HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *STACHYTARPHETA INDICA* ON WISTAR STRAIN RATS

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Summary

The present study appraised the hepatoprotective activity of alcoholic extract of *stachytarpheta indica*(whole plant) on Wistar strain rats. Liver damage was induced by intraperitonial administration of carbon tetrachloride (1ml/kg) for 15 days. The extent of damage was studied by assessing biochemical parameters. The alcoholic extracts of *Stachytarpheta indica*(300mg&600mg/kg) were administered orally to the animals treated with carbon tetrachloride and its effects on biochemical parameters were compared with standard drug silymarin (100mg/kg, p.o). *Stachytarpheta indica* showed significant reduction of serum enzymes-AST, ALT, ALP, TP & Bilirubin (Aspartate Transminase, Alanine Transminase, Alkaline Phosphatase, Total Protein & Total bilirubin) when compared to control rats. The hepatoprotective effect of *Stachytarpheta indica* was comparable with the standard drug Silymarin. It was confirmed by histopathological study. The effect of extract 600mg/kg was almost equal to that of standard drug.

Keywords: *Stachtarpheta indica*, carbon tetrachloride, hepatoprotective, Silymarin

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Introduction

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment to its functions may lead to many implications on one's health. Hepatic dysfunction due to inhalation of hepatotoxin is increasing world wide[1,2]. Among the various mechanisms involved in the hepatotoxicity by hepatotoxin, one is oxidative damage through free radical generation[3,4]. Management of liver disease is still a challenge to the modern medicine. Conventional medicine is now pursuing the use of natural products such as herbs to provide the support that the liver needs on a daily basis[5]. *Stachytarpheta indica* is an herbal drug which is being extensively used in the Indian traditional system of medicine for diabetes & liver components. It belongs to the family, Verbenaceae. The plant is widely used throughout the Amezon[6]. It is a snake weed which is native to tropical America & Asia and commonly called as Indian snake weed. Leaves are simple, not lobed or divided, opposite, stalked, elliptic or ovate, dentate, apex acute and pinnately veined[7]. It is reported for its antidiarrhoel effect[8] and cardiovascular effects[9]. The plant contains flavanoids, terpenes & phenol contents[10]. In the present study the Hepatoprotective effect of ethanolic extract of *stachytarpheta indica* is investigated in a scientific manner to validate its use as alternative and complementary herbal drug.

Materials and Methods

Drugs and Chemicals

Carbontetrachloride from S.D.Fine chemicals Ltd., Mumbai; Serum enzymes-ALT, AST, ALP, TP & Bilirubin kit were from Span Diagnostic Ltd., Bangalore; Silymarin from Mark drugs Pvt Ltd, Bangalore. All chemicals used in the study were of analytical grades.

Plant Material

The whole *Stachytarpheta indica* plant was collected from the Government Sidha Medical College, TamilNadu and stored at room temperature in a dry place prior to use. The plant was authentified as *Stachytarpheta indica* by Professor Chelladurai Research Botanist, Palayamkottai, Tamilnadu, India.

Extract Preparation

The dried S.indica plant powder (75g) was extracted in Soxhlet apparatus with 450 ml of 95% ethanol at controlled temperature. The collected extract was concentrated under reduced pressure (<45^{0}C) using a vacuum pump for complete removal of the solvent. Pure organic part of the sample thus prepared was stored at 4-5^{0}C until used. The extract was then subjected to qualitative phytochemical investigation for the identification of phytoconstituents viz., sterols, alkaloids, glycosides, saponins, tannins, carbohydrates and flavanoids[11].

Animals

Adult albino (Wistar strain) rats weighing between 150-200gm (2-3 months) were used for the study. The animals were procured from 'The Animal house' of SRM College of Pharmacy. The use of animals was approved by the 'Institutional Animal Ethical Committee'. Throughout the experimental period, the animals were housed in cages under room temperature (20±2^{0}c); relative humidity (60- 70%) and were exposed to 12:12h light: dark cycle. The food and water were available *ad libitum*.

Acute Toxicity Study

Minimal lethal dose (MLD) in Wistar albino mice in group of 10 each for each dose was calculated for the extract by the method of Litchfield and Wilcoxon[12]. The animals were administered oral graded dose of the extract. MLD for the extract was 3000mg/kg.

Experimental Procedure

The experiment was carried out after obtaining clearance from Institutional Animal Ethical Committee. The animals were divided in to 5 groups of 6 animals each. The animals from Group I which served as control received vehicle 1%Acacia at a dose of 1mg/kg p.o for 14 days and olive oil (1ml/kg p.o) for 10 days. Group II - V received 1ml/kg/day p.o of ccl_4 for 10 days[13]. The standard drug Silymarin (100mg/kg p.o.) was administered to Group III animals for 14 days. Group IV & V received ethanolic extract of *Stachytarpheta indica* in the dose for 14 days respectively. The ccl_4, Silymarin & the extracts were administered concomitantly to the respective group of animals.

On 14^{th} day, blood was collected through retro orbital vein and serum was separated by centrifugation at 2500 rpm for 10 minutes. Serum was used for the assay of hepatic marker enzymes – Total protein, Total bilirubin, Serum Aspartate Transminase, Serum Alanine Transminase, and Alkaline Phosphatase[14]. The animals were sacrificed; liver was dissected immediately and used for histopathological studies.

Histopathological Studies

The tissue of the liver was fixed in 10% formalin and embedded in paraffin wax. Sections of 4-5µ thickness were made and stained with haematoxylin-eosin. Histological observations were made under light microscope[15].

Statistical Analysis

The values were expressed as mean ±SEM. The statistical analysis was carried out by One way Analysis of Variance (ANOVA) followed by students Newman-keuls test. P values<0.01 were considered significant.

Results

Acute toxicity studies

Stachytarpheta indica produces 50% of mortality at 3000mg/kg. Thus two doses (300 and 600 mg/kg p.o.) which were found to be safe were employed for further pharmacological studies.

Biochemical estimations

The results for the effect of *Stachytarpheta indica* on serum enzymes ALT, AST, ALP, TP, Bilirubin are shown in Table:1. The administration of ccl_4 resulted in a marked increase of ALT, AST, ALP & Bilirubin levels in serum. However, the total protein level was decreased. The toxic effect of ccl_4 was significantly controlled ($p<0.01$) in the animals treated with ethanolic extract of *S. indica* by way of restoration of the levels of liver function biochemistry similar to that of standard drug silymarin. The animals treated with 600mg/kg of the extract showed significant results which were almost equal to that of silymarin.

Histopathology

Histopathological profile of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Fig.1). Group II animals exhibited disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration (Fig.2). The liver sections of the rats treated with ethanol extract of *S.indica* and silymarin followed by CCl4 intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Fig. 3, 4 & 5).

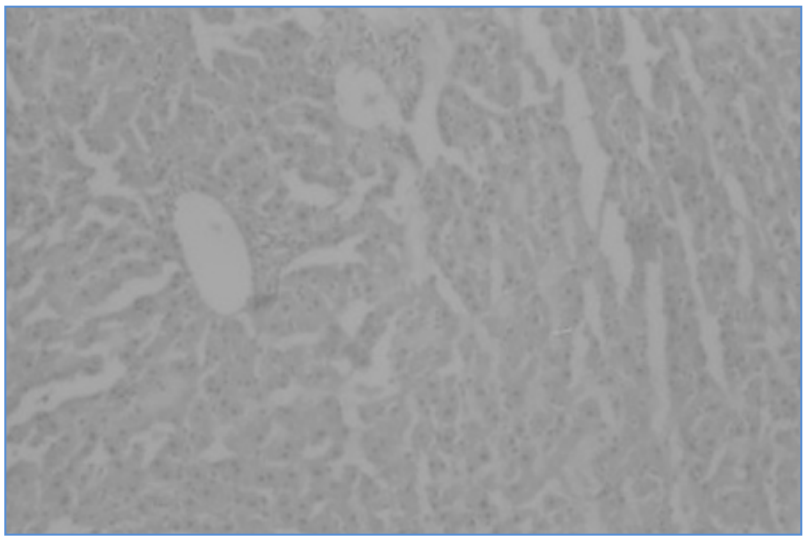
Table 1: Effect of *Stachytarpheta indica* plant extracts on

ALT, AST & ALP, TP, Bililrubin

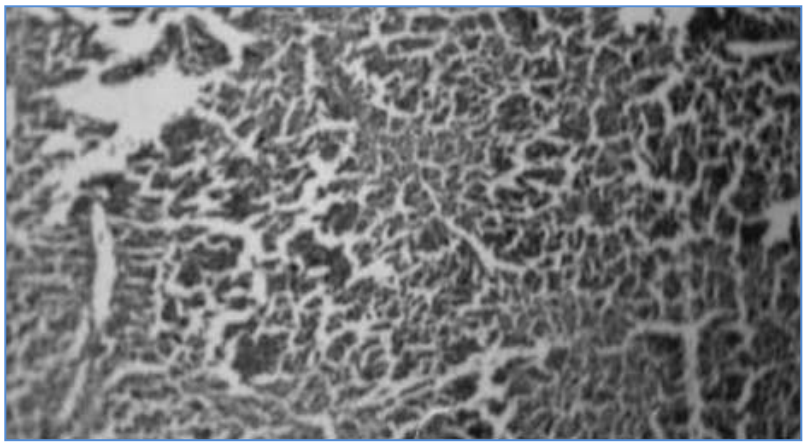
Groups	Treatment	ALT (U/ml)	AST (U/ml)	ALP (U/ml)	TP (g/dl)	Bilirubin (mg/dl)
I	Aacacia+Olive oil (1ml+1ml/kg p.o)	55.84±4.51	43.22±3.11	36.22±4.57	7.46±0.36	0.72±0.3
II	CCl_4(1ml/kg p.o)	108.22±3.57*	103.22±4.01*	87.54±3.03*	2.18±0.25*	4.26±1.50*
III	CCl_4+Silymarin (100mg/kg p.o)	74.53±4.21**	68.28±2.55**	44.25±2.02**	6.86±0.48**	1.06±0.46**
IV	CCl_4+ SI- extract (300mg/kg p.o)	69.23±2.54**	62.14±3.52**	51.00±5.46**	3.72±0.52**	2.15±0.86**
V	CCl_4+ SI- extract (600mg/kg p.o)	63.01±2.57**	48.35±2.88**	40.51±4.51**	6.12±0.22**	1.02±0.97**

Values are mean±SEM; n=6, by one way ANOVA followed by student-Newman-Keuls test; *P<0.05Vs group I, **P<0.05Vs group II; ALT-Alanine Transaminase; AST-Aspartate Transaminase; ALP-Alkaline Phosphatase; TP-Total protein

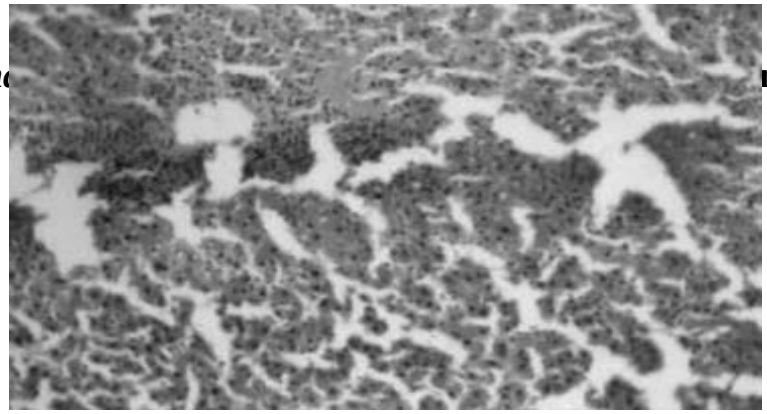
Slides showing histopathology of liver tissues



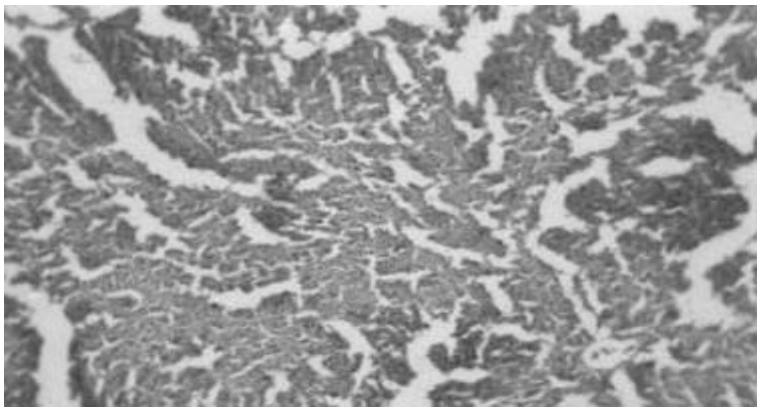
Section of the liver tissue of control rats showing normal histology



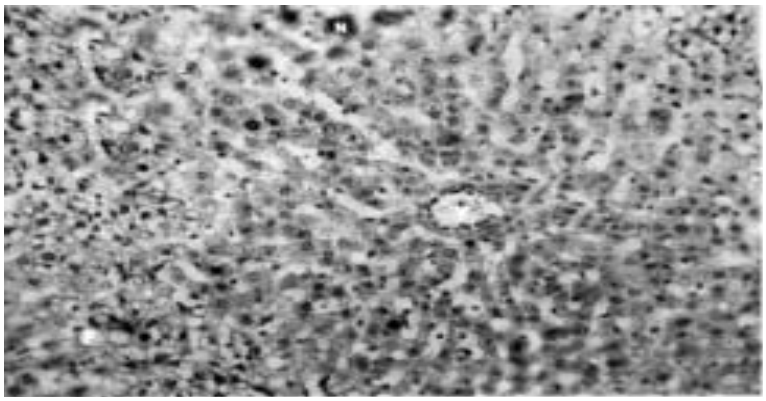
Section of the liver tissue of rats treated with CCl4 showing necrosis & fatty



Section of the liver tissue of silymarin-treated rat showing normal hepatocytes & hepatic duct



Section of the liver tissue of ethanol extract (300mg/kg) treated rat showing normal arrangements of hepatocytes around the central vein



Section of the liver tissue of ethanol extract (600mg/kg) treated rat showing normal arrangements of hepatocytes around the central vein, absence of necrosis

Discussion

Carbon tetrachloride is the one of the most commonly used hepatotoxins in the experimental study of liver diseases[16] .It induces liver cell necrosis and apoptosis and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration[17]. The hepatotoxic effect of carbon tetrachloride is mainly due to its active metabolite, trichloromethyl radical[18]. This activated radical bind covalently to the macromolecules and induce lipid peroxidation and forms lipid peroxides which produce damage to the membrane[19]. The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in transaminases and alkaline phosphatase which are cytoplasmic in location and released into circulation after cellular damages was the clear indication for the loss of functional integrity of the cell membrane[20,21]. Amino transferases are present in high concentration in liver, an important class of enzymes linking carbohydrate and amino acid metabolism. Alanine amino transferase and aspartate amino transferase are well known diagnostic indicators of liver disease. In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the blood stream[22]. In the present study, the activities of these enzymes were found to increase in the hepatotoxic animals, and were significantly reduced in groups of ethanolic extract of *Stachytarpheta indica* administered rats as compared to that of toxicant rats. This confirms the protective effect of ethanolic extract of *Stachytarpheta indica* against carbon tetrachloride induced hepatic damage. The effect was more pronounced with 600mg/kg extract. A possible mechanism of the *Stachytarpheta indica* extract as hepatoprotective may be due to its anti-oxidant effect or inhibition of cytochrome P450[23]. This might be due to the higher contents of flavonoids present in the extract which could have reduced the accumulation of toxic CCl3 derived metabolites[22].

Histopathological examination of the liver section of the rats treated with toxicant showed intense centrilobular necrosis

and vacuolization. The rats treated with silymarin and extracts along with toxicant showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cards and absence of necrosis and vacuoles.

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