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Evaluation of anti-ulcer activity of methanolic extract *Combretum paniculatum* Vent. in rats and mice using pylorus –ligation induced model

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Abstract

Combretum paniculatum (CP) vent is popularly used in traditional medicine to treat peptic ulcer disease. The anti-ulcer activities of 80% methanol leaf extract of CP was evaluated in rats and mice. The effects of CP extract on gastric ulcer in rats and mice in pylorus ligation –induced model was studied using varying concentrations (200, 400 and 800 mg/kg body weight). Omeprazole (20mg/kg body weight) was used as the reference drug. Parameters such as volume, PH of gastric fluid, ulcer sore, percentage ulceration, percent inhibition of the ulcer sore, ulcer index and percent inhibition of ulcer index were determined. Histopathological study was also conducted. Data were analyzed using One-way analysis of variance followed by Tuckey's post hoc test and $P< 0.05$ was considered as statistically significant as well as $P< 0.01$ as statistically highly significant. The oral median Lethal dose (LD50) was found to be greater than 2000mg/kg, phytochemicals such as flavonoids, tannins, phenols, alkaloids, terpenoids, glycosides and steroids were found to be present while saponins were found to be absent. CP highly significantly ($P<0.01$) reduced gastric ulcer index by 49.6%, 62.4% and 87.6% , CP posseses both dose-dependent and time dependent anti-ulcer activities. This study validates the anti-ulcer pharmacological activities of this plant, further investigation should be carried out to isolate specific phytochemicals as well as authenticate the mechanisms of action responsible for these activities.

Keywords: *Combretum paniculata*; Anti-ulcer activity; Omeprazole; Pylorus –Ligation Induced model

1. Introduction

Peptic Ulcer disease (PUD) is a disease of the gastrointestinal tract (GIT) which comprises both gastric and duodenal ulcers. It is characterized by imbalance between offensive (pepsin, gastric acid and *Helicobacter pylori*) and defensive (prostaglandin, bicarbonate ion, mucin, growth factors and nitric oxide) factors [1].

The corrosion of the linings of stomach by the acid peptic juices causes duodenal ulcers , millions of people worldwide are affected by gastric hyperacidity globally [2]. Gastric hyperacidity is associated with elevation of the stomach acid levels in the GIT, this excess acid secretion can lead to inflammation, irritation or erosion of stomach mucosa which is known as gastritis that can be acute or chronic [2].

The gastric mucosa is continuously exposed to various precipitating factors such as acid, pepsin, bile acid, bacterial products and drugs. These can lead to gastric acid and pepsin secretion reducing synthesis and inhibiting gastric blood flow and gastric motility [3].

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Medicinal plants have always been the main sources of new drugs candidates for the treatment of gastric ulcer, 75-80% of the world population in the developing countries still use herbal medicines for primary health care because of better cultural acceptability, better compatibility with human body and lesser side effects [4].

The combretaceae is a large family of herbs, shrubs and trees comprising about 20 generals and 600 species tropically distributed in Africa and Asia. CP is used extensively in folk medicine for the treatment of various diseases such as malaria, inflammation, infections, diabetes, stomach pain, cancer, wounds and diarrhoel [5].

Histamine (H_2) receptor antagonists , proton pump inhibitors and antimuscarinics drugs are drugs used currently for treating peptic ulcer [4]. These drugs are marked by adverse reactions like hypersensitivity, arrhythmia, impotence, gynaecomastia and blood disorders [6].

Investigation of synthetic drugs indicates that there are incidences of relapses and danger of drug interaction during treatment. The inability of the current conventional medicine to treat ulcer necessitate for more research to overcome this problem [4].

There is need for better protection and reduce the incidence of relapse, thus the search for new and ideal anti-ulcer drug continues and herbal drugs are not left out for new and novel drugs. In addition to this, herbal drugs increase the defensive factors such as mucin secretion, cellular mucus, bicarbonate secretion, mucosal blood flow and cell turnover [7]

Since plant derivatives are among the suitable drugs for the treatment and prevention of gastric ulcer, therefore warranting for new and safer drugs with fewer side effects. This study was therefore carried to evaluate the gastro protective activity of the methanolic extract of CP.

2. Material and methods

2.1. 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, mortal and pestle, separating funnel, beakers, cotton wool, retort stand, PH meter, rotary Evaporator and soxhlet apparatus.

2.2. Animals

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

2.3. Experimental animals

Adult female Wistar Albino rats and Swiss Albino mice of either sex bred in the animal care unit of the 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 light, humidity and room temperature (19-25^{0}C ; 12 hrs light and dark cycles) standard pellet and distilled water were provided ad libitum. Ethical Approval was sought from Bingham University Research Ethics Committee.

2.4. Grouping of animals and dosing

Thirty (30) female wistar albino rats of 150-250g and Swiss Albino mice of 20-30g 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 extract of 200mg/kg (CP 200), 400mg/kg (CP 400) and 800 mg/kg (CP 800) respectively. Group V was treated with a 30mg/kg Omeprazole (OM20).

2.5. 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, UNN with the voucher no PCG/UNN/0987.

2.6. 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 2.5kg of the powdered plant material with 7.5L of methanol. The powder was allowed to macerate for 48hrs in methanol by vigorous shaking at room temperature using soxhlet apparatus. The mixture was filtered after 48hrs and the filterate subjected to dryness using rotary evaporator. The evaporation flask was heated evenly and materials with a lower boiling point, the solvent stream was recycled in the receiving flask, following cooling by the glass condenser [8]. The percentage (%) yield was determined according to this formula [9]

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

2.7. Quanlitative and quantitative phytochemical analysis

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

2.8. Acute toxicity study

Three (3) female Swiss albino mice were randomly grouped and kept in a cage. After being fasted for 2 hours, 1000mg/kg of the extract dissolved in distilled water was administered to one mouse and observed for any signs of toxicity for 24hours.The following day, the second mouse received 1500mg/kg and the remaining mouse was administered 2000mg/kg of the extract and observed for any gross changes for 14 days according to OECD 425 guideline 2008 [11].

2.9. Pyloric –ligation induced ulcer model

The rats and mice were deprived of food but had free access to water for 1 day before pyloric ligation was carried out, pylorus ligation was performed in the animals for the induction of gastric ulcers 1 hour after the last administration of rapidly different test solutions on fasted rats. The abdomen was opened by using a small incision below the xiploid process after induction of anesthesia by ketamine hydrochloride (150mg/kg, Intraperitoneal) and xylazine (10mg/kg).

The stomach was exposed and a thread was placed around the pyloric sphincter and tied in a tight knot. The stomach was placed back carefully and the abdominal wall was closed with sutures. The animals were deprived of food and water during the postoperative period and the animals were sacrificed 5 hours after pylorus ligation by overdose of xylazine and ketamine.

The stomachs were removed and the contents were drained into tubes and centrifuged at 1000rpm for 10 minutes. The supernatant was then subjected to analysis for gastric volume, gastric PH, free acidity, total acidity and pepsin content according to [12] .

Each stomach was examined for ulcer and the percentage ulceration was determined using Image J software. The percentage inhibition of ulceration was computed and compared with the control.Ulcer index (UI) and % ulcer protection were calculated using the method of using the formula below.

$$\text{\% Ulcer protection} = \frac{\text{UI in control –UI in test}}{\text{UI in control [12]}} \text{ X } 100$$

2.10. Histopathological analysis

2.10.1. Tissue preparation

Sections of the stomach were collected for histopathogical examination. The samples were fixed in 10% phosphate buffered formalin for a minimum of 48 hours. 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, 5Um thick with a rotary microtone, floated in water bath and incubated at 60^{0}C for 30 minutes. The 5um thick sectioned thick were subsequently cleaned in 3 grades of alcohol (90%, 80% and 70%).The sections were then stained with haematoxyllin for 15 minutes, blueing was done with ammonium chloride, differentiation was done with 1% acid alcohol before counter staining with eosin. Permanent mounts were made on degreased glass slides using a mountant (DPX) [13].

2.10.2. 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 (Motic ™) 5.0 mega pixels microscope camera at X 160 magnification [13].

2.11. Statistical analysis

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

3. Results

3.1. Qualitative phytochemical test

Phytochemicals such as flavonoids, phenols, tannins, alkaloids, terpenoids, glycosides and steroids were found to be present in the methanol extract of CP while saponin was absent as indicated in table 1.

Table 1 Qualitative Phytochemical Screening of Methanolic Extract Of Cp

Phytochemicals	Remarks
Flavonoid	+
Phenol	+
Tannin	+
Alkaloid	+
Saponin	-
Terpenoid	+
Glycoside	-
Steroid	+

KEY= PRESENT, - ABSENT

3.2. Quantitative phytochemical test

Table 2 indicates the amount of phytochemicals present in the methanol extract of CP. The extract was mostly dominated by phenols (54.7±1.49) followed by flavonoids (18.6±0.47) and tannins (16.8±2.57).

Table 2 Quantitative Phytochemical Screening of Methanolic Extract Of Cp

Phytochemicals	Quantity (mg/200g)
Flavonoids	18.6±0.47
Phenol	54.7±1.49
Tannins	16.8±2.57
Alkaloid	0.80±0.08
Saponins	0.00±0.00
Terpenoids	2.81±0.04
Glycoside	0.00±0.00
Steroid	2.02±0.02

3.3. Acute toxicity study

Acute toxicity study was conducted based on the limit test dose of 2000 mg/kg as described by OECD 425 guideline 2008. The extract of CP at the dose of 2000mg/kg; body weight has no toxic effect in mice.

3.4. Pylorus ligation induced ulcer model in rats

The extract significantly ($p<0.05$) reduced the ulcer index from 8.46 ± 0.08, 6.32 ± 0.03 and 2.09± 0.04 (800mg/kg) in the pylorus ligation induced ulceration group and also exhibited dose-dependent percentage ulcer inhibition from 49.6%, 62.4% and 87.6% was comparable with that of omeprazole as illustrated in figure 1 and figure 2.

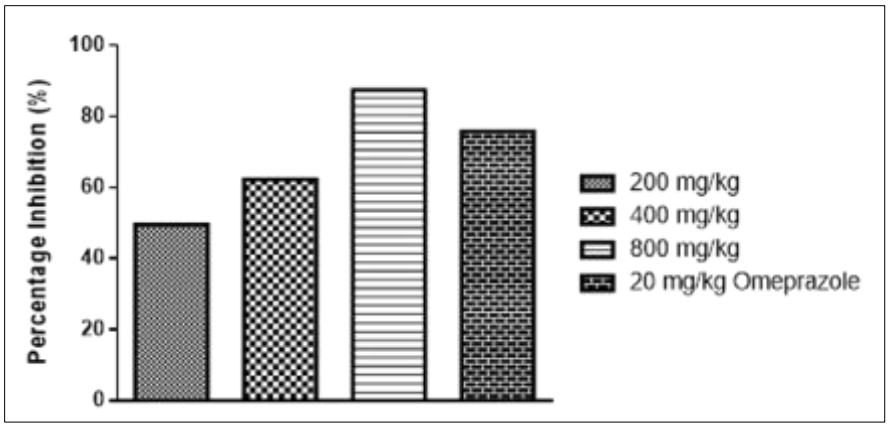


Figure 1 Effect of Methanolic Extract of Cp on Percentage Inhibition

Values are represented as mean ± S.E. M, n=5, *P<0.05, significantly different from control (Distilled water)

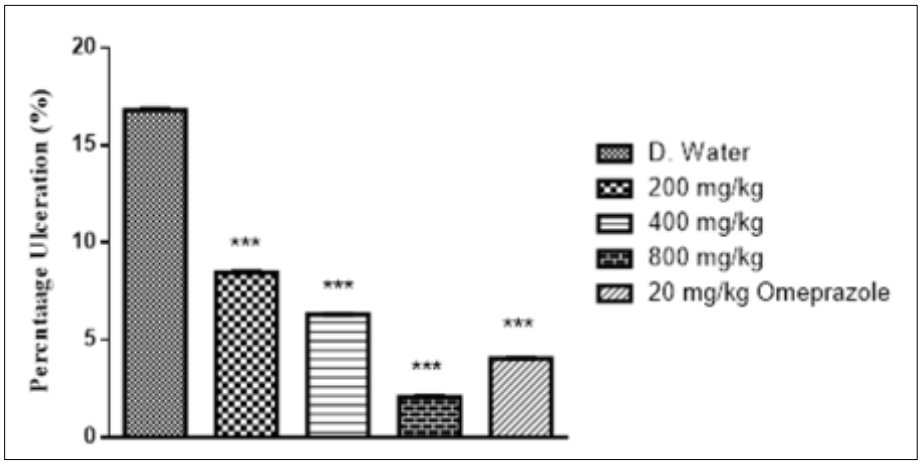


Figure 2 Effect of Methanolic Extract Of Cp On Percentage Ulceration

Values are represented as mean ± S.E. M, n=5, *P<0.05, significantly, ** *P<0.01 highly significant different from control (Distilled water)

3.5. Gastric volume and gastric ph

The extract highly significantly P< 0.01 reduced the gastric volume from 1.57± 0.19, 1.13± 0.15 and 0.67± 0.13 (800mg/kg) while the gastric PH was increased from 3.16± 0.36, 3.18± 0.27 and 5.80± 0.70 was comparable with that of omeprazole as shown in table 1.

Table 1 Effect Of Methanolic Extract Of Cp On Gastric Volume And Ph

Treatment (mg/kg)	Volume (ml)	Ph
200	1.57± 0.19	3.16± 0.36
400	1.13± 0.15**	3.18± 0.27**
800	0.67± 0.13**	5.80± 0.70**
20 (Omeprazole)	0.87± 0.32**	4.64± 0.30**
2 ml (Distilled water)	1.92± 0.42	2.67± 0.25

Values are represented as mean ± S.E. M, n=5, *P<0.05 significantly, **P< 0.01 highly significantly different from control (Distilled water)

3.6. Free acidity and total acidity

Figure 3 shows the effect of free acidity and total acidity of methanol extract of CP. The extract displayed reduction dose-dependent inhibition of free acidity and total acidity which was more remarkable at the dose of 800mg/kg comparable to the standard drug (20mg/kg omeprazole).

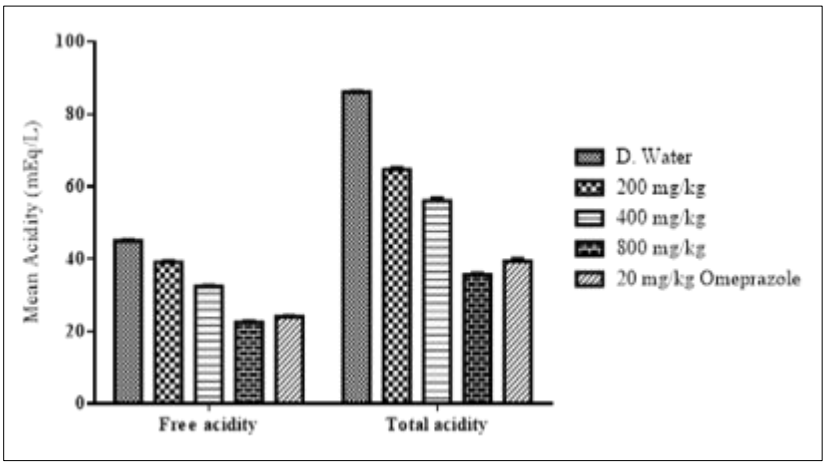


Figure 3 Effect of Methanol Extract Of Cp On Free Acidity And Total Acidity

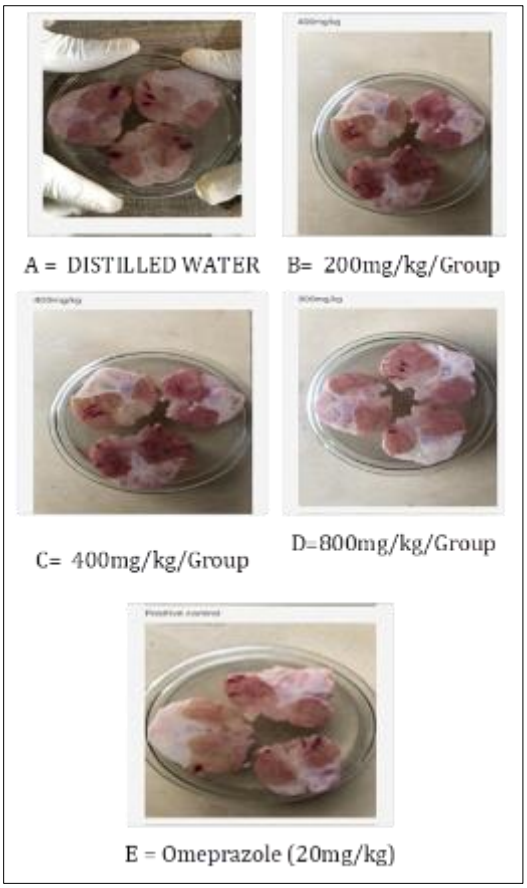


Figure 4 Photomicrographs of The Stomach Ulcers

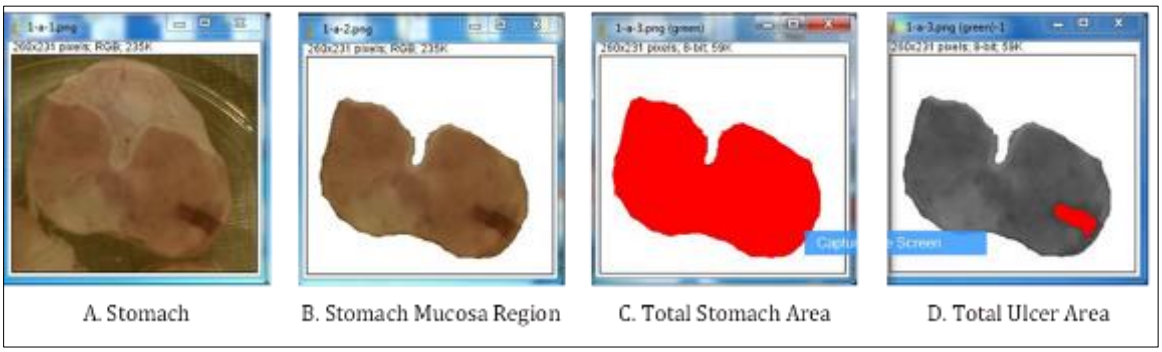
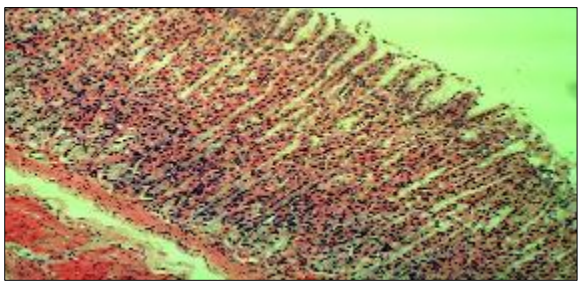


Figure 5 Photomicrograph of The Normal Stomach And Stomach Ulcer

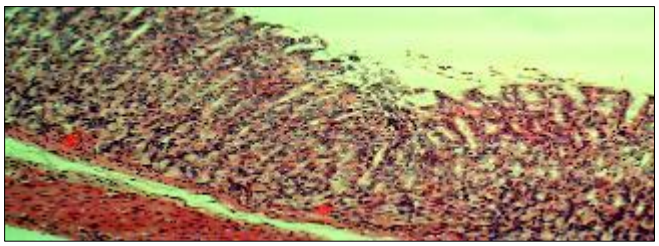


A= NORMAL STOMACH

Sections of the normal stomachs indicated normal histology and morphology of gastric mucosa as well as the fundic mucosa of the glandular stomach.

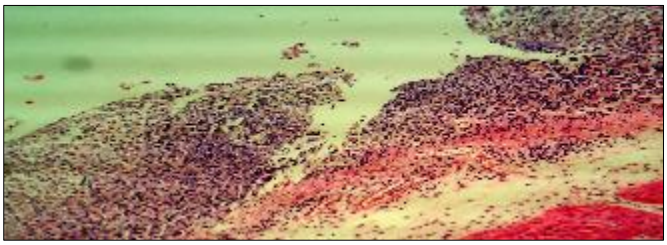
A= DISTILLED WATER.

Sections of the stomachs treated with distilled water indicated very mild multifocal areas of mucosal necrosis, the affected areas are few, limited to the upper layers of the mucosa.



B= OMEPRAZOLE (20 MG/KG)

Sections of the stomachs treated with 20mg/kg omeprazole showed mild multifocal areas of mucosal necrosis, the affected areas are few and seem to be limited to the upper areas of the mucosa.



C= CP METHANOL EXTRACT (400MG/KG)

The sections of the stomachs treated with 400mg/kg CP methanol extract exhibited a multifocally-widespread necrosis of the mucosal structures with variable infiltration of inflammatory cells. Clusters of inflammatory cells are also present in the submucosa.

D= CP MTHANOL EXTRACT (800 MG/KG)

The sections of the stomachs treated with 800mg/kg CP methanol extract indicated normal gastric histology and morphology similar to the normal stomachs

Figure 6 Photomicrograph of Histopathological Study

4. Discussion

Herbal products are medicinal drugs derived from plants and are believed to have lesser side effects as a result of their various possible health benefits [14]. Phytochemicals are phy-toconstituents produced by plants, they have both protective and disease-preventive activities. Phytochemical screening of the methanol extract of CP indicated the presence of tannins, phenols and flavonoids which are known to have cytoprotective activity for which anti ulcerogenic efficacy has been extensively confirmed [15]. In the quantitative phytochemical analysis, phenols were the most abundant active constituents, researches have shown that phenolic compounds participate significantly in the gastroprotection of a great number of vegetal extracts [4].

Flavonoids and tannins are also phenolic compounds characterized by both protein precipitating and vasoconstriction effects, thus may prevent ulcer development. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants [16, 17]. It has been suggested that these compounds will be able to stimulate mucous, bicarbonate and the prostaglandin secretion. Furthermore, they have been reported to counteract the deleterious effects of reactive oxidants in gastrointestinal lumen [18].

The acute toxicity of the methanol extract of CP was investigated to determine any adverse effect that may arise as a result of a single contact or multiple exposures in a short time within 24 hours' period [4]. However, the LD_{50} was found to be 2000mg/kg, this probably explains its wide use in the treatment of ulcer, malaria, cancer, infections, diabetes and wounds.

The anti-ulcer activity was carried out in pylorus ligation induction model, the percentage ulcer protection was observed in this model and the percentage of ulcer protection varies with the standard omeprazole when compared to methanol extract of CP.

In pyloric ligation model, ulcer was induced as a result of accumulation of gastric acid which resulted in auto-digestion of mucosa and breakdown of the gastric mucosal barrier, consequently leading to upper gastrointestinal damage including lesions, life threatening perforation and hemorrhage. Anti-ulcer activity is supported by decrease in the aggressive factors like gastric volume, free acidity and total acidity as well as increase in the resistance factors like pH, as a result the anti-secretary mechanism [19].

The anti-ulcer activities of methanol extract of CP were analyzed histologically on the fourteenth day similar to studies conducted by [20], In this the most significant changes occur during the first week of ulcer healing, this correlates with the wound healing study conducted by [21].

The conversion of fibroblasts into myofibroblasts was observed during the healing process. Extracellular matrix components such as collagen types I and III were synthesized from myofibroblasts. Internal wound healing such as ulcer was assessed by an indicator such as fibroblast proliferation [22]. The major cell type found in the granulation of wound tissues is fibroblast. They are very essential in this type of wound healing including secretion of a series of growth factors that generates angiogenesis, proliferation and matrix deposition [23].

Phytochemicals such as phenols, tannins and flavonoids are antioxidants with remarkable anti-ulcer activities, the antioxidant activities in relation to anti-ulcer activities of various plants have been investigated by researchers [4, 20] and these activities have been attributed to the presence of phenols, tannins and flavonoids which are free radical scanvergers [8]. These bioactives have been reported to possess pharmacological properties such as antimicrobial, antioxidant, analgesic, and anti-inflammatory activities which enhance the internal wound-healing process such as ulcer mainly due to wound contraction and increased rate of epithelization [24].

5. Conclusion

The methanol extract of CP possesses significant anti-ulcer activity in pylorus ligation induced animal model. It has gastric antisecretory and acid neutralizing effect that are comparable to reference drug omeprazole. The anti-ulcer activity is probably due to the presence of bioactive compounds like flavonoids, tannins and terpenes. Further studies are required to confirm the exact mechanism responsible for the ulcer healing and protecting activity of the extract and to identify the chemical constituents responsible for it.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

Ethical approval was sought from Bingham University Ethics Research Committee.

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