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Hepatoprotective activity of *Lagenaria siceraria* fruit extracts against carbontetrachloride-induced hepatic damage in rats

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ABSTRACT

Evaluation of the hepatoprotective activity of Lagenaria siceraria fruit extracts against carbontetrachloride (CCl4)-induced hepatotoxicity was done on rats. Hepatotoxicity was induced in male Wistar rats by intraperitoneal injection of CCl_4 (1 ml/kg/day for 7 days). Lagenaria siceraria ethanol extract (LSEE) and vacuum dried Lagenaria siceraria juice extract (LSJE) were administered to the experimental rats (400 mg/kg/day, p.o. for 10 days). The hepatoprotective effect of these extracts was evaluated by the assay of liver function biochemical parameters (total bilirubin, serum protein, alanine aminotransaminase, aspartate aminotransaminase, and alkaline phosphatase activities), liver weight and histopathological studies of the liver. In LSEE-treated animals, the toxic effect of CCl_4 was controlled significantly by restoration of the levels of serum bilirubin, protein and enzymes as compared to the normal and the standard drug silymarin- treated groups. Histology of the liver sections of the animals treated with the extracts showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration, which further evidenced the hepatoprotective activity. In conclusion LSEE and LSJE possess significant hepatoprotective activity.

Keywords: Bottle gourd; serum marker enzymes, ethanol extract, fresh juice extract

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INTRODUCTION

The liver which occupies the pivotal position in body plays an essential role in drug and xenobiotic metabolism and in maintaining the biological equilibrium of the organism. The role played by this organ in the removal of substances from the portal circulation makes it susceptible to a persistent attack by offending foreign (xenobiotic) compound culminating in liver dysfunction. Despite the tremendous strides in modern medicine, there are few drugs that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells. However many herbal formulations are available for liver disorders in the Indian market based on Ayurvedic principles [1]. Herbal drugs are frequently considered to be less toxic and free from side effects than synthetic drugs. Medicinal plants like Andrographis paniculata, Boerhaavia diffusa [2] Hibiscus rosasinensis [3], Phyllanthus amarus [4], are well known for their hepatoprotective effects.

The plant, Lagenaria siceraria (Mol.) Standl. (Family: Cucurbitaceae), known as bottle gourd, is a common fruit vegetable used throughout the India. Since time immemorial the fruit is used as diuretic, cardio-tonic, cardio-protective and nutritive agent. The fruit is also reported to have good source of vitamin B complex and choline along with fair source of vitamin C and β-carotene. It is also reported to contain cucurbitacins, fibers and polyphenols [5-8]. LS fruit has been reported to possess antioxidant activity [9], hypolipidemic and antihyperlipidemic effects in normocholesterolemic and triton-induced hyperlipidemic rats [10]. HPLC analysis of methanol extract from plant shows the presence of flavone-C glycosides [11]. Lagenin, a novel protein has been isolated from lyophilized extract of seeds [12]. Literature reviews indicated that the hepatoprotective activity of these species has not been clinically evaluated so far. An active and safe drug is needed for the treatment of hepatitis. In view of this, the present study was aimed at evaluating the hepatoprotective activity of the fruit of L.siceraria against carbon tetrachloride (CCl_4)-induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of the analytical grade. Silymarin was purchased from Ranbaxy labs Ltd. Kits for the examination of total bilirubin, total protein, serum transaminases and serum alkaline phosphatase were purchased from Span Diagnostics Kits Ltd., India.

Preparation of extract

Lagenaria siceraria fruits were collected from the local farms of Rangareddy District, Andhra Pradesh in the month of October-November, the botanical authentication was done by the authority of Department of Botany, Osmania University, Hyderabad and voucher specimen is lodged in our research laboratory for the future reference. The fresh and semi-ripe fruits were sliced using a home slicer and the slices obtained were shade-dried, pulverized and passed through a 20 mesh sieve. The dried, coarsely powdered plant material was extracted

with 99% ethanol using a Soxhlet apparatus. The solvent was evaporated under vacuum which gave semisolid mass (23% w/w) with respect to the dried powder. Also the fruits were cut into small pieces and fed to a juicer to collect the juice and the collected juice was filtered and vacuum dried to obtain the L. siceraria fruit juice extract (LSJE, yield: 17 % w/w).The preliminary phytochemical screening was carried out to detect the chemical constituents of both fresh fruit juice extract as well as ethanol extracts (LSEE) which revealed the presence of flavonoids, steroids, saponins and polyphenols, carbohydrates, proteins. Both the extracts were stored in tight containers in dessicator.

Drug formulations:

Oral suspensions containing 400mg/ml of the LSEE and LSJE were prepared in 1% w/v gum acacia.

Animals:

Albino Wistar rats weighing 150-200 g of either sex, 4 months of age were used for this study. The experimental animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3° C and 35-60% humidity). Standard pelletized feed and tap water were provided ad libitum. The Institutional Animal Ethical Committee (IAEC) of Malla Reddy College of Pharmacy, Hyderabad, with Reg. No. 1217/a/08/CPCSEA, approved the study.

Acute toxicity study

The acute toxicity of the ethanol extract and dried fresh juice extracts was determined using albino mice of either sex (20-30g), those maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OECD Guideline No. 420) method of CPCSEA was adopted for the toxicity studies. No mortality was observed even at the dose of 2000mg/kg for both the extracts. $1/5^{th}$ of LD_{50} dose i.e. 400mg/kg for both the extracts was selected for the screening of hepatoprotective activity.

Hepatoprotective activity

Five groups of animals containing six each were used for the study. The animals of Group I served as the control and received the vehicle 1% w/v gum acacia. Groups II-V received 0.25 mL of $CC1_4$ in liquid paraffin (1:1) 1 mL kg^{-1} body weight intraperitoneally (IP) for 7days [13]. The standard drug Silymarin (Ranbaxy Lab. Dewas) was administered to Group III animals in the dose of 100 mg/kg/day p.o. for 10 days. While, Groups IV and V were treated with LSEE and LSJE of L.siceraria in the dose of 400 mg/kg/day, p.o. (as per acute toxicity studies) for 10 days, respectively. The CCl_4, silymarin and the extracts were administered concomitantly to the respective groups of animals. All the animals were killed on day 11 and the blood samples were collected by cardiac puncture method into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and

biochemical investigations were carried out to assess liver function viz ., total bilirubin, total protein, serum transaminases and serum alkaline phosphatase. Weight of the livers was also calculated.

Histopathology

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5 mm thickness, processed in alcohol-xylene series and were stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathological changes.

Statistical analysis

All the values are expressed as mean ±SEM and data was analyzed by one-way ANOVA, using Graph pad INSTAT. The post hoc analysis was carried out by Dunnett's multiple comparison test to estimate the significance of difference between individual groups. $p< 0.05$ was considered statistically significant.

RESULTS

Table 1: Effect of Lagenaria siceraria fruit extracts on biochemical parameters in rats subjected to CCl_4 -induced hepatotoxicity.

Groups	Total Bilirubin	Total Protein	AST	ALT	ALP	Liver
	mg/dl	mg/dl	(IU/l)	(IU/l)	(IU/l)	weight(g)
1	0.79±0.52	6.21±0.45	21.94±2.54	46.54±4.57	6.83±0.32	2.85±0.98
2	2.28±0.57*	3.07±0.59*	61.83±1.72*	95.08±2.34*	13.14±0.90*	4.59±0.67*
3	1.65±0.25*	5.91±0.36**	18.27±2.11**	56.88±0.84**	7.35±0.84*	3.12±0.23*
4	0.86±0.91**	6.81±0.35**	25.25±1.64**	51.56±1.62**	6.61±0.73*	3.27±0.43**
5	1.87±0.62*	5.53±0.47**	44.88±2.09*	64.36±1.54**	9.83±0.62**	3.37±0.89*

Values are expressed as mean±SEM. n=6 rats in each group. *P<0.05significant as compared to control, **P<0.05 significant as compared to CCl4 treated group, statistical test employed is ANOVA followed by dunnet's t test.

The administration of CCl_4 to the animals resulted in a marked increase in total bilirubin, serum amino transaminases (AST and ALT) and serum alkaline phosphatase activities. However, the serum total protein level was decreased. The toxic effect of CCl_4 was controlled in the animals treated with the LSEE and LSJE by way of restoration of the levels of the liver function biochemistry similar to that of the standard drug silymarin (Table-1). Among the extract-treated groups, significant hepatoprotective activity was observed in those treated with ethanol extract. Histological profile of the control animals showed normal hepatocytes (Figure-1). Group-II animals exhibited intense centrilobular necrosis, vacuolization and macrovesicular fatty change (Figure-2). The sections of liver taken from the animals treated with standard drug

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silymarin showed the hepatic architecture, which was similar to that of control. The animals treated with LSEE exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration (Figure-3). However, moderate accumulation of fatty lobules, presence of vacuoles and granuloma (Figure-4) was noticed in the sections of animals treated with LSJE.

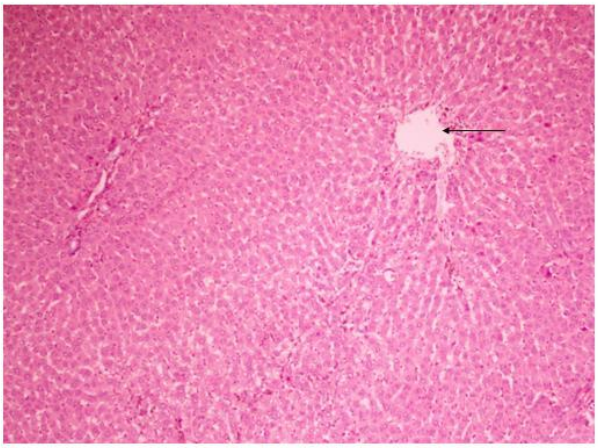

Fig-1: Liver tissue of control animal showing normal histology. Stain H and E, magnification 100X

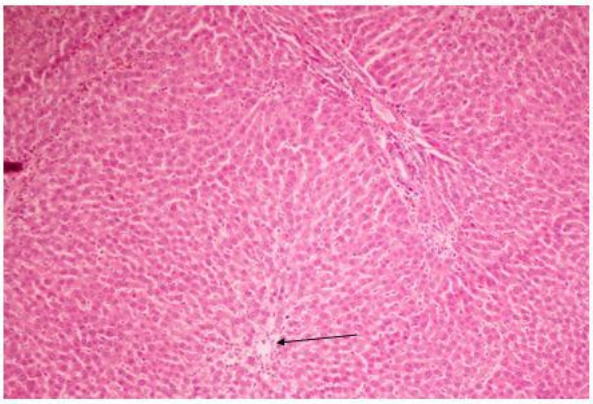

Fig. 2: Liver tissue of animal treated with CCl4 showing necrosis and periportal round cell collection. Stain H and E, magnification 100X.

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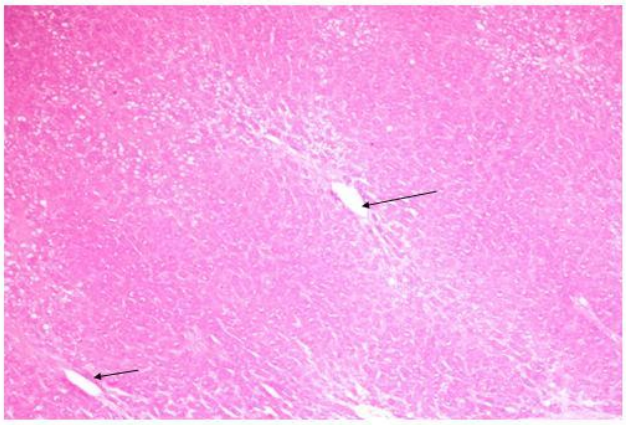

Fig. 3: Liver tissue of ethanol extract-treated animals showing normal arrangement of hepatocytes around the portal vein and Panlobular Macrovacuoles. Stain H and E, magnification 100X.

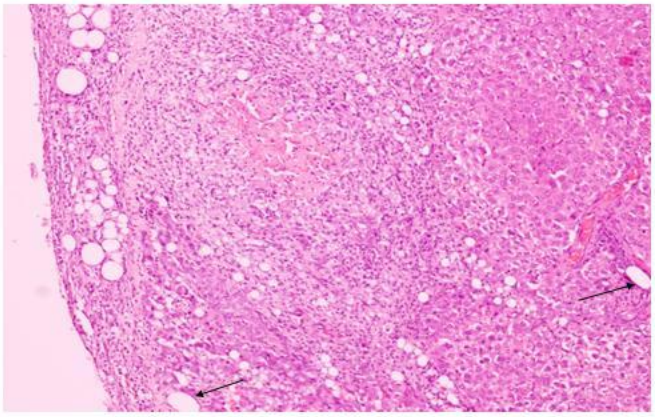

Fig. 4: Liver tissue of fresh juice extract-treated animals showing normal arrangement of hepatocytes with minimal Granuloma. Stain H and E, magnification 100X.

DISCUSSION

Carbon tetrachloride has been used as a tool to induce hepatotoxicity in experimental animals. This toxic chemical caused peroxidative degradation in adipose tissue resulting in fatty infiltration of the hepatocytes. The increase in transaminases and alkaline phosphatase was a clear indication of cellular leakage and loss of functional integrity of cell membrane [14]. The increase in the level of serum bilirubin reflected the severity of jaundice [15]. Carbon tetrachloride, which is an intrinsic hepatotoxin, was used to induce hepatic damage in this

study since it has previously been shown to exert its toxic effects on the liver [16]. Administration of CCl_4 causes severe injury in rats` livers. This damage is recognized by an increase in serum levels of the hepatic enzymes SGOT and SGPT, which are indices of liver cell damage [17]. The biochemical mechanisms involved in the development of CCl_4 hepatotoxicity have long been investigated. It is generally believed that it is due to lipid peroxidation caused by carbon trichloromethyl radical ($CCl^{\bullet}_3$). CCl_4 is biotransformed by cytochrome P-450 to the trichloromethyl-free radical that induces membrane lipid peroxidation and disturbs Ca^{2+} homeostasis to produce hepatocellular injury [18]. The administration of fruit extracts showed significant hepatoprotective activity, which was comparable with the standard drug silymarin. It is known that SGOT can be found in the liver, cardiac muscle, kidney, brain, pancreas, lungs, skeletal muscle, leukocytes and erythrocytes (in decreasing order of concentration) [19]. Whereas SGPT is present in highest concentration in the liver. In tissues, SGPT occurs in two locations, the cytosol and mitochondria [20]. SGPT appears to be a more sensitive and specific parameter of acute hepatocellular damage than SGOT [15]. Therefore, the possible hepatoprotective mechanism of the extracts of Lagenaria siceraria on the CCl_4-induced liver injuries may be through the following actions; inhibition of the cytochrome P-450 activity, prevention of the process of lipid peroxidation, stabilization of the hepatocellular membrane and enhancing the protein synthesis [21].

Administration of LSEE and LSJE showed significant hepatoprotective activity, which was comparable with the standard drug silymarin. The effect was more pronounced with ethanol extract. On the basis of the obtained results in this study, it can be concluded that Lagenaria extracts have preventive effect against CCl_4-induced hepatocellular damage in rats. The present liver-protective effect of Lagenaria extracts is presumably due to its contents of sulphurated, phenolic and terpenoid compounds. The effect of these compounds could be through preventing the accumulation of excessive free radicals and protecting the liver against CCl_4 intoxication. The protection of liver by Lagenaria extracts against CCl_4-induced toxicity might be related to glutathione-mediated detoxification. The findings of the present study support the claims of ayurvedic traditional medicine practitioners on the usefulness of Lagenaria siceraria in liver ailments caused by various etiologies.

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