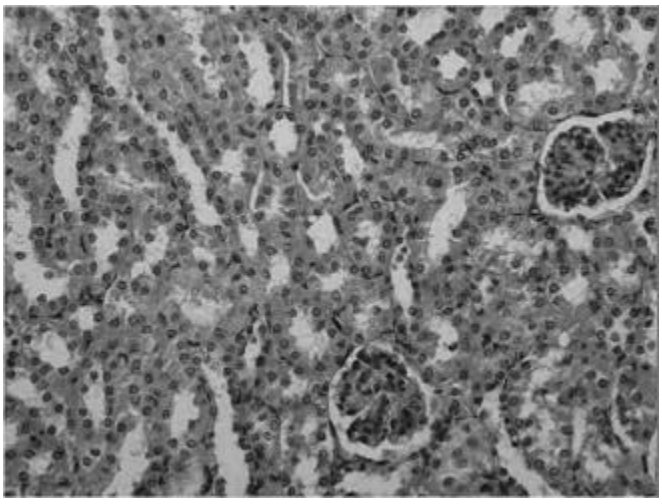

Fig. 5: Section of kidney of control mice showing normal architecture of glomeruli in Bowman's capsule. The Convoluted Tube (CT) and distal tubules (dt) are well arranged. This denotes the normal function of kidney

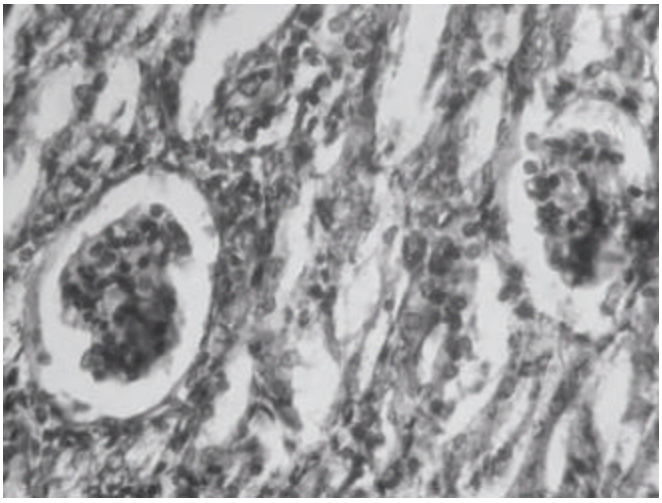

Fig. 6: Section of kidney of profenofos treated group showing high degree of degeneration in the glomeruli as well as Bowman's capsule. Vacoulation in the entire cytoplasm can be observed as the endothelial cells, convoluted tubules (CT) and distal tubules (dt) appeared to be completely distorted. This denotes the abnormal functioning of the kidney

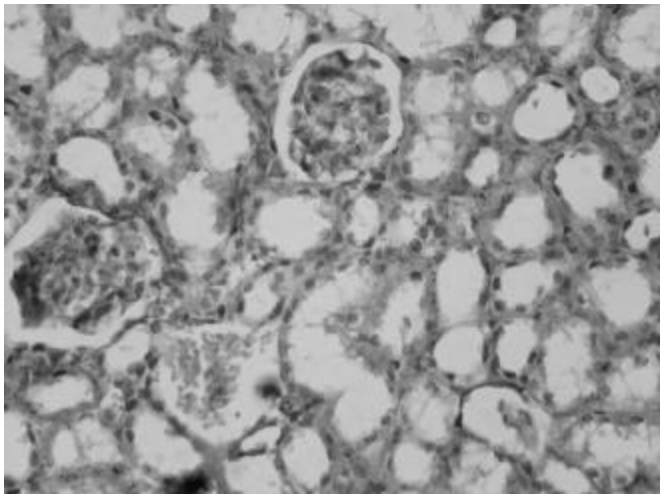

Fig. 7: Section of kidney of *A. filiculoides* administrated to profenofos pre-treated group showing restoration in the glomerulus with Bowman's capsule is clearly observed as the architecture of convoluted and distal tubules, is clearly observed

DISCUSSION

Kidney is the major organ of metabolism which eliminates various metabolic wastes along with waste of exogenous chemicals. Profenofos is an insecticide, are widely used to increase the productivity of crops by killing the pests. Profenofos easily enters into animal system via food and water. It is largely known to cause toxicity in various organs, such as the liver and brain. It also induces swelling of Bowman's capsules and tubular degeneration in the kidney (Amer *et al*., 2007). In the present study, profenofos (Organophosphate) treated to mice showed deleterious effect on the kidney function test as well as at cellular level.

In Kidney Function Test, serum urea level accounts for the protein metabolism i.e., catabolism of amino acids while uric acid is the most abundant aqueous antioxidant, particularly effective in quenching the hydroxyl and superoxide anion radicals. Production of uric acid via adenosine nucleotide turnover may occur in every tissue in body and uric acid may be increased throughout the body. A high amount of uric acid level quenches free radical countered toxicity thereby preventing the lipid peroxidation and tissue damage. Creatinine is a non protein nitrogenous substance formed during muscle metabolism of creatinine and phosphocreatinine and filtered by glomerular of the kidney. Accumulation of creatinine in the blood is used as significant marker for toxicity in kidneys (Finco, 1997).

In the present study, there was many fold inclination in the serum levels of urea, uric acid and creatinine but after the administration of *A. filiculoides* there was significant decrease in their levels denotes that *A. filiculoides* has nephron-protective properties which normalizes the physiology of kidney thence its function. Similar studies on antidotes, have been well established as *Cuminum cyminum* and *Coriandrum sativum* antidote effect against profenofos induced nephrotoxicity and hepatotoxicity in Swiss albino mice (Kumar *et al*., 2011a, b).

Profenofos is an organophosphate pesticide which leads to production of reactive oxygen species in cells. Malondialdehyde (MDA) concentration is a major oxidative product of per-oxidize polyunsaturated fatty acid which is considered to be an important indicator of Lipid Peroxidation

(LPO). In the present study the serum malondialdehyde concentration increased many folds after exposure of profenofos in mice but after treatment with *A. filiculoides* there was significant decrease in levels which denotes the normalization in the cells.

In the histopathological study of kidney the study showed high degree of degeneration in the nephrocytes as degenerated convoluted tubules, distal tubules, glomeruli and Bowman's capsule were significantly observed. The epithelial cells of these tubules appear to loose their integrity as the agglutination in the epithelial cells nucleus was observed. But, after the administration of *A. filiculoides* there was significant restoration in the cellular architecture of convoluted tubules, distal tubules, Glomeruli with Bowman's capsule.

The study thus, reveals that *A. filiculoides* extract has the nephro-protective as well as antioxidant property which not only normalizes the physiological activities of the body but also restores the cellular integrity against the profenofos induced toxicity.

CONCLUSION

Thus, from the entire study, it can be concluded that although the entrance of these hazardous pesticide (Profenofos) into our body cannot be checked but by the use of the *A. filiculoides* extracts as potent antidote can solve the problem at much extent by normalizing the physiology of the body and maintaining the cellular integrity.

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