

## Antiulcer activity of *Dodonaea viscosa* leaf extract in aspirin induced albino rats

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### Abstract

*Dodonaea viscosa* is being used in Ayurvedic and Malay traditional medicine for the treatment of various diseases including gastric ulcer. Considering the above claims, the present work was undertaken to validate the antiulcer potential of the Ethanolic leaves extract of *Dodonaea viscosa* against in vivo aspirin induced gastric ulcer. The extract (500 mg/body weight) significantly ( $P < 0.001$ ) reduced the ulcer index, volume, free and total acidities of gastric juice while at the same time increased the pH and normalized the pepsin action. *Dodonaea viscosa* also enhanced the level of reduced glutathione and reduced the MDA level. In conclusion, the present study provides preliminary data on the antiulcer potential of *Dodonaea viscosa* leaves and support the traditional uses of the plant for the treatment of Gastric ulcer.

### INTRODUCTION

An ulcer is defined as disruption of the mucosal integrity of the stomach and or duodenum leading to a local defects or excavation due to active inflammation. Ulcers occur within the stomach and /or duodenum and are often chronic in nature. Peptic disorders are very common in India, with 4 million individuals (new cases and recurrences) affected per year. Life time prevalence of PUD in the India is approximately 12% in men and 10% in women. Moreover an estimated 15,000 deaths per year occur as a consequence of complicated PUD. The financial impact of these common disorders has been substantial, with an estimated burden on health care costs of > \$15 billion per year in India<sup>[1]</sup>.

Peptic ulcer is a term used to refer to a group of ulcerative disorders of the upper gastrointestinal tract involving principally the most proximal portion of the duodenum and the stomach, which have in common the participation of acid and pepsin in their pathogenesis. The major forms of common peptic ulcer are duodenal ulcer and gastric ulcer, both of which are chronic diseases.

Although there are many drugs used for the treatment of gastric ulcers, most of these produce several adverse reactions. Several plants and herbs have also been employed in the treatment of gastrointestinal disorders, including gastric ulcers. On the basis of these, we analysed the antiulcer activity of *Dodonaea viscosa* on aspirin induced toxicity in rats.

*Dodonaea viscosa* Linn.-is a shrub belonging to family Sapindaceae. The plant has many medicinal properties and has been used by native peoples from all regions where it is found. It is a traditional medicine worldwide, administered orally or as poultice to treat a great variety of ailments<sup>[2]</sup>. Leaves are used to relieve itching, fever, swelling, aches and can be used as a antispasmodic agent<sup>[3]</sup>, leaves and roots as a painkiller to soothe toothaches and headaches<sup>[4]</sup>. In India, seeds are used as fish poison<sup>[5]</sup>.

Previous chemical studies on this species resulted in the isolation and characterization of several flavonoids<sup>[6]</sup>, diterpenoid acids<sup>[7,8]</sup>, some biologically active saponins<sup>[9,5]</sup> and plant acids<sup>[10]</sup>, a

novel p-coumarine acid esters<sup>[11]</sup>, essential oils<sup>[12]</sup>, sterols<sup>[13,7]</sup> and tannins<sup>[10]</sup> from the arial parts of *Dodonaea viscosa* and saponin esters from the seeds of *Dodonaea viscosa*<sup>[5]</sup>.

### MATERIALS AND METHODS

#### Animals

Male albino rats of wistar strain approximately weighing 100-125g were used in this study. They were healthy animals, purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature  $27 \pm 2^\circ \text{C}$  and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The animal feed composition is crude protein (22.3%), crude oil (4.01%), crude fibre (4.02%), Ash (8.02%) and sand silican (1.02%).

#### Chemicals

Thiobarbituric acid (TBA), 2,4, Dinitrophenylhydrazine (DNPH), reduced glutathione, aspirin were purchased from Sigma Chemical Company, Mumbai. All other chemicals and reagents used in this study were of analytical grade with high purity and obtained from Glaxo Laboratories and Sisco Research Laboratories, Mumbai, India.

#### Collection and preparation of plant extract

The leaves of *Dodonaea viscosa* was purchased from local traditional medical shop at Thanjavur. Leaves were cut into small pieces and shade dried at room temperature and was soaked with ethanol (50%) for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The alcoholic extract was dissolved in distilled water just before oral administration.

#### Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the

**Table 1.** Details of forward and reverse primer used for PCR amplification

Phytochemical constituents	Result of qualitative tests
Alkaloids	+
Triterpenoids	+
Saponins	—
Tannins	+
Flavonoids	+
Phenolic compound	+
Steroids	+
Phlobatannins	—
Carbohydrates	+
Cardiac glycosides	+
Anthroquinones	—

(+) positive result, (-) negative result

constituents<sup>[14,15]</sup>.

### Aspirin induced ulcer

The gastric ulcers were induced in rats by administering aspirin (600mg/kg body weight) daily for three consecutive days<sup>[16]</sup>.

### Experimental design

Body weight of animals were recorded and they were divided into four groups of six animals each as follows.

Group I: Normal animal received with standard fed and water to allow *ad libitum*.

Group II: Animals received oral administration of aspirin (600mg/kg body weight) daily for three consecutive days.

Group III: Ulcer induced rats treated with *Dodonaea viscosa* (500mg/kg bwt) for three consecutive days.

Group IV: Ulcer induced rats treated with standard drug Ranitidine (150mg/kg bwt) for three consecutive days.

After completed the experimental regimen, the rats were sacrificed and the stomach was removed. The gastric content was collected and centrifuged for 5 min at 2000 x g and the supernatant was separated. The volume<sup>[17]</sup>, pH, and total acidity and free acidity of gastric fluid<sup>[18]</sup>, pepsin activity<sup>[19]</sup>, ulcer index and % inhibition of ulcer index, were determined. Serum was also separated for the determination of MDA<sup>[20]</sup> and GSH<sup>[21]</sup> level.

### Statistical Analysis

The results were represented as mean  $\pm$  SEM. Data was statistically analyzed using student “t” test. P.values set as lower than 0.001 was considered as statistically significant.

### RESULTS

In this study, The phytochemical compounds were screened and analyzed the antiulcer activity of *Dodonaea viscosa* against aspirin induced ulcer. The results indicates the presence of tannins, flavonoids, steroids, cardiac glycosides, terpenoids, alkaloids, triterpenoids, carbohydrates, phenol and absence of saponin, phlobatannins, and anthroquinones (Table 1).

Table 2 shows the total gastric volume, P<sup>H</sup>, activity of pepsin, free and total acidity, ulcer index of control and experimental groups. In aspirin induced ulcerative rats (group-II)

**Table 2.** Effect of *Dodonaea viscosa* on MDA and GSH in experimental rats

Parameters	Serum		Stomach	
	MDA( $\mu$ m/L)	GSH(mg/dl)	MDA((nmole/mg)	GSH(( $\mu$ g/mg)
Group I	1.8 $\pm$ 0.86	8.13 $\pm$ 0.50	1.6 $\pm$ 1.24	8.71 $\pm$ 16.21
Group II	4.28 $\pm$ 1.20	4.01 $\pm$ 1.81	7.06 $\pm$ 1.24	4.61 $\pm$ 8.24
Group III	1.14 $\pm$ 1.18*	8.38 $\pm$ 1.22*	1.20 $\pm$ 2.37*	8.24 $\pm$ 2.48*
Group IV	1.9 $\pm$ 0.90*	8.25 $\pm$ 0.70*	8.24 $\pm$ 2.48*	8.75 $\pm$ 17.31*

Results are mean  $\pm$  SEM (n=6)

\*P<0.001 was considered statistically significant when compared with aspirin treated group

**Table 2.** Effect of *Dodonaea viscosa* on pH, Ulcer Index, Volume, Total and Free acidity, and % inhibition of ulcer index experimental rats

Parameters	Volume (ml)	pH	Pepsin	Total acidity (mEq/L)	Free acidity (mEq/L)	Ulcer index (Score)	% inhibition
Group I	4.08±0.81	1.70±0.14	33±2.68	52.09±0.64	38.21±0.57	1.64±0.11	00.00
Group II	2.03±0.16	4.70±0.14	45±2.65	85.12±0.17	52.18±0.84	3.10±0.21	00.00
Group III	4.06±0.16*	1.17±0.83*	33±2.68*	51.06±0.87*	38.72±0.64*	1.6±0.22*	43.54
Group IV	4.8±0.23*	1.71±0.14*	32±2.65*	52.21±0.71*	39.23±0.59*	1.70±0.15*	45.16

Results are mean ± SEM (n=6)

\*P&lt;0.001 was considered statistically significant when compared with aspirin treated group.

increased the gastric volume, total and free acidity compared to control group. At the same time the activity of pepsin was enhanced due to decreased  $P^H$ . Administration of aspirin produced ulcer index when compared to control. All the above parameters were reversed when the rats treated with *Dodonaea viscosa* (group III) and standard drug ranitidine treated rats (group-IV) when compared to ulcerative rats ( $P<0.001$ ).

The table 3 showed the level of MDA and GSH in serum and tissue of control and experiment rats. In aspirin treated group, there was an increase in the MDA level and decrease in GSH level compared to group-I. Administration of *Dodonaea viscosa* brought about a significant reduction ( $p<0.001$ ) in MDA level and an increase in the level of reduced glutathione when compared to group-II. Standard drug was also found to produce similar result as that of group-III.

## DISCUSSION

Ulcer is a major health hazard both in terms of morbidity and mortality. The pathophysiology of gastric ulcer has generally focused on imbalance between aggressive and protective factors in the stomach, such as acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins and epidermal growth factors<sup>[22]</sup>. The reactive oxygen species especially hydroxyl radical plays a major role in causing oxidative damage of mucosa in all types of ulcers<sup>[23]</sup>.

Preliminary phytochemical screening of *Dodonaea viscosa* revealed the presence of steroids, flavanoids, tannins, saponins, carbohydrates and alkaloids (Table 1). Phytopharmacological studies of flavanoids have opened new vistas in ulcer research. Quercetin is reported to have free radical scavenging<sup>[24]</sup> and does dependent antiulcer activity. It is reported to be mediated by endogenous PAF<sup>[25]</sup>.

In the present study, increase in the volume of gastric juice and their free and total acidity and decreased  $P^H$  of gastric juice were observed in aspirin treated ulcerogenic rats as compared to normal rats. The severity in terms of volume, total acidity and free acidity showed marked decrease in *Dodonaea viscosa* treated rats when compared to those in ulcerogenic rats. The restored  $P^H$  of gastric juice was also observed in *Dodonaea*

*viscosa* treatment rats as compared with ulcerogenic rats. The increase in volume in the ulcerogenic rats is undoubtedly due to increased production of hydrochloric acid as is evident from the total acidity and decrease  $P^H$  value of gastric juice. The decrease in volume of the gastric juice and concomitant decrease in the acidity and restored  $P^H$ , thus providing the antiulcer activity of *Dodonaea viscosa*.

Pepsin is most active in acidic environments between 37°C and 42°C at  $P^H$ , 1.5 to 2<sup>[26]</sup>. Pepsin exhibits maximal activity at pH 2.0 and is inactive at pH 6.5 and above, however pepsin is not fully denatured or irreversibly inactivated until  $P^H$  8.0. Therefore pepsin in solution of upto  $P^H$  8.0 can be reactivated upon re-acidification. Pepsin may be inhibited by high  $P^H$ <sup>[27]</sup>. The present study revealed that the decreased activity of pepsin due to increase the of g  $P^H$  gastric juice. Administration of *Dodonaea viscosa* to aspirin induced rats restored the activity of pepsin due to restore the optimum  $P^H$ .

Present study demonstrated the potential of *Dodonaea viscosa* to significantly reduced gastric ulceration as indicated by the reduction in ulcer (%inhibition of ulcer index) in the aspirin-induced assays. Chronic use of anti-inflammatory drugs and stress are some of the main causes of gastric ulcers<sup>[28]</sup>, and since *Dodonaea viscosa* exerted significant antiulcer activity under experimental models that mimic those conditions.

Results suggested that *Dodonaea viscosa* posses anti-secretory potency as well as acid neutralizing effect. Furthermore, based on findings<sup>[29]</sup>, the anti-secretory effect is suggested to be one of the mechanism through which the extract of plant was able to protect the stomach mucosa from aspirin induced damage. It was well known that inhibition of prostaglandin synthesis, which is essential for mucosal integrity and regeneration, will trigger and mucosal lining damage. It is also believed that the extract exert its antiulcer by increasing the synthesis of endogenous prostaglandins, which in turn promote mucus secretion and enhance the mucosal barrier against the actions of various damaging agents<sup>[30]</sup>.

Oxygen derived free radicals cause lipid peroxidation, which leads to membrane fluidity, resulting in reduced membrane integrity of surface epithelial cells, thereby causing gastric

ulcers<sup>[31]</sup>. It has been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration and scavenging these free radicals can play an appreciable role in healing gastric ulcers<sup>[32]</sup>. Our study revealed that the aspirin induced ulcer severity and lipid peroxidation were aggravated, which is also indicated by increased MDA content under aspirin induction as compared to control rat, whereas inhibition of lipid peroxidation in *Dodonaea viscosa* administration indicates the anti lipid peroxidative effect which could have prevented lipid peroxidation mediated ulcerative damage to gastric mucosa. Hence, it can be suggested *Dodonaea viscosa* provides antiulcer activity in rats. It may act as gastric cytoprotective agent by modulating scavenging of free radicals.

Reactive oxygen species are involved in the pathogenesis of aspirin induced ulcer. Results of the present study indicates alterations in the antioxidant status after aspirin induced ulcers. Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation<sup>[33]</sup>. Administration of *Dodonaea viscosa* resulted in a significant increase in reduced glutathione levels as compared to ulcerated rats which suggested its efficacy in preventing free radical induced damage.

## CONCLUSION

It can be concluded that *Dodonaea viscosa* possess anti ulcerogenic principles which inhibit gastric acid, pepsin and/or stimulate mucous secretions. However, further investigations are required to isolate the active principles of these plant drugs and to elucidate their mechanism of antiulcerogenic activity.

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