Available online at www.pharmacie-globale.info ISSN 0976-8157

Research Article

PHARMACIE GLOBALE

INTERNATIONAL JOURNAL OF COMPREHENSIVE PHARMACY

# HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *Stachytarpheta indica* ON WISTAR RATS

Gayatri G*, Ramesh Babu J, Sumalatha N, Venkateswarao J and S Vidyadhara

Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, Andhra Pradesh, India.

Received: 17 November 2010; Revised: 28 December 2010; Accepted: 2 January 2011; Available online: 11 January 2011

## ABSTRACT

The objective of the present study appraised the hepatoprotective activity of ethanolic extracts of S*tachytarpheta indica* (whole plant) on Wistar rats. Liver damage was induced by intraperitonial administration of carbon tetrachloride (1ml/kg,b.w,p.o) for 7 days. The extent of damage was studied by assessing biochemical parameters. The ethanolic extracts of *Stachytarpheta indica* (300mg & 600mg/kg,b.w,p.o) were administered respectively to the animals treated with carbon tetrachloride and its effects on biochemical parameters were compared with standard drug solitarian (100mg/kg,b.w 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.

## 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 thehepatotoxicity 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

### Collection of 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.

### Plant Authentification

The plant was authentified as *Stachytarpheta indica* by Professor Chelladurai, Research Botanist, Palayamkottai, Tamilnadu, India.

### Extract Preparation

The dried *Stachytarpheta 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 (<450C) using a vacuum pump for complete removal of the solvent. Pure organic part of the sample thus prepared was stored at 4-50C 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 M/S Mahavir enterprises, Hyderabad. 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±20c); relative humidity (60- 70%) and were exposed to 12:12h light: dark cycle. They were fed with standard laboratory diet supplied by M/S Rayans biotechnologies Pvt Ltd; Hyderabad and water *ad libitum*.

### Determination of 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

***Corresponding Author:**
Gayatri G
Chebrolu Hanumaiah Institute of Pharmaceutical Sciences,
Chowdavaram, Guntur, Andhra Pradesh, India.
Contact no: +91-9949063122, +91-9701467657
Email: ramesh.janga@yahoo.com

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 and olive oil (1ml/kg p.o) for 7 days. Group II-V received 1ml/kg/day p.o of $CCl_4$ for all 7 days.[13] The standard drug Silymarin (100mg/kg p.o.) was administered to Group III animals for 7 days. Group IV & V received ethanolic extract of *Stachytarpheta indica* in the dose for 7 days respectively. The $CCl_4$, Silymarin & the extracts were administered concomitantly to the respective group of animals. On 7th 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 3000 mg/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 ccl4 resulted in a marked increase of ALT, AST, ALP & Bilirubin levels in serum.

**Table 1. Effect of *Stachytarpheta indica* plant extracts on ALT, AST, ALP, TP and Bilirubin**

| Groups | Treatment | ALT (U/ml) | AST (U/ml) | ALP (U/ml) | TP (g/dl) | Bilirubin (mg/dl) |
|---|---|---|---|---|---|---|
| I | Acacia+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 | $CCl_4$+Siliymarin (100ml/kg p.o) | 74.53±4.21** | 68.28±2.55** | 44.25±2.02** | 6.86±4.8** | 1.06±0.46** |
| IV | $CCl_4$+SI-extract (300ml/kg p.o) | 69.23±2.54** | 62.14±2.52** | 51.00±5.46** | 3.72±0.52** | 2.15±0.86** |
| V | $CCl_4$+SI-extract (600ml/kg p.o) | 63.01±2.57** | 48.35±2.88** | 40.51±4.51** | 6.12±0.22** | 1.02±0.97** |

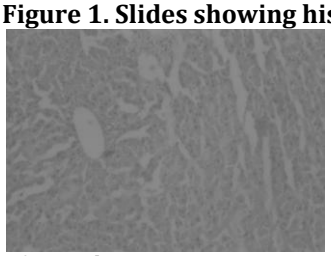
ValuesAre Mean±SEM: n=6, by ANOVA followed by student-Newman –keuls test; *p<0.05 vs group-I,**p<0.05 vs group-II: ALT-Alanine Transaminase, AST-Aspartate; Transaminase; ALP-Alkaline Phosphatase; TP-Total protein

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 600 mg/kg of the extract showed significant results which were almost equal to that of silymarin.

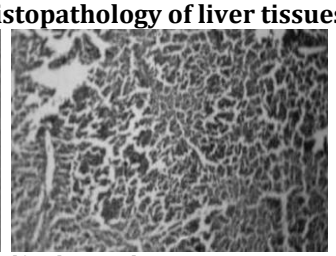
### Histopathology

Histopathological profile of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure **1a**). Group II animals exhibited disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration (Figure **1b**). The liver sections of the rats treated with ethanol extract of *S. indica* and silymarin followed by $CCl_4$ intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Figure **1c**, **1d** & **1e**).

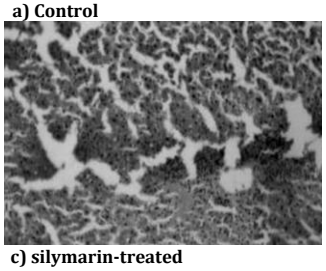
**Figure 1. Slides showing histopathology of liver tissues**



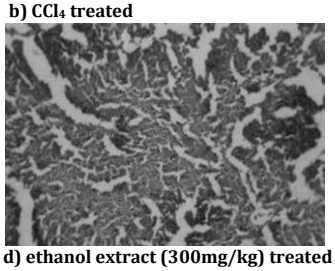
**a) Control**



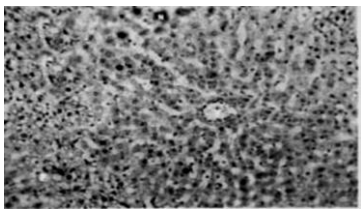
**b) $CCl_4$ treated**



**c) silymarin-treated**



**d) ethanol extract (300mg/kg) treated**



**e) ethanol extract (600mg/kg) treated**

**a)** Section of the liver tissue of control rats showing normal histology
**b)** Section of the liver tissue of rats treated with $CCl_4$ showing necrosis & Fatty vacuole
**c)** Section of the liver tissue of silymarin-treated rat showing normal hepatocytes & hepatic duct
**d)** Section of the liver tissue of ethanol extract (300mg/kg) treated rat showing normal arrangements of hepatocytes around the central vein
**e)** 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 $CCl_3$ derived metabolites.[24] 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.

## CONCLUSION

Hepatoprotective activity of the ethanolic extracts (300.600mg/kg) of *stachytarpheta indica* was studied. In this study, the drug treated animals showed decreased serum enzymes like ALT,AST,ALP and increased liver enzymes like SOD, Catalase and GSH. This confirms the protective effect of ethanolic extract of *Stachytarpheta indica* against carbon tetrachloride induced hepatic damage. A possible mechanism of the *Stachytarpheta indica* extract as hepatoprotective may be due to its anti-oxidant effect or inhibition of cytochrome P450. This might be due to the higher contents of flavonoids present in the extract which could have reduced the accumulation of toxic $CCl_3$ derived metabolites. 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. Thus it was concluded that the extract exhibited significant dose dependent hepatoprotective activity. The effect of the extract 600mg/kg was almost equal to that of standard drug used.

## ACKNOWLEDGMENT

Authors are grateful to Dr. S Vidyadhara, Principal for providing necessary facilities to carry out this work. I commend on behalf of humanity to those rats that give their supreme sacrifice for the palliation of human sufferings.

## REFERENCES

1. Reddy P B, Reddy C K, Rambhau D et al. Indian J Pharm Sci 1993; 55:134.
2. Preussmann R; Hepato carcinogens as potential risk for human liver cancer. In: Remmer H, Bolt HM, Bannasch P, eds. Primary liver tumors. Lancaster, MTP press, 1978;11-29.
3. Montesano R, Kirby G M; Chemical carcinogens inhuman liver cancer. In: Brechot, eds. Primary liver cancer-Etiological and progression factors. CRC, Boca Raton, 1994; 57-77.
4. DeLeve L D, Kaplowitz N; Mechanisms of drug induced liver disease. Gastroenterol clin N Am 1995; 24:787-810.
5. Sherlock S, Dooley J; Disease of liver & biliary system. Blackwell scientific publications 2002; 11; 322-356.
6. Handa S S, Sharma A, Chakraborti K K; Natural products and plants as liver protecting drugs. Filoterapia 1986; 57:307-45
7. Holm L et al. A geographical atlas of world weeds. Atlas W Weed 1979.
8. Otinmeyin O Sunday, Vguru O M, Akanbi B E; Antidiarroheal effect of aqueous extract of *Momordica balsamnia* and *Stachytarpheta indica* in rats. J Natural products2008; 1:36-45
9. Idu M Omogbai, Amaechina F, Ataman J E; Some cardiovascular effects of the aqueous extract of the leaves of *Stachytarpheta jamaicensis.* I J Pharmacol 2006; 163-165.
10. Rosenther L; The Chemical investigation of Plants. G Bell & Sons Ltd, London 1930.
11. Shas B S, Quadry J S; A Text Book of Pharmacognosy. B.S.Prakashan, New Delhi 1980; 16&24.
12. Litchfield J T, Wilcoxan F; A simplified method of evaluating dose-effect experiments. J Endocriol 1943; 33:297-299.
13. Jaiprakash B, Aland R, Karadi R V et al. Hepatoprotective activity of fruit pulp of *Balanites aegyptiaca*. Indian Drugs 2003; 40:296-297.
14. Mankani K L, Jagadeesh Singh S D, Manohara Y N et al. Evaluation of hepatoprotective activity of stembark of *Pterocarpus marsupium* Roxb. I J Pharmacol 2005;37:165-168.
15. Sethuraman M G, Lalitha K G, Rajkapoor B; Hepatoprotective activity of *Sarcostemma brevistigma* against carbon tetrachloride induced hepatic damage in rats. Current Science 2003;84:1186-1187.
16. Johnson D E, Kroening C; Mechanism of early Carbontetrachloride toxicity in cultured rat hepatocytes, Pharmacol Toxicol 1998;83:231-9 17.
17. Shi G F, Q Li; Effects of oxymetrine on experimental hepatic fibrosis and its mechanism *in-vivo.* World J Gastroenterol 2005;11:268-271.
18. Srivastava S P, Chen N O, Holtzman J L; The *in-vitro* NADPH dependent inhibition by ccl4 of the ATP dependent calcium uptake of hepatic microsomes from male rats-Studies on the mechanism of inactivation of the hepatic microsomal calcium pump by the ccl3. radical. J biol chem 1990;265:8392-9
19. Mujeep M, Aeri V, Bagri P et al. Hepatoprotective activity of methanolic extract of *Tylophora indica* leaves. I J Green Pharmacy 2009;125-127.
20. Saraswat B, Visen P K, Patnaik G K et al. Anticholestic effect of picroliv, active hepatoprotective principle of *Picrorhiza kurrooa* against carbon tetrachloride induced cholestatis. Indian J Exp Biol 1993; 31:316-8.
21. Sallie R, Tredger J M, William R; Drugs and the liver. Part I. Testing liver function. Biopharm Drug Disp 1991; 12:251-259.
22. Chaudhari B P, Chaware V J, Joshi Y R et al. Hepatoprotective activity of Hydroalcoholic extract of

*Momordica charantia* Linn. leaves against Carbon tetrachloride induced Hepatopathy in Rats. I J ChemTech Res 2009; 1(2):355-358.

23. Recknagel R O, Glende E A, Dolak J A et al. Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther 1989; 43:139-154.