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EVALUATION OF ANTI-ULCER ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *SANTALUM ALBUM* ON ALBINO RATS

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ABSTRACT

Back round: Ulcer is the most common prevalent gastro intestinal disorder, which affects approximately 10 -15% of people in the world. It makes major global health problem today. Ulcer is an open sore, it can be developed inside the inner lining of the stomach (gastric ulcer) or the small intestine (duodenal ulcer). Both the ulcers are also commonly referred to as peptic ulcers. **Objectives:** The present study was carried out to investigate the anti-ulcer activity of Aqueous and ethanolic extract of *Santalum album* on albino rats. **Materials and Methods**: The present study was carried out by NSAID induced ulcer models in albino rats. The antiulcer activity of aqueous and ethanolic extracts of *Santalum album* 250 mg and 505 mg/kg p.o. 5 days) was compared with standard drug (Lansoprazole). In NSAID induced ulcer model, the studied parameters were ulcer index and percentage protection. **Results:** In NSAID induces ulcer model, the ulcer index was significantly reduced at $p<0.01$ in 500mg/kg of aqueous and ethanolic extract of *Santalum Album* treated groups as compared with control. All the doses of AESA and EESA showed the dose-dependent antiulcer effect as well as significant ($p<0.01$) increase in percentage protection of ulcer as compared to control group in all the experimental models. The aqueous extract of AESA at 500 mg/kg has more potent antiulcer activity than 250 mg/kg of aqueous and ethanolic extract of *Santalum album* and 500mg/kg of EESA. **Conclusion**: The results of the study indicate that the AESA has better potential against ulcer which supports the traditional claims in folklore medicine**.** Phytochemical investigation suggests the presence of, tannins which may be responsible for the anti-ulcer activity.

KEYWORDS: Antiulcer Effect, Santalum album, AESA,EESA, NSAID induced ulcer, Ulcer Index.

INTRODUCTION

Ulcer is an open sore that develops on the inside lining of the stomach (a gastric ulcer) or the small intestine (a duodenal ulcer). Both types of ulcers are also referred to as peptic ulcers. The most common symptom of a peptic ulcer is a burning or gnawing pain in the center of the abdomen (stomach). In the past, it was mistakenly thought that the main causes of peptic ulcers were lifestyle factors, such as diet, smoking, alcohol, and stress. While these factors may play a limited role, it is now known that the leading cause of peptic ulcers is a type of bacteria called H. pylori. It can infect the stomach and small intestine; and in some people, the bacteria can irritate the inner layer of the stomach and small intestine, leading to the formation of an ulcer.[1]

Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and Helicobacter pylori) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors.[2] Painkillers known as non-steroidal anti-inflammatory drugs (NSAIDs), which include aspirin and ibuprofen, are the second most common cause of peptic ulcers. These types of painkillers can irritate the lining of the stomach and small intestine in some people, particularly if they are taken on a long-term basis. A number of drugs, including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents, are available for the treatment of peptic ulcer, but most of these drugs produce several adverse reactions, including toxicities, and may even alter biochemical mechanisms of the body upon chronic usage.[3] The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs and also reduces the offensive factors serving as a tool in the prevention of peptic ulcer.[4] In this modern era also, 75-80% of the world populations still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The chemical constituents present in the herbal medicine or plant are a part of the physiological

functions of living flora and hence they are believed to have better compatibility with human body.[5]

Santalum album is a hemi parasitic tree, native to semi-arid areas of the Indian subcontinent It is now planted in India, China, Sri Lanka, Indonesia, Malaysia, the Philippines. The height of the evergreen tree is between 4 and 9 metres. The tree is variable in habit, usually upright to sprawling, and may intertwine with other species. The plant parasitizes the roots of other tree species, with a haustorium adaptation on its own roots, but without major detriment to its hosts. An individual will form a non-obligate relationship with a number of other plants. The reddish or brown bark can be almost black and is smooth in young trees, becoming cracked with a red reveal. The heartwood is pale green to white as the common name indicates. The leaves are thin, opposite and ovate to lanceolate in shape. Glabrous surface is shiny and bright green, with a glaucous pale reverse. Fruit is produced after three years, viable seeds after five. These seeds are distributed by birds. Alkaloids, Flavonoids, Tannins, Phenolic compounds are present in this plant. Traditionally it was used as Analgesic, Anti spasmodic, Digestive, Diuretic, Astringent, Antiseptic.[6-7]

MATERIALS AND METHODS

Plant material *Santalum album* was collected from in and around chembarambakkam, Chennai, India and authenticated by Dr.P.Jayraman, Director of Plant Anatomy Research Centre, Chennai. The fresh bark of *Santalum album* was identified and deposited at Department of Pharmacognosy, Sree Sastha Pharmacy College, Chembarambakkam, Chennai with the voucher number SSCPDPCOG/IT/2020. The fresh bark was separated and kept for shade drying. Dried bark material was powdered using a mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an airtight container.

Extraction of Plant material

The barks of *S.album* were washed thoroughly in water to remove foreign matter and allowed to shade dry with a relative humidity of 40–45%. Then, barks were powdered in roller grinder and passed through a sieve (No. 60). Then, the fine powder (Approx. 150 gm) was defatted with petroleum ether and extracted with water and 1 litre of 95% ethanol at room temperature by using Soxhlet apparatus for 72 hours. The resultant extract was filtered and concentrated in a rotary evaporator under reduced pressure to obtain a thick semi-solid which was stored at −20°C until required. The yield of the extract was found to be 7.2 %w/w.

Chemicals and Drugs

Lansoprazole and Anaesthetic ether were purchased from Sigma Co. (Sigma St. Louis, MO). Absolute ethanol was of analytical grade and was purchased from Merck (German). The other reagents were of analytical grade.

Animals

Albino wistar rats of either sex weighing between 150-250 gm maintained in the Animal house facility of the Department of Pharmacology, Sree Sastha Pharmacy College were used in these experiments. The animals were maintained on standard small animal feeds (Excel feed, Ilorin) and water ad libitum. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) under the reference no. 1332/DPCG//20 /CPCSEA and CPCSEA guidelines adhered to during the maintenance and experiment. This research was carried out in accordance with the rules governing the use of laboratory animals as accepted internationally. The experiment was conducted between the hours of 900 h and 1600 h. The experimental groups consisted of six animals. They were maintained at constant room temperature (22° ± 1 °C) and submitted to12 h light/dark cycle with free access to food and water.

Experimental Procedure

Acute oral toxicity study

Acute oral toxicity was conducted as per OECD guidelines (Organisation of Economic Cooperation and Development) 423 (Acute toxic class method). The acute toxic class method is a stepwise procedure of three animal of a single-sex per step. Depending on the mortality and/or moribund status of animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for the acceptable data-based scientific conclusion. The method uses defined doses, (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for the classification of chemicals which causes acute toxicity. The method previously described by Lorke was adopted.[8]

Antiulcer activity

NSAID's induced ulcer (Aspirin induced ulcer)[9]

Albino wistar rats of either sex weighing between (150-200gms) were divided into six groups of six animals in group.

Group-I – Control (Aspirin 200mg/kg p.o)
Group-II – Standard (Lansoprazole 8mg/kg in 2% gum acacia p.o).
Group-III – Aqueous extract *Santalum Album* (250mg/kg p.o.).
Group-IV – Aqueous extract *Santalum Album* (500mg/kg p.o.).
Group-V – Ethanolic extract *Santalum Album* (250mg/kg p.o.).
Group-VI – Ethanolic extract *Santalum Album* (500mg/kg p.o.).

On day 5, Aspirin at dose of 200mg/Kg was administered to the animals of all the groups (I to VI) one hour after the administered of last dose of the extract/ Lansoprazole. Four hours after the administered

of Aspirin, the animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of stomach. Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer, and sample was sending to further Histopathological study.

Score the ulcers as below
0-Normal coloured stomach
0.5- Red coloration
1- Ulcers spot
1.5- Haemorrhagic stress
2- Ulcers ≥ 3 but ≤ 5
3- Ulcers > 5

Mean ulcer score for each animal is expressed as ulcer index.[10]
Ulcer index (UI) was measured by using following formula: $UI = UN + US + UP \times 10^{-1}$
Where UI (Ulcer Index);
UN (Average number of ulcers per animal);
US (Average number of severity score);
UP (Percentage of animals with ulcers
The percentage protection was calculated using the formula,

$$\text{Percentage protection} = 100 - \frac{U_t}{U_C} \times 100$$

Where, U_t = ulcer index of treated group
U_c = ulcer index of control group

Statistical analysis
The results were expressed as mean ± SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Dennett's' test P value less than <0.05 was considered to be statistically significant. *P<0.05, **<0.01 and ***<0.001, when compared with control and toxicant group as applicable.

RESULTS

In NSAID induces ulcer model, the ulcer index was significantly reduced at p<0.01 in 500mg/kg of aqueous (4.16 ± 0.30) and ethanolic (4.45 ± 0.39) extract of *Santalum Album* treated groups as compared with control. All the doses of AESA and EESA showed the dose-dependent antiulcer effect as well as significant (p<0.01) increase in percentage protection of ulcer as compared to control group in all the experimental models. The aqueous extract of AESA at 500 mg/kg(4.16 ± 0.30) has more potent antiulcer activity than 250 mg/kg of aqueous and ethanolic extract of *Santalum album* and 500mg/kg of EESA.

The influence on the ulcer index in aspirin induced ulcer of Lansoprazole (8mg/kg); aqueous and ethanolic extract *Santalum Album* 250, 500mg/kg. Along with the percentage protection that had significantly increased are summarized in table.

Table 1: Effect of aqueous and ethanolic extracts of *Santalum album* on aspirin induced ulceration in rats.

Gr.no.	Treatment	Dose	Ulcer index	% Protection
1	Control (Aspirin)	200 mg/kg p.o.	5.87 ± 0.32	--
2.	Lansoprazole	8 mg/kg p.o.	1.15 ± 0.11***	80.40
3.	AESA	250 mg/kg p.o.	5.21 ± 0.33	11.24
4.	AESA	500 mg/kg p.o.	4.16 ± 0.30**	29.13
5.	EESA	250 mg/kg p.o.	5.42 ± 0.22	7.66
6.	EESA	500 mg/kg p.o.	4.45 ± 0.39**	24.19

Values are the mean ± S.E.M. of 6 rats / treatment Significant *P < 0.05, **P < 0.01 and ***P < 0.001 compared with Control.

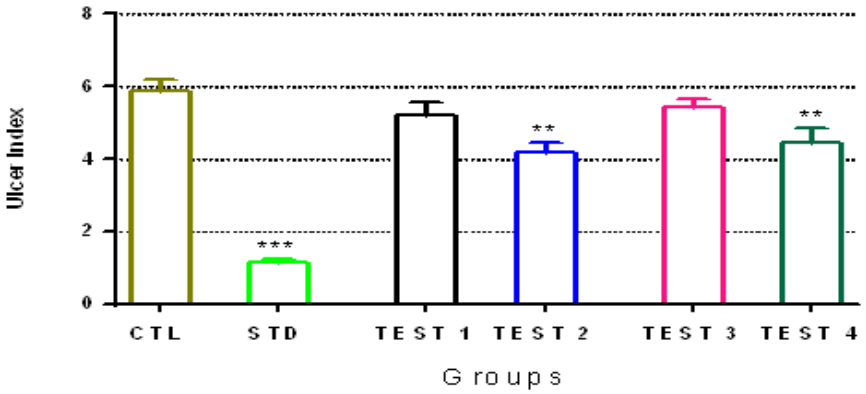
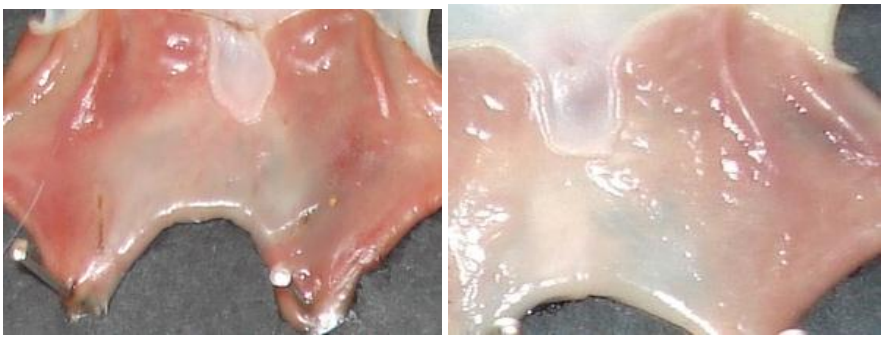
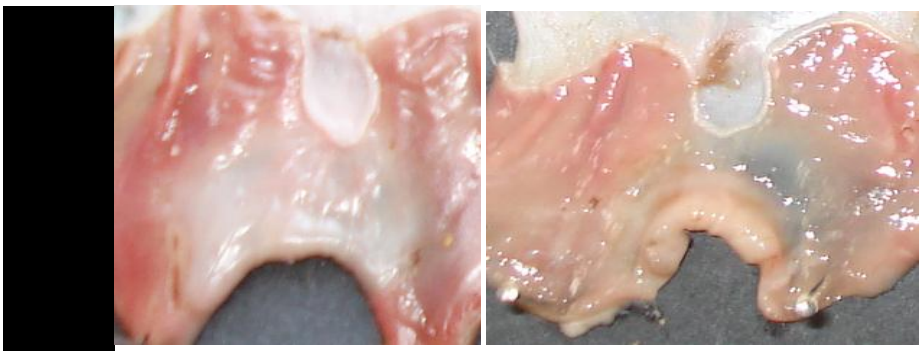


Fig. 1: Effect of Aqueous and Ethanolic extracts of *Santalum Album* on ulcer index following aspirin induced ulcer on rats.

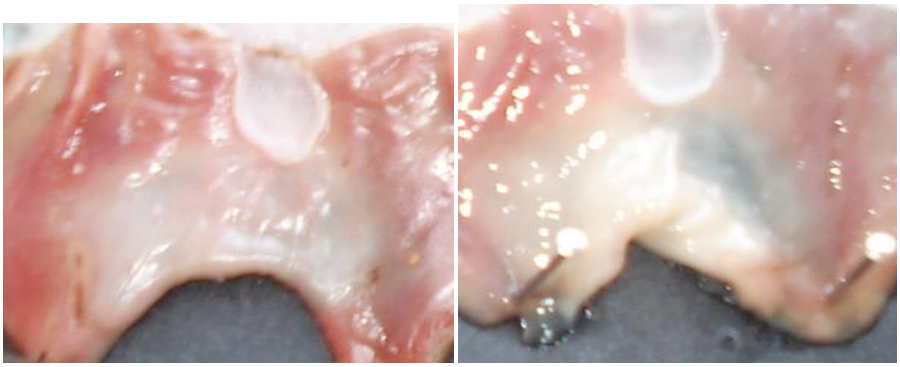
CTL: Control
STD: Standard (Lansoprazole 8 mg/kg p. o.)
TEST 1: Aqueous extract of *Santalum Album* (250 mg/kg p.o.)
TEST 2: Aqueous extract of *Santalum Album* (500 mg/kg p.o.)
TEST 3: Ethanolic extract of *Santalum Album* (250 mg/kg p.o.)
TEST 4: Ethanolic extract of *Santalum Album* (500 mg/kg p.o.)



Control Standard (Lansoprazole)



AESA 250mg/kg AESA 500mg/kg



EESA 250mg/kg EESA 500mg/kg

Fig. 2: Effect of Aqueous and Ethanolic extracts of *Santalum Album* on ulcer index following aspirin induced ulcer on rats.

DISCUSSION

The acute toxicity study revealed that the plant extract was safe in rats at a limit dose of 2000 mg/kg and that the median lethal dose (LD50) of the extract is above 2000 mg/kg. This finding supports the work done on rats in another study.[11] The gastric ulcer is caused due to stress-induced increase in gastric acid (HCl) secretion and these acid secretions promote ulceration due to exposure of the unprotected lumen of the stomach to the accumulating acid.[12-14] Aspirin causes a dose dependent reduction in mucosal prostaglandins - PGE2 and PGI2 bio-synthesis accompanied by an increase in the mean area of gastric ulcerations.[15] Aspirin is known to inactivate irreversibly the PG synthetase system, which mediates synthesis of prostaglandin in the mucosa. An Increase in acid secretion and back diffusion at H^+ ions is also noticed. It is reasonable to assume that the observed gastric mucosal lesions induced by aspirin are due to a deficiency of mucosal prostaglandin.[16] Aspirin induced ulcer is mediated through tissue damaging free radicals, which are produced from the conversion of hydroperoxyl to hydroxyl fatty acids, which leads to cell destruction. The hydroperoxyl fatty acids are generated from the

degeneration of mast cells and generalized lipid per oxidation accompanying cell damage.[17] Aqueous and Ethanolic extract of *Santalum album* prevents the ulcer may be by the antisecretory and cytoprotective property. The preliminary phytochemical analysis of *S.album* extract showed the presence of flavonoids, terpenoids, saponin, tannins, and glycosides. The antioxidant components from many plant extracts have been extensively confirmed for their antiulcerogenic efficacy.[18] It is suggested that these active compounds would be able to stimulate prostaglandin secretion and counteract the deteriorating effects of reactive oxidants in gastrointestinal lumen.[19-20] Flavonoids are thought to increase mucosal prostaglandin content, decrease histamine secretion from mast cells by inhibition of histidine decarboxylase, inhibit Helicobacter pylori growth, act as free radical scavengers, and inhibit H+/K+-ATPase.[21-22] Saponin may activate mucous membrane protective factors, and tannins render the outermost layer of the mucosa less permeable, for instance, to chemical irritation.[23] In addition, terpenoids are also reported to have potent activity against gastric ulcers.[24-25] Therefore aqueous and extract of *Santalum album* possesses antiulcer activity, may be due to the presence of saponins,flavonoids, tannins, and terpenoids.

CONCLUSION

The present study concluded that the antiulcer activity of aqueous and ethanolic extract of *Santalum album* may be attributed to antisecretory and antioxidant properties. The bioactivity-guided phytochemical screening of AESA and EESA revealed that the presence of flavonoids, tannins, saponin, and terpenoids, which may be responsible for the anti-ulcer effect and can be further fractionated and investigated for their role and utility in any of the anti-ulcer mechanisms.

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Competing interests

Author have declared that no competing interests exist.

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