INVESTIGATION INTO ANTI-OXIDANT AND ANTINUCLEAR EFFECT AND MECHANISM OF SESAMUM INDICUM LEAVES IN A RODENT MODEL

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ABSTRACT

Objective: An ethanolic extract of Sesamum indicum Linn was used to test for phytochemicals, antioxidant activity, and gastroprotective effects.

Study Design: Cross-Sectional Study

Place of Study: Rashid Latif Medical College Lahore.

Duration of Study: July 2021 to March 2022

Materials and Methods: This study ulcerated Wister albino rats using pylorus ligation and indomethacin-induced ulcer screening. Ulceration required pylorus ligation. These ulcer screening models were used. An ethanolic S. indicum leaves (EESIL) at 100, 200, and 400 mg/kg (orally for 7 days) was compared against omeprazole, a common ulcer medication. The trial lasted seven days. Throughout the course of the experiment, readings were taken for the following variables: stomach content; pH; total acidity; pepsin activity ulcer score; free acidity; ulcer index (UI); percentage of inhibition of ulcers; mean mucin, pepsin, and total protein content; and ulcer index (UI).

Results: In comparison to the control group, the pylorus ligation model resulted in a decrease in pepsin activity, free acidity, pepsin content, ulcer score, total protein content, and % ulcer inhibition. Moreover, the overall acidity level was lower (P< 0.05 and P< 0.01). EESIL-tested groups had higher mean mucin and stomach content pH (P 0.05 and P 0.01). Both findings were statistically significant. EESIL dosages (100, 200, and 400 mg/kg) had dose-dependent gastroprotective effects. Compared to the control group, stomach metrics like Ul and ulcer score dropped significantly (P< 0.05 and P< 0.01), gastric pH increased, and ulcer percentage inhibition increased. Increased ulcer inhibition percentage. Moreover, stomach pH increased and ulcer percentage decreased.

Conclusion: Antioxidant, anti-ulcer, EESIL, and EESIL compounds have antioxidant action depending on concentration. Antioxidant is connected. The study found that the EESIL's increased defensive and decreased offensive components increased its antiulcer potential. This was due to the study's revisions. The research's offensive and defensive design caused this.

Keywords: Antioxidant, anti-ulcer, ethanolic extract of sesame indicum leaves, indomethacin, pylorus ligation, ulcer index

INTRODUCTION

Peptic ulcers are a form of pathological lesion that can develop in any part of the digestive tract that has been subjected to acid and activated pepsin. Peptic ulcers can be caused by both chronic and acute exposure to acid and pepsin. The term "peptic ulcer disease" refers to one of the conditions that affect the greatest number of individuals in today's world (PUD). Because there is a disparity between the components that are protective and the factors that are aggressive, it has become a pervasive threat to the health of people all over the world. Dyspepsia, which includes pain, discomfort, bloating, fullness, nausea, heartburn, and regurgitation, are the general symptoms of gastric ulcers. At this time, the majority of patients who have gastric ulcers require long-term treatment with medications that block gastric acid discharges. PUD is one of the leading causes of preventable death in the United States. An H2-receptor antagonist like ranitidine and a proton pump inhibitor like pantoprazole are two examples of the types of medications that fall under this category. [4]

The plant with the scientific name Sesamum indicum L. also goes by the common names til, beniseed, gingelly, and sim-sim. It is a member of the Pedaleaceae family. Other common names for the plant include sesamum and gingelly. People have been making use of and learning about this particular oilseed plant for a very long time, making it one of the most well-known and ancient oilseed plantings. Although it is widely acknowledged in the academic literature that numerous local cultivars of sesame do exist, it is often assumed that just a single species of the genus Sesamum is capable of being cultivated. [5] The cultivation of sesame seeds for the production of edible oil is the oldest oilseed crop that is known to have been practised by humans. There are many different kinds of sesame, the most major of which are broad in size. The sub-Saharan region of Africa is home to the vast majority of the widely dispersed varieties that belong to the genus Sesame. It was found out through an extensive range of different types of research that Sesamum indicum was the country that pioneered the cultivation and discovery of the plant that would later be known as sesame. In order to develop therapies and management methods for ulcers, it is absolutely necessary to recognize phytoconstituents that are derived from plants. This treatment approach for ulcer care and therapy has made use of a wide array of herbal treatments over the course of its history. [8] On the other hand, a complete review of the available research showed that sesame leaves do not possess any antiulcer activity. This conclusion was reached after the leaves were tested. As a consequence of this, we sought to carry out research into the mechanism or mechanisms of action that are responsible for the antioxidant and antiulcer effects of S. indicum leaves extract in experimental rats.

MATERIALS AND METHODS

Extract preparation: After being given a thorough cleaning, the leaves were allowed to dry in the shade before being ground into a powder. Using an electric blender, a total of 0.5 kilogrammes of air-dried leaves were reduced to a powdery consistency and mashed into a finer consistency. Following the defatting of pulverised leaves (0.5 kg) with petroleum ether using a Soxhlet system, the resulting slurry was filtered, and the residue was air-dried after 24 hours' time. After that, 400 grammes of the defatted and dried residue (out of a total of 0.5 kilogrammes) were subjected to 15 cycles of continuous extraction with one litre of ethanol that had a volume-to-volume ratio of 95% using a Soxhlet apparatus at a temperature ranging from 60 to 70 degrees Celsius. This process was carried out at a temperature of between 60 and 70 degrees Celsius. An extract that had been collected was put through an oven that had a hot air dryer in it so that it could be dried. Following the removal of the fat and the drying of the residue, we were left with a total of 42.3 grammes of extract. After the brownish
extract had been moved into a clean bottle, the bottle was put into a desiccator to ensure that it would remain dry until it was needed again.

In vitro study: Diphenyl-1-pirclyhydradyl assay: We were able to evaluate how effective EESIL was at scavenging free radicals by employing the DPPH test method. [8]

The hydroxyl radical is a particular type of ROS that is exclusive to the biological system and contributes greatly to the potency of the system as a whole. Damage to the cells is brought on by the interaction of the hydroxyl ion with the polyunsaturated fatty acid molecules that are found in the phospholipids of the cell membranes. This interaction is what is known as the "redox reaction." [9]

Phenolic content in extract: The Folin-Ciocalteu method was utilised in order to carry out a colorimetric analysis with the objective of measuring the total quantity of phenolics that were present in the sample. This was done in order to determine whether or not the sample was suitable for consumption. In conjunction with the Folin-Ciocalteu phenol reagent, an aliquot of extract from either the bark or the leaf of the plant was utilised. The volume of the aliquot was 0.3 millilitres. This was done in order to facilitate a comparison of the outcomes (2.25 mL). The solution was stored in 2.25 millilitres (mL) of sodium carbonate that had a concentration of 6%, and it was allowed to sit at room temperature for a period of five minutes after it had been prepared. After that, we let it sit for the advised amount of time, which was one hundred and ten minutes, before continuing. The experiment that was conducted to assess the absorbance of the mixture employed a wavelength of 725 nm as its focal point. The findings were reported as the amount of gallic acid equivalent (GAE) in milligrammes per gram of the isolated material. These values were obtained from a standard calibration curve of gallic acid that covered a concentration range of 0 to 200 mg/mL. [10]

Flavonoid content in extract: After making certain tweaks to the aluminium colorimetric method, the total flavonoid content of the sample was determined using quercetin as the reference standard. A range of quercetin concentrations ranging from 0 to 200 mg/mL was produced so that a calibration curve could be generated from the data. In a nutshell, in two separate test tubes, 0.1 mL of 10% aluminium chloride, 80% methanol, 1 M potassium acetate, and distilled water were added to and mixed with 0.5 mL of standard and 0.5 mL of extract. This was done so that the concentration of both the extract and the standard could be determined more accurately. In order to make a blank, the same technique was carried out; however, 0.5 mL of distilled H2O was used in place of the standard or sample, and the quantity of aluminium chloride was also deducted from the overall amount. After placing each tube in the incubator, it was allowed to remain there at room temperature for a period of thirty minutes. [10]

Experimental animals: Guidelines for Experiments on Animals were adhered to during the entirety of the experiment, including its upkeep. Every animal was kept in a regular husbandry environment that provided them with food, water, and access to any liberties they might require.

Acute oral toxicity study: In order to prepare ready for the experiment, the last part of the previous night was spent depriving three adult female albino rats of their usual food supply. This was done in preparation for the experiment. This was done in advance of the experiment so that we would be prepared. After giving each animal a different dose of an ethanolic extract of S. indicum, which ranged from 50 to 2000 mg/kg, the behaviour of the animals was observed continuously for one hour, then at semi-intervals for four hours for any significant change in behaviour, and finally for another 72 hours for any mortality, followed by 14 days for any mortality. The range of the doses used was from 50 to 2000 mg/kg.

This was carried out in accordance with Oecd's Guideline 425, which is a document that was developed by the Organization for Economic Co-operation and Development (OECD). [11] Changes in the skin, fur, mucus membrane (nose), eyes, and autonomic salivation, lacrimation, sweat, and dilatation of blood vessels, urine incontinence, and faeces) are checked once daily in the cage, as are changes in the central nervous system. Changes in the skin, fur, mucus membrane (nose), and eyes, as well as autonomic salivation, lacrimation, sweat, and dilatation of blood vessels are also checked. Alterations in the skin, fur, mucus membrane (the nose), and eyes could be symptoms of a more serious condition (tiredness, gait, tremors, and convulsions). According to the findings of study that was carried out on the topic, it is safe to consume leaf extract from S. indicum up to a dosage of 2,000 milligrammes per kilogramme of body weight. As a result of this, the doses of 100, 200, and 400 mg/kg that will be used in the clinical trials of antiulcer medications were decided upon. [11]

Grouping of animals and antiulcer study treatment plan: In every single one of the several versions of the experiment, albino rats were utilised, and those rats were separated into five groups of Five individuals each. The animals had unlimited access to water throughout the twenty-four hour fasting period that came before the testing. This interval was conducted to ensure accurate results. Animals in Group I was given distilled water as a vehicle control. Animals in Groups II, III, and IV were administered EESIL at scaled doses (100, 200, and 400 mg/kg) for 7 days (once daily, respectively). Animals in Group V were given omeprazole 20 mg/kg as a control group. The results of this study are presented in the following table.

Pylorus ligation induced ulcers: [Table 1] presents, for the purpose of this model, a classification of the animals in accordance with the level of antiulcer activity that each animal possesses. This involves a total of four unique species represented within each of the four categories that were established for the animals to be placed in. The I Group was used as a control and received a dose of saline equivalent to 1 milliliter per kilogramme of body weight. This dose was taken orally. The following amounts of EESIL were respectively given orally to the Groups II, III, and IV: 100 mg/kg, 200 mg/kg, and 400 mg/kg respectively. The last group received a dose of omeprazole equivalent to 20 milligrammes per kilogramme taken orally. In the thirty-six hours leading up to the pylorus ligation, the animals were fasted so they would not be affected by the procedure. [12] A minute incision was made right below the xiphoid process in order to expose the abdominal cavity of the animal while it was under the influence of ether for the little time that it was under the influence of this substance. After first slightly elevating it, the pyloric region of the stomach was ligated so that it could keep receiving blood supply despite the manipulation. After the stomach had been carefully adjusted in preparation for the treatment, the abdominal wall was then sewed back up. [13] Oral administration of the medication took place not too much longer after the ligation of the pylorus had been performed. After the pylorus ligation had been conducted for a period of six hours, the rats were given an overdose of ether anaesthesia and then shocked. During the postoperative phase, the animals were not permitted to consume any food or water. As the stomach was cut open, a glass jar was placed underneath it to collect any fluids that may have been released. Following the measurement of the total volume of the contents, the sample was put through a centrifuge for 10 minutes at a speed of 2,000 revolutions per minute. In order to evaluate the pH, free acidity, total acidity, and peptic concentration of the supernatant, aliquots were used in the measuring process (1 ml each). On the other side, we utilised the precipitate, and using that, we were able to determine the total proteins. After determining whether or not any lesions were present in the foragnet of each individual stomach, the severity of any lesions that were found was evaluated. [14]

Using the ulcer score for each animal, we were able to determine an ulcer index, which was calculated by measuring the surface area of the ulcer. This was done in order to facilitate a comparison of the outcomes (2.25 mL). The so

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Where UI, UP (the proportion of animals that have ulcers), UN (the average number of ulcers associated with one animal), and US (the percentage of animals that have ulcers) are numbers that represent the percentage of animals that have ulcers (average severity score). After performing the calculation necessary to estimate the percentage of ulceration that was prevented, the results were compared to those obtained from the control group.

NSAIDs (indomethacin) induced gastric ulcer: Indomethacin was given to animals orally at 20 mg/kg BW. This dosage was given to the animals exactly one hour after the last dose of the medicine being studied. The rats received nonsteroidal anti-inflammatory medications for six hours of the 10-hour trial (NSAIDs). The rats were then sedated with an overdose of anaesthetic. Dissection followed stomach removal. After dissecting the stomach along its major axis, the interior was cleaned with serum physiological solution (0.9% NaCl) to remove debris. After stomach opening, this was done. After that, the mucosa was peeled to measure the Ul, stomach volume, and pH. Each measurement used the stomach. [16]

Statistical analysis: We made use of both the mean and the standard error of the mean in order to adequately convey the assessments that we made on the observable data. In order to make a comparison of the means, a one-way analysis of variance was performed. In order to establish the statistical significance, it was necessary to check that P was lower than 0.05.

RESULTS

Preliminary phytochemicals: In accordance with the procedures that were supplied, the EESIL was subjected to a series of different phytochemical tests. EESIL was found to have a wide range of phytochemicals, which were identified as carbohydrates, flavonoids, glycosides, amino acids, steroids, alkaldoids, proteins, and tannins.

<table>
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<th>Table 1: Grouping of Animals</th>
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<tr>
<td>Groups</td>
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<td>EESIL 100 mg/kg</td>
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EESIL (Etthanol extract of Sesamum indicum leaves)

In vivo anti-ulcer activity study: The effect of an ethanolic extract of the leaves of Sesamum indicum in a model of gastric ulcers that has a pylorus ligation: For rats whose pyloruses were tied off, the secretion of gastrointestinal content, stomach pH, total acidity, pepsin activity, UI, free acidity, ulcer score, percent ulcer inhibition, pepsin content, mean mucin, and total protein content are reported in , [Table 2], and [Table 3]. The findings are detailed in [Table 2], [Table 3], and [Table 4], respectively. [Table 1] summarises the findings. As contrasted with the first group, not only did the content of the stomach and the UI decrease by a sizeable amount, but so reduced the amount of pepsin activity, free acidity, pepsin content, total acidity, and total protein content (P 0.05 and P 0.01, respectively). On the other hand, all three of the variables—the pH of the gastric content, the mean mucin, and the percentage ulcer inhibition—showed substantial increases (P 0.05, and P 0.01, respectively). The percentages of inhibition showed by EESIL (100, 200, and 400 mg kg1) were respectively 24.5+0.4, 48.0+1.0, and 67.4+2.6, while the percentage of inhibition shown by omeprazole, a traditional drug, was 78.5+2.4. When coupled with omeprazole, EESIL showed evidence of having an antulcer effect at a dose of 400 mg/kg.

DISCUSSION

It has been shown that S. thermophilus as a gastroprotectant, as well as the antioxidant actions of S. thermophilus. In experimental rats with pyloric ligation and indomethacin-induced stomach ulcers, indicum extract was studied, along with the possible underlying processes that are involved in the actions that these circumstances have. Prior to conducting the pharmacological activity assessment, the preliminary phytochemicals analysis of test extracts (EESIL) was conducted out first. This was done before the pharmacological activity evaluation. PUD is a common illness that manifests in the gastrointestinal tract and can cause a variety of symptoms. Anti-ulcer medications are linked to a sizeable number of undesirable adverse effects [18]. Several plant species have been shown to be beneficial in the treatment of gastroduodenal problems by clinical investigations. These studies have been conducted. The formation of a gastric ulcer can be traced back to an increase in hydrochotic acid (the acid produced by the stomach) that was brought on by stress. An increase in hydrochotic acid output contributes to ulceration because it makes the lumen of the stomach, which is not
protected, more vulnerable to the acid that has built up. This ultimately leads to the development of an ulcer in the stomach. pylorus is another example of a medication that has the potential to create ulcers in the stomach. Because it reduces the level of bicarbonate in the stomach, generates reactive oxygen species (ROS), initiates lipid peroxidation, induces stomach cell apoptosis and necrosis, and suppresses the creation of prostaglandins, indomethacin is the cause of gastric ulcers. Flavonoids are known to possess antioxidant capabilities due to the fact that they are able to bind metal ions such as copper and iron, scavenge free radicals, and inhibit enzymes that are responsible for the generation of free radicals.

CONCLUSION

The findings lend support to the idea that EESIL exhibits an antioxidant activity, which may be seen here. The oral delivery of S did not result in any of the observed fatalities that were possible. Indicum was still efficacious even when administered at the maximum dose of 2,000 mg/kg. In the pyloric ligation model, it was found that EESIL greatly suppressed stomach volume, acidity, pepsin activity, ulcer score, UI, pepsin content, and total protein content. This finding was made possible by the fact that EESIL reduced the length of the pyloric ligated. Also, it had a mucin mean, a percentage suppression of ulcers, and a gastric pH. It was found that EESIL was able to considerably raise the pH of the stomach in ethanol-induced ulcers, stop the growth of ulcers, and reduce the quantity of ulcerative inflammation (UI), ulcer score, and gastric content. These results were observed. It was found that EESIL significantly improved stomach pH and prevented a significant percentage of ulcers in patients with indomethacin-induced ulcers, all while reducing the amount of gastric content, score, and index. This was seen to be the case in both instances. The findings of the study revealed that the EESIL possessed greater antiulcer capability as a consequence of a reduction in the number of offensive factors and an increase in the number of defensive aspects. These changes were brought about as a direct result of the investigation.

REFERENCES


