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Research Article

PROTECTIVE EFFECT OF *SALIX TETRASPERMA* ROXBURGH LEAF EXTRACTS AGAINST CARBON TETRACHLORIDE INDUCED LIVER INJURY IN RATS

J.H.Virupaksha *[1], Ramarao Nadendla [2], A.Narendra Babu [3], M.Komali [4]
[1]Research Scholar, Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India
[2]Principal, Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India
[3]Professor, Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India
[4]Assistant Professor, Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India
*Corresponding Author Email: jhvirupaksha@gmail.com

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ABSTRACT

The present study was undertaken to investigate the protective effect of ethanolic and aqueous leaf extracts of *Salix tetrasperma* Roxburgh against carbon tetrachloride induced hepatotoxicity in rats. The test extracts administration resulted in significant elevation of biochemical parameters like viz. serum SGOT, SGPT, ALP, direct bilirubin and total bilirubin levels, while albumin is found to be decreased compared to normal group. Pre-treatment with silymarin, ethanolic and aqueous extract of *Salix tetrasperma* Roxburgh significantly prevented and biochemical changes induced by these hepatotoxins. Histopathology of liver confirmed our finding as the treatment with the extracts resulted in minor liver cell damage compared to toxic control group. Our result clearly indicates hepatoprotective effect offered by STEtOH (400 mg/kg, p.o.) was found to be significantly greater than STAQ (400 mg/kg, p.o.) and standard (silymarin 50 mg/kg, p.o.) group.

Keywords: *Salix tetrasperma* Roxburgh, biochemical parameters, Chloroform, hepatotoxity, Silymarin.

INTRODUCTION

Traditional herbs are wonderful remedies for the treatment of devastating disorders. Recently, there has been a change in a universal fashion from synthetic to herbal medicine, which is like homecoming to nature. In the present situation, the dietary changes lead to livers disorders like non-alcoholic and alcoholic fatty liver disorders.

Liver is the principal organ which actively involves in metabolic functions. Liver performs an important function that detoxifies those hepatotoxicants, which can cause hepatic injury during metabolic reaction. Oxidative stress is considered as the imbalance between reactive oxygen species production and antioxidant protective mechanism. It is principal cause of the development of various hepatic disorders [1]

CCl_4, a well-known hepatotoxin, has been widely used as a model to evaluate hepatotoxicity[2]. CCl_4 induces hepatotoxicity by increased oxidative stress, and a connection between oxidative stress and lipid peroxidation has been reported[3].Firstly, CCl_4 is metabolized by action of cytochrome P450 oxygenase system to convert the trichloromethyl free radical, CCl_3. Secondly, CCl_3. radical reacts with some biological molecular such as proteins, nucleic acids and lipids. Furthermore, the CCl_3. radical is converted into the trichloromethyl peroxy radical (CCl_3OO.) when it reacts with oxygen. This radical is still more reactive and is capable to initiate the process of lipid peroxidation[3]. CCl_4 induces liver injury progressing from steatosis to centrilobular necrosis, and develops fibrosis and cirrhosis[4].

The genus Salix comprises about 500 species that mainly distributed in temperate region worldwide and also in higher altitudes of tropics. Information given by traditional medical practitioners in Western Ghats indicates that the leaf of *Salix tetrasperma* Roxburgh is used to treat hepatotoxicity. However, there is no scientific data to prove the traditional use of *Salix tetrasperma* Roxburgh in the treatment of hepatotoxicity. So the present investigations was undertaken to determine the effects of the ethanolic and aqueous extract resulting in hepatoprotective activity in Chloroform induced hepatotoxicity in rats.

MATERIALS AND METHODS

Preparation of plant extract

The leaves of *Salix tetrasperma* Roxburgh were dried in shade and powdered was subjected to successive solvent extraction in Soxhlet extractor as per standard procedure using petroleum ether, ethyl acetate and ethanol as solvent. The marc obtained from the ethanol extraction utilized for aqueous extraction, by maceration for 48 hr. During maceration period, few drops of chloroform were added to avoid fungal growth. The extract was filtered, concentrated and the solid marc was weighed. The extracts were stored in airtight containers stored in refrigerator further use.

Animals

Wister albino rats of either sex, weighing around 200-250 g. were used in the present experimental study. They were obtained from in house breed animals of Chalapathi Institute of Pharmaceutical Sciences, Guntur. The rats were provided standard rat pellet with supplied water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Chalapathi Institute of Pharmaceutical Sciences, Guntur, A.P (Approval No. 27/IAEC/CIPS/2014-15; dt 21/02/2015) and care of the animals was taken as per guidelines of the CPCSEA.

Acute toxicity test

Acute oral toxicity study for the *Salix tetrasperma* extracts was carried out using OECD guideline-425 (modified, adopted March 23, 2006), the sequential test that uses a maximum of five animals. A test dose of 2000 or exceptionally 5000 mg/kg may be used in situation where experiment has information indicating that the test material is likely to be nontoxic[5].

Drugs and reagents

Silymarin was purchased from Sigma Aldrich, Bangalore, India. Carbon tetrachloride and formalin was obtained from Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, CCl_4 was administered intraperitoneally (i.p) in rats. SGOT, SGPT, ALP, total bilirubin were obtained from Span Diagnostics, Surat, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

Experimental groups

The efficacy of *Salix tetrasperma* Roxburgh ethanolic extract was compared with aqueous extract by evaluating in vivo hepatoprotective activity in rats against liver fibrosis[2].
Six groups, each comprising of five wistar rats, were employed in the present study.

Group I: Normal control received normal saline for 14 days orally.
Group II: Group II (CCl_4-treated control group): Rats were administered CCl_4 (3ml/kg, i.p in 1:1dilution with liquid paraffin) on the day 14.
Group III: (Silymarin + CCl_4-treated group): Rats were treated with silymarin (50 mg/kg, orally) for 14 days. On the 14th day Silymarin was administered 60 min prior the administration of CCl_4.
Group IV (STEtOH + CCl_4-treated group): Rats were treated with STEtOH (200mg/kg body weight) for 14 days. On 14th day STEtOH was administered 60 min before the administration of CCl_4.
Group V (STEtOH +CCl_4 -treated group): Rats were treated with STEtOH (400mg/kg body weight) for 14 days. On 14th day STEtOH was administered 60 min before the administration of CCl_4.
Group VI: (STAQ + CCl_4-treated group): Rats were treated with ST AQ (400 mg/kg body weight) for 14 days. On 14th day ST AQ was administered 60 min before the administration of CCl_4.

Biochemical Parameters evaluated

Blood biochemical parameters: albumin (g/dl), total bilirubin (mg/dl), direct bilirubin (mg/dl), indirect bilirubin (mg/dl), SGOT (U/lit), SGPT (U/lit), and ALP (U/lit).

After the last dosing of fourteen day after 24 h the blood sample were collected by puncturing retro-orbital plexus and serum was separated by centrifugation. Rats were sacrificed; liver were excised, rinsed clean in saline and preserved in 10% formalin for histopathological study.

Histopathological studies

Portions of liver from all the experimental groups were fixed in 10% formalin, dehydrated in alcohol and then embedded in paraffin. Microtome sections (5 µm thick) were prepared from liver sample and stained with haematoxylin-eosin dye. The sections were examined for the pathological findings.

Statistical analysis

The results are expressed as mean ± standard error of means (S.E.M.). For determination of significant differences (p value <0.05) one-way analysis of variance (ANOVA) followed by Tukey's multiple range test using graph pad prism version 3.0 was used.

RESULTS

Effect on blood biochemical and urine analysis parameters

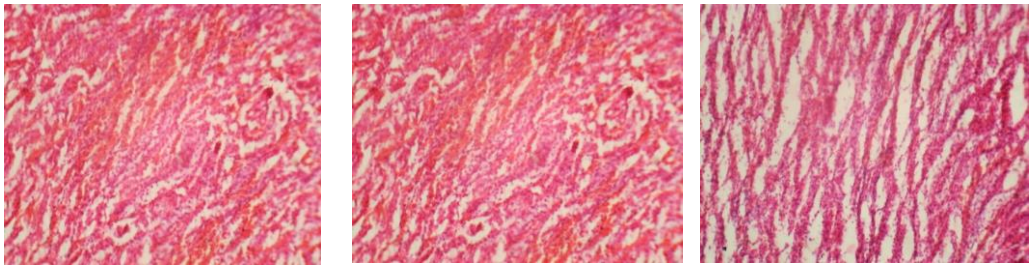
In CCl_4 induced hepatotoxicity (3ml/kg, i.p.) the activities of serum marker enzymes showed significantly increased levels of all blood parameters except decreased direct bilirubin levels, when compared to normal control group. STEtOH and STAQ treatment groups showed significant hepatoprotective effect ($p<0.001$) against CCl_4 group as described in table 1.

Silymarin (50 mg/kg, orally) pre-treated animals showed significant difference in SGOT, SGPT and in ALP which is evident from table1. STEtOH (400 mg/kg body weight) pre-treated animals showed significant increased levels of all blood biochemical parameters except ALP, STAQ (400 mg/kg body weight) to pre-treated animals showed significant increased levels of all blood biochemical parameters.

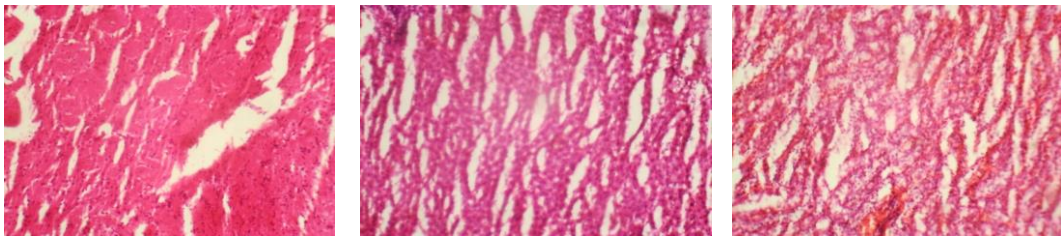
Histopathological examinations of showed defects ranging from massive necrosis, congested central vein, fatty degenerations and infiltration by inflammatory cells in rats treated with CCl_4 alone in fig 1. However, histological profile of rats pre-treated with *Salix tetrasperma* Roxburgh exhibited significant liver protection against the toxicant as evidenced by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration Figure 1.

Table 1: Protective effect of ST EtOH and STAQ against carbon tetra chloride induced hepatotoxicity

S.N	Parameters	Control	Positive Control	Silymarin (50 mg/kg)	STEtOH 200 (mg/kg)	STEtOH 400 (mg/kg)	STAQ 400 (mg/kg)
1.	Albumin (g/dL)	4.74±0.15	5.4±0.57	5.29±0.07	4.31±0.31	4.44±0.41	4.22±0.38
2.	Total Bilirubin (mg/dL)	0.64±0.09	0.79±0.01	0.66±0.08	0.78±0.07	0.76±0.18	1.68±0.54
3.	Direct bilirubin (mg/dL)	0.32±0.07	0.30±0.04	0.22±0.07	0.22±0.04	0.44±0.15	0.66±0.22
4.	Indirect bilirubin (mg/dL)	0.30±0.05	0.46±0.09	0.36±0.02	0.56±0.04	0.86±0.29	1.02±0.36
5.	SGOT (U/lit)	294.8±21.73	785.6±19.94	170.4±20.06	458.6±22.77***	338.8±28.05***	496.8±27.90***
6.	SGPT (U/lit)	181.6±10.94	573.4±10.14	162.0±17.31	359.0±43.01**	306.8±21.78***	345.6±11.82***
7.	ALP (U/lit)	64.8±1.93	89.0±3.16	79.6±2.29	82.8± 3.62*	80.8± 4.38*	76.4± 3.08*



Control liver **Positive control liver** **Slilymarin treated**



Treatment with S.E.200 mg/kg **Treatment with S.E.400 mg/kg** **Treatment with S.A.400 mg/kg**

Figure 1: Histology slides of the isolated liver of various treatment groups showing haematoxylin and eosin stained cells

DISCUSSION

Carbon tetrachloride toxicity is an established model for the evaluation of hepatic damage. CCl_4 will accumulates in liver parenchyma cells and metabolically activated by the cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical ($*CCl_3$) which binds with cellular lipids and proteins in the presence of oxygen to influence lipid peroxidation[6]. These resulted in alteration of structures of the endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, increased levels of serum biochemical marker enzymes like AST, ALT, and ALP, reduction of protein synthesis, increased lipid peroxidation and destruction of Ca^{2+} homeostasis[7].The weakened in the transport function of the hepatocytes causes the leakage of enzymes from cells due to changes permeability of membrane, which ensures in lowered levels of AST, ALT and ALP in the hepatic cells and an elevated level in serum. For such evaluation of liver damage by CCl_4 hepatotoxic, the enzyme levels such as AST and ALT are largely considered[8]. Serum ALP and bilirubin levels on the other hand, are related to the function of hepatic cell. An elevated serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure. Bilirubin is one of the most useful clinical parameter for assesses severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte[9]. Depletion in total serum protein observed in the CCl_4 treated group may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein[10]. In CCl_4-induced liver injury in rats, obtained data suggest that the treatment with ethanolic and aqueous extract of *Salix tetrasperma* Roxburgh and its different fractions significantly reduced the enhanced level of serum AST, ALP which seems to offer the protection and maintain the functional integrity of hepatic cells. Effective control of bilirubin level and alkaline phosphatase activity by different doses of the extract and its fractions points towards an early improvement in the secretory mechanism of the hepatic cell. The significant raise in protein levels suggests the stabilization of endoplasmic reticulum leading to protein synthesis[11].

The hepatoprotective activity of *Salix tetrasperma* Roxburgh has not been previously studied and the mechanism by which it occurs not fully understood. The chemical investigation of *Salix tetrasperma* Roxburgh revealed the presence of flavonoids, polyphenolic compounds. The qualitative phytochemical analysis of *Salix tetrasperma* Roxburgh shows positive test for flavonoids, polyphenolic compounds, tannins, triterpenes, steroids, saponins.This is further confirmed by HPLC, HPTLC chromatograms (unpublished data), L.C-ESI-M.S.studies[12].Hence further work is necessary to elucidate the exact chemical constituent responsible for hepatoprotective activity along with mechanism of action.

In several studies it has been shown that antioxidant action of plant extracts play an important role protection against CCl_4 induced hepatic cell injury. Antioxidant enzymes like SOD, ABTS, and DDPH activity were involved in several defence processes against oxidative damage protects cells against free radical peroxides and various poisonous substances[12,13].The histopathological results indicate that *Salix tetrasperma*

Roxburgh preserved the structural integrity of liver tissue which was damaged by CCl_4- intoxication.

These results indicate that the ethanolic and aqueous leaf extracts of *Salix tetrasperma* Roxburgh preserved structural integrity of the hepatocellular membrane and showed dose dependant protective effect.

CONCLUSION

On the basis of the results obtained from this investigation, we can conclude that the *Salix tetrasperma* Roxburgh leaf extracts have significant hepatoprotective activity as evident by significant reduction in biochemical parameters. It may be logically to suggest that it may be useful as hepatoprotective agent. Further detailed investigations required, including isolation and characterization of active compounds and mechanism of action.

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