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RESEARCH ARTICLE

Evaluation of Anti-ulcer Activity of Aqueous Extract *Combretum paniculatum* Vent in Mice and Rats using Acidified Ethanol-induced Ulcer Model

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ABSTRACT

The present study was carried out to investigate the anti-ulcer activity of 80% aqueous leaf extract of *Combretum paniculatum* (CP) in rats and mice. Various concentrations of CP aqueous extract (200, 400, and 800 mg/kg body weight) were used to determine the anti-gastric ulcer activities of methanol extracts of CP in mice and rats in an acidified ethanol-induced model. Cimetidine (150 mg/kg) was used as a reference drug. The following parameters, such as gastric fluid volume, PH, ulcer score, percentage ulceration, and percentage inhibition of ulcer index, were determined. An acute toxicity study was carried out, and the LD_{50} was determined as well as the histopathological study. Data were analyzed using one-way analysis of variance followed by a Tuckey post hoc test, and $P < 0.05$ was considered significant while $P < 0.01$ was considered highly significant. The CP aqueous extract highly significantly $P < 0.01$ reduced the gastric index by 41.8%, 51.3%, and 82.3% in the acidified ethanol-induced ulcer model with reference to the standard drug. The oral median lethal dose (LD_{50}) was found to be >2000 mg/kg body weight. CP aqueous extract possesses both dose-dependent and time-dependent anti-ulcer activities. Bioactives such as flavonoids, tannins, phenols, alkaloids, terpenoids, glycosides, and steroids were present, while saponins were found to be absent. This study confirms the anti-ulcer pharmacological activities of CP aqueous extract; we suggest that further investigations should be carried out to isolate specific phytochemicals as well as to determine the mechanisms of action.

Keywords: Acidified ethanol-induced ulcer model, Anti-ulcer activity, Cimetidine, *Combretum paniculatum*

INTRODUCTION

Combretum paniculatum (CP) belongs to the *Combretaceae* family. It is a cadent shrub with tailing branches. The leaves of the plants are used in traditional medicine for the treatment of various diseases such as stomach pain, malaria, infections, wounds, ulcers, cancer, and diarrhea (Banskota *et al*., 2000; Abera, 2014).[1,2]

Peptic ulcer (PU) is a gastrointestinal disorder that affects about 10% of the world's population.[3] The disease is characterized by inflammation of the stomach or duodenal lining; these are ulcers on the digestive tract membrane. PU disease is categorized into duodenal ulcers affecting the duodenum and gastric ulcers, which affect the stomach.[4] PU disease is an imbalance between gastric offensive

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factors such as acid and pepsin and defensive mucosal factors like environmental and host factors.[5] Several factors are also associated with the occurrence of PU, including a stressful lifestyle, alcohol consumption, use of steroidal and non-steroidal anti-inflammatory drugs, *Helicobacter pylori* infections, smoking, lower socio-economic status, and family history.[6]

PU disease is complicated by gastrointestinal bleeding, perforations, penetration of ulcers into adjacent organs, and gastric outlet obstruction.[7] Anti-ulcer drugs such as proton pump inhibitors, histamine 2 receptor antagonists, cytoprotectants, demulcents, anticholinergics, antacids, and prostaglandin analogs are used for the treatment of ulceration.[8] Clinical evaluation of these drugs shows that there are incidences of relapses, adverse effects, and the danger of drug interactions during ulcer therapy.[6]

Medicinal plants have always been the main sources of novel drugs for the treatment of gastric ulcers because the majority of the world population relies on herbal medicines for primary health care due to their better cultural acceptability, better accessibility, and lesser side effects.[9]

In this study, the gastroprotective activities and phytochemical composition of CP methanol extract were evaluated in order to authenticate the anti-ulcer activities of this plant.

MATERIALS AND METHODS

Chemicals and reagents

Methanol (BDH Limited, Poole, England), ethanol (BDH Limited, Poole, England), chloroform, formaldehyde, hydrochloric acid, distilled water, sodium hydroxide, phenolphthalein, omeprazole (Greenlife Pharmaceuticals Ltd., Lagos, Nigeria), ketamine, and xylazine.

Centrifuge, measuring cylinder, mortar and pestle, separating funnel, beakers, cotton wool, retort stand, PH meter, rotary evaporator, and soxhlet apparatus.

Collection of plant material

Fresh leaves of CP were collected in June 2022 from Owere Obukpa in Nsukka LGA, Enugu State. The plant material was identified and authenticated by a taxonomist (Mr. Felix) in the department of pharmacognosy and environmental medicine at UNN with the voucher number PCG/UNN/0987.

Preparation and extraction of plant material

The plant materials were separated from unwanted materials, air dried, and ground to powder. Cold maceration was adopted to extract 2.5 kg of the powdered plant material with 7.5L of distilled water. The powder was allowed to macerate for 48 h in distilled water by vigorous shaking at room temperature using a soxhlet apparatus. The mixture was filtered after 48 h, and the filtrate was subjected to dryness using a rotary evaporator. The evaporation flask was heated evenly, and materials with a lower boiling point, the solvent stream were recycled in the receiving flask, following cooling by the glass condenser.[9] The percentage (%) yield was determined according to this formula.[10]

$$\text{Yield}\ (\%) = \frac{\text{Weight of the extract yield}}{\text{Weight of the plant material}} \times 100$$

Qualitative and quantitative phytochemical analysis

The phytochemical composition of the CP extract was determined according to standard procedures,[11] while the quantities of various phytochemicals present in the CP extract were estimated according to.[10]

Animals

Wistar albino rats (150–250 g) and Swiss albino mice (20–30 g).

Experimental animals

Adult female Wistar Albino rats and Swiss Albino mice of either sex bred in the animal care unit of Bingham University were used for the study. The animals were housed in polypropylene plastic cages with wood shavings as bedding material and maintained under standard conditions such as

light, humidity and room temperature (19–25˚C; 12 h light and dark cycles). Standard pellets and distilled water were provided ad libitum. Ethical approval was sought from the Bingham University Research Ethics Committee.

Grouping of animals and dosing

Thirty (30) female Wistar albino rats of 150–250 g and Swiss Albino mice of 20–30 g were randomly divided into five different groups of six rats or mice in each group. The negative control (NC) group was given distilled water (Group 1). Treatment groups II, III, and IV were given CP crude extracts of 200 mg/kg (CP 200), 400 mg/kg (CP 400), and 800 mg/kg (CP 800), respectively. Group V was treated with 150 mg/kg of cimetidine.

Acute toxicity study

Three female Swiss albino mice were randomly grouped and kept in a cage. After being fasted for 2 h, 1000 mg/kg of the extract dissolved in distilled water was administered to one mouse and observed for any signs of toxicity for 24 h. The following day, the second mouse received 1500 mg/kg, and the remaining mouse was administered 2000 mg kg of the extract and observed for any gross changes for 14 days according to the OECD 425 guideline 2008.[12]

Acidified ethanol-induced ulcer

Thirty Swiss albino mice (20–30 g) of either sex were randomly divided into five groups of six mice in each group. The mice were fasted for 24 h with free access to water only prior to the experiment. Group 1 (NC) received 10 mL/kg of distilled water. Group 2 (the positive control) received Cimetidine (150 mg/kg), while groups 3,4, and 5 received 200,400, and 800 mg/kg of the aqueous extract of CP, respectively. One hour after the pretreatment, ulcers were induced with acidified ethanol (10 mL/kg) administered orally. After another hour, the animals were sacrificed under an overdose of xylazine and ketamine anesthesia. The abdomens were opened by a midline incision below the xiphoid process, and the stomachs were removed, and opened by cutting along the greater curvature. The stomachs were removed and the contents were drained into tubes and centrifuged at 1000 rpm for 10 min. The supernatant was then subjected to analysis for gastric volume, gastric PH, free acidity, total acidity, and pepsin content, according to.[13]

The stomachs were rinsed under a stream of water and the picture of each stomach was taken for the analysis using ImageJ software. The percentage ulceration was determined using ImageJ software and the percentage inhibition was computed using the following formula.

Ulcer protection (%) was calculated using the relation.

$$\%\text{ulcer protection} = \frac{(\text{UIc} - \text{UIt})}{\text{UIc}} \times 100$$

Histopathological analysis

Tissue preparation

Sections of the stomach were collected for histopathological examination. The samples were fixed in 10% phosphate-buffered formalin for a minimum of 48 h. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70%, 80%, 90%, and 100% alcohol), cleared in 3 grades of xylene, and embedded in molten wax. On solidifying, the blocks were sectioned, 5 μm thick, with a rotary microtone, floated in a water bath, and incubated at 60°C for 30 min. The 5 μm thick sectioned were subsequently cleaned with 3 grades of alcohol (90%, 80%, and 70%). The sections were then stained with hematoxyllin for 15 min, bluing was done with ammonium chloride, and differentiation was done with 1% acid alcohol before counterstaining with eosin. Permanent mounts were made on degreased glass slides using a mountant (DPX).[14]

Slide examination

The prepared slides were examined with a compound light microscope (Motic™) using X4, X 10, and X40 objective lenses. The photomicrographs were taken using a Motic^{Tm} 5.0 megapixel microscope camera at X 160 magnification.[14]

Statistical analysis

The results were expressed as inhibition against ulceration in percentage and the standard error of the mean. One-way analysis of variance and the Student's *t*-test were used to analyze the data using the Software Package for Windows (SPSS) (Version 16). Also, statistically significant differences ($P < 0.05$) and ($P < 0.01$) were used to determine the differences between the groups in the study.

RESULTS

Acute toxicity study

No mortality was observed in the different groups of mice that received the aqueous extract of the CP; they only indicated vigorous paw licking and dullness. Therefore, the aqueous extract of CP at the dose of 2000 mg/kg body weight has no toxic effect in mice.

Acidified ethanol-induced ulcers in mice

To ascertain the anti-ulcer properties of the aqueous extract of the CP, the ethanol-induced ulcer model method was adjusted with acid. The most widely used medication to assess the gastroprotective properties of extracts, fractions, or medications in mice is ethanol.

Effect on free acidity and total acidity

$$\%\,Ulceration\,(Ulcer\ Index) = \frac{Total\,Ulcer\ Area}{Total\ stomach\ Area} \times 100$$

Sections of the normal stomachs indicated normal histology and morphology of the gastric mucosa as well as the fundic mucosa of the glandular stomach.The sections of the stomachs treated with distilled water showed a multifocally-widespread necrosis of the mucosal structures. The affected areas showed an influx of inflammatory cells and the laying down of collagenous fibers.

Sections of the stomachs treated with 150 mg/kg cimetidine exhibited mild multifocal areas of mucosal necrosis [Table 1].

Sections of the stomachs treated with 400 mg/kg CP aqueous extract showed mild multifocal areas of mucosal necrosis with marked inflammatory cellular infiltration [Table 2]. The affected areas are few and seem to be limited to the upper areas of the mucosa. Sections of the stomach presented in this group showed mild multifocal areas of mucosal necrosis with marked inflammatory cellular infiltration. The affected areas are few and seem to be limited to the upper areas of the mucosa.

DISCUSSION

Ethanol is the most widely used agent in experimental models for the evaluation of antiulcerative activity in animals.[14,15] It represents a form of gastric irritation resulting from the inhibition of prostaglandin synthesis and ethanol necroses in the superficial cells of the gastric mucous membrane by precipitation of the cytoplasmatic components, interrupting the function of the cell mucous membranes, with the participation of vasoactive mediators released, such as leukotriene C4 (LTC4) and histamine.[15]

The ethanol-induced ulcer model method was modified with acid to determine the anti-ulcer activities of the aqueous extract of the CP. Ethanol is the most commonly used drug for evaluating the gastroprotective activities of extracts, fractions, or drugs in animals.[15,16] It causes gastric irritation due to the inhibition of prostaglandin synthesis, necrosis of ethanol, precipitation of cytoplasmic components as a result of the superficial cells of the gastric mucous membrane, resulting in the function of the cell mucous membranes, and the release of vasoactive mediators such as LTC4 and histamine.[16]

Acute toxicity study of the aqueous extract of CP showed no mortality up to 2000 mg/kg body weight. The absence of LD_{50} also indicated that the LD_{50} is >2000 mg/kg, which could probably explain various reasons for ethnomedicinal uses such as ulcers, malaria, cancer, diabetes infections, and wounds.

Plants produce secondary metabolites, which are non-nutritive phytochemicals characterized by protective (or disease-preventive) activities to

protect themselves. Recent research has also shown that many phytochemicals can protect humans against diseases.[10] Phytochemical studies carried out in the genus Combretum have demonstrated the occurrence of many classes of phytoconstituents, including triterpenes and flavonoids. Additionally, phytochemical analysis of the methanol extract of *Combretum paniculata* indicated the presence of active compounds such as flavonoids, tannins, phenols, alkaloids, terpenoids, glycosides, and steroids, according to research conducted.[17] These phytochemicals have been attributed to the gastroprotective activities of some combretum species, such as *Combretum apiculatum* and *Combretum Dolichopetalum,* as well as other plant species, which include *Parkia biglobosa*, *Aloe vera*, *Mangifera indica*, *Zingiber officinale,* and so on.[5,10,17,18]

Extraction was carried out to eliminate impurities or recover a desired product by dissolving the plant material in a more polar solvent, such as methanol, which has a higher polarity than distilled, in order to extract a wide polarity range of chemical components, as illustrated by a study conducted on the methanol extract of CP. The methanol extract of CP displayed more potent anti-ulcer activities than the crude extract.[19]

The acidified ethanol induces the solubilization of the mucous constituents in the stomach, increases the flow of sodium and potassium in the lumen, increases the pepsin released, and decreases the tissue levels of DNA, RNA, and proteins, leaving the mucous membrane unprotected, leading to an injury in the tissue.[20] Since ethanol causes gastric ulcers by lowering protective factors in the gastric mucosa, ulcer induction is characterized by heavy bleeding due to immediate stasis in the blood flow.[15] It is possible that the aqueous extract of CP contains bioactives that can enhance protective factors and restore gastric blood circulation.

In addition to this, the anti-secretory activities of this extract may also be attributed to anti-vasoactive mediator activities, which block the release of mediators such as LTC4 and histamine,[15] which may prevent the formation of edema, which may contribute to the increase of lesions in this model.[21-23]

Histopathological study indicated that most significant changes occur during the 1st week of ulcer healing, which correlates with the wound healing study.[24] This also followed the conversion of fibroblasts into myofibroblasts, which was observed during the healing process, including the synthesis of extracellular matrix components such

Table 1: Effect of aqueous extract of Cp on percentage ulceration and percentage inhibition

Treatment (mg/kg)	Percentage ulceration (%)	Percentage inhibition (%)
200	11.6±2.40	41.8±042
400	9.69±1.27*	51.3±1.20
800	3.51±1.37**	82.3±1.31
150 (cimetidine)	9.02±2.87**	54.6±2.78
10 mL (distilled water)	19.9±4.74	

Table 2: Effect of aqueous extract of CP on free acidity (Meq/l) and total acidity (Meq/l)

TREATMENT (mg/kg)	Free acidity (MEQ/L)	Total acidity (MEQ/L)
200	39.2±0.14	64.7±0.55
400	32.4±0.41	56.1±0.81
800	22.5±0.33	35.7±0.30
150 (cimetidine)	24.0±0.12	39.4±0.72
10 (distilled water)	45.0±0.35	86.2±0.12

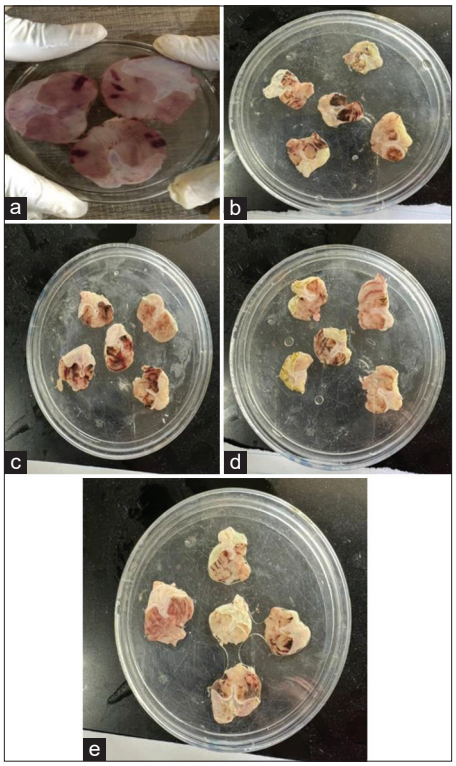

Figure 1: Photomicrograph of the stomach ulcers, (a) Distilled Water, (b) 200MG/KG, (c) 400MG/KG, (d) 800MG/KG, (e) 150MG/KG Cimetidine

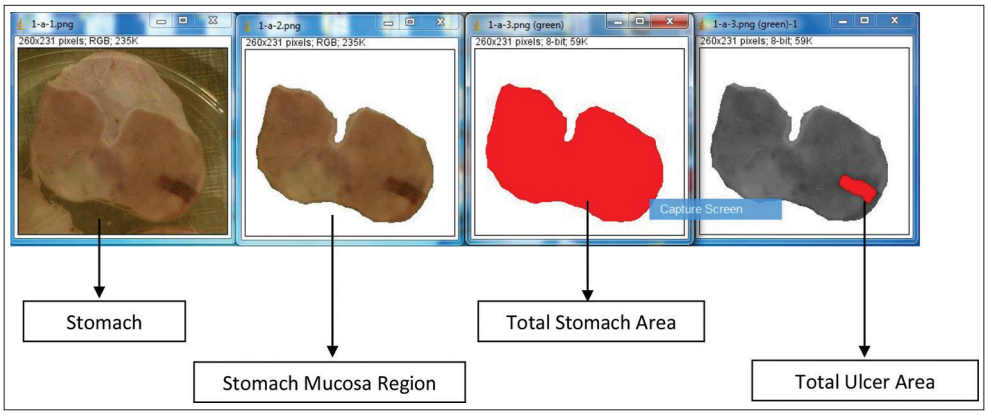

Figure 2: Photomicrographs of normal stomach and stomach ulcers

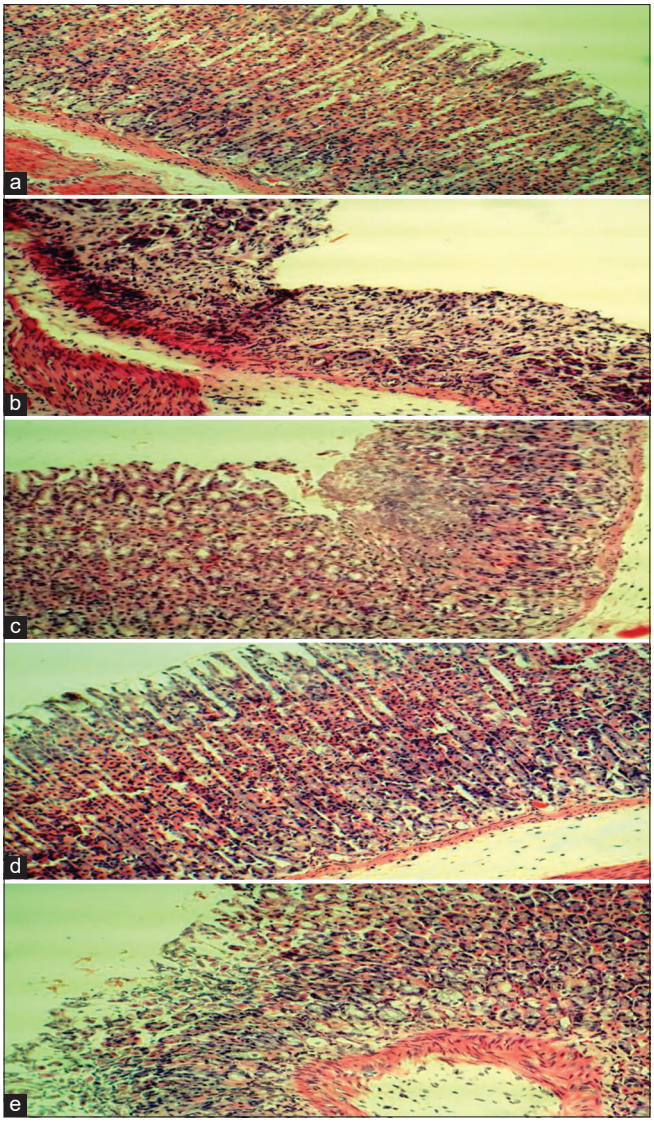

Figure 3: Photomicrographs of histopathological study, (a) normal stomach, (b) distilled water, (c) 150MG/KG Cimetidine, (d) Aqueous extract CP (400MG/KG), (e) Aqueous extract (800MG/KG)

as collagen types I and III from myofibroblasts.[25] Fibroblast, which is the major cell type found in the granulation of wound tissues, is important for the healing of internal wounds such as ulcer. It secretes a series of growth factors that generate angiogenesis, proliferation, and matrix deposition.[26]

The anti-ulcer activities of the aqueous extract of CP were analyzed histologically on the fourteenth day, similar to the studies conducted.[17]

The antioxidant effect of the aqueous extract of CP may be related to another mechanism that contributes to its anti-ulcer activity. Studies have shown that ethanol enhances the production of reactive oxygen species (ROS). In ischemia and reperfusion experiments, lesions appear in the cells of the gastric mucous membrane due to the formation of ROS [Figures 1-3].[5]

Several researches have demonstrated that the antioxidant activity of crude extract, methanol fraction of African locust bean tree and methanol extract of CP were due to the presence of flavonoids, tannins and phenols.[11,17] Flavonoids, tannins and phenols are phenolic compounds and plant phenolics are major group of compounds that act as primary antioxidants or free radical scanvergers.[5,9,11,17]

CONCLUSION

The aqueous extract of CP exhibited significant gastroprotective activity in a modified ethanol-induced ulcer model. It has gastric antisecretory and acid-neutralizing effects that are comparable to the reference drug cimetidine. Further studies should be carried out to identify the exact phytochemicals and mechanisms of action responsible for these activities.

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