

INVESTIGATION OF HEPATOPROTECTIVE ACTIVITY OF SPONDIAS PINNATA

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ABSTRACT

Spondias pinnata (Anacardiaceae) stem heart wood are well known in India as Jangliaam (Hindi), Adavimaamidi, Kondamaamidi (Telugu), Common hog plum, Indian mombin (English) of ethyl acetate and methanolic extracts was prepared and tested for its hepatoprotective effect against carbon tetrachloride induced in rats. Alteration in the levels of biochemical markers of hepatic damage like SGPT, SGOT, ALP, bilirubin were tested in both treated and untreated groups. Carbon tetrachloride has enhanced the SGPT, SGOT, ALP and bilirubin levels. Treatment with ethyl acetate extract of *S. pinnata* stem heart wood (100, 200 and 400 mg/kg) has brought back the altered levels of biochemical markers to the near normal levels in the dose dependent manner. This was evident from significant reduction in serum enzyme, SGOT, SGPT, ALP and Total bilirubin (TB). Various pathological changes like centribular necrosis and vacuolization were observed in CCl₄ treated rats, which were significant protective activity in groups treated with SP and silymarin. It was concluded from the study that ethyl acetate and methanolic extracts of SP possess hepatoprotective activity against CCl₄ induced hepatotoxicity in rats.

Keywords: Hepatoprotective, *Spondias pinnata*, Carbontetrachloride, Silymarin.

INTRODUCTION

Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, ALP and bilirubin levels are elevated[1,2]. However there are several herbs/herbal formulations claimed to possess beneficial activity in treating hepatic disorders.

Spondias pinnata (Anacardiaceae) stem heart wood are well known in India as Jangliaam (Hindi), Adavimaamidi, Kondamaamidi (Telugu), Common hog plum, Indian mombin (English)[4]. It was found that this plant contains sterols, flavonoids and gums[3]. There are reports which showed that fruits are astringent and antiscorbutic, also used in bilious dyspepsia. Bark astringent and refrigerant, used in diarrhea and dysentery; a paste of it applied in rheumatism. Roots employed for regulating menstruation[4]. Previously isolated compounds are β -Amyrin, oleanolic acid and amino acids(alanine, leucine) from *S. Pinnata*[5]. However, there are no significant basis or reports in the modern literature regarding its usefulness as hepatoprotective agent. Thus the present study was conducted to evaluate the hepatoprotective activity of the ethyl acetate and methanol extract of the *S. pinnata* stem heart wood by using CCl₄ induced hepatic injury in rats.

MATERIALS AND METHODS

Plant Material Collection:

Spondias pinnata (Anacardiaceae) stem heart wood were collected from the Salur, Vizianagaram district area, India in the month of December 2007 and authenticated by the taxonomist, Department of Botany, Andhra University and the specimen voucher no AUCP/BGR/2007/A56 was preserved in the Department.

Acute toxicity studies:

Acute toxicity studies were performed for extracts of selected plant according to the toxic classic method as per guidelines. None of these extracts showed mortality even at a dose of 1000mg/kg and therefore considered safe.

Toxicological studies were conducted in mice (N=6) for all the extracts as per the Irvin's method [6] at the doses of 100, 300 and 1000 mg/kg, no mortality was observed.

Materials:

All the materials used for this experiment are of Pharmacopoeial grade. Carbon tetrachloride (E. Merck), silymarin (Sigma Chemical Co.) and olive oil were purchased from the local supplier. Diagnostic kits for the estimation of SGOT, SGPT, SALKP and serum bilirubin were purchased from local supplier (Sai chemicals)

manufactured by Ranbaxy Diagnostics Ltd., New Delhi, India. Water represents the double distilled water, standard orogastric cannula was used for oral drug administration.

Animals:

Wistar albino rats of either sex weighing between 200-250 gm were obtained from M/s. Mahavir Enterprises, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of $22 \pm 1^\circ\text{C}$ with an alternating 12 h light–dark cycle and relative humidity of $60 \pm 5\%$), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and by the Regulatory body of the government (Regd no. 516/01/a/CPCSEA). They were fed with standard laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, Andhra Pradesh, India. Food and water was allowed *ad libitum* during the experiment.

CARBONTETRACHLORIDE-INDUCED HEPATOTOXICITY:

The animals were divided into nine groups of six animals each. Group-I served as normal control received 5% acacia mucilage (1 ml/kg.p.o) daily once for 7 days. Group-II served as toxic control and received CCl_4 (1 ml/kg i.p) daily once for 7 days [7]. Group-III was treated with the standard drug Silymarin (50 mg/kg .p.o) and followed by CCl_4 (1 ml/kg i.p) daily once for 7 days[8]. Groups IV-VI were treated with ethyl acetate extract of *S. pinnata* stem heart wood at doses of 100, 200 & 400mg/kg p.o. in acacia mucilage respectively followed by CCl_4 (1 ml/kg i.p) daily once for 7 days. Groups VII-IX were treated with methanol extract of *S. pinnata* stem heart wood at doses of 100, 200 & 400mg/kg p.o. in acacia mucilage respectively followed by CCl_4 (1 ml/kg i.p) daily once for 7 days. After completion of treatment blood was collected, serum was separated and used for determination of biochemical parameters.

COLLECTION OF BLOOD SAMPLES

All the animals were sacrificed on 7 th day under light ether anesthesia. The blood samples were collected separately in sterilized dry centrifuge tubes by puncture retro-orbital plexes and allowed to coagulate for 30 min at 37°C . The clear serum was separated at 2500rpm (Microcentrifuge) for 10min and subjected to biochemical investigation viz., serum glutamic oxaloacetate transe aminase (SGOT), serum glutamic Pyruvate transe aminase (SGPT), Alkaline phosphatase (ALP) and Total Bilirubin (TB).

ASSESSMENT OF LIVER FUNCTION

The Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by UV kinetic method in which both SGOT and SGPT were assayed based on enzyme coupled system; where keto acid formed by the aminotransaminase reacts in a system using NADH. The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm for SGOT malate dehydrogenase (MDH) reduces to malate with simultaneous oxidation of NADH to NAD. The rate of oxidation of NADH is measured, where as SGPT [9] the pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase. Estimation of Alkaline phosphate (ALKP)[10] involves hydrolysis of P-nitrophenyl phosphate by alkaline phosphatase to give P-nitrophenol, which gives yellow color in alkaline solution. The increase in absorbance due to its formation is directly proportional to alkaline phosphate (ALKP) activity. Estimation of total bilirubin (TB) [11] involved the reaction of bilirubin with diazotized sulphanic acid to form an azocompound, the color of which is measured at 546 nm. All the estimations were carried out using standard kits in semi auto analyzer Screen Master 3000.

STATISTICAL ANALYSIS:

Results of biochemical estimation were reported as mean \pm SEM for determination of significant inter group difference was analyzed separately and one-way analysis of variance (ANOVA) was carried out[12]. Dunnet's test was used for individual comparisons [13].

RESULTS AND DISCUSSIONS:

Serum levels of SGOT, SGPT, SALKP and total bilirubin were significantly increased ($p < 0.01$) in carbon tetrachloride treated Group-2 rats. Group-3 rats treated with Silymarin produced significant reduction ($p < 0.01$) in SGOT, SGPT, SALKP and total bilirubin levels.

In Groups: 4-6 treated with ethyl acetate extract of *Spondias pinnata* at doses of 100, 200 and 400mg/kg; p.o respectively, there is significant decrease in SGOT, SGPT, SALKP and total bilirubin levels when compared to Group-2 rats. The activity of the extracts is found to be dose dependant. The results were given in Table-(1 & 1.1) and Figure-1.

TABLE- 1: Effect of ethyl acetate and methanol extracts of *S. pinnata* on biochemical estimation of SGOT, SGPT, SALKP and total bilirubin of CCl₄ induced toxicity in rats.

Groups	SGOT (IU/ml)	SGPT (IU/ml)	SALKP (IU/ml)	Total Bilirubin (mg/dl)
Control 1ml/kg	160.5 ± 0.62	96.95 ± 1.34	179.5 ± 0.99	0.82 ± 0.06
CCl ₄ 1ml/kg	295.5 ± 0.39 ⁺	269.5 ± 1.8 ⁺	296.5 ± 1.45 ⁺	2.02 ± 0.03 ⁺
Silymarin 50mg/kg	174.8 ± 1.88***	107.5 ± 1.45***	187.7 ± 2025***	0.89 ± 0.04***
SPEE 100 mg/kg	241.3 ± 2.84*	200.83 ± 0.60**	206.5 ± 3.40*	1.34 ± 2.42*
SPEE 200 mg/kg	228.2 ± 7.40**	184.23 ± 2.84**	197.5 ± 1.91***	1.27 ± 1.27**
SPEE 400 mg/kg	188.9 ± 2.35***	131.03 ± 1.38***	195.4 ± 3.05***	1.06 ± 1.43***
SPME 100 mg/kg	253.1 ± 1.09*	228.3 ± 2.13**	246.5 ± 3.40*	1.48 ± 3.26*
SPME 200 mg/kg	231.3 ± 4.73**	214.0 ± 3.26**	237.3 ± 2.24**	1.37 ± 2.31**
SPME 400 mg/kg	212.4 ± 2.24***	190.3 ± 2.16***	231.3 ± 4.21***	1.24 ± 1.07***

Values are mean ± SEM for six observations

P: ⁺<0.001 Compared to respective control group-1

P: *<0.05, **<0.01, ***<0.001 Compared to respective control CCl₄ group-2

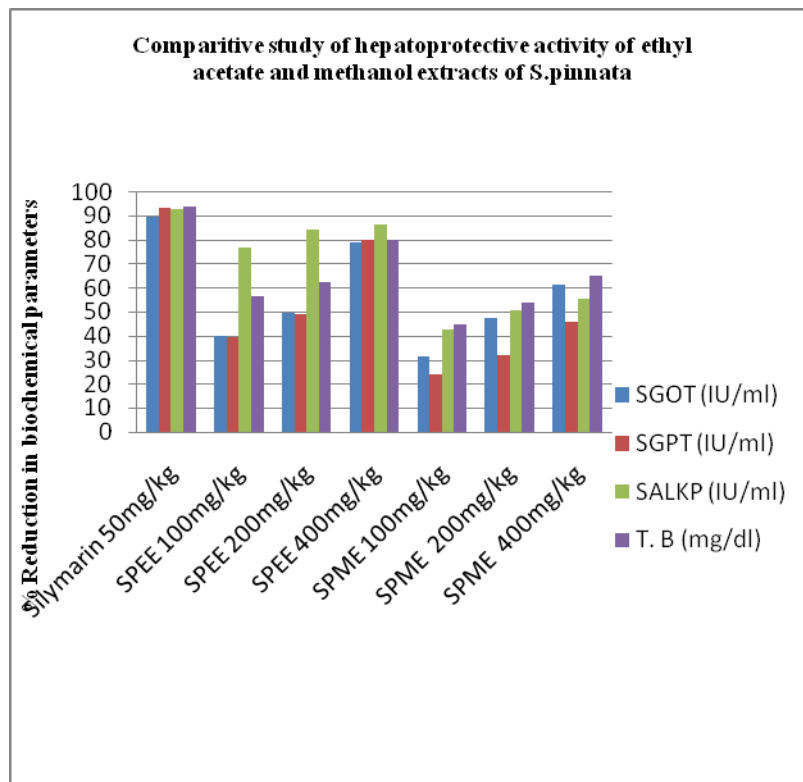
SPEE- *Spondias pinnata* ethyl acetate extract, SPME- *Spondias pinnata* methanol extract

TABLE-1.1: Percentage Reduction Of Various Biochemical parameters Due To Treatment With Ethyl acetate And Methanol Extracts Of *S. pinnata* Against CCl₄ Induced Hepatotoxicity In Rats.

TREATMENT	SGOT (IU/ml)	SGPT (IU/ml)	SALKP (IU/ml)	T. B (mg/dl)
Silymarin 50mg/kg	89.63	93.8	92.99	94.16
ALEE 100mg/kg	31.33	37.89	57.6	50.83
ALEE 200mg/kg	64.81	56.66	71.79	64.99
ALEE 400mg/kg	75.85	78.27	82.05	71.66
ALME 100mg/kg	33.77	39.5	60.68	53.33
ALME 200mg/kg	67.62	57.64	79.48	69.16
ALME 400mg/kg	77.92	81.46	83.76	75.83

The comparative efficacy of the extracts tested for their hepatoprotective activity, the relationship between dose and percentage reduction in each case were depicted in the form of a bar diagram as shown(fig-1).

Figure-1: Effect of ethyl acetate and methanol extracts of *S. pinnata* on biochemical estimation of SGOT, SGPT, SALKP and total bilirubin of CCl_4 induced toxicity in rats.



Carbontetrachloride (1ml/kg.i.p) intoxication in normal rats produced elevated levels of serum biochemical parameters significantly SGOT(160.5 ± 0.62 , 295.5 ± 0.39), SGPT(96.95 ± 1.34 , 269.5 ± 1.8), ALKP(179.5 ± 0.99 , 296.5 ± 1.45), T.B(0.82 ± 0.06 , 2.02 ± 0.03) indicating acute hepatocellular damage and biliary obstruction. The percentage reduction of various serum biochemical parameters in case of standard drug Silymarin(50mg/kg.p.o) in CCl_4 intoxicated rats revealed a significant reduction ($p < 0.01$) in the levels of SGOT(89.63%), SGPT(93.8%), ALKP(92.99%) and T.B(94.16%).

When compared to the CCl_4 toxic control group, the group treated with the ethyl acetate extracts of *Spondias pinnata*, at doses of 100, 200 and 400mg/kg; p.o in CCl_4 intoxicated rats exhibited a significant reduction ($p < 0.01$) of SGOT(40.18%, 49.85%, 78.96%), SGPT(39.80%, 49.42%, 80.24%), ALKP(76.92%, 84.61%, 86.41%) and T.B(56.66%, 62.49%, 79.99%) levels respectively.

In Groups: 7-9 treated with methanol extract of *Spondias pinnata* at doses of 100, 200 and 400mg/kg; p.o respectively, there is significant decrease in SGOT, SGPT, SALKP and total bilirubin levels when compared to Group-2 rats. The activity of the extracts is found to be dose dependant.

The comparative efficacy of the extracts tested for their hepatoprotective activity, the relationship between dose and percentage reduction in each case were depicted in the form of a bar diagram as shown.

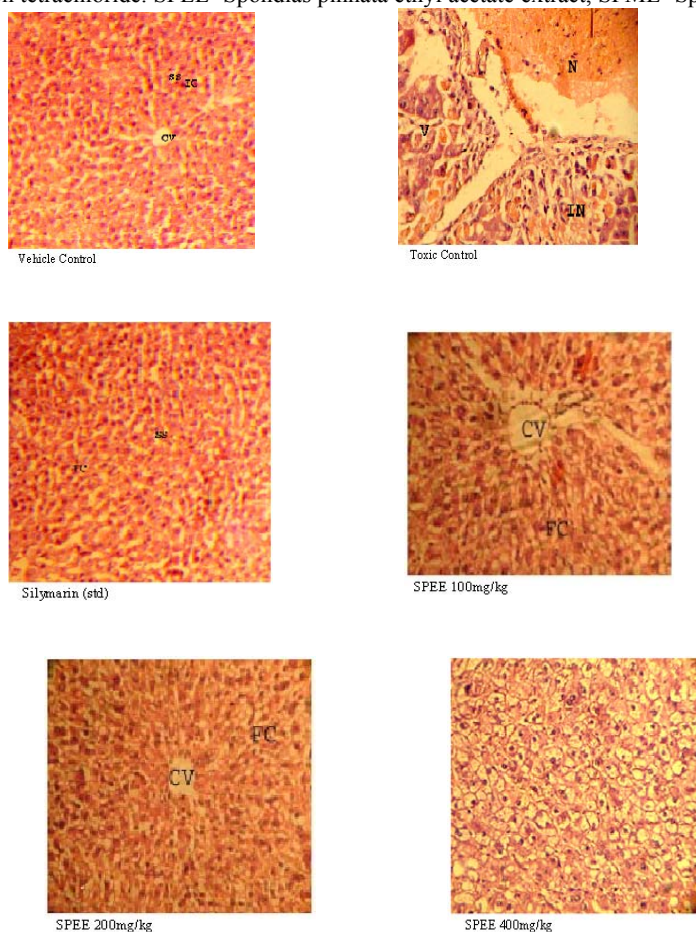
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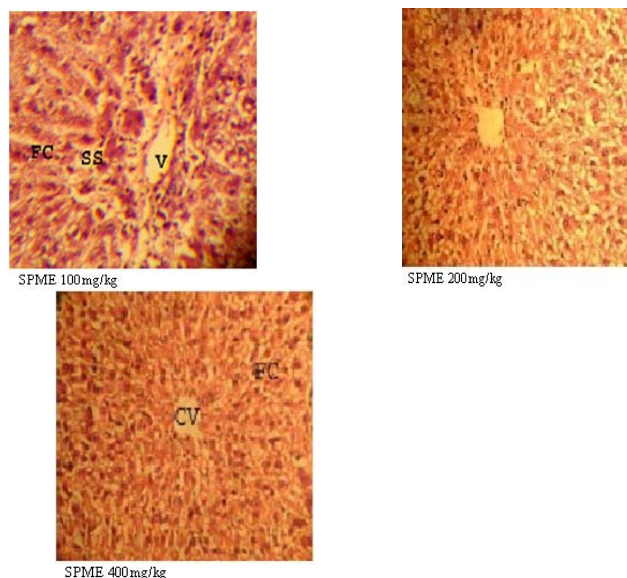
When compared to the CCl₄ toxic control group, the group treated with the methanol extracts of *Spondias pinnata*, at doses of 100, 200 and 400mg/kg; p.o in CCl₄ intoxicated rats exhibited a significant reduction ($p < 0.01$) of SGOT(31.40%, 47.55%, 61.55%), SGPT(23.87%, 32.15%, 45.88%), ALKP(42.73%, 50.59%, 55.72%) and T.B(44.99%, 54.16%, 64.99%) levels respectively.

Though both the extracts were recorded with significant hepatoprotective activity with same “p” value ($p < 0.01$). The ethyl acetate extract was found to be more potent than methanol extract because of effect on percentage reduction in elevated levels of biochemical parameters and effect was dose dependant.

The effect of ethyl acetate and methanol extracts of stem on CCl₄ induced liver damage in rats with reference to biochemical changes in serum was shown. Percentage decrease or increase was calculated by Histopathology of liver tissues. Group I (vehicle control)—section shows central vein surrounded by hepatic cord of cells (normal architecture). Group II (toxic control)—section shows patches of liver cell necrosis with inflammatory collections, around central vein. Group III (standard silymarin)—almost near normal. Group IV (SPEE 100mg/kg)— less inflammation around central vein. Group V (SPEE 200mg/kg)— less inflammatory cellular infiltration. Group VI (SPEE 400mg/kg)— minimal inflammatory cells Almost normal liver. Group VII (SPME 100mg/kg)— inflammatory collections around central vein and focal necrosis. Group VIII (SPME 200mg/kg)— inflammation decreasing around central vein. Group IX (SPME 400mg/kg)— less inflammatory cells around central vein, absence of necrosis (Fig-1A).

Figure-1A: Representative photographs of histopathological changes showing effect of the test material on the rats intoxicated with carbon tetrachloride. SPEE- *Spondias pinnata* ethyl acetate extract, SPME- *Spondias pinnata* methanol extract





CONCLUSION:

The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects or in maintaining the normal hepatic physiological mechanism, which have been imbalanced by a hepatotoxin.

Orally administered doses of 100, 200 and 400mg/kg of methanol of stem heart wood of *S. pinnata* produced significant decrease in SGOT, SGPT, SALKP and total bilirubin levels. The activity of the extracts is found to be dose dependant. In CCl_4 induced toxic hepatitis, toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures [14]. Administration of methanolic extracts of *S. pinnata* showed recovery against the toxic effects of CCl_4 . The hepatoprotective effect of the drugs was further concluded by the histopathological examinations of the liver sections which reveal that the normal liver shape was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with ethyl acetate extract and methanolic extract and intoxicated with CCl_4 the normal cellular shape was retained as compared to silymarin, thereby confirming the protective effect of the extracts of *S. pinnata*

The hepatoprotective activity of *S. pinnata* could be due to the presence of bioflavonoids which have hepatoprotective properties [15-17]. The result of this investigation indicated that the methanolic extract of stem heart wood of *S. pinnata* possess hepatoprotective activity against CCl_4 induced liver damage in rats. Attempts are being made to isolate and characterize the active principle to which the hepatoprotective activity can attribute.

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