RESEARCH ARTICLE

Received on: 04/02/2016
Published on:22/04/2016

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Conflict of Interest: None Declared !

Hepatoprotective activity of *Streblus asper* leaf extracts in CCl_4 induced albino rats

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ABSTRACT

Liver is one of the vital organs in the human body. It plays a major role in several metabolic reactions. Cirrhosis, hepatitis, jaundice, liver cancer are some of the disorders due to which the liver diminishes. The available drugs are not completely curbing the disorders and mostly they are fetching side effects. Medicinal plants can combat to these problems and can be used for the therapy. The plant *Streblus asper* is collected from the village area of Kesamudram, Warangal district. The plant leaves were dried, powdered and used for the extraction. Methanol and aqueous were used as solvents to prepare the extract through maceration technique. The albino rats were divided into the following groups for studying the hepatoprotective activity. Group- I control, group-II- CCl_4 (1ml /kg 1:1 in coconut oil- i.p.,), group- III - CCl_4+ Liv – 52 (5ml/kg), group-IV – CCl_4+ SAALE (*Streblus asper* Aqueous Leaf Extract) 200mg/kg, , group-V- CCl_4+ SAALE 400mg/kg, group-VI- CCl_4+ SAMLE-(*Streblus asper* Methanol Leaf Extract) 200 mg/kg and group-VII- CCl_4+ SAMLE 400 mg/kg. 8 rats were maintained in each group, the treatment of the extract and liver tonic was done through oral with gastric gavage for 14 days. On 15th day the blood of the rats was collected through retro orbital plexus. The serum samples were separated for the serological tests. The rats were sacrificed and livers were processed for the histological sections. The serum parameters like increased bilirubin, SGOT, SGPT, ALP, LDL, VLDL, Cholesterol, triglycerides and decreased levels of protein, HDL were noticed in the CCl_4 induced group-II rats. The normal levels were seen in the both extracts administered rats. Results were compared significantly with group-III. Reformed hepatocytes in the extracts treated rats were also supported the hepatoprotective activity of the extracts. The SAMLE showed better results than to the SAALE.

Key words: CCl_4, SGOT, SGPT, ALP, Hepatoprotective activity.

Introduction

Liver is a vital organ in the human body, plays a great role in different metabolic reactions. As it is a xenobiotic organ it may prone to several harmful substances. Consuming of alcohol, junk food, intaking more drugs like analgesics, antipyrectics may cause the drastic damage to the liver. Several hepatic failures are being noticed like hepatiits, cirrhosis, hepatomegaly, hepatic steatosis, hepatocarcinoma etc in the regular human life. Several synthetic drugs are available in treating the liver disorders. Most of the synthetic drugs fetch side effects like nausea, drowsyness, and also affecting the other vital orgnas of the human body. Indian ancestry elucidated the importance of plants for their therapeutic nature. In that consort, many of the plants are unveiled by the pharmacologists. The liver markers in serum like SGPT (Serum Glutamate Pyruvate Transaminase), SGOT (Serum Glutamate Oxaloacetate Transaminase), ALP (Alkaline Phosphatase) etc explain the functional nature of the liver. Disturbances in these markers helpful in the diagnosing the liver disorders. Free radicals like

super oxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) generate in the body cause oxidative stress and can damage the organs of the body.

Literature review:

Since ancient times plants are involved in human life. The plants have been using by human for treating several disorders. The literature is also supported that several medicinal plants were screened for their hepatoprotective activity. Among them some of the plants like- *Andrographis paniculata, Anoectochilus formosanus, Azadirachta indica, Cassia roxburghii, Coccinia grandis, Colchicum autumnale, Foeniculum vulgare, Flacourtia indica, Indigofera tinctoria, Prostechea michuacana, Lepidium sativum, Rubia cordifolia, Orthosiphon stamineus, Solanum nigrum, Scutellaria rivularis, Terminalia catappa* are some of the plants that have already proved for their hepatoprotective activity[1].

Materials and Methods:

Collection of plant material:

The medicinal plant *Streblus asper* was collected from the village area of Kesamudram, Warangal District, Telangana State. It was identified and authenticated by Prof. V.S. Raju (Retd.), Department of Botany, Kakatiya University, Warangal. The plant was stored in the herbarium of the lab by allocating voucher number.

LITERATURE OF THE PLANT

Classification of the plant:

Kingdom: Plantae
Class: Angiosperms
Order: Rosales
Family: Moraceae
Genus: *Streblus*

Species: *asper Streblus asper* Lour (Family : Moraceae) is a small tree which is observed in tropical countries such as India , Srilanka , Malaysia , the Philippines and Thailand. It is known by various names, e.g. Barrinka, Berrikka , Rudi, Sheora, Koi, Siamese rough bush and tooth brush tree. In India it is known by its several names, the most commonly used ones being Shakhotaka (Sanskrit), Siora (Hindi), Sheora (Bengali) and Piray (Tamil). It is used traditionally in leprosy, piles, diarrhea, dysentery, elephantiasis and cancer. It is a rigid shrub; branchlets tomentoseor pubescent. Leaves are 2-4 inch, rigid, elliptic, rhomboid, ovate or obovate, irregularly toothed; petiole 1/12 inch. Male heads globose, solitary or tonate, sometimes androgynous; short scab rid, flowers minute. Female flowers longer peduncle, fruit pisiform; perianth yellow. It is found in the drier parts of India.

Preparation of extracts:

The collected plant material leaves were dried in shade for about 15 days. The leaves were powdered with electrical grinder. The collected coarse powder then passed through No.10 mesh and the fine powder was used for the extraction.

Maceration technique was employed to prepare the extract from leaf powder of the plant. Solvents like methanol and aqueous were used to get the extract. 50g of powder was taken in Stoppard conical flasks; it was mixed with 250ml of solvent and allowed for 24hrs at room temperature with random shaking. Then the filtrate-I was collected and the marc dissolved in 250ml of solvent for 24 hrs and collected the filtrate-II. Then the filtrates (I&II) were subjected to distillation to get extracts and stored in well closed amber glass containers at refrigerator temperature prior to use.

Animal model for the study and their maintenance:

Wistar albino rats of either sex of weighing 200 to 220g were selected for the experiment. The albino rats were brought from the Mahaveer Enterprises (Reg. No. 146/1999/CPCSEA), Hyderabad. The rats were housed in polypropylene cages and acclimatized to the well conditioned house. The house was maintained with the temperature 25±5^{0}C and relative humidity 50 to 60% and 12:12 hr light and dark cycle (IAEC/03/UCPSc/KU/10). They were fed with standard rat pellet (Hypro Nutrients, Pune), water *ad libitum.* The husk was used as bed to animals. Before keeping the animals, the polypropylene cages were sterilized along with water feeding bottles.

Toxic study of the extracts:

The acute toxic study of the extract was performed by the stair case method[2]. The albino rats of either sex were divided into 6 groups 8 in each and the SAMLE (*Streblus asper* Methanol leaf extract). SAALE (*Streblus asper* aqueous leaf extract) extracts were administered through oral with the doses 200, 400 and 600mg/kg to each group. The rats were observed for 72 hrs[3]. There was no mortality of the rats up to 400 mg/kg. 200mg/kg and 400mg/kg were

selected as the doses for carrying the experiments of hepatoprotective activity.

Experimental Design for hepatoprotective studies

Group – I = Control

Group – II = CCl_4 induced (1ml/kg)

Group – III = CCl_4 + Liver tonic (5ml/kg)

Group –IV = CCl_4 + SAALE 200mg/kg (*Streblus asper* Aqueous leaf extract)

Group –V = CCl_4 + SAALE 400mg/kg (*Streblus asper* Aqueous leaf extract)

Group –VI = CCl_4 + SAMLE 200mg/kg (*Streblus asper* Methanol leaf extract)

Group –V II= CCl_4 + SAMLE 400mg/kg (*Streblus asper* Methanol leaf extract)

All of the groups were maintained with 8 rats. The CCl_4 was induced to all other groups (except Control group) with the dose of 1 ml/kg i.p. by 1:1 dilution in coconut oil. The CCl_4 induction was done on 1st day and 7th day of the experiment. The Liv -52 syrup (Himalaya Company) was used as positive drug with the dose 5ml/kg by oral [4, 5]. The SAALE was also administered to the Group –IV and Group – V groups with dose 200 mg/kg and 400 mg/kg body weight and SAMLE was administered to the Group – VI and Group - VII rats with dose 200 mg/kg and 400 mg/kg body weight respectively. The treatment of extracts and liver tonic to the CCl_4 damaged groups (except group-II) was carried out through oral for 14 days [2].

On 15th day before sacrifice of the rats, the blood samples were collected through retro orbital plexus. The blood samples allowed for the centrifugation at 3000rpm for 15 minutes to separate serum for the serological tests (by commercially available kits). The liver tissues were used for the histological process.

All values were expressed in mean±SD, with n=8, the values were analyzed with one way ANOVA followed by Dunnett multiple comparison test. **p<0.01 compare to group-I, *p<0.05compare to group-I, ns= Not significant compare to group-I.

RESULTS:

Serological parameters:

The serological tests like SGOT, SGPT, ALP, Bilirubin, Triglycerides, Cholesterol, HDL, LDL VLDL, and Protein were observed to analyze the hepatoprotective activity of the prepared extracts.

The vital liver marker enzymes like SGOT, SGPT and Alkaline Phosphate were increased in the CCl_4 induced hepatotoxic group. The reduced levels of these enzymes were noticed in the group-III .The normal values were also observed in the all extract treated group from IV to VII. (Table No.1).

The reduction of these enzymes was more in the SAMLE treated groups than to the SAALE treated groups.

GROUP	NAME	SGOT (U/L)	SGPT (U/L)	ALP (IU/L)
I	CONTROL	40.29±2.80	39.89±4.52	186.17±3.33
II	CCl_4 INDUCED	142.19±3.98**	103.75±4.33**	277.41±4.49**
III	CCl_4+ LIV-52	41.81±2.78 ns	59.87±4.96**	175.68±4.04**
IV	CCl_4 + SAALE 200 mg/kg	137.12±4.74**	104.18±3.94**	273.55±4.89**
V	CCl_4 + SAALE 400 mg/kg	116.81±5.50**	97.05±3.36**	209.47±5.93**
VI	CCl_4 + SAMLE 200 mg/kg	90.11±4.78**	83.30±5.70**	220.71±3.72**
VII	CCl_4+ SAMLE 400 mg/kg	67.39±5.40**	71.92±4.00**	208.18±4.72**

Table-1 - **Serological tests- SGOT, SGPT and ALP**

GROUP	NAME	Total Bilirubin (mg/dl)	Total Protein (g/dl)
I	CONTROL	0.66±0.13	5.27±1.10
II	CCl_4 INDUCED	1.57±0.44**	3.08±0.70**
III	CCl_4 + LIV-52	0.88±0.45 ns	5.67±0.55 ns
IV	CCl_4 + SAALE 200 mg/kg	1.33±0.20**	2.81±0.59**
V	CCl_4 + SAALE 400 mg/kg	1.06±0.14*	3.03±0.39**
VI	CCl_4 + SAMLE 200 mg/kg	0.92±0.27 ns	3.94±0.48**
VII	CCl_4 + SAMLE 400 mg/kg	0.71±0.10 ns	5.41±0.36 ns

Table-2-**Serological tests- Total Bilirubin &Total Protein**

GROUP	NAME	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)
I	CONTROL	125.44±4.48	78.34±4.08	63.02±4.56
II	CCl_4 INDUCED	184.95±4.37**	108.60±4.96 **	133.83±6.62**
III	CCl_4+ LIV-52	118.88±4.01*	75.14±4.28 ns	63.32±5.94 ns
IV	CCl_4 + SAALE 200 mg/kg	180.81±4.90**	106.15±4.91**	137.60±6.96**
V	CCl_4 + SAALE 400 mg/kg	172.78±5.51**	93.29±3.55**	127.36±6.30**
VI	CCl_4 + SAMLE 200 mg/kg	160.59±5.82**	94.00±4.58 **	119.32±8.68**
VII	CCl_4 + SAMLE 400 mg/kg	147.26±4.94**	78.76±2.07 ns	97.36±7.54**

Table-3- **Serological tests- Total Cholesterol and Triglycerides and LDL**

GROUP	NAME	HDL (mg/dl)	VLDL (mg/dl)
I	CONTROL	46.80±3.39	15.59±0.84
II	CCl_4 INDUCED	27.96±5.68**	21.68±0.96**
III	CCl_4 + LIV-52	40.63±3.81ns	15.02±0.85 ns
IV	CCl_4 + SAALE 200 mg/kg	21.29±4.60**	21.22±0.98**
V	CCl_4 + SAALE 400 mg/kg	26.79±4.02**	18.63±0.69**
VI	CCl_4 + SAMLE 200 mg/kg	22.47±4.94**	18.79±0.91**
VII	CCl_4 + SAMLE 400 mg/kg	34.15±5.57**	15.75±0.41 ns

Table- 4- Serological tests- HDL and VLDL

All values were expressed in mean±SD, with n=8, the values were analyzed with one way ANOVA followed by Dunnett multiple comparison test. **p<0.01 compare to group-I, *p<0.05compare to group-I, ns= Not significant compare to group-I.

The bilirubin levels were increased in the CCl_4 group. The lowered values were recorded in the group- III. The normal levels were also observed in the group- IV to VII. (Table No. 2).

The protein levels of all groups were reported in the table no 2. The total proteins were significantly increased in the extract treated groups. As they were decreased in the group-II (Table No.2).

The Cholesterol, LDL, VLDL and Triglycerides levels were increased in the group-II (CCl_4).They observed with normal values in the Liv-52 administered group- III. The decreased Cholesterol, LDL, VLDL and Triglycerides were noticed in extracts treated groups. The effect of the SAMLE was more in the serological parameters of group VI and VII. The HDL values were also decreased in the group-II rats, where as they were increased in the group-III and extracts treated rats (Table-3, 4).

Histological sections:

The necrosis of hepatic cells were observed in the liver CCl_4 toxic group rats Fig. 2.The reformation of hepatocytes were seen in the cross sections of liver of the group - III. The normal histology observed in the group-I. The reformation of hepatocytes was seen more in the group- V and VII. The normal histology was also observed in the group –VII, as the necrotic tissue was become normal in the SAMLE 400 mg/kg treated group. (Fig.1to 5).

DISCUSSION:

SGOT, SGPT, Bilirubin, ALP can work as the liver functioning markers, due to damage of the liver these marker enzymes release into the circulation of the blood [6].

The trichloromethyl free radicals, trichloromethyl peroxy free radicals which were formed from the CCl_4 cause damage to the membranes of lipids of endoplasmic reticulum[7]. These enzymes release into circulation due to loss of membrane integrity.

The increased levels of SGOT, SGPT, ALP and Bilirubin were noticed in CCl_4 induced group might be because of the necrosis of liver. The Liv-52 administered CCl_4 rats showed the normal levels of these parameters. The normal function of the liver reveals the normal values of the SGOT, SGPT, ALP and Bilirubin. The SAMLE showed effective hepatoprotective activity than to the SAALE treated CCl_4 administered rats. Similar results were identified in the CCl_4 treated rats with *Momardica charantia* extracts[5].

In addition to the changes in liver, significant changes were also noticed in the serological parameters like SGPT, SGOT, ALP and bilirubin. Similar results were achieved in rats treated with aqueous extract of *Capparis deciduas*[8]. In the present investigation these parameters were decreased for SAMLE and SAALE in treated rats.

Serum bilirubin is the protein with the highest concentration in plasma and it is synthesized by the liver. It transports many small molecules in the blood (for example calcium and progesterone). It also prevents the fluid in the blood from leaking out into the tissues. The present study revealed significant decrease in the levels of serum bilirubin in SAMLE and SAALE treated rats when compared to CCl_4 treated group rats. Decreased serum bilirubin may arise from liver protection. Similar results were achieved in rats treated with aqueous extract of *Argemon mexicana* leaf extract by[9].

The total proteins of serum were decreased in the group II rats. The liver failure results the reduced levels of serum proteins also because of the hepatotoxicity. The normalized values of protein were seen in the group III to VII, which was because of the rejuvenating or repairing of liver. The SAMLE and SAALE extract may have the capability to reform the liver cells by increasing protein values of serum. The similar results were observed in the poly herbal tablet treated CCl_4 induced rats [10].

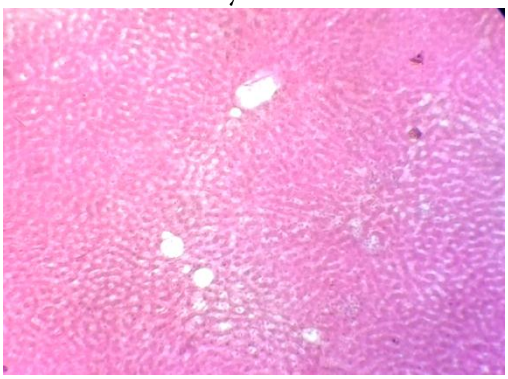

Fig-1- Group-I rat's liver cross section

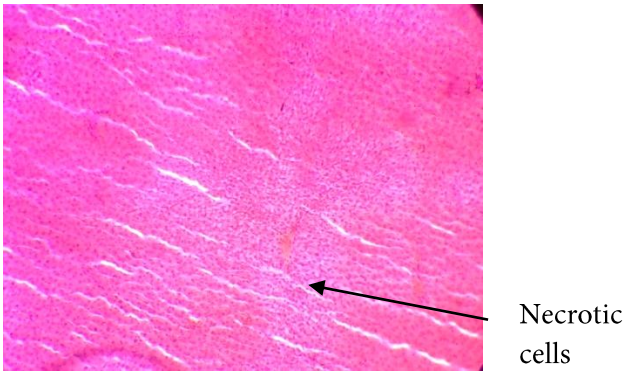

Fig-2- Group- II rat's liver cross section

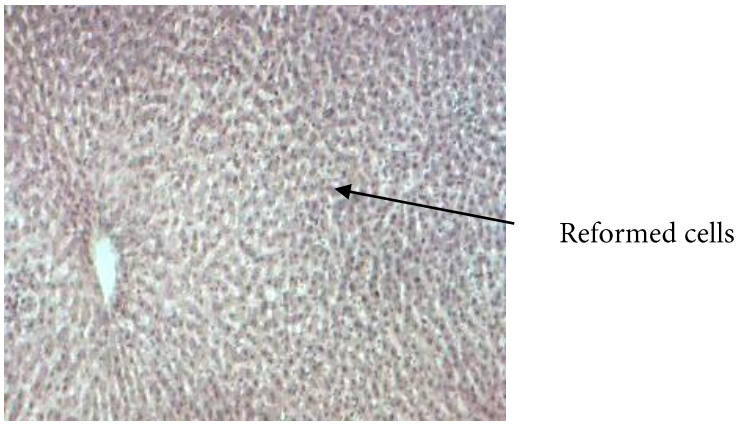

Fig-3- Group-III rat's liver cross section

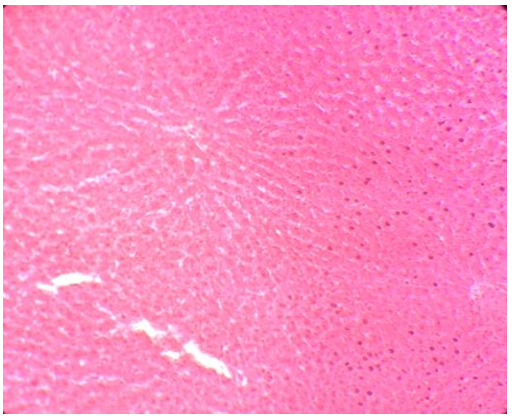

Fig-4- Group-V rat's liver cross section

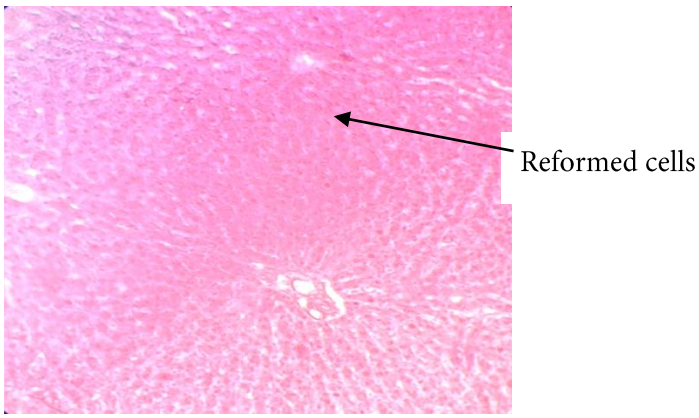

Fig-5- Group- VII rat's liver cross section

The high levels of cholesterol, VLDL, LDL reveal the chordiac diseases. There is the possibility of development of atherosclerosis and other cardiac diseases due to elevated cholesterol, VLDL, LDL, levels. The reduced HDL levels were also noticed in the CCl_4 induced liver rats. The increased values of HDL explain the cardioprotecive activity. The normal values of HDL, VLDL, LDL and cholesterol were noticed in the Liv-52 treated rats. The increased values of the HDL were also observed in the SAALE& SAMLE given rats. The activity of the SAMLE was more effective than to the SAALE.

The total cholesterol levels were increased in group II because of the cholestasis of the liver which generally occurs due to elevated levels of ALP. The elevated LDL levels and reduced HDL cholesterol levels were also seen in the group II rats. The reduced total cholesterol and LDL levels were seen in the group III. The SAMLE, SAALE treated rats also observed having decreased levels of LDL and total cholesterol. The increased HDL levels were seen in the group III to VII. The increased levels of HDL explain the capacity of the extract to fight against atherosclerosis and other coronary artery diseases. The similar reduced levels of serum cholesterol and increased levels of HDL were also found in the *Polygala arvensis* treated CCl_4 rats [11].

Triglycerides of the CCl_4 administered hepatotoxic rats showed altered values. They were normalized in the SAALE, SAMLE and LIV-52 treated rats. The triglycerides of serum observed to be elevated in the group II. It may be because of the cardiovascular disturbances. The recovery of the triglycerides was seen in the group III to VII.

The vital antioxidant enzymes might have counteracted the free radicals caused by the CCl_4 and protected the liver against oxidative stress.

The presence of phytochemicals like Flavonoids, Tannins, Steroids, Phenols in the extracts also play their role in stabilizing the antioxidant enzymes for their effective hepatoprotective activity [2]. Phenolic acid also express the antioxidant property [12], to fight against the free radicals.

Investigation on various plant phytochemicals revealed that the presence of flavonoids, phenolic compounds has the potential free radical scavenging activity [13].

The restore of all the serological parameters, biochemical parameters supported the hepatic cells to rejuvenate against the CCl_4 caused lipid peroxidation. The necroses of liver tissue were noticed in the CCl_4 induced group. The normal arrangement of sinusoids, hepatocards were found in the LIV-52, SAALE and SAMLE administered rats.

The histological sections are also revealed that the hepatocellular damage in the CCl_4 induced hepatotoxic group (group- II). The SAMLE + CCl_4 (400mg/kg) i.e., group-VII liver section was shown the rearrangement hepatic cells. The SAALE + CCl_4 group - V (400mg/kg) were also shown recovery of the tissue compare to the hepatocytes of the CCl_4 damaged group. The histology can be easily comparable with the CCl_4 + liver tonic group rats.

The dose SAMLE 400mg/kg decreased serum enzymes effectively than to the dose of SAALE 400mg/kg in the treated animals. The hepatoprotective activity was increased as the administration of extracts dose increased.

The plant extracts of SAMLE (*Streblus asper* methanol leaf extract) and SAALE (*Streblus asper* aqueous leaf extract) were shown hepatoprotective activity in CCl_4 induced hepatotoxic rats. SAMLE has shown better results than to the SAALE. However, further studies are needed in isolating the active compounds which are responsible for the hepatoprotection.

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Cite this article as:

R. Vijay Kumar, V. Rama, G. Venkat Raji Reddy and M. Krishna Reddy. Hepatoprotective activity of *Streblus asper* leaf extracts in CCl_4 induced albino rats. Asian Journal of Pharmacology and Toxicology, 04(14), 2016, 01-07.