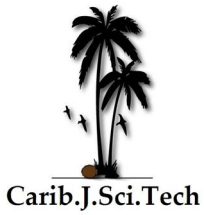

HEPATOPROTECTIVE AND ANTIOXIDANT EFFECT OF AZOLLA FILICULOIDES ON PROFENOFOS INDUCED HEPATOTOXICITY IN SWISS ALBINO MICE

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Keywords: Profenofos, organophosphate,Hepatotoxicity , azolla, antidote.

ISSN 0799-3757

http://caribjscitech.com/

ABSTRACT

In present study the organophosphate was administered at the rate of 15mg/kg of body weight to Swiss albino mice for 45 days to observe the effect on liver of mice. After that, the mice were administered with Azolla filiculoides at the rate of 10 mg/kg of body weight for 30 days. The liver function test (LFT) and lipid peroxidation was evaluated after Profenofos exposure and then Azolla treated groups. The study reveals that Profenofos causes deleterious effect on biochemical parameters as increased in its levels was observed. But, after Azolla administration for 30 days there was significant normalization in the levels of LFT and lipid peroxidation denotes the hepato protective and antioxidant activity of Azolla filiculoides.

Introduction

Liver, the major organ of the body comprising 2-3% of the total grown-up body weight, is primarily concerned with the metabolic activity of organisms.[1] Liver is liable for detoxifying the chemical substances in the blood and in this process it is exposed to high concentrations of toxicants and toxic metabolites making it susceptible to injury.[2] Liver is an organ of principal importance and its disorders are abundant with no valuable remedies. Therefore, search for new medicines is mandatory. In recent years, much interest has been urbanized in therapeutic valuation of traditionally used herbals with that of the recent concept of evidence based assessment.[3]

Profenofos (O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate) is a toxic organophosphorous insecticide.[4-5] Profenofos is a broad spectrum insecticide extensively used in agricultural and household applications, causing rigorous environmental pollution, with adverse impacts on human, wildlife and environment.[6] Effect of Profenofos on animal occurs through food and water.[7] Profenofos causes diverse symptoms of toxicity and biochemical changes in the enzyme activity of the liver and brain following two sub lethal doses of profenofos in mice.[8] Organo phosphorous can also induce the oxidative stress by generating free radicals and changeable antioxidant levels of the free radical scavenging enzyme activity.[9-12] The toxicity caused by profenofos appeared fatal even at a fairly low plasma concentration as recorded in a case of fatal human poisoning where high concentrations of metabolites were detected suggests profenofos is rapidly metabolized.[13] Profenofos is bio activated, perhaps by phosphorothioate oxidation with microsomal enzyme and NADPH.[14,15] Several in vitro studies have been conducted to see the toxicity profile of profenofos. The lymphocytes from peripheral blood samples of healthy human donors via comet assay revealed single strand breaks in DNA as comet tail lengths which indicate genetic damage.[16] Preceding studies have shown that at low doses, organophosphorous pesticides not only act as genotoxic agent but can also, affect several other biochemical pathways. Profenofos induced apoptosis and necrosis in cultured human peripheral blood lymphocytes under in vitro conditions using the DNA diffusion assay is well reported.[17] Amelioration by other plants Cuminum cyminum and Coriandrum sativum on profenofos induced liver toxicity in mice has been well studied.[18] The chronic effect of profenofos on mammals can be eliminated by giving suitable antidote to the affected one. The present study, deals with the vital role of the Azolla filiculoides extracts in reducing the hepatotoxicity at the cellular and biochemical levels. The Azolla filiculoides may be a suitable antidote against chronic toxicity of profenofos on mammals.

Material and methods

2.1 Animals

Thirty female swiss albino mice (28g to 32g) were obtained from animal house of Mahavir Cancer Institute & Research Centre, Patna, India (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008). The research work was approved by the IAEC (Institutional Animal Ethics Committee) 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 hrs light/dark cycle at 22 ± 2^{o} C.

2.2 Chemicals

Commercially existing 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 market. Commercially available kit for Chemical analyses like SGPT, SGOT, Alkaline Phosphatase and Bilirubin was used of crest coral clinical system, Goa, India. Analytical grade TCA, TBA and SDS were purchased from Sigma Aldrich company.

2.3 Medicinal plant

Azolla filiculoides (Red Azolla) is a small free floating fresh water fern, green to reddish brown or red at edges. The nitrogen-fixing capability of Azolla has led to Azolla being widely used as a biofertiliser, especially in parts of Southeast Asia. Indeed, the plant has been used to strengthen agricultural productivity in China for over a thousand years. Azolla filiculoides have the potential to remove the certain heavy metals from waste waters.[19]

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 hours and finally extracted with 5% absolute ethanol using soxhlet apparatus for 6 -8 hours and the residue was concentrated and dried at 37^0C. The dose was finally made to 10 mg/kg body weight for oral administration.

2.4 Treatment Protocol

The animals were grouped into three groups- control, profenofos treated and A. filiculoides treated. The profenofos treated group was administered at the rate 15mg/body weight for 45 days to observe the Profenofos induced hepatotoxicity. Upon profenofos treated group then A.filiculoides at the rate 10mg/Kg body weight was administered for 30 days.

2.4.1 Biochemical Analyses

After the entire treatment protocol the experimental animals were sacrificed. Blood was collected by orbital sinus puncture method. Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetate Transaminase (SGOT), alkaline Phosphatase and bilirubin activities were measured according to the method described.[20-22]

2.4.2 Lipid peroxidation (LPO)

LPO refers to the oxidative humiliation of lipids. Thiobarbituric acid reactive substances (TBARS), as a marker for LPO, were determined by the double heating method.[23] 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 tri chloroacetic acid (TCA) solution was added to 0.5 ml serum in a centrifuge tube and incubated for 15 min at 90^0C. After cooling at room temperature (RT), the mixture was centrifuged at 3000g for 10min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test tube and again incubated for 15 min at 90^0C. The solution was then cooled at RT and its absorbance was measured using Thermo Scientific UV-10 (UV –Vis) spectrophotometer (USA) at 532nm.

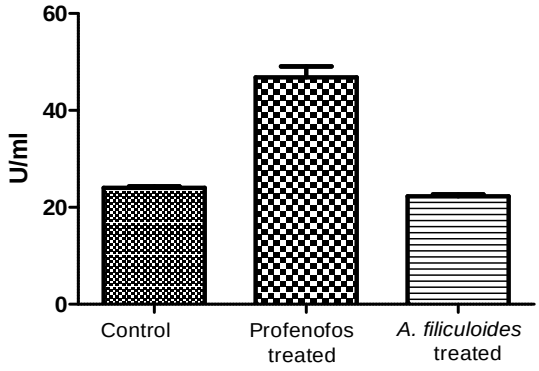
2.5 Statistical Analysis

Results are presented as mean ± S.D 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 't' test at 99.9% ($p < 0.05$) confidence level. Calculations were performed with the GraphPad Prism Program (GraphPad Software, Inc., San Diego, USA).

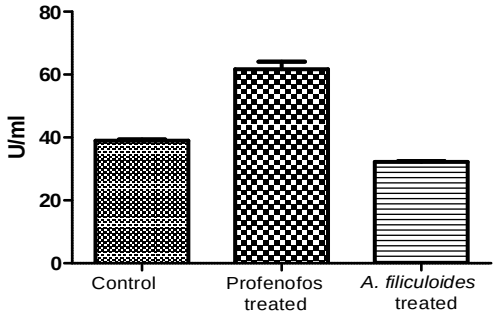
Results

Morbidity and mortality: The mice after profenofos exposure (15mg/Kg body weight/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.

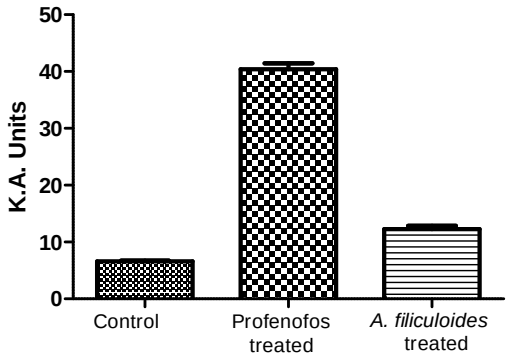
Biochemical changes : The SGPT, SGOT, Alkaline phosphatase, Bilirubin and Lipid peroxidation activity showed significant increase ($p < 0.05$) in profenofos treated group in comparison to control mice group. But, these values are significantly lowered ($p < 0.05$) in A. filiculoides treated group. The biochemical assessment thus shows the hepatoprotective activity of A. filiculoides (Graph fig.1-5).



Graph Fig1: Effect of A. filiculoides on profenofos induced toxicity showing SGPT activity (n=6, values are mean± S.D)



Graph Fig2: Effect of A. filiculoides on profenofos induced toxicity showing SGOT activity (n=6, values are mean± S.D)



Graph Fig3: Effect of A. filiculoides on profenofos induced toxicity showing alkaline phosphatase activity (n=6, values are mean± S.D)

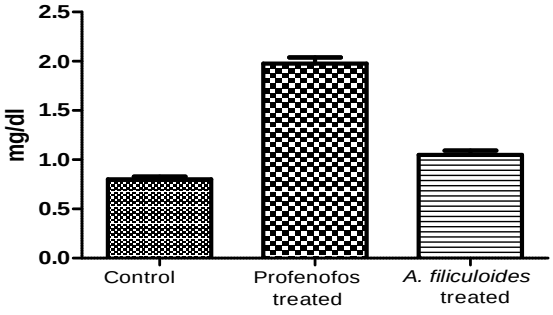
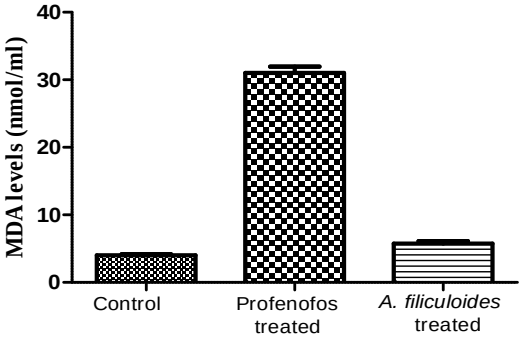


Fig4: Effect of A. filiculoides on profenofos induced toxicity showing bilirubin activity (n=6, values are mean± S.D)



Graph Fig 5: Effect of A. filiculoides on profenofos induced toxicity showing lipid peroxidation activity (n=6, values are mean± S.D)

Discussion

Pesticides are used to kill pests in the fields are now reaching the human body through various food chains. Liver is one of the important and major site of metabolism, resulting in inactivation of exogenous chemicals or xenobiotics to non-toxic metabolites. As, due to high metabolic capability and the portal blood supply, toxic responses occur relatively frequent in the liver compared to other organs. In the present study, serum transaminases, alkaline phosphatase and bilirubin were used as an index of hepatocellular injury in study of profenofos induced intoxication. Generally, the serum transaminase level reflects the level of hepatic necrosis. The present study shows increased serum transaminase, alkaline phosphatase and bilirubin levels denotes the level of degeneration. Similar, findings have been well studied, shows that profenofos causes different symptoms of toxicity, revealed some biochemical changes especially in the enzymes activity of the liver and brain by administration of two sub lethal doses of profenofos in mice.[8] Organophosphorous pesticides can induce oxidative stress by generating free radicals and altering antioxidant levels of the free radical scavenging enzyme activity.[9, 24-32] In the present study also, there was immense increase in the levels of lipid peroxidation denotes that it has lead to improper functioning of the liver enzymes.

But after administration of A. filiculoides there was complete reversal in the liver function as the serum transaminase, alkaline phosphatase and bilirubin levels showed decreased levels denotes normalization in the function of the liver. Thus, A. filiculoides bears hepatoprotective activity against xenobiotics. Furthermore, there was decrease in the levels of the lipid peroxidation denotes the antioxidant activity of A. filiculoides. There has been no study, reporting the hepatoprotective or antioxidant activity of A. filiculoides. Although, other studies show that it has the property to absorb the heavy metals from soil.[33-35] Further studies at compound level are still required for concrete confirmation.

Conclusion

From the present study it is evident that A. filiculoides not only eliminates the toxic effects of Profenofos but also acts as a suitable antioxidant normalizing the function of the liver.

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