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Ulcero-protective Potentials of Methanolic Extract of *Acacia ataxacantha* Leaves in Indomethacin and Stress Induced Gastric Ulcer Models

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Authors' contributions

Author TCA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ROA and OJO managed the analyses of the study. Author ITI managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This study was carried out to investigate the ulcero–protective activity of methanolic extract of *Acacia ataxacantha* leaves (MEAAL) against indomethacin and stress induced gastric ulcer in experimental rats.
Study Design: Administration of MEAAL at the dose of 100mg/kg body weight and 200mg/kg body weight and evaluation of its ulcero-protective activity.
Place and Duration of Study: The experiments were conducted at the Department of Biochemistry, University of Ilorin between September 2012 to May 2013.
Methodology: *Acacia ataxacantha* leaves were extracted with 95% methanol. MEAAL at the dose of 100 and 200mg/kg body weights were administered to male albino rats 30 minutes before the administration of indomethacin and subjecting to stress. Ranitidine was used as a standard antiulcer drug. Animals were then sacrificed and various gastric parameters assessed were gastric ulcer indices, gastric pH levels, gastric ulcer percentage inhibition, which were done in order to explore the ulcero-protective potential of

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the plant.
Results: Induction of ulcer by the intraperitoneal administration of indomethacin and forcing rats to undergo stress by swimming resulted in increased ulcer index and decreased pH. Rats pretreated with MEAAL (100 and 200mg/kg body weights) showed significant reduction in ulcer index to indomethacin and stress induced ulcer models in a dose dependent manner when compared to the negative control group. Also, the significant decrease in the gastric pH levels of both ulcer models, were normalized by MEAAL. The various percentages of gastric ulcers inhibition were statistically significant (P<.05) in the groups pretreated with MEAAL. The overall effect of the extract was comparable to that of the standard drug (ranitidine) used.
Conclusion: These findings validated the potentials of *Acacia ataxacantha leaves* as an ulcero-protective agent and provides a scientific rationale for the use of *Acacia ataxacantha* in Senegalese folk medicine.

Keywords: Acacia ataxacantha; ulcero-protective; gastric ulcer; indomethacin; ranitidine.

1. INTRODUCTION

The term ulcer was first introduced by Quike in 1882 [1] and it is now regarded as the new "plague" of the 21st century [2]. A peptic ulcer is a benign lesion in the lining of the stomach or duodenum, where acid and pepsin bathes the surface. Gastric mucus is the highly hydrated viscoelastic gel that protects the epithelium from mechanical stress, as well as from erosion by acid and pepsin [3]. It is mostly found in people who keep themselves in hurry, become worry and consume curry [4]. Factors such as stress, smoking, alcohol usage, nutritional deficiencies and frequent ingestion of non-steroidal-anti inflammatory drugs such as indomethacin (NSAIDs) have been shown to contribute to gastric ulcer incidences [5]. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium [6]. Gastric ulcers are also associated with considerable morbidity related to chronic epigastric pain, nausea, vomiting, and anemia [7]. Causes of gastric ulcer disease due to NSAIDs include factors that increase acid secretion, reduction of gastric mucosal blood flow, inhibition of prostaglandin synthesis, disruption of mucosal barrier, inhibition of mucus and bicarbonate secretion in the gastro intestinal mucosa [8,9]. Development of peptic ulcers has been linked to "*Helicobacter pylori*" and NSAIDs. However, other causes of this disease have increased particularly affecting the management of peptic ulcers [10]. On rare occasions, a gastric ulcer may become malignant. Ulcer therapy has progressed from vagotomy to anticholinergic drugs, histamine H_2 receptor antagonists, antacids and to proton pump inhibitors [11]. A widely used drug associated with rare idiosyncratic hepatotoxicity is the histamine H_2 receptor antagonist ranitidine (RAN) [12]. It is available over the counter for oral administration or by prescription for parenteral administration for treatment of gastric ulcers, hypersecretory diseases, and gastroesophageal reflux disease. Idiosyncratic RAN occurs in few people taking the drug [13]. Most liver reactions are mild and reversible; however, extensive liver damage have occurred in individuals undergoing RAN therapy [14,15]. Thus, there is need for more effective and safe antiulcer agents. These needs have prompted researchers to investigate the pharmacological features of natural compounds with more effective, less side effects and less expensive for the treatment of gastric ulcer disease [16]. There are many plant extracts used in folk medicine for the treatment of gastric ulcer.

Acacia ataxacantha or Flame thorn is an African tree species with conspicuous red pods and numerous hooked prickles. It is widespread in sub-Saharan Africa from Senegal in the west to Sudan in the east, Namibia, Botswana, Zimbabwe, and in the Transvaal and Kwazulu-Natal and northern part of Nigeria. The genus Acacia belongs to the family Fabaceae [17]. Ethnobotanically, *Acacia ataxacantha* leaves and roots have been used for vitamins, minerals, digestive system disorders, chest ailments infestations, pain, respiratory system disorders. The roots used to protect infants from witchcraft. The roots have been documented for its use in Kenya as a treatment for joint and back ache [18]. *Acacia ataxacantha* pods and seeds have also been documented to be used as a stomachic herbal drug and for dysentery in Abeokuta, southwestern Nigeria [19]. Some Senegalese herbal practitioners claim that the leaves are used in the treatment of gastric ulcer. Inspite of its broad spectrum ethnomedicinal uses, there is a dearth of scientific information published in literature on its acclaimed medicinal potentials especially for gastric ulcer. However, there are also several experimental models for evaluating ulcero-protective activity either for plant crude extracts or pure natural and synthetic compounds [20]. The aim of the present study was to evaluate the ulcero-protective potential of MEAAL in indomethacin and stress induced gastric ulcer models.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *Acacia ataxacantha* were collected in September, 2012 from a farmland in Uruan, Kano State, Nigeria. The plant was identified and authenticated by Mr. Bolu, a taxonomist in the Department of Botany, University of Ilorin, Nigeria. Herbarium specimen voucher no 892 was deposited at Department of Botany of same institution for future references.

2.2 Extract Preparation

Fresh leaves (2kg) of the plant were dried under room atmospheric temperature for two weeks. The leaves were pulverized into fine powder. The powder was macerated in 95% methanol (100g/300ml) for 72hrs under sterile conditions to avoid fermentation. The extract was cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotary evaporator and kept under refrigeration at –4℃ till further use.

2.3 Preliminary Phytochemical Screening

The methods described by Odebiyi and Sofowora [21] were used to test for the presence of saponins, tannins, alkaloids, flavonoids, polyphenols, terpenoids and glycosides in the test extract.

2.4 Acute Oral Toxicity Study

In the acute toxicity test carried out in male rats, we took four doses and 10 rats in each dose of MEAAL at 500,1000,2000,3000mg/kg body weights.

2.5 Ulcero-protective Study

2.5.1 Animal subjects

Male albino rats (*Rattus norvegicus*) with average weight of (130–150) grams were obtained from the Animal Holding Unit of the Faculty of Science, University of Ilorin, Ilorin, Nigeria. Male rats were used because the upper and lower portions of the rumen are analogous to the body of the stomach in man both anatomically and functionally [22]. The animals were housed in clean metabolic cages, placed in well-ventilated house conditions (Temperature: 28-31℃; photoperiod: 12h natural light and 12h dark; humidity: 50-55%). They were also allowed free access to pelletized rat feeds (Bendel Feeds and Flour Mills Ltd., Kwara State, Nigeria) and water *ad libitum*. The cages were cleaned of waste once daily. The experimental protocol has been approved by the Institutional Animals Ethics Committee with the permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), University of Ilorin, Ilorin, Nigeria. Animals were allowed two weeks of acclimatization.

2.5.2 Indomethacin-induced ulcer

Thirty male rats were randomized into five groups of six rats each. Food was withdrawn 24hrs and water 2hrs before the commencement of experiment [23]. Normal saline served as the vehicle of oral administration of MEAAL, indomethacin was administered intraperitoneally.

Animals in group I served as control and received 2ml normal saline orally.
Animals in group II received indomethacin and served as negative control.
Animals in group III received 100mg/kg body weight of the extract orally.
Animals in group IV received 200mg/kg body weight of the extract orally.
Animals in group V received the standard drug, ranitidine 20mg/kg body weight orally.

Ranitidine at the dose of 20mg/kg body weight has been successfully used in previous study as a standard drug in the treatment of ulcer [24]. Hence, the drug at the dose of 20mg/kg body weight was chosen. After 30 minutes, indomethacin was administered at a dose of 20mg/kg body weight to group III, IV and V. After 3hrs, the animals were sacrificed with excess anaesthetic ether. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored [25]. Ulcer index (UI) and gastric ulcer inhibition percentage of each of the groups pretreated with extract were calculated using standard methods [25,26]. Collection of the gastric juice was from oxyntic cells of the stomach which was put into a graduated test tube and then centrifuged at 1000r for 10min. The supernatant was however collected and pH was determined in gastric juice [27].

2.5.3 Stress induced ulcer

Thirty male rats were randomized into five groups of six rats each. Stress ulcers were induced by forcing the male albino rats to swim in a glass cylinder containing water to the height of 35cm maintained at 25℃ for 3hrs [28]. Animals were fasted for 24hrs prior to the experiment. Normal saline served as the vehicle of oral administration of extract.

Animals in group I served as control and received 2ml of normal saline orally.
Animals in Group II served as negative control.
Animals in group III received 100mg/kg body weight of the extract.
Animals in group IV received 200mg/kg body weight of the extract.
Animals in group V received the standard drug, ranitidine 20mg/kg body weight orally.

After 30 minutes, the animals in groups II, III, IV and V were forced to swim in a glass cylinder containing water. After this, the animals were sacrificed with excess anaesthetic ether. Each stomach was opened along the greater curvature and examined for gastric erosions under a dissecting microscope (10×). Gastric juice was collected into centrifuge tubes and centrifuged at 1000r/min for 10 minutes. The pH of the gastric juice was recorded using a pH meter. The ulcer index(UI) and gastric ulcer percentage inhibition was determined. This was quantified by a scoring technique whereby normal gastric mucosa was scored 0, punctuate haemorrhage (pinpoint ulcers) was scored 0.5, one or two small hemorrhage ulcer was scored 1.0, and ulcer greater than 3 mm in diameter was scored 2.0. Ulcer index: The number of ulcers was counted and scoring was undertaken according to the reported method [29]. The ulcer index was determined by using the formula:

Ulcer index = 10/X
Where: X = Total mucosal area/Total ulcerated area

2.6 Statistical Analysis

All the values were expressed as mean ± standard error of the mean (S.E.M) of six animals each across the groups. Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) with the spss soft-ware (version 16) Duncan Multiple Range Test (DMRT). *P* value<.05 was considered to be statistically significant.

3. RESULT

3.1 Preliminary Phytochemical Screening

The preliminary phytochemical screening indicated the presence of alkaloids, polyphenols, flavonoids, saponins, tannins, terpenoids. However, glycosides were completely absent.

3.2 Acute Oral Toxicity Study

MEAAL showed a lethal effect in the dose range of 1000 to 3000mg/kg as seen in changes in skin and fur, eye colour and mucous membrane, and also respiratory, circulatory, behavioral patterns of the male rats. So we had taken MEAAL at a minimum and maximum dose of 100mg/kg and 200mg/kg of body weight of rats for further studies.

3.3 Indomethacin-Induced Gastric Ulceration

Intraperitoneal administration of indomethacin induced severe gastric ulceration. MEAAL pretreatment and ranitidine groups showed significant reduction (*P*<.05) in the incidence and severity of ulceration. MEAAL and ranitidine showed significant increase (*P*<.05) in pH also a significant reduction (*P*<.05) in ulcer indices relative to the negative control group were

observed. The effect of MEAAL was comparable to that of the standard drug, ranitidine. These results are shown in Table 1.

Table 1. Effect of MEAAL pretreatment on pH, ulcer indices and inhibition percentage in indomethacin - induced gastric ulceration

Group	pH	Ulcer indices	Inhibition %
I (Control)	2.49±0.21[a]	-	-
II (Negative control)	1.98±0.11[b]	14.88±1.20[a]	-
III MEAAL (100mg/kg)	3.48±0.21[c]	1.89±0.16[b]	65
IV MEAAL (200mg/kg)	3.52±0.11[c]	1.87±0.08[b]	69
V Ranitidine (20mg/kg)	3.45±0.23[c]	1.92±0.18[b]	68

Data are mean±S.E.M of six determinants. Values carrying letters different from the negative control for each parameter are significantly different (P<.05)

3.4 Stress-Induced Gastric Ulceration

Forcing male albino rats to swim in a glass cylinder containing water for 3 hrs induced severe gastric mucosal lesions. MEAAL pretreatment, at a dose of 100mg/kg body weights and 200mg/kg body weights, showed significant ulcero-protective potential against these lesions (P<.05). Standard drug ranitidine at 20 mg/kg body weight included in the study exhibited significant protection as well (P<.05). There was also a significant (P<.05) dose-dependent increase and reduction in the pH and ulcer indices respectively relative to negative control group in the MEAAL pretreatment groups. The effect of MEAAL was comparable to that of the standard drug, ranitidine. These results are shown in Table 2.

Table 2. Effect of MEAAL pretreatment on pH, ulcer indices and inhibition percentage in stress-induced gastric ulceration

Group	pH	Ulcer indices	Inhibition %
I (Control)	2.51±0.18[a]	-	-
II (Negative control)	1.50±0.14[b]	32.88±3.16[a]	-
III MEAAL (100mg/kg b.w)	2.69±0.19[c]	9.53±0.76[b]	52
IV MEAAL (200mg/kg b.w)	2.76±0.11[c]	5.39±0.31[c]	71
V Ranitidine (20mg/kg)	2.79±0.15[c]	5.52±0.18[c]	85

Data are mean±S.E.M of six determinants. Values carrying letters different from the negative control for each parameter are significantly different (P<.05)

4. DISCUSSION

Indomethacin is an indole derivative act not only as anti-inflammatory but also analgesic and antipyretic. This drug has better ulcerogenic potential than other NSAIDS [30]. Indomethacin reduces the prostaglandins PG by inhibiting both COX enzymes, that impares the mucosal barrier thus rendering gastric mucosa more susceptible to injury [31,32]. In our study, indomethacin caused damage on the glandular mucosa of high ulcer index consequently leading to a fall in pH level by the induction of H^+/K^+ ATPase in gastric parietal cells. In contrast to it, pre-treatment with MEAAL at the dose of 100 mg/kg and 200mg/kg body weights significantly decreased (P<.05) the ulcer index which was comparable to the effect exerted by standard antiulcerogenic drug ranitidine as evident in Table 1. The fall in pH levels in indomethacin induced gastric ulcer in albino rats, were increased with MEAAL pre-treatment at the dose of 100mg/kg body weight and 200mg/kg body weights as shown in

Table 1. Suppression of acid remains the mainstay treatment for this disease, as maintaining a gastric pH over 3 for 18–20h day ensures the healing of most of the gastric ulcers after 8 weeks of treatment [33,34]. The MEAAL protected in a dose-dependent manner, the gastric mucosa from indomethacin - induced ulcers with significant protection (P<.05). Ranitidine, substance orally used as a standard drug in this experiment, at a dose of 20mg/kg, provided similar protection against the gastric ulcers induced by indomethacin. There was a significant increase (P<.05) in the gastric ulcer inhibition percentages relative to the negative control of the MEAAL pretreated and ranitidine groups as shown in Table 1. The mechanism of action through which the extract inhibited ulcerogenesis by indomethacin is very unclear because indomethacin inhibits cyclooxygenase and produces the well recognized prostaglandin cytoprotective deficiency [35], which contributes to the pathogenesis of gastric ulcers.

Forcing albino rats to swim in water is one of the best models in rats to induce ulcer. The model provides both emotional stress as well as physiological stress to the animal. Stress-induced gastric damage is generally considered to be the result of an imbalance between aggressive and defensive mucosal factors. The imbalance determines the outcomes of gastric lesions under the exposure to noxious etiologies represented with either a relative increase in aggressive factors or a considerable decrease in protective factors [36,37]. The stress model used here increases acid secretion and decreases gastric mucosal pH [38]. Brozozowski et al. [39] observed that exposure of rats to 3hrs of stress induced by cold and restraint produced gastric lesions, and that this effect was accompanied by a decrease in prostaglandin (PGE_2) generation and a marked fall in gastric blood flow. In the present study, as shown in Table 2, MEAAL pre-treatment at the dose of 100mg/kg and 200 mg/kg body weights increase the pH when compared to the negative control. MEAAL at the dose of 100mg/kg and 200mg/kg body weights have also shown significant ulcero-protective activity in a dose dependent fashion, by decreasing the high ulcer index evident in the negative control group as shown in Table 2. This is also similar to the action of the standard ulcer drug ranitidine. This is also made evident, when MEAAL at a dose of 100mg/kg and 200mg/kg body weights also increase the gastric inhibition percentages when compared to the negative control. The results of the present study indicated that MEAAL pre-treatment displays a significant cytoprotective activity, since it significantly reduced gastric lesion induced by a non-steroidal, anti-inflammatory drug, Indomethacin and stress induced ulcers. The ulcero-protective potential of the extract is probably due to a reduction in gastric acid secretion since it caused an upsurge of gastric pH, thus, decreasing ulcer indices. The preliminary phytochemical analysis indicated the presence of alkaloids, polyphenols, flavonoids, saponins, tannins, terpenoids. However, glycosides were completely absent. These secondary metabolite classes are related to ulcero - protective activity. Many studies have releaved the antiulcerogenic properties of flavonoids [40,41]. The formation of lesions by different necrotic agents are inhibited by the flavonoids, they are also said to protect the mucosa [42]. Selective inhibition of prostaglandin F2ά and protection of gastric mucosa as been linked to the saponins from previous study making it to exhibit an ulcero protective action [43,44]. Considering the presence of these secondary metabolites, the ulcero–protective activity elucidated by the *Acacia ataxacantha* leaves may be connected to the presence of these phytoconstituents.

5. CONCLUSION

In conclusion, this study validated the ulcero-protective potentials of *Acacia ataxacantha* leaves in indomethacin and stress induced gastric ulcer using rat models. The result shed light and gives a scientific rationale on the reasons for its use in Senegalese folk medicine.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "principles of laboratory animal care" (nih publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee."

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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