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Research Article

PROTECTIVE EFFECT OF *PISONIA ALBA* IN ATRAZINE TOXICITY ON BIOCHEMICAL MARKER ENZYMES IN THE LIVER TISSUE OF ALBINO WISTER RAT *RATTUS NORVEGICUS*

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ABSTRACT

The goal of this study was to see if *Pisonia alba* might protect the albino wister rat *Rattus norvegicus* from the herbicide atrazine's toxicity effects on AST, ALT, ACP, and ALP. *Rattus norvegicus* were inebriated with a sublethal dose of atrazine (0.25 mg of atrazine) for 28 days in this experiment. When compared to the control, the biochemical manufacturing enzymes in the liver were found to be higher. During the treatment of atrazine-intoxicated rats with *P. alba*, they were returned to a near-normal level (Group III and IV). The outcomes that were noticed were thoroughly explained.

Keywords: Atrazine, *Rattus norvegicus*, *Pisonia alba* and Biochemical makers enzymes.

INTRODUCTION

Atrazine (IUPAC: 6-chloro-N2-ethyl-N4-isopropyl-1, 3, 5-triazine-2, 4-diamine) is an atriazine herbicide commonly used in corn, sugar cane, and sorghum production. It is widely used in non-EU nations and is one of the most widely used pesticides in the United States. The discovery of atrazine in ground water, surface water, and drinking water (WHO, 1993) has piqued interest in its biological consequences. The irrational use of pesticides is one of the main causes of environmental contamination (Mehdi *et al*., 2005). Pesticides are distinct from other chemical substances in that they are released into the environment on purpose. The liver is a vital organ that helps the body's metabolism of both endogenous and foreign chemicals. The majority of xenobiotics are detoxified in the liver by a series of activation, conjugation, and clearance pathways (Rouimi *et al*., 2012). ATR has the ability to cause oxidative stress by increasing the concentration of reactive oxygen species and oxidative damage products like lipid peroxides, and thus influencing antioxidant enzyme activity (Abarikwu, 2014).

Enzymes such as ACP, ALP, AST, and ALT have been employed as bioindicators to evaluate pollution exposure (Lavanya *et al*., 2011). AST and ALT are two of these enzymes that are commonly employed to diagnose tissue damage and act as stress indicators in aquatic biomonitoring (Jung *et al*., 2003). ALT and AST are commonly measured clinically as part of a diagnostic evaluation of hepatocellular injury to determine liver health (Wang *et al*., 2012). The aminotransferases or transaminases (AST and ALT) and the phosphatases: total, acid, and alkaline are the major enzymes of clinical significance in liver function (Raju, 2005). *Pisonia alba* is known in Tamil as Leechi kottai kerai (Khare, 2007). *Pisonia alba* is an evergreen glaborous garden tree with minutely puberulous new branches that belongs to the Nyctaginaceae family. It is native to Hawaii and has spread throughout India. *Pisonia alba* leaves are used in alternative medicine as an analgesic, anti-inflammatory, and diuretic (Radha *et al*., 2008). Hypoglycemic and antifungal agent (Sripathi & Poongothai, 2008; Sunil,

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2009). It can be used to cure ulcers, diarrhoea, and snake bites, among other things. The leaves are edible and used to cure wounds, rheumatism, and arthritis (Prabu *et al*., 2008). Kim and colleagues (Kim *et al*., 2002). Leaves are also used as a vegetable and salad ingredient, as well as for cattle feed. The various parts of *Pisonia grandi* are widely used in tribal folk medicines and as an Indian traditional medicine for anti-diabetic (Sunil, 2009), anti-inflammatory (Jayakumar *et al*., 2006; Radha *et al*., 2008; Thillaivanan *et al*., 2013), wound healing (Prabu *et al*., 2008), diuretic (Prabu *et al*., 2008), wound healing (Elumalai & Prakash, 2012). Natural remedies derived from traditional herbs are used to treat hepatotoxicity, and they are regarded to be effective and safe (Fakurazi *et al*., 2012; Huang *et al*., 2010). The use of medicinal plants as a complement to conventional treatment has recently piqued international interest. Extracts from medicinal plants and other natural sources contain a wide range of chemicals with significant biological activity (Watanabe *et al*., 2001). As a result, an attempt was made to investigate the effects of atrazine given at a sublethal dose for 28 days to Albino Wistar rats (*Rattus norvegicus*), as well as the chelating activities of *Pisonia alba* against atrazine toxicity.

MATERIALS AND METHODS

Experimental animal

The present study used adult male albino Wistar rats (*Rattus norvegicus*) weighing 150-200 g from the Central Animal House, Rajah Muthiah Medical College (Reg No.160/1999/CPCSEA, Proposal number: 1096/2014), Annamalai University. The Ethics Committee on Animal Experiment, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, India, accepted the study protocol.

Experimental chemical

The experimental chemical atrazine was bought from Rallis India Limited in Mumbai (TATA Atrataf 50 percent WP).

Preparation of Ethanolic Extract

The dried powder was extracted (*Pisonia alba*) in a Soxhlet apparatus using ethanol at a temperature range of 55 °C to 60 °C. In a vacuum evaporator, the filtrate was evaporated to dryness under decreasing pressure.

Preparation of samples

Using a homogenizer, a 10% (w/v) tissue homogenate was produced in 50 mM Tris HCL (PH 7.4). The homogenate was centrifuged at 10,000 rpm for 10 minutes at 40°C to obtain post mitochondrial supernatant (PMS). The particle was discarded, and the resulting supernatant was dubbed PMS. The homogenate and post mitochondrial supernatant of rat liver tissue were tested for a variety of biochemical parameters.

Enzymatic Assay

Tissue aspartate aminotransferase was measured using a diagnostic kit based on Reitman and Frankel's approach (1957). Alanine aminotransferase was measured using a diagnostic kit based on Reitman and Frankel's approach (1957). The diagnostic kit was used to calculate tissue alkaline phosphatase using Kind and King's approach (1954). Tissue acid phosphatase was calculated using King's technique and a diagnostic kit (1956).

Experimental design

A total of 24 animals will be divided into 4 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: *Pisonia alba* (1 g/kg bw)

Statistically Analyses

The results of this study were expressed as means SE, percentage changes, and statistically assessed using the student t-test (Milton and Tsokos, 1983) to compare the means of treatment and control data, with the results being judged significant at the (P0.05) and (P0.01) levels.

RESULTS AND DISCUSSION

Hence an attempt has been made to investigate the biochemical makers enzymes activities such AST, ALT, ACP and ALP of liver tissue in albino wister rat *Rattus norvegicus* against atrazine exposed group I, atrazine along with *P. alba* (group III) alone *P. alba* alone (group IV) for the period of 1, 7, 14, 21 and 28 days.When compared to the control group I, the activity of aspartate aminotransferase (AST) in the liver of *Rattus norvegicus* after atrazine administration was considerably lower in group II. - 19.814, - 22 were the percent changes. 435, minus 26 187, minus 32. For the periods of 1, 7, 14, 21, and 28, respectively, 951 and -36.150 were used. In the rat treated with atrazine and *Pisonia alba* extract, group III showed a substantial rise in comparison to group II. For periods of 1 to 28 days, the percent changes were 1.422, 2.221, 12.769, 15.983, and 16.824 correspondingly. When a single dose of *Pisonia alba* extract was given to group IV, the AST activity was higher than in group III, and it was close to that of the control group. For periods of 1 to 28 days, the percent changes were -13.045, -14.226, -14.220, -14.213, and -14.214, respectively. At 1% and 5% levels, the reported AST activity in liver tissue for all four groups was statistically significant (Table 1).

When compared to the control group I, the Alanine aminotransferase (ALT) activity in the liver of *Rattus norvegicus* a after atrazine administration was considerably lower in group II. For periods of 1, 7, 14, 21, and 28 days, the % changes were -29. 394, -35.086, 40.949, -45.400, and -19.940, respectively. In the rat given atrazine and *Pisonia*

alba extract in group III, the level of atrazine was substantially higher than in group II. For the periods of 1 to 28 days, the percent changes were 26. 588, 19.695, 5.649, 12.135, and 19.940, respectively. When the extract of *Pisonia alba* was given to group IV alone, the ALT activity was higher than in group III, and it was close to that of the control group. For the periods of 1 to 28 days, the % changes were 24.702, 19.750, 14.797, 9.843, and 9.834, respectively. At 1% and 5% levels, the reported ALT activity in liver tissue for all four groups was statistically significant (Table 2).

When compared to the control group I, the acid phosphatase (ACP) activity in the liver of Rattus norvegicus after atrazine administration was reduced in group II. For the periods 1, 7, 14, 21, and 28, the percent changes were -6.130, -18.480, -26.238, -30.663, and -39.585, respectively. In the rat given atrazine and *Pisonia alba* extract in group III, the level of atrazine was substantially higher than in group II. For the period of 1 to 28 days, the percent changes were 1.548, 14.955, 10.645, 9.116, and 19.034 correspondingly. When a single dose of *Pisonia alba* extract was given to group IV, the ACP activity was higher than in group III, and it was close to that of the control group. For the period of 1 to 28 days, the percent changes were 0.072, 0.120, 0.217, 0.241, and 0.433, respectively. At 1% and 5% levels, the reported ACP activity in liver tissue for all four groups was statistically significant (Table 3). When compared to the control group I, the activity of alkaline phosphatase (ALP) in the liver of *Rattus norvegicus* after atrazine administration was considerably lower in group II. For periods of 1, 7, 14, 21, and 28 days, the % changes were 111.403, 102.631, 101.754, 102.631, and 93.859, respectively. When rats were given atrazine and *Pisonia alba* extract, the concentration of atrazine in group III was substantially higher than in group II. For the periods of 1 to 28 days, the percent changes were -41.908, -37.229, -43.478, -42.857, and -40.271, respectively. When a single extract of *Pisonia alba* was given to group IV, the ALP activity was higher than in group III, and it was close to that of the control group. For the periods of 1 to 28 days, the % changes were - 8.771, -8.781, -8.761, -8.791, and -8.753, respectively. At the 1% and 5% levels, the reported ALP activity in liver tissue for all four groups was statistically significant (Table.4).

Table 1. Chances in the Aspartate Amino Transferase (AST) (IU/L) activities in the liver tissue of albino Wister rat *Rattus norvegicus* administered to atrazine followed by the extract of *Pisonia alba* administered to 28 days**.**

	Groups	Treatment Duration in Days				
		1	7	14	21	28
AST	Group-I Control	84.288 ±2.363	84.285± 2.244	84.280± 2.196	84.277± 1.963	84.275 ± 1.972
	Group-II atrazine	67. 587± 2.006** -19.814	65.375± 1.872** -22. 435	62. 209 ± 1.960** -26. 187	56.505 ± 2.786** -32. 951	53.809 ±2.509** -36.150
	Group-III atrazine + *Pisonia alba*	83.089 ± 2.472** 22.936	82.413± 7. 904* 26.068	73.518 ± 2.680** 18.179	70.807 ± 2. 872** 25.311	70.096± 2.387** 30.268
	Group-IV *Pisonia alba*	73.292 ± 2.804* -13.045	72.294± 2.009** -14.226	72.295±2.373** -14.220	72. 298± 2.904** -14.213	72.296 ± 2.707** -14.214

(Values are mean S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+,-), NS-Non-significant.

Table.2.Chances in the Alanine Amino Transferase(ALT) (IU/L) activities in the liver tissue of albino Wister rat *Rattus norvegicus* administered to atrazine followed by the extract of *Pisonia alba* administered to 28 days.

	Groups	Treatment Duration in Days				
		1	7	14	21	28
ALT	Group-I Control	40.473± 1.863	40.469± 1.096	40.467±2.084	40.464±2.273	40.461± 1.896
	Group-II atrazine	28.576±2.086** -29. 394	26.270± 2.112** -35. 086	23.896± 1.544** -40. 949	22.093± 1.709** -45. 400	21.544± 2.305** -46.753
	Group-III atrazine+ *P. alba*	20.978± 2.226* 26. 588	21.096±2.315** 19.695	22.546± 2.096 5.649	24.774± 1.880** 12.135	25.840±1.196** 19.940

	Group-IV	30.475± 1.715**	32.476± 2.804	34.479±2.317	36.481± 2.326	36.482± 2.445
	Pisonia alba	24.702	19.750	14.797	9.843	9.834

(Values are mean S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+,-), NS- Non-significant.

Table 3. Changes of Acid Phosphatase (ACP) (µmole/min/mg protein) activities in the liver of albino Wister rat *Rattus norvegicus* administered to atrazine followed by the supplementary feed of *Pisonia alba* administered to 28 days.

	Groups	Treatment Duration in Days				
		1	7	14	21	28
ACP	Group-I Control	4.127 ± 0.034	4.134 ± 0.036	4.139 ± 0.044	4.145 ± 0.046	4.148 ± 0.028
	Group-II atrazine	3.874** ± 0.048 -6.130	3.370** ± 0.037 -18.480	3.053** ± 0.039 -26.238	2.874** ± 0.031 -30.663	2.506** ± 0.044 -39.585
	Group-III atrazine +*P.alba*	3.934** ± 0.029 1.548	3.874** ± 0.037 14.955	3.378** ± 0.052 10.645	3.136** ± 0.043 9.116	2.983** ± 0.036 19.034
	Group-IV *Pisonia alba*	4.130^{NS} ± 0.054 0.072	4.139^{NS} ± 0.046 0.120	4.148^{NS} ± 0.037 0.217	4.155^{NS} ± 0.039 0.241	4.166^{NS} ± 0.054 0.433

(Values are mean S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+,-), NS- Non-significant.

Table 4. Changes of Alkaline Phosphatase (ALP) (µmole/min/mg protein) activities in the liver of albino Wister rat *Rattus norvegicus* administered to atrazine followed by the extract of *Pisonia alba* administered to 28 days.

	Groups	Treatment Duration in Days				
		1	7	14	21	28
ALP	Group-I Control	114. 908 ± 3.004	114.914 ± 4.052	114.921 ± 3.037	114.927 ± 4.061	114.935 ± 3.056
	Group-II atrazine	241.208 ± 6.055** 111.403	231. 915 ±4.052** 102.631	231..212 ±4.044** 101.754	231.007 ± 3.057** 102.631	221.870± 4.053** 93.859
	Group-III atrazine+*P. alba*	140.344 ± 3.061** -41.908	145.126 ± 3.028** -37.229	131.877± 4..039** -43.478	132.334 ± 3.033** -42.857	132.154 ± 4.044** -40.271
	Group-IV *Pisonia alba*	104. 915 ± 4.062* -8.71	104. 923 ±4.046* -8.781	104.931± 5.055* -8.761	104. 943± 4.045* -8.791	104. 957 ± 3.036* -8.751

(Values are mean S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+,-), NS Non-significant.

The pervasive pollution of the environment caused by chemical chemicals such as pesticides is a severe problem for all living things, including people 38-40. Various chemical substances that enter animal bodies are transported to and expelled by organs responsible for detoxification, such as the liver and kidney. The calculation of antioxidant potential and hepatocyte damage are valuable tools in the assessment of liver toxicity in the current study. An experiment with rats revealed that pesticide had oxidative and inflammatory effects on the brain system and livers of mammals. More than 100 human diseases are caused by oxidative stress (Kosecik *et al*., 2005), and liver diseases are still one of the most important health problems in the world (Huang *et al*., 2010). Antioxidants obtained from nature combat the oxidative stress caused by several hepatotoxins (Sundararajan *et al*.,

2006). Enzyme markers are more accurate indications of an organ's health. Many environmental contaminants, including as pesticides and metals, can cause the production of reactive oxygen species (ROS) (Glusczak *et al*., 2007).

Any ingested toxicant in an animal or human has a significant target organ in the liver. This could be related to the principal site for all chemical metabolisms (Thangapandiyan & Miltonprabu, 2013). These findings imply that ethanolic extract functions as a natural antioxidant and anti-inflammatory medium (Huang *et al*., 2010). However, the aspartate trasaminase (AST) level in the exposed groups' livers fell at first, and then increased after some time. Lysozymes secrete ALP, which is regarded as a key integrity marker of cellular injury. Bromadiolone-treated rats had significantly higher levels of AST, ALT, ALP, LDH, and bilirubin, which is consistent with a recent report. As a result, changes in these enzymes' activity are often related to the severity of cellular damage. Aspartate aminotransferase (AST) and aspartate aminotransferase (ALT) are often evaluated clinically to determine liver health as part of a diagnostic evaluation of hepatocellular damage. In clinical research, aspartate aminotransferase is a good indication of liver injury. AST was discovered to be secreted into the bloodstream during hepatocellular damage. These enzymes seep into the bloodstream from dead or damaged cells (Mansour & Mossa, 2010). Elevated ALT and AST levels in this study hint to active amino acid consumption in energy-generating metabolic pathways such gluconeogenesis. In order to escape pesticide-induced oxidative stress, it also represents a genetic aberration in production (Bhushan *et al*., 2013). Enhanced levels of serum enzymes like AST and ALT suggested increased permeability and hepatocyte injury or necrosis. The current findings are consistent with those who found higher hepatic markers. These findings show that atrazine acts as an enzyme inhibitor, impairing hepatic metabolism, at the dosages used in this study. As the concentration of petrol rises, so do the levels of AST, ALT, and ALP. While Hepatotoxicity was observed in male and female rats exposed to Premium Motor Spirit (PMS) blend unleaded gasoline (UG) vapours.

The treated rats' livers had higher levels of alkaline and acid phosphatises. It's been suggested that an increase in ACP levels is caused by liver cell destruction (Vohra & Khera, 2013). Similarly, an increase in the specific activity of ACP in rat tissues only validated the pesticide-induced increase in the dephosphorylation potential within the rat cells. According to there was a corresponding increase in liverenzyme activity. (Revathi *et al*., 2016:Tamizhazhagan & Pugazhendy, 2017; Tamizhazhagan *et al*., 2017a, 2017b) *Pisonia alba* supplementation provides anti-oxidant and anti-inflammatory properties against atrazine-induced oxidative stress. It also protects against hepatic dysfunction caused by atrazine and plays a modulatory role in atrazine-induced free radical production. When compared to the control group I, *Rattus norvegicus* showed a recovery response in enzymological activity after 28 days of supplemental group III (atrazine together with *Pisonia alba*), which was drastically restored from the atrazine exposure (group II).

CONCLUSION

As a result of our research, it is obvious that after supplementing the atrazine-treated rat with *Pisonia alba*, the activity levels of AST, ALT, ACP, and ALP in the test tissue showed a trend toward normalcy. Vitamin A, Vitamin C, thiamine, riboflavin, nicotinic acid (Vitamin B3), alkaloids, proteins, and fats were found in the active ingredient of *P. alba*, as well as alkaloids, proteins, and lipids. Vitamin C is one of four dietary antioxidants, with Vitamin E, Vitamin A precursor-carotene, and Selenium being the others.

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