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Evidence for gastroprotective, anti-inflammatory and antioxidant potential of methanolic extract of *Cordia dichotoma* leaves on indomethacin and stress induced gastric lesions in Wistar rats

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ABSTRACT

The *Cordia dichotoma* (CD) is having anticancer and other pharmacological effects as it contains mainly flavonoids. The present study was aimed to demonstrate the gastroprotective effect of methanolic extract of CD leaves (MECD) obtained using Soxhlet extractor. In this study the qualitative phytochemical analysis of MECD revealed the presence of bioflavonoids and determination of quercetin was confirmed by HPLC analysis. The MECD was administered orally at doses 50 mg/kg, 100 mg/kg and 200 mg/kg against indomethacin induced gastric ulceration and stress-induced gastric ulceration in Wistar rats. Omeprazole at 10 mg/kg orally was used as the reference standard. The various parameters like gastric volume, gastric pH, total acidity, ulcer index, percent protection were estimated for assessment of anti-secretory and gastroprotective effects of MECD. At the same time antioxidant parameters like superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) in addition to that inflammatory parameters such as tumor necrosis factor-α (TNF-α), interleukin-6 and interleukin-10 were also estimated according to their respective method of estimation using analyzing kit. The MECD have reduced gastric volume, total acidity and gastric mucosal damage in both the experimental models significantly and dose dependently as compared with control group. Similarly the antioxidant enzymes like SOD and CAT were increased while MDA levels were decreased significantly, at the same time TNF-α and IL-6 levels were decreased and anti-inflammatory IL-10 levels were increased significantly in MECD treated groups. Thus the pretreatment with MECD has shown significant gastroprotective potential probably due to its antioxidant and anti-inflammatory properties.

1. Introduction

The gastric ulcerative condition is histologically defined as a damage of the mucous membrane that affects superficially or deeper muscularis mucosa of the stomach. It is most probably due to excessive gastric acid secretion or aggressive pepsin activity [1,2]. Ulcers more commonly affect the stomach and proximal duodenum and less frequently occur in distal duodenum, lower esophagus or in the jejunum. Gastric ulcerative disorders influence the quality of life and productivity of the affected patients [2,3]. The major etiological factors of this disorder are stress, alcohol, smoking, nutritional deficiencies, *Helicobacter pylori*, and frequent and indiscriminate use of nonsteroidal anti-inflammatory drugs (NSAIDs) [3]. The conventional therapy of gastric ulcer includes antacids, histamine receptor antagonist, proton pump inhibitors, prostaglandin analogs and the surgical vagotomy, however, these are limited by gastrointestinal toxicity or causing any other morbidities [4,5]. Numerous synthetic antiulcer drugs are presently available and some of these like cimetidine, misoprostol, ranitidine, omeprazole, and esomeprazole are employed to manage and cure the gastric ulcer. However, each of these drugs confers simpler to severe side effects, prompting a search for non-toxic, easily accessible, and affordable antiulcer medication [6–8].

Oxygen free radicals are deleterious to the integrity of biological tissues and mediate their injury. The mechanism of damage involves lipid peroxidation, which destroys cell membranes with the release of intracellular components, such as lysosomal enzymes, leading to further tissue damage. The radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism and DNA damage [9]. Pattanaprateep et al. described that the generation of the superoxide anion

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(free radicals) is the key factor in cellular damage and it is shown in different models of acute and chronic injury. This cascade of cellular insult was determined using various indicators or markers like superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) levels in stomach tissue [10].

Plant-derived medicines are considered to be the first line of defense in maintaining health and combating diseases. Every country around the world uses traditional medicines to some extent, while the peoples of developing countries accounted for 70–95% for primary care. Minimum 25% of all present medicines are derived either directly or indirectly from natural sources [11,12]. The phytoconstituents from the plant will be identified and used as a principal source of new drugs to treat various diseased conditions. *Cordia dichotoma*, a small to moderate size plant of family *Boraginaceae*, also known as bhokar, Indian cherry, gonda, lasura, and shlesmataka. The plants are reported to cure diseases of blood, heart, liver, kidney and gastrointestinal disorders [4,13]. Different plant parts such as fruit, bark, leaves and seed of the plants are used as an antipyretic, antianemic, antioxidant, antidiabetic, antiulcer, immuno-modulatory, anticancer activity and has been used as a remedy for impotency, gastric pain, asthma, mouth ulcers, bronchitis, diarrhea, diabetes, cardiovascular diseases, rheumatism, and dental caries [14–16]. Screening of fruit, leaves, seed and bark has shown the presence of pyrrolizidine alkaloids, flavonoids, saponins, terpenes and sterols [17,18].

This study aimed to investigate the gastroprotective effects of MECD against indomethacin and stress-induced gastric ulcers in Wistar rats using omeprazole as a standard drug. In addition, the probable mechanism by which MECD deserve its efficacy was elucidated in terms of oxidative stress and inflammatory measures using indicators like SOD, CAT, MDA and TNF-α, IL-6 (proinflammatory) as well as IL-10 (anti-inflammatory) respectively.

2. Materials and methods

2.1. Plant material and chemicals

The leaves of *Cordia dichotoma* were collected from Dhule district region (Maharashtra, India) in June 2017, and authenticated. All other chemicals were of analytical grade and purchased from Sigma Aldrich unless specified.

2.2. Preparation of extract from the plant

CD leaves were collected from the botanical garden, School of Pharmacy and Technology Management, Shirpur. Leaves were properly cleaned with water and shade dried under room temperature and ensured complete drying. Dried leaves were coarsely powdered using a mechanical mixer. This powdered crude drug (100.0 g) was extracted using methanol in soxhlet apparatus for four hours [19]. After extraction, the solvent was evaporated with the help of rotary evaporator. The vacuum dried Methanolic extract was dissolved in purified water and administered to rats at doses 50 mg/kg, 100 mg/kg, and 200 mg/kg.

2.3. Experimental animals

Wistar rats of either sex weighing 160–180 g were selected and acclimatized for 5 days under standard laboratory conditions and kept in environmentally controlled rooms (25 ± 2 °C, 12 h light, and dark cycle), animals were having free access to standard diet and water. The entire experimental work was carried out in accordance with CPCSEA guidelines [20]. The study was approved by the Institutional Animal Ethics Committee (NMIMS, School of Pharmacy & Technology Management, Shirpur, Maharashtra). IAEC Approval no: SPTM-IAEC/Dec-17/02/19.

2.4. Phytochemical analysis of the extract

The preliminary phytochemical screening of the crude MECD was carried out in order to determine the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins, tannins, terpenoids, coumarins, saponins according to standard procedures [21].

2.5. Characterization/identification of quercetin by HPLC

The quercetin (flavonoid) in the MECD was identified by reversed phase, HPLC-UV method using quercetin as the standard. 500 mg of extract dissolved in 50 ml of organic solvent (methanol) and sonicated for 15 min at ambient temperature. The resultant solution was allowed to stand for 30 min thereafter filtered through 0.45 µm membrane filter then filtered was diluted with methanol and solution was injected in to HPLC system. The chromatographic condition used as per Ang, L.F, et al. [22]. The chromatograph was obtained and compared with the standard solution of quercetin.

2.6. Acute toxic study

The acute toxicity study for MECD was carried out in Wistar rats (170–200 g) by the fixed dose method as per OECD guideline No. 420, 2001. The extract was administered at the dose 5, 50, 300, 2000 and 5000 mg/kg body weight, to determine mortality rate. The animals were observed for fourteen consecutive days [23].

2.7. Experimental design

Animals were divided into following groups, each group contains six rats. Pre-treatment with the different doses of MECD was given for 21 days prior to indomethacin and stress-induced ulceration. The extract was administered orally once daily using oral feeding tube of 18 gauge with the ad libitum provision of food and water throughout the experimental period.

2.8. Antiulcer activity of MECD in indomethacin-induced gastric ulceration in Wistar rats

Group I	Healthy
Group II	Control received only indomethacin 30 mg/kg on the 21st day
Group III	Standard received omeprazole 10 mg/kg + indomethacin 30 mg/kg on the 21st day
Group IV	Pre-treated with MECD (50 mg/kg) for 21 days + indomethacin 30 mg/kg on the 21st day
Group V	Pre-treated with MECD (100 mg/kg) for 21 days + indomethacin 30 mg/kg on 21st day
Group VI	Pre-treated with MECD (200 mg/kg) for 21 days + indomethacin 30 mg/kg on the 21st day

2.9. Antiulcer activity of MECD in stress-induced gastric ulceration in Wistar rats

Group I	Healthy
Group II	Control received no treatment and stress induced by continuous 8 h swimming on 21st day)
Group III	Standard received omeprazole 10 mg/kg + stress induced by continuous 8 h swimming on the 21st day
Group IV	Pre-treated with MECD (50 mg/kg) for 21 days + stress induced by continuous 8 h swimming on the 21st day