Gastro-protective effect of crude hexane leaf extract of *Sesamum Indicum* in Rabbits

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Summary: The gastroprotective effect of the hexane extract of the leaves of *Sesamum indicum* (HESI) was investigated in twenty-eight male rabbits. Gastroprotective activity against necrotizing agent induced ulceration was studied. Gastric wall mucus was determined by standard methods. Anti-secretory activity was determined in twelve pyloric-ligated rabbits. Phytochemical and acute toxicity tests were performed. Results showed that the hexane extract of *S. indicum* significantly reduced gastric ulcers induced by necrotizing agent when compared with values obtained with the negative control (p<0.01). Pre-treatment with graded doses of HESI significantly increased gastric mucous in a dose dependent manner when compared with negative control (p<0.001). Misoprostol significantly protected rabbits from necrotizing agent induced ulceration by 99.9% when compared with negative control (p<0.01). Misoprostol also significantly increased mucous turnover with mean gastric mucous of 597.75 ± 4.70µg Alcian blue/g wet tissue when compared with the negative control (p<0.001). Additionally, treatment with HESI resulted in a significant increase in gastric fluid after histamine stimulation in pyloric-ligated rabbits when compared with the negative control (p<0.001). The extract of *S. indicum* also significantly decreased titratable acidity of histamine treated rabbits and increased gastric pH when compared with negative control (p<0.001). HESI significantly protected rabbits from ulceration after histamine administration in 4 hour pyloric ligated rabbits with a percentage protection of 79.3% (p<0.001). The extract also increased the volume of gastric fluid significantly after 4 hr pyloric ligation (p<0.001). Cimetidine protected pyloric-ligated rabbits from ulceration (92.1%) (p<0.001) and also raised the gastric pH (p<0.001), protein concentration in the gastric fluid (p<0.001), and reduced the titratable acidity (p<0.001) when compared with the negative control. The leaves of *S. indicum* possess gastroprotective properties.

Keywords: Gastroprotection, Antisecretory, *Sesamum indicum*, Ulcers, Necrotizing agent, Histamine.

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INTRODUCTION

Gastric ulcer also known as stomach ulcer is an excoriated area in the lining of the stomach. Gastric ulcers among other types of ulcers are illnesses that affect a considerable number of people in the world, some of the causes of this disorders are: stress, smoking, nutritional deficiencies bacterial infection and ingestion of non-steroidal anti inflammatory drugs (Nash *et al.*, 1994). Its pathogenesis is influenced by factors such as acid-pepsin secretion, mucosal barrier, mucous secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermal growth factor) (Sales, 1990). Gastric ulcers emanate from an imbalance between aggressive and protective factors (Al-kofahi and Attah, 1999). The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs. (Peskner and Maricic, 1998).

Various herbal preparations from plants notably *Musa sapientum*, *Tectona grandis*, *Rhamnus procumbens*, *Rhammis triqueria-Wall*, *Withania somnifera*, *Dhatura fastuosa* and *Asparagus racemosus* have been tried for their ulcer protective effect both experimentally (Goel and Sairam, 2002) and clinically (Arora and Sharma, 1990).These plants and many more synthesize substances that are useful to the maintenance of integrity of gastric mucosa in humans and animals. The use of herbs to treat diseases is almost universal among non
industrialized societies and many of these herbs used by humans yield useful medicinal compounds (Lai and Roy, 2004). Sesamum indicum Linn belong to the family pedaliaceae. The plant morphology has been described (Heine, 1963). The constituents of Sesamum indicum (compositions of the seeds, leaves and roots) which includes: flavonoids, vitamins, proteins-sesamin and sesamolin are responsible for the astringent, diuretic, emollient, nutritive and medicinal uses (Brown, 1995).

The drugs that are currently available for the treatment of gastric ulcers are not devoid of side effects. Moreover, pharmaco-economic considerations have made the use of these drugs difficult for common rural dwellers. Therefore, the search for an ideal anti-ulcer drug continues and has been extended to herbal drugs for their better protection, easy availability, low cost, and safety. The purpose of this study is to evaluate the gastroprotective effect of the hexane extract of the leaves of Sesamum indicum in rabbits.

MATERIALS AND METHODS

Plant collection & Taxonomy
The leaves of Sesamum indicum were collected from a field in University of Nigeria, Enugu campus. The leaves were authenticated by a taxonomist at Department of Botany, University of Calabar, Cross-River State, Nigeria. The fresh leaves were dried under a shade and powdered with an electric blender or Mill grater (MS 223, Taiwan).

Animals
Twenty-eight (28) male rabbits weighing 0.7-1.2 kg were procured from the Animal House of the Department of Pharmacology & Toxicology, University of Nigeria, Nsukka. The animals were kept in clean stainless steel cages at the Animal House of the College of Medicine, University of Nigeria, Enugu Campus under standard condition of temperature (25 ± 5ºC) and a light/dark periodicity of 12:12 hrs. These animals were fed with commercially available rabbit pellets (Guinea feed Nig., Plc) and had unrestricted access to clean water ad libitum. All the animals were handled in this work according to Institutional and international guidelines on animal experimentation.

Preparation of Plant Extracts
1500g of powdered leaves were macerated in 5 Litres of n-hexane for 48 hrs. The extract obtained was strained through muslin and subsequently filtered through a Whatman no. 1 filter paper. The aqueous extract was concentrated by freeze drying and the concentrate stored in the refrigerator until required. The filtrate was evaporated to dryness on a rotary evaporator (Model 349/2 Corning, England). The residue obtained was stored in the refrigerator (4°C ± 2°C). The yield of the hexane extract of Sesamum indicum (HESI) was 14.8%. 40g of HESI was dissolved in 0.85% NaCl and made up to 100ml with the same solvent. Appropriate dilutions were made from this for the study.

Acute toxicity test (Median Lethal dose, LD50)
This was performed on mice and the Lorke (1983) procedure of LD50 determination was used.

Phytochemical test
Phytochemicals of the extracts were identified by qualitative chemical tests (Trease and Evans, 2002).

Gastroprotective activity

Necrotizing agent induced ulceration
Sixteen (16) male rabbits were employed and grouped into four groups of 4 rats each. The animals were fasted for twenty-four hours but had free access to water. The animals in groups A to D were given Saline (5ml/kg), 200mg/kg HESI, 400mg/kg HESI, & Misoprostol (200µg/kg) respectively. Administration was by the oral route. Thirty minutes later, each animal was given 10ml/kg body weight of the necrotizing agent (80% ethanol in 0.1N HCl) which is known to produce gastric lesions (Al-Bekairi et al, 1992). After 30 minutes, the animals were euthanized and their stomach opened along the greater curvature. The stomach of each animal was rinsed under a stream of saline and pinned flat on a cork board. The ulcers were viewed with the aid of a magnifying lens(x 10) and each given a severity rating (Main & Whittle, 1975) as follows:

\[
<1\text{mm} = 1\text{(pin point)} \\
1\text{mm}<2\text{mm} = 2 \\
2\text{mm}<3\text{mm} = 3 
\]

The overall total divided by a factor of 10 was designated as the ulcer index (UI) for that stomach. The percentage ulcer protection was calculated as follows (Suzuki et al, 1976):

\[
\% \text{ ulcer protection} = 1 - \left( \frac{\text{ulcer index for test agent}}{\text{ulcer index for negative control}} \right) \times 100
\]

Gastric wall mucus determination
The glandular segments of the stomach were removed and weighed, to determine gastric wall mucus in rabbits after receiving necrotizing agent only or necrotizing agent plus HESI (Corne et al, 1974). Each segment was transferred immediately to 1% Alcian blue solution (dissolved in 0.16M sucrose solution, buffered with 0.05M sodium acetate adjusted to pH 5.8) for 2 hrs, and the excess dye was removed by two successive washes of 15 and 45 minutes in 0.25M sucrose solution . The dye complexed with gastric wall mucus was extracted or eluted by immersion in 0.5M magnesium chloride solution for 2 hrs. A 4ml aliquot of blue extract was
then shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of glandular tissue (net) was then calculated from a standard curve of Alcian blue SGX.

**Determination of anti-secretory activity**

To determine anti-secretory activity, twelve rabbits were grouped into three groups (A to C) of four rabbits each. The rabbits were fasted for 36 hrs prior to the test. The 36-hr fasted rabbits were anesthetized under diazepam/ketamine hydrochloride anesthesia, the abdomen cut open, the stomach brought out and the pylorus ligated. Care was taken to avoid bleeding or occlusion of the blood vessels. The incisions were carefully sutured after doses of HESI (250mg/kg), saline (5ml/kg), and Cimetidine (100mg/kg) were administered intraperitoneally, immediately after pyloric ligation (Shay et al., 1945). Histamine (0.5mg/kg) was administered intra-peritoneally thirty minutes after extract administration. The animals were sacrificed 4 h after the pyloric ligation. The stomachs were removed, contents collected, measured, centrifuged, and subjected to analysis for pH, & titratable acidity against 0.01 N NaOH at pH 7 with neutral red indicator (Ochei & Kolhatkar, 2004). The pH was measured using a digital pH meter (Consort P 107, Belgium). The protein content of the gastric juice was measured with the biuret method of Weichselbaum.

**Statistical Analysis**

The results were expressed where appropriate as Mean ± Standard error of mean. The mean values of test groups were compared with those of the control group and considered significant at p< 0.01, and 0.001 using the student's t-test.

**RESULTS**

Result of acute toxicity studies revealed that the hexane extract of the leaves of Sesamum indicum had an oral LD$_{50}$ >5000 mg/kg in rats. Preliminary phytochemical tests revealed the presence of flavonoids, glycosides, saponins, reducing sugars, carbohydrates and a neutral pH (7.0). The hexane extract of *Sesamum indicum* significantly reduced gastric ulcers induced by necrotizing agent (p<0.01) at 200 and 400 mg/kg body weight compared to values obtained in the negative control (Table 1). Pretreatment with HESI (200 and 500 mg/kg body wt.) significantly increased the gastric mucous (p<0.001) in a dose dependent manner when compared with negative control (Table 1). Mistoprostol (positive control) significantly protected rabbits from necrotizing agent induced ulceration by 99.9% when compared with negative control (p<0.01). Mistoprostol also significantly increased mucous turnover with mean gastric mucous of 597.75 ± 4.70µg Alcian blue/g wet tissue of the negative control (p<0.001). Additionally, treatment with HESI resulted in a significant increase in gastric fluid after pyloric ligation and histamine stimulation compared with the negative control (p<0.001).

The extract of *Sesamum indicum* also significantly decreased titratable acidity of histamine treated rabbits when compared with negative control (p<0.001, Table 2). The HESI (250mg/kg body wt)

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**Table 1**

Effect of *Sesamum indicum* on necrotizing agent induced ulceration in rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Ulcer index (UI)</th>
<th>%Ulcer protection(%U.P)</th>
<th>Gastric mucous (µg Alcian blue/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A saline-5ml/kg</td>
<td>35.00±3.00</td>
<td>--</td>
<td>298.25±18.52</td>
</tr>
<tr>
<td>B 200mg/kg SI</td>
<td>18.50±1.19**</td>
<td>47.1</td>
<td>413.25±9.34**</td>
</tr>
<tr>
<td>C 400mg/kg SI</td>
<td>5.25±1.11**</td>
<td>99.9</td>
<td>497.75±15.33**</td>
</tr>
<tr>
<td>D 200µg/kg MP</td>
<td>0.25±0.25**</td>
<td>99.9</td>
<td>597.75±4.70**</td>
</tr>
</tbody>
</table>

**P<0.01; *** P<0.001; n = 4; SI = Sesamum indicum hexane extract; MP = Misoprostol**

**Table 2**

Effect of *Sesamum indicum* on gastric secretion, titratable acidity, gastric pH, protein concentration of gastric fluid and ulceration in 4 hrs pylorus ligated rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Ul (%U.P)</th>
<th>Vol. of Juice (ml)</th>
<th>Gastric pH</th>
<th>Protein conc (µg/100ml)</th>
<th>Titratable acidity (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Saline)</td>
<td>91.75 ±6.06</td>
<td>13.50±0.54</td>
<td>1.65±0.17</td>
<td>500.00±68.18</td>
<td>207.7±7.76</td>
</tr>
<tr>
<td>B (HESI)</td>
<td>19.00±1.29 (79.3 %**)</td>
<td>21.25±1.42*</td>
<td>4.13±0.10**</td>
<td>1155.00±61.31**</td>
<td>68.25±5.20**</td>
</tr>
<tr>
<td>C (Cimet.)</td>
<td>7.25 ±1.11 (92.1 %**)</td>
<td>24.50±0.98**</td>
<td>4.62±0.14**</td>
<td>1491.75±75.06**</td>
<td>38.50±7.03**</td>
</tr>
</tbody>
</table>

*p<0.01; **p<0.001; n = 4; HESI = hexane extract of Sesamum indicum (250mg/kg b.w), Cimet. = Cimetidine (100mg/kg b.w)
significantly protected rabbits from ulceration after histamine administration in a 4 hour pyloric ligated rabbit with a percentage protection of 79.3% (p<0.001; Table 2). The extract also increased the volume of gastric fluid significantly after a 4 hr pyloric ligation (p<0.01). Cimetidine (H₂-receptor antagonist) protected pylor-ligated rabbits from ulceration (92.1%) significantly (p<0.001) and also raised the gastric pH (p<0.001), protein concentration in the gastric fluid (p<0.001), and reduced the titratable acidity (p<0.001) when compared with the negative control (Table 2).

DISCUSSION
The result of this study showed that oral administration of HESI prevented gastric mucosal injuries by ethanol in hydrochloric acid (Necrotizing agent) and this is the most commonly employed test in the evaluation of anti-ulcer and cytoprotective activities (Borrelli and Izzo 2000; Konturek et al., 1998). It has been suggested that oxygen radical may contribute to the formation of ethanol – induced gastric mucosal lesions and anti-oxidants are protective against the damage caused by these oxidants (Toma et al., 2005). It has been reported that medicinal plants sometimes exhibit their cytoprotective action through their mild irritant property (Al-Yahya et al., 1990; Rafatullah et al., 1990; Rafatullah et al., 1995). Robert et al. (1983) have also described the ability of a mild irritant in protecting gastric mucosa against strong irritants, a protection referred to as “adaptive protection”. These findings were supported by the increase in mucous turnover of the stomach and this together with the reduced ulcer indices confirms the cytoprotective ability of the extract. The cytoprotection may be attributed to alkaloids which are present in S. indicum leaves. Many alkaloids such as hyosine-N-methyl bromide (Buscopan®) have been shown to suppress acid secretion (BNF, 2000). Moreso, alkaloids from Mikania cordata leaves are reported to significantly reduce gastric ulceration induced by diaclofenac sodium (Paul et al., 2000). Sesamum indicum contains phenols, flavonoids, anthocyanins, cyanoergic glycosides, polyacetylene alkaloids and peptides (Chakraborthy et al., 2008). The plant also contains vitamins like riboflavin, nicotinic acid tocopherol, pantothenic acid, thiamine, niacin, and trace amount of ascorbic acid (Chakraborthy et al., 2008). Earlier studies show that antioxidants play a major role in protection against cellular damage by scavenging free radical formation (Szabo, 1989). Furthermore, several flavonoids prevent gastric mucosal lesions produced by various models of experimental ulcer and protect the gastric mucosa against various necrotic agents. They are the most important plant constituents associated with anti-ulcer activity (Martin et al., 1993). Flavonoids also stabilize membranes and this may be the mechanism by which they inhibit ulcer induction. Flavonoids have effect on arachidonic acid metabolism (Akah et al., 1997).

Sesamum indicum is used for the treatment of diarrhoea (Brown, 1995). Drugs used for the management are agents that reduce gastric hypermotility and these have been shown to be beneficial anti-ulcer agent (Mersereau and Hincheay, 1998). Drugs affecting motility, frequency and consistency of diarrhoea also affect secretion (Di Carlo et al., 1994). The significant reduction of histamine induced gastric secretion and inhibition of gastric ulcers by HESI may involve direct reduction of gastric secretion through one or more of possible mechanisms (Murakami et al., 1985; Howden, 1994). It has been demonstrated that certain antulcer drugs increase the amount of gastric mucous secretion in gastric mcosa (Bolton et al., 1976; Robert et al., 1984). This mucous consist of mucin-type glycoproteins which can be detected by amount of Alcian blue (Bolton et al., 1978). The gastric wall mucous depletion induced by 80% ethanol in 0.1N HCl is also one of the mechanisms responsible for gastric lesions (Koo et al., 1986). Alcian blue binds negatively charged particles. The increase in bound Alcian blue indicates the protective effect of orally administered HESI, which may occur through the formation of protecting complexes between the HESI suspension and the mucous that acts as a barrier against agents introduced into the stomach (Clamp et al., 1978). The extract could also have exerted its effect by direct antagonism of H₂ –receptor stimulation by histamine; however, this was not investigated in this work.

The present study supported the anti-ulcer and anti-secretory properties of the hexane extract of Sesamum indicum leaves substantiating its use for the treatment of gastric ailments by traditional medical practitioners in south eastern Nigeria.

Aknowledgement
Our profound gratitude goes to the Laboratory staff of Department of Medical laboratory sciences, University of Nigeria, Enugu campus, Mr Charles Ugwu who made available the plant used, the entire staff of the Animal House of the College of Medicine, UNEC and the Taxonomist, Pastor Frank I. Apejoye, of the Department of Botany, University of Calabar for authenticating the plant material.

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