

## Anti-hepatotoxic activity of Fruit pulp of *Momordica dioica* Roxb. (Cucurbitaceae)

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### SUMMARY

The Hexane Extract (HE) and Ethyl Acetate Soluble Fraction of the Methanolic Extract (EASFME) of the fruit pulp of *Momordica dioica* Roxb. (Cucurbitaceae) was evaluated for its anti-hepatotoxic activity in rats. Acute hepatotoxicity was induced by administering paracetamol (2 g/kg, p.o.) for 3 days. The extracts, at a dose of 400 mg/kg (p.o.) administered for 7 days exhibited a significant therapeutic effect by lowering Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP) and Serum bilirubin and increasing the serum protein levels. These biochemical observations were supplemented by histopathological examination of the liver sections. The activity of extract was also comparable to the standard drug Silymarin, which is a well-known natural anti-hepatotoxic drug.

**Key words:** *Momordica dioica* Roxb; Cucurbitaceae; Anti-hepatotoxic

### INTRODUCTION

*Momordica dioica* Roxb. (Cucurbitaceae) is a perennial dioecious climber with tuberous roots found throughout India (Sastri, 1962). The fruits are used in the treatment of inflammation caused by lizard excretion (Nadkarni, 1976) as well as mental and digestive disorders (Satyavati, 1987). The whole plant is known for its use in the treatment of eye disease, poisoning and fever (Satyavati, 1987). There is paucity of data about the pharmacological activities of *M. dioica*, which prompted us to pursue this pharmacological evaluation of *M.dioica* fruit pulp to verify the medicinal properties. The most important constituents of the root of the plants are fatty acid (Stearic acid) sterol (Spinosterol) triterpenes (Bryonolic acid, Gypsogenin, Hederagenin) alkene (Hentriacontene) (Mohdali *et al.*, 1998), and

the fruit pulp consists of proteid (Lectin) and triterpene (Ursolic acid).

In the present study, vacuum dried, HE and EASFME of *M.dioica* fruit pulp was evaluated for its anti-hepatotoxic activity.

### MATERIALS AND METHODS

#### Plant material

*Momordica dioica* fruits were collected and seed was separated mechanically during November 2001 from Virudhunagar district of TamilNadu, India and identified by Dr. Jayaraman, Taxonomist, Retired Professor, Presidency College, Chennai. A Voucher specimen was deposited at the Department of pharmacognosy, S.R.M. College of Pharmacy, kattankulathur, India for future reference. The fruit pulp was shade dried, pulverized using a cutter mill and stored in an airtight, light-resistant container for further use.

#### Preparation of the extract

The pulverized fruit pulp was extracted by maceration

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successively with hexane and methanol for 48 hours. Then the extract was vacuum dried using rotary vacuum flash evaporator to yield a solid residue of the respective extract viz. hexane extract and methanol extract. To the methanolic extract, ethyl acetate was added and ethyl acetate soluble fraction was separated and concentrated under vacuum to get dried residue. (Yield 0.5% w/w and 2.6% w/w respectively on dried weight basis) and stored in a desiccator. For pharmacological experimentation, a weighed amount of dried extract was freshly suspended in 2% v/v aqueous Tween-80 solution.

#### Preliminary photochemical screening

On systematic qualitative analysis, HE gave positive test for steroids, alkaloids, flavanoids and fatty acids and EASFME showed positive tests for carbohydrates, proteins, steroids, flavanoids and triterpenes (Kokate, 1990; Evans, 1997; Harborne, 1998).

#### Dose and route of administration

For inducing acute hepatic damage, paracetamol suspension was administered for 3 days at a dose of 2 g/kg body weight, p.o. (Ashok Shenoy *et al.*, 2002), followed by the standard drug silymarin (200 mg/kg, p.o) and two extracts for 7 days (400 mg/kg, p.o).

#### Animals

Albino rats (Wistar strain) of either sex weighing 150-250 g were obtained from the Tamil Nadu Veterinary and Animal Science University, Madhavaram, Chennai, India. They were placed in polypropylene cages with wire-net floors in a controlled room environment ( $25^{\circ}\pm 2^{\circ}\text{C}$ ), were provided with standard laboratory food and boiled water *ad libitum* and were maintained at a natural day-night cycle. The animals were randomly divided into five groups of six rats each, the groups being balanced for sex and body weight. The animals were fasted for 24 hrs before experimentation but allowed free access to boiled water.

#### Chemicals used

Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT),

Serum alkaline phosphatase (ALP), Serum bilirubin, total proteins and albumins were estimated by using standard kits from M/S Ranbaxy Laboratories Ltd., New Delhi, India. All the reagents used were of analytical grade. Silymarin (Silybon, M/S Micro Labs) was used as standard drug.

#### Experimental procedure

The animals were divided into five groups of six rats each. A suspension of paracetamol was prepared in 2% v/v Tween 80 and administered orally at the dose of 2 g/kg body weight. Silymarin and extracts were also administered in a similar way.

- Group I consisted of control rats, which received 2% v/v Tween 80 for 10 days
- Group II received paracetamol orally once daily for 3 days
- Group III received paracetamol orally for 3 days followed by silymarin 200 mg/kg orally for the next 7 days.
- Group IV received paracetamol orally for 3 days followed by HE 400 mg/kg orally for the next 7 days.
- Group V received paracetamol orally for 3 days followed by EASFME 400 mg/kg orally for the next 7 days

At the end of the treatment (on the 11<sup>th</sup> day) period, rats of each groups were anaesthetized using anesthetic ether, and then blood samples were collected by direct cardiac puncture, and centrifuged at 2000 rpm at 4°C for 10 mts to separate the serum for different biochemical analysis. The rats were sacrificed by cervical dislocation. The livers were immediately excised to study its histopathology.

#### Enzyme assay

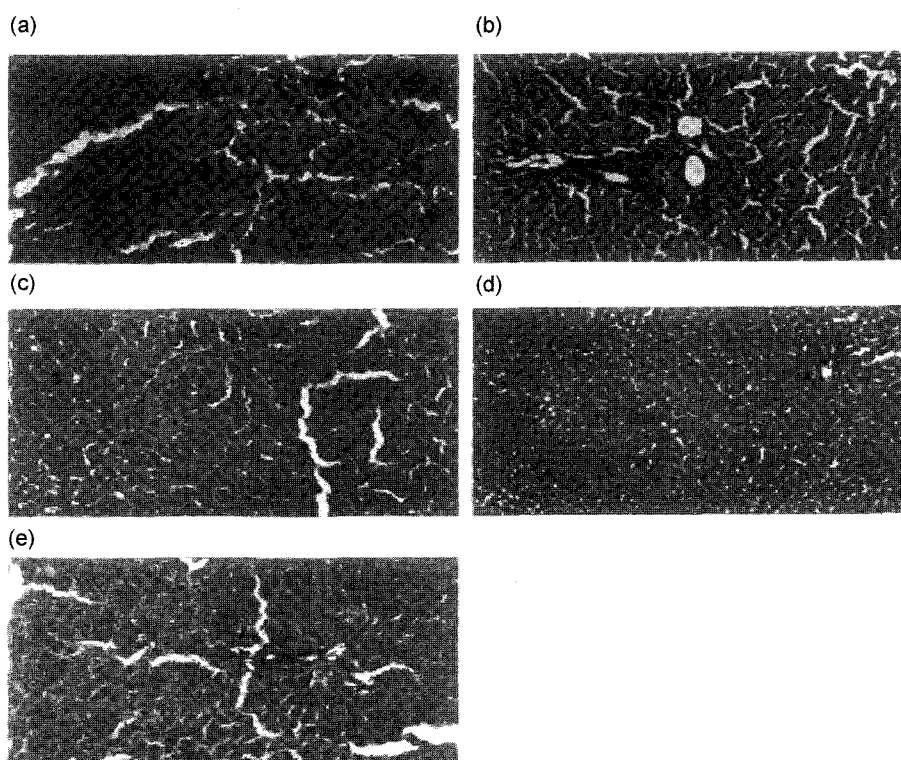
SGOT and SGPT were determined by the Reitman and Frankel method (1957). Serum bilirubin was estimated by the Malloy and Evelyn method (1937) and Serum ALP was estimated by the Kind and King method (1954) (Table 1).

#### Protein estimation

The serum was also used to determine the levels of total protein, albumin, globulin and the albumin-globulin ratio. Total protein was estimated by the

**Table 1.** Effect of *Momordica dioica* fruit pulp on serum biochemical parameters during paracetamol induced liver damage in rats [Mean±SEM (P Value)]

Parameters	Group I Control Normal Saline (0.1 ml/kg)	Group II Paracetamol (2 gm/kg)	Group III Sily- marin (200 mg/kg)	Group IV HE (400 mg/kg)	Group V EASFME (400 mg/kg)
SGOT (IU/L)	49.81 ± 1.31	133.66 ± 11.03	57.65 ± 1.95 <sup>a</sup>	71.23 ± 6.43 <sup>a</sup>	106.55 ± 4.45 <sup>a</sup>
SGPT (IU/L)	40.50 ± 2.13	102.42 ± 10.09	51.4 ± 5.38 <sup>b</sup>	63.01 ± 3.33 <sup>a</sup>	84.38 ± 8.00
Alkaline phosphatase (IU/L)	111.28 ± 11.59	453.96 ± 18.98	215.06 ± 18.44 <sup>a</sup>	164.68 ± 14.65 <sup>a</sup>	293.62 ± 19.93 <sup>b</sup>
Bilirubin (mgm/dl)	2.95 ± 0.39	3.52 ± 0.64	2.68 ± 0.16 <sup>a</sup>	2.90 ± 0.25	2.90 ± 0.19
Total protein (gm/dl)	10.47 ± 0.10	10.01 ± 0.44	11.35 ± 0.41	10.99 ± 0.29	10.18 ± 0.77
Albumin (gm/dl)	7.66 ± 0.07	7.0 ± 0.13	7.72 ± 0.09	7.71 ± 0.14	7.60 ± 0.11
Globulin (gm/dl)	2.81 ± 0.04	3.01 ± 0.08	3.63 ± 0.15	3.28 ± 0.12	2.58 ± 0.17
Albumin globulin ratio	2.72 ± 0.09	2.32 ± 0.14	2.13 ± 0.11	2.35 ± 0.09	2.94 ± 0.14

<sup>a</sup> $p < 0.001$ , <sup>b</sup> $p < 0.05$ 

- a. Liver section of a normal rat showing normal hepatic cell architecture.  
 b. Liver section of a rat with paracetamol induced hepatotoxicity showing severe focal necrosis.  
 c. Liver section of a rat induced with paracetamol + standard drug Silymarin showing almost normal hepatic cell architecture.  
 d. Liver section of a rat induced with paracetamol + HE treated group showing almost normal cell architecture.  
 e. Liver section of a rat induced with paracetamol + EASFME treated group showing mild focal necrosis.

method of Lowry *et al.*, (1951) and the albumin was estimated by the method of Doumas *et al.*, (1971) (Table 1).

#### Histopathological examination

Small fragments of the liver was washed in ice-cold

saline, fixed in 10% formalin solution, dehydrated with ethanol (50%), embedded in paraffin and cut into 5 µm thick sections using a microtome. The sections were stained with eosin-haematoxylin dye for photo microscopic observation (Gray, 1964) of necrosis, steatosis and fatty change of hepatic cells

(Figs. a, b, c, d and e).

### Statistical analysis

The experimental results were expressed as the Mean  $\pm$  SEM (Standard Error Mean). The Dunnetts test was used to make a statistical comparison between groups. Results with  $P < 0.05$  were considered statistically significant.

## RESULTS AND DISCUSSION

Acute administration of paracetamol (2 g/kg, p.o) lead to increase in SGOT, SGPT ALP and serum bilirubin concentration, which are used as reliable markers of hepatotoxicity (Harper, 1961). Oral administration of *M.dioica* fruit pulp extract at a dose of 400 mg/kg, body weight, p.o markedly prevented the paracetamol-induced elevation of SGOT, SGPT, ALP, serum bilirubin and also increased the levels of total proteins and albumins (Table 1).

Paracetamol, a widely used over-the-counter analgesic and antipyretic, produces hepatic necrosis when ingested in very large doses. It is metabolized in the liver primarily to glucuronide and sulphate conjugates. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P<sub>450</sub> (Ashok Shenoy *et al.*, 2002). Induction of cytochrome P<sub>450</sub> or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity. Therefore the anti-hepatotoxic activity of the drug may be due to: inhibition of cytochrome P<sub>450</sub>, promotion of glucuronidation, stimulation of hepatic regeneration, activation of the functions of the reticuloendothelial systems or inhibition of protein biosynthesis (Rao *et al.*, 1997).

Oral administration of *M. dioica* fruit pulp of HE (400 mg/kg) exhibited a significant reduction in paracetamol-induced levels of serum SGOT, SGPT, ALP and serum bilirubin and also increase the levels of total proteins and albumins value remarkably to the normal group. This increase in total proteins and albumin value shows further evidence for the protective effect of HE.

The EASFME reduced the elevated marker enzyme levels to only certain extent and bilirubin level has been reduced to the normal value. EASFME has increased the total protein content

and albumin level remarkably. Both HE and EASFME were compared with the standard herbal drug silymarin with a dose of 100 mg/kg, bodyweight, p.o. The silymarin has provide a better inhibition of the elevated level of SGOT, SGPT, ALP and serum bilirubin and also increased the protein content and albumin level than the normal value. Overall the activity exhibited by HE was comparable with the standard drug silymarin.

## CONCLUSIONS

The results obtained from the levels of the hepatic marker enzymes showed significant anti-hepatotoxic activity of the HE of *M.dioica* when compared to the standard drug silymarin. However, further studies are required to provide a detailed phytochemical examination of the active extract of *M. dioica* to identify the principle(s) responsible for the activity and to elucidate their mechanism of action.

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