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**Chemical Investigation and Antihepatotoxic Activity of the Fruits of *Lagenaria Siceraria***

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The antihepatotoxic activity of different fractions of the ethanolic extract of *Lagenaria siceraria* fruit, administered orally to different groups of rats was evaluated using the CCl<sub>4</sub>- induced hepatotoxicity test. All fractions tested, in a dose of 250 mg/kg showed significant activity, with the petroleum ether fraction exhibiting comparatively higher activity. Two steroids were isolated from the petroleum ether fraction and they were identified as fucosterol and campesterol.

**L**AGENARIA *siceraria* (Cucurbitaceae) considered to be indigenous to India, is a vegetable grown throughout the year. The tender bottle gourds are used as a vegetable and is a good source of vitamin B and fair source of Vitamin C. The fruit pulp is known to possess cooling, diuretic and antibilious properties.<sup>1</sup> As no studies on the antihepatotoxic activity of the fruit pulp have been reported so far, in the present study, an attempt has been made to study the activity of the petroleum ether, ether and ethyl acetate fractions of the ethanol extract using CCl<sub>4</sub> - treated rats as the model of liver injury. The isolation and characterization of phytochemical constituents are also discussed.

## EXPERIMENTAL

### Preparation of plant extracts

The fruits of *Lagenaria siceraria* were procured from the local market of Udupi on October 1993. Its botanical identity was confirmed at the Dept. of Botany, Poorna Prajna College, Udupi. A herbarium specimen has been deposited in the College of Pharmaceutical Sciences, Manipal.

The dried powdered fruit pulp (5 kg) was extracted with ethyl alcohol for 2.5 h. This extract was

concentrated *in vacuo* to a syrupy consistency and then evaporated to dryness (yield 760 g). The ethyl alcohol fraction was then fractionated into petroleum ether soluble fraction (68 g), ether soluble fraction (47 g) and ethyl acetate fraction (40 g). The different fractions were subjected to chemical and pharmacological studies.

### Chemical Studies

The petroleum ether extract (56 g) was saponified with 20% ethanolic KOH (500 mg) for 2 h. The unsaponifiable portion was then extracted with ether, washed with distilled water and dried over anhydrous sodium sulfate to give a yellowish residue (9.7 g). The residue was dissolved in chloroform (20 ml), loaded onto a neutral alumina column (150 g) prepared in petroleum ether (60- 80°). The column was eluted with petroleum ether (60- 80°), 10%, 25% and 50% benzene in petroleum ether, then with benzene followed by graded mixtures of benzene and chloroform, chloroform and finally with chloroform and methanol (95:5; 90:10). The elution of different components was monitored by TLC (Si-gel; benzene: petroleum ether, 1:1; visualised with methanolic sulfuric acid).

The 50% benzene in petroleum ether and the benzene eluates were combined as they were found

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to give identical spots on TLC (Si-gel; benzene:ethyl acetate, 85:15; visualised in the iodine chamber). A white solid deposited on concentration. It responded to the LB test for sterols and was designated as Compound LS-1 (35 mg).

The 50% chloroform in benzene eluate on concentration yielded a white solid that responded to the LB test for sterols and was designated as Compound LS-2 (22 mg).

**Compound LS-1** m.p. 123-125°,  $[\alpha]_D^{29}$  -38° (CHCl<sub>3</sub>). It analysed for C<sub>29</sub>H<sub>48</sub>O (found C 82.75%, H 11.7%; requires C 84.38%. H 11.72%).

IR  $\nu_{\text{max}}^{\text{KBR}}$  cm<sup>-1</sup>: 3400 (br, OH), 2920, 2850 (C-H stretch of CH<sub>2</sub>) 1630 (C=C stretch), 1450, 1380 (C-O stretch), 800 (C-H of the olefin).

<sup>1</sup>H-NMR (CDCl<sub>3</sub>):  $\delta$  5.4 (1H, d, J 2Hz, allylic coupling), 5.1 (t, 1H), 3.4 (quintet, 1H, C-OH), Acetate m.p. 118-119°.

**Compound LS-2** m.p. 156-159°  $[\alpha]_D^{23}$  - 32° (CHCl<sub>3</sub>). It analysed for C<sub>28</sub>H<sub>48</sub>O (found C 82.5%, H 12.1%; requires C 83.9%, H 12.08%).

IR  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 3390 (br, OH), 2950, 2920 (C-H str in CH<sub>3</sub> and CH<sub>2</sub>), 1475 (CH def in CH<sub>3</sub>), 1390, 1380 (C-H def in gem dimethyl), 1080 (C-O str of sec. alcohol).

EIMS m/z (rel. int.): 400 (M<sup>+</sup>, 100%), 385 (M<sup>+</sup>-Me, 37%), 382 (M<sup>+</sup>-H<sub>2</sub>O, 16%), 367 (M<sup>+</sup>-Me-H<sub>2</sub>O, 11.5%), 315 (M<sup>+</sup>-85, 60.4%), 289 (M<sup>+</sup>-111, 51%), 255 (M<sup>+</sup>-side chain-H<sub>2</sub>O, 25%), 231 (M<sup>+</sup>-side chain - 42, 95%) 213 (M<sup>+</sup>-side chain - 42-H<sub>2</sub>O, 95%). Acetate m.p. 137-139°.

### Pharmacological Studies

Male wistar albino rats weighing between 200 to 250 g were used for the study. They were housed in polypropylene cages in an adequately ventilated

room. The rats were fed standard rat feed pellets supplied by Hindustan Lever Co., Bombay and water *ad libitum* throughout the course of the study.

### Acute toxicity studies

Animals weighing between 200 to 250 g were used for the study. These were divided into 6 groups of 5 animals each. The test extract was administered orally as a solution in propylene glycol (5 ml/kg) to different groups in increasing dose levels of 125, 250, 500, 1000 and 2000 mg/kg body weight.

The animals were then observed continuously for 1 h, then frequently for 24 h and thereafter once daily for 14 days. During this period, the animals were observed for gross behavioural and morphological profiles.

### Hepatoprotective activity

Forty male albino rats were used for the evaluation of the hepatoprotective activity of each extract. These were divided into 5 groups of 8 animals each. **Group-1** animals served as normal control. They were orally dosed with propylene glycol (5 ml/kg weight), daily for five consecutive days. **Group-2** animals served as CCl<sub>4</sub> control. They were injected intraperitoneally with CCl<sub>4</sub> (0.5 ml/kg) followed after 10 min by the oral administration of propylene glycol, once daily for five consecutive days. **Group-3** animals were injected with CCl<sub>4</sub> (0.5 ml/kg) i.p. followed after 10 min by the petroleum ether fraction (250 mg/kg) as a suspension in propylene glycol, once daily for five consecutive days. **Group-4** animals were injected with CCl<sub>4</sub> (0.5 ml/kg) i.p. followed after 10 min by the ether fraction (250 mg/kg) as a suspension in propylene glycol, once daily for five consecutive days and **Group-5** with CCl<sub>4</sub> (0.5 ml/kg) i.p. followed after 10 min by the ethyl acetate fraction (250 mg/kg) as a suspension in propylene glycol, once daily for five consecutive days.

### Biochemical studies

On the sixth day, 24 h after administration of the last test dose, blood samples were withdrawn from the animals of all 5 groups by direct cardiac puncture of the unanaesthetised animals. The serum was separated by centrifugation and kept at  $-15^{\circ}$ . Using an autoanalyser, Alanine transferase (ALT), Aspartate transferase (AST), Alkaline Phosphatase, Total Proteins and Albumin were studied.

### Histopathological studies

Two animals from each group were sacrificed on the sixth day. The excised liver was sliced and kept in 10% formalin. Liver sections, stained with haematoxylin and eosin were then observed for degenerative and necrotic changes which were graded as follows.

(a) Degeneration: 0 : No degeneration; + : Few vacuolated cells per lesion; ++ : More than 10 cells per lesion; +++ : More than 2 rows of vacuolated cells around necrotic zone per lesion.

(b) Necrosis: 0 : No necrosis; + : Focal necrosis of one or two cells per lesion; ++ : Focal necrosis of more than 2 cells per lesion; +++ : Massive centrilobular necrosis.

### Statistical analysis

The results of the biochemical estimations<sup>3,4</sup> were expressed as mean  $\pm$  S.E.M. The variations in a set of data were estimated by performing the one-way analysis of variance (ANOVA) followed by the Studentised Range procedure, resulting in the "Allowance Value" (A) at 95% confidence level.

## RESULTS AND DISCUSSION

### Chemical Studies

**Compound LS-1 (Fucosterol)** gave characteristic colour reactions for a sterol. The yellow colour

obtained with tetranitromethane confirmed unsaturation in the molecule. The  $^1\text{HNMR}$  signals at  $\delta$  5.1 and 5.4 assigned to two vinylic protons, were indicative of two double bonds. The multiplet at  $\delta$  3.4 was assigned to the single proton (H of H-C-OH) indicating a secondary alcoholic grouping. Thus the identity of Compound LS-1 as fucosterol was confirmed.

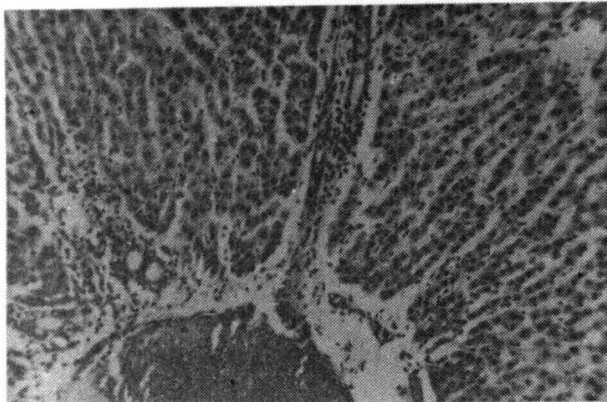
**Compound LS-2 (Campesterol)** answered the LB test. The yellow colour obtained with tetranitromethane confirmed unsaturation in the molecule. The  $\text{M}^+$  peak at 400, followed by fragments typical of  $\Delta^5$  sterols ( $\text{M}^+ -85$ ,  $\text{M}^+$ -side chain  $-\text{H}_2\text{O}$  and  $\text{M}^+ -\text{R}-42$ ) helped to characterise the sterol as campesterol.

### Pharmacological Studies

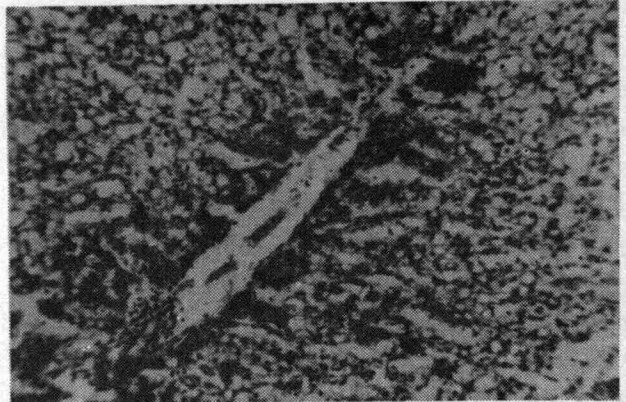
The petroleum ether, ether, and ethyl acetate fractions administered orally in doses upto 2 g/kg did not produce any mortality in rats when observed upto 14 days after administration.

The results of the present investigation are represented in Table 1. The various fractions (Group 3, 4, and 5) of the ethanol extract of *L. siceraria* administered to rats, treated with  $\text{CCl}_4$  were compared with  $\text{CCl}_4$  control group.  $\text{CCl}_4$  is a widely used hepatotoxin known to cause liver damage due to free radical formation during its metabolism by hepatic microsomes<sup>5,6</sup> which in turn causes the peroxidation of cellular membranes leading to the necrosis of hepatocytes. The activity of the different fractions was monitored by estimating serum transaminases which give a fairly good idea about the functional state of the liver.<sup>7</sup>

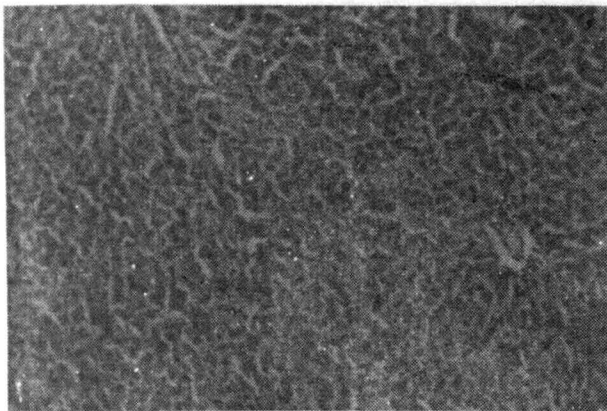
The statistical analysis of the results of the biochemical estimation reveal that all the groups tested i.e. petroleum ether fraction (Group 3), ether fraction (group 4), and ethyl acetate fraction (Group 5) exhibited statistically significant protection against  $\text{CCl}_4$  induced increase in the level of serum transaminases



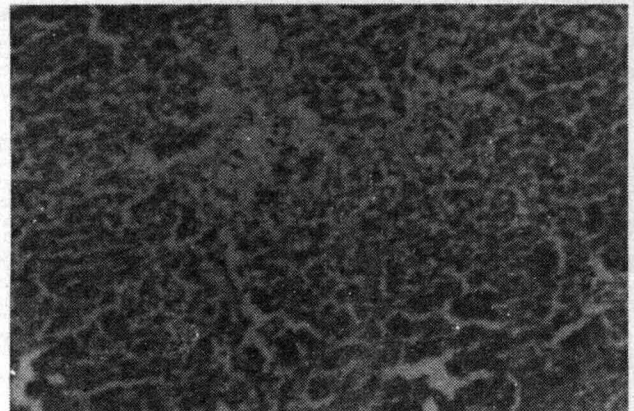
**Fig. 1** Liver of Group 1 rat showing normal structure of the hepatic lobules. Haematoxylin and eosin, 100 X.



**Fig. 2** Liver of Group 2 rat showing such intense fatty degeneration that the necrotic areas are not particularly visible; there is collection of polymorphs around the vein. Haematoxylin and eosin, 100 X.



**Fig. 3** Liver of Group 3 rat showing recovery of hepatic parenchyma. No degeneration is observed, Haematoxylin and eosin, 100 X.



**Fig. 4** Liver of Groups 4 rat showing degeneration with rows of vacuolated cells around necrotic zone, Haematoxylin and eosin, 100 X.

in rats. The administration of the petroleum ether fraction to the CCl<sub>4</sub> treated rats led to near normalisation of the elevated serum transaminase levels. This is evident from the statistical equivalence between the mean levels of transaminases in the CCl<sub>4</sub> control (Group 2) and Group 3. There was no significant alteration in the protein and albumin levels of any of the groups.

The histopathological studies support the biochemical findings. Hepatotoxicity induced by CCl<sub>4</sub> manifested itself by the sixth day with the livers

showing massive degeneration enveloping the not so visible necrotic areas (Fig 2). The liver sections of rats treated with petroleum ether fraction (Fig 3) appeared normal on microscopical examination. The livers of rats of Group 4 and Group 5 (see Fig 4 and 5 i.e. Ether and Ethyl Acetate fractions) revealed moderate to severe degeneration with rows of vacuolated cells around necrotic zone.

All the fractions tested in our study showed significant hepatoprotective activity with the petroleum

**Table 1: Effect of various fractions of the ethanol extract of the fruits of *Lagenaria Siceraria* on CCl<sub>4</sub>-induced Biochemical and histopathological changes in male albino rats**

Group	Total Proteins (TP) g/dL	Albumin (ALB) g/dL	Alanine Transferase (ALT) U/L	Aspartate Transferase (AST) U/L	Alkaline Phosphatase (ALK.PH.) U/L	Histopathology	
						D	N
1. Control	7.81±0.14	3.20±0.04	48.3±2.7	189.6±24.2	619.2±29.4	0	0
2. CCl <sub>4</sub> treated	7.36±0.04	3.11±0.04	1389±155	1630±166.6	800.9±20.3	+++	+++
3. Petroleum Ether	7.48±0.04	3.08±0.04	47.13±4.37	289.1±16.6	663.0±44.1	+	0
4. Ether	7.38±0.03	3.05±0.02	257.00±38.4	479.4±46.0	725.8±41.0	+++	+
5. Ethyl Acetate	7.48±0.04	3.15±0.03	110.5±27.2	330.5±31.5	669.5±47.7	++	+
6. Value	6.9	3.9	61.4	54.1	3.3		
7. Allowance Value	0.32	0.16	341.8	375.9	178.8		

n = No. of animals in each group

D = Degeneration

N = Necrosis

All values are expressed as Mean ± standard error of the Mean



**Fig. 5 Liver of Group 5 rat showing degeneration with rows of vacuolated cells around necrotic zone. Haematoxylin and eosin, 100 X.**

ether fraction exhibiting comparatively higher activity. Studies on the hepatoprotective activity of plant sterols are relatively few. The use of carpesterol<sup>8</sup> in completely preventing CCl<sub>4</sub> induced pentobarbitone sleep prolongation and lowering serum transaminases is an example. There are also reports on the

antihepatotoxic activity of triterpenes<sup>9</sup> of the oleanane and dammarane series.

Though it cannot be concluded with certainty, it seems possible that the better activity of the petroleum ether fraction in our study may be due to sterols like campesterol and fucosterol present therein.

### ACKNOWLEDGEMENTS

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