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Impact of *Pongamia Pinnata* extract on Lead acetate mediated Toxicity in Rat Liver

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Abstract: The hepato protective effect of the methanolic extract of *Pongamia Pinnata* flowers, an indigenous. Ayurvedic medicinal plant in India, was studied in rats with lead acetate induced hepatotoxicity. Administering 160 mg/kg b.wt/day of lead acetate for 90 days to male albino rats resulted in significantly elevated levels of ALT, AST, GGT, ALP, Bilirubin and protein were also observed on lead acetate administration as compared with those of the experimental control rats. Methanolic *Pongamia Pinnata* flowers was administered at a dose of 150 mg/kg b.wt / day for the 90 days of experiment to lats with lead acetate induced liver injury, which significantly decreased the levels of ALT, AST, ALP, GGT, Bilisubin and protein compared to that untreated lead acetate administered rats. Thus, the data indicate that treatment with methanolic *Pongamia Pinnata* flowers against toxicity in liver of animals with lead acetate induced liver injury.
Key words: Hepatic markers, lead acetate, methanol, *Pongamia Pinnata*, liver, carvedilol.

Introduction

Heavy metals constitute a heterogeneous group of elements widely varied in chemical properties, biological function and are placed under environmental pollutant category due to their toxic effects on plants, animals and human beings. Exposure to toxic metals has become an increasingly recognized source of illness worldwide[1]. Heavy metals are persistent in nature, therefore get accumulated in soil and plants. Dietary intake of many heavy metals through consumption of plants has long-term deterimental effects on human health[2]. Contamination of heavy metals in the environment is a major global concern because of their toxicity and threat to human life and environment[3,4]. The earlier reports reveals that on heavy metal contamination in soils from various anthropogenic sources such as industrial wastes[5],automobile emissions[6], mining activity[7] ,and agricultural practices[8].The group of heavy metals are about 65 and are defined in a number of criteria such as their cationic-hydroxide formation, specific gravity greater than 5 g/ml, complex formation, hard-soft acids and bases, etc.,

Carvedilol is metabolized extensively in animals and humans, and its pharmacokinetics were described previously for monkeys and humans[9,10].Fujimaki and Hakusui[11,12] showed that, in rats, carvedilol metabolites were secreted primarily in bile, and those authors described the two major biliary metabolites, which were formed by aromatic ring hydroxylation and subsequent glucuronidation. Hydroxylation of carvedilol in rats occurred with some stereoselectivity[13]and the adiolabeled carvedilol metabolites were shown to undergo enterohepatic recycling[11].Search for hepatoprotective agents has

made man turn to alternative sources viz. indigenous system of medicine.It is a well-documented fact that most medicinal plants are enriched with bioflavonoids, which have hepatoprotective property. *Pongamia pinnata* (L.) Pierre (Fabaceae) is one such plant containing a number of bioactive compounds.

Pongamia pinnata (Linn.) is a medium sized glabrous tree popularly known as karanja in hindi, Indian Beech in English and Pongam in Tamil[14]. Most of the Indian system of traditional medicine Ayurveda and Siddha used to treat *P.pinnata* for various kinds of diseases including diabetes mellitus[15].*P.pinnata* also called as Derris indica, is a monotypic genus and grows abundantly along the coasts and riverbanks in Myanmar. The seeds are reported to contain on an average about 28 – 34% oil with high percentage of polyunsaturated fatty acids[16]. Historically, *Pongamia* has been used as folk medicinal plant, particularly in Ayurvedha and Siddha systems of Indian medicine[17]. 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, diarrhoea[18] etc., 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. More recently, the effectiveness of *P. pinnata* as a source of biomedicines has been reported[19] specifically as antimicrobial and therapeutic agents. The objectives were considered for the present investigation. are the changes in the activity of hepatic marker enzymes (AST, ALT, ALP and GGT), protein and bilirubin level in the serum.

Materials and Methods

Experimental Animals

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 (160-180 g) were selected and housed in polypropylene cages layered with husk and kept in a semi-natural light/dark condition (12 h light/12 hours dark). The animals were allowed free access to water and standard pellet diet (Amrut Laboratory Animal Feed, Pranav Agro Industries Ltd., 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).

Source of chemical

All other chemicals and solvents were of analytical grade and purchased from S.D. Fine Chemicals, Mumbai and Himedia Laborateries Pvt.Ltd., Mumbai, India.

Experimental induction of hepatotoxicity

Hepatotoxicity was induced by the oral administration of freshly prepared of lead acetate solution (160 mg/kg b.wt./day) [20].

Preparation of the *Pongamia pinnata* extract

The flowers were collected and 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.

Experimental design

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 *Pongamia 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. Blood was collected in a cleaned tube with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of various biochemical parameters.

Analysis of Blood and Tissue samples

Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 40 min. Serum was separated by centrifugation at 2000 rpm for 10 minutes.Activities of AST and ALT were assayed by the method of Reitmann and Frankel, (1957) [21]. Alkaline phosphatase was assayed by the method of King and Armstrong, (1934) [22]. The enzyme activity was assayed according to the method of Rosalki and Rau, (1972) [23]. The protein content was estimated by the method of Lowry *et al*., (1951) [24]. Serum bilirubin was estimated by the method of Malloy and Evelyn, (1937) [25].

Table 1: Changes in the concentration of hepatic marker enzymes of serum AST, ALT, ALP GGT total bilirubin and total protein of control and treated rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	Total bilirubin (mg/dl)	Total Protein (g/dl)
Group I	95.48 ± 9.19[b]	34.94 ± 3.36[b]	19.33 ± 1.86[b]	3.38 ± 0.32[a]	1.59±0.15[c]	5.56±0.53[a]
Group II	167.93± 16.16[a]	135.31±13.02[a]	96.65 ± 9.20[a]	9.49 ± 0.91[d]	2.38±0.23[a]	4.40±0.42[b]
Group III	103.97±10.00[b,c]	42.28 ± 4.07[b,c]	22.52 ± 2.16[b]	4.49 ± 0.43[b]	1.46±1.14[c]	5.44±0.52[a]
Group IV	116.51±11.21[c]	55.75 ± 5.36[d]	35.55±3.42[c]	5.28±0.50[c]	2.17±0.20[d]	5.00±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).

Statistical Analysis

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

Hepatic Marker enzymes

AST

The treatment of astragalin, MeOH extract of *P.pinnata* on lead acetate administration resulted a significant ($p<0.05$) increase the activity of serum AST when compared to control. Administration of MeOH extract of *P.pinnata*, significantly reduced the toxicity of lead acetate on liver and retained the AST concentration as to that of normal (**Table.1).**

ALT

There was a significant elevation of serum ALT than normal controls, it indicates that the lead acetate induced damage on the hepatic cells. A significant ($p<0.05$) reduction was observed in ALT in rats treated with carvedilol and MeOH extract of *P.pinnata* than lead acetate treated group. The decrease was maximum ($p<0.05$) in rats which received a dose of 20 mg/kg of astragalin Table 1.

ALP

The activity of ALP was decreased in serum of lead acetate treated groups (77 %) when compared with those of the control. there were slight variations in the ALP activity on MeOH extract of *P. pinnata* administrated serum than control rats (54%) (Table 1).

GGT

The concentration of GGT was significantly reduced in rats intoxicated with lead acetate, compared to control. The co-administration of carvedilol and MeOH extract of *P.pinnata* were able to restored the activity of GGT, similar to that of control (Table 1).

Total Bilirubin

Lead acetate intoxication in normal rats significantly elevated the level of total bilirubin in serum, whereas there was a significant decrease in the level of total bilirubin by the administration of carvedilol and MeOH extract of *P.pinnata* (Table 1).

Total Protein

The level of protein were significantly decreased in lead acetate treated group when compared to control group (Table 1).Administration of carvedilol and MeOH extract of *P.pinnata* significantly increased the level of total protein.

Discussion

Effect on Liver function Markers

Generally heavy metals may exert their cytotoxic effects by damaging cell membranes. When liver cell plasma membrane is damaged, variety of enzymes normally located on the cytosol is released into the blood stream. The present results indicates that lead acetate ingestion induced a significant elevation of serum ALT, AST and ALP in lead acetate treatment. Their estimation in serum is a useful quantitative marker of the extent and type of hepatocellular damage[26]. Since aminotransferases (ALT and AST) are an important class of enzymes linking carbohydrate and amino acid metabolism, the relationship between the intermediates of the citric acid cycle is well established[27]. Hassanin, (1994)[28] reported that the elevated serum ALT significantly more than AST on lead exposure which indicates liver damage[29] and development of fibrosis[30]. Lead binds to plasmatic proteins, where it causes alterations in a high number of enzymes.

Treatment with MeOH extract of *P.pinnata* significantly reduced the activities of the liver marker enzymes in lead acetate treated rats. This indicates that MeOH extract of *P.pinnata* tends to prevent liver damage by maintaining the integrity of the plasma membrane, thereby suppressing the leakage of enzymes through the membranes, exhibiting hepatoprotective activity. It can also perturb protein synthesis is hepatocytes[31] (Georing, 1993). The observed decrease in protein content of plasma of rats treated with Pb may be due to decreased hepatic DNA and RNA[29]. The decrease in the level of total protein observed in lead actate treated rats may be associated with the decrease in the number of hepatocytes, which inturn may result in decreased capacity to synthesize protein. An increased level of serum bilirubin was observed it could be due to the toxicity of lead on hemoglobin. El-Zayat *et al*. (1996) [32] and Hassanin, (1994) [28] reported that the decrease in hepatic total protein content is in response to lead intoxication and the serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. It provides useful information on how well the liver is functioning[33]. Bilirubin, a chemical breakdown product of hemoglobin, is conjugated with glucuronic acid in hepatocytes to increase its water solubility.

Hepatic marker enzymes AST, ALT, ALP, GGT,total bilirubin and total protein also elevated in the serum of lead acetate intoxicated rats, showing the functional abnormality of liver. The co-administration of MeOH extract of *Pongamia Pinnata* with lead acetate represents its ability to maintain the normal functional status of liver. The protective effect of MeoH extract of P.P was almost equal to that of the standard drug carvedilol, with respect to biochemical parameters, and, histopathological observations and proinflamatory cytokines in the liver.

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