

Antioxidant and Antiulcer Activity of Sesamum Indicum Leaves Extract in Experimental Rats: An in-Vitro Study**Vikas Kumar**

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Conflict of interest: Nil

Abstract**Aim:** The aim of the present study was to estimate phytochemical screening, in vitro antioxidant activity, and gastroprotective activity of Sesamum indicum Linn ethanolic extract.**Methods:** The study was conducted at Narayan Medical College and Hospital, Sasaram, Bihar, India**Results:** The high dose of S. indicum extracts (group-5) significantly reduced gastric mucosal lesion. The low dose test group (group-4) showed significantly reduced gastric mucosal lesion. The statistical significant changes noted only in ulcer size, number and index. The high dose of S. indicum extracts (group-5) significantly reduced mucin content, volume of gastric juice, gastric pH, free and total acidity (group-3) when compared to positive control group. The low dose test group (group-4) showed significantly reduced mucin content, volume of gastric juice, gastric pH, free and total acidity (group-3) when compared to positive control group. The high dose of S. indicum extract (group-5) showed comparable results in parameters like free acidity and total acidity with the standard group.**Conclusion:** Although the high dose sesame (1mg/kg) group showed significant gastric protection against ulcer induced by cold restraint method. However no clear inference can be drawn at this stage and hence we consider the work for further extensive research.**Keywords:** Antioxidant, anti-ulcer, ethanolic extract of sesamum indicum leaves, indomethacin, pylorus ligation, ulcer indexThis is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided original work is properly credited.**Introduction**

Peptic ulcer diseases are a group of heterogeneous disorders, which manifests as a break in the lining of gastrointestinal mucosa bathed by pepsin and acid. Among the diseases of gastro-intestinal diseases, peptic ulcer is the most predominant with a worldwide prevalence of about 40% in the developed countries and 80% in the developing countries. The pathology of the gastric ulcer is lack of equilibrium between gastric aggressive factors and the mucosal defensive factors. [1] The present modality of treatment is the use of histamine H₂-receptor antagonists, anticholinergic drugs, antacids and PPI's. From olden days, there have been use of plant sources in the treatment of various disease modalities due to their perceived lower side effects, ease of accessibility and affordability. Plants are some of the most attractive sources for the discovery of new drugs and some have been shown to have promise for the treatment of gastro duodenal ulcer with minimum side effects. [1]

Plants with traditional ethno medicinal uses in peptic ulcer management thus need to be screened

for potential antiulcer activity. The sesame oil contain the lignans, sesamol, sesamin, pinoresinol and lariciresinol. Sesamol (SES), is a component of traditional health food in various Asian countries. It protects against atherosclerosis, hypertension, and aging. It has also been explored for wound healing, antioxidant, anti-inflammatory, and free radical scavenging activity. [2] A localized loss of gastric as well as duodenal mucosa leads to the formation of peptic ulcer. It arises when the normal mucosal defensive factors such as mucus, mucosal blood flow, formation of bicarbonate ions and prostaglandin E₂ are impaired or over powered. Also by the aggressive factor includes acid, pepsin, NSAIDs and Helicobacter pylori. [3] Gastric ulcer resulted from persistent erosions and damage of the stomach wall that might become perforated and developed into peritonitis and massive hemorrhage as a result of inhibition in the synthesis of mucus, bicarbonate and prostaglandins. [4] Medicinal plants have always been the main sources of new drugs candidates for the treatment of gastric ulcer. [5]

Ulcer therapy has progressed from vagotomy to anti-cholinergic drugs, histamine-H₂ receptor antagonists, antacids and more recently to proton pumps inhibitors which revolutionized the treatment of peptic ulcers and other gastro intestinal disorders, but there is still no complete cure for this disease. It has been shown that long term use of these drugs leads to various adverse and side effects. Relapses of the malady, ineffectiveness of different regimens and even resistance to drug are emerging. In recent years, there has also been growing interest in alternative therapies and the use of natural products, especially those derived from plants. [6] Plant extracts are shown to have more attractive sources of new drugs and results for the treatment of gastric ulcer. Thus, there is an urgent requirement to identify more effective and safe anti-ulcer agent. [7] Though the advances in modern medicines are significant, there remains an ever increasing demand for herbal medicines. Effective and potent herbal medicines require evaluation by standard scientific methods so as to be validated for the treatment of diseases.

The aim of the present study was to estimate phytochemical screening, in vitro antioxidant activity, and gastroprotective activity of *Sesamum indicum* Linn ethanolic extract.

Materials and Methods

The study was conducted at department of Pharmacology, Narayan Medical College and Hospital, Sasaram, Bihar, India for six months

Chemicals/ drugs/ instruments used

Pantoprazole, *S. indicum* seed extract, ketamine, orcinol 1.6%, sulphuric acid 60%, phenolphthalein, topfer's reagent, 0.5% carboxymethyl Cellulose, distilled water, surgical kit, pH meter.

Plant Preparation and Extraction

The fresh sesame seeds were purchased from the local market and seeds were grinded to fine powder. The powder was filled in to filter paper bag and placed in the soxhlet apparatus for extraction. The soxhlet apparatus is connected to round bottom flask which was filled by ethanol (90%) solvent and water bath to maintain temperature. The ethanol was boiled at 40 °C for

over a period of 24 hours. The extract obtained was 10 % and was stored in desiccator at room temperature.

Experimental Animals

The animals were taken from central animal house - male/female albino rats of Wistar strain, weight - 200- 250gm. The animals were housed under standard condition, housed individually with normal water and food granules, 12:12 hours light dark cycle, 50% humidity and 28°C temperature and provided with standard food granules and water ad libitum.

Animal grouping and treatment

Ulcer inducing method

The procedure for inducing ulcers is with the stress- induced ulcer model, which include animals being fasted for a period of 18-24 hours prior to the experiment. Ulcers are then induced by placing animals individually in a restricted cage for 8 hours and in restraint cold ventilated refrigerator at a temperature of 2-3°C for 2-4 hours. [1]

Drug Dosing

- Group I - Negative control, 0.9% normal saline - no ulcers induced x 7 days
- Group II - Positive control 0.9% saline + ulcer induced rats x 7 days
- Group III - Standard - pantoprazole 30mg/kg x 7 days
- Group IV - Test 1 - sesame extract 0.5mg/kg x 7 days
- Group V - Test 2 - sesame extract 1mg/kg x 7 days

Day 1 - The ulcers were induced by the Stress-Induced Ulcer Model. From Day 3 - 9: Standard and test drugs are given via oral route via a feeding tube and animals are dosed once a day, with 0.5% carboxy methyl Cellulose (vehicle) for 7days, with the dosage mentioned above. On day 10 - the animals are euthanized using ketamine, the abdomen is dissected then the stomach was removed for the assessment of gastric mucosal damage.

Table 1: Animals were divided in to 5 treatment groups 4 rats in each group

Group no.	Group name	Drugs to be administered	No. of rats
I	Negative Control	0.9% Normal saline	4
II	Positive Control	0.9% Normal saline + ulcer induced rats	4
III	Standard	Pantoprazole(30mg/kg)	4
IV	Test dose 1	<i>S. indicum</i> extract (0.5mg/kg) for 7 days	4
V	Test dose 2	<i>S. indicum</i> extract (1mg/kg) for 7 days	4
		Total rats	20

Measurement of Ulcer

The stomach was dissected along the greater curvature and fixing on a board or transparent glass examination can be carried out with hand lens macroscopically and by a tracing on the transparent paper and the paper on to graph sheets and size of ulcers and the ulcer index was calculated as per the method of Rao et al.⁸

Ulcer index = Ulcer size X ulcer number

Mucin content determination: (Winzler method)

Diluted sample orcinol (1.6%) and sulphuric acid (60%) are added, vortexed and boiled for 10 min mixtures are cooled in ice-cooled water to stop reaction and absorbance studied at 425 nm.

Measurement of volume of gastric juice

Gastric juice from the stomach was drained into a centrifuge tube after the animals were sacrificed. The tube was centrifuged at 3000rpm for 10 minutes and the Centrifuged sample was decanted and analysed for the volume of gastric juice. [8]

Measurement of pH of gastric juice

pH of the centrifuged sample of gastric juice is measured using a digital pH Meter, type DPH-100 (Dalal instruments).

Measurement of Free and Total Acidity

The free and total acidity is measured by titrating 0.1 ml of gastric juice with 0.01 NaOH using topfer's reagent and phenolphthalein as INDICATORS (HAWK, 1965). Orange yellow and point with topfer's reagent is for the free acid content and the pink endpoint with phenolphthalein gives a measure of total acid content. Acidity is expressed as mEq/L per 100 grams body weight.

Statistical Analysis

The results were analyzed using one way ANOVA in SPSS 21 Software for Microsoft. The statistical significant value for any measure was set to $p < 0.05$ at a confidence interval of 95%. The results expressed are in mean \pm standard error mean.

Results

Table 2: Effect of *S. indicum* on gastric ulcer

Group	Mean ulcer Size (mm)	Mean ulcer number	Mean ulcer index
Group I	-	-	-
Group II	24.6 \pm 0.48	12.6 \pm 0.74	296 \pm 18.4
Group III	1.5 \pm 0.4	2 \pm 0.6	3.16 \pm 0.7
Group IV	10.5 \pm 0.5	9.4 \pm 0.5	104.4 \pm 0.8
Group V	1.7 \pm 0.3	2.8 \pm 0.62	5.3 \pm 1.3

The high dose of *S. indicum* extracts (group-5) significantly reduced gastric mucosal lesion. The low dose test group (group-4) showed significantly reduced gastric mucosal lesion. The statistical significant changes noted only in ulcer size, number and index.

Table 3: Effect of *S. indicum* on Mucin Content, pH and Vol. of Gastric Juice

Group	Mucin	pH	Volume of gastric juice (ml)
Group I	356.34 \pm 3.7	4.46 \pm .05	2.04 \pm 0.06
Group II	223.7 \pm 1.8	2.58 \pm 0.02	7.23 \pm 0.15
Group III	332.2 \pm 3.7	4.58 \pm 0.72	3.07 \pm 0.08
Group IV	342.8 \pm 2.2	4.6 \pm 0.07	3.7 \pm 0.12
Group V	316 \pm 8.6	4.2 \pm 0.18	4.4 \pm 0.26

The high dose of *S. indicum* extracts (group-5) significantly reduced mucin content, volume of gastric juice, gastric pH, free and total acidity (group-3) when compared to positive control group. The low dose test group (group-4) showed significantly reduced mucin content, volume of

gastric juice, gastric pH, free and total acidity (group-3) when compared to positive control group. When compared with the standard group it showed significant changes only in ulcer size, number and index but not in other laboratory parameters.

Table 4: Effect of *S. indicum* on free acidity and total acidity

Group	Free acidity (mEq/ L/Hr/100gm)	Total acidity (mEq/ L/Hr/100gm)
Group I	6.07 \pm 0.03	23.7 \pm 1.5
Group II	20.5 \pm 0.08	84.6 \pm 0.36
Group III	6.94 \pm 0.04	32.4 \pm 0.38
Group IV	7.5 \pm 0.12	28.6 \pm 0.52
Group V	6.84 \pm 0.07	28.2 \pm 0.50

The high dose of *S. indicum* extract (group-5) showed comparable results in parameters like free acidity and total acidity with the standard group.

Discussion

Peptic ulcers are pathological lesions in any part of the gastrointestinal system exposed to acid and activated pepsin. Peptic ulcer disease (PUD) is one of the most frequent illnesses in today's world. Due to an difference between protective (mucus, prostaglandins and bicarbonate) and aggressive (acidity and reactive oxygen species [ROS]) factors, it has developed into a widespread global health offender with increasing incidence and pervasiveness. [9] The probable number of about 16,500 deaths occur every year due to PUD. [10] The general signs of gastric ulcers are dyspepsia, including pain, discomfort, bloating, fullness, nausea, heartburn, regurgitation, and whistling in the abdominal region. [11] In the present era, the prevalence of daytime pain remains a common phenomenon along with the increased prevalence of night time pain due to lifestyle changes, increased stress, and longer daytime working hours. Currently, most patients with gastric ulcer need long-term treatment with drugs that inhibits gastric acid secretions, such as ranitidine (H₂-receptor antagonist) and pantoprazole (proton-pump inhibitor). [12] The plant of Sesame (*Sesamum indicum* L. Family: Pedaleaceae) is commonly known as till, beniseed, gingelly, sim-sim, and sesamum. It is a popular oldest oilseed plant used and understood by human beings. Sesame has different local cultivars as well known in the literature, but it is said the genus *Sesamum* has only one cultivated species. [13]

The high dose of *S. indicum* extracts (group-5) significantly reduced gastric mucosal lesion. The low dose test group (group-4) showed significantly reduced gastric mucosal lesion. The statistical significant changes noted only in ulcer size, number and index. The pathology of the stress ulcer is not clear, the possible hypotheses for genesis of stress ulcers is Ischaemic hypoxic injury to the mucosal cells and depletion of the gastric mucus barrier rendering the mucosa susceptible to attack by acid-peptic secretions. [14] In the present study the ulcer induction was done using restraint cage and placing the rats in cold ventilated freezer for 2 hours. Edward et al [15], studied the Synergism between cold and restraint for rapid production of stress ulcers in rats and they observed that restraint and exposure to cold acted synergistically to produce gastric ulcers. Sesame (*Sesamum indicum*) belongs to family Pedaliaceae with the chemical compositions of lignans, sesamol, sesamin, pinoresinol and lariciresinol. Sesamol (SES), is a component of traditional health food in various Asian countries. It protects against

atherosclerosis, hypertension, and aging. It has also been explored for wound healing.

The high dose of *S. indicum* extracts (group-5) significantly reduced mucin content, volume of gastric juice, gastric pH, free and total acidity (group-3) when compared to positive control group. The low dose test group (group-4) showed significantly reduced mucin content, volume of gastric juice, gastric pH, free and total acidity (group-3) when compared to positive control group. When compared with the standard group it showed significant changes only in ulcer size, number and index but not in other laboratory parameters. The high dose of *S. indicum* extract (group-5) showed comparable results in parameters like free acidity and total acidity with the standard group. As evidenced by pylorus ligation ulcers, autodigestion of the stomach mucosa and degeneration of the gastric mucosal wall result in superior gastrointestinal damage, with lesions, hemorrhage potentially catastrophic rupture, and ulcers. Stomach ulcers are caused by the stomach's pyloric blockage, which causes gastric acid accumulation. Agents that lower stomach acid output while increasing mucus secretion can help prevent ulcers from developing due to this surgery. Omeprazole, like ranitidine, is an antiulcer medication that inhibits stomach secretion and pepsin activity via an anti-secretory mechanism. Auto-digestion and stomach wall breakdown are caused by the digestive effects of retained gastric fluids (including pepsin and gastric acid), disruption of gastric blood flow, and enhanced construction of free radicals. [16]

Conclusion

Although the high dose sesame (1mg/kg) group showed significant gastric protection against ulcer induced by cold restraint method. However no clear inference can be drawn at this stage and hence we consider the work for further extensive research.

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