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Research Article
Enhanced Gastric Mucus Production by *Syzygium guineense* Leaf Extract Mediates is Antiulcer Properties

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Abstract

Background and Objective: *Syzygium guineense* (Myrtaceae) is a leafy forest tree with edible fruits and leaves found in many parts of Africa, where a decoction of its leaves have been used to treat wounds, diarrhea, rheumatism and infections. This study was performed to investigate the anti-ulcerogenic activity of *Syzygium guineense* ethanol leaf extract (SGLE) in experimental models of gastric ulceration. **Materials and Methods:** The gastro protective effect of SGLE (100, 200, 400, 800 and 1000 mg kg^{-1}) was evaluated in acidified ethanol-induced ulcers while doses of 400, 800 and 1000 mg kg^{-1} SGLE were investigated in piroxicam induced ulcers. Its effect on gastric mucus concentration and gastric wall mucus was also determined using alcian blue, a mucus binding dye. Misoprostol (150 µg kg^{-1}) was used as control in all the experimental models. **Results:** At doses of 800 and 1000 mg kg^{-1}, SGLE produced significant ($p<0.001$) protective activity against acidified ethanol-induced gastric ulcers. It also caused a reduction in mean ulcer index and produced 58.1-88.6% protection against piroxicam-induced ulceration. These effects were comparable to those produced by misoprostol. SGLE was also observed to stimulate gastric mucus production, evoking 65.45-84.03% increase of mucus concentration in gastric content and 54.17-85.42% increase in mucus barrier of stomach. Histopathological findings showed a reduction of epithelial and vascular damage of stomach tissue in SGLE-treated group. **Conclusion:** *Syzygium guineense* possesses anti-ulcer effects and gastric mucus barrier and secretion may be implicated in these effects. It may also serve as a useful starting point for anti-ulcer drug development.

Key words: Anti-ulcer, ethanol-induced ulcer, gastric wall mucus, gastroprotective, *Syzygium guineense*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Peptic ulcer disease (PUD) has been a major threat to the world's population over the past two centuries, with a high morbidity and substantial mortality[1]. PUD affects about 4 million people globally and prevalence has shifted from predominance in males to similar occurrence for sexes[2]. Today, the increasing demands of work, family and other socio-economic expectations predispose men and women to high stress levels which can trigger the release of gastric acid in the stomach and duodenum. In some cases, some people also seek to relieve stress by social drinking of alcoholic beverages and smoking, which further aggravates the deleterious effect of stress on the stomach wall. In addition to these, the use of non-steroidal anti-inflammatory drugs for treatment of pain and inflammation in different diseases can be occasioned by the unwanted side effects of gastric ulceration and bleeding. Gastric ulcers ultimately develop due to continuous exposure of the gastric surface to gastric acid and other gastro-aggressive agents, arising from an imbalance between the levels of these agents and that of gastric cytoprotective factors[3]. Aggressive agents may be endogenous (gastric acid, pepsin, bile acid reflux) or exogenous (alcohol, caffeine, tobacco, *Helicobacter pylori*, stress, non-steroidal anti-inflammatory drugs)[4]. Protective factors include a continuous mucus barrier, prostaglandins, an unimpaired cellular regenerative ability, bicarbonate secretion, mucosal vascularization and endogenous anti-oxidants[5]. Nitric oxide has also been recognized to play a vital regulatory role in gastric acid secretion and mucosal protection[6]. Aggressive factors may overwhelm protective factors, leading to injury on gastric epithelia which develops on the inside mucosal lining of the stomach and initial portion of the small intestine.

Drugs used in treating peptic ulcer include proton-pump inhibitors, H_2 receptor antagonists, antibiotics, cytoprotectants like sucralfate, prostaglandin analogues, bismuth salts and antacids. Some of these conventional drugs may however produce undesirable side effects and may be less or equally effective compared to medicinal plants[7]. Such medicinal plants containing active chemical constituents are considered safer, owing to a long history of use in prevention and treatment of various diseases[8]. The anti-ulcer effects of medicinal plant extracts have been attributed to their ability to block acid secretion, increase the endogenous production of protective nitric oxide, prevent free radical-induced damage and stimulate production of gastric mucus[9-11]. *Syzygium guineense* (Myrtaceae) is a small tree with edible fruits, its leaves are often used as source of food in famine[12]. In Mali, Senegal and Sierra Leone a decoction of its leaves have been used in traditional medicine for the treatment of ulcers, wounds, diarrhea, rheumatism and infections[12]. It is widespread in sub saharan Africa where its bark is traditionally used to treat gastrointestinal upset and diarrhea[13,14]. Preliminary phytochemical analysis of its crude extract revealed the presence of triterpenes and anti-bacterial properties[15]. No research report was found on the effect of *Syzygium guineense* on experimental ulcers in literature.

This study was conducted to investigate the effects of the leaf extract of *Syzygium guineense* against acidified ethanol and piroxicam-induced ulceration in rats. It also aims to evaluate its effect on gastric mucus secretion and justify its use as a traditional antiulcer remedy.

MATERIALS AND METHODS

Plant material and extraction: Fresh leaves of *S. guineense* were collected from Suleja, Niger state, Nigeria in August, 2016. The leaves were identified by a plant taxonomist in the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen of the plant material was prepared and deposited in NIPRD herbarium (voucher number: NIPRD/H/6644). The leaves were air-dried under shade for 2 weeks and milled mechanically to coarse power. The powdered plant material was extracted by maceration with ethanol, 80% (v/v) and filtered after 48 h with Whatman filter paper. The filtrate obtained was concentrated under vacuum and concentrated extract obtained was dried to a constant weight on a hot water bath maintained at 50°C. The dry extract obtained was transferred to an air-tight glass container and refrigerated until required for use.

Animals: Adults Wistar rats (80-100 g) of both sexes obtained from Animal Facility Centre, NIPRD were used. The animals were maintained in steel cages at 25±1°C with free access to standard rodent feed and water. They were acclimatized for at least one week prior to each experiment. All applicable institutional standard operating procedures and international guidelines for the care and use of animals were adhered to[16].

Drugs and reagents: Misoprostol (Misoclear®, Marie Stopes International, UK) piroxicam (Felvin 20®, Osaka Pharmaceuticals Pvt., Ltd., India), hydrochloric acid, chloroform, ethanol, sodium hydroxide, sucrose, sodium acetate, alcian blue 8GX (Sigma Aldrich, Germany). All other chemicals, drugs and reagents used were of analytical grade.

Evaluation of extract against ulceration

Acidified ethanol-induced ulcers: This test was performed using the model described by Mizui and Doteuchi[17]. After an overnight 18 h fast, rats were randomized into 7 groups (n = 5). Group 1 was treated orally with 1% tragacanth suspension (5 mL kg^{-1}), while groups 2-7 received different doses of the extract (100, 200, 400, 800 and 1000 mg kg^{-1}) and 150 µg kg^{-1} misoprostol, respectively. One hour after the treatment, each rat received 0.6 mL of 60% ethanol in 0.3 M HCl to induce gastric ulcers. After 30 min, the animals were sacrificed under chloroform anesthesia and their stomachs were dissected out, opened along the greater curvature, distended between two glass plates for better visualization and photographed with a camera. Ulcers were scored based on Best's Ulcer staging index as follows[18]:

- Deep linear ulcer>10 mm = 4
- Deep linear ulcer<10 mm = 2
- Circular ulcer 1-3 mm = 1
- Circular ulcer<1 mm = 0.5
- Fraction of stomach showing evidence of hemorrhage = 2

The results were expressed in terms of percentage inhibition of the induced ulceration. The stomach tissues were preserved for histopathological analysis.

Piroxicam-induced gastric ulceration: Rats were fasted for 48 h and randomized into 5 groups (n = 5) then treated orally with the normal saline, SGLE (400, 800, 1000 mg kg^{-1}) and 150 µg kg^{-1} misoprostol. One hour later, gastric ulcers were induced by oral administration of piroxicam (250 mg kg^{-1}) to the rats. The rats were sacrificed under chloroform anesthesia 6 h later and their stomachs were removed, opened along the greater curvature, distended between two glass plates for better visualization and photographed. The lengths of lesions were measured and scored.

Mucus concentration in gastric content and gastric wall: This assay was carried out in rats by adopting the method described by Adzu *et al.*[19] with slight modification. The rats were fasted for 24 h and then randomized into five groups (n = 6). Group 1 was treated with the 1% tragacanth suspension, groups 2-4 with three dose levels of SGLE (400, 800 and 1000 mg kg^{-1}) while group 5 received 150 µg kg^{-1} misoprostol. Each rat was given 0.3 mL acidified ethanol (60% ethanol in 0.3 M HCl), 1 h after the treatment. After 30 min, the rats were sacrificed under chloroform anesthesia. Gastric content from their stomachs were immediately evacuated into tubes containing 10 mL solution of 0.02% alcian blue in 0.16 M sucrose solution prepared with 0.05 M sodium acetate buffer adjusted to pH 5.3 with 1 M hydrochloric acid. The mixture was incubated at 20°C for 24 h and afterwards, centrifuged at 2500×g for 10 min. The absorbance of the supernatant of each sample was measured at 598 nm using a UV-visible spectrophotometer. The concentration of Alcian blue was calculated by linear regression with a calibration curve obtained from standard serial dilutions of different concentrations of the dye ($y = 4.1941 \times -0.0386$, $R^2 = 0.9958$) and results expressed in mg mL^{-1} of alcian blue.

Separately, the stomach of each rat from was transferred into tubes containing 10 mL of 0.02% alcian blue in 0.16 M sucrose solution in acetate buffer and incubated at 20°C for 24 h. After incubation and the mixture was centrifuged at 2500×g for 10 min and absorbance of the supernatant of each sample was measured at 598 nm and to determine gastric barrier mucus concentration, expressed in mg mL^{-1} of alcian blue.

Histopathological analysis: Ulcerated tissues were preserved in 10% formal saline solution and dehydrated with mixtures of increasing grades of ethanol, clarified in xylene using a tissue processor and embedded in paraffin. After processing, two sections of tissues were made from each block, stained with haematoxylin/eosin and observed under a microscope (×100) for characterization of pathological changes.

Statistical analysis: Results were expressed as mean±SEM. Statistical analysis was carried out using one-way analysis of variance (ANOVA) using Graph Pad Prism 5.0 software. The data obtained was further subjected to dunnet's *post hoc* test, differences between treated groups and the untreated control were accepted as significant at $p<0.05$.

RESULTS

Effect of extract on acidified ethanol-induced ulceration: Treatment with SGLE offered significant ($p<0.001$) protection against the development of gastric ulcers following administration of acidified ethanol as shown in Table 1. A dose-dependent anti-ulcer activity was produced by the extract, with a preventive ratio of 10-73.8% relative to the untreated control group. The effect elicited by 800 and 1000 mg kg^{-1} doses of SGLE were statistically significant ($p<0.001$) and comparable to that of misoprostol.

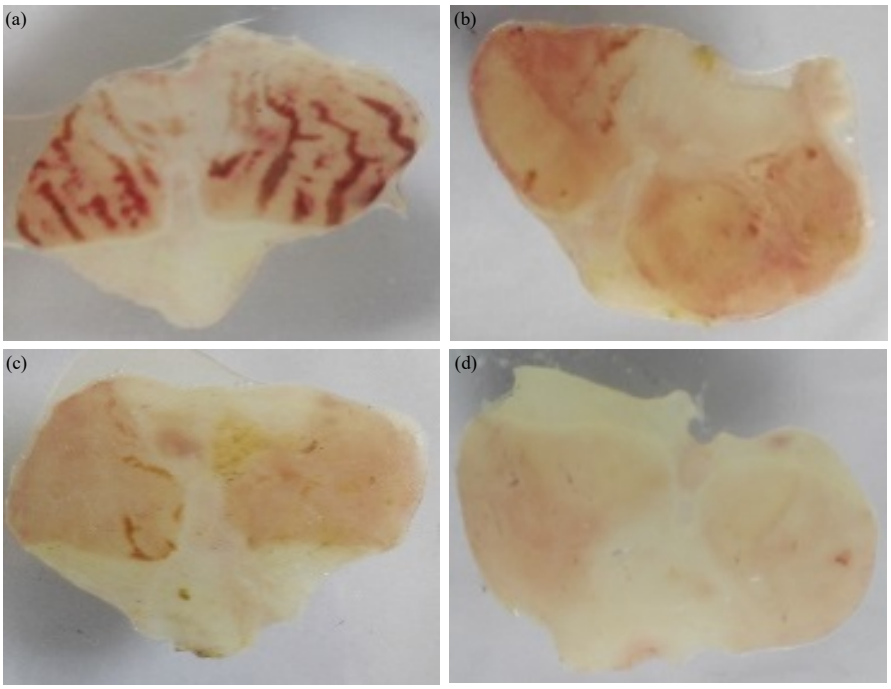

Fig. 1(a-d): Effect of *S. guineense* leaf extract on acidified ethanol-induced ulcers in rats
(a) Vehicle-treated, (b) 800 mg kg^{-1} SGLE-treated, (c) 1000 mg kg^{-1} SGLE-treated and (d) 0.150 mg kg^{-1} misoprostol-treated

Table 1: Effect of SGLE on acidified ethanol induced ulcer in rats

Treatments	Dose (mg kg^{-1})	Mean ulcer index	Preventive ratio (%)
Vehicle	-	25±2.7	-
SGLE	100	21±3.1	10.0
	200	17±4.6	25.0
	400	15±2.6	37.0
	800	6.1±2.1***	73.8
	1000	6.4±1.7***	72.5
Misoprostol	0.150	0.0±0.0***	100.0

Mean ulcer index presented as mean±SD of five numbers of animals in each group (n = 5). Multiple comparisons between treatment groups were performed by Dunnet's test. ***Significantly different from control at p<0.001

Table 2: Effect of SGLE on piroxicam induced ulcer in rats

Treatments	Dose (mg kg^{-1})	Mean ulcer index	Preventive ratio (%)
Vehicle	-	4.375±2.4	-
SGLE	400	1.833±1.8	58.1
	800	0.500±0.2	88.6
	1000	1.160±0.93	73.3
Misoprostol	0.150	0.160±0.17	96.2

Mean ulcer index presented as mean±SD of five numbers of animals in each group (n = 5)

Effect of extract against piroxicam-induced ulceration: Administration of piroxicam (250 mg kg^{-1}) to the vehicle-treated group evoked discoloration and loss of folds, with a mean ulcer index (UI) of 4.375. These features were reduced in SGLE-treated (UI<1.833) and misoprostol-treated (UI = 0.16) groups. The extract produced non-dose dependent anti-ulcer activity against piroxicam-induced ulceration, inhibiting ulceration by 58.1-73.3% at 400-1000 mg kg^{-1} doses. A dose of 800 mg kg^{-1} SGLE yielded a maximum percentage of inhibition (88.6%) and was similar to misoprostol (96.2%) in this regard (Table 2).

Effect of extract on mucus concentration in gastric content and gastric wall: SGLE was observed to stimulate gastric mucus production in a non-dose dependent manner at the doses used (Table 3). Mucus production was observed to increase by 84.03% in the 1000 mg kg^{-1} SGLE-treated group and this was higher than that produced by misoprostol (61.11%). The same trend was seen in Table 4 as SGLE-treated groups increased stomach wall mucus (43.7-85.42%); while stomach wall mucus was observed to increase by 60.42% in the misoprostol-treated group (Table 4).

Histopathology: Histopathological findings revealed epithelial injury in the vehicle-treated group characterized by disruption of membrane, lesions within the membrane, infiltration of inflammatory cells and alterations in cell organization (Fig. 2a). Figure 2b-e shows that in SGLE-treated groups showed the epithelial membrane was preserved with mild inflammatory cell infiltration.

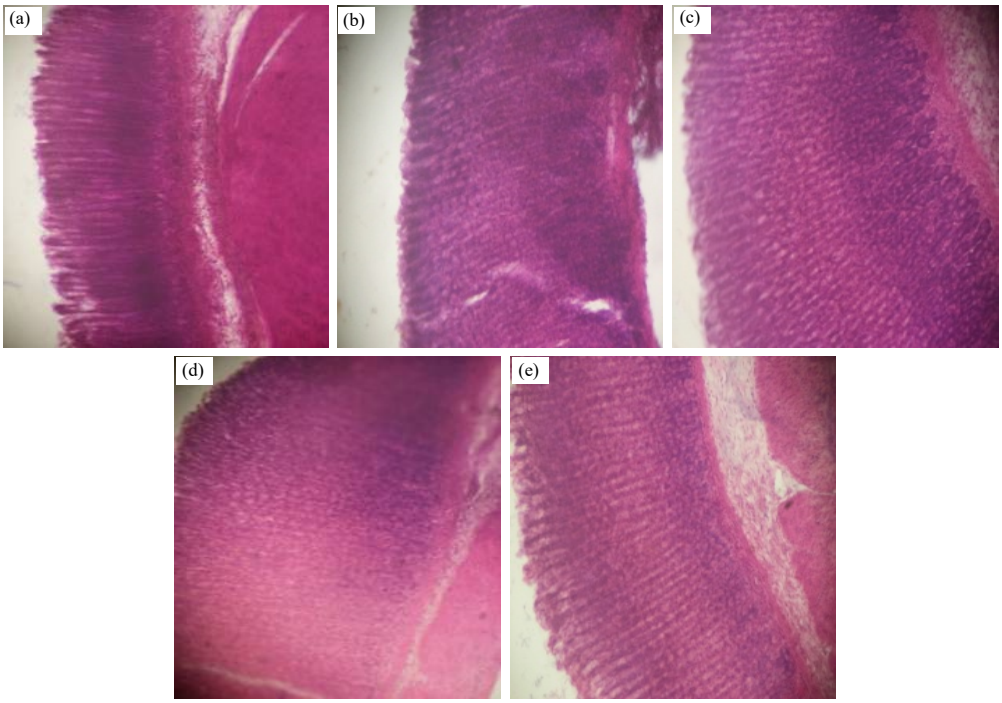

Fig. 2(a-e): Histopathological analysis of stomach tissue of experimental groups. Magnification: ×100. Stain: haematoxylin/eosin
(a) Vehicle-treated, shows ulceration and lesions occurring within the mucous membrane, increased interepithelial spaces, disruption of epithelial lining, (b) 400 mg kg^{-1} SGLE-treated, epithelium of the intestine showing mild thickening as seen in the reduction of interepithelial spaces, (c) 800 mg kg^{-1} SGLE-treated, mucosal gland pattern slightly distorted and mild erosion of mucous membrane, (d) 1000 mg kg^{-1} SGLE-treated, no significant evidence of gastric damage and (e) Misoprostol-treated group

Table 3: Effect of SGLE on gastric mucus content in acidified ethanol-induced ulceration in rats

Treatments	Dose (mg kg^{-1})	Alcian blue (mg mL^{-1}) in gastric content	Increase (%) of mucus in gastric content
Vehicle	-	0.0576±0.023	-
SGLE	400	0.0953±0.026	65.45
	800	0.0667±0.012	15.80
	1000	0.1060±0.027	84.03
Misoprostol	0.150	0.0928±0.028	61.11

Table 4: Effect of SGLE on stomach mucus barrier in acidified ethanol-induced ulceration in rats

Treatments	Dose (mg kg^{-1})	Alcian blue (mg mL^{-1}) in stomach barrier	Increase (%) of mucus barrier
Vehicle	-	0.048±0.007	-
SGLE	400	0.074±0.045	54.17
	800	0.069±0.024	43.75
	1000	0.089±0.033	85.42
Misoprostol	0.150	0.053±0.006	60.42

DISCUSSION

Physiologic gastro-protection is mediated mainly by mucus and bicarbonate production. Prostaglandin E_2 (PGE_2) protects the gastric mucosa by inhibiting secretion of hydrochloric acid and stimulating secretion of mucus and bicarbonate. The extract used in this study stimulated gastric mucus secretion as determined in gastric wall and contents. These likely accounts for its significant gastroprotective effect against ethanol-induced ulcer formation. The protective effect of extract against ethanol-induced gastric lesions may be due to a stimulatory effect on PGE_2-mediated gastric mucus, which produces a protective barrier against noxious agents. The protective role of gastric mucus was shown in a previous study by Vendramini-Costa *et al.*[20], which reported that the antiulcer effects of a styryl lactone, gonithalamin was reversed by a sulfhydryl-blocking agent which inhibits mucus generation. Earlier investigation of the extract showed that it possessed significant antioxidant properties[21] and this may also contribute to ameliorating the ethanol-induced free radical gastric damage. Antioxidants have been reported to play a significant role in the protection of the gastric mucosa against various necrotic agents[22]. Oxygen derived free radicals play an important role in the pathogenesis of various diseases, including PUD. It was reported that some drugs and formulations which possess potent antioxidant action were effective in healing experimentally induced gastric ulcers and the extract used in this study may act in similar manner[23].

Non-steroidal anti-inflammatory drugs (NSAIDs) generally act via inhibition of the enzyme, cyclo-oxygenase (COX)-1

involved in synthesis of prostaglandins responsible for the normal physiological protection of gastric mucosa. The Inhibition of prostaglandin synthesis by NSAIDs also simultaneously activates the lipoxygenase pathway leading to increased synthesis of leukotriene and other pro-inflammatory mediators that can mediate gastrointestinal injury and damage[24]. Piroxicam like other NSAIDs is a non-selective COX inhibitor possessing anti-inflammatory, analgesic and antipyretic properties, employed as an ulcerogen in experimentally-induced ulcers[25]. It causes gastrointestinal bleeding and ulceration, by reversibly inhibiting COX-1 thereby blocking prostaglandin production and generation of reactive oxygen species. Thus, the gastroprotective activity of SGLE against piroxicam-induced ulcer development may be through a reversal of the action of piroxicam on COX-I, which ultimately minimizes piroxicam-induced ulcerative effects[26].

Alcian blue was used to determine the gastroprotective ability of SGLE based on its specificity to bind gastric mucus. A reduction in dye recovery indicates depletion of mucus barrier[19]. Gastric mucus is important in the lubrication of food masses so as to enable movement within the stomach and to facilitate the formation of a protective layer over the epithelium lining of the stomach. The continuous mucus layer consists of an adherent inner layer that covers the mucosa and a thicker loose layer that is continually removed by movement of the luminal content[27]. It plays a significant role as a first line defense against gastrointestinal damage. Ulceration is usually facilitated by the dissipation of the mucus gel and phospholipid layer which usually leads to mucosal injury. Hence, the stimulation of mucus secretion by SGLE may facilitate its protective effect against gastric ulceration. Receptor-mediated gastric mucus exocytosis can be induced by a wide range of secretagogues including PGE2, secretin and some physiologic neurotransmitters via cyclic AMP accumulation in gastric epithelial cells[28]. Gastric mucus can also be secreted by apical expulsion, or throughexfoliation[29]. The extract likely acts a receptor-mediated mucus secretagogue or through other non-receptor-mediated mucosecretory mechanisms.

CONCLUSION

This study demonstrates the anti-ulcerogenic activity of *S. guineense*. It also substantiates its potential as antiulcer drug prototype. Gastric mucus production is a likely mechanism through which *S. guineense* exerts its ulcer-preventive properties and gastro-protective function. Further work is needed to ascertain the specific mechanism underlying its gastric mucoprotective action and other possible mechanisms of anti-ulcer effects.

SIGNIFICANCE STATEMENT

This study discovered the gastric ulcer-preventive action of *S. guineense* that can be beneficial for treatment of ulcers induced by stress or drugs. The findings of this study will help other researchers to uncover the critical areas of novel natural remedies that many researchers were not able to explore. Thus a new theory on beneficial effects of natural plant extracts may be arrived at.

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