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Antiulcer activity of *Wrightia tinctoria* (Roxb.) R.Br

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ABSTRACT

The purpose of the present study was aimed at evaluating the antiulcer activity on leaves of Wrightia tinctoria (Roxb) R.Br (Family Apocynaceae) on albino rat. The antiulcer activity of the Wrightia tinctoria methanolic extract (TM) and Wrightia tinctoria 70% ethanolic extract (T70E) were compared with carboxy methyl cellulose (CMC), pylorus control, Aspirin and standard famotidine which was evaluated by employing aspirin plus pylorus ligation induced ulcer model. The Biochemical parameters like volume of gastric juice secretion, pH, free acidity, total acidity, ulcer index and percentage inhibition were studied at the concentration of 200 mg/kg body weight. The plant methanolic extract showed significant gastro protective activity of 65.89% when compared with the standard drug famotidine (20 mg/ kg) which showed 75.34%. The result suggested that the methanolic extract of Wrightia tinctoria leaves possesses anti-ulcer effect. The observed effect may be due to the presence of bioactive constituents.

Keywords: Biochemical parameters, Ulcer index, Percentage inhibition, Aspirin plus Pylorus ligation, Famotidine.

INTRODUCTION

Numerous plants and herbs are used to treat gastrointestinal disorders in traditional medicine. Peptic ulcer is one of the major gastrointestinal disorder in clinical practice [1] which occurs due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors [2] consequently, reduction of gastric acid production as well as re-inforcement of gastric nucosal production has been the major approaches for therapy of peptic ulcer disease[3]. Considering the several side effects of modern medicine indigenous drugs with fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer [4]. The type of drugs varies from being protein pump inhibitor to H_2 antagonist or a cytoprotective agent. At the same time, each of these drugs confers simpler to several side effects like arrhythmias,

impotence, gynaecomastia, enterochromaffin- like cell, hyperplasia and haemopoeitic changes [5].

Identification of new drugs from plants or herbs for use in various disease and aliments is contemporarily an ideal approach. The practices of herbal medicine dates back to the very earliest period of know human history. There is evidence of herbs having been used in the treatment of diseases and for revitalizing body system in almost all ancient civilizations. The herbs are preferred because they do not produce any adverse effect with respect to their popularity and therapeutic utility.

*Wrightia tinctoria (*Roxb) R. Br (Family: Apocynaceae) exhibit in plains and slopes of the shevaroy hills. Different parts of this plant are used in Ayurveda, Siddha and other traditional systems of medicine for curing various ailments. In Ayurveda, it is known as Mathura rasam. The plant is used as antimicrobial, parakeratosis, psoriasis,astringent, stomachic, tonic febrifuge and seeds are used for kudal vriddhi and pittavayu diseases. In Unani is used as uterine sedative of vayu [6,7,8].The reported constituents are alkaloids, terpenes, wrightial[9], Tryptanthrin [10], Indole and flavonoids [11]. The objective of the present study was to investigate the antiulcer activity of various extracts like TM and T70E of the leaves of *Wrightia tinctoria.*

MATERIALS AND METHODS

Animals

Albino rats of Wistar strain of either sex weighing about 150-200 g were used. Pregnant animals were excluded. They were housed in standard cages at room temperature (25±2°C) and provided with food and water *ad libitum*. The animals were deprived of food for 24 h before experimentation, but had free access t drinking water. The study was conducted after obtaining institutional Animals ethical committee clearance bearing the number CPCSEA/265/09-11.

Drugs and Chemicals

Aspirin was obtained from German Remedies Ltd., Mumbai, India and Famotidine from Glenmark Pharmaceuticals Ltd., Mumbai. All other chemicals used in this study were obtained commercially and were of analytical grade.

Plant Material

Wrightia tinctoria leaves were collected from Shevaroy Hills at Salem district and it was identified and authenticated (BSI/SC/5/23/08-09/Tech-741) by taxonomist of the Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore.

Preparation of Extracts

The leaves of *Wrightia tinctoria* were collected and dried in shade. The leaves were then powdered and extracted individually with 70% Ethanol and Methanol in a Soxhlet extractor [12,13,14]. The extracts were then concentrated, dried and stored in a desiccator.

Acute toxicity studies

Albino Rats were kept overnight fasting prior to drug administration. A total of ten animals of two group five each were used which received a single oral dose (2000mg/kg b.w.) of *Wrightia tinctoria* methanolic extract(TM) and *Wrightia tinctoria* 70% ethanolic extract (T70E). After the administration of TM & T70E, food was withheld for further 3-4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h

(with special attention during the first 4h) and daily thereafter for a period of 14 days. Mortality, if any was determined over a period of 2 weeks [15].

Selection of dose of the extract

LD_{50} was done as per OECD guidelines for fixing the dose for biological evaluation. The LD_{50} of the extracts as per OECD guidelines falls under class for values with no signs of acute toxicity at 2000 mg/ kg. $1/10^{th}$ of LD_{50} cut off was taken as screening dose, and the dose were selected at 200 mg/ kg b.w. for both TM and T70E.

Aspirin plus Pylorus Ligation – induced Gastric ulcer Model

TM, T70E, aspirin and standard antiulcer drug famotidine [16,17] were prepared in 0.5% Sodium Carboxy Methyl Cellulose (CMC) suspension as vehicle and administered orally once daily at a volume of 10ml/ kg body weight. The animals were divided into five groups, consisting of six each. Group I received (0.5% CMC).Group II received aspirin alone (200mg/kg p.o.) Groups III and IV received aspirin orally as an aqueous suspension at a dose of 200 mg/kg (TM and T70E) body weight respectively for 7 days. Group V received famotidine orally at the dose of 20 mg/kg body weight for 7 days [18]. From days 5 to 7, animals of groups II – V received aspirin orally as an aqueous suspension at a dose of 200 mg/kg, 2 h after the administration of respective drug treatment [19, 20].

Animals in all the groups were fasted for 18 h after the respective assigned treatment and were anaesthetized with anaesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process and pylorus portion of stomach was lifted out and ligated [21]. Precaution was taken to avoid traction to the blood supply. The stomach was sutured with interrupted sutures. Four hours after pylorus ligation the rats were sacrificed and the stomach was removed. The gastric contents were collected, centrifuged and the volume of the supernatant was expressed as ml/100 g body weight.

Free and Total acidity

Free and total acidity were determined by titrating with 0.01 N NaOH using Topfer's reagent and phenolphthalein as indicator [22]. The free and total acidity were expressed as μ equiv/100 g/ 4h. The stomach was then incised along the greater curvature and observed for ulcers.

Ulcer Index

After the incision of the stomach at the greater curvature the ulcers were observed. And the number of ulcers was counted using a magnifying glass and the diameter of the ulcers were measured using vernier calipers. The following arbitrary scoring system [23] was used to grade the incidence and severity of lesions.

i) Score 10 = denuded epithelium;
ii) Score 20 = petechial and flank haemorrhages;
iii) Score 30 = one or two ulcers;
iv) Score 40 = multiple ulcers and
v) Score 50 = perforated ulcer.

Ulcer index (UI) was then calculated from the above scoring as follows:-

Eq .1: $UI = U_N + U_S + U_P \times 10^{-1}$

Where U_N is the average of number of ulcers per animal, Us is the mean severity of ulcer score and Up is the percentage of animals with ulcer incidence. The percentage inhibition was calculated by the following formula:

Eq .2: % inhibition = [UI control – UI treated / UI control] x 100

Statistical Analysis

Results were expressed as mean ± S.E.M. Data were analyzed for statistical significance by one – way analysis of variance (ANOVA) followed by Dunnett's tests with the level of significances set at $P<0.01$.

RESULTS

Acute Toxicity studies

In LD_{50} studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at the dose of 200 mg/ kg body weight.

Aspirin plus Pylorus Ligation – Induced Gastric Ulcer Model

Animals in Aspirin Plus Pylorus ligation group showed a significant ($P<0.01$) increase in the ulcer index and acid secretary parameters like gastric volume, pH, free and total acidity when compared with those of vehicle and treated group. In the rats of this group, a number of perforated ulcers (score 50) were also observed. Administration of TM andT70E produced significant ($P<0.01$) decrease in ulcer index. All the ulcers were of scores 10 and 20 and no perforated ulcers were observed. The (TM and T70E) extracts also significantly reduced the gastric volume, total and free acidity and increased the pH of the gastric fluid, proving its antisecretory activity. TM and T70E at a dose of 200 mg/kg body weight showed protection index of 65.89% and 55.29%, respectively, where as famotidine showed protection index of 75.34 % at a dose of 20 mg/kg body weight Table (1).

Table : 1 Effect of test compounds on Aspirin plus Pylorus Ligation – induced ulcer model.

Group	Treatment	Dose mg/kg	Volume of Gastric juice (ml/100 g)	pH	Free acidity (µ equiv./100g/4h)	Total acidity (µ equiv./ 100g/4h)	Ulcer Index	% inhibition
I	Control	5 ml/ kg	3.75±0.34[a]	3.82± 0.2[a]	8.92±1.18[a]	5.32±0.98[a]	-	-
II	Pylorus control	200(p.o)	5.1±0.12[a]	2.3±0.15[a]	86.71±2.72[b]	97.68±2.66[b]	42.4±1.50[b]	
III	T70E	200(p.o)	4.33±0.33[a]	2.8±0.27+a	36.73±2.32[a]	50.31±1.30[a]	19.4±1.66[a]	55.29
IV	TM	200(p.o)	4.21±0.19[a]	2.98±0.18[a]	26.71±2.01[a]	43.48±1.40[a]	14.8±1.26[a]	65.89
V	Famotidine	20 (p.o)	3.35±0.33[a]	3.95±0.28[a]	14.81±1.70[a]	27.66±1.27[a]	10.7±1.34[a]	75.34

Results are expressed as the mean ±SEM; n=6 in each group

[a]P <0.01 Vs Group II, [b]P<0.01 Vs Group I (One way ANOVA followed by Dunnett's test)

DISCUSSION

Peptic ulcer results due to over production of gastric acid (or) decrease in gastric mucosal production. Aspirin plus Pylorus Ligation (APL) induced ulcers occur because of an increase in acid pepsin accumulation due to pylorus obstruction and subsequent mucosal digestion [24].

In APL induced gastric ulcer model the methanolic and 70% ethanolic extracts of *Wrightia tinctoria* (Roxb) R.Br. leaves attenuated the gastric volume, pH, free acidity, total acidity and ulcer index thus showing the antisecretary mechanism. Famotidine is standard control used here to test antisecretary mechanism.

The present study reveals that TM and T70E treated groups showed a significant ($P<0.01$) increase in gastric juice pH, reduces the gastric volume, free acidity and total acidity when compared to control. This effect was similar to famotidine treated group. TM decreased the ulcer index more effectively than the T70E. These results show that the antiulcer activity of TM might be due to its ant secretory activity.

CONCLUSION

The results of our study prove that the crude extract of *Wrightia tinctoria* possess antiulcer activity against experimentally induced acute gastric ulcer model. Hence, it can be suggested that the antiulcer activity of the extract may be attributed to its antisecretory activity.

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