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Curative Potential of *Hyptis suaveolens* Aqueous Extract on Ethanol-Induced Gastric Ulcer in Albino Wistar Rats

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ABSTRACT

Objective: *This study was carried out to investigate, the curative potential of the aqueous extract of H. suaveolens (AEHS) against ethanol-induced gastric ulcer.* ***Materials and methods:*** *Total 25 Wistar rats were randomly divided into 5 groups consisting of 5 rats each in A, B, C, D, and E. Group A served as normal control group and was given distilled water. Group B served as the ulcer control group, the ulcer was induced but was not treated. Group C served as positive control, the ulcer was induced and treated with cimetidine (20 mg/kg). Group D and E served as extract treated groups, the ulcer was induced but treated with 250 mg/kg and 500 mg/kg body weight of the aqueous extract respectively. The experiment lasted for 3 weeks and after the drug administration the animals were sacrificed and the stomach was harvested for gross and histological studies.* ***Results:*** *It was observed that there was a reduction in all ulcer index in both the groups that received the extract and the group that received the standard drug. The standard drug and the extract reduced all ulcer index to 0.00 ± 0.00, 0.07 ± 0.07 and 0.22 ± 0.09, $p \leq 0.014$, $p \leq 0.001$, $p \leq 0.002$, respectively. The gross study showed complete healing in the group that received the standard drug and incomplete healing in the extract treated groups. There was a regeneration of tissues in both the extract treated group and the standard drug, as observed in the histological studies.* ***Conclusion:*** *In conclusion, the aqueous extract of H. suaveolens (AEHS) has a curative effect against ethanol-induced a gastric ulcer.*

Keywords: Gastric ulcer, *H. suaveolens*, Cimetidine, Ethanol, Histology

INTRODUCTION

Recently, the incidence of gastric ulcer disease has been on the increase both in developed and underdeveloped countries. It is considered a serious health problem that poses a threat to the economy and thus has been subjected to many investigations both clinically and experimentally [1]. Gastric ulcer disease is the most common gastrointestinal disorder and occurs whenever any factor causes the stomach secretion of aggressive factors (acid and pepsin) to overwhelm that of the digestive factors (mucus and bicarbonate) resulting to the damage of the stomach mucosal layer [2-4]. Such factors include too much consumption of non-steroidal anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* infection, smoking and stress [5-8].

There are many available orthodox antiulcer drugs such as proton pump inhibitor, H2-blockers, and antibiotics but the major challenge remains the adverse effects and resistance of these drugs with prolonged use [9,10]. Thus, there is a need to investigate the effectiveness and toxicity of medicinal plant formulations used in traditional medicine for the prevention, treatment, and management of gastric ulcers. Many medicinal plants have been shown to possess antiulcer potentials in animal studies [10,11]. One of the medicinal plants that may have a great prospect in the treatment of gastric ulcer is *Hyptis suaveolens*.

Hyptis suaveolens (*H. suaveolens*) commonly known as wild spikenard is an important ethnobotanical medicinal plant which belongs to the family Lamiaceae. It is an erect and strappingly aromatic herb spreading along roadsides, rail tracks, wasteland, watercourses, pasture and open forests where the soil is well drained [12]. Almost all parts of this plant are used in traditional medicine to treat various diseases. Infusion of the leaves is used to treat swellings, abscesses, hemorrhoids, uterine infection, colic, and stomach ache. The decoction of the roots is highly valued as an appetizer and is also used to treat a headache [13]. Phytochemical screening of the plant showed the presence of essential oils, alkaloids, flavonoids, phenols, saponins, terpenes and sterols [14].

PATIENTS AND METHODS

Plant Collection and Authentication

The fresh leaves of *H. suaveolens* were collected within the premises of the College of Health Sciences, Ebonyi State University, Abakaliki and was identified and authenticated by a taxonomist, Nwankwo, O. E in the Department of Biological Sciences, Ebonyi State University, Abakaliki where a herbarium specimen exists.

Preparation of Aqueous Extract

The fresh leaves of *H. suaveolens* were air dried and blended into powder using an electric blender of the model: NG-99, China. About 200 g of the powder was dissolved in 1000 ml of distilled water for 48 hours and shook thoroughly at 12 hours interval to ensure adequate extraction. The mixture was filtered through Whatman No. 1 filter paper and the filtered extract was concentrated using a rotary evaporator maintained at 45°C and thereafter, stored in a refrigerator at 4°C for proper preservation. Prior to oral administration, the extract was reconstituted in distilled water to give the required doses of 250 mg/kg and 500 mg/kg body weight [15].

Chemicals and Drugs

Ethanol, diethyl ether, and cimetidine were purchased from Godal Pharmacy, Abakaliki, Ebonyi state.

Ethical Approval

The experimental procedures and technique used in the study were in accordance with accepted principles for laboratory animal use and care. All protocols used were approved by the Ethics Committee of Faculty of Basic Medicine, Ebonyi State University.

Experimental Animals

Total 25 Wistar rats weighing between 130-180 g were purchased from the animal house of the Department of Anatomy, College of Health Sciences, Ebonyi State University, Abakaliki. They were maintained under standard laboratory conditions in the animal house of the College of Health Sciences, Ebonyi State University, Abakaliki and allowed free access to standard feed and water.

Animal Grouping

The 25 Wistar rats were randomly allotted into 5 groups consisting of 5 rats each in a group.

- Group A served as a negative control group and was given distilled water
- Group B served as positive control group and was given absolute (95%) ethanol (1 ml)
- Group C served as reference control group and was given cimetidine (20 mg/kg)
- Group D and E served as the test groups and received 250 mg/kg and 500 mg/kg body weight of the aqueous extract respectively

All the rats in each group were well feed throughout the experiment. All administrations were through the oral route using stainless steel intubation needle. The rats in Group B to E were fasted for 18 hours to ensure an empty stomach before gastric ulcer induction using Ubaka model [11,16].

Induction of Ulcer

1 ml/kg body weight of 95% ethanol was orally given to rats in Group B to E and ulceration was established after 1 hour of induction by sacrificing one rat out of the 5 rats in each group [10]. The stomach was removed and sectioned along the lesser curvature to observe the ulcerations.

Treatment

The animals in the experimental Group D and E were treated with 250 mg/kg body weight and 500 mg/kg body weight respectively, while Group C received the reference standard drug cimetidine (20 mg/kg), for 3 weeks.

Animal Sacrifice

The animals were sacrificed after 3 weeks of drug administration; the stomach was harvested and studied for ulcer index using the method described by Kulkarni, as shown below [17]:

- 0: Normal coloration
- 0.5: Red coloration
- 1.0: Spot ulcers
- 1.5: Hemorrhagic streaks
- 2.0: Deep ulcers
- 3.0: Perforation

The ulcer index was expressed as the sum of scores given to the gastric lesions as described by Tan [16].

Gross Evaluation of Gastric Mucosa

The mucosal layer of the stomach of each rat was rinsed with normal saline to remove gastric contents and blood clots and then observed for any changes in the anatomy of the mucosa. Photographs of the gross structure of gastric mucosa were taken for proper observation and documentation.

Histological Studies

The stomach was fixed in 10% formol saline and processed using routine histological techniques.

Statistical Analysis

Data from the experiment were expressed as mean ± S.E.M (standard error of the mean). For data comparison, one-way analysis of variance (ANOVA) was used. Differences between groups were considered statistically significant at $p \leq 0.05$. All the tools were from International business machine statistical package for social sciences (IBMSPSS) version 20.

RESULTS

Ulcer Indices after 21 Days Treatment

The ulcer control group was shown to have mean ± SEM of 1.07 ± 0.15 while reference control showed mean ± SEM of 0.00 ± 0.00. Group D indicated mean ± SEM of 0.07 ± 0.07 while group E had mean ± SEM of 0.22 ± 0.09 (Table 1).

Table 1 Descriptive Statistics of the various groups after treatment

Groups	Ulcer index (Mean ± SEM
Group B (Ulcer but untreated)	1.07 ± 0.15
Group C (positive control)	0.00 ± 0.00
Group D (Ulcer+250 mg/kg of extract)	0.07 ± 0.07
Group E (Ulcer+500 mg/kg of extract)	0.22 ± 0.09
$p \leq 0.014$; $p \leq 0.001$; $p \leq 0.002$	

Comparative Gross Appearance of the Stomach of Wistar Rats at the Beginning and the End of the Experiment

The macroscopic appearance of the normal mucosa and control mucosa on day 1 and after 21 days is shown in Figures 1-5.

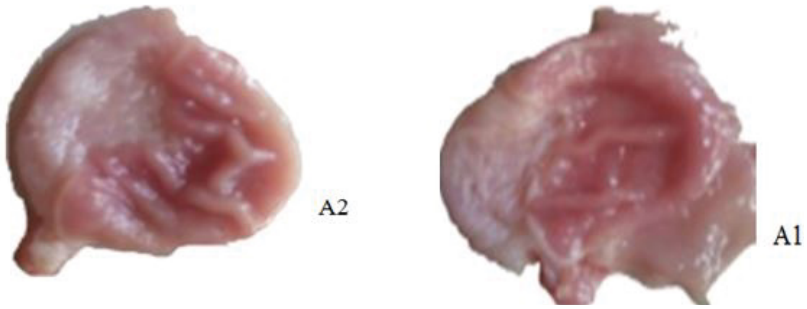

Figure 1 Macroscopic appearance of normal mucosa (normal control) in day one (A1) and after 21 days (A2): The mucosa appeared in folds

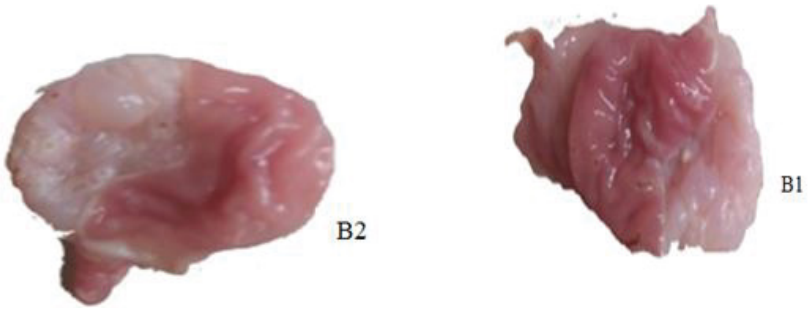

Figure 2 Macroscopic appearance of ulcerated mucosa (ulcer control) in day one (B1) and after 21 days (B2): Mucosal ulcerations were seen in day one which persists after 21 days

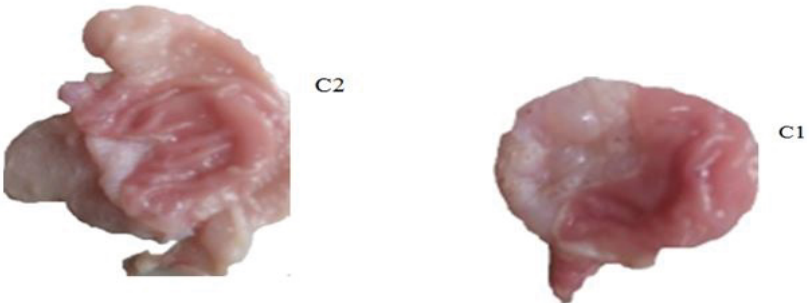

Figure 3 Macroscopic appearance of the mucosa of the reference control group in day one (C1) of ulceration and after 21 days treatment (C2): Ulcerations were seen in the mucosa on day one which was completely healed after 21 days treatment

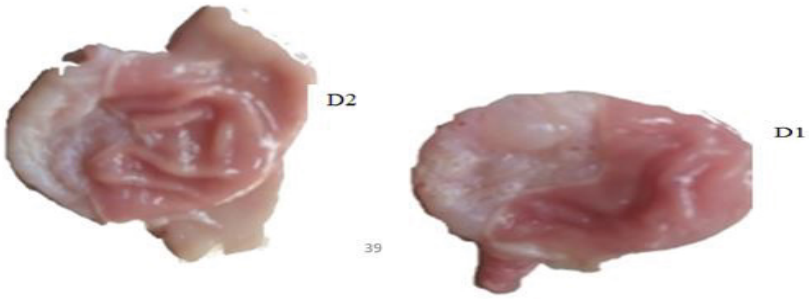

Figure 4 Macroscopic appearance of the mucosa of the test group (250 mg/kg) in day one (D1) and after 21 days treatment (D2): There was ulceration in the mucosa in day one with little or no ulcerations after 21 days treatment

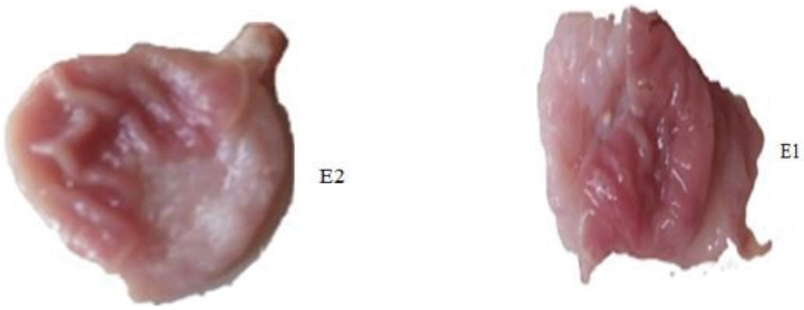

Figure 5 Macroscopic appearance of the stomach of the test group (500 mg/kg) in day one (E1) and after 21 days treatment (E2): There was ulceration in the mucosa in day one which healed a little after 21 days treatment

Histology

In Figure 6 the photomicrographs of group A normal control group section of the stomach is shown.

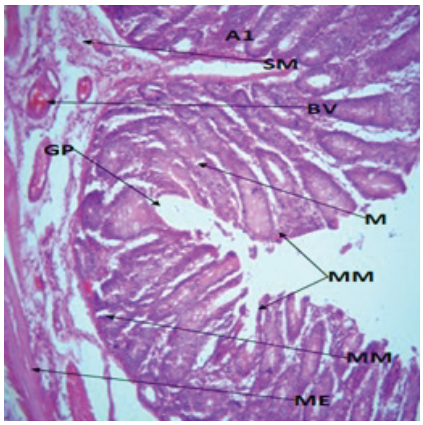

Figure 6 Photomicrographs of group A normal control group section of the stomach (x150)(H/E) shows normal stomach with muscular external (ME), mucosa (M), gastric pit (GP), the muscularis mucosa (MM), the submucosa (SM) and the blood vessels (BV)

Figure 7 shows the photomicrograph of group B section of stomach administered with 1 ml ethanol.

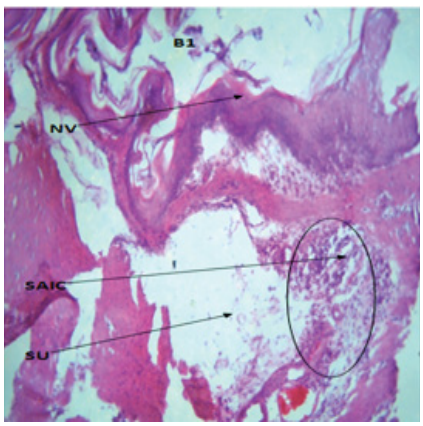

Figure 7 Photomicrograph of group B section of stomach administered with 1 ml ethanol shows severe effect with severe ulceration within the submucosa (SU), necrotic (N) appearance of the mucosa layer (N) and a severe aggregate of inflammatory cell (SAIC)

Figure 8 explains the photomicrograph of group C section of stomach administered with 20 mg/kg cimetidine.

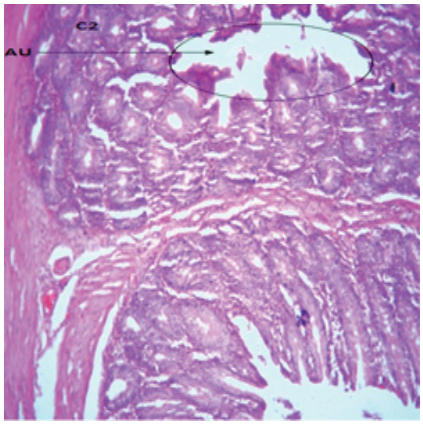

Figure 8 Photomicrograph of group C section of stomach administered with 20 mg/kg cimetidine shows moderate regeneration with mild focal ulceration (MFAU)

Figure 9 shows the photomicrograph of group B section of stomach administered with 1ml ethanol.

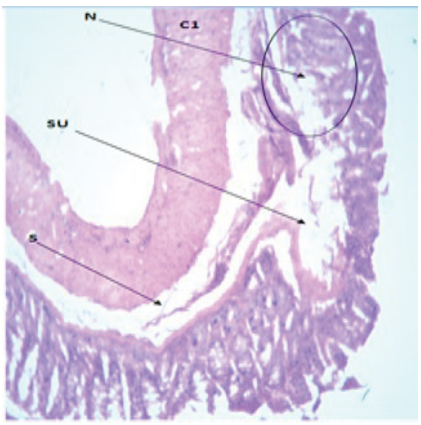

Figure 9 Photomicrograph of group B section of stomach administered with 1ml ethanol shows severe effect with severe dilation and ulceration within the submucosa (MU/LSM) and the mucosa

Figure 10 shows the photomicrograph of group D section of stomach administered with 250 mg/kg extract.

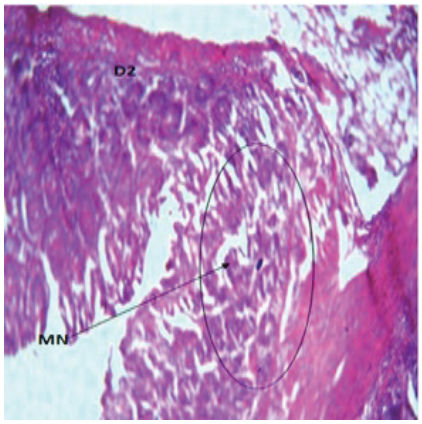

Figure 10 Photomicrograph of group D section of stomach administered with 250mg/kg extract shows moderate regeneration with a moderate focal area of necrosis (N) within mucosa layer

Figure 11 shows the photomicrograph of group E section of stomach administered with 500 mg/kg extract.

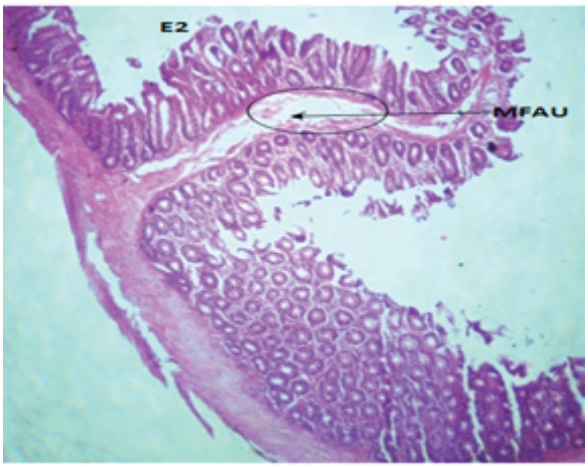

Figure 11 Photomicrograph of group E section of stomach administered with 500 mg/kg extract shows moderate regeneration with mild ulceration (MFU) within the submucosa

DISCUSSION

Gastric ulcer is a benign lesion of the stomach occurring preferentially along the small curvature in the transition zone between the body and the antrum where the mucosa epithelium is exposed to aggressive factors [18]. According to the statistics of 2005, the incidence of gastric ulcer was up to 80%, especially in the western world [19]. Ethanol has been implicated with gastric ulcer and penetrates the stomach mucosa rapidly, damaging the plasma and cell membrane which leads to increase in the permeability of the intracellular membrane to water and sodium resulting to damage of the gastric mucosa [20]. It disrupts the mucosal barrier of the stomach by dissolving the gastric mucus thereby causing backflow of acid [21]. It stimulates the production of free radicals which causes an increase in lipid peroxidation destroying the mucosal membrane.

There are many products used for the treatment of gastric ulcers, including H2-blockers (Cimetidine), M1-blockers, proton pump inhibitors which decrease secretion of acid, and sucralfate and carbenoxolone, which provide mucosal defense. The efficacy of this drugs is limited by their numerous side effects [18]. The results of this study showed that cimetidine reduced all ulcer index to zero, which indicate complete healing and agree with previous findings that it is used in the treatment of gastric ulcer.

In the present study, following oral administration of ethanol to the rats, ulcerations of different sizes were observed on the gastric mucosa of all the groups. Groups treated with AEHS significantly decreased the mucosal damage grossly and the ulcer index when compared with the ulcer control group. The antiulcer properties of *H. suaveolens* (AEHS), could be attributed to the presence of phytochemicals such as flavonoids and triterpenoids [22]. Flavonoids and triterpenoids have been reported to possess cytoprotective effect [23,24]. The study revealed a significant reduction of all ulcer index by *H. suaveolens* (AEHS), it was more effective at extract dose of 250 mg/kg when compared to extract dose of 500 mg/kg. The macroscopic anatomy of the stomach of the extract treated groups showed significant healing. The healing was more in the lower dose (250 mg/kg) when compared to the higher dose (500 mg/kg) [25].

CONCLUSION

The extract of *H. suaveolens*, has a curative potential against ethanol-induced gastric ulcer.

DECLARATIONS

Conflict of Interest

It is affirmed that this manuscript is an honest, accurate, and transparent account of the study being reported, no important aspects of the study have been omitted. All authors and co-authors worked honestly.

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