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Full Length Research Paper

Antioxidant activity of methanolic extract of *Pongamia pinnata* on lead acetate induced hepatic damage in rats

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The antioxidant effect of methanolic extract of *Pongamia pinnata* was evaluated against lead acetate induced hepatic damage in male albino rats. Methanolic extract from the flowers of *P. pinnata* at a dose level of 150 mg/kg b.wt/day was administered orally daily once for 90 days. The substantially decreased enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) and non-enzymatic antioxidants like reduced glutathione (GSH), Vitamins C and E due to lead acetate treatment were restored towards normalization. Carvedilol at a dose level of 5 mg/kg b.wt/day was used as a standard reference also exhibited significant antioxidant activity against lead acetate induced hepatotoxicity. The results of this study strongly indicate that methanolic extract of *P. pinnata* has got a potent antioxidant action against lead acetate induced hepatic damage in rats.

Key words: Hepatoprotective, antioxidant enzymes, methanolic extract of *Pongamia pinnata,* lead acetate, carvedilol.

INTRODUCTION

Liver is an important organ and is actively involved in many metabolic functions and is the frequent target for a number of toxicants (Meyer and Kulkarni, 2001). Hepatic damage is associated with distortion of these metabolic functions (Wolf, 1999). Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Guntupalli et al., 2006). In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders (Chatterjee, 2000). In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity (Kirtikar and Basu, 1995). A single drug cannot be effective for all types of severe liver diseases (Shahani, 1999). Therefore, an effective formulation has to be developed using indigenous medicinal plants, with proper pharmacological experiments and clinical trials. With the above scenario, the methanolic extract of flowers of *Pongamia pinnata* was subjected to various assays in order to evaluate their antioxidant effect against lead acetate in albino rats. *P. pinnata* (Linn.) is a medium sized glabrous tree popularly known as karanja in hindi, Indian Beech in English and Pongam in Tamil (Krishnamurthi, 1969). *P. pinnata* is also called as Derris indica, is a monotypic genus and it grows abundantly along the coasts and riverbanks in Myanmar.

The seeds are reported to contain an average of about 28 to 34% oil with high percentage of polyunsaturated fatty acids (Sarma et al., 2005). Historically, *Pongamia* has been used as folk medicinal plant, particularly in Ayurvedha and Siddha systems of Indian medicine

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Abbreviations: SOD, Superoxide dismutase; **CAT,** catalase; **GPx,** glutathione peroxidase; **GR,** glutathione reductase; **GST,** glutathione S-transferase; **GSH,** glutathione; **CPCSEA,** committee for the purpose of control and supervision on experimental animals; **SPSS/PC,** statistical package for social sciences, personal computer; **DMRT,** Duncan's multiple range test; **ROS,** reactive oxygen species.

(Meera et al., 2003) for various kinds of diseases including diabetes mellitus (Punitha and Mahoharan, 2006). More recently, the effectiveness of *P. pinnata* as a source of biomedicines has been reported (Brijesh et al., 2006) specifically as antimicrobial and therapeutic agents. All parts of the plant have been used as a crude drug for the treatment of tumours, piles, skin diseases, itches, abscess, painful rheumatic joints wounds, ulcers, diarrhea, etc., (Meera et al., 2003; Shoba and Thomas, 2001). Besides, it is well known for its application as animal fodder, green manure, timber and fish poison. It has also been recognized to possess applications in agriculture and environmental management, with insecticidal and nematicidal activity (Brijesh et al., 2006). The objectives for the present investigation are to evaluated activity of the methanolic extract of *P. pinnata* against lead acetate toxicity when compared with carvedilol a well known antioxidant agent.

MATERIALS AND METHODS

Healthy adult male albino Wistar rats, bred and reared in Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used for the experiment. The weight of the animals ranged (160 to 180 g) were selected and housed in polypropylene cages layered with husk and kept in a semi-natural light/dark condition (12 h light/12 h dark). The animals were allowed free access to water and standard pellet diet (Amrut Laboratory Animal Feed, Pranav Agro Industries Limited, Bangalore, India). The experimental procedures were approved by the Institutional Animal Ethics Committee, Annamalai University, (Registration Number: 166/1999/CPCSEA, Pro. No. 491) and animals were cared in accordance with the "Guide for the care and use of laboratory animals" (NIH, 1985) and "Committee for the purpose of Control and Supervision on Experimental Animals" (CPCSEA, 2004).

All other chemicals and solvents were of analytical grade and purchased from S.D. Fine Chemicals, Mumbai and Himedia Laboratories Pvt. Limited., Mumbai, India. Hepatotoxicity was induced by the oral administration of freshly prepared of lead acetate solution (160 mg/kg b.wt./day) as described (Banu et al., 2006). The fresh flowers of *P. pinnata* was collected from the local gardens of STET Women's College, Mannargudi, Tamilnadu, India and voucher specimen are deposited submitted in the STET Herbarium at the Department of Botany and Microbiology, STET Women's College, Mannargudi, Tamil Nadu, India and then dried in shade for 15 days and made to coarse powder. The powder was passed through sieve No.40 to achieve uniform particle size and then used for extraction process. A weighed quantity of the powder was subjected to continuous hot extraction in soxlet apparatus with methanol, The extract was evaporated under reduced pressure using rotovac evaporator until all solvent was removed to give a molten extract. Those extract of *P. pinnata* was used for the study. Rats were divided into the following groups:

Group 1. Control rats.
Group 2. Rats continued to receive lead acetate and considered as toxic control.
Group 3. Rats were administered carvedilol (5 mg/kg b.wt/ day with 0.5 % methyl cellulose to facilitate dissolution and absorption) along with lead acetate.
Group 4. Rats were administered methanolic extracts of *P. pinnata* (150 mg/kg b.wt./ day) along with lead acetate.

After 90 days of treatment, the animals were fasted for 12 h and sacrificed by cervical dislocation. The liver tissues were dissected out, weighed and washed using ice cold saline solution. Liver tissues (250 mg) were sliced into pieces and homogenised (10% w/v) in Tris-HCl buffer (0.1 M; pH 7.4) and centrifuged at $3000x_g$ for 20 min at 4°C. The resulting supernatant was used for various biochemical assays. The antioxidant parameters such as SOD were assayed by the method of Kakkar et al. (1984). The activity of CAT was determined in tissue homogenate by the method of Sinha (1972). The activity of GPx was assayed in tissue homogenate by the method of Rotruck et al. (1973). GSH in tissues were estimated by the method of Boyne and Ellman (1972). Vitamin C was measured according to the method of Roe and Kuether et al. (1943). Vitamin E in tissues was estimated by the method of Desai (1971). GR (EC 1.6.4.2; GR) were assayed according to the methods of Rotruck et al. (1973) and Carlberg and Mannervik (1975). GST (EC 2.5.1.18; GST) activity was assayed according to the method of Habig et al. (1974). All quantitative measurements were expressed as means ± SD for control and experimental animals. The data were analyzed using one way analysis of variance (ANOVA) with the help of SPSS/PC (statistical package for social sciences, personal computer) and the group means were compared by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the p value is less than 0.05.

RESULTS

The SOD, CAT and GPx activity in the liver of control experimental animals are given in Table 1. SOD, CAT and GPx activities in the liver of rats treated with lead acetate (Group 2) were significantly lowered compared with control rats (group 1) ($P<0.05$), whereas admnini-stering methanolic extract of *P. pinnata* and carvedilol to lead acetate treated rats (Group 4) significantly elevated SOD, CAT and GPx activities compared to those animals on lead acetate treatment alone (Group 2). The activities of GR and GST in the tissues of both the control and experimental animals are given in Table 2. The levels of GR and GST were significantly lowered in the kidney of animals treated with lead acetate (Group 2) compared with the control rats (Group 1) ($P<0.05$). methanolic extract of *P. pinnata* at a dose of 150 mg/kg b.wt/day and carvedilol 5 mg/kg together with treatment significantly elevated the activities of GST and GR kidney tissues compared with those of the unsupplemented lead acetate treated rats (Group 2). As shown in Table 3, the concentration of GSH, Vitamin C and E were significantly lower in kidney of rats receiving lead acetate as compared to control rats. Treatment with methanolic extract of *P. pinnata* administered rats (Group 4) significantly elevated GS, vitamin C and E levels as compared to those on lead acetate administration along (Group 2).

DISCUSSION

In the present study, the activities of antioxidant enzymes like SOD, CAT and GPx, in rat liver was dramatically decreased by the treatment of lead. This decrease could

Table 1. Effect of methanolic extract of *P. pinnata* on liver SOD, CAT and GPx normal and lead acetate administered rats.

Group	SOD	CAT	GPx
Control	5.26 ± 0.50 [a]	58.69 ± 5.64 [a]	11.20 ±1.07 [a]
Lead acetate	0.49 ± 0.04 [b]	38.18 ± 3.67 [b]	5.32 ± 0.51 [b]
Lead acetate + Carvedilol	5.38 ± 0.51 [a]	56.42 ± 5.43 [a]	10.64 ± 1.02 [a]
Lead acetate+methanolic extract of *P. pinnata*	5.93 ± 0.57 [a]	57.28 ± 5.51 [a]	10.34 ± 0.99 [a]

Values are expressed as mean ± SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ DMRT. For SOD the units are: Enz. Req. for 50% inhibition of NBT rdn / min/ mg protein. For CAT the units are: µ moles of hydrogen peroxide utilized /min / mg protein. For GPx the units are: µg of GSH utilized /min / mg protein.

Table 2. Effect of methanolic extract of *P. pinnata* on the activities of liver, GR and GST of normal and lead acetate administered rats.

Group	GR	GST
Control	17.99 ± 1.73[a]	6.54 ± 0.63 [a]
Lead acetate	8.44 ± 0.81 [b]	3.61 ± 0.34 [b]
Lead acetate + Carvedilol	16.58 ± 1.59 [a]	5.78 ± 0.33 [a]
Lead acetate+methanolic extract of *P. pinnata*	14.19 ± 1.36 [a]	5.03 ± 0.48 [a]

Values are expressed as mean ± SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ DMRT. For GR the units are: µ moles of NADPH utilized / min/ mg protein. For GST the units are: µ moles of CDNB – GSH conjugate formed /min / mg protein.

Table 3. Effect of methanolic extract of *P. pinnata* on the activities of liver GSH, Vitamin C and Vitamin E of normal lead acetate administered rats.

Group	GSH (mmol/mg tissue)	Vitamin C (mg/dl)	Vitamin E (mg/dl)
Control	14.01 ± 1.34 [a]	0.67 ± 0.06 [a]	5.22 ± 0.50 [a]
Lead acetate	9.11 ± 0.87[b]	0.42 ± 0.04[b]	3.42 ± 0.33[b]
Lead acetate + Carvedilol	13.09 ± 1.26 [a]	0.73 ± 0.07 [a]	5.08 ± 0.48 [a]
Lead acetate + methanolic extract of *P. pinnata*	11.50 ± 1.10 [a]	0.60 ± 0.05 [a]	4.10 ± 0.39 [a]

Values are expressed as mean ± SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ DMRT.

be due to a feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS generation. The generation of H_2O_2 may also lead to inactivation of this enzyme (Ohata et al., 2004). SOD requires copper and zinc for its activity. The reduced activity of SOD inpresence of lead acetate may cause accumulation of $O_2^{\cdot-}$, H_2O_2 or the products of its decomposition. Balasubashini et al. (2004) reported that the SOD plays an important role in protecting tissues against oxygen free radicals. Copper ions play functional role in the reaction by undergoing alternate oxidation whereas zinc ions seem to stabilize the enzyme. Both the metal ions are replaced by lead, which decreases the activity of SOD.

CAT, which acts as preventive antioxidant and plays an important role in protection against the deleterious effects of lipid peroxidation (Dinkova-Kostova, 2002). The inhibition of CAT activity may be due to enhanced production of O_2^- and peroxyl radicals during the chronic administration of lead. Inhibition of heme synthesis by lead is well reported and since CAT is a heme-containing enzyme, its activity decreases (Mylroie et al., 1984). GPx is a selenium containing metalloenzyme, partially located within cellular membranes, which can remove hydrogen peroxide by converting, reduced GSH into oxidized GSH.GPx, can also terminate the chain reaction of lipid peroxidation by removing lipid hydroperoxides and H_2O_2 from the cell membrane (Roberta and Timothy, 1995).

GR, the enzyme responsible for recycling of GSH from the oxidized form (GSH disfulfide; GSSG) to the reduced form (reduced GSH) is also deprived by lead (Hunaiti et al., 1995). The decrease of GR enzyme activity during lead administration may be due to the inhibiting activity of cell membrane bound enzyme by the lead acetate. The present study reveals that decreased levels of GR in lead treated groups might be due to increased reactive

oxygen species (ROS) generation. Administration of methanolic extracts of *P. pinnata* and the reference drug carvedilol-improved the GR level protected the organs from the oxidative damage of tissues by reacting with ROS. The antioxidant treatment after lead administration helped to maintain the erythrocyte GSH content (Flora et al., 2004; Flora et al., 2003). GST is a multifunctional enzyme which utilizes GSH in the detoxification of xenobiotic compounds by enhancing the formation of GSH conjugates (Benson et al., 1978; Jakoby, 1978). GSH is an important constituent of intracellular protective mechanisms against various noxious stimuli including oxidative stress (Ross, 1988). The replenishment of GSH level in the methanolic extract of *P. pinnata* treated rats may be related to the antioxidant and free-radical scavenging effects of methanolic extract of *P. pinnata*.

Vitamin C protects plasma lipids against lipid peroxidation and has an important role in the regeneration of α-tocopherol (Patra et al., 2001). Vitamin E reacts with lipid peroxide to terminate the radical chain reaction in the membrane lipids (Hochslein and Rice-Evans, 1982). Thus it acts as a chain terminator and protects the cell against oxidative stress (Yoshito et al., 1991). In agreement with these reports, the depletion of tissue level of non enzymatic antioxidants in lead acetate intoxicated rats, which leads to the participation of free radicals in mediating the lead acetate induced oxidative injury. The direct free radical scavenging activity of methanolic extract of *P. pinnata* may improve the non enzymatic antioxidants reduction. The ability of methanolic extract of *P. pinnata* to enhance the levels of antioxidant activity suggest that this extract might be potentially useful in counteracting free radical-mediated injuries involved in the development of tissue damage caused by lead acetate. Hence, it merits further development for exploitation as a therapeutic agent and further research on the mechanism of action of *P. pinnata* is underway.

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