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Hepatoprotective activity of *Marrubium vulgare* against paracetamol induced toxicity

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ABSTRACT

Aim: The present work was carried out to evaluate the hepatoprotective activity of *Marrubium vulgare* against paracetamol toxicity in Wistar rats.

Methods: Hepatoprotective properties of the methanol extract of whole plant were evaluated on paracetamol induced hepatotoxicity. Hepatotoxicity was induced in albino Wistar rats by the administration of paracetamol (2 g/kg), p.o. for 7 days. Methanol extract of *Marrubium vulgare* was administered at the doses 100 and 200 mg/kg/day, p.o. for 7 days. Serum analysis was performed to estimate the levels of ALT, AST, ALP, albumin, total bilirubin, and triglycerides. The liver was solated and homogenized for the estimation of glutathione and malondialdehyde. Histopathology studies were also performed on the catalase liver samples.

Results: The toxic effects of paracetamol were significantly controlled in the extract treated groups which was manifested by the restoration of serum biochemical parameters to near normal levels.

Conclusions: From the study it was concluded that *M. vulgare* possess significant hepatoprotective properties.

1. Introduction

Medicinal plants have been used throughout the world for ages to treat various ailments of mankind. *Marrubium vulgare* L. (Lamiaceae) one such plant commonly known as "horehound" in Europe, or "Marute" in the Mediterranean region, is naturalized the latter and Western Asia and America. In the Mediterranean, *M. vulgare* is frequently used in folk medicine to cure a variety of diseases. The plant is reported to possess cytotoxic,[1] antiprotozoal,[2] antioxidant and antigenotoxic[3,4] antimicrobial,[5,6] antibacterial,[7] antispasmodic,[8] immunomodulatory[9] activity. *M. vulgare* in particular has been reported to posses antidiabetic,[10] molluscicidal,[11] antibacterial and cytotoxic,[12] and gastroprotective.[13]

More than 87 medicinal plants have been used in different combinations in the preparation of 33 patented herbal formulations in India.[14,15] Herbal formulations (Liv 52, Livergen, Livokin, Octogen, Stimuliv and Tefroliv) have been found to

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produce marked beneficial effects in the studied pharmaco-logical, biochemical and histological parameters against acute liver toxicity in mice model induced by paracetamol (PCM).[16] Despite of tremendous advances in modern medicine, there are no effective drugs available that offers protection to the liver from damage or stimulate the liver functioning. Aiming these factors the present investigation was undertaken to evaluate the hepatoprotective activity of methanolic extract of *M. vulgare* (MEMV).

2. Materials and methods

2.1. Drugs and chemicals

Paracetamol and enzymatic diagnostic kits were procured from S.D. Fine Chemicals New Delhi and E-Merk, Germany. Silymarin was purchased from Sigma Co. New Delhi, India. All other chemicals used in this study were of analytical grade.

2.2. Source of plant material and preparation of extracts

The plant material was collected from local area of Srinagar of Jammu and Kashmir, India in the month of July 2010. The collected plant material was duly identified and voucher specimen (No. 2580/2010) is deposited in the herbarium of the institute for future reference.

The whole plant material was dried in the shade at 30 ± 2 °C. The dried plant material (500 g) was ground into a powder using mortar and pestle and passed through a sieve of 0.3 mm mesh size. It was then subjected to extraction with methanol (3×4.0 L) at room temperature after defating with petroleum ether 60—80 °C (3×3.5 L) for 24 h at room temperature. The methanolic extract was concentrated under reduced pressure in rotavapour to yield a crude gum type extract. The extract was stored in refrigerator for further use.

2.3. Phytochemical screening

The preliminary qualitative phytochemical screening of *M. vulgare* was conducted for the presence and/or absence of alkaloids, glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oils, cyanogenic glycosides, coumarins, sterols and/or triterpenes.

2.4. Determination of total phenolic content

Total phenolic content of MEMV was determined by the Folin—Ciocalteu reagent assay. The total phenolic concentration in the extract was expressed as gallic acid equivalents (GAEs) and was measured according to the method described by Singleton and Rossi.[17]

2.4.1. Animals
Male Wistar rats weighing between 150 and 200 g were used for this study. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 26 °C and relative humidity of 30—70%. A 12:12 light:day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). The Institutional Animal Ethics Committee approved (Project No. 864) the animal experiments and the guidelines for animal care were followed, as recommended by the Indian National Science Academy. Test materials were administered as mg/kg body weight of animals.

2.5. Paracetamol-induced liver toxicity

Rats were divided into 5 groups (G-I to G-V) of six each. G-I served as normal control and received 0.5% (CMC) carboxy methyl cellulose suspension (1 ml/kg) once daily for 7 days. G-II served as PCM control, received paracetamol (2 g/kg) for seven days. G-III served as reference control, received silymarin (200 mg/kg) once daily for 7 days along with PCM (2 g/kg). G-IV and G-V were treated with MEMV (100 mg/kg and 200 mg/kg respectively) once daily for 7 days along with PCM (2 g/kg). All the test drugs and PCM were administered orally by suspending in 0.5% CMC solution. After 24 h of last dose of PCM, the blood was collected from retro plexus, after blood collection, the animals were sacrificed by cervical dislocation and the liver was dissected out and used for biochemical studies and histological examination.

2.6. Estimation of liver marker enzymes and bilirubin

The blood collected from the rats was used for biochemical analysis. The blood was allowed to clot and centrifuged (Remi, Mumbai) for separation of serum. The serum was separated and used for assay of Alanine amino transferase (ALT), Aspartate amino transferase (AST), Alkaline phosphatase (ALP) by standard methods using enzyme assay kits. Albumin, triglycerides and serum bilirubin were also measured by kits method according to the instructions provided by the company (E—Merck, Germany).

2.7. Tissue biochemical assay

The catalase activity was measured according to method of Sinha et al.[18] The level of lipid peroxidation in liver homogenate was determined by the method of Buege and Aust.[19] Hepatic reduced glutathione (GSH) level was determined by the method of Ellman modified by Jollow et al.[20]

2.8. Histopathological examination

Liver pieces preserved in 10% formaldehyde solution were used for histopathological study. The liver tissues were placed in plastic cassettes and immersed in neutral buffered formalin for 24 h. The fixed tissues were processed routinely, embedded in paraffin, cut into 4 μm-thick sections and stained with hematoxylin and eosin (H&E). The extent of paracetamol-induced hepatic damage was evaluated by assessing the morphological changes in the liver sections.

2.9. Statistical analysis

The statistical analysis was carried out by one-way analysis of variance (ANOVA). The values are represented as mean ± SE. Comparison of mean values of different groups treated with