Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (7):241-249
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Hepatoprotective and antioxidant activities of *Limnophila heterophylla*

Raja Sundararajan and Ravindranadh Koduru

GITAM Institute of Pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh, India

---

ABSTRACT

Limnophila heterophylla, a medicinal plant native to tropical and subtropical South and Southeast Asia (Indomalaya), including southern China and it is commonly used in folk medicine to treat various diseases. The aim of the present study was to evaluate the hepatoprotective and in-vivo antioxidant activities of Limnophila heterophylla. The hepatoprotective activity of methanol extract of leaves of Limnophila heterophylla was evaluated against carbon tetrachloride + olive oil (1:1) induced hepatic damage in rats. The methanol extract of leaves of Limnophila heterophylla at dose of 250 and 500 mg/kg were administered orally once daily for seven days. Serum enzymatic levels of serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), Lactate dehydrogenase (LDH), total bilirubin (TB), and total protein (TP) were estimated. In-vivo antioxidant activity of methanol extract of Limnophila heterophylla was evaluated by various assays including superoxidedismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRD), reduced glutathione, and malondialdehyde (MDA) levels in liver tissues. The results of the carbon tetrachloride -induced liver toxicity experiments showed that rats treated with the methanol extract of Limnophila heterophylla 500 mg/kg showed a significant decrease in SGOT, SGPT, ALP, LDH, and TB levels, which were all elevated in the CCl_4 + olive oil group ($p < 0.01$). Limnophila heterophylla leves extracts therapy also protective effects against histopathological alterations. The extract showed potent activities on SOD, CAT, GRD, GPx, reduced glutathione and MDA levels. Further histopathological examination of the liver sections was carried out to support the induction of hepatotoxicity and hepatoprotective efficacy. The results of the present study strongly reveal that methanol extract of leaves of Limnophila heterophylla had hepatoprotective and antioxidant activities against carbon tetrachloride - induced hepatic damage in experimental animals.

Keywords: *Limnophila heterophylla*, Carbontetrachloride, Hepatoprotective, Antioxidant activity.

INTRODUCTION

Limnophila heterophylla [Syononyms: *Columnea heterophylla Roxb*., *Limnophila reflexa* Benth., *Limnophila heterophylla* var. reflexa (Benth.) Hook. f., *Limnophila roxburghii* G. Don] is an aquatic herb, mainly submerged, but with shoots that often emerge above the water surface, rooting at nodes. This plant is listed as a “serious” weed in India and a “common” weed in Thailand and regarded as among the most problematic weeds of deep-water rice in West Bengal, India (1). The plant finds lot of applications in the traditional system of medicine against various ailments (2). The plant leaves are crushed with coconut oil and applied on the wound to quicken healing (3) and is established as a source of flavonoids, terpenoids. The isolated phytochemicals as well as different extracts exhibited some kind of pharmacological activities such as antibacterial, anti fungal (4), wound healing (5) and COX-Inhibitor (6). Exhaustive research regarding isolation of more phytochemicals and pharmacology study on this medicinal plant is still necessary so as to explore the plant regarding its medicinal importance. Therefore, the aim of this

review was to boost up present day researchers in this direction to undertake further investigation of this plant. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as in traditional systems of medicines in India (7). Hepatic fibrosis is a common condition in which major amounts of liver parenchyma cells are replaced by fibrous connective tissue. Liver diseases remain one of the serious health problems and it is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury (8). Experimentally hepatic fibrosis is formed by the administration of CCl_4, paracetmol, thioacetamide etc. Scavenging of free radicals by antioxidant could reduce the fibrosis process in the tissue. As per the literature survey, the hepatoprotective effect of *Limnophila heterophylla* leaves against CCl_4 + Olive oil induced liver injury in rat has not been demonstrated. Hence, the present study focused on evaluating the hepatoprotective and antioxidant effects of methanol extract from *Limnophila heterophylla* leaves on CCl_4 + Olive oils-induced liver injury in rat.

MATERIALS AND METHODS

Plant material

The plant material was collected from Tirupati (Andhra Pradesh) and further identified, confirmed & authenticated by Dr. Madavchetty, Professor, Botany department, Sri Venkateswara University, Tirupati. Voucher specimen No (GIP-Plant No-006) has retained in GITAM Institute of Pharmacy, GITAM University. The collected leaves of *Limnophila heterophylla* were washed with tap water. The leaves were cut in to small pieces and air-dried thoroughly under shade (at room temperature) for one month to avoid direct loss of phytoconstituents from sunlight. The shade dried material was powdered using the pulverizer and sieved up to 80 meshes. It was then homogenized to fine powder and stored in air-tight container for furthers analysis.

Chemicals

All chemicals used in the study were of analytical grade. CCl_4 was procured from Krishna Chemicals Pvt. Ltd., Gujarat, and Silymarin from GVK Bio sci, Hyderabad.

Preparation of *Limnophila heterophylla* extract

The leaves of *Limnophila heterophylla were* refluxed with methanol in a soxhlet extractor for 72 hrs. The excess solvent was removed from the extract by vacuum rotary flash evaporator and concentrated over the hot water bath. Finally dried extract was stored in desiccators for hepatoprotective and antioxidant activities.

Preliminary phytochemical screening

The methanol extract was subjected to various phytochemical studies to identify the presence of various phytoconstituents like alkaloids, steroids, flavonoids, glycosides, tannins, resins, carbohydrates, amino acids, proteins and terpenes (9).

Safety Evaluation

The toxicity study was carried out using OECD guide lines No. 423. Three female mice of the same age group and weight were taken in a single dose up to the highest dose of 2000 mg/kg B/W orally. The animals were observed for 1 hr continuously and then hourly for 4 hr, and finally after every 24 hr up to 15 days for any mortality or gross behavioral changes (10).

In-vivo hepatoprotective activity

Experimental animals

Swiss albino rats (200–250gm) were used for the experiments. Animals were housed under standard conditions (i.e. at 22 ± 2^0C; humidity: 50–55% and 12 h natural light/dark cycle) in polypropylene cages and fed with standard laboratory diet and water ad libitum at least 1 week prior to experiment. All the animal treatment was conducted in accordance with the guideline approved by Institutional Animal Ethics Committee (IAEC) and CPCSEA.

Experimental design

A total of 35 rats were divided into5 groups of 7 rats each. Group I served as normal control and received only the vehicle (1mL/kg/day orally).Group II received CCl_4 1mL/kg (1:1 of CCl_4 in olive oil) i.p. once daily for 7 days. Group III received CCl_4 1mL/kg (1:1 of CCl_4 in olive oil) i.p. and silymarin 100 mg/kg orally (p.o.) for 7 days. Groups IV, V were administered methanol extract of leaves of *Limnophila heterophylla* at 250 and 500 mg/kg body

weight p.o., respectively and dose of 1mL/kg i.p. of CCl_4 (1:1 of CCl_4 in olive oil) for 7 days. All rats were sacrificed by cervical dislocation 24hrs after the last treatment.

Biochemical parameters

The blood samples were allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation at 3500 rpm at 37^0C for 15min and analyzed for various biochemical parameters such as serum glutamic oxalo acetate transaminases (SGOT), serum glutamic pyruvate transaminases (SGPT), alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), total bilirubin (TB) and total protein (TP).

Antioxidant parameters

For estimating antioxidant activity, animals were sacrificed and liver was excised, rinsed in ice –cold normal saline followed by 0.15M Tris-HCl (pH-7.4) blotted dry and weighed. A 10% w/v of homogenate was prepared in 0.15M Tris-HCl buffer and processed for the estimation of lipid peroxidation (TBARS). A part of homogenate after precipitating proteins with trichloroacetic acid (TCA) was used for estimation of glutathione. The remaining homogenate was centrifuged at 1500 rpm for 15 min at 4^0C. The supernatant thus obtained was used for the estimation of superoxide dismutase, GPx, GSH and catalase.

Estimation of hepatoprotective parameters

SGOT & SGPT

Serum transaminases (GOT and GPT) were determined by the method developed by Reitman and Frankel (11). Each substrate (0.5mL) [either α -L-alanine (200mM) or L-aspartate (200mM) with 2mM α - ketoglutarate] was incubated for 5 min at 37°C. A 0.1mL of serum was added and the volume shall be adjusted to 1.0mL with sodium phosphate buffer (pH 7.4; 0.1M). The reaction mixture was incubated for 30 and 60 min for GPT and GOT, respectively. A 0.5mL of 2, 4-dinitrophenyl hydrazine (1mM) was added to the reaction mixture and left for 30 min at room temperature. Finally, the color was developed by the addition of 5mL NaOH (0.4 N) and the product formed was read at 505nm. Data were expressed as IUL^{-1}.

Alkaline phosphatase

Alkaline phosphatase (ALP) was assayed by the method of Kind and King (12). The reaction mixture of 3.0 ml containing 1.5 ml of buffer (carbonate-bicarbonate buffer, 0.1M, pH 10.0), 1 ml of substrate and requisite amount of the enzyme sources was incubated at 37^oC for 15minutes. The reaction was arrested by the addition of 1.0 ml of Folins phenol reagent. The control tubes were received the enzyme after arresting the reaction. The contents was centrifuged and to the supernatant, 1.0 ml of 15% sodium carbonate solution, 1.0ml of substrate and 0.1ml of magnesium chloride (0.1M) was added and mixture was incubated for 10 minutes at 37^0C. The colour was read out 640 nm against the blank.

Lactate dehydrogenase activity

Lactate dehydrogenase (LDH) activity was estimated in serum by the standard method (13, 14). The reaction mixture consisted of 0.1mL of nicotinamide adenine dinucleotide (NADH)-reduced disodium salt (0.02 M), 0.1mL of sodium pyruvate (0.01 M), 0.1mL of serum, and made up to 3mL with sodium phosphate buffer (0.1M; pH 7.4). The changes in the absorbance was recorded at 340nm at 30s interval each for 3min and the enzyme activity was calculated using a molar extinction coefficient of $6.220M^{-1}$ cm^{-1} and it was expressed as nanomoles NADH oxidized min^{-1} mg^{-1} protein.

Total Bilirubin

Total bilirubin (TB) content was estimated by method of Malloy and Evelyn (15). The two test tubes were taken and each into was added 0.2ml of serum sample and 1.8 ml of distilled water. To the unknown, 0.5 ml of diazo reagent and to the blank, 0.5 ml of 1.5% hydrochoric acid was added. Finally, to each tube, 2.5 ml of methanol was added and then allowed to stand for 30 minutes in ice and absorbance was read at 540nm. For a standard curve, the above standard was diluted 1in 5ml methanol. The amount of direct reacting bilirubin was determined similarly by substituting 2.5ml of water for 2.5ml of methanol. The values were expressed as mg/dl.

Total Protein

Total protein (TP) content in the tissue was determined by earlier method reported by Lowry et al., (16), using

bovine serum albumin (BSA) as the standard.

Estimation of antioxidant parameters

Superoxide dismutase assay

Superoxide dismutase (SOD) activity was analyzed by the method described by Rai et al. (17). Assay mixture contain 0.1mL of supernatant, 1.2mL of sodium pyrophosphate buffer (pH 8.3; 0.052M), 0.1mL of phenazine methosulfate (186 mM), 0.3mL of nitroblue tetrazolium (300 mM), and 0.2mL of NADH (750 mM). Reaction was started by the addition of NADH. After Incubation at 30°C for 90s, the reaction was stopped by the addition of 0.1mL of glacialacetic acid. Reaction mixture was stirred vigorously with 4.0mL of n-butanol. Color intensity of the chromogen in the butanol was measured spectrophotometrically at 560nm and the concentration of SOD was expressed as Umg^{-1} of protein.

Catalase assay

Catalase activity (CAT) was measured by the method of Bergmeyer (18). A 0.1mL of supernatant was added to cuvette containing 1.9mL of 50mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0mL of freshly prepared 30mM H_2O_2. The rate of the decomposition of H_2O_2 was measured spectrophotometrically at 240 nm. Activity of CAT was expressed as Umg^{-1} of protein.

Glutathione reductase assay

Glutathione reductase (GRD) activity was assayed by the method of Mohandas et al. (19). The assay system consist of 1.65mL sodium phosphate buffer (0.1M; pH 7.4), 0.1mL EDTA (0.5mM), 0.05mL oxidized glutathione (1mM), 0.1mL NADPH (0.1mM), and 0.05mL supernatant in a total mixture of 2mL. The enzyme activity was quantified by measuring the disappearance of NADPH at 340nm at 30s intervals for 3min. The activity was calculated using a molar extinction coefficient of 6.22 X $10^3M^{-1}cm^{-1}$ and was expressed as nanomoles of NADPH oxidized min^{-1} mg^{-1} protein.

Glutathione peroxidase assay

Glutathione peroxidase (GPx) activity was determined by the method described by Wendel (20). The reaction mixture consist of 400µL of 0.25M potassium phosphate buffer (pH- 7.0), 200 mL supernatant, 100 µL GSH (10 mM), 100 µL NADPH (2.5mM), and 100µL GRD ($6UmL^{-1}$). Reaction was started by adding 100µL hydrogen peroxide (12mM) and absorbance was measured at 366nm at 1min intervals for 5 min using a molar extinction coefficient of 6.22X $10^3M^{-1}cm^{-1}$. Data was expressed as mU mg^{-1} of protein.

Reduced glutathione assay

Reduced glutathione (GSH) was measured according to the method of Ellman (21). The equal quantity of homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.01 ml of this supernatant, 2ml of phosphate buffer (pH 8.4), 0.5 ml of 5'5-dithio, bis (2-nitrobenzoic acid) and 0.4ml double distilled water was added. Mixture was vortexed and the absorbance read at 412nm within 15min. The concentration of glutathione was expressed as µg/mg of protein.

Lipid peroxidation assay

Lipid peroxidation (LPO) was measured by the method of Zhang (22). Acetic acid 1.5mL (20%; pH 3.5), 1.5 of TBA (0.8%), and 0.2mL of sodium dodecyl sulfate (8.1%) was added to 0.1ml of supernatant and heated at 100°C for cooled and 60 min. Mixture was cooled, and 5mL of n-butanol: pyridine (15:1) mixture and 1mL of distilled water was added and vortexed vigorously. After centrifugation at 1200g for 10min, the organic layer was separated and the absorbance was measured at 532nm using a spectrophotometer. Malonyldialdehyde (MDA) is an end product of LPO, which reacts with TBA to form pink chromogen–TBA reactive substance. It was calculated using a molar extinction coefficient of 1.56 X 10^5M^{-1} cm^{-1} and shall be expressed as nanomoles of TBARS mg^{-1} of protein.

Histopathological studies

A portion of the left lobe of the liver was preserved in 10% neutral formalin solution for at least 24 h, processed and paraffin embedded as per the standard protocol. Sections of 5µm in thickness were cut, deparaffinized, dehydrated,

and stained with haematoxylin and eosin (H&E) for the estimation of hepatocyte necrosis and vacuolization. Morphological changes were observed including cell gross necrosis, sinusoidal congestion, fatty change, ballooning degeneration, inflammatory infiltration.

Statistical analysis

The data were expressed as mean ± SE, whose biochemical and physiological parameters were analyzed statistically using one-way ANOVA followed by Dunnet's t-test using the SPSS statistical software for comparing with the control group and CCl_4 + Olive oil -treated group. $P < 0.05$ was considered as significant.

RESULTS

Percentage yield

The percentage yield of the methanol extract of *Limnophila heterophylla* was found to be 23.2% and mentioned in the Table No.1.

Table No 1. Percentage yield of methanol extract of *Limnophila heterophylla*

Extract Name	% Yield (w/w)
Methanol extract of *Limnophila heterophylla*	23.2

Preliminary phytochemical screening of *Limnophila heterophylla*

It was observed that the preliminary phytochemical screening of *Limnophila heterophylla* showed the presence of steroids, triterpinoids, phenolics, tannins, and flavonoids in methanol extract, where as absence alkaloids, oils, fats, glycosides and saponins. The preliminary phytochemical screening for various functional groups is tabulated as Table No. 2.

Table No. 2. Qualitative analysis of methanol extract of *Limnophila heterophylla*

Name of phytochemical test	Observation report of methanol extract of *Limnophila heterophylla*
Carbohydrates	-
Steroids	+
Flavonoids	+
Tannins	+
Alkaloids	_
Triterpinoids	+
Phenolics	+
Saponins	-
Glycosides	-
Oils & fats	-

"+" indicates positive; "-" indicates negative

Toxicity study

When rat fed with methanol extract of *Limnophila heterophylla* up to 2000 mg kg^{-1},p.o. exhibited no mortality or any sign of gross behavioral changes when observed initially for 24hrs, and finally up to 15 days.

Carbon tetrachloride induced hepatotoxicity

The impact of CCl_4 + Olive oil on the levels of SGOT, SGPT, ALP, LDH, bilirubin, and total protein in the serum were summarized in Table 3. The serum levels of above biochemical parameters were significantly ($P < 0.05$) increased; however, a significant decrease in the levels of total protein was observed in CCl_4 + Olive oil treated control compared to normal control. Treatment with methanol extract of *Limnophila heterophylla* and silymarin prior to CCl_4 + Olive oil intoxication, afforded protection by lowering of the above serum markers as well as by increasing the total protein content. Better protection was observed with the higher dose (500 mg/kg) of the extract.

***In vivo* antioxidant assays**

As shown in the table 4, CCl_4 + Olive oil intoxication produced significant ($P < 0.05$) reduction in SOD, CAT, GRD, GPx, and GSH activities along with significantly increased lipid peroxidation level (expressed as MDA) when compared to normal control. Treatment with methanol extract of *Limnophila heterophylla* at doses 250 and 500 mg/kg b.w for 7 days showed significant higher levels of SOD, CAT, GRD, GPx, and GSH in addition to significant lower levels of hepatic MDA as compared to CCl_4 + Olive oil intoxicated rats.

Table: 3. Hepatoprotective parameters of methanol extract of *Limnophila heterophylla* against CCl_4 + Olive oil induced damaged.

Biochemical Parameters	Control group	CCl4 + Olive oil treated group	*Limnophila heterophylla* methanol extract(mg/kg)		Silymarin (25mg/kg)
			250	500	
SGOT (IUL^{-1})	46.9±74	186.8±11.4	121.4±5.6	66.8±11.6	52.7±76
SGPT (IUL^{-1})	106±9.5	254±15.4	196 ±12.6	147±8.5	102±9.5
ALP (KA Units)	42.8±1.18	74.6±0.99	44.7±0.65	42.4±1.39	45.2±1.25
LDH (nanomoles NADH oxidized min^{-1} mg^{-1} protein)	375.6±11.4	524.8±14.5	446.8±2.5	446.2±0.05	404.7±0.13
Total bilirubin (mg/dl)	2.7±0.04	4.24±0.14	2.0±0.17	3.2±0.24	2.2±0.05
Total protein (mg/dl)	9.7±0.15	4.28±0.06	6.5±0.02	8.7±0.37	8.2±0.13

*Data are given as mean SD of six animals. * Significant difference ($p < 0.05$) from control or CCl4 + Olive oils -treated rats. ALP = alkaline phosphatase; BIL=bilurubin, LDH= Lactate dehydrogenase; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase.*

Table.4. Effect of methanol extract of *Limnophila heterophylla* on biochemical parameters

S.NO	Treatment	SOD (u/mg of protein)	CAT (u/mg of protein)	GRD (nanomoles of NADPH oxidized min-1 mg-1 protein.)	GPx (mU mg^{-1} of protein)	GSH (µg/mg of protein)	MDA (nanomoles of TBARS mg^{-1} cm of protein)
1	Control	59.4±6.5	189.6±0.06	18.6±1.39	276.6±0.99	9.2±07	4.6±03
2	Toxic Control	24.6±0.34	46.5±07	7.4±1.16	167.4±0.85	3.4±09	13.6±02
3	Standard	59.8±0.45	179.4±15	15.2±0.04	259.2±18	8.8±08	5.8±03
4	MECG 250 mg/kg	43.8± 03	132.6±1.3	11.4±0.37	211.6±0.17	6.8±2.3	9.4±0.05
5	MECG 500 mg/kg	54.6±015	159.8±11	13.8±0.11	224.8±0.18	7.4±1.4	6.4±04

*Data are given as mean SD of six animals. * Significant difference ($p < 0.05$) from control or CCl_4 + Olive oil -treated rats. SOD= Super oxide dismutase; CAT= Catalase, GPx= Glutathione peroxidase; GRD = glutathione reductase; MDA = Malonyldialdehyde; RD = Reduced glutathione*

Histopathological observations

The histopathological studies of the liver showed fatty changes, swelling, necrosis, cell vacuolization, degenerated nuclei and inflammatory, infiltration with loss of hepatocytes in CCl_4 + Olive oil intoxicated rats (II) in comparison with normal rats (I). The liver sections of rats treated with the lower (IV) and higher (V) of the extract showed reduced degeneration of hepatocytes, normalization of fatty changes, decrease in vacuolization and necrosis of the liver. Silymarin treated group showed considerable reduction in necrosis and damage of liver cells (III).

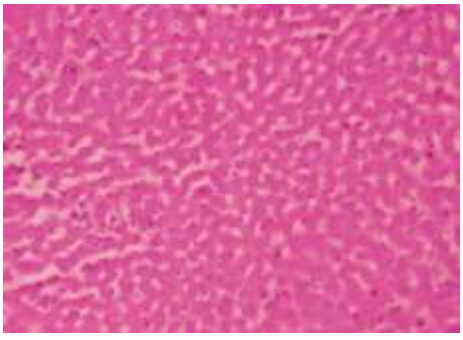

Figure I:-Normal

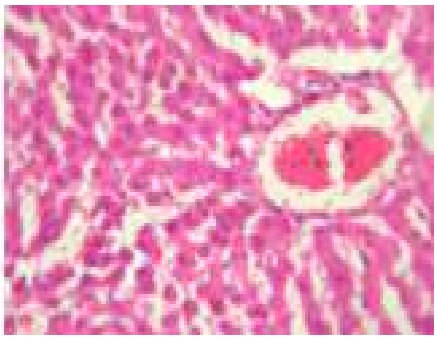

Figure II- CCl4- Olive oil (1:1, 1 ml/kg)

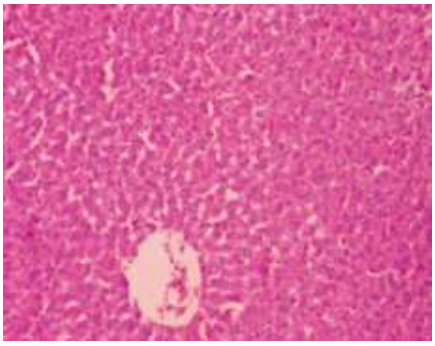

Figure III-Standard (Silymarin 25mg/kg)

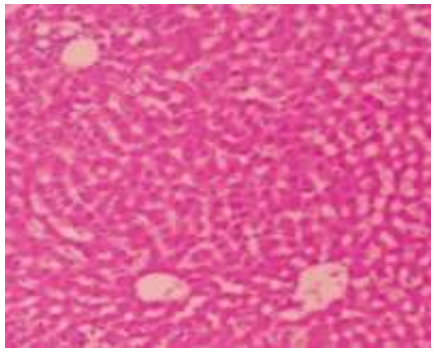

Figure **IV-Low dose (250 mg/kg)**

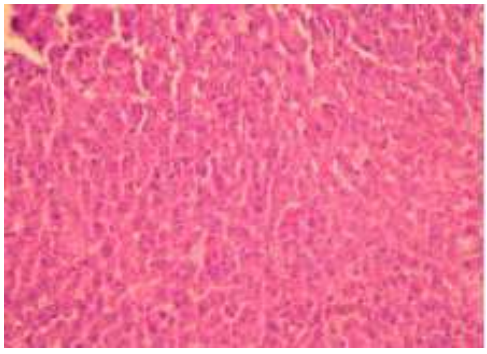

Figure V-High dose (500 mg/kg)

DISCUSSION

Phytochemical screening of the *Limnophila heterophylla* shows the presence of steroids, alkaloids, triterpinoids, phenolics, tannins, flavonoids and saponins in methanol extract. Acute toxicity studies revealed the non-toxic nature of the methanol extract of *Limnophila heterophylla* up to a dose level of 2000mg/kg body weight in rats. There was no lethality or toxic reaction found at any of the doses selected during the study.

The present study was undertaken to assess the in vivo antioxidant effects of methanol extract of *Limnophila heterophylla* on CCl_4 + Olive oil induced hepatotoxicity in rats. The hepatotoxicity induced by CCl_4 + Olive oil is due to its metabolite $CCl_3^{\bullet}$, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage. Hepatocellular necrosis leads to elevation of the serum marker enzymes such as SGPT, SGOT, bilirubin, ALP, and LDH which are released from the liver into blood (23). The present study revealed a significant increase in the activities of SGPT, SGOT, bilirubin, ALP, and LDH levels on (24, 25), indicating considerable hepatocellular injury. Administration of *Limnophila heterophylla* methanol extract at different doses level (250 and 500 mg/kg) attenuated the increased levels of the serum enzymes, produced by CCl_4 + Olive oil and caused a subsequent recovery towards normalization comparable to the control groups animals.

The hepatoprotective effect of the *Limnophila heterophylla* methanol extract was further accomplished by the histopathological examinations. *Limnophila heterophylla* methanol extract at different dose levels offers hepatoprotection, but 500mg/kg is more effective than the lower dose. In CCl_4 + Olive oil induced hepatotoxicity, the balance between ROS production and these antioxidant defenses may be lost, 'oxidative stress' results, which through a series of events deregulates the cellular functions leading to hepatic necrosis. Hence result showed that the activities of CAT, SOD, GRD, GSH and GPx in group treated with CCl_4 + Olive oil declined significantly along with significantly increased lipid peroxidation level (expressed as MDA) than that of normal group. Co-administration of methanol extract of *Limnophila heterophylla* at a dose of 250 and 500 mg/kg for 7 days markedly prevented these CCl_4 + Olive oil induced alteration and maintained enzymes level near to normal values. Standard (silymarin) treated group also significantly increased the level of CAT, SOD, GRD, GSH and GPx in CCl_4 + Olive

oil induced toxic rats. CAT considered as most important H_2O_2 removing enzyme and also a key component of anti oxidative defense system. Here CAT activity was increased and then restored to normal levels on administration of ethanolic extract of *Limnophila heterophylla*. SOD plays an important role in the elimination of ROS and protects cells against the deleterious effects of super oxide anion derived from the peroxidative process in liver and kidney tissues and the observed increase in SOD activity suggests that the methanol extract of *Limnophila heterophylla* has an efficient protective mechanism in response to ROS.

The reduced activities of GRD and GPx observed point out the hepatic damage in the rats administered with CCl_4 +Olive oils but the treated with, 250 and 500mg/kg of *Limnophila heterophylla* methanol extract groups showed significant increase in the level of these enzymes, which indicates the antioxidant activity of the *Limnophila heterophylla*. Regarding non enzymic antioxidants, GSH is a critical determinant of tissue susceptibility to oxidative damage and the depletion of hepatic GSH has been shown to be associated with an enhanced toxicity to chemicals, including CCl_4 + Olive oil. Furthermore, a decrease in hepatic tissue GSH level was observed in the CCl_4 + Olive oil (26) treated groups. The increase in hepatic GSH level in the rats treated with, 250 and 500 mg/kg of *Limnophila heterophylla* methanol extract may be due to de novo GSH synthesis or GSH regeneration.

The level of lipid peroxide is a measure of membrane damage and alterations in structure and function of cellular membranes. In the present study, elevation of lipid peroxidation in the liver of rats treated with CCl_4 + Olive oil was observed. The increase in LPO levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. Treatment with *Limnophila heterophylla* methanol extract significantly reversed all the changes. Hence, it is possible that the mechanism of hepatoprotection of *Limnophila heterophylla* may be due to its antioxidant activity.

CONCLUSION

A methanol extract of *Limnophila heterophylla*, in the dose of 500mg/kg, p.o., has improved the biochemical (SGPT, SGOT, TB, ALP, LDH and TP) and antioxidant parameters (CAT, SOD, GRD, GSH, GPx and MDA) levels significantly, which were comparable with silymarin. On the basis of the study it can be conclude that methanol extract of *Limnophila heterophylla* possesses both hepatoprotective and *in-vivo* antioxidant activities.

Acknowledgement
The authors are thankful to UGC (New Delhi, India) for providing financial assistance to GITAM institute of pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh, India.

REFERENCES

[1] R. Sahu, *Indian Agriculturist* **1992**, 36(1), 49-51.
[2] K.S. Mukherjee, D. Gorai, S.M.A. Sohel, D. Chatterjee, B. Mistri, B. Mukherjee, G. Brahmachari, *Fitoterapia* **2003**, 74, 188-190.
[3] P.N. Arul Manikandan, *Ancient Science of Life* **2005**, 25 (1), 21-27.
[4] R.H. Padiya, E.D.Patel, R.N. Acharya, *International Journal of Ayurvedic Medicine* **2013**, 4(1).
[5] G.B.S Reddy, A.B. Melkhani, G.A.Kalyani, J.V. Rao, A. Shirwaikar, M. Kotian, R. Ramani, K.S. Aithal, A.L. Udupa, G. Bhat, K.K. Srinivasan, *International Journal of Pharmacognosy* **1991**, 29, 145-153.
[6] G. Brahmachari, S.K. Jash, L.C. Mandal, A. Mondal, R. Roy, *Rasayan Journal of Chemistry* **2008**, 1(2), 288-291.
[7] A. Subramonium, D.A. Evance, S. Rajasekharan, P. Pushpagandhan, *Indian Journal of Experimental Biology* **1998**, 36, 385-389.
[8] B.H. Babu, B.S. Shylesh, J. Padikkala. *Phytotherapy* **2001**, 72, 272-276.
[9] C.K. Kokate, Practical Pharmacognosy, New Delli: Vallabh prakashan; **1997,** 107-11.
[10] B.K. Chandan, A.K. Saxena AK, S. Shukla, N. Sharma N, D.K. Gupta, K.A. Suri, J. Suri, M. Bhadauria and B.Singh, *Journal of Ethnopharmacology* **2007**, 111, 560–6.
[11] S. Reitman, S.A. Frankel, *American Journal of Clinical Pathology* **1957,** 28, 56–63.
[12] P.R. Kind and E.J. King, *Journal of Clinical Pathology* **1954,** 7, 322-326.
[13] A. Kornberg, S.P. Colowick, N.O. Kaplan N.O, *Methods in Enzymology* **1955**, 1, 441-443.
[14] S. Raja, K.F.H. Nazeer Ahamed, V. Kumar, M. Kakali, A. Bandyopadhyay, K. Pulok K. Mukherjee, *Journal of Ethnopharmacology* **2007**, 109, 41–47.

[15] H.T. Malloy, K.A. Evelyn, *Journal of Biological Chemistry* **1937**, 119, 481-490.
[16] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randal, *Journal of Biological Chemistry* **1951,** 193: 265–275.
[17] S. Rai, A. Wahile, K. Mukherjee, B.P. Saha, P.K. Mukherjee, *Journal of Ethnopharmacology* **2006,** 104, 322-327.
[18] H.U. Bergmeyer, K.Goweh, H. Grassel, *Methods of enzymatic analysis* **1974**, 438-439.
[19] J. Mohandas, J.J. Marshall, G.G. Duggin, J.S. Horvath, D. *Tiller Cancer Research* **1984** 44: 5086–5091.
[20] A. Wendel, *Methods in Enzymology* **1981,** 77: 325–33.
[21] G.L. Ellman, *Archives of Biochemistry and Biophysics* **1959**, 82, 70–77.
[22] X.Z. Zhang, Crop Physiology Research Methods **1992**; 131–207.
[23] H. Zeashan, G. Amresh, S. Singh, CH.V. Rao, *Food Chemistry and Toxicology* **2008**, 46: 3417-3421.
[24] K. Ashok Shenoy, S.N. Somayaji, K.L. Bairy, *Indian Journal of Pharmacology* **2001**, 33, 260-266.
[25] L. Wang, Q. Ran, D.H. Li, H.Q. Yao HQ, Y.H. Zhang, S.T. Yuan, Chinese *Journal of Natural Medicine*, **2011,** 9 (3), 194-198.
[26] R.P. Hewawasam, K.A.P.W. Jayatilaka, C. Pathirana, L.K.B, *Mudduwa Journal of Pharmacy and Pharmacology*, **2003**, 55, 1413-1418.