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(Research article)

ANTI - ULCER ACTIVITY OF *SYZYGIUM ALTERNIFOLIUM* AGAINST ETHANOL AND NSAID INDUCED ULCER IN RATS

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ABSTRACT

Syzygium alternifolium a member of the Myrtaceae family, is used in fruit is used for curing stomach ache, ulcers and management of rheumatic pains; seeds as anti-diabetic agents; leaves to treat dry cough and dysentery; and stem bark as antiseptic. The purpose of the present study is to investigate the acute oral toxicity and anti-ulcer profile of the Ethanol Extract of *Syzygium alternifolium* (ESA) extract in albino rats. No toxicity of extract up to 2000mg/kg body weight orally as per OECD guidelines No.423. ESA at the doses of 250 and 500 mg/kg body weight orally was administered to evaluate anti-ulcer activity by using Ethanol and indomethacin, induced gastric ulcer models in Albino rats. Ethanol Extract of *Syzygium alternifolium dose* dependent inhibition in ethanol induced gastric lesions, causing 68.04% protection at 500 mg/kg, and 54.74% protection at 250 mg/kg, ESA dose dependent inhibition in indomethacin induced gastric lesions, causing 65.14% protection at 500 mg/kg and 54.13% protection at 250 mg/kg, All the results are found to be statistically significant ($p \leq 0.05$). Hence we suggest that Ethanol Extract of the fruit of *Syzygium alternifolium could* decrease the acidity and to increase the mucosal defense in the gastric areas, thereby justifying its use as an anti ulcerogenic agent.

Keywords: *Syzygium alternifolium,* Anti-ulcer activity, Ethanol, Indomethacin,

INTRODUCTION

Peptic ulcer disease is a very common global health problem today. Peptic ulcer is a lesion of the gastric or duodenal mucosa. Duodenal ulcers are more common in adult males. Gastric ulcers occur commonly at old age and in lower socio-economic class of individuals. Although the exact cause of ulceration is not known, hydrochloric acid and pepsin are responsible for maintaining the lesion once it is produced. Peptic ulceration occurs only in areas, which are bathed by the acidic gastric juice. Therefore, the term peptic ulcer refers to ulceration of the areas which might be acted upon by acid peptic juice namely the stomach and the first portion of the duodenum. Peptic ulcers also occur at the lower end of the esophagus, on the jejunal side of a gastroenterostomy and in Meckel's diverticulum.

The current therapeutic approach to gastric ulceration is to achieve inhibition of gastric secretion, promotion of gastric protection, blockage of apoptosis, and epithelial cell proliferation for effective healing3. In recent years, focus on plant research has increased worldwide, and several studies had shown immense potential of medicinal plants. Herbal medicines derived from plant extract, are increasingly being recognized in treating various clinical diseases, with relatively little knowledge of their modes of action [1-4].

Syzygium alternifolium (Wt.) (Family: Myrtaceae) is a tree or shrubs, evergreen, usually with essential oils-containing cavities in foliage, branchlets, and flowers. Stipules absent or small and caducous. Leaves opposite, occasionally alternate, occasionally ternate or pseudo-whorled; leaf blade with secondary

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veins pinnate or basal, often with intramarginal veins near margin, margin usually entire. Inflorescences axillary or terminal, cymose but variously arranged, one to many flowered. Flowers bisexual, sometimes polygamous, actinomorphic. Hypanthium usually adnate to ovary and prolonged above it. Calyx lobes (3 or) 4 or 5 or more, distinct or connate into a calyptra. Petals 4 or 5, sometimes absent, distinct or connate into a calyptra, sometimes coherent and pseudo calyptrate. Stamens usually numerous, in 1 to several whorls; filaments distinct or connate into 5 bundles opposite petals; anthers 2-celled, dorsifixed or basifixed, dehiscing longitudinally or rarely terminally; connectives usually terminating in 1 or more apical glands. Ovary inferior, semi-inferior, or very rarely superior, carpels 2 to more, locules 1 to many, pseudoseptum sometimes present, placentation usually axile but occasionally parietal; ovules one to be several per locule. Style single; stigma single. Fruit a capsule, berry, drupaceous berry, or drupe, one to be many-seeded. Seeds without endosperm or endosperm sparse and thin; testa cartilaginous or thinly membranous, sometimes absent; embryo straight or curved. About 130 genera and 4500-5000 species: Mediterranean region, sub-Saharan Africa, Madagascar, tropical and temperate Asia, Australia, Pacific islands, tropical and South America; 10 genera (five introduced) and 121 species (50 endemic, 32 introduced treated here) in China. Many Myrtaceae are cultivated garden ornamentals, street trees, or plantation trees. Some members of the tribe Syzygieae is grown as fruit crops. *Syzygium alternifolium* fruit is used for curing stomach ache, ulcers and management of rheumatic pains; seeds as anti-diabetic agents; leaves to treat dry cough and dysentery; and stem bark as antiseptic [5]. From the source of literature documentation and relevant traditional approaches on plant drugs, the present investigation was carried out to investigate the anti-ulcer profile of the Ethanol Extract of *Syzygium alternifolium* fruit (ESA) is being reported here.

MATERIALS AND METHODS

Plant material

The fruit of *Syzygium alternifolium* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. *Madhava Chetty, K.*, Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

Preparation of plant extract

The collected fruit was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with Ethanol (90%) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extract is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used in the experiment. The percentage yield of prepared extracts was around 10.5%w/w.

Animals Used

Albino rats (180–200 g) of either sex were maintained in a 12 h light/dark cycle at a constant temperature 25 ◦C with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of six rats. Moreover, the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute toxicity study

The acute toxicity of Ethanol extracts of *Syzygium alternifolium* fruit was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg 2000mg/kg doses. Hence, $1/10^{th}$ (250mg/kg) and $1/5^{th}$ (500mg/kg) of this dose was selected for further study [6].

ANTI-ULCER ACTIVITY

Ethanol induced gastric ulcer

Animals were randomly divided into four groups each of 6 rats. Group I treated with 4% v/v aqueous tween 80 (10 ml/kg p.o), Group II & III treated with Ethanol extract of *Syzygium alternifolium* (250and 500mg/kg p.o) respectively for 14 days and Group IV treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 14^{th} day, Gastric ulcers were induced with ethanol at a dose of 8ml/kg [7] administered to all groups by orally. The animals were anaesthetized 6 h with ether, and stomachs were incised along the greater curvature and the ulcer index for each rat was taken as the mean ulcer score.

Indomethacin induced gastric ulcer

Animals were divided into four groups each of six rats. Group I treated with 4% v/v aqueous tween 80 (10 ml/kg p.o). Group II & III treated with Ethanol extract of *Syzygium alternifolium* (250and 500mg/kg p.o) respectively for 14 days and Group IV treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the 14^{th} day, gastric ulcers were induced with indomethacin (40 mg/kg p.o) administered to all groups after fasting for 24 h. The animals were

sacrificed 4 h after treatment with the ulcerogenic agent [8] to assess the anti-ulcer activity and ulcer index were examined on the dissected stomachs as described below.

Measurement of ulcer index

The stomachs were excised and were examined for hemorrhagic lesions in the glandular mucosa. Immediately, after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature, and the mucosa was rinsed with cold normal saline to remove the blood contaminants, if any. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%*I*) was calculated as described by Nguelefack et al. (2005) [9] using the following formula:

$$\% I = \frac{(USc - USt)}{USc} \times 100$$

Where USc = ulcer surface area in control and
USt = ulcer surface area in treated animals.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences between the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

RESULTS

Acute toxicity study

Acute toxicity study in which the animals treated with the Ethanol Extract of *Syzygium alternifolium* at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Effect of Ethanol Extract of Syzygium alternifolium on gastric ulcer induced by Ethanol

The Ethanol Extract of *Syzygium alternifolium* showed the significant anti-ulcer effect against ulcers induced by Ethanol in a dose-dependent manner. In ethanol induced ulcer model, Ethanol Extract of *Syzygium alternifolium* at a dose of 250 and 500 mg/kg body weight showed the protective effect of 54.74 and 68.04%, respectively, where as Omeprazole showed the protection index of 80.60% at a dose of 20 mg/kg body weight (Table -1).

Effect of Ethanol Extract of Syzygium alternifolium on gastric ulcer induced by Indomethacin

The Ethanol Extract of *Syzygium alternifolium* showed the significant anti-ulcer effect against ulcers induced by *Indomethacin* in a dose-dependent manner. In Indomethacin induced ulcer model, Ethanol Extract of *Syzygium alternifolium* at a dose of 250 and 500 mg/kg body weight showed the protective effect of 54.13 and 65.14%, respectively, where as Omeprazole showed the protection index of 77.02% at a dose of 20 mg/kg body weight (Table -2).

DISCUSSION & CONCLUSION

The results from this study show that the Ethanol extracts from the fruit of *Syzygium alternifolium* exert protective effects against ethanol, indomethacin, pylorus ligation and cold restraint stress-induced gastric mucosal damage. The anti-ulcer effect of *Syzygium alternifolium* was tested against gastric lesions induced by ethanol, the experimental model related to lesion pathogenesis with production of reactive oxygen species. Reactive oxygen species are involved in the pathogenesis of ethanol-induced gastric mucosal injury in vivo [10-12]. *Syzygium alternifolium* prevented the mucosal lesions induced by ethanol. Results in the present study also indicate similar alterations in the anti-oxidant status after ethanol induced ulcers. The gastric mucosal protection against ethanol can be mediated through a number of mechanisms that include enhancement of the gastric mucosal defense through the increase in mucus and/or bicarbonate production, reducing the volume of gastric acid secretion or by simply neutralizing the gastric acidity [13].

ESA may either reduce the gastric acid secretion or enhance the barrier defense of the mucosal wall. ESA dose dependent inhibition in ethanol induced gastric lesions (Table -1). Histopathological studies suggest that the ethanol damage to the gastrointestinal mucosa starts with microvascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting [14].

Their anti-ulcerogenic potency was tested against indomethacin-induced ulcer. Indomethacin is a cyclooxygenase inhibitor which suppresses gastroduodenal bicarbonate secretion, reduces endogeneous prostaglandin biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow in animals [15]. It is also well known that prostaglandins synthetized in large quantities by the gastrointestinal mucosa can prevent experimentally induced ulcers by ulcerogens. Thus, when the ulcers lesions are induced by indomethacin, the cytoprotective effect of the anti-ulcer agent can be mediated through endogeneous prostaglandins [16]. The results obtained show that the mean ulcer index was significantly reduced in the Ethanol extracts

from the fruitof *Syzygium alternifolium* treated groups, compared to their respective controls. *Syzygium alternifolium* extracts may stimulate the secretion of prostaglandins or possess prostaglandins like-substances (Table -2).
The Ethanol extracts of *Syzygium alternifolium* at a dose of 500mg/kg showed similar activity to that of omeprazole (a proton pump inhibitor, which is used to heal stomach and duodenal ulcers). The gastro protective effect of omeprazole is mediated through the block of acid secretion by inactivation of H+/K+-ATPase [17,18]. This study reveals that the aqueous and methanol extracts from the fruit of *Syzygium alternifolium* are potent inhibitors of gastric mucosal lesions caused by ethanol, indomethacin, pylorus ligation and cold-restraint stress in rats.

Further, our results fortify the ethano pharmacological importance of ESA as an anti-ulcer agent. Etiology of ulcers produced in different ulcer models is diverse. Since ESA has been found effective in various models depicting its anti-ulcerogenic activity. ESA and its active constituents may emerge as the more effective therapeutic agent to counter gastric ulcer incidence. However, more experimentation, detailed phytochemical and experimental analysis are required for a definitive conclusion.

Table 1: Effect of Ethanol Extract of Syzygium alternifolium (ESA) in ethanol (8 ml/kg) induced gastric ulcer in rats

Group	Design of Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Control (4% v/v aqueous tween 80, 10 ml/kg b.w) p.o	20.46 ± 1.14	---
II	ESA (250mg/kg b.w) p.o	9.26 ± 0.18*	54.74
III	ESA (500mg/kg b.w) p.o	6.54 ± 0.17**	68.04
IV	Omeprazole (20mg/kg b.w) p.o	3.97 ±0.59**	80.60

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. *P < 0.01 and **P < 0.001 as compared to control (n = 6 in each group).
ESA = Ethanol Extract of *Syzygium alternifolium*
b.w= Body weight.

Table-2: Effect of Ethanol Extract of Syzygium alternifolium (ESA) in indomethacin (40 mg/kg) induced gastric ulcer in rats.

Group	Design of Treatment	Ulcer Index	Percentage Inhibition (% I)
I	Control (4% v/v aqueous tween 80, 10 ml/kg b.w) p.o	18.14 ± 0.42	---
II	ESA (250mg/kg b.w) p.o	8.32 ± 0.22*	54.13
III	ESA (500mg/kg b.w) p.o	6.33 ± 0.32**	65.14
IV	Omeprazole (20mg/kg b.w) p.o	4.17 ± 0.21**	77.02

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. *P < 0.01 and **P < 0.001 as compared to control (n = 6 in each group). ESA = Ethanol Extract of *Syzygium alternifolium.* B.W=Body weight.

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