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Protective effect of hydro-alcoholic extract of *Ruta graveolens* Linn. leaves on indomethacin and pylorus ligation-induced gastric ulcer in rats

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ABSTRACT

Background: The search for an ideal and new antiulcer drug has been extended to herbals for novel molecules that decrease the incidence of relapse and afford better protection.
Objective: The present study was designed to investigate the protective effect of hydro-alcoholic extract of *Ruta graveolens* (RGE) Linn. leaves on indomethacin (IND) and pylorus ligation-induced gastric ulcer in Wistar rats.
Materials and methods: The rats of all the six groups were deprived of food for 24 h. Then, the first group received 1 ml/kg/day p.o. of 1% carboxymethylcellulose calcium (CMC), second group 1 ml/kg/day p.o. of 1% CMC and third group 20 mg/kg/day p.o. of IND. Fourth and fifth groups received RGE 200 and 400 mg/kg/day p.o., respectively; while the sixth group 10 mg/kg/day p.o. omeprazole. After 30 min, last three groups received 20 mg/kg/day p.o. of IND also. All these treatments after food deprivation were repeated each day for 5 consecutive days. Pylorus ligation was performed on 6th day in last five groups. After 4 h, stomach by sacrifice of the rats was examined for ulcer index (UI) and gastric mucus. Gastric juice was assessed for acidity, pH and pepsin; while gastric tissues were assessed for thiobarbituric acid reactive substance (TBARS) and glutathione (GSH).
Results: Fifth group showed significant decrease in UI (10.33 ± 0.67), TBARS (0.33 ± 0.03 mmol/mg), free acidity (48.78 ± 5.12 meq/l/100 g), total acidity (99.33 ± 9.31 meq/l/100 g), and pepsin activity (8.47 ± 0.41 μg/ml) levels while it showed significant increase in mucus (412.4 ± 21.6 μg/g), GSH (57.9 ± 4.8 mmol/mg) and pH (3.32 ± 0.27) compared to third group. Percent protection in RGE 400 mg was found to be 63.32 compared to indomethacin.
Conclusion: RGE possesses antiulcerogenic activity as it exhibits protective effect on gastric ulcer in rats.

1. Introduction

Peptic ulcer is a sore on the lining of the stomach or duodenum. It may also develop in the esophagus just above the stomach. A person can have both the duodenal and gastric ulcers at the same time [1]. The duodenal ulcer is many times more common than gastric ulcer and is mainly a disease of men [2]. The lifetime risk for developing a peptic ulcer is approximately 10%. It can develop more than 1 time in the lifetime of a man. It is quite common. It is developed in about half a million people each year in the United States and can be expected to develop in 5–10% of the adult population during the lifetime in Western countries [1,3]. It was found that the highest incidence (56.5%) of peptic ulcer in India is among the semiskilled workers and the lowest (2.5%) in managerial and professional groups [4]. The lifetime prevalence of peptic ulcer is 0.75% in Madras, 0.69% in Chandigarh and 0.61% in Delhi [5–7].

Peptic ulcer is a disease characterized by the imbalance between gastric offensive factors like pepsin secretion, acid, nitric oxide, lipid peroxidation and defensive mucosal factors like mucin secretion, glycoproteins, mucosal cell shedding, proliferation, antioxidant enzymes such as superoxide dismutase, glutathione (GSH), and catalase levels [8]. The long-term use of the pain relievers (nonsteroidal anti-inflammatory drugs) is the most common cause of the disease [9]. Alcoholism, spices, and smoking add

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to the severity of the ulcer that often precipitates serious complications of ulcer like perforation, stenosis or bleeding [10].

Most of the commonly used drugs such as proton pump inhibitors (rabeprazole, pantoprazole, and omeprazole), M_1 blockers (telenzepine, pirenzepine) and H_2 blockers (famotidine, ranitidine) decrease the secretion of acid while drugs like carbenoxolone and sucralfate promote mucosal defenses. These drugs balance the defensive factors (cell turnover, mucosal blood flow, mucin secretion, bicarbonate secretion, and cellular mucus) and aggressive factors (bile salts, pepsin, acid, and *Helicobacter pylori*) [11]. However, there are incidences of danger of drug interactions and relapses during the therapy of ulcer by synthetic drugs. Further, herbal drugs mostly augment the defensive factors such as bicarbonate secretion, mucosal blood flow, cell turnover, cellular mucus, and mucin secretion [10]. Hence, the search for an ideal and new antiulcer drug continues, and it has been extended to herbals in search for novel and new molecules which decrease the incidence of relapse and afford better protection.

Ruta graveolens Linn. belonging to family Rutaceae is commonly known as garden rue. It contains quinolone alkaloids, glycosides, flavonoids (rutin and quercetin) and furanocoumarins (psoralens and methoxy psoralens) [12]. Above-ground parts of the plant have the highest rutin content at the beginning of blooming that decreases after blooming [13]. Leaves of the plant are collected in early summer just prior to the beginning of blooming [14]. Phytoconstituents alcohol, aliphatic ketones, and acids were also isolated from its volatile oil [15]. Volatile oil obtained from *R. graveolens* is being used as flavoring agent and also being used for therapeutic purposes. In Unani system of medicine, it is reported as abortifacient, anti-vitiligo and on local application, it increases blood supply and has anti-inflammatory property, relieve joint and gouty pain. It is also an ingredient of Unani formulations such as jawarish kamuni, safoof muhazzil, and majoon halteet [16]. The jawarish kamuni is carminative, digestive, stomachic and relieves stomach pain, and colitis whereas safoof muhazzil is used for weight loss [17]. According to homeopathy, fresh leaves of *R. graveolens* are useful in rheumatism, arthritis, neuropathic pain, and varicose vein [12]. It is used as antispasmodics, digestive and for intestinal gases in Ayurvedic system of medicine [18]. Hence, keeping in view the effects of *R. graveolens* on gastrointestinal tract in traditional system of medicines such as Ayurveda and Unani, an attempt has been made in this study to evaluate antiulcer activity of hydro-alcoholic extract of *R. graveolens* (RGE).

2. Materials and methods

2.1. Collection and authentication of crude drug

The leaves of *R. graveolens* Linn. were obtained from Sami Labs Limited., Bengaluru and authenticated by Dr. Shekhar Chaturvedi, botanist and manager at Sami Labs Limited. The voucher specimen was deposited for future reference (Ref. no.: slab/cif/0359/02).

2.2. Special reagents or instruments

5,5-dithiobis-2-nitro benzoic acid (DTNB, Sigma-Aldrich), albumin (Sd fine), Alcian blue (Sd fine), ethylene diamine tetra acetate (EDTA, Sd fine), indomethacin (IND, Jagsonpal), omeprazole (Sd fine), phosphate buffer pH-6 (prepared as per IP, 2010), thiobarbituric acid (TBA, Spectrochem), Topfer's reagent (Sd fine), tris buffer (Sd fine), double beam UV spectrophotometer (UV-1700, Shimadzu), centrifuge (Almicro).

2.3. Evaluation of hydro-alcoholic extractive value of R. graveolens

Air-dried leaves of *R. graveolens* Linn. were powdered. About 100 g of dry coarse powder was taken in a closed flask and defatted with petroleum ether. The marc was dried under shade and extracted with hydro-alcoholic azeotropic mixture (ethanol:water – 70:30) by Soxhlet extractor. The extract was filtered and concentrated to a semisolid mass in a rotavapor. Finally obtained hydro-alcoholic RGE Linn. leaves were weighed and extractive value was calculated. RGE was stored in a cool place for its use in research [19].

2.4. Preliminary phytochemical screening of the extract

Phytochemical screening of RGE in favor of carbohydrates (Benedict's test), protein (Biuret test), alkaloid (Maeyer's test), steroid (Lieberman–Burchard's test), saponins (Foam test), phenolics (ferric chloride test) and flavonoids (Shinoda test) was carried out according to standard methods [20].

2.5. Experimental animal

Wistar rats of either sex weighing between 200 and 250 g were procured from the animal house facility, Faculty of Pharmacy, Integral University, Lucknow and kept in polypropylene cages as six rats in each cage under standard laboratory environment of 12/12 h light and dark cycle with free access to standard pellet diet with drinking water *ad libitum*. They were randomized into experimental and control groups. The animal house was maintained at 22 ± 2 °C temperature and 50 ± 15% relative humidity. Ethical clearance was obtained from Institutional Animal Ethics Committee, Faculty of Pharmacy, Integral University (IU/Pharm/M.Pharm/CPCSEA/12/07).

2.6. Acute toxicity study

The procedure was followed as per the Organization for Economic Cooperation and Development-423 guidelines (acute toxic class method). Wistar rats of either sex selected by random sampling were used [21]. They were deprived of food (but not water) for overnight, after which the extract was administered orally at 5 mg/kg body weight (bw) and changes in the behavior of rats were observed for 24 h after RGE administration. For any signs of toxicity and mortality, rats were observed for 14 days. If, mortality was observed in two out of three rats, then the dose administered was assigned as toxic dose. If, mortality was observed in one rat then the same dose was repeated again to confirm the toxic dose. If, mortality was not observed, the procedure was repeated for higher doses (200, 500 and 2000 mg/kg bw).

2.7. Experimental protocol

The antiulcerogenic activity of hydro-alcoholic RGE leaves was evaluated using six groups of Wistar rats with each group consisting of six rats [22]. Rats were deprived of food (but not water) for 24 h prior to being subjected to ulcerogens. The first group (negative control) received 1 ml/kg/day p.o. of 1% carboxymethylcellulose calcium (CMC), second group (positive control) 1 ml/kg/day p.o. of 1% CMC and third group 20 mg/kg/day p.o. of IND. Fourth and fifth groups received 200 and 400 mg/kg/day p.o. of RGE, respectively while the sixth group 10 mg/kg/day p.o. of standard omeprazole. After 30 min, last three groups received 20 mg/kg/day p.o. of IND also. All these treatments after food deprivation were repeated each day for 5 consecutive days. Pylorus ligation was performed on the 6th day in last five groups under ether anesthesia