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Research Article

Hepatoprotective effect of *Pisonia alba* and *Cardiospermum halicacabum* in atrazine toxicity on biochemical parameters in the liver tissue of albino wister rat *Rattus norvegicus*

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Abstract

The present study was undertaken to evaluate the hepatoprotective effect of *P. alba* and *C. halicacabum* against the toxicity effects of herbicide atrazine on total bilirubin, AST, ALT and ALP in the albino wister rat *Rattus norvegicus*. In the present experimental study, *Rattus norvegicus* were intoxicated to sub lethal dose of atrazine (0.25 mg of atrazine) for four weeks. The biochemical parameters in the liver were evidence by increased compared to the control. During the treatment of *P. alba* and *C. halicacabum* (1gm) against atrazine intoxicated rats were restored near normal level (Group III and IV). The observed results were discussed in detail.

Keywords: Atrazine, *Rattus norvegicus*, *P. alba*, *C. halicacabum* and Biochemical parameters.

Introduction

Atrazine is a herbicide and inhibits the photosynthesis in the target plants. It is water-soluble and can be transported in dissolved form [Humburg, 1989]. It has been detected consistently in water bodies [Thurman *et al*., 1992]. It is quite susceptible to leaching or runoff. Atrazine has also been reported in precipitation, so it can lead to contamination of pristine water resources. Approximately 1 to 6% of the applied herbicides are released to the aquatic environment. Aged and persistent herbicides can become recalcitrant due to increased sorption and decreased bioavailability over time (Felsot and Dzantor, 1997).

ALT and AST are commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health (Wang *et al*., 2012). Aspartate and alanine amino transferases (AST and ALT), may serve as strategic links between protein and carbohydrate metabolisms, which is known to alter under several physiological and pathological conditions (Shivakumar, 2005). ALP act as a serum indicate tissue damage or toxic effects in liver (Klassen and Plaa, 1966).

Cardiospermum halicacabum (Linn), family Sapindaceae, is a deciduous, branching, herbaceous climber, which is distributed throughout the India. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite (Chopra and Chopra, 1986); its roots for nervous diseases, as a diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific; its leaves and stalks are used in the treatment of diarrhea, dysentery and headache (Kurian, 1995) and as a poultice for swellings (Chopra and Chopra, 1986). Phytochemical constituents such as flavones, aglycones, triterpenoids, glycosides and a range of fatty acids and volatile ester have been reported from the various extracts of this plant (Srinivas *et al*., 1996).

Pisonia grandis (Synmyn: *Pisonia alba*) commonly known as Leechikottai kerai in Tamil, Velati salet in Hindi (Khare, 2007). The plant *Pisonia grandis*, belonging to the family Nyctaginaceae, is an evergreen glaborous garden tree with young shoots are minutely puberulous. It is native of Hawai island and naturalized throughout India. In the alternative system of medicine *Pisonia grandis* leaves are used as analgesic, anti-inflammatory, diuretic (Radha *et al*., 2008) hypoglycemic agent (Sunil *et al*., 2009) and antifungal (Shubashini and Poongothai, 2010). It is also used in the treatment of ulcer, dysentery and snake bite. The leaves are edible and mostly used to treat wound healing, rheumatism and arthritis (Prabu *et al*., 2008). Leaves also consumed as vegetable and salad, fed to cattle (Chatterjee and Prakashi, 1997).

Hence an attempt has been made to investigate the effect of administration of *P. alba* and *C. halicacabum* crude extract on rats exposed to a sub lethal dose of atrazine. Physiological parameters, including the values of total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were chosen as specific indicators of animal response to the experimental treatments.

Materials and Methods

Experimental animal

Adult male albino Wistar rat (*Rattus norvegicus*) weighing (150-200 g) were obtained from Central Animal House, Rajah Muthiah Medical College, (Reg No. 160/1999/CPCSEA, Proposal number: 1096/2014), Annamalai University were used for the present investigation. The study protocol was approved by the Ethics Committee on Animal Experiment, Faculty of Science, Annamalai University, Annamalainagar.

Experimental chemical

Experimental chemical atrazine was purchased from (TATA Atrataf 50% WP) manufactured by Rallis India Limited, Mumbai.

Supplementary feed

Healthy disease free leaves of *Cardiospermum halicacabum* and *Pisonia alba* were collected from in and around Chidambaram and Perunthottam, the plant was identified. The leaves were washed in running tap water for 10 minutes leafs were dried, aerial parts (1kg) of *Cardiospermum halicacabum* and *Pisonia alba* were macerated thrice at room temperature and prepared in powdered condition.

Preparation of samples

A 10% (w/v) tissue homogenate was prepared in 50mM Tris HCL (PH 7.4) using homogenizer. Post mitochondrial supernatant (PMS) was prepared by centrifuging the homogenate at 10,000 rpm for 10 min at 40°C. The pellet was discarded and supernatant thus obtained was referred to as PMS. Various biochemical parameters were assayed in the homogenate and post mitochondrial supernatant of rat liver tissue.

Biochemical analysis

The bilirubin was performed based on the modified method of Malloy and Evelyn (1937). AST and ALT were assayed colorimetrically according to the method of Reitmann and Frankel (1957). Tissue alkaline phosphatase was estimated by using the diagnostic kit based on Kind and King's method (1954).

Experimental design

A total of 30 animals will be divided into 5 groups of 6 in each.

Group 1: Control animals

Group 2: atrazine alone (0.25mg/kg bw)

Group 3: atrazine (0.25mg/kg bw) + *Pisonia alba* (1 g/kg bw)

Group 4: atrazine (0.25mg/kg bw) + *Cardiospermum halicacabum* (1 g/kg bw)

Group 5:atrazine (0.25mg/kgbw) + *Pisona alba* (1 g/kg bw) + *Cardiospermum halicacabum* (1 g/kg bw)

Statistical analysis:

Results were expressed as mean ± S.E. Statistical analysis was performed using Student's t-test and p-values < 0.05 were considered statistically significant. Differences between means were

evaluated by one-way analysis of variance (ANOVA).

Results

In the present experimental observed that the liver tissue biochemical parameter such as total bilirubin, AST, ALT and ALP levels were increased significantly at 5 % level ($p<0.05$) in the treated group II (Table 1). At the end of four weeks the total bilirubin, AST, ALT and ALP levels were increased when compared to control group I. In the group III and IV total bilirubin, AST, ALT and ALP levels were restored when compared to group II. In the group V total bilirubin, AST, ALT and ALP levels there was no changes observed when compared to group II and which was near to control group I.

Effect of crude extract of *P. alba* and *C. halicacabum* on atrazine induced hepatotoxicity in albino wister rat *Rattus norvegicus*

Group	Total bilirubin mg/dl	AST IU/L	ALT IU/L	ALP IU/L
Group I Control	0.492 ±0.028	51.07±0.61	148.9±0.36	173.61±2.56
Group II Atrazine treated	2.38±0.12	139.25±25.3	219.33±45.36	423.3±15.19
Group III Atrazine + *P. alba*	0.504±0.02	89.04±0.36	205.05±24.21	181.67±20.18
Group IV Atrazine + *C. halicacabum*	0.589±0.02	164.63±0.83	240.39±6.39	151.9±6.38
Group V Atrazine + *P. alba* and *C. halicacabum*	0.496±0.05	52.18±0.62	149.36±1.39	180.35±1.58

One way anova

F	234.3	233.2	267.7	185.6
dF	7.40	7.40	7.40	7.40
P	<0.001	<0.001	<0.001	<0.001

N: 6 animals in each group
F: <0.001 indicates significant compared to control values are expressed as mean + SE

Discussion

Herbal drugs are playing an important role in health care campaign worldwide and there is a resurgence of interest in the herbal medicine for the treatment of various aliments like hepatitis, epilepsy, isomers etc. for which there is no specific treatment available. many authors have reported the hepatoprotective activity of number of medicinal plants like *Phyllanthus ninrri* (Venkateswaran *et al*.,1987), *Picrorluza karooa* (Dwivedi, 1990), *Croton ablongifollus* (Ahmed *et al*., 2002) and *Nigella sativa* (Mohideen *et al*.,2003).

In the present study, the estimation of serum markers like total bilirubin, total protein enzymes like aspirate amino transaminase, alanine amino transaminase and alkaline phosphatase activities indicate the functional status of liver. When there is a damage to the lipid membranes of the hepatocyte by the release of abnormal concentration of serum markers in to the blood stream. The increase may be the clear indicate of cellular leakage and loss at functional integrities of the cell membrane (Sarasvathi *et al*., 1993).

In the present investigation, the animals of group II (atrazine treated) showed significant loss of appetite and reduction in their body weight when compared the control. The estimation of serum bilirubin is the most sensitive test because it confirms the intensity of hepatic damage. In toxic hepatitis the extent of excretion of bilirubin through the intestine is very less. The bilirubin is excreted in to the canaliculi and then regurgitated in to the blood stream. Hence hyperbirubinemia is common in hepatitis condition. In the present investigation it was observed that concomitant treatment of the animals with all the crude extract showed significant reduction in the levels of serum bilirubin.

In the case of *Pisonia alba* showed therapeutic effect by significant lowering the levels of bilirubin while the effect of the aqueous extract was comparatively less. The effect of other indigenous medicinal plants *Cassia angustifalia* (Ilavarasan *et al*., 2001), *Pistacia lentiscus* (Janaki *et al*., 2002), *Foeniculum vulgare* (Ozbek *et al*., 2003), *Wrightia unctri* (Chandrasekar *et al*., 2004) etc. On vectoring the level of serum bilirubins have been reported.

In toxicity studies, a variety of biochemical parameters are measured to evaluate a broad range of physiological and metabolic functions affecting target organ identification and tissue injury assessment (Akhtar *et al.,* 2012). In the present investigation the atrazine treated group was increase the activity of bilirubin, AST, ALT and ALP. More over the group IV (atrazine along with *C. halicacabum*) decreased the levels of these biochemical parameters. Because the supplementary group of plant having such a wound healing property compounds like pinitol, apigenium, luteolin, chrysoeriol, vitamin E and rutin. But the group III (atrazine along with *P. alba*) decreased activity was observed.

Wachukwu *et al.* [2004] reported that there was a corresponding increase in the activity of the liver enzymes, AST, ALT and ALP with increase in the concentration of petrol while` Uboh *et al*. [2009] reported that exposure of male and female rats of Premium Motor Spirit (PMS) blend unleaded gasoline (UG) vapors caused hepatotoxicity. Also previous studies observed that gasoline vapors induced proatherogenic changes in serum lipid profile and signs of hepatic oxidative stress [2005] in male and female rats.

Krishan and Veena (1981) also observed increase in levels of AST, ALT and ALP in serum of fish exposed to 2,3,4 – triaminoazo benzene resulting to hepatocellular damage. Mohssen (1997) was studies that indicated increase in the activity of liver enzyme following liver damage albino mouse exposed to toxic substances.

Shrivastava *et al*. (1989) reported that ASAT and ALAT levels were increased significantly in plasma, liver, kidney, lung, brain, heart, intestine and muscle of rat treated with dichlorvos and suggested that these results might be due to cellular damage or increased permeability of plasma membrane. Similar increase in the tissues and plasma levels of these enzymes have also been reported in various species of animals given acute and sub-acute doses of other organo phosphorus (op) insecticides (Snow and Watson, 1993). ASAT and ALAT enzymes are involved in amino acid metabolism and an increase in these enzymes together with AKP in serum indicate tissue damage or toxic effects in liver (Klassen and Plaa, 1966).

Bilirubin is formed from haemoglobin in the reticulo endothelial system, and then circulates attached to plasma albumen. Approximately 80% of circulating bilirubin is derived from red blood cells; the remaining 20% bilirubin is formed from in effective erythropoiesis resulting from destruction of erythroid cells in bone marrow (Tortora and Grobawiski, 2003). Cypermethrin-induced inhibition of this enzyme might also have contributed towards hyperbilirubinemia in cypermethrin intoxicated rats (Pande, 2001). Since liver plays a key role in bilirubin metabolism, any damage to liver cells, which probably was inflammation in the present study resulting in disturbed bile excretion, might be responsible for hyperbiliribinemia in atrazine intoxicated rats.

Conclusion

This study has shown that atrazine as a free radical causes hepatotoxicity while feeding on *P. alba* and *C. halicacabum* reversed the hepatotoxicity. Both of these two plants the *C. halicacabum* having the more therapeutic properties compared to *P. alba.*

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