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Antiulcerogenic Effects of *Gymnosporia rothiana* (Celastraceae) against Different Experimental Models

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ABSTRACT

Gymnosporia rothiana is used in Indian folk medicine as an antiulcerogenic agent. Despite of its promising use, there has been no scientific report present regarding its antiulcer activity. Therefore, this study was intended to evaluate the antiulcer property of various extracts of leaves of *Gymnosporia rothiana* at different dose levels in ethanol induced and indomethacin induced gastric ulcer models. It was observed that oral administration of all the extracts shows significant reduction in ulcer lesion index as well as increase in volume and pH of gastric content in both experimental models, being petroleum ether extract the most effective at dose of 250 mg/kg; it significantly reduced gastric lesion index (70.06%), in comparison to omeprazole (71.20%) and methanolic extract at a dose of 500 mg/kg (67.22%). Increased gastric mucosal defense mechanism by petroleum ether extract is probably due to its high levels of terpenoids like β amyrin, lupeol, friedelin. The present results clearly shows antiulcer effect of *Gymnosporia rothiana* against various irritants has been mainly due to cytoprotective effect mediated through prostaglandin and partly due to free radical scavenging activity.

KEYWORDS: *Gymnosporia rothiana*, antiulcer, cytoprotective, terpenoids

INTRODUCTION

Peptic ulcer is the most common GIT disorder in the present day life of the industrialized and civilized world. The changing pattern of clinical evaluation and regulatory requirements for merits and demerits of drugs will be highlightened for future challenges and advances in antiulcer drug development (1). In this aspect, the plant kingdom might provide a useful source of new antiulcer compounds for the development as pharmaceutical entities or alternately, as simple dietary adjuncts to existing therapies.

The celastraceae family comprises approximately 50 genus and 800 species distributed mainly tropical and subtropical regions (2, 3). The *Gymnosporia* genus is the largest one of the celastraceae family. Many biological activities of this genus were determined experimentally as an anticancer, antiulcerogenic, analgesic, antinociceptive, anti-inflammatory, antioxidant, antimalarial (4,5). *Gymnosporia rothiana* (walp) Lawson, commonly known as Henkal, is a large armed shrub, growing western peninsular region of India (6). Its leaves are used in Indian folk medicine for treatment of number of ailments including cancer, ulcer, and rheumatism (7). It is also used as an anti-inflammatory, antioxidant, antiseptic, antidiarrhoeal drug. Its leaves are reported to contain n-octacosanol, β amyrin (8). Despite the popular use of this species as a medicinal plant, there is no more data available about its phytochemistry and pharmacological effect. Therefore, present study has been aimed to investigate the antiulcer effects of various extracts of leaves of *Gymnosporia rothiana* at different dose levels.

MATERIALS AND METHODS

Plant material

Leaves of *Gymnosporia rothiana* were collected from Satpuda valley, Dist: Nandurbar, Maharashtra, India. The plant was authenticated at Department of Botany, S.S.V.P.S's College of Science, Dhule, Maharashtra. The voucher specimen has been kept at Pharmacognosy Department, R.C.Patel College of Pharmacy, Shirpur. The leaves were shed dried, ground and sieved with a 40 mesh sieve.

Preparation of extracts

About 1 kg of leaves powder was subjected to hot extraction using soxhlet extractor, successively with petroleum ether, chloroform and methanol All the extracts were filtered and concentrated under reduce pressure by using rotary flash vacuum evaporator and then dried by using vacuum dryer, giving GRPE (2.3 %), GRCL (1.8 %) and GRME (15.6 %) respectively.

Extraction of plant material for GC-MS analysis

100 gm leaves powder of *Gymnosporia rothiana* were extracted with ethyl acetate. After filtration, the acidic compounds were extracted out with 5% aqueous KOH (three times) followed by the extraction of the basic compounds with 5% aqueous HCl (three times). The organic fraction, which contain the neutral compounds, was washed with water to pH- 7 and concentrated in rotary vacuum evaporator to 50 ml. Suspended particle were removed by centrifuging the concentrated extract for 10 min at 6000 rpm. Then solvent was evaporated to dryness, giving a residue, which was dissolved in chloroform for GC-MS analysis.

Chromatographic analysis

GC-MS analysis was performed on a Hewlett-Packard 5890 gas chromatograph, with a split injector (1:50) at 280 °C and a Hewlett-Packard 5970 mass selective detector (MSD), with the GC-MS interface temperature at 280 °C. The injection volume was 2µl. Hydrogen was employed as carrier gas, at a pressure of 60 kPa. A HP-1 25m × 0.25 mm × 0.33 µm methylpolysiloxane cross-linked capillary column was employed with temperature programming from 100 °C (held for 2 min) to 280 °C (held for 30 min) at a ratio of 4 °C/min.

Animals

The study was performed with male wistar rats (150–200 g), housed in standard environmental conditions (25 ± 3°c and humidity 60 ± 5%) under a 12h dark: 12h light cycle. During maintenance animals received a diet of food pellets (Amrut Labs, Pune) and water ad libitum. Before the experiments, the animals were deprived of food for 24hrs. Experimental protocol were designed to meet the "Guidelines of animal experimentation", approved by the ethical committee of the institute.

Antiulcer activity

Antiulcer activity was evaluated using two different assay models for induction of gastric lesions: NSAID- induced (indomethacin) and absolute ethanol- induced gastric lesions (10). For sake of comparison, animals were treated with omeprazole and cimetidine, depending on the experiment. At the end of each experiment, the animals were sacrificed by cervical dislocation; the stomach was removed and its gastric content was collected. Then stomach opened along the greater curvature and fixed between two glass plates. Free acidity, total acidity, volume and pH of gastric content were calculated as per standard methods. Ulcer lesion index calculated by severity of gastric mucosal lesion (11), graded as follows: 1) Loss of normal morphology, discoloration of mucosa, mucosal damage, hemorrhagic streaks (1 point each) 2) petechial point (< 10, 2 points, ≥10, 3 points) and 3) No. / Size of the ulcers (number of ulcer until 1mm × 2 points, larger than 1 mm × 3 points) 4) perforated ulcer (number of ulcer × 4 points). The percentage determination is as follows:

Ethanol induced gastric lesion

After 24 h fasting, the rats were divided into eight groups of six animals each. The group I served as a normal control, given 1% CMC in water (5 ml/kg, p.o), Group II was treated orally with omeprazole (30 mg/kg), Group III, V, and VII orally received 250 mg/kg of petroleum ether, chloroform, and methanol extract respectively while Group IV, VI, and VIII orally received 500 mg/kg of petroleum ether, chloroform, and methanol extract respectively. After 45 min, ulceration was induced by oral administration of 1 ml of absolute ethanol. Animals were sacrificed after 1h following the administration of absolute ethanol (12, 13).

Indomethacin induced gastric lesion

After 24 h fasting, the rats were divided into eight groups of six animals each. The group I served as a normal control, given 1% CMC in water (5 ml/kg, p.o), Group II was treated orally with cimetidine (100 mg/kg). Remaining groups (group III – VIII) were treated as previously described. After 45 min, ulceration was induced by oral

Table 1: The mass fragment of identified components from Gymnosporia rothiana by GC-MS.

Compound	Retention Time	M+	Main Fragments	%[a]
Friedelin	51.39	426	411, 341, 302, 273, 205, 125, 109,69	4.75
Hop-22(29) en-3 beta-ol	44.21	426	278, 193, 123, 109, 97, 81, 71, 57, 43	5.10
Lupeol	42.10	426	257, 220, 218,203,189, 175,147,121,105	38.44
β amyrin	40.63	426	218,203, 189, 135, 105	11.40
Campesterol	40.21	400	381, 255, 231, 199, 161,145, 121, 91, 69	8.45
Ergosterol	38.54	400	245, 213, 157, 89, 55, 43	2.28
Y- Sitosterol	37.88	414	381, 283,213,189,147, 133, 121, 109, 95	7.28
Squalene	26.88	410	367, 257, 231, 177, 161, 135, 121,81, 69	1.71
Phytol	19.96	296	278, 193, 123, 109, 97,81,71,57,43	3.39
Palmitic acid	18.56	256	241, 213, 157, 101, 88, 69, 57	2.65

[a]*The area of GC-MS peak depend not only on concentration of corresponding compound, but also on the intensity of their mass spectral fragmentation, so the data given in table is not true quantitation but can be used for comparison between two samples*

administration of 100 mg/kg of indomethacin. Animals were sacrificed after 1h following the administration of indomethacin (14).

Statistical analysis

All the results are reported as mean ± SEM. The statistical analysis was carried out using one-way ANOVA followed by Dennett's multiple comparison and p value < 0.05 were considered statistically significant.

RESULTS

Phytochemical studies

Phytochemical investigations of petroleum ether extract and chloroform extract revealed the presence of terpenes and steroids while methanol extract shows the presence of condensed tannins, flavanoids, saponin glycosides, alkaloids, proteins & amino acids.

GC-MS analysis

The result of GC-MS analysis of *Gymnosporia rothiana* were given in Table 1, which shows GC-MS experimental data, retention time (RT), and mass fragment of compounds for terpenoids and steroids of *Gymnosporia rothiana*. Individual compound were identified from RT, mass data and by comparison of the data of standard compounds with those of in the literature. Ten compounds viz. Friedelin, Hop-22(29)-en-3.beta-ol, lupeol, β amyrin, campesterol, ergosterol, sitosterol, squalene, phytol, palmitic acid were identified.

Table 2: Effect of different doses of extracts on ethanol induced gastriculcer

Treatment	Dose (mg/kg)	n	Ulcerative Lesion Index (ULI)	ULI Inhibition (%)
Control	5	6	31.67 ± 1.84	----
Omeprazole	30	6	9.12 ± 1.05[c]	71.20
GRPE	250	6	9.48 ± 1.57[c]	70.06
	500	6	4.93 ± 0.46[c]	84.43
GRCL	250	6	24.55 ± 0.12[a]	22.48
	500	6	19.31 ± 2.14[b]	39.02
GRME	250	6	14.83 ± 1.62[b]	53.17
	500	6	10.38 ± 1.19[b]	67.22

Significant difference compared to control group
[a] *p< 0.05*,
[b] *p< 0.005*,
[c] *p< 0.001*

Table 3: Effect of extracts on pH, volume of gastric fluid, free acidity and total acidity in ethanol induced gastriculcer

Treatment	Dose (mg/kg)	n	Gastric volume	pH	Free acidity	Total acidity
Control	5	6	2.15 ± 0.1	2.1 ± 0.11	30.43 ± 0.84	48.33 ±0.49
Omeprazole	30	6	3.68 ± 0.42[c]	4.6 ± 0.19[c]	10.83 ± 0.54[c]	16.87 ± 0.60[c]
GRPE	250	6	3.61 ± 0.23[c]	4.2 ± 0.14[c]	12.02 ± 0.76[c]	19.11 ± 0.34[c]
	500	6	4.73 ± 0.49[c]	5.4 ± 0.40[c]	7.83 ± 0.30[c]	11.50 ± 0.51[c]
GRCL	250	6	2.19 ± 0.18[a]	2.3 ± 0.14[a]	30.19 ± 1.12[a]	45.63 ± 0.92[a]
	500	6	2.67 ± 0.57[b]	2.6 ± 0.21[a]	28.31 ± 0.63[a]	40.27 ± 0.74[a]
GRME	250	6	3.19 ± 0.71[b]	3.3 ± 0.18[b]	17.67 ± 0.81[b]	24.45 ± 0.27[b]
	500	6	1.78 ± 0.19[b]	4.0 ± 0.26[b]	14.19 ± 0.42[b]	19.66 ± 0.53[b]

Significant difference compared to control group
[a] *p< 0.05*,
[b] *p< 0.005*,
[c] *p< 0.001*

Table 4: Effect of different doses of extracts on indomethacin induced gastric ulcer

Treatment	Dose (mg/kg)	n	Ulcerative Lesion Index (ULI)	ULI Inhibition (%)
Control	5	6	17.19 ± 2.43	----
Cimetidine	100	6	2.75 ± 1.42c	84.00
GRPE	250	6	5.34 ± 1.29c	68.95
	500	6	4.27 ± 0.79c	75.15
GRCL	250	6	13.02 ± 2.16b	24.25
	500	6	12.37 ± 1.84b	28.03
GRME	250	6	10.28 ± 1.35b	40.19
	500	6	8.41 ± 1.64b	51.07

Significant difference compared to control group
a$p< 0.05$
b$p< 0.005$
c$p< 0.001$

Evaluation of antiulcer activity

All the extracts of G*ymnosporia rothiana* showed a dose dependant gastroprotective effect against ethanol induced (Table 2 and 3), and indomethacin induced gastric ulcer (Table 4 and 5). Petroleum ether extract at dose of 250 mg /kg significantly protected mucosal damage (70.06%), in comparison to omeprazole (71.20%) and methanolic extract at a dose of 500 mg/kg (67.22%).

DISCUSSION

Peptic ulcer occurs when there is an imbalance between the damaging effects of gastric acid and pepsin and the defense mechanisms, which protects gastric mucosa from these substances. Therefore, effective drug against peptic ulcer are those which basically act either by reducing the aggressive factors or by stimulating mucosal defense. The genesis of ethanol induced gastric ulcer is associated with disturbances in gastric secretion, damage to gastric mucosa, alteration in permeability, gastric mucus depletion and also with free radical production, leads to increased lipid peroxidation which in turn causes damage to cell and cell membrane (15,16). While non steroidal anti-inflammatory drugs like indomethacin are known to induce ulcer by inhibiting prostaglandin synthetase through cyclooxygenase pathway. In the stomach, prostaglandin plays a vital protective role, stimulating the secretion of bicarbonate and mucus, regulating mucosal cell turnover and repair. Thus the suppression of prostaglandin synthesis by NSAIDS results in increased susceptibility to mucosal injury and gastric ulcer (17).

Phytochemical investigations revealed the presence of terpenes and steroids like Friedelin, Hop-22(29)-en-3.beta-ol, lupeol, β amyrin, campesterol, ergosterol, sitosterol, squalene and phytol. Methanol extract shows the presence of condensed tannins, flavanoids, saponin glycosides, alkaloids, proteins & amino acids. A review on antiulcer drugs of plant origin shows that Triterpenes like β amyrin, lupeol acetate, ursolic acid, glycerrhetinic acid and sterols like β sitosterol exert their antiulcer effect by strengthening defensive factors such as stimulation of mucus synthesis or maintenance of prostaglandin contents of gastric mucosa at high levels (18). In addition, these compounds act as antioxidant which protects gastric mucosa against oxidative damage (19). Furthermore extracts containing flavanoids also shows antisecretory, cytoprotective and antioxidant activity (20).

It was observed that oral administration of petroleum ether, chloroform and methanol extracts of *Gymnosporia rothiana* produces significant reduction in ulcer lesion index as well as increase in volume and pH of gastric content in both ethanol and indomethacin induced gastric ulcer models, being petroleum ether extract the most effective, it increased the gastric mucosal defense mechanism due to its high levels of terpenoids.

CONCLUSION

Present results clearly shows antiulcer effect of extracts of *Gymnosporia rothiana* against various irritants has been mainly due to cytoprotective effect mediated through

Table 5: Effect of extracts on pH, volume of gastric fluid, free acidity and total acidity in indomethacin induced gastric ulcer

Treatment	Dose (mg/kg)	n	Gastric volume	pH	Free acidity	Total acidity
Control	5	6	1.98 ± 1.03c	2.0 ± 0.42	27.11 ± 1.67	42.10 ± 1.26
Cimetidine	100	6	4.13 ± 0.57^c	5.8 ± 0.76^c	6.14 ± 1.12^c	11.04 ± 0.72^c
GRPE	250	6	3.64 ± 0.23^b	3.9 ± 0.14^b	12.19 ± 0.42^b	18.61 ± 0.97^b
	500	6	3.88 ± 0.49^a	4.6 ± 0.30^b	10.63 ± 0.84^b	16.57 ± 0.60^b
GRCL	250	6	2.07 ± 0.12^a	2.0 ± 0.92^a	25.57 ± 0.71^a	39.43 ± 1.42^a
	500	6	2.08 ± 0.19^b	2.1 ± 0.87^b	25.04 ± 0.48^b	36.19 ± 1.63^b
GRME	250	6	2.97 ± 0.63^b	2.9 ± 0.61^b	20.81 ± 0.94^b	28.45 ± 0.71^b
	500	6	3.18 ± 0.81^a	3.2 ± 0.27^b	17.83 ± 0.81^b	25.69 ± 0.26^b

Significant difference compared to control group
[a] $p< 0.05$,
[b] $p< 0.005$, [c] $p< 0.001$

prostaglandin and partly due to free radical scavenging activity. Thus the study provides for the first time evidence that showed antiulcer effect of *Gymnosporia rothiana* which correlate with its folklore claim as an antiulcer drug.

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