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Hepatoprotective Activity of the Fruits of *Piper longum* Linn

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Ethanol extract of *Piper longum* fruits and five different crude fractions, petroleum ether (40-60°), solvent ether, ethyl acetate, butanol and butanone were subjected to preliminary qualitative chemical investigations. The ethanolic extract and all other fractions were screened orally for hepatoprotective activity in adult Wistar rats. The ethanolic extract and butanol fraction have shown significant activity, lowering the serum enzymes glutamic oxaloacetic transaminase and glutamic pyruvic transaminase in rats treated with carbon tetrachloride when compared to control and Liv-52-treated rats.

Piper longum Linn. is an important medicinal plant, which finds uses in Ayurveda and Unani systems of medicine[1,2]. It is usually called as Indian Long Pepper. It occurs in hotter parts of India from central Himalayas to Assam, Mikir Hills, lower hills of West Bengal and evergreen forest of Western Ghats from Konkan to Kerala. It is an aromatic, climber, stems creeping jointed and become attached to other plants; leaves 5-9 cm wide, sub acute, entire, glabrous, cordate with broad rounded lobes at the base, spikes cylindrical pedunculate, upright 1.3 to 2.5 cm long and 4 to 5 mm in diameter. Fruits are ovoid, yellowish orange sunk in freshly spike. The fruit has warm taste; useful in asthma, bronchitis, tumours, spleen disorders, inflammations, leprosy, insomnia, jaundice, piles and tuberculosis, the roots are used as laxative, anthelmintic; useful in bronchitis, abdominal pains, tumours and disease of spleen[3].

Literature survey revealed, piperine (4-5%) isolated from *Piper longum* possesses significant hepatoprotective activity[4]. Apart from this, it also contains pipalartene, piper longumine, volatile oil (1%), starch (40%), resins, gums, fatty oil and inorganic matter. Ethanolic extract of *Piper longum* fruits possesses significant antiulcer action[5]. Piperlongumine, an alkaloid present in the root and stem bark possesses significant insecticidal activity against *Musca domestica*[6]. The antianginal property is exhibited by the volatile oil content present in the *Piper longum* fruit[7]. *Piper longum* fruit showed the antibacterial activity against gram positive and gram-negative bacteria[8]. Ethanolic extract of fruits of *Piper longum* possesses antiamoebicidal activity[9].

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MATERIALS AND METHODS

The *Piper longum* fruits were collected from a medicinal garden of K. L. E. S's B. M. K. Ayurvedic Medical College and authenticated at the department of *Dravya Guna* of the Ayurvedic College. A voucher specimen has been kept in our laboratory for future reference. LIV-52 syrup of Himalaya Drug Company, Bangalore, was purchased from local market, carbon tetrachloride (CCl_4) was procured from E. Merck (India) Ltd. Mumbai and Olive oil (Olio Sarro, Italy) was also procured from local market.

Extraction and Fractionation:

In the present study, around 3.0 kg of the shade dried fruits of *Piper longum* were reduced to a fine powder which was subjected to hot continuous extraction with ethanol (95%) in 10 batches of 300 g of each in a Soxhlet extractor. After complete extraction, the solvent was distilled off and concentrated on a water bath to dry residue. The concentrated ethanol extract (about 250 g) was dispersed in 250 ml of distilled water and subjected to fractionation by using

petroleum ether (40-60°), solvent ether, ethyl acetate, butanol and butanone in succession. Each fraction was washed with water, then dried over anhydrous Na_2SO_4 and concentrated to a small volume and then evaporated to dryness. The dried ethanolic extract and its fractions were stored in a dessiccator[10]. All these extracts (i.e. ethanolic extract and petroleum ether, solvent ether, ethyl acetate, butanol and butanone fractions) were dried at 50° and were screened for toxicity and hepatoprotective activity.

Toxicity Studies:

Wistar rats of either sex (150-200 g) were used for the hepatoprotective study. Swiss albino mice were used for toxicity study were obtained from the experimental animal house, Department of Livestock Production, Government Veterinary College, Hebbal, Bangalore. They were maintained under standard housing conditions. The animals were given standard laboratory feed and water *ad libitum*. The study was cleared by the Institutional Animal Ethics Committee. (Registration no. 221/CPCSEA/BGM)

All the extracts were administered orally to different groups of mice in doses ranging from 200-3000 mg/kg. There was no lethality in any of the groups. Mice, which received extracts in doses above 2000 mg/kg exhibited ptosis (dropping of upper eyelids) and were found lethargic. One tenth of the maximum dose of the extracts tested for acute toxicity was selected for evaluation of antihepatotoxic activity i.e., 300 mg /kg p.o[11].

Experimental protocol:

Rats were randomly divided into seven groups of six rats each. The groups were given the following treatments, animals in group I received 5% gum acacia 1ml/kg orally for 4 d with 2 ml of olive oil given subcutaneously on day 2 and 3. This group served as the control group. Animals in group II received 5% gum acacia 1 ml/kg orally for 4 d with 1:1 CCl_4 in olive oil 2 ml/kg given subcutaneously on day 2 and 3. This group served as CCl_4 treated. Animals in group III received ethanol extract of fruits of *Piper longum* 300 mg/kg orally for 4 d with 1:1 CCl_4 in olive oil 2 ml/kg given subcutaneously on day 2 and 3. Animals in group IV received petroleum ether fraction of fruits of *Piper longum* 300 mg/kg orally for 4 d with 1:1 CCl_4 in olive oil 2 ml/kg given subcutaneously on day 2 and 3. Animals in group V received butanol fraction of fruits of *Piper longum* 300 mg/kg orally for 4 days with 1:1 CCl_4 in olive oil 2 ml/kg given subcutaneously on day 2 and 3. Animals in group VI received butanone fraction of fruits of *Piper longum* 300 mg/kg orally for 4 d with 1:1 CCl_4 in olive oil 2 ml/kg given subcutaneously on day 2 and 3. Animals in group VII received LIV-52 syrup for 4 d with 1:1 CCl_4 in olive oil 2 ml/kg given subcutaneously on day 2 and 3. This group served as standard group.

Assessment of hepatoprotective activity:

The method of Handa *et al.*[12] was used to evaluate CCl_4-induced hepatotoxicity. The rats were sacrificed on day 5 under light ether anesthesia. Blood collected from the carotid artery was allowed to coagulate at 37° for 30 min and the serum was separated by centrifugation at 2,500 rpm and analysed for biochemical investigations i.e. serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT)[13]. Liver was processed immediately after removal for histological investigations.

Assessment of Liver Function:

Biochemical parameters such as SGOT and SGPT in Table 1 were analyzed according to reported methods[14] to determine the functional state of the liver. Histological investigation of the liver slices were processed for paraffin embedding for 48 h in 10% formalin, following the standard micro technique[15]. Five-micron sections of the livers stained with alum–haematoxylin and eosin were observed for histopathological changes under a light microscope.

TABLE 1: EFFECT OF THE EXTRACT OF *PIPER LONGUM* FRUITS ON HEPATIC DAMAGE INDUCED BY CCL_4.

Group (n)	Biochemical Parameters Mean±S.D.	
	SGOT IU/L	SGPT IU/L
Control (6)	128±15.3	37.5±12.4
CCl_4 treated (6)	327±51.4	277±25.5
Ethanol extract (6)	156±16.0*	98.2±6.20*
Petroleum ether fraction (6)	207±27.9	244±22.3
Butanol fraction (6)	191±13.0*	171±15.0*
Butanone fraction (6)	204±16.7	188±16.2
Standard drug LIV-52 (6)	143±12.0*	86.6±9.9*

*P<0.001 indicates significant compared to control. n denotes the number of animals used.

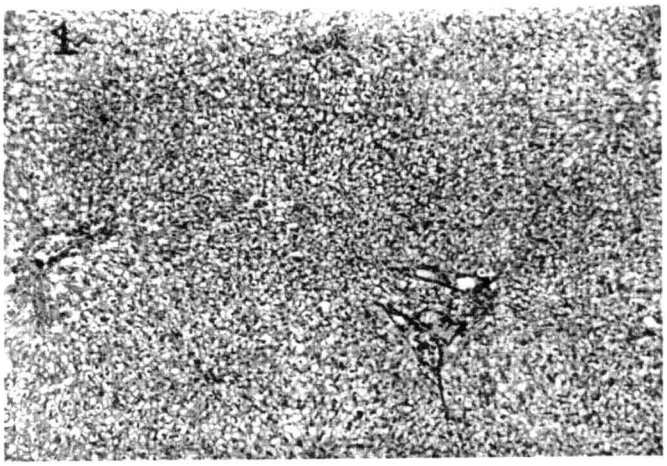

Fig.1: Liver tissue of control rats showing normal histology.

Liver tissue of control rats. Normal histology

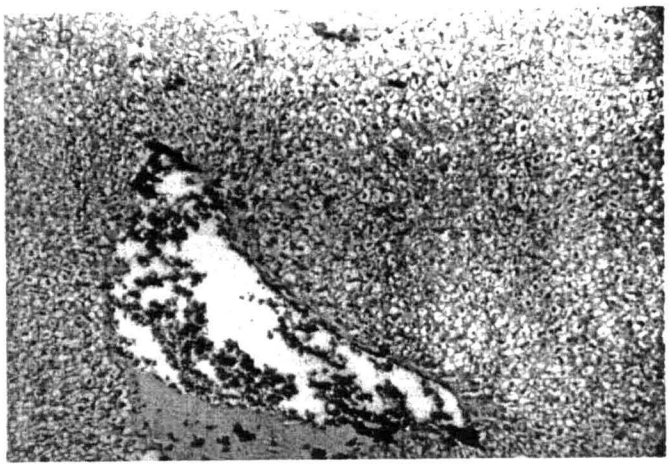

Fig.2: Liver tissue of CCl_4 treated animals.

Liver tissue of CCl_4 treated animals showing intense centrilobular necrosis and vacuolization, fatty degeneration in centrilobular is also seen.

Statistical Analysis:

Results of biochemical estimations were reported as mean±SD for determination of significant inter group differences; each parameter was analyzed separately, and a one-way analysis of variance (ANOVA) was carried out[16] followed by Dunnet's 't' test[17] for individual comparisons.

RESULT AND DISCUSSION

The ethanol extract and butanol fraction have shown marked decrease in both SGOT and SGPT activity and an equal degree of activity as compared to that of LIV-52. (Table 1). Histopathological profile of the normal untreated rat liver is shown in fig. 1. The hepatotoxic effect of CCl_4 resulted in intense centrilobular necrosis and vacuolisation[18] as shown in fig. 2. Fatty degeneration was also observed in centrilobular areas. Livers obtained from rats treated with ethanol extract and butanol fraction of fruits of *Piper longum* (figs. 3 and 4) showed significant signs of amelioration of CCl_4–induced liver injury as evident from the presence of normal hepatic cords, absence of necrosis and less degree of infiltration. Liver of rats treated with other extracts showed signs of protection against CCl_4 injury to some extent but it

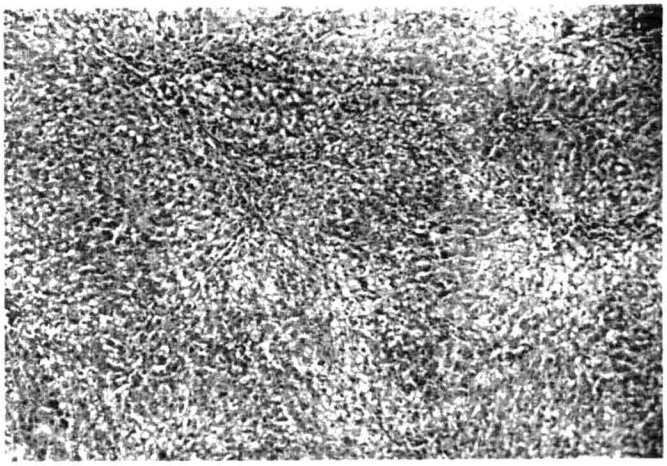

Fig.3: Liver tissue of rats treated with ethanol extract of *Piper longum* fruits.

Liver tissue of rats treated with ethanolic extract of *Piper longum* fruits showing significant signs i.e. absence of necrosis and less degree of infiltration.

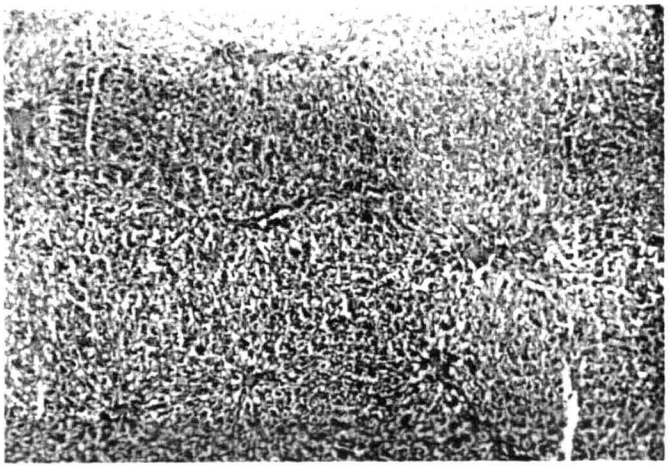

Fig.4: Liver tissue of rats treated with butanol fraction of *Piper longum* fruits.

Liver tissue of rats treated with butanol fraction, showing significant signs i.e. less hepatocellular degeneration and mild macro and micro fatty change.

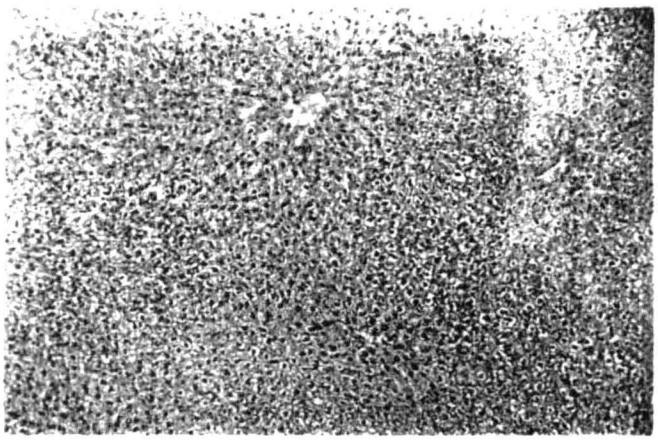

Fig. 5: Liver tissue of rats treated with standard LIV-52.

Liver tissue of rats treated with standard LIV-52 showing significant signs of amelioration as evident from the presence of normal hepatic cords, absence of necrosis and less degree of infiltration.

is not comparable with standard drug LIV-52 (fig. 5). Hepatotoxicity induced by CCl_4 is attributed to generation of trichloromethyl free radical during metabolism by hepatic microsomes, which in turn cause peroxidation of membrane lipids. Hepatoprotective action of fruits of *Piper longum* may be due to its ability to induce microsomal enzymes, which accelerates the excretion of CCl_4 or by inhibition of lipid peroxidation by CCl_4[19]. Decrease in the activity of SGOT and SGPT enzymes shows the reversal of induced toxicity of liver. Biochemical and histopathological observation reveals that ethanolic extract and butanol fraction of fruits of *Piper longum* exhibit significant hepatoprotective activity. In summary, we can conclude that ethanolic extract and butanol fraction of ethanolic extract of fruits of *Piper longum* exert a clear protective action against carbon tetrachloride induced hepatic damage.

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