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RESEARCH ARTICLE

Hepatoprotective Activity of Fruit Extract of the Plant Sapindus Trifoliatus against CCl_4 induced Hepatic Damage in Rats

Prasenjit Mishra[1], Bibhuti Bhusana Panigrahi[*1], Rjendra Sekhar Rath[1], Biswaranjan Sarangi[1], Nilima Shukla[2]

[*1]*Hi-Tech College Pharmacy, Hi-Tech Medical College & Hospital Campus, Bhubaneswar-751025, Odisha, India.*

[2]*Sri Jayadev College of Pharmaceutical Sciences, Naharkanta, Bhubaneswar, Odisha, India.*

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ABSTRACT

To investigate the effect of ethanolic and aqueous extract of pericarps of *Sapindus trifoliatus* (ST) in *In - vivo* models. (Family: Sapindaceae) Ethanol and aqueous fruit extracts of *S. trifoliatus* demonstrated hepatoprotective activity against carbon tetrachloride induced liver damage in rats. The parameters studied were serum total bilirubin, total protein, alanine transaminase, aspartate transaminase and alkaline phosphatase activities. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Results of the biochemical studies of blood samples of CCl_4 treated animals showed significant increase in the levels of serum markers and decrease in total protein level reflecting the liver injury caused by CCl_4. Whereas blood samples from the animals treated with ethanol and aqueous fruit extracts showed significant decrease in the levels of serum markers and increase in total protein indicating the protection of hepatic cells. The results revealed that ethanolic extract followed by aqueous extract of *S. trifoliatus* fruit could afford significant protection against CCl_4 induced hepatocellular injury.

KEYWORDS

Hepatoprotective activity, *S. trifoliatus* (S.T.), SGOT (S.A.T.), SGPT, ALP, T.P

INTRODUCTION

The plant S. trifoliatus (family-sapindaceae) is a medium size tree plentily available in forest division, Malkangiri District in Orissa and throughout India. The plant is locally known as ritha. It is known locally as soapnut tree. The plant has been reported for its high content of saponin, flavonoids and sugars[2,3] as depicted by *Arulomozhi et. al*. The saponin moeity is characterized as the hederagenin[2] group of glycosides after the phytochemical test/evaluation.

***Address for Correspondence:**
Dr. Bibhuti Bhusana Panigrahi
Professor of Pharmaceutics, Hi-Tech College of Pharmacy,
Hi-Tech Medical College & Hospital Campus,
Pandra, Raulgarh, Bhubaneswar-751025, Odisha, India
E-Mail Id: drbbpanigrahi@gmail.com

The pericarp is reported for its various medicinal property. It is regarded as a tonic, stomachic, spermicidal and is used in the treatment of hemicrania (migraine, hysteria, epilepsy etc).

MATERIALS AND METHOD

The dried pericarps of the fruits of *S. trifoliatus* (Family: Sapindaceae) were collected from the local market and were authenticated by Dr. S. P. Rath, Botanist Deptt. of Botany, Utkal University, Bhubaneswar, matched with the specimen with the existing herbarium No.109. Ethanolic extraction was done with according to *Arulomozhi et. al.* order following

continuous/successive extraction method. Aqueous extract of S.T fruit was prepared and reported.[3,4]. In ethanolic extract hederagenin, flavonoids, alkaloids were found[2,3]. Briefly one hundred gram of the pericarp soaked in 400ml of distilled water for 16h. The percolate was then decanted, centrifuged and filtered through Whatman (No.1) filter paper to obtain clear extract (300ml). This process of extraction was repeated again with the same volume of distilled water. The percolates were pooled and lyophilized which yielded a brown colored powder (70% yield). Acid hydrolysis of the extract yielded only one glycone, which was identified as hederagenin[2,3]. Therefore estimation of this saponin present with extract was calculated as hederagenin[2,3]. The content of hederagenin was estimated in the extract by boiling it with 50% methanolic hydrochloric acid. The entire mixture was evaporated to dryness and reconstituted in methanol. The concentration of hederagenin was found to be between 5.65-6.5% by weight of the extract. The whole extract had been taken for phytochemical evaluation and confirmed the compound as saponin, flavonoids and alkaloids[2,3.] Rest 93.5 to 94.35 of confirmed compound flavonoids and alkaloids.

Animals

Adult male Swiss albino mice (18-22g) and Wister rats (175-200g) were obtained from Orissa University of Agriculture & Technology, Animal Husbandry Division (OUAT) Bhubaneswar. On arrival they are randomly divided into various treatment groups in polypropylene cages with paddy husk as bedding or paddy husk bud. Animals were housed at a temperature 24±2^{0}C at relative humidity of 30.70%. A 12: 12, light: dark cycle was followed. All animals had free access to water filtered through aqua guard and standard pelleted laboratory animal diet. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee, Hi-Tech Medical College & Hospital, Bhubaneswar, Odisha and were in accordance with the guidelines of Committed for the purpose of control and supervision of Experiment on Animals. (CPCSEA), Ministry of Forests and Environment, Government of India.

Acute Toxicity Studies

Acute toxicity study was conducted for both the extracts by stair case method[4] following OECD guidelines 2002. The LD_{50} found to be 350-mg/kg body weight P^o in rat, one tenth of this i.e. (35 mg/kg, P^o) was selected as maximum dose for the evaluation of antihepatotoxic activity[5]. (Table-1)

Table 1: Acute toxicity study of the fruit extract of the plant *Sapindus trifoliatus* in rats

Dose (Mg/kg body weight)	No. of Animals Died / Survived	
	Aqueous extract	Ethanolic extract
50	0/10	0/10
100	0/10	0/10
150	0/10	0/10
200	1/9	2/8
250	2/8	3/7
300	4/6	4/6
350	*5/5	*5/5
400	8/2	10/0
450	10/0	10/0

→ *50% mortality is considered as lethal dose (LD_{50})

→ There was no motality or any adverse symptoms upto 350Mg/Kg of aqueous & ethanolic extract of *Sapindus trifoliatus* fruits.

→ Onward to the 350Mg/Kg dose all dose shows motality.

→ 1/10Th of LD_{50} was selected for antihepatotoxic activity.

→ The actual contents of Hendergein (15 –20 Mg), and the Rest Part Of alkaloids, flavoniods.

Evaluation of Hepatoprotective Activity

The animals were divided into 5grs of 6 rats each. The animals in Group-I served as control and received the vehicle (1ml/kg / day of 5% w/v Carboxy methyl cellulose P^o) for 14 days. All the animals of Group-II to V received 0.1 ml/kg / day CCl_4 IP (E-Merck, Mumbai, India) for 14 days[6]. Group-III animals received the standard drug Silymarin (100mg/kg / day P^o, Ranbaxy Lab Dewas) for 14 days. Both Aqueous and ethanolic fruit extract (35mg/kg / day, P^o) of *S. trifoliatus* were administered to the animals of Group-IV and -V respectively for 14 days. The CCl_4 silymarin and the extracts were administered concomitantly to the respective group of animals.

The animals of all the groups were sacrificed by light ether anesthesia on 14th day. The blood sample of each animal was collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30min at 37^0C. The clear serum was separated at 1000g for 10min and was subjected to biochemical investigation viz. total bilirubin[7], total protein[8], serum alanine transaminase, serum aspartate transaminase[9] and alkaline phosphatase[10].

Results of biochemical estimations were reported as Mean ± SEM of 6 animals in each group. The data were subjected to one-way ANOVA followed by students *t*- test. $P \leq 0.01$ was considered as statistically significant.

Histopathology

The liver sample were excised from the experimental animals of each group and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin for 48hr and then with bovine solution for 6hr and they were processed from paraffin embedding. The sections were taken at 5mm thickness using microtome & processed in alcohol-Xylene series and were stained with alum hematoxylin and eosin[11].

The sections were examined microscopically for the evaluation of histopathological changes.

RESULTS

Effects of aqueous and ethanolic fruit extract of *S. trifoliatus* on CCl_4 induced liver damage in rats with reference to biochemical changes in serum are shown in Table-2.

As per the procedure followed At the end of 14, 17, 20 days treatment Mehendale et. al., blood samples of CCl_4 treated animals showed significant increase in the levels of total bilirubin, alanine transminase, aspartate transminase and alkaline phosphatase compared to normal control groups but the total protein level decreased reflecting the liver injury caused by CCl_4.

Whereas blood samples from the animals treated with aqueous and ethanol fruit extract of *S. trifoliatus* showed significant decrease in the levels of serum markers and significant increase in total protein to the near normal which are comparable to the values registered in the standard drug treated (silymarin) groups of animals, indicating the protection of hepatic cells against CCl_4 induced hepatic damage was more pronounced in ethanol extract treated group of animals.

Histological profile of control animal showed normal hepatocytes (Fig.1), the section of liver of the Group-II (CCl_4 induced) animals exhibited severe intense centrilobular necrosis (N), vacuolization and macro vesicular fatty changes (Fig.2).

The liver section of silymarin treated animals showed normal hepatic architecture (Fig.3). The liver section of the animals treated with ethanol extract exhibited significant liver protection against CCl_4 induced liver damages as evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration (Fig.4). However, moderate accumulation of fatty lobules (Fig.5) was observed in the liver sections of aqueous extract treated animals.

Table 2: Effect of aqueous & ethanolic fruit extract of *S. trifoliatus* on CCl_4 induced hepatotoxicity in rats.

Group (W)	Total Bilirubin (Mg / dl)	Total protein (Gm%)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control (1% w/v gm, tragacanth, PO)	0.472±0.015	9.223±0.83	153.25±0.456	55.75±1.055	178.15±1.438
CCl_4 (0.1ml/kg/day, IP)	2.461±0.103[a]	5.715±0.313[a]	218.9±5.155[a]	139.6±2.245[a]	495.53±2.880[a]
CCl_4 + Silymarin (0.1ml/kg/day, IP + 100mg/kg/day, PO)	0.575±0.002[b]	8.95±0.035[b]	209.35±0.266[b]	78.15±0.338[b]	210.15±0.495[b]
CCl_4 + Aqueous extract (0.1ml/kg/day, IP + 35mg/kg/day, PO upto14 days)	1.010±0.013[b,c]	8.015±0.009[b,c]	273.5±0.35[b,c]	169±0.195[b,c]	278.3±0.298[b,c]
CCl_4 + Aqueous extract (0.1ml/kg/day, IP + 35mg/kg/day, PO upto20 days)	0.875±0.025[b,c]	8.000±0.105[b,c]	240.5±0.451[b,c]	153± 0.185[b,c]	208.3±0.225[b,c]
CCl_4 + ethanol extract (0.1ml/kg/day, IP + 35mg/kg/day, PO upto 14 days)	0.913±0.008[b,c]	8.215±0.019b,c	267.8±0.245[b,c]	95.5±0.333[b,c]	250.5±0.355[b,c]
CCl_4 + ethanol extract (0.1ml/kg/day, IP + 35mg/kg/day,POupto20days)	0.855±0.005[b,c]	7.210±0.035b,c	260.8± 0.24[b,c]	103.5±0.315[b,c]	242.5±0.255[b,c]

[Values are mean±SEM from 6 animals in each group]
P value: < 0.01: Compared to [a]Control, [b]CCl_4, [c]Silymarin
AST = Aspartate transaminase
ALT = Alanine transaminase
ALP = Alkaline phosphatase

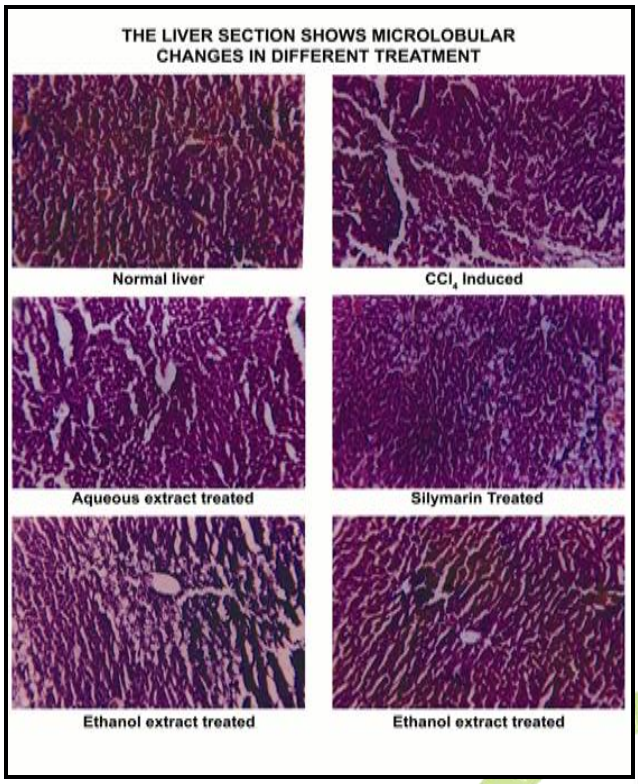

Figure 1: The liver section shows microlobular changes in different treatment

DISCUSSION

CCl_4 induced hepatic injury is the common model used for hepatoprotective drug screening.[12] In CCl_4 induced hepatic toxicity a toxic reactive-metabolite trichlormethyl (CCl_3) was produced by the microsomal oxidase system cytochrome P450[13] through bioactivated radicals' binds covalently to the macro molecules at the lipid membrane of the adipose tissue and causes per oxidative degradation. As a result, fats from the adipose tissues are translocated and accumulated in the hepatocytes[13.] Several plants viz., *Sarcostemma bresistigma*[14,16], *Murraya koenigii*[15], *Balanites augyptica*[16], *Glycyrrhiza glabra*[17] etc. have been reported for their efficacy in controlling the CCl_4 induced hepatic damage. The extent of hepatic damage in assesed by the elevated level of biochemical parameters which is attributed to the generation of trichloromethyl free radical which in turn causes per oxidation of lipids of cellular membrane[18].

Hepatocellular necrosis leads to very high level of Aspartate transaminase and Alanine transaminase released from liver to blood. Between the two alanine transaminase is a better index of liver injury, as its activity represents 90% of total enzyme present in the body. This decrease hi-serum transaminase concentration indicates the stabilization of plasma membrane and protection of hepatocytes against the damage caused by CCl_4[19]. ALP activity on the other hand is related to the functioning of hepatocytes and increase in its activity is due to its increased synthesis in presence of increased biliary pressure[20]. The data in Table-2 reveal the decreased level of serum transaminase in animals treated with ethanolic fruit extract of *S. trifoliatus* indicating the stabilization of plasma membrane and hepatic potentiation against the effect of CCl_4 and decreased ALP concentration evidences the normal functioning of hepatic cells.

Further, in the present investigation preliminary phytochemical analysis of fruit extracts, revealed the presence of large amount of flavonoids, alkaloids, tannins, saponin present. Flavonoid[21], alkaloid[21], saponin[22] are well known for their antioxidant and hepatoprotective activities. In this study ethanol extract showed protective effect against toxicity induced by CCl_4 which may be attributed to the individual or combined effect of phytoconstituents present in it. Several phytoconstituents have the ability to induce microsomal enzymes thereby accelerating the excretion of CCl_4 or inhibiting the lipid per oxidation induced by CCl_4or liver protection is because of decrease trichlorocarbon radicle (i.e. CCl_4 bioactivation). Further through the aqueous extract showed positive tests to only three groups of phytoconstituents viz., saponins (hendergenin), alkaloids & flavonoids, it exhibited considerable liver protection against CCl_4 induced hepatic damage. This may be attributed to the antioxidant and hepatoprotective activity of saponins, alkaloids & flavonoids present in it.

Based on the above results of the pharmacological investigation test it can be concluded that the ethanolic extract of *S. trifoliatus* fruits possesses more significant

hepatoprotective activity. The reason for the variation in the potency of the extracts may be due to the presence of additional phytoconstituents like alkaloids, flavonoids in the ethanol extract of the plant. The present finding provides scientific evidence to the ethno medicinal value of this plant used by the tribal group in Mayurbhanj District and Malkangiri tribal area in treating hepatitis and jaundice.

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