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Gastro-protective Effect of *Ageratum conyzoides (Linn)* Leaf Extract on Aspirin-Induced Ulcer in Albino Rats

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Abstract

This study examined the protective effects of *Ageratum conyzoides* leaf extract on ulcers in albino rats. Thirty-two male rats were divided into two groups: twelve for the acute toxicity study and twenty for the ulcer experiment, divided into four groups. Group A (normal control) received 2ml of distilled water and 0.03ml Tween 80. Group B (negative control) was administered 400mg/kg aspirin. Group C was treated with 20mg/kg omeprazole and 400mg/kg aspirin, while Group D received 500mg/kg of *Ageratum conyzoides* extract and 400mg/kg aspirin. Rats were fasted for 24 hours with access to water, and Groups C and D received their respective treatments 30 minutes before aspirin. No mortality was recorded in the acute toxicity test, even at 5000mg/kg. Gastric juice volume significantly increased in Group B but decreased in Groups C and D ($p<0.05$). pH levels dropped significantly in Group B ($p<0.05$), while free acidity, total acidity, and pepsin activity increased, indicating ulceration. These values were reduced in Groups C and D. Ulcer index increased in Group B, but the percentage inhibition in Groups C and D were 62% and 66%, respectively, compared to Group B. The anti-ulcerogenic effects of *Ageratum conyzoides* could be linked to its bioactive compounds. The novelty of the study is that Ageratum conyzoides (500 mg/kg) provides better gastro-protection (66% inhibition) than omeprazole (62%) in aspirin-induced ulceration. It also emerges as a potent, natural, and cost-effective alternative to synthetic anti-ulcer drugs. This study supports its further development for ulcer management.

Keywords: *Ageratum conyzoides,* Aspirin, Omeprazole, Ulcer, Bioactive

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1. Introduction

An ulcer is an open sore or lesion usually found on the skin or mucosal membranes of the body tissue. A peptic ulcer is a sore in the lining of the stomach or duodenum, the first part of the small intestine. If a peptic ulcer is found in the stomach, it is called a gastric ulcer. If it occurs in the duodenum, it is called a duodenal ulcer. The gastro-duodenal mucosa constantly secretes mucin, which acts as a barrier that prevents self-digestion by enzymes and secretions such as acids, and in ulcers, the protective cover is compromised [1]. This may be due to an imbalance between impaired mucosal defensive factors (mucus, bicarbonate, prostaglandins, nitric oxide, and growth factors) and aggressive factors (acid, pepsin, *Helicobacter pylori,* and bile salts) in the mucosal membrane of the stomach and duodenum [2]. Gastric ulceration has been attributed to various causes such as stress, hormones, drugs, alcohol, smoking, and ingestion of certain foods [3]. Recently, *Helicobacter pylori* has been implicated in antral gastritis, peptic ulcer, and gastric malignancy [4-5]. Non-steroidal anti-inflammatory drugs (NSAID) are a class of drugs that are grouped and provide antipyretic (fever-reducing) effects, analgesic and anti-inflammatory effects. Examples include aspirin, ibuprofen, Naproxen, and Diclofenac, etc [6]. These classes of drugs have been reported to induce ulcer formation in over 40–50% of patients with rheumatoid arthritis and cardiovascular diseases.

Aspirin, also referred to as acetylsalicylic acid (ASA), is a drug often used as an analgesic to relieve minor aches and pain, as an antipyretic to reduce fever, and as an anti-inflammatory medication. It is used long-term at low doses, to help prevent heart attacks, strokes, and blood clot formation in patients. Aspirin inhibits the activities of cyclooxygenase (COX) enzymes, and it does so in irreversible NSAIDs. Aspirin affects both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (Cox-2) [7]. The inhibition of Cox-1 may cause gastrointestinal ulcers and bleeding [8-

10]. These side effects are caused by overdosing the systemic circulation with aspirin without a medical or doctor's prescription. *Ageratum conyzoides*, commonly known as goat weed or Billy Goat weed, is an annual herb with a long history of traditional medicinal uses in many countries in the world, especially in the tropical and subtropical regions [11]. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities [12-13]. *Ageratum conyzoides* have been used in various parts of Africa, Asia, and South America for curing various diseases. It is used as a purgative, treatment for ophthalmia, colic, ulcer, and wound dressing [14]. In some African countries, the plant is indicated for the treatment of mental and infectious diseases as well as headache and dyspnea [15-16]. Here in Nigeria, apart from its use in ulcer treatment, it is also used in the treatment of skin diseases, diarrhea, and wound dressing [12-16]. However, there are several medicinal uses of the plant [17].

2. Materials and Methods

2.1. Ethics statement

The Ethics Committee of the School of Health Sciences, Abia State University, Uturu, approved the study (ABSU/SOHT/2024/039). All animals received care following the WHO's ethical code for the animal experimentation [18].

2.2. Animals

A total of thirty-two male albino rats weighing 160-250g were used for the study. The rats were purchased from the animal house, department of Physiology, College of Medicine, University of Nigeria, Enugu campus. Then they were kept in the animal house, department of Biochemistry, Faculty of Biological and Physical Science, Abia State University, Uturu to acclimatize for 14 days (2 weeks) under standard laboratory conditions with free access to commercial feed and water adlibitum.

2.3. Plant materials

The leaves of *Ageratum conyzoides* were harvested from a bush located at Okigwe, Imo state Nigeria. The plant was identified and authenticated by a taxonomist at the Department of Plant Science and Biotechnology, Abia State University, Uturu. A voucher specimen was kept in the herbarium for future reference.

2.4. Plant Preparations

The leaves of the plant were dried under shade and milled using an electric blender. Thereafter, 1000g of the powdered leaves of *Ageratum conyzoides were* soaked in 3 liters of chloroform for 24 hours, then strained with a muslin cloth, and filtered using Whatman number (1) filter paper. The filtrate was allowed to evaporate to dryness in the open air and thus a greenish color extract was formed. The concentration of the extract will be made in distilled water for the experiment. Then twenty 20g of the extract was dissolved in 10 ml of 0.03% Tween 80 and made up to 100 ml with distilled water.

2.5. Acute Toxicity Test

Twelve (12) Male albino rats will be used to determine the LD_{50} as described by Lorke, (1983) [19].

2.6. Animal Grouping

At the completion of the two weeks acclimatization period, the twenty (20) rats were randomly placed into four groups of five rats each.

Group A: Normal control (Rats that received 5ml distilled water and 0.03ml Tween 80)

Group B: Negative control (Rats that received 400mg Aspirin only).

Group C: Positive control (Aspirin- induced ulcer rats treated with a reference drug Omeprazole).

Group D: Test group **(**Aspirin-induced ulcer rats treated with leaf extract of *Ageratum conyzoides*)

2.7. Ulcer Induction

The animals were starved 24 hours before the commencement of the experiment but had free access to clean water. The animals in group A were given 5ml/kg of distilled water mixed with 0.03ml 3% Tween 80 only, while Group C and D received the reference drug (20mg Omeprazole) and 500mg/kg of *Ageratum conyzoides* leaf extract respectively. Then after thirty minutes, all animals in groups B, C, and D were given 400mg Aspirin except Group A which is the normal control. At the end of the experiment, which lasted for four hours, animals were anesthetized with chloroform and sacrificed per the National Institute of Health Guidelines for their care and use of laboratory animals [20]. The stomachs of the animals removed, cut open through the greater curvature, and then washed in normal saline. The stomachs spread and pinned flat on plywood using thumbtacks. Thereafter, a magnifying glass used to observe their stomachs using Main and Whittle [21] method described below.

2.8. Calculation of Ulcer Scores/ Index

Normal stomach =0
Red coloration=0.5
Spout ulcer =1
Hemorrhagic streaks=1.5
Ulcer>3mm<5mm=5
Ulcer >5mm=3
The total score divided by a factor of 10 was designed as an ulcer index for their stomach.
This is as follows;

$$\text{Ulcer index} = \frac{UA+US+UP}{10}$$

Where; UA= Average number of ulcers per animal
US= Ulcer severity of score
UP=% of animals with ulcers.

2.9. Gastric juice collection

After sacrificing the animals, the stomachs were excised carefully by keeping the esophagus closed, and they were opened along the greater curvature which enhanced the collection of the gastric juice.

2.10. Gastric juice volume

The volume of gastric juice was determined by the method described by Deshphande [22].

2.11. Determination of P^H

The PH of the gastric juice was measured by using a P^H meter.

2.12. Pepsin Activity

This was determined by the method described by Debnath [23].

2.14. Percentage Inhibition

The percentage of ulcer inhibition in the animals was calculated using the formula of Suzuki [25] as follows;

Percentage ulcer inhibition = $_1$ - Ulcer index for the test agent / Ulcer index for the control $\times_{100}$

2.15. Statistical Analysis

The data were represented as mean ± triplicate determinations of (n=5), using analysis of variance (ANOVA) for a completely randomized block design, and the group mean was compared for significance with Turkey Multiple Test at $P< 0.05$ using SPSS version 11.

3. Results and discussion

The acute toxicity test LD_{50} result showed no mortality recorded even at a high dose of 5000mg/kg administration of the leaf extract (Table 1). The study results showed a significant increase of $P<0.05$ in the gastric juice volume in group B but a significant decrease of $p<0.05$ was seen in C and D respectively. There was a significant reduction in p^H level in group B compared to group A while a significant increase was observed in groups C and D respectively. Free acidity, total acidity, and pepsin activity increased significantly in group B but had a reduction in C and D when compared to group A. More also, ulcer index showed an increase in group B 1.32±0.00, which indicates ulceration of the gastric mucosa. However, a decrease $P<0.05$ was observed in group C 0.49±0.00 and D 0.44±0.00 respectively when compared with group B (negative control), while the percentage inhibition of groups C and D were 62% and 66% respectively. The percentage inhibition showed a significant increase of $p<0.05$ in C when compared with B as shown in Table 2. The reference drug (Omeprazole) was able to help in inhibiting and reducing negative effects of Aspirin in the rats. The leaf extract of *Ageratum conyzoides* observed to have provided stronger protection to rat's gastro mucosa, thus preventing ulcerations of animals in that group. Aspirin-induced gastric ulcer is a useful model to induce severe ulceration in experimental animals [26-27].

Scientists have established the relationship between acid production, pH, volume of gastric juice, and gastric ulcers [28-30]. The essential criteria that determine the status of the mucosal defense barrier against the offensive assault of

2.13. Free Acidity and Total Acidity

Free acidity and total acidity were measured by the method described by Kulkarrni [24].

acid pepsin is the quality and quantity of mucus secretion. Increased mucosal secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms including lessening stomach friction during peristalsis and acting as an effective barrier to back diffusion of hydrogen ions. Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H^+ ions [31]. The depletion in mucus concentration in gastric stomach tissue can be attributed to the depletion of mucosal prostaglandins by inhibiting COX activity. The inhibition of mucosa prostaglandin production occurs rapidly following oral administration of aspirin. This is correlated with the rapid absorption of these drugs through the mucosa [32]. Previous studies have also shown that deficiency of prostaglandins plays a key role in aspirin-induced gastrointestinal side effects [33]. Table 2 shows effects of *Ageratum conyzoides* leaf extracts on ulcerative indices of the aspirin-induced rats.

In Group B, Aspirin caused a significant increase in the ulcer indices. The results of the study showed a significant increase $P<0.05$ in the gastric juice volume in group B but a significant decrease of $p<0.05$ was seen in C and D respectively. There was a significant reduction in p^H level in group B compared to group A while a significant increase was observed in groups C and D respectively. Induced ulcers are thought to be caused due to increased acid or pepsin in the stomach. The leaf extract at doses of 500mg/kg and Omeprazole 20mg/kg respectively, significantly decreased the ulcer index, pepsin activity, free and total acidity ($p<0.05$) when compared to their respective negative controls. It also significantly increased the gastric barrier mucus (percentage inhibition) to 66% and 62% respectively when compared to their controls. Most interestingly, *Ageratum conyzoides* leaf extract at 500mg was able to provide adequate protection to the mucosa against ulceration. However, the Omeprazole at 20mg administration also demonstrated a protective ability in ameliorating the effects of aspirin although not as potent as *Ageratum conyzoid*. The high potency of protection observed in leaf extract of Ageratum could be attributed to bioactive components found in plants [34]. Plants are known to contain lots of antioxidants, which are capable of inhibiting activities of free radicals and also reduce imbalance caused by the aggressive factors which give rise to the ulceration [35-36].

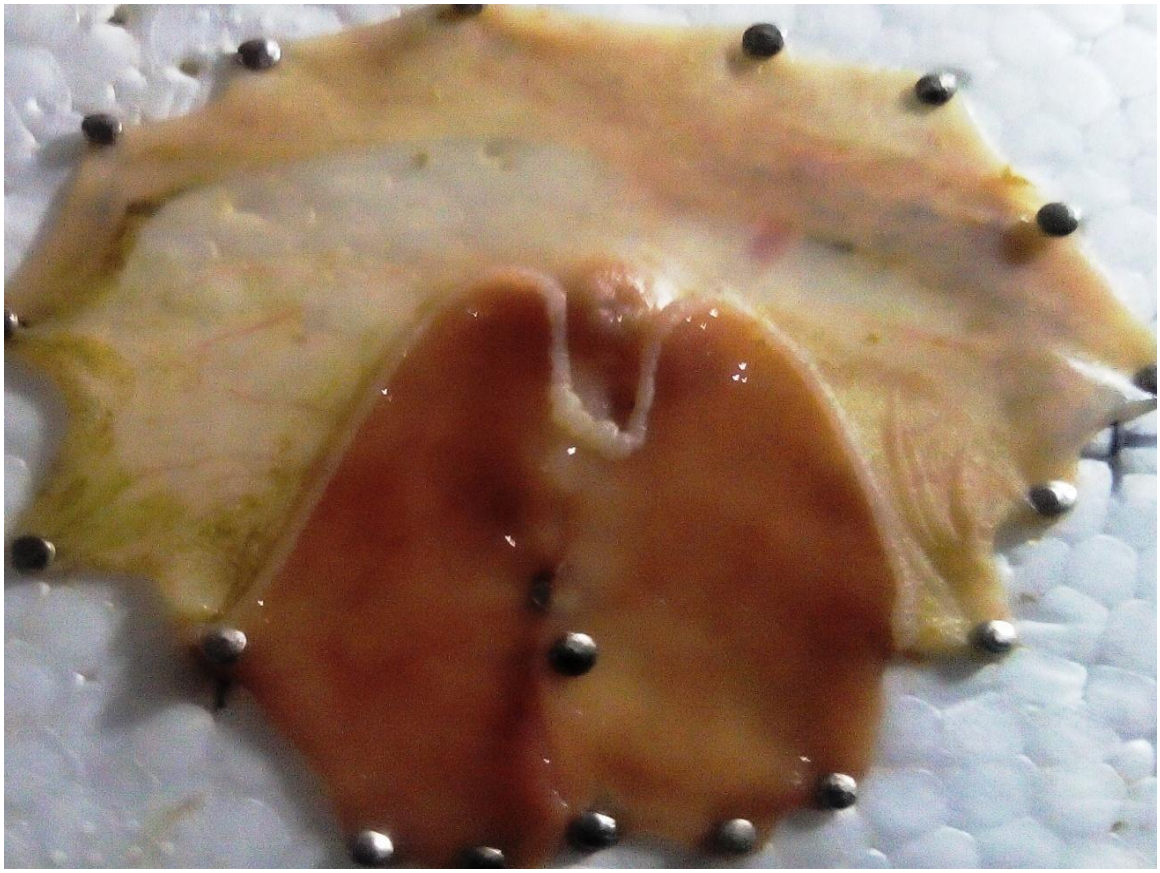

Plate 1: Group A (positive control), showing normal stomach, no erosion or laceration

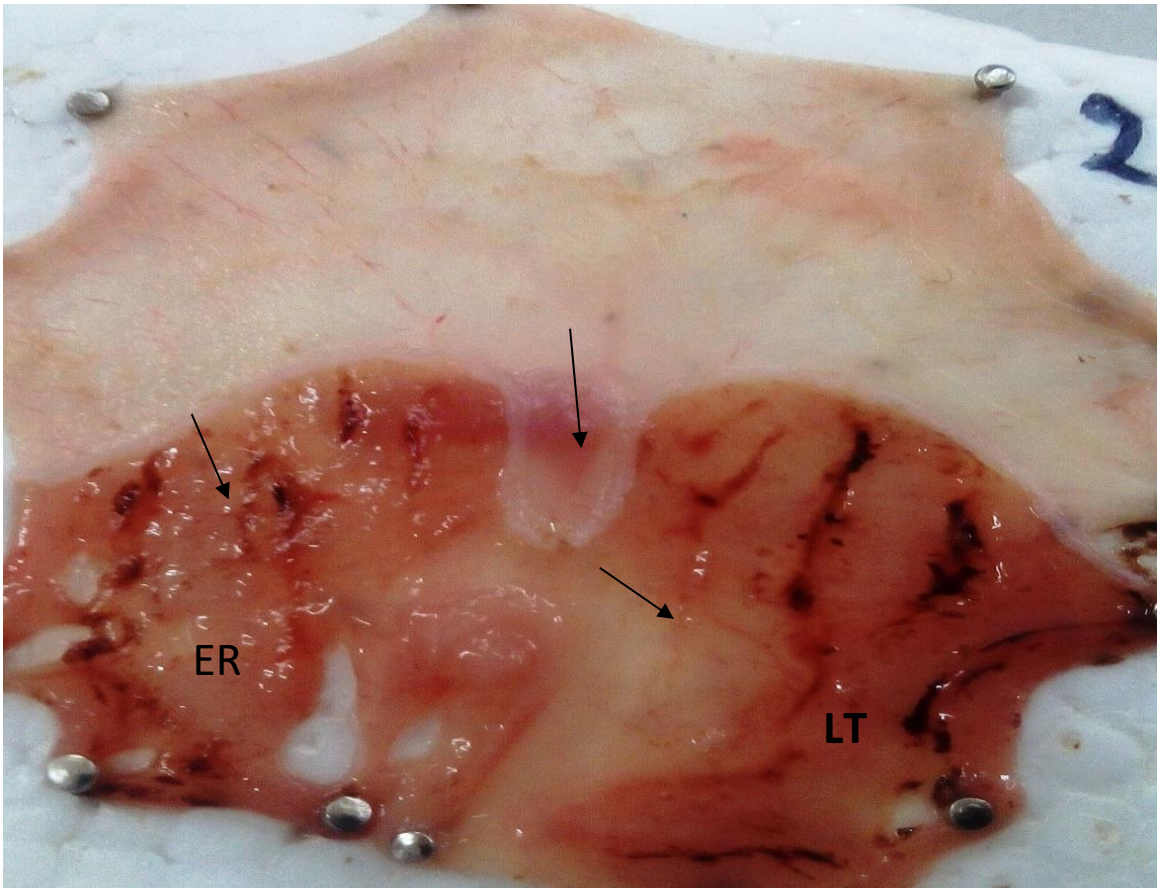

Plate 2: Group B (Negative control), showing laceration and erosion of the mucosal walls of the stomach
LT: = laceration and ER= Erosion

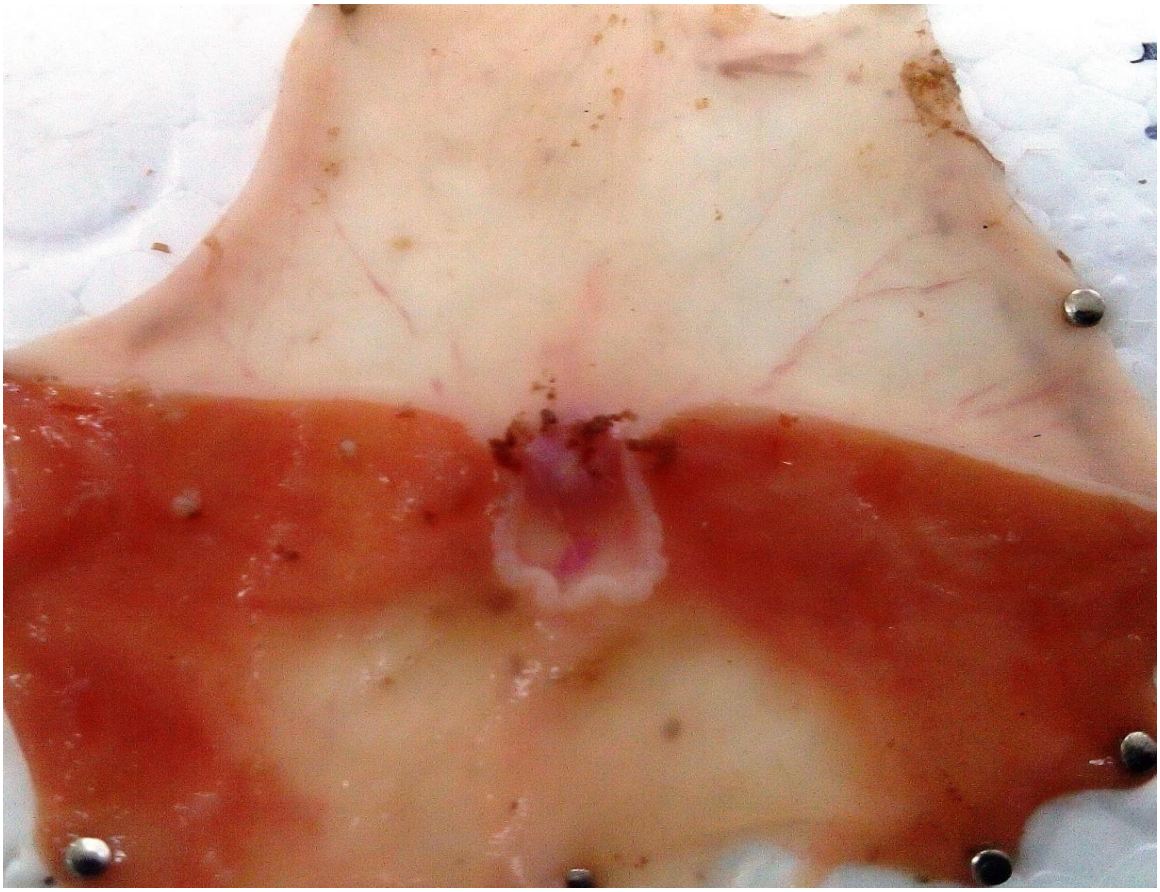

Plate 3: Group C: Stomach of Reference drug control rat after treatment with 20mg Omeprazole

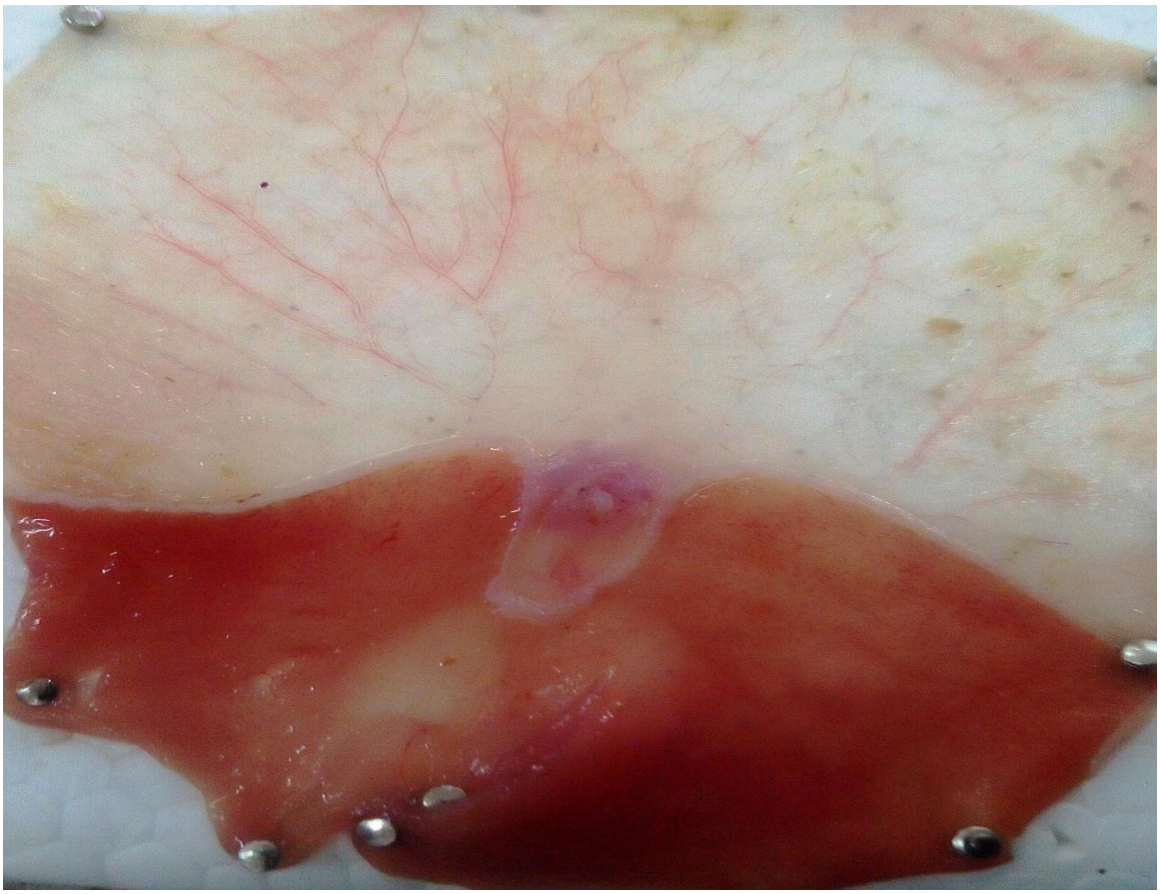

Plate 4: Group D: Showing stomach of the plant extract test group after treatment with *Ageratum conyzoides*

Table 1: Result for acute toxicity evaluation of *Datura metel* leaf extract

Groups	No of mice	Mortality
Phase 1		
I	**3**	**None**
II	**3**	**None**
III	**3**	**None**
Phase 2		
IV	**3**	**None**
V	**3**	**None**
VI	**3**	**None**

Table 2: Effects of *Ageratum Conyzoides* leaf extract on ulcerogenic indices of aspirin-induced ulcer

|Groups	Gastric Juice vol. (ml/4hr)	P^H	Free Acidity (mEq/L)	Total Acidity (mEq/L)	Pepsin (µmolThyro/ml)	Ulcer index	% Inhibition
A Normal control 5m/kg+0.03ml Tween 80	1.0±0.14[a]	2.99±0.04[a]	116.47±2.82[a]	137.11±1.77[a]	239.91±26.61a	0.00	-
B Negative control 400mg/kg Aspirin	2.27±0.22[c]	1.95±0.07[b]	199.83±5.67[d]	198.71±9.13[b]	327.87±19.99[b]	1.32±0.00[a]	0.00[a]
C Positive control(Reference drug)20mg/kg Omeprazole+400 Aspirin	1.16±0.50[b]	3.04±0.37[a]	145.43±4.98[c]	149.65±4.98[c]	89.57±05.21[a]	0.49±0.00[b]	62[b]
D Test Extract (*Ageratum Conyzoid*)500mg +400mg Aspirin	0.75±0.09[a]	4.53±0.49[c]	130.40±3.07[b]	128.30±2.87[a]	132.84±3.73[a]	0.44±0.00[b]	66[c]

The results represented are Mean ± SD of triplicate determinations (n =5). Mean with the same superscript, in the same column are not significantly different ($P < 0.05$).

4. Conclusion

Results from this study largely show that the leaf extracts of *Ageratum conyzoides* could help in providing protection against ulceration and ameliorating the negative effects of Aspirin and other NSAIDs which could cause inflammation. It also provides proof of great potency and better performance compared to some anti-ulcer agents as seen in Table 2. The anti-ulcerogenic and protective properties observed in the leaf extracts of *Ageratum conyzoides* could be attributed to the presence of the bioactive constituents such as flavonoids etc. found in plant. Therefore, the efficacy of the plant extract observed in the study justifies its use in traditional medicine for the treatment of disease.

Statements and Declarations

The authors declare that no funds, grants, or other support were received during preparation of this manuscript.

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