Research Article

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SCREENING OF ETHANOLIC EXTRACT OF *STACHYTARPHETA INDICA* L. (VAHL) LEAVES FOR HEPATOPROTECTIVE ACTIVITY

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ABSTRACT

Petroleum ether, Chloroform, Ethanol and Aqueous extract of shade dried leaves of *Stachytarpheta indica L. (Vahl)* were subjected for phytochemical studies. Ethanolic extract showed the presence of Carbohydrates, Glycosides and Flavanoids. Most of the active constituents were present in Ethanolic extract and hence extract was further evaluated for hepatoprotective activity. Hepatoprotective activity of Ethanolic extract of *Stachytarpheta indica L. (Vahl)* was evaluated by carbon tetrachloride induced toxicity with standard hepatoprotective drug as Liv-52. Ethanolic extract of *Stachytarpheta indica L. (Vahl)* produced reduction in CCl_4 induced elevated levels of SGPT, SGOT, SALP and Serum bilirubin and reversed total protein in rats indicating hepatoprotective activity at the dose of 200mg/kg body weight and was comparable to that of standard drug Liv-52(1ml/kg body weight).Further histopathological studies indicated that the animals pretreated with ethanolic extract of *Stachytarpheta indica L. (Vahl)* was minimal with distinct preservation of structures and architectural frame of the hepatic cells, and is comparable to standard Liv-52.

KEYWORDS- *Stachytarpheta indica L. (Vahl),* Ethanolic extract, Carbon tetrachloride, Hepatoprotective activity.

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INTRODUCTION

Stachytarpheta indica L.(Vahl) (Family- Verbenaceace),an annual herb or shrub, hairy or glabrous, high, stems erect, dichotomously branched, leaves elliptic, obtuse or acute, coarsely serrate, glabrous or nearly so, base much tapering and decurrent into petioles which are consequently obscure[1,2]. In Brazil, hot tea prepared from the leaves of *Stachytarpheta indica L. (Vahl)*, routinely used for dyspepsia, fever, chronic liver problems, and constipation and as diuretic. Hence the present study is planned to evaluate the extent of hepatoprotective activity of leaves of *Stachytarpheta indica L. (Vahl)* by using Ethanolic extract [3, 4].

MATERIALS AND METHODS

Materials

All the solvents are of Analytical grade. The materials used in this study are dried powdered leaves of *Stachytarpheta indica L. (Vahl)*, Ethanol, Tween-80 (SD-Fine Chemicals, Mumbai) and Liv-52 syrup (Gift sample from The Himalaya drug company, Makali Bangalore). Carbon tetrachloride (CCl_4) was used as hepatotoxins to induce hepatotoxicity (Thomas Bakar Chemicals Ltd, Mumbai). Animals used are Albino mice (20-30gm of either sex) and Wistar strain Albino rats (150-200gm of either sex). Both the animals were procured from Animal house, Department of Pharmacology, Karnataka Medical College, Hubli and Ethical Committee Clearance was obtained from K.L.E College of Pharmacy Hubli.

Methods

Collection of the plant

The leaves of *Stachytarpheta indica L. (Vahl)* were collected from the Botanical gardens of Karnataka University, Dharwad during the month of May. The same was authenticated by Dr.G.R.Hegde, Professor in Department of Botany, Karnataka University, Dharwad (GRH/KUD-1472004) and the leaves of *Stachytarpheta indica L. (Vahl*) were collected and shade dried.

Preparation of the extract

The dried leaves were subjected to successive extraction [5, 6, 7, 8] by using different solvents of ascending polarity i.e. Petroleum ether, Butanol, Ethyl acetate and Ethanol in a Soxhlet apparatus. Aqueous extraction was carried out by maceration method. The ethanolic extract was collected as most of the constituents were extracted in ethanol. It was concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in desiccator.

Phytochemical studies

Preliminary qualitative chemical investigations [5, 7, 9, 10, 11, 12,13] of Ethanolic extract of leaves of *Stachytarpheta indica L. (Vahl)* were performed to know the presence of Carbohydrates, Glycosides, Alkaloids, Tannins and Flavonoids. Further thin layer chromatography was performed to confirm their presence.

Acute toxicity

Albino mice of either sexes weighing between 20 and 30gms were used in present investigation. The animals were fasted overnight prior to the experimental procedure. The Up and Down or 'Staircase' method [14] was adopted, and accordingly dose of alcoholic extract was calculated.

Two mice were injected with particular dose, say 50mg/kg and observed for a period of 24 hr for any mortality. The subsequent doses are then increased by a factor of 1.5, if the dose was tolerated or decreased by a factor of 0.7 if it was lethal. The maximum nonlethal and minimum lethal doses are thus determined using only about 10 mice. Once the approximate LD_{50} or the range between the maximum non lethal and minimum lethal doses is found, a final or more reliable LD_{50} assay is planned using at least 3 or 4 dose level within this range with large number of animals in each group. LD_{50} is expressed in terms of mg/kg.

In addition, source of the animal, sex, age, body weight, injection time, route and solvent, and the presence and absence of any immediate reactions were also recorded for further references.

Assessment of Hepatoprotective activity

Hepatoprotective activity was carried out using Albino rats (150-200gm) of either sex [15, 16, 17]. The animals were grouped into four of six animals each and maintained on standard diet and water *ad libitum.*

Tween-80 (1%) was given to I & II group as a vehicle for 10 days by oral route. Liv-52 was administered to III group at the dose of 1ml/ kg body weight by oral route for 10 days. Ethanolic extract was administered to IV group at a dose of 200mg/kg body weight by oral route for 10 days. CCl_4 at a dose of 0.7ml/kg body weight was injected to II, III and IV groups on 3rd, 6th and 10th day by intraperitonial route as shown in following Table No.1.

On 10th day, one hour after the last dose of CCl_4 injection, animals were sacrificed by cervical dislocation and the blood was collected from the Carotid artery, serum was separated and used for the estimation of various biochemical parameters like SGOT, SGPT, SALP, Serum Bilirubin and reversed total protein in rats. Livers were excised and fixed in formalin for assessment of Histopathological studies and Liver weight.

Histopathological study

Processing of isolated liver

The isolated organ was cut into small pieces and preserved into formalin (10% solution) for at least 2 days. The liver pieces were washed in running water for about 12 hours. This was followed by dehydration with alcohol of increasing strength (70, 80 and 90%) for 12 hours each. Then the final dehydration was done using absolute alcohol 3 times for 12 hours each. Again the tissue is cleaned by using xylene 2 times for 15 to 20 min. each. After cleaning the organ pieces were subjected to paraffin infiltration in automatic tissue processing unit [17, 18, 19].

Embedding in paraffin

Hard paraffin was melted and was poured into square-shaped blocks. The liver pieces were then dropped into the liquid paraffin quickly and allowed to cool.

Sectioning

The blocks were cut using microtome to get sections of thickness 5 microns. The sections were taken on a microscopic slide on which egg albumin (sticky substance) was applied. The sections were allowed to remain on the sticky substance for three days till it sticks firmly onto the slide. The section should be dried completely before staining

Staining

Eosin is in acidic stain and hematoxylin is a basic stain, which are used for staining.

Observation

All the slides were observed for changes in histopathological characteristics and photographs were taken.

RESULTS AND DISCUSSION

The phytochemical studies of Ethanolic extract of *Stachytarpheta indica L. (Vahl)* revealed the presence of Carbohydrates, Glycosides and Flavonoids. In acute toxicity studies the maximum nonlethal dose was found to be 2000mg/kg body weight. Hence 1/10th of the dose was taken as effective dose (200mg/kg body weight) for Ethanolic extract. Hepatoprotective activity of Ethanolic extract of *Stachytarpheta indica L. (Vahl)* was evaluated by carbon tetrachloride induced toxicity. The results are tabulated in Table No.2. The results indicate reduction in CCl_4 induced elevated levels of SGPT, SGOT, SALP and Serum bilirubin and reversed total protein in Test group treated with Ethanolic extract of *Stachytarpheta indica L. (Vahl)*, indicating hepatoprotective activity at the dose of 200mg/kg body weight and was comparable to that of standard drug Liv-52(1ml/kg body weight). Further histopathological studies indicated that the animals pretreated with Ethanolic extract of *Stachytarpheta indica L. (Vahl)* was minimal with distinct preservation

of structures and architectural frame of the hepatic cells, and is comparable to standard Liv-52. The results are shown in figures 1 to 4. Further, it has been reported that flavanoid constituents of the plant possess antioxidant activity and was found useful in the treatment of liver damage [20, 21, 22, 23]. Hence the significant activity shown by Ethanolic extract of *Stachytarpheta indica L. (Vahl)* may be attributed to the present flavanoids.

CONCLUSION

The Qualitative phytochemical investigations of Ethanolic extract of *Stachytarpheta indica L. (Vahl)* have shown the presence of Flavanoids. Ethanolic extract of *Stachytarpheta indica L. (Vahl)* offers protective effect against CCl_4-induced hepatotoxicity in experimental rats. The mechanism of action is yet to be investigated but may be due to the antioxidant effects of Flavonoids found to be present in the extract.

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Table No.1: Schedule for administration of CCl_4 and test substances to the rats

Groups	**Dose/kg body weight**	**No. of Days/Route**
Group-I Normal control	Tween 80 (1%)	For 10 days/Oral.
Group-II Hepatotoxic Control	CCl_4 0.7ml/kg Tween 80 (1%)	For 3rd, 6th, and 10thday/ Intraperitonial For 10 days/Oral
Group-III Standard (Liv-52)	CCl_4 0.7ml/kg Liv-52-1ml/kg	For 3rd, 6th, and 10th day/Intraperitonial. For 10 days/Oral.
Group-IV Test (Ethanolic extract)	CCl_4 0.7ml/kg Ethanolic extract-200mg/ body weight.	For 3rd, 6th, and 10th day/ Intraperitonial For 10 days/Oral.

Table No.2: Biochemical parameters of different groups for CCl_4 induced hepatotoxicity

Treatment Groups	**SGPT (U/ml)**	**SGOT (U/ml)**	**ALP (KU/mL)**	**Serum (Bilirubin (mg/mL)**	**Total Proteins (mg/mL)**
Normal Control	158.2±3.50	227.4±7.05	12.32±0.07	0.74±0.04	3.51±0.30
Hepatic Control.	273.2±3.45	297.4±6.56	15.22±0.20	1.40±0.07	2.61±0.21
Standard (Liv-52)	160.8±2.13	226.6±2.44	12.72±0.16	0.78±0.02	3.11±0.23
Test (Ethanolic extract)	160.4±3.00	226.4±3.45	12.60±0.16	0.69±0.02	3.01±0.30

n=6, ANOVA,
*P value for test <0.0001 against Normal Control;
*P value for Hepatic control <0.0001 against Normal Control;
*P value for Standard <0.0001 against Normal Control;
*P value for Test (Ethanolic extract) <0.0001 against Normal Control.

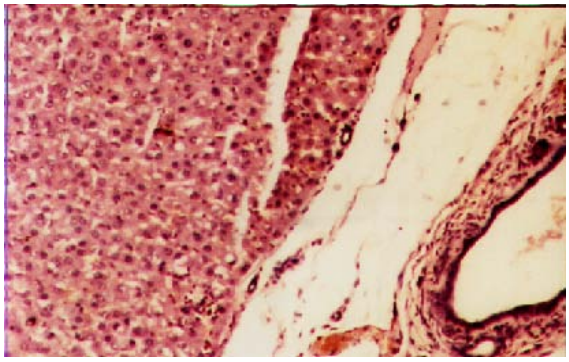

Figure 1: Normal Liver Biopsy (Normal group)

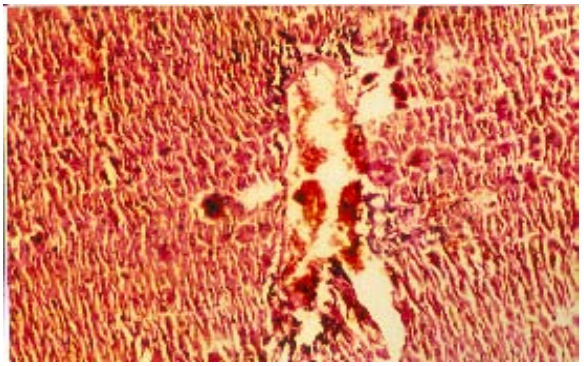

Figure 2: Necrosis of Hepatocytes (CCl_4 group)

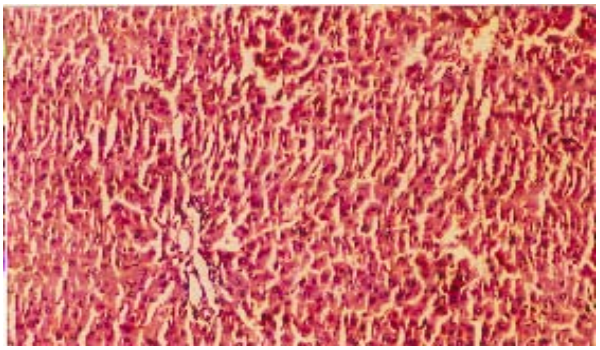

Figure 3: Regeneration of Hepatocytes (Liv-52 group)

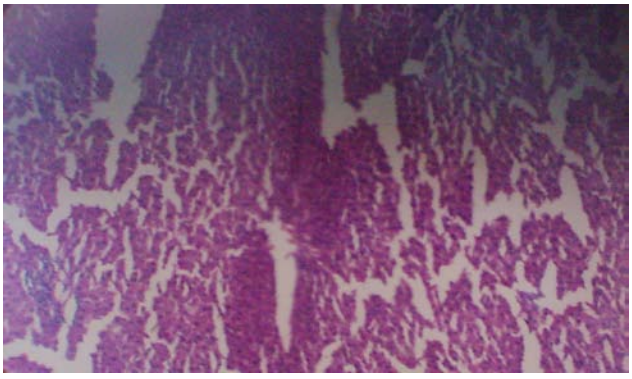

Figure 4: Regeneration of Hepatocytes (Ethanolic extract)

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