Convalescence consequence of *Pisonia alba* and *Cardiospermum halicacabum* aligned with the atrazine inebriated on antioxidant enzymes and histological changes in liver tissue of *Rattus norvegicus*

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**Abstract**

Atrazine, 2-chloro-4-(ethyl amino)-6-(isopropyl amino)-s-triazine, is a widely used herbicide for the control of grass and broad leaf weeds in crops such as sorghum, maize, sugarcane, lupins, pine and eucalyptus plantations. Therefore, this study was designed to determine the protective effect of *P. alba* and *C. halicacabum* on atrazine induced oxidative liver injury in albino rats. Thirty male rats were divided into five groups of 6 animals each. Changes in liver dysfunction parameters represented by SOD, CAT, GSH and LPO were determined in liver as indicators of oxidative damage. Meanwhile atrazine administration decreased SOD, CAT and GSH in animals. In addition, liver LPO level was increased. Liver histological studies have confirmed the changes observed in enzymological parameters and proved the beneficial role of *P. alba* and *C. halicacabum*. With *P. alba* or *C. halicacabum* administration during intoxication of atrazine, corrective effects on atrazine induced oxidative stress in the liver have been observed, while *P. alba* and *C. halicacabum* together assured a more efficient protection of the organ against the noticed oxidative stress.

**Keywords:** *Rattus norvegicus*, SOD, CAT, GSH, liver, *P. alba*, Histology, Enzymology and *C. halicacabum*

**Introduction**

Human and animal exposure to chemicals is rarely limited to a single chemical. Individuals are exposed daily to a variety of chemicals in food, drink, cosmetics and indoor and outdoor pollutants. In recent years, an assortment of environmental problems has led to augment concern about potential toxicity from exposure to multiple chemicals, including pesticide residues detected in food or water (Yang *et al.*, 1989). Atrazine is a triazine herbicide used in the control of broadleaf and some grass weeds in agriculture, particularly during the cultivation of corn crops. Atrazine is one of the most profoundly used herbicides in the world, with approximately 34,000 tonnes used in the USA in 2007 and approximately 800 tonnes in Canada in 2002 (US EPA, 2011).

Oxidative stress is defined as a disruption of prooxidant-antioxidant balance, leading to potential damage. Oxidative stress results from disruption of the prooxidant and antioxidant balance by reactive oxygen species (ROS) and other radicals or oxidants (Prior, 2004). While xenobiotics are able to increase ROS levels, the capacity to induce oxidative stress depends on the overwhelming of antioxidant defenses. Aerobic organisms have developed antioxidant defense mechanisms that scavenge ROS or prevent ROS-
mediated cellular damage (Valavanidis et al., 2006), including enzymes sensitive to free radical proliferation (Palace and Brown, 1994) such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) (Droge, 2002). Among the main ROS-inflicted damages is lipid membrane oxidation, known as lipid peroxidation (LPO). The reduced glutathione (GSH) antioxidant system is the principal protective mechanism of cells and is a crucial factor in the development of the immune response by immune cells reduced glutathione (GSH) comprise a system that maintains a reduced intracellular environment and acts as a primary defense against excessive generation of harmful ROS (Sheela et al., 2005).

Recently, there has been worldwide interest in the role of medicinal plants in complementary medicine. Extracts prepared from medicinal plants and other natural sources contain a variety of molecules with potent biological activities (Watanabe et al., 2001). Pisonia grandis (Synnym: Pisonia alba, Pisoniamor indifolia) is commonly known as Leechikottaikerai in Tamil. In the alternative system of medicine P. grandis leaves are used as analgesic, anti-inflammatory, diuretic, hypoglycemic agent and antifungal. 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 (Shubashini and Poongothai, 2010). Cardiospermum halicacabum is commonly known as Mudakkathan in Tamil. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite; 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 diarrhoea, dysentery and headache and as a poultice for swellings (Prabu et al., 2008).

Wistar rat has been used as tamed laboratory animal throughout the world. Biological studies on these have proved valuable and more knowledge we have gained from these tiny rodents. Considering these entire factors wistar rats was found to be the most suitable animal for the present investigation. Further liver is a site where detoxification of a xenobiotic compound occurs. Induced enzymological changes after atrazine treatment and supplementary feed Pialaba and C. halicacabum in the liver tissue which may serve as a tool in the assessment of the additional mode of action. Therefore in the present investigation an attempt has been made to investigate the impact of orally administered sublethal dose of atrazine and possible, alleviate effects of P. alba and C. halicacabum on SOD, CAT, GSH, LPO and histopathological alteration of rat liver tissues.

Materials and Methods

Experimental animal

Adult male albino Wistar rat (Rattus norvegicus) weighing (150-200 g) was obtained from the Central Animal House, Rajah Muthiah Medical College, 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) by Rallis India Limited, Mumbai.

Supplementary feed

Healthy disease free leaves of C. halicacabum and P. alba were collected from in and around Chidambaram and Perunthottam. The leaves were washed in running tap water for 10 minutes and dried, aerial parts (1kg) of C. halicacabum and P. alba were
maceration thrice at room temperature and prepared in powdered condition.

**Preparation of Ethanolic extract**

The dried powder was extracted (P. alba and C. halicacabum) in a Soxhlet apparatus using ethanol at a temperature range of 55°C to 60°C. The filtrate was evaporated to dryness at reduced pressure in a vacuum evaporator.

**Preparation of samples**

A 10% (w/v) tissue homogenate was prepared in 50mM Tris HCl (pH 7.4) using a 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.

**Enzymatic assay**

**Superoxide dismutase (SOD)**

Superoxide dismutase activity was determined following the procedure of Kakkar et al. (1984). At alkaline pH, superoxide anion O$_2^-$ causes the autooxidation of epinephrine adenochrome; while completing the reaction of SOD decreased the adenochrome formation and changes in absorbance were recorded at 480 nm, measured at 10sec intervals for 1 min in spectrophotometer.

**Catalase (CAT)**

The activity of catalase (CAT) was assayed by the method of Sinha (1972). The decomposition of hydrogen peroxide was followed directly by measuring the decrease in absorbance at 240 nm, at 10 sec intervals for 1min in spectrophotometer (Hitachi model, U-2001). The catalase activity was expressed as n moles of hydrogen peroxide metabolised /mg ptn/min.

**Reduced glutathione (GSH)**

Reduced glutathione was estimated by the method of Ellman (1959). Samples were deprotenised by sulfsalicylic acid followed by the react 9+ ion of sulphydryl groups of glutathione with DTNB (5,5′- dithiobis-2-nitrobenzoic acid) to produce a yellow coloured product 5-thio-2-nitrobenzoic acid. The reaction was read at 412nm. The results were expressed as n mol GSH/mg protein.

**Lipidperoxidation (LPO)**

The levels of lipid peroxidation in tissues were determined by quantitating the TBARS by in the tissues was measured in terms of malondialdehyde (MDA; a product of lipid peroxidation content) and determined by using the thiobarbutaric acid reagent (TBA) reagent. The organic layers were transferred into a clear tube and its absorbance was measured at 532 nm. The rate of lipid peroxidation was expressed as moles of malondialdehyde formed/g wet wt. of tissue.

**Histopathological studies**

The standard histological technique was followed by the method of Gurr (1959). The influence of the tested pesticides on the histopathology of liver (the essential organ for drug metabolism) was investigated. At the end of this experiment (30 days), liver from each sacrificed rat were dissected out, trimmed of excess fat and weighed (the relative weight of the organ equals the weight of the organ divided by the weight of the whole rat body). Then, the liver were fixed in 10% neutral formalin and prepared for histopathological examination.

**Experimental design**

A total of 30 animals were 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) + P. alba (1 mg / kg bw)
Group 4: atrazine (0.25mg/kg bw) + *C. halicacabum* (1 mg/kg bw)

Group 5: atrazine (0.25mg/kg bw) + *P. alba* (1 mg/kg bw) + *C. halicacabum* (1 mg/kg bw).

**Statistical analysis**

The data obtained from the quantitative study were expressed as the mean ± S.E. The 't' values were calculated by using the formula (Trivedy and Goel, 1984).

**Results**

The SOD, CAT and GSH activities of control and treated rat and also *P. alba* and *C. halicacabum* dosed rat are represented in Table - 1. The level of SOD in the liver tissue significantly decreased in the atrazine treated group II compared to the control group I. In the atrazine treated with *P. alba* combined group III the level of SOD, CAT and GSH significantly decreased when compared to the atrazine treated group. In the atrazine treated with *C. halicacabum* combined group IV the level of SOD and CAT significantly increased when compared to the atrazine treated group. But group V SOD, CAT and GSH activity was increased when compared to group II and it was near to normal. The activity of SOD, CAT and GSH in the liver tissue at 5 different groups showed significant at 1% and 5% level.

A highly significant elevation in liver lipid peroxidation (LPO) level in atrazine intoxicated rat group II was noticed in Table - 1. In combined treatment of atrazine with *P. alba* supplementary group III significantly increased in compared to the treated group II. The treatment of atrazine along with *C. halicacabum* group IV the LPO level significantly decreased compared to control group II. But group V the LPO activity was decreased when compared to group II and it was near to normal. The LPO activity in 5 different groups was statistically significant at 1% and 5% level.

**Histology studies**

The liver of rat exposed to sublethal dose of atrazine shows drastic damages in the cellular architecture compared to the untreated control (Fig. 1 and 2). The outer membrane of the liver is ruptured at many points. Variation in the space of hepatocytes is evident in different regions of the liver. The blood vessels are destroyed leaving large spaces (vacuoles) with damaged red blood cells (Fig. 1 and 2). Deterioration of cellular architecture with marked necrosis is also observed. The hepatocytes are found usually in groups with conspicuous spaces between them. Hepatocytes possess a markedly thickened nuclear and cytoplasmic membrane. This is obliterated at some places (Fig.-3 and 4). The hepatic ducts have become larger in size. Inter hepatic spaces have been dilated and orientation of nuclei is promiscuous. Nuclear pycnosis, cytoplasmic lysis and karyolysis were observed in different regions (Fig. 5 and 6). The degenerated paranchymal cells showed abnormal enlargement and these cells are called hypertropic sized cells, which have increased the volume of the liver. In group III and IV (atrazine along with *P. alba* and atrazine along with *C. halicacabum*) administered rat shows slight cytoplasmic vacuolization, lateralization and condensation of the nuclei (Fig. 7 and 8). In the group V the liver showed the histological pattern to near normalcy (atrazine along with *P. alba* and *C. halicacabum*) (Fig. 9 and 10).

**Discussion**

In the present investigation the atrazine treated group exhibited decline in the activity of SOD, CAT and GSH, except LPO. More over the group IV (atrazine along with *C. halicacabum*) augmented the levels of these enzymes. Because the supplementary...
Table - 1. Hepato protective effect of *P. alba* and *C. halicacabum* against atrazine induced toxicity in *Rattus norvegicus*

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>SOD (U/min/mg protein)</th>
<th>CAT (U/min/mg protein)</th>
<th>GSH (µg/mg protein)</th>
<th>LPO (nmole/mg protein)</th>
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<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>7.15 ± 0.51</td>
<td>72.62 ± 1.45</td>
<td>6.06 ± 0.13</td>
<td>4.18 ± 0.16</td>
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<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
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<tr>
<td>Atrazine</td>
<td>4.68 ± 0.18*</td>
<td>39.72 ± 0.90*</td>
<td>3.71 ± 0.15*</td>
<td>13.21 ± 0.19*</td>
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<tr>
<td></td>
<td>– 34.54</td>
<td>– 45.30</td>
<td>– 38.77</td>
<td>+ 216.06</td>
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<tr>
<td><strong>Group III</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Atrazine + <em>P. alba</em></td>
<td>0.742 ± 0.045**</td>
<td>30.148 ± 1.92*</td>
<td>0.231 ± 0.018**</td>
<td>28.46 ± 1.72**</td>
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<tr>
<td></td>
<td>– 26.7</td>
<td>– 30.11</td>
<td>+ 50.9</td>
<td>+ 33.0</td>
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<tr>
<td><strong>Group IV</strong></td>
<td></td>
<td></td>
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<tr>
<td>Atrazine + <em>C. halicacabum</em></td>
<td>6.40 ± 0.13**</td>
<td>61.43 ± 0.92*</td>
<td>5.03 ± 0.19*</td>
<td>12.03 ± 0.16*</td>
</tr>
<tr>
<td><strong>Group V</strong></td>
<td></td>
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</tr>
<tr>
<td>Atrazine + <em>P. alba</em> and <em>C. halicacabum</em></td>
<td>7.28 ± 0.15**</td>
<td>73.44 ± 1.26**</td>
<td>6.29 ± 0.18*</td>
<td>4.69 ± 0.11**</td>
</tr>
<tr>
<td></td>
<td>+ 1.8</td>
<td>+ 1.12</td>
<td>+ 3.79</td>
<td>+ 12.20</td>
</tr>
</tbody>
</table>

– or + indicate % over control, *Significant at 1% level, **Significant at 5% level. Values are Mean ± SE of 5 individual observations.

Fig. - 1&2. But group V (X 100 and X 400) shows the image of rat liver in control group

Fig. - 3 & 4 (X 100 and X 400) shows the images of atrazine treated group
Fig. 5 & 6 (X 100 and X 400) shows the images of atrazine along with *P. alba* treated tissue

Fig. 7 & 8 (x 100 and x 400) shows the atrazine along with *C. halicacabum* treated tissue

Fig. 9 & 10 (X 100 and) shows the atrazine along with *P. alba* and *C. halicacabum* treated tissue

HC - Hepatic cells; VA – Vacuoles; S – Sinusoids; PN - Pycnotic nucleus, H – Hepatocytes; N – Nucleus
group of plant having such a wound healing property compounds like pinitol, apigenium, luteolin, chrysoeriol and rutin. But the group III (atrazine along with P. alba) a decreased activity was observed. The levels of the oxidized lipids were increased while SOD activity and GSH concentrate decreased relative to those of control and this is in agreement with Upasani and Balaraman (2003) who found significant increase in the lipid peroxidation and decrease in the level of endogenous antioxidants in the liver of lead exposed animals.

In an another study Flora et al. (2003) have shown that lead exposure produced significant depletion of hepatic GSH activity pointing to hepatic oxidative stress. Ito et al. (1985) have found to inhibition of SOD activity by exposure to lead in the manufacturing processes. The principal toxic effects of atrazine involve interactions with large number of cellular processes of including the formation of complexes with free thiols and protein thiol groups, leading to oxidative stress (Stacey and Kappas, 1982).

Antioxidant enzymes such as superoxide dismutase (a Cu$_{2+}$ dependent enzymes) and catalase (in which NADPH protect it against inactivation by its substrate H$_2$O$_2$) play a major role in the intracellular defense against oxygen radical damage to aerobic cells. Chung et al. (1982) demonstrated that Hg caused time dependent decrease in the activities of the enzymes of the glutathione (GSH) metabolism pathway in the rat. Therefore increase in the formation of ROS by atrazine may induce membrane biochemical functional alterations and thus induced liver cell damage.

Glutathione lead complexes also reduce intracellular damage by preventing atrazine from entering tissue and cells and becoming intracellular toxin. The elevated level of SOD, CAT and GSH by the influence of P. alba and C. halicacabum as compared to the atrazine may have facilitated the conjunction reaction of xenobiotics metabolism and may have increased the availability of non-critical nucleophilic for inactivation of electrophiles and therefore might be playing a major role in metallo-protection. Some of the active constituents of have been reported to possess strong antioxidant activity and provokes free radical scavenging enzyme system. Antioxidants are compounds that can delay or inhibit oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction (Velioglu et al., 1998).

In the present evaluation the treated group has been increased the lipid peroxidation level compared to control group. The increased concentration of LPO, variation in antioxidant status and modified activities of cellular enzymes makes oxidative stress more evident in pesticide exposure (Lopez et al., 2007). It has also been reported that chlorpyrifos generate oxidative stress in liver through heightened levels of reactive oxygen species (ROS), hydrogen peroxide, nitrate and nitrite (Mehta et al., 2009).

All the possible mechanism of atrazine toxicity may lead to the formation of reactive oxygen species (ROS) as found in the present investigation. Therefore increase in the formation of ROS by lead acetate may induce membrane biochemical functional alterations and thus induced liver cell damage. It was observed that P. alba and C. halicacabum when given in combination with lead acetate significantly increases the liver GSH level, SOD, CAT activity as antioxidant potential and thereby declines the level of lipid peroxidation.

In the present study, atrazine induce hepatotoxicity manifests itself in disorganization of the hepatic cords, cytoplasmic vacuolization and invading
of infiltrative inflammatory cells (El-Sokkary et al., 2005). Since, atrazine toxicity effect on a range of cellular enzymes particularly those involved in energy production and associated with massive dilated mitochondria leading to hydropic degeneration appear as cytoplasmic vacuolization (Buchheim et al., 1998). However, mild and to a lesser extent, moderate degrees of hydropic degeneration and mild portal cellular infiltration were seen in 50 - 60% of rats exposed to atrazine along with C. halicacabum.

The histological examination of the liver tissue of the animals treated with atrazine revealed severe histopathological changes typical to those reported in the literature. Similar observations were reported by Kubo et al. (1996). Moreover, recent studies have shown that lead (Pb) induce DNA damage (Xu et al., 2003).

Ethanolic extract of C. halicacabum extract repressed the TNF-α induced DNA binding activity of NF-κB. These indicate the anti-inflammatory activity of the plant (Sheeba and Asha, 2009). These results suggest that ethanolic extract act as a natural antioxidant and anti-inflammatory mediator (Huang et al., 2010). Lately, the anti-inflammatory role of rutin has been recognized in this plant (Babu and Krishnakumari, 2005). The gamma-glutamyl transpeptidase and phospholipase A2 activity to reduce the lipid peroxide content when compared to exposed group. At the same time the P. alba have certain important medicinal properties but when compared to the C. halicacabum it was less. These bioactive compounds present in C. halicacabum and P. alba which may give recovery to rat in the presence of toxic stress.

**Conclusion**

Based on the results it may be concluded that P. alba and C. halicacabum supplementation has a preventive and protective effects on atrazine induced oxidative stress. Moreover, it protects from atrazine induced hepatic dysfunction and executes its modulatory role in atrazine induced free radical production. Among the two plants, C. halicacabum possess more therapeutic properties than P. alba.

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**Reference**


Sheeba, M.S and Asha, V.V. 2009. *Cardiospermum halicacabum* ethanol extract inhibits LPS induced COX-2, TNF-α and iNOS expression, which is mediated by NF-κB regulation, in RAW 264.7 cells. *J. Ethnopharmacol.*, 124 : 39 - 44.


