

Anti Ulcer Effect of *Avicennia officinalis* Leaves in Albino Rats

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Abstract: The plant extract of *Avicennia officinalis* herbal preparation are traditionally used in the treatment of various diseases, as anti tumor, anti microbial, wound healing agents and anti oxidant activity. In the present study the gastro protective effect of *A. officinalis* leaves in a model of NSAID – induced ulcer in rat was analysed. The lyophilized extract was given by oral gavages (125 and 62.5mg/kg) three times at 12 h intervals before administering Diclofenac sodium at 100mg/kg. Pretreatment with the extract significantly decreased the ulcerated area. The volume and acidity of the gastric juice decreased in the pretreated rats. The plant extract elevated the gastric juice in untreated rats, showing near normal levels in the pretreated rats. In conclusion, *A. officinalis* was able to decrease the acidity and increase the mucosal defense in the gastric areas, there by justifying its use as an antiulcerogenic agent.

Key words: NSAID-Diclofenac • Gastric Ulcer • Gastric juice • Ranitidine • Hot water extract • Cold water extract

INTRODUCTION

A peptic ulcer is a sore in the lining of stomach or duodenum the first part of small intestine. If peptic ulcers are found in the stomach, they are called gastric ulcers. If they are found in the duodenum, they are called duodenum ulcers [1]. Gastric ulceration has been attributed to various causes such as stress, hormones, drugs, alcohol, smoking and ingestion of certain foods [2]. Recently, *Helicobacter pylorus* has been implicated in the antral gastritis, peptic ulcer, gastric malignancy and the non-ulcer dyspepsia [3]. With the increasing use of non steroidal anti inflammatory drugs (NSAIDs) and the possibility for co-infection with *Helicobacter pylori*, the prevalence of gastric ulcers is estimated to be as high as 4%, with a 10% lifetime risk [4]. Regardless of the general appearance of a gastric ulcer at the time of endoscope, histological evaluation is generally considered to be warranted to rule out the possibility of malignancy. The determination of risk factors to stratify those ulcers that pose the greatest risk for malignant transformation would provide important clinical information and permit a more cost-effective strategy for performing surveillance upper endoscope [5]. The treatment of peptic ulcers with plant products and herbs as used in folk medicine was reported [6 - 10]. The protection of induced gastric ulcer

in laboratory animals using medicinal plants was reported [11]. *A. officinalis* is a commonly available as white mangrove plant in almost all the coastal states of India. It is a folklore medicinal plant used mainly against rheumatism, paralysis, asthma and snake-bites, skin disease, ulcer. A decoction of the plant with sugar candy and cumin is used in dyspepsia with acid eructation's [12, 13]. The fruits are plastered onto tumors in India [14]. Indian mangrove is a folk remedy for boils and tumors [15, 16] suggest that the roots are aphrodisiac. Unripe seeds are poulticed onto abscesses, boils and smallpox sores. Indochinese uses the bark for skin afflictions, especially scabies. A resinous substance exuded from the bark acts as a contraceptive and apparently can be taken all year long without ill effects [17]. Philippines use the seed for ulcers, the resin for snakebite.

The present study was carried out to provide scientific validation for anti ulcer activity of *A. officinalis*.

MATERIALS AND METHODS

The leaves of *A. officinalis* were collected from Parangipettai (South east coast of India), Tamil Nadu and India. The leaves were cut into small pieces and shade dried for the experimental studies. Dried leaves were powdered and then extracted in cold water extract by

keeping the leaf powder in cold water (1:50 W/v) for 48 hours and then it was filtered with the help of Whatmann paper No. 1 filter paper and filtrate was lyophilized. The hot water extract was prepared by boiling leaf powder in distilled water (1:10 w/v) at 90°C for one hour. Then it was filtered by whatman No.1 filter paper and the filtrate was lyophilized. Both the lyophilized samples were stored at dry place.

Animal Model: Healthy Female albino Wister rat of 110-200 g were used throughout the study. They were maintained in a controlled environmental condition of temperature and humidity. All animals were fed with standard pellet diet and water *ad libitum*. Animal experiments were conducted according to the guidelines of institutional animal ethical committee. All the animals were grouped into seven groups. Each group had 3 animals. Group-1 was negative control (without any treatment fed with normal water). Group-2 was positive control treated with NSAID. Group - 3 included pretreated animals with low dose of cold water extract and then treated with NSAID. Group - 4 had pretreated animals with high dose of cold water extract and then treated with NSAID. Group-5 contained pretreated animals with low dose of hot water extract and then treated with NSAID. Group-6 was with pretreated animals with high dose of hot water extract and then treated with NSAID. Group-7 was standard control pretreated with Ranitidine and then treated with NSAID following the dose and mode of administration [18].

All the animals were by oral gavages with the help of feeding tube. The doses determined as low dose at the rate of 62.5 mg/kg of the body weight and high following dose at the rate of 125 mg/kg of the body weight for both the sample. Then non steroidal anti-inflammatory drug, diclofenac sodium was used as the ulcerogenic agent at the dose of 100 mg/kg of body weight. Standard anti ulcer drug ranitidine used at the rate of 20 mg/kg of body weight. *In vivo* Protocol [18] All the groups of animals were kept overnight fasting, fed only with the tap water. The animals of group III, IV, V and VI were treated with the sample extract at different doses. This treatment was given thrice at the 12 hours interval. Animals of the group VII were treated with ranitidine simultaneously. After one hour of last administration of sample extract the NSAID was given by oral gavages to the group II to group VII animals. After 6 hours of NSAID administration, the animals were sacrificed by cervical dislocation.

The animals were dissected and the stomach was taken out. Finally the ulcers were observed macroscopically. The observation was made for any bulging or inflammation in the stomach. The stomachs were opened along the greater curvature and the mucosa was exposing for evaluation. The ulcer scores (US) were calculated as the arithmetic mean for each treatment. Scarifying the rat, stomach was removed and opened along the greater curvature and washed it slowly under top water, but it on the glass slide and observed naked eye.

Collection of Gastric Juice: The stomach was carefully keeping the esophagus closed opened along the greater curvature and the gastric contents were removed the gastric contents were collected in plain tubes and centrifuged at 3000 rpm for 5 min the volume of the supernatant was expressed as ml/100 gm body weight the mucosa was flushed with saline and observed for gastric lesions using the macroscopic structure.

RESULTS

Diclofenac sodium caused damage on the glandular mucosa (3.35±0.25). In contrast to it, the pretreatment with hot water and cold water extract of *A. officinalis* leaves at doses at 62.5 and 125mg/kg of the body weight decreased the ulcerated area to 2.10±0.20 and 2.35±0.15, respectively, which was comparable to the effect exerted by Ranitidine 1.45±0.35. Administration of leaves extract in *A. officinalis*, Decreased of gastric volume in comparison with rats treated with ranitidine. The gastric volume gets decreased simultaneously the gastric acidity also decreased significantly.

DISCUSSION

The present study showed that pretreatment with the leaf extract (both hot water and cold water) of *A. officinalis* caused a beneficial effect on NSAID-induced gastric ulcer in rats as evidenced by the reduction in the ulcer score. The defense mechanism of the gastrointestinal mucosa against aggressive factors such as HCl, bile acid and NSAIDs, mainly consisting of functional, humoral and neuronal factors, while prostaglandins and nitric oxide act as humoral factors. In recent experiments, it has been found that heat shock proteins (HSPs), specifically HSP70 and HSP47 are involved in the gastric production. The HSC 70 (a constitutive form of HSP 70) is co precipitated with COX-1 and the neuronal form of nitric

Tabel 1: Ulcer Score at different concentration of Plant extract

Group No.	Group Name	Concentration (mg/kg of body weight)	Ulcer Score (Mean \pm SD)	Volume of gastric juice (ml)	pH of gastric juice
I	Negative control	-	-	-	-
II	Positive control	100.0	3.35 \pm 0.25	1.60	2.3
III	Low dose (Cold extract)	62.5	2.45 \pm 0.10	1.05	3.5
IV	High dose (Cold extract)	125.0	2.35 \pm 0.15	0.95	3.2
V	Low dose (Hot extract)	62.5	2.35 \pm 0.20	0.85	4.1
VI	High dose (Hot extract)	125.0	2.10 \pm 0.20	1.20	4.5
VII	Standard control	20.0	1.45 \pm 0.35	1.15	5.3

oxide synthase after treatment with mild irritants (20% ethanol). A positive relationship between enhanced interaction of HSC70 with either cyclooxygenase -1 or nitric oxide synthase and mucosal defence mechanisms and ulcer healing, most probably through protecting key enzymes related to cytoprotection [19].

Pretreatment with the mangrove *A. officinalis* increased PGE₂ production in spite of NSAID-induced depletion, at the considerably high dose of diclofenac used (100 mg/kg) [18]. These results may be attributed to the poly phenolic compounds found in the mangrove plants. Phenols stimulate PGE-2 formation based on their action as co substrates for the peroxidase reaction. Oral dose at 500mg/kg body weight gave the highest level of gastric production. Mucus content increased, accompanied by a proportional increase in proteins. The genus *Indigofera* (Fabaceae) is used in folk medicine to treat gastro intestinal pain, however the anti-ulcerogenic properties the plant by the oral administration of MeOH extract did not produce any single of acute toxicity.

The anti-ulcerogenic activity was proved whereas in the present study *A. officinalis* leaf extracted in cold and hot water. These results indicate the *A. officinalis* has anti-ulcer compounds. Which have anti secretory and cytoprotective effects that may be related to the presence of flavonoids detected by phytochemical analysis [20]. On the other hand, tannins may prevent ulcer development due to their protein precipitating and vasoconstricting effects [21]. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants [22]. This preparatory to bind with proteins also explains the fact that polyphenols inhibit enzymes tested *In vitro* [23]. The major active principles of the red mangrove are polyphenols, represented in their majority by polymeric tannins (80%) and hydrolysable tannins (20%) and catechin. These substances are characterized by their polyphenolic nature, with cytoprotective properties [24] which are associated to antiulcerogenic activity in other plants [25, 26]. The *Rhizophora mangle* also showed the Mucus content

was increased and it was accompanied by a proportional increase in proteins. The group, which received cimetidine, showed no effect on the mucus secretion induced in this experimental model [27, 28].

Since *Excoecaria agallocha* also has shown same but less anti ulcer activity in comparison to *Rhizophora mangle* [29]. *A. officinalis* showed good anti ulcer activity. Probably due to the presence of tannins or polyphenolic compounds in fair quantity.

Based on the comparison with the *R. mangle* and *E. agallocha*, it is expected that the wound healing capacity of *A. officinalis* during ulcer is due to several mechanisms, such as coating the wound, forming complexes with proteins of cell wall, chelating free radicals and reactive oxygen species, stimulating the contraction of the wound and increasing the formation of new capillaries and fibroblasts [19].

These results indicate that the high dose and hot water extract has anti secretory activity. The present study showed that pretreatment with the leaf extract (both hot water and cold water) of *A. officinalis* caused a beneficial effect on NSAID-induced gastric ulcer in rats as evidenced by the reduction in the ulcer score.

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