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Antiulcerogenic Activity of Various Extracts of *Dodonaea viscosa* (L) Jacq. Leaves

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Preliminary phytopharmacological investigations of ethanol extract of *Dodonaea viscosa* leaves revealed the presence of flavonoids, tannins, sterols and saponins and showed promising antiulcer activity. In view of this we conducted bioactivity-guided fraction studies of *D. viscosa* using various experimental gastric ulcer models and offensive and defensive gastric mucosal factors in rats. Among the tested extracts, ethyl acetate extract exhibited higher ulcerative lesion index, increased serum calcium level and decreased alkaline phosphatase activity in all experimental models. The antiulcer activity of the extracts may be attributed to cytoprotective and healing activity of flavonoids.

Gastroduodenal ulcer occurs due to acid secretion, pepsin activity and breakdown of mucosal defense mechanism[1]. Efforts are being made to discover an effective antiulcerogenic drug, which will not only heal the peptic ulcer but also prevent their recurrence. Search for a suitable drug for the treatment of peptic ulcer from natural products has been an ongoing process. Recent studies have implicated the role of free radicals and lipid peroxidation in the development of ulcers[2]. Literature search revealed that herbs rich in flavonoids show several biological activities including antiulcerogenic activity[3]. One of the major drawbacks associated with the use of NSAIDs in the treatment of post–operative pain, edema, rheumatoid arthritis and fever is the gastric irritation or ulceration. These ulcerogenic agents may retard healing of gastric ulcer as observed in case of aspirin and indomethacin. The setback is attributed to inhibition of cyclooxygenase responsible for synthesis of cytoprotective prostaglandins. In view of all these facts present studies were undertaken.

Dodonaea viscosa (L). Jacq., popularly known as *aliar* and *vilayati mehandi* in India, is an evergreen shrub abundantly available in Western Ghats of Karnataka. The species has been used in traditional medicine for snakebite and as febrifuge. It is also used to heal the simple ulcer[4], fracture, sore and painful gum and teeth for immediate relief[5,6]. *D. viscosa* has been reported to posses local anaesthetic, smooth muscle relaxant[7], antimicrobial and antiinflammatory activity[8]. Surveys conducted in tribal areas of Western Ghats of Karnataka revealed the use of leaves of this plant as a remedy in the treatment of snakebite, wounds and ulcers. In spite of its abundant use in trible medicine, systematic studies on phytochemical and pharmacological activities of this plant are not reported.

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MATERIALS AND METHODS

The leaves of *Dodonaea viscosa* (L). Jacq. were collected in their growth phase in areas around of Dharwad, Karnataka, in the month of Sept, 2002. The plant was authenticated at the Department of Botany, Karnataka University, Dharwad. The shade dried powdered leaves (1 kg) were extracted exhaustively with 95% ethanol (3 cycles/h) in a Soxhlet apparatus by continuous heat extraction. The total ethanol extract was concentrated *in vacuo* to a syrupy consistency (yield 200 g) and used for antiulcerogenic studies.

Bioactivity-guided fraction study:

The coarse powder of the leaves of *D. viscosa* (900 g) was used for sequential extraction with different solvents in

their increasing order of polarity. The extracts obtained were petroleum ether (60-80°) extract (PE), benzene extract (BE), chloroform extract (CE), acetone extract (AE), ethyl acetate extract (EAE), ethanol extract (EE) and chloroform:water extract (ACE)[9,10]. The dried extracts were subjected to various chemical tests to detect the different phytoconstituents. Selected extracts based on phytochemical screening were used for antiulcerogenic studies.

Healthy male Wistar rats weighing between 150-200 g were used for the study and housed individually in polypropylene cages maintained under standard conditions (12:12 h light and dark cycles; 25±2°. 35-60% humidity). The rats were fed with animal feed pellets manufactured by Hindustan Lever (India) Ltd., Mumbai. The animals were kept on fasting for 48 h before test but water was given *ad libitum*. The animal experimentation protocols were approved by the Institutional Animal Ethics Committee. The chemicals used for study were procured from S. D. Fine-Chem. Ltd. Mumbai and Ranbaxy Fine Chemicals Ltd, New Delhi.

Acute toxicity study:

Healthy albino mice of either sex weighing 20-25 g and of 90 d age were used to determine the safer dose by up and down staircase method[11]. The animals were starved overnight before the experimental procedure. Extracts were suspended in 1% CMC in water and administered intraperitoneally.

Induction of acute gastric mucosal damage:

Antiulcerogenic activity was evaluated using three different assay models. Aspirin plus pylorus-ligation and administration of indomethacin and ethanol in different set of animals were employed for induction of acute gastric mucosal lesion.

Aspirin plus pylorus-ligated gastric ulcer[12]:

Aspirin was suspended in 1% CMC suspension in water and administered orally at a dose of 200 mg/kg in rats once daily for 5 d. Standard drug/extract treatment was carried out 30 min prior to aspirin treatment on each day. On d 6 the 24 h fasted rats were subjected to pylorus-ligation under ether anaesthesia. The sixth dose of drug/extract was given 30 min prior to pylorus-ligation. After 4 h of pylorus ligation, the animals were sacrificed and the stomach was isolated after suturing the lower oesophageal end. Gastric juice was collected and filtered through glass wool in a measuring cylinder and stomach was opened along the greater curvature. The gastric contents were centrifuged at 3000 rpm for 5 min. The supernatant was collected and its volume is expressed as ml/100 g of body weight[12]. Free acidity and total acidity were measured as per the reported method[13]. Both the free acidity and total acidity are expressed as mEq/l. The ulcerative lesion index (ULI) of each animal was calculated by adding the values mentioned in Table 1. Percent inhibition was calculated by using the formula, control mean ULI-test mean ULI/control mean ULI.

Indomethacin-induced ulcer:

Animals were divided in to 6 groups of 6 animals each. Control group was treated with 1% CMC in water (5 ml/kg). Test groups of rats received different extracts of *D. viscosa* leaves (375 mg/kg p.o). Standard group was given with ranitidine (40 mg/kg p.o). Ulceration was induced by oral administration of 30 mg/kg of indomethacin after 45 min of the treatment. The animals were sacrificed 6 h. following administration of an ulcerogen. The stomach was isolated and opened along the greater curvature. The ulcerative lesion index and percentage inhibition of ulcer of each animal was calculated[14]. Blood samples were collected from the marginal tail vein of indomethacin- treated rats and serum alkaline phosphatase[15] and serum calcium[16] levels in the blood samples were estimated.

Ethanol-induced ulcer:

Animals were divided in to 6 groups of 6 animals each. Control group was treated with 1% CMC in water (5 ml/kg p.o). Test groups of rats received different extracts of *D.*

TABLE 1: VALUES CONSIDERED FOR CALCULATION OF ULCERATIVE LESION INDEX

Parameter	Score
Loss of normal morphology	1 point
Discolouration of mucosa	1 point
Mucosal edema	1 point
Haemorrhagic streaks	1 point
Petechial points (until 9)	2 points
Petechial points (more than 10)	3 points
Ulcer up to 1 mm	number of ulcers x 2 points
Ulcer greater than 1 mm	number of ulcers x 3 points
Perforated ulcers	number of ulcers x 4 points

viscosa leaves (375 mg/kg p.o). Standard group was given with ranitidine (40 mg/kg p.o). Ulceration was induced by oral administration of 1 ml of absolute ethanol per rat after 45 min of the treatment. The animals were sacrificed 6 h. following the administration of ethanol. Ulcerative lesion index and percentage inhibition of ulcers were evaluated[14].

Statistical analysis:

All the results are reported as Mean±SEM. Statistical significance was analyzed employing one-way ANOVA followed by Post-hoc Dunnett's test using Graph Pad Prism version 4.0. Values of P less than 0.05 were considered significant.

RESULTS

Preliminary phytochemical screening of *D. viscosa* revealed the presence of sterols, flavonoids, tannins, saponins, carbohydrates and alkaloids. The dose of the extracts determined by up and down staircase method was found to be 375 mg/kg body weight in mice. Preliminary investigations with ethanol extract of the leaves exhibited a significant ($P<0.05$) ULI in all the models and further bioactivity guided fraction studies showed the ULI in the order of EAE>ACE>EA>PE. In the group of animals in which ulcer was induced using aspirin plus pylorus-ligation, EAE showed significant activity in all the selected parameters (Table 2) with 56.8% inhibition of ulcers and a significant reduction in free acidity, total acidity and ULI ($P<0.05$). pH of the gastric contents in this group was found to be similar to that found with ranitidine (pH 4.0). Further ACE showed good antiulcer activity with 52.8% inhibition of ulcer. The extract significantly altered pH (3.8) and volume of gastric juice (1.13 ml), respectively, while a significant effect ($P<0.05$) on free acidity, total acidity and ULI was observed.

The results of indomethacin-induced gastric ulcer in rats are summarized in Table 3. The data suggests that all the extracts showed significant reduction ($P<0.05$) in ULI compared to control animals, but less reduction when compared to ranitidine-treated animals (14.5±0.89). Among tested extracts, EAE showed a good percent inhibition of ulcers (54.9%). All the extracts showed significant activity ($P<0.05$) in ethanol-induced ulcer model. PE treated animals showed activity at $P<0.05$.

Biochemical estimations in indomethacin-induced ulcers were carried out and the details are depicted in Table 4. All the extracts showed significant decrease ($P<0.05$) in serum alkaline phosphatase concentration with EAE showing maximum reduction in enzyme concentration 25.1±1.5 (U/l) while control showed 48.5±0.5 (U/l). Further, a significant increase in serum calcium level was observed ($P<0.05$) with EAE treated animals (15.6±0.6 U/l).

DISCUSSION

The antiulcerogenic activity proposed for the extracts of leaves of *D. viscosa* was in line with the observations made. Gastric ulceration is related with integrity of mucosal layer and is mainly dependent on the arachidonic acid metabolism. The ulcerogens like NSAID's induce the effect by interfering with cyclooxygenase pathway. Alcohol can affect the mucosal blood flow, platelet thrombi[17] and can increase the production of free radicals leading to increased lipid peroxidation, which can damage the cell and cell membranes[18]. While pylorus-ligation increases the presence of acid and pepsin in the stomach[17].

TABLE 2: EFFECT OF *DODONAEA VISCOSA* ON ASPIRIN PLUS PYLORUS-LIGATED GASTRIC ULCERS

Treatment P.O.	Dose	Gastric Juice (ml)	$_{P}H$	Free Acidity (mEq/l)	Total Acidity (mEq/l)	ULI	% Inhibition of ulcer
Control	5 ml/kg	1.68±0.09	2.1	32.2±2.85	48.8±1.83	39.8±1.58	-
Ranitidine	40 mg/kg	0.97±0.06	4.4	12.5±1.40	19.2±0.65	15.5±1.26	61.1
EE	375 mg/kg	1.27±0.08	3.4	18.5±1.50	22.2±0.60	23.3±1.75	41.5
PE	375 mg/kg	1.43±0.12	2.9	24.3±1.58	40.3±0.95	27.7±2.23	30.4
EAE	375 mg/kg	1.03±0.13	4.0	13.7±1.20	20.5±0.92	17.2±1.62	56.8
ACE	375 mg/kg	1.13±0.07	3.8	15.0±1.41	21.7±0.90	18.8±1.64	52.8

$P<0.05$ vs. control (one-way ANOVA followed by Post-hoc Dunnett's test). Each value is a mean±SEM (n=6). ULI stands for ulcer index.

TABLE 3: EFFECT OF *DODONAEA VISCOSA* ON THE ACUTE ULCERS INDUCED BY INDOMETHACIN AND ETHANOL IN RATS

Treatment p.o.	Dose	Indomethacin (30 mg/kg, p.o)		Ethanol (1 ml/rat)	
		ULI	% Inhibition of ulcer	ULI	% Inhibition of ulcer
Control	5 ml/kg	41.7± 2.44	-	32.7±1.36	-
Ranitidine	40 mg/kg	14.5± 0.89	65.2	18.3±0.56	44.0
EE	375 mg/kg	22.0± 2.63	47.2	22.5±2.96	31.2
PE	375 mg/kg	24.2± 2.59	42.0	25.3±1.86	22.6
EAE	375 mg/kg	18.8± 1.85	55.0	19.8±1.25	39.4
ACE	375 mg/kg	20.2± 1.22	51.6	20.7±1.48	36.7

P<0.05 vs. control (one-way ANOVA followed by Post-hoc Dunnett's test). Each value is a mean±SEM. (n=6). ULI is ulcer index.

TABLE 4: EFFECT OF *DODONAEA VISCOSA* ON SERUM ALKALINE PHOSPHATASE AND SERUM CALCIUM IN INDOMETHACIN-INDUCED ULCER

Treatment p.o	Serum alkaline phosphatase (U/lit)	Serum calcium (U/lit)
Control	48.51±0.58	9.68±0.53
Ranitidine	24.23±1.19	16.18±0.62
EE	29.31±1.49	14.45±0.69
PE	35.73±2.24	12.67±0.70
EAE	25.12±1.55	15.68±0.63
ACE	27.35±1.38	15.15±0.59

P<0.05 vs. control (one-way ANOVA followed by Post-hoc Dunnett's test). Each value is mean±SEM. (n=6).

The extract of *D. viscosa* is reported to contain tannins, saponins, traces of alkaloids and flavanoids like quercetin[19]. Phytopharmacological studies of flavanoids have opened new vistas in ulcer research. Quercetin is reported to have free radical scavenging[3] and dose-dependent antiulcer activity. It is reported to inhibit the mucosal content of platelet aggregating factor (PAF), suggesting the protective role of these substances to be mediated by endogenous PAF[20]. Further, the antiulcer activity of flavanoids is attributed to their free radical scavenging effect and their ability to inhibit the cyclooxygenase responsible for synthesis of inflammatory prostaglandin. The pathophysiology of tissue necrosis has been attributed to the alkaline phosphatase activity. Biochemical investigations are reported with increased alkaline phosphatase activity in gastrointestinal ulceration, liver and bone disease[21]. Moreover calcium ions are reported to maintain the integrity of cell membrane and regulate the cell adhesion. The animals treated with extracts have exhibited an increase in serum calcium level and decrease in alkaline phosphatase activity. Thus it can be concluded that the antiulcerogenic activity of the plant may be due to the cytoprotective and healing property of flavanoids. Determination of exact mode of action is subject of our further research interest.

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