Protective Effect of Root Extract of *Operculina turpethum* Linn. Against Paracetamol-Induced Hepatotoxicity in Rats

S. V. SURESH KUMAR*, C. SUJATHA, J. SYAMALA, B. NAGASUDHA AND S. H. MISHRA[1]
Department of Pharmacognosy, Sri Padmavathi School of Pharmacy, Tirupati-517 501. [1]Pharmacy Department, Faculty of Engineering & Technology, M. S. University of Baroda, Vadodara-390 001, India.

The ethanolic extract obtained from roots of *Operculina turpethum* (Convolvulaceae) were evaluated for hepatoprotective activity in rats by inducing liver damage by paracetamol. The ethanol extract at an oral dose of 200 mg/kg exhibited a significant protective effect by lowering serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase and total bilirubin. These biochemical observations were supplemented by histopathological examination of liver sections. Silymarin was used as positive control.

Operculina turpethum, which is commonly known as *trivit,* is a large stout perennial twinner with milky juice and fleshy branched roots[1]. It is one of the plants mentioned in the literature having claims of activity against liver disorders[2]. It also has anthelmintic expectorant, antipyretic, anti-inflammatory and purgative properties[2]. It contains a wide variety of phyto constituents, which are useful in treatment of different ailments and includes glycosidic resin, coumarins, beta-sitosterol, and essential oils[1-4]. The present study was undertaken to evaluate the hepatoprotective activity of root extract of this plant in experimental animals against paracetamol-induced hepatotoxicity and is reported here.

MATERIALS AND METHODS

Roots of *Operculina turpethum* were obtained from Srinivasa Ayurvedic Pharmacy, Tirupati, Andhra Pradesh, as a gift sample and their identity was confirmed by specimen species preserved at the Department of botany herbarium, S. V. University, Tirupati. The roots are air-dried, powdered and used for further studies. All the chemicals used for glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALKP) and total bilirubin (TBL) determinations are analytical grade of E. Merck, Mumbai.

***For correspondence**
E-mail: sureshsolleti@yahoo.co.in

Preparation of extract:
The powdered roots are extracted with petroleum ether (40-60°) to remove lipids and then again extracted with ethanol in Soxhlet extractor. The extract was concentrated under vacuum to get the residue. The residue was dried in vacuum desiccator. The extractive yield of ethanol was found to be more, and it was selected for hepatoprotective screening. All the test suspensions (100 mg/ml) were prepared in the vehicle, i.e., 5% w/v acacia mucilage and were administered in the dose of 200 mg/kg orally.

Toxicity studies:
Wistar rats weighing 150-175 g of either sex, maintained under standard husbandry conditions, were used for all sets of experiments in groups of six animals. Animals were allowed to take standard laboratory feed and tap water. The ethanolic extract was administered to different groups of rats in doses ranging from 100-2000 mg/kg. There is no lethality in any of the groups. One tenth of the maximum dose of the extract, tested for acute toxicity, was selected for evaluation of hepatoprotective activity, i.e., 200 mg/kg[5]. The experiments were performed after the experimental protocols had been approved by the Institutional Animal Ethics Committee, M. S. University of Baroda, Vadodara.

Effect on normal liver functions:
The ethanolic extract was evaluated for its effect on

normal liver functions by studying its effect on normal serum biochemical parameters. The rats were divided into control and test groups, each comprising of six animals. The control group received vehicle (5% acacia mucilage, 1 ml/kg orally) at 0, 24 and 48 h intervals, and the test group received ethanolic extract (200 mg/kg orally) at 0, 24 and 48 h intervals. After 72 h of first dose administration, blood was collected by puncturing the retro-orbital plexus and was allowed to clot at room temperature. Serum was separated by centrifuging at 2500 rpm. The serum obtained was used for the determination of SGOT[6], SGPT[6]. Serum ALKP was assayed by phenyl phosphate method[7] and TBL assay was carried out according to the method of Michaelssohons modification of Jendrassik and Grof[8].

Paracetamol-induced hepatotoxicity[9]:

Rats were divided into four groups of six each: control, hepatotoxin, test and positive-control groups. The control group received vehicle at 0, 24 and 48 h orally. The animals in the hepatotoxin group received the vehicle at 0, 24 and 48 h, followed by paracetamol at a dose of 3 g/kg orally. The test group received the first dose of extract at 0 h; the second dose of extract at 24 h; and at 48 h, the third dose of extract followed by a dose of paracetamol. The animals in the positive-control group received the first dose of silymarin (200 mg/kg orally)[10] at 0 h; the second dose of silymarin at 24 h; and at 48 h, the third dose of silymarin followed by a dose of paracetamol. After 96 h, blood was collected from all the groups and was allowed to clot, for the separation of serum. Serum was utilized for estimation of SGOT, SGPT, ALKP and TBL by reported methods to assess liver functions.

Histopathological studies:

One animal from the treated groups showing maximal activity as indicated by improved biochemical parameters from each test, positive control, hepatotoxin and control groups were utilized for this purpose. The animals were sacrificed, and the abdomen was cut open to remove the liver. Then, 5 mm thick pieces of the liver were fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5 ml of glacial acetic acid) for 12 h and then embedded in paraffin, using conventional methods[11], and cut into 5 μm thick sections and stained, using haematoxylin-eosin dye, and finally mounted in diphenylxylene. Then the sections were observed under microscope for histopathological changes in liver architecture, and their photomicrographs were taken.

Statistical analysis:

The mean values±SEM were calculated for each parameter. Percentage reductions against the hepatotoxin by test samples were calculated by considering enzyme level difference between the hepatotoxin treated and the control group as 100% levels of reduction. For determining the significant inter-group difference, each parameter was analyzed separately, and one-way analysis of variance (ANOVA)[12] was carried out .Then the individual comparisons of the group mean values were done using Dunnet's Procedure[12].

RESULTS

In the study of the effect of the ethanol extract of *Operculina turpethum* on normal liver functions, it was found to be non-toxic at the selected dose (200 mg/kg) since the parameters SGOT, SGPT, ALKP and TBL were within the limits like that of control (Table 1). Paracetamol intoxication in normal rats elevated the levels of SGOT, SGPT, ALKP and TBL significantly, indicating acute centrilobular necrosis. The rats treated with ethanolic extract of *Operculina turpethum* showed a significant reduction in all the four biochemical parameters elevated by paracetamol (Table 2). This reduction in biochemical parameters exhibited by ethanol extract is similar when compared with that of silymarin. The percentage reductions of all four biochemical parameters against hepatotoxin by ethanol extract and positive control are given in Table 3.

The histopathological profile of the rat treated with

TABLE 1: EFFECT OF ETHANOLIC EXTRACT ON LIVER FUNCTIONS

Group	Biochemical parameters mean±SEM			
	SGOT (U/ml)	SGPT (U/ml)	ALKP (KA units/100ml)	TBL (mg/dl)
Control	53.3±4.3	80.7±10.9	67.5±7.1	0.9±0.1
Ethanolic extract	60.3±3.7	78.2±9.3	70.8±5.4	1.0±0.1
F calculated	1.5	0.03	0.1	1.3
5% Allowance	14.7	37.0	22.9	0.3

F critical = 4.96 (P<0.05). Mean values are average of six determinations.

TABLE 2: EFFECT OF *OPERCULINA TURPETHUM* ROOTS ON PARACETAMOL-INDUCED HEPATOTOXICITY

Group	Biochemical Parameters mean ± SEM			
	SGOT (U/ml)	SGPT (U/ml)	ALKP (KA Units/100ml)	TBL (mg/dl)
Control	53.3±4.4	89.3±9.3	73.7±5.8	1.0±0.1
Paracetamol	149.0±13.6	188.7±21.5	174.6±12.4	1.94±0.1
Ethanolic extract	94.8±8.5*	77.3±5.8*	84.7±7.1*	1.6±0.1*
Silymarin	50.7±6.2*	72.0±6.4*	80.5±7.0*	0.95±0.1*
F Calculated	27.0	19.5	32.0	93.7
5% Allowance	31.7	44.6	30.2	0.2

F critical = 3.10 (P<0.05). *Significant reduction compared to paracetamol. Mean values are average of six determinations.

TABLE 3: PERCENT REDUCTION OF BIOCHEMICAL PARAMETERS BY ETHANOLIC EXTRACT AND SILYMARIN

GROUP	SGOT	SGPT	ALKP	TBL
Ethanolic extract	56.6	112.1	89.1	82.1
Silymarin	102.7	117.4	93.2	104.2

ethanolic extract (Fig. 1) showed no visible changes confirming the safety of the extract at selected dose regimen. Histopathological examination of liver sections of control group (Fig. 2) showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. In the liver sections of the rats intoxicated with paracetamol (Fig. 3), there is disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis extending to mid-zone and sinusoidal haemorrhages and dilatation. There was chronic inflammatory cell infiltrate in the portal tracts.

The liver sections of the rats treated with ethanolic extract and intoxicated with paracetamol (Fig. 4) and rats treated with silymarin and intoxicated with paracetamol (Fig. 5) showed less vacuole formation, reduced sinusoidal dilation, and less disarrangement and degeneration of hepatocytes, indicating marked regenerative activity. The intensity of centrilobular necrosis was less.

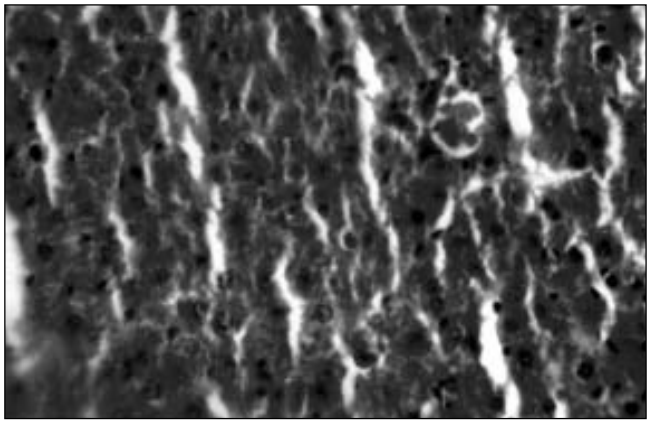

Fig. 1: Liver section of rat treated with ethanol extract.
Liver section of the rat showing no visible changes confirming the safety of the extract. 400X. Haematoxylin-eosin stain.

DISCUSSION

Paracetamol, an analgesic and antipyretic, is assumed to be safe in recommended doses; overdoses, however, produce hepatic necrosis. Small doses are eliminated by conjugation followed by excretion, but when the conjugation enzymes are saturated, the drug is diverted

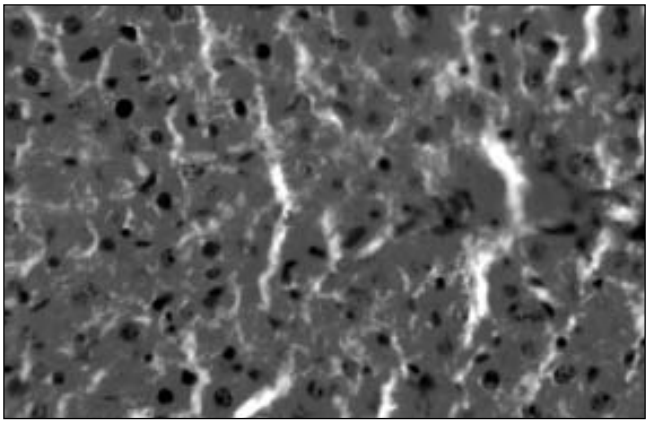

Fig. 2: Normal rat liver section.
Liver section of the rat showing normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. 400X. Haematoxylin-eosin stain.

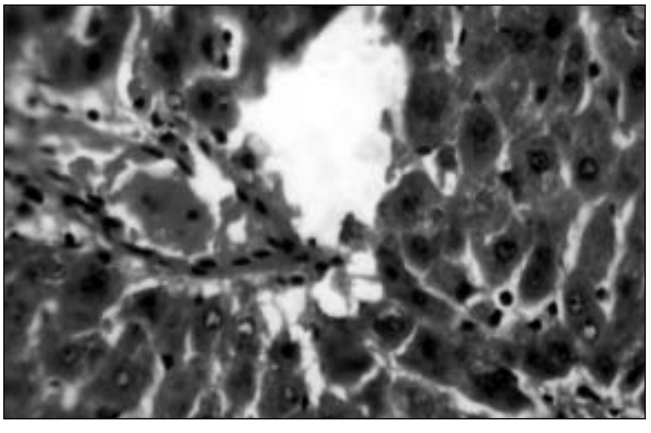

Fig. 3: Liver section of rat intoxicated with paracetamol.
Liver section of the rat showing disarrangement and degeneration of normal hepatic cells with centrilobular necrosis extending to mid zone and sinusoidal hemorrhages and dilation. 400X. Haematoxylin-eosin stain.

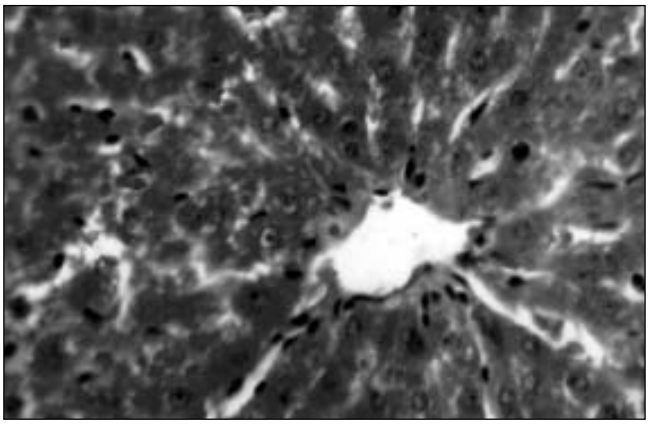

Fig. 4: Liver section of rat treated with ethanolic extract and intoxicated with paracetamol.
Liver section of the rat showing less vacuole formation reduced sinusoidal dilation, less disarrangement and degeneration of hepatocytes. 400 X. Haematoxylin-Eosin stain.

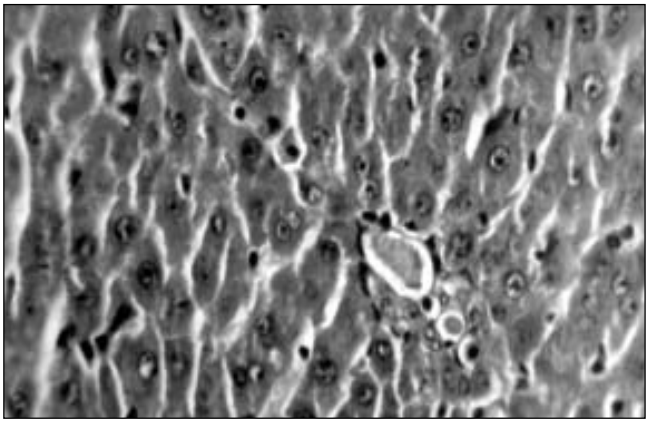

Fig. 5: Liver section of rat treated with silymarin and intoxicated with paracetamol (Positive Control).
Liver section of the rat showing less vacuole formation reduced sinusoidal dilation, less disarrangement and degeneration of hepatocytes. 400X. Haematoxylin-eosin stain.

to an alternative metabolic pathway, resulting in the formation of a hydroxylamine derivative by cytochrome P_{450} enzyme. The hydroxylamine derivative, a reactive electrophillic agent, reacts non-enzymatically with glutathione and detoxifies. When the hepatic reserves of glutathione depletes, the hydroxylamine reacts with macromolecules and disrupts their structure and function. Extensive liver damage by paracetamol itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes. Induction of cytochrome P_{450} or depletion of hepatic glutathione is a prerequisite for paracetamol-induced toxicity[9]. The ethanol extract of *Operculina turpethum* reduced the elevated levels of all the four biochemical parameters by paracetamol.

Paracetamol-induced liver necrosis was inhibited significantly by *Operculina turpethum* root extract, which confirms the protective action of the ethanolic extract of *Operculina turpethum* against experimentally induced liver damage in rats. SGOT, SGPT, ALKP and TBL are the most sensitive tests employed in the diagnosis of hepatic disease[13]. The elevated levels of these parameters were significantly reduced by the treatment of *Operculina turpethum* root extract. It can be concluded from this investigation that roots of *Operculina turpethum* possess hepatoprotective activity. Further detailed studies may, however, confirm the utility profile of this drug.

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