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Original Research Article

Gastroprotective Effect of Methanol extracts of *Sphenocentrum jollyanum* Pierre and *Curculigo pilosa* (Schumach. & Thonn.) Engl. in Wistar Rats

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ABSTRACT

Curculigo pilosa (Schumach. & Thonn.) Engl. and *Sphenocentrum jollyanum* Pierre are commonly used plants in traditional medicine in the management of several ailments. The rhizomes of *C. pilosa* and seeds of *S. jollyanum* are traditionally used for treating gastrointestinal diseases. There is need to justify the medicinal claim of the selected plants as having anti-ulcerogenic activity to ensure efficacy and safety. In this study, the gastroprotective effect of methanol extracts of *Sphenocentrum jollyanum* Pierre and *Curculigo pilosa* (Schumach. & Thonn.) Engl. in Wistar Rats was evaluated. Lorke's method was used for the toxicity study. Animals were grouped and pre-treated with varied doses of extracts and standard drug (cimetidine) for 7 days and gastric ulcer was induced experimentally using 40 mgkg^{-1} *b.w.* indomethacin on day 8 of treatment. Gastric ulcer index, biochemical, and histological evaluation were investigated. The LD_{50} of both extracts were > 5000 mgkg^{-1} *b.w.* as no lethality was recorded up to this level. Cimetidine (100 mgkg^{-1} *b.w.*), *C. pilosa* (50 mgkg^{-1} *b.w.*), and *S. jollyanum* (200 mgkg^{-1} *b.w.*) gave inhibitions of 97.5%, 82.5%, and 72.5% respectively, compared to 0% of the ulcer untreated. A significant increase in antioxidant parameters was observed compared with ulcer untreated group. Histological evaluation of the gastric tissue from ulcerated untreated group revealed severe lesions, which were ameliorated in *C. pilosa*, *S. jollyanum* extracts and cimetidine treated groups. *Curculigo pilosa* and *Sphenocentrum jollyanum* extracts demonstrated gastroprotective activity probably via increased antioxidant activities against indomethacin-induced gastric ulcer thereby justifying their ethno-medicinal claim.

Keywords: Gastric ulcer, Gastroprotection, Indomethacin, *Curculigo pilosa*, *Sphenocentrum jollyanum*, Histopathology,

Introduction

Peptic ulcer disease is a gastrointestinal disorder affecting about 4 million people worldwide annually, which requires urgent attention.[1] Disparity between protective factors (such as prostaglandins, circulation of mucosa blood, mucin, bicarbonate surface, and nitric oxide) and aggressive factors (gastric acid, ethanol, Non-steroidal Anti-inflammatory Drugs (NSAIDs), *Helicobacter pylori*) results in gastric ulcers.[2] The NSAIDs are known for managing arthritis and other painful inflammatory conditions in the body. These classes of drugs include indomethacin, diclofenac, aspirin, ibuprofen, naproxen, among others. However, the major side effect of NSAIDs is upper gastrointestinal tract injury such as peptic ulcer disease, slight to severe dyspeptic signs, abdominal pain, and hemorrhage. These NSAIDs have been used in developing stomach ulcer models in experimental rats. It is significant in exploring possible efficacy of cytoprotective and anti-secretory mediators as the fundamental pathophysiology includes gastric acid secretion and synthesis of prostaglandin mucosa. In gastroprotection research, the NSAIDs induced gastric ulcer is the most commonly used anti-ulcer model.[3]

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Herbal medicine being the main therapeutic remedy for treating numerous diseases including gastric ulcer in many developing countries, it is pertinent to justify the medicinal claim of selected plants as having anti-ulcerogenic activity to ensure efficacy and safety. Some currently used anti-ulcer drugs have side effects such as depression, central nervous system disorder and endocrine effects such as elevations of serum prolactin, galactorrhea, loss of libido, impotence and reductions in sperm count.[4] The frequency of adverse and side effects of orthodox medicine necessitates the use of medicinal plants with fewer side effects as alternative. For most of the world's population, medicinal plants are a very important source of traditional medicine. About 70% of people globally depend frequently on herbal medicines for their crucial healthcare needs.

Curculigo pilosa, family Hypoxidaceae is a medicinal plant indigenous to Africa and found in abundance in southwestern Nigeria. It is commonly called African crocus and "epakun" among the 'Yorubas'. The rhizomes of *C. pilosa* are used as food and possess medicinal properties.[5] High amylolitic activity in *C. pilosa* extracts describes its popular use in the preparation of baby foods that are easily digestible and conventional sorghum beer preparation process. The antioxidant activities and dietary composition of *C. pilosa* have been previously reported.[5] The plant's rhizome is used locally in the management of heart and gastrointestinal ailments. Phytochemical screening of *C. pilosa rhizome* has been reported to have bioactive phytochemicals such as saponins, tannins, flavonoids, and alkaloids.[6]

Sphenocentrum jollyanum, family Menispermaceae is a plant known for various biological activities. This plant is locally known as "Akerejupon" in southwestern Nigeria and commonly used locally in managing several ailments. The fruit is edible and taken against fatigue, the flower parts are also used in combination with *Piper*

guineense Schumach. & Thonn., family Piperaceae and lime juice in the management of coughs, chronic wounds, and fever.[7] The plant's roots stimulate appetite, relieve constipation, and increase food digestion once chewed in Nigeria. Phytochemical screening of seeds of *S. jollyanum* showed the abundance of tannins, flavonoids, saponins, and alkaloids.[8] Previous studies also reported some isolated compounds from *S. jollyanum*.[9,10] Despite the numerous biological activities of these plants, their gastroprotective properties have not been reported. Previous ethnobotanical survey reported that *Curculigo pilosa* and *S. jollyanum* plants are traditionally used for the management of gastric ulcer in southwestern Nigeria.[11] This study was thus designed to assess the anti-ulcer activities of *C. pilosa* rhizomes and *S. jollyanum* seeds methanol extracts to validate the traditional claim of them as antiulcerogenic plants.

Materials and Methods

Chemicals and reagents
Absolute methanol (redistilled), Cimetidine tablet (Rx Nigeria), Indomethacin tablet (Vinay Pharma, Mumbai, India), Distilled water, Normal saline, Hand Gloves, Methylated spirit, Sodium hydroxide, NaOH, Thiobarbituric acid (TBA), Hydrochloric acid (HCl), Hydrogen peroxide (H_20_2) (BDH chemicals, England), Conc. HCl, Lead acetate solution, Glacial acetic acid, 3, 5 – dinitrobenzoic acid, Conc. Sulphuric acid, 10% H_2SO_4, p-anisaldehyde, Cerric sulphate, Pepsin enzyme (BDH Laboratory Supplies, Poole, England).

Plant materials, collection and authentication
The rhizomes of *C. pilosa* and seeds of *S. jollyanum* were collected in May, 2016, at their local habitat in Odo ona area, Apata, Ibadan, Oyo State, Nigeria. Identification and authentication were done by Mr. Tope Soyewo at Forest Herbarium, Ibadan (FHI) located at Forestry Research Institute of Nigeria (FRIN), where voucher specimens were deposited with FHI numbers 111263 and 110510, respectively.

Extraction of powdered samples
The rhizomes of *C. pilosa* were washed thoroughly to eliminate adhering particles and cut into bits before drying. The seeds of *S. jollyanum* and rhizomes of *C. pilosa* were dried in shade for three weeks and pulverized into powder. One kilogramme (1 kg) of powdered samples of each plant was macerated with 100% methanol (8000 mL) for five days stirring daily using a glass rod. Maceration was done three times using fresh solvent for the wet residue for maximum extraction. The filtrates were combined and evaporated *in vacuo* at 40 °C.

Animals
A total of seventy-seven (77) Wistar rats were used for the study of acute toxicity and indomethacin-induced gastric ulcer; thirty-two animals for acute toxicity study and forty-five animals for indomethacin-induced gastric ulcer. The rats (120 – 150 g) were acquired at the University of Ibadan Central Animal House.

Ethical consideration on use of experimental animals
This study obtained an approval from Animal Care and Research Ethics Committee of the institution with UI-ACUREC/19/0018.

Housing and feeding
Experimental animals were reserved in the Animal House at 26.0-28.0 °C in compact bottom cages under pathogen-free conditions, with relative humidity of 70-80%. The animals were acclimatized for two weeks while maintaining normal situations (12 h light and dark), nourished with standard rat pellet feed and given access to fresh water *ad. libitum*. Animal management and procedures complied with National Institute of Health recommendations[12] for animal care and use in the laboratory.

Animal groups
Animal Groups: Healthy Wistar rats (n = 45) were separated randomly into 9 groups (n=5), made up of group A (cimetidine group), group B (ulcer untreated), groups C, D, and E: (*C. pilosa* methanol extracts at 50, 100, and 200 $mgkg^{-1}$), groups F, G, and H (*S. jollyanum* methanol extracts at 50, 100, and 200 $mgkg^{-1}$), and group I (normal control).

Toxicity study
Acute toxicity studies were performed on male Wistar rats according to the method of Lorke[13] to establish the safe effective dose. Sixteen animals were used for each of the extracts to give a total of thirty-two animals. The animals were randomly grouped into four (n=3) and orally given 10, 100, 1000 $mgkg^{-1}$ *b. w.* of *C. pilosa* or *S. jollyanum* methanol extracts, respectively, while the fourth group served as control. Since there was no death, 1600, 2900 and 5000 $mgkg^{-1}$ doses were given to a new group of rats at one rat per dose having the last animal as control to determine lethality. The animals were observed for a 24 h period for signs and symptoms of toxicity and death.[13] The surviving animals were kept under close observation then euthanized 14 days after; histopathological assessment was done on the heart, liver, and kidney of the animals to determine the level of toxicity damage.

Indomethacin-induced gastric ulcer
Animals were pre-treated with cimetidine (100 $mgkg^{-1}$), *C. pilosa* or *S. jollyanum* at varied doses (50, 100, and 200 $mgkg^{-1}$ *b.w.*) orally for 7 consecutive days. Slightly modified method of Akpamu *et al.*[14] was used to induce gastric ulcer where indomethacin (40 $mgkg^{-1}$ *b.w*) was administered to the animals, 1 h after cimetidine or extract dosing. The animals in group B (ulcer untreated group) were administered indomethacin only in the final day, while the animals in group I (normal control) were not administered either drug or indomethacin. Indomethacin was given 24 h after fasting the animals on day 7. After gastric ulcer induction, euthanasia by cervical dislocation was done where their stomachs were excised. The stomachs were washed off any food remains by gently rinsing in a cold phosphate buffer solution and cautiously spread on a waxed paper.

Quantification of ulcers
Gastric Ulcer index (UI) of each animal was determined as average of ulcers in each group[15] where:
No lesion = 0, Bleeding and Slight lesions (0.5 – 1.0) mm = 1, Moderate Lesions (1.0 – 1.5) mm = 2, severe lesions (1.5 – 2.5) mm = 3, and perforated ulcers (2.5 – 3.5) mm = 4.
Percentage inhibition was calculated using:

$$\% \text{ Inhibition} = \frac{^{UI}\text{ ulcer untreated} - {}^{UI}\text{ treated}}{^{UI}\text{ ulcer untreated}} \times 100$$

A segment of the gastric tissue was kept for biochemical analysis, while the other half was fixed in formalin (10%) for histological assessment.

Biochemical assays
The removed stomach in cold phosphate buffer solution was homogenized, centrifuged at 4000 rpm for 10 min at 4°C, the supernatant decanted and kept at 4°C for biochemical assays.

Total gastric protein concentration
The concentration of protein in the stomach tissue was measured [16]. Briefly, 0.1 mL of 2N NaOH was added to 0.1 mL of sample/standard. The solution was hydrolyzed for 10 min at 100°C in a boiling water bath. The hydrolysate was cooled to room temperature and 1 mL of freshly mixed complex-forming reagent was added. The solution was allowed to stand at room temperature for 30 min. The absorbance was taken at 750 nm and Bovine serum albumin (2.5-15µg/mL) was used as standard.[16]

Determination of gastric nitric oxide (NO)
The amount of stomach nitric oxide was calculated.[17]

Gastric antioxidant activity
Superoxide dismutase (SOD) was quantified using the method of Marklund and Marklund.[18] Briefly, aliquots of supernatant were mixed

with 1 Mm pyrogallol and buffer solution (Tris HCl 1Mm-EDTA 5mM, pH 8.5). The reaction was incubated for 20 min and stopped with 1 N HCl addition then centrifuged at 1400 rpm for 4 min. The supernatant absorbance was measured at 405 nm using the spectrophotometer. One unit of SOD activity defines the amount of SOD that inhibited the oxidation of pyrogallol by 50%, relative to control. The SOD activity was expressed as u/mg of protein.

The catalase (CAT) activity was evaluated according to the method of Aebi.[19] Aliquots of supernatant were mixed with a solution containing 30% H_2O_2 milli-Q water and buffer 5 mM Tris EDTA pH 8.0. Spectrophotometry was used to determine the absorbance at 240 nm for 60 s and the CAT enzyme activity was expressed in mol/min/mg of protein.

Reduced glutathione (GSH) levels in the gastric mucosa were measured using the method described by Moron *et al.* [20] Briefly, aliquots of tissue homogenate were mixed with 12.5% trichloroacetic acid and centrifuged for 4000 rpm at 4°C for 15 mins. The supernatant absorbance including TRIS buffer (0.4 M, Ph 8.9) and 5, 5-dithiobis-2-nitrobenzoic acid (DTNB, 0.01M) was measured at 420 nm in a spectrophotometer. The values are expressed as µGSH/g of tissue.

The malondialdehyde (MDA) concentration in the gastric tissue homogenate was determined using a method of Varshney *et al*.[21] Briefly, thiobarbituric acid (TBA) reacts with MDA present in the sample (which are in acidic medium, at 95°C for 30 min) to form TBA-reactive products (TBARS). The absorbance was then measured using spectrophotometer at 534 nm and the MDA levels calculated.

Histological assessment

The stomach samples in 10% formalin were processed and fixed in paraffin wax. Sections (5 µm) were prepared and stained with Haematoxylin and Eosin (H&E). Histopathological changes were examined and photographed using a digital camera.

Statistical analysis

Data were expressed as Mean ± SEM, analyzed using one-way ANOVA and Dunnet multiple comparison test. Statistical differences was significant at $p<0.05$.

Results and Discussion

Plant extraction

The weights of *Curculigo pilosa* and *S. jollyanum* methanol extracts were 88.6 g and 49.0 g with 8.87% and 4.9% yield respectively.

Acute toxicity study

Single dose oral administration of 10, 100, and 1000 mgkg^{-1} *b.w.* of *C. pilosa* and *S. jollyanum* crude extracts appeared to be safe visually as no death or noticeable signs of toxicity were observed in treated animals for the first 24 h and by the end of 48 h observation. No late toxicological effect was observed up to 14 days after treatment. Therefore, the extracts are said to have LD_{50} of > 5000 mgkg^{-1}.

The toxicity result supports the report of Mbaka *et al*.[22] where acute toxicity study of *S. jollyanum* leaf extract displayed no mortality when given up to 11 g/kg *b.w.* orally. Acute toxicity study of *C. pilosa* is reported for the first time. Toxicity studies are vital in setting safety limits for potential drugs and are also often used to determine potential health hazards from plant extracts.[23]

Histological analysis

The histopathology result of the heart, liver and kidney is shown in Figures 1-6 to determine the level of toxicity.

Effect of C. pilosa on the heart, kidney, and liver

No visible lesion was found in the kidney of control animals (A), 10 mgkg^{-1} (B), and 100 mgkg^{-1} (C). However, mild diffuse tubular regeneration and shrinkage of glomeruli was observed in animals administered with 1000 mgkg^{-1} (D), 1600 mgkg^{-1} (E) and 2900 mgkg^{-1} (F) animals which became more severe at 5000 mgkg^{-1} (G) treated animals (Figure 1). Photomicrograph of heart of control animals, 10, 100, 1000, 1600, 2900, up to 5000 mgkg^{-1} extract treated animals showed no visible lesion (Figure 2). Photomicrograph of a section of liver tissues showed no visible lesions in the control animals up to 1000 mgkg^{-1} treated animals. However, severe portal congestion, diffuse vacuolar degeneration of hepatocytes was observed in 1,600 mgkg^{-1}, 2900 mgkg^{-1}, and 5000 mgkg^{-1} treated animals (Figure 3).

Effect of S. jollyanum seed extract on the kidney, heart, and liver

No observable lesion was found in control animals (A), 10 mgkg^{-1} (B), 100 mgkg^{-1} (C), up to 1000 mgkg^{-1} treated animals. However, mild diffuse tubular regeneration was noticed in high dosages of 1600, 2900 and 5000 mgkg^{-1} treated rats (Figure 4). Photomicrograph of a section of heart tissues in control animals, 10, 100, up to 1000 mgkg^{-1} showed no visible lesions but doses at 1600, 2900, and 5000 mgkg^{-1} treated rats revealed several foci of perivascular cellular infiltration, the affected vessels are moderately to severely congested (Figure 5). No observable damage was found in liver tissues of normal control, animals treated with 10, 100, and 1000 mgkg^{-1} while slight portal and central venous congestion, with moderate periportal cellular infiltration was detected in the liver tissues of 1600, 2900, and 5000 mgkg^{-1} treated animals (Figure 6).

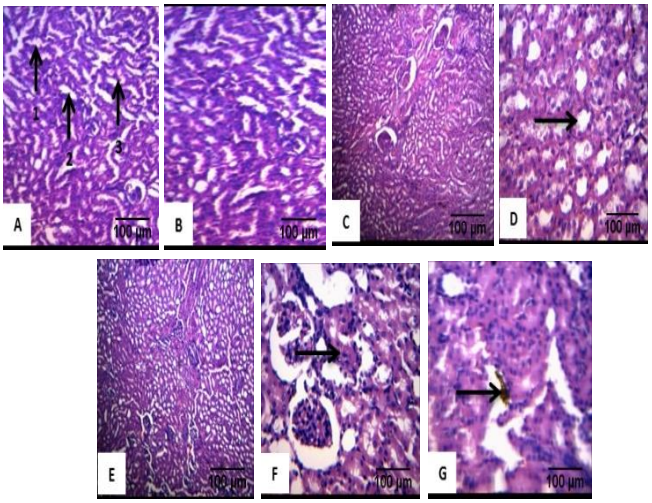

Figure 1: Photomicrograph of kidney of animals administered with *C. pilosa* extracts, Magnification: X400 except in C and D (X100); Bars =100 µm. (A) Control animals: 1: Bowman's capsule, 2: Renal tubules; 3: Glomerulus (B) 10 mgkg^{-1} (C) 100 mgkg^{-1} (D) 1000 mgkg^{-1} (E) 1600 mgkg^{-1} (F) 2900 mgkg^{-1} (G) 5000 mgkg$^{-1.}$ Arrows in D, F, and G showing diffuse tubular regeneration and shrinkage of glomeruli

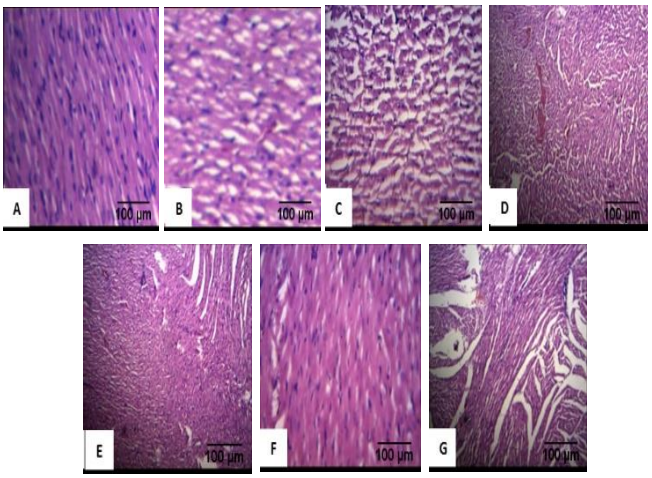

Figure 2: Photomicrograph of heart of animals administered with *C. pilosa* extracts, Magnification: X400 except in D and E (X100); Bars =100 µm. (A) Control animals (B) 10 mgkg^{-1} (C) 100 mgkg^{-1} (D) 1000 mgkg^{-1} (E) 16000 mgkg^{-1} (F) 2900 mgkg^{-1} (G) 5000 mgkg^{-1}

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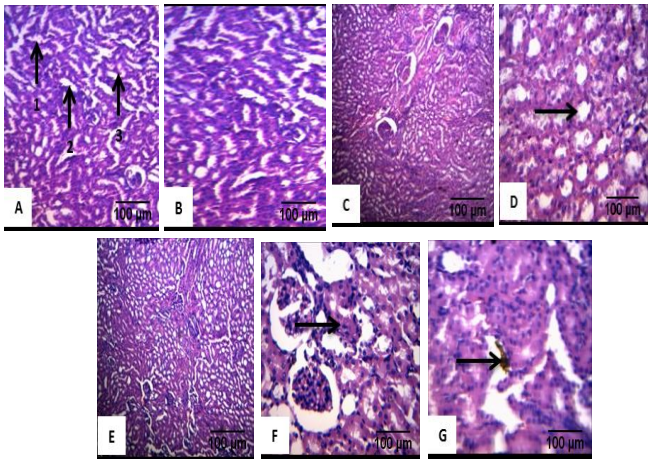

Figure 3: Photomicrograph of the liver of animals administered with *C. pilosa* extracts, Magnification: X400 except in C, D and G (X100); Bars =100 µm. (A) Control animals: 1: Normal hepatocytes, 2: Interstitial spaces (B) 10 mgkg^{-1} (C) 100 mgkg^{-1} (D) 1000 mgkg^{-1} (E) 1600 mgkg^{-1} (F) 2900 mgkg^{-1} (G) 5000 mgkg^{-1}. Arrows in E, F, and G show severe portal congestion and diffuse vacuolar degeneration of hepatocytes

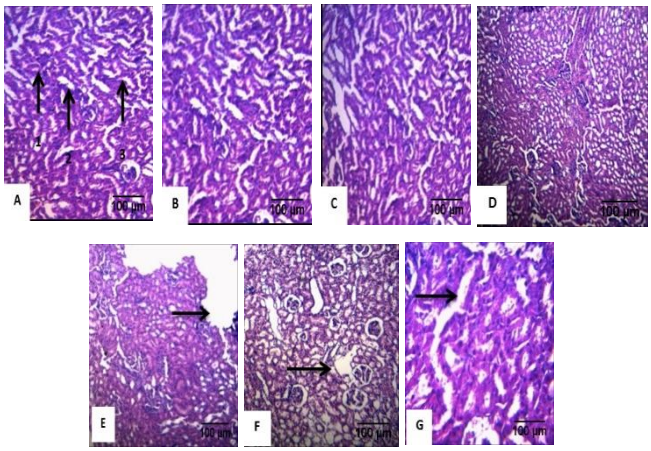

Figure 4: Photomicrograph of kidney of animals administered with *S. jollyanum* extracts, Magnification: X400 except in D and E (X100); Bars =100 µm. (A) Control animals: 1: Bowman's capsule; 2: Renal tubules; 3: Glomerulus (B) 10 mgkg^{-1} (C) 100 mgkg^{-1} (D) 1000 mgkg^{-1} (E) 1600 mgkg^{-1} (F) 2900 mgkg^{-1} (G) 5000 mgkg^{-1}. Arrows in D, E and F show mild diffuse tubular regeneration

Effect of methanol extracts of C. pilosa and S. jollyanum on change in body weight

There was a general considerable body weight increase in all the treated groups by day 8 when compared with day 1 (Figure 7).

The body weight increase in treated animals might be due to the presence of tannins in both *C. pilosa* and *S. jollyanum* extracts. Studies showed that medicinal plants with small amounts of tannins when administered induced a rise in body weight.[24]

Effect of C. pilosa and S. jollyanum methanol extracts on stomach weight

The stomach weight of treated and untreated groups did not differ significantly on day 8 of the experiment (Table 1).

Indomethacin-induced gastric ulcer

Reduction in severity of lesions caused by indomethacin-induced gastric ulcer

The standard (cimetidine) treated group significantly reduced the gastric ulcer index from 8.00 ± 2.72 in the ulcer untreated group to 0.20 ± 0.20, while the *C. pilosa* (50 mgkg^{-1} *b.w*) treated group significantly reduced the ulcer index from 8.00 ± 2.72 to 1.40 ± 0.97. *S. jollyanum* (200 mgkg^{-1} *b.w*) treated group also showed significant reduction in gastric ulcer index from 8.00 ± 2.72 to 2.20 ± 1.24. *C. pilosa* (100 and 200 mgkg^{-1} *b.w*) treated groups significantly decreased the gastric ulcer index from 8.0 ± 2.72 to 5.60 ± 2.37, and 3.80 ± 1.46, respectively, while 50 and 100 mgkg^{-1} *b.w.* extracts of *S. jollyanum* caused a significant reduction of gastric ulcer index from 8.0 ± 2.72 to 5.60 ± 0.92 and 3.80 ± 1.39, respectively. *C. pilosa* extract at 50 mgkg^{-1} *b.w* showed the most significant activity amongst the extracts (Table 1). Percentage inhibition of indomethacin-induced gastric mucosal damage for all the groups pre-treated with cimetidine revealed 97.5% inhibition. This study also revealed that *C. pilosa* at 50, 100, and 200 mgkg^{-1} *b.w* gave percentage inhibition of 82.5, 30.0, and 52.5%, respectively, while *S. jollyanum* at 50, 100, and 200 mgkg^{-1} *b.w* gave percentage inhibition of 30.0, 52.5, and 72.5%, respectively. Cimetidine (100 mgkg^{-1} *b.w)* gave the highest percentage inhibition followed by *C. pilosa* 50 mgkg^{-1} *b.w* extract, which gave 82.5% inhibition (Table 1).

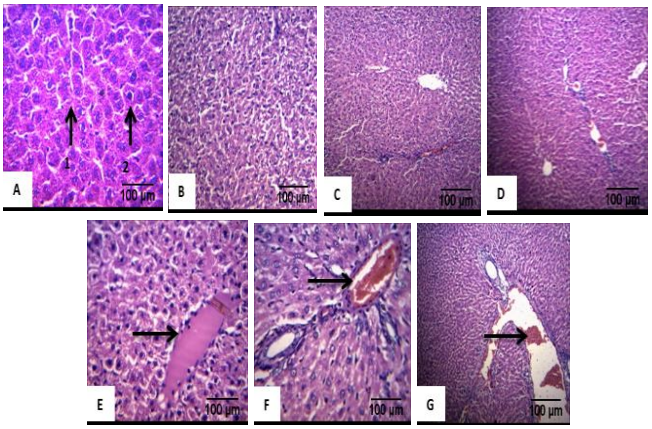

Figure 5: Photomicrograph of heart of animals administered with *S. jollyanum* extracts, Magnification: X100 except D (X400); Bars = 100 µm. (A) Control animal (B) 10 mgkg^{-1} (C) 100 mgkg^{-1} (D) 1000 mgkg^{-1} (E) 1600 mgkg^{-1} (F) 2900 mgkg^{-1} (G) 5000 mgkg^{-1}. Arrows showing several foci of perivascular cellular infiltration

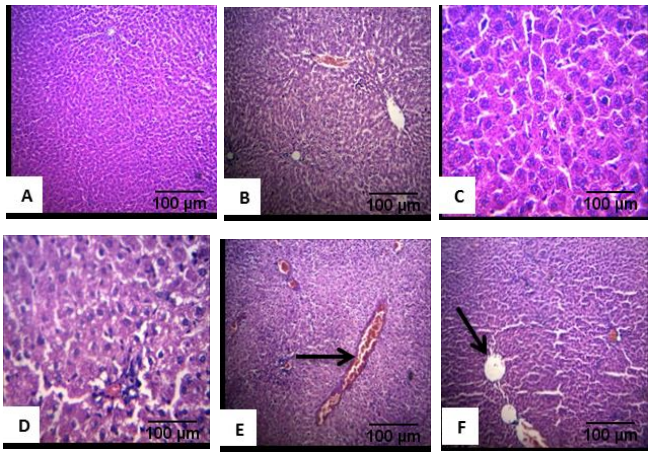

Figure 6: Photomicrograph of liver of animals administered with *S. jollyanum* extracts, Magnification: X100 except in C and D (X400); Bars =100 µm. (A) Control animals: 1: Normal hepatocytes, 2: Interstitial spaces (B) 10 mgkg^{-1} (C) 100 mgkg^{-1} (D) 1000 mgkg^{-1} (E) 1600 mgkg^{-1} (F) 2900 mgkg^{-1}. Arrows showing central venous congestion, with modest periportal cellular infiltration.

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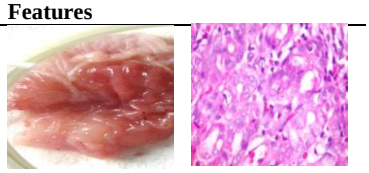
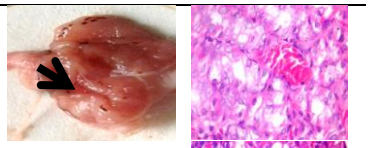
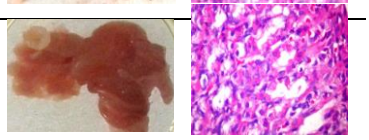
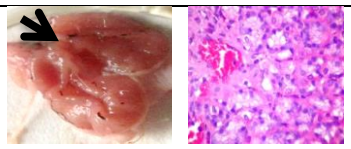
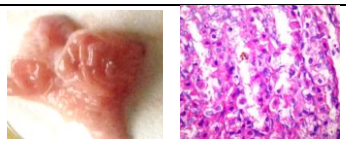
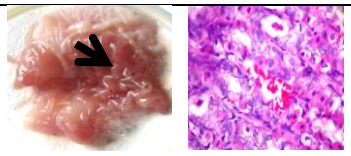
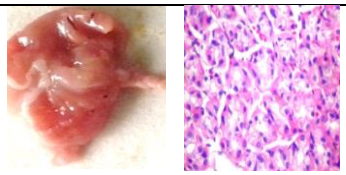
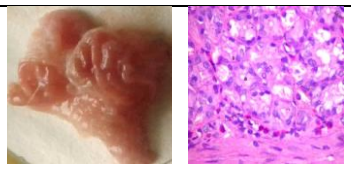
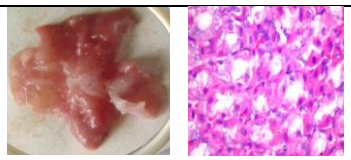
Table 1: Effect of methanol extracts of *C. pilosa* and *S. jollyanum* on ulcer index in indomethacin-induced gastric ulcer

Groups	Ulcer index (Mean ± SEM)	% Inhibition	Mean Stomach Weight (g)	Macroscopic and Microscopic Features
A: Cimetidine 100 mg/kg *b.w.*	0.20 ± 0.20[a]	97.5	0.92 ± 0.03	
B: Ulcer untreated	8.00 ± 2.72	0	0.84 ± 0.02	
C: C.P 50 mg/kg *b.w.*	1.40± 0.97[a]	82.5	0.81 ± 0.04	
D: C.P 100 mg/kg *b.w.*	5.60 ± 2.37[c]	30.0	0.84 ± 0.02	
E: C.P 200 mg/kg *b.w.*	3.80 ± 1.46[b]	52.5	0.87 ± 0.03	
F: S.J 50 mg/kg *b.w.*	5.60 ± 0.92[c]	30.0	0.93 ± 0.03	
G: S.J 100 mg/kg *b.w.*	3.80 ± 1.39[b]	52.5	0.87 ± 0.01	
H: S.J 200 mg/kg *b.w.*	2.20 ± 1.24[a]	72.5	0.84 ± 0.01	
I: Normal control	0.00 ± 0.00[a]	100	0.94 ± 0.04	

Keys: Ulcerated areas are indicated with arrows, S.J: *Sphenocentrum jollyanum*, C.P: *Curculigo pilosa*. [a] $p < 0.05$ when compared with normal control, [b] $p < 0.05$ when compared with ulcer untreated, [c] p when compared with cimetidine

Curculigo pilosa and *Sphenocentrum jollyanum* plant extracts possess numerous medicinal values with reported pharmacological activities. [25, 26] Several drugs are marketed for management of gastric ulcers, which include antacids, inhibitors of proton pump, anti-histamines and anti-cholinergic. Many of these drugs possess severe side effects, such as altered bowel function when antacids are used [27], toxicity of the liver and kidney in the case of anti-histamines.

The result showed that *C. pilosa* at 50 $mgkg^{-1}$ possesses the capacity of reducing gastric injury induced by indomethacin, while *S. jollyanum* at 200 $mgkg^{-1}$ exhibited a reduction in gastric damage.

The gastroprotection activity exhibited by *Curculigo pilosa* at the lowest concentration (50 $mgkg^{-1}$) could be the function of potency of the plant extract. Higher doses could have caused some biological effects which may interfere with the efficacy results. The present investigation revealed that the seeds of *S. jollyanum* and rhizomes of *C. pilosa* showed anti-ulcer activity indicated by lowering ulcer index or increasing percentage inhibition. Indomethacin is a well-recognized NSAID used for treatment of pain, fever, and joint stiffness caused by arthritis.

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Indomethacin and other NSAIDs act on stomach tissue to interrupt the defensive coating of the mucus layer.[28] There is higher ulcerogenic potential in indomethacin than other NSAIDs therefore the choice for gastric ulcer experiment. The histopathology showed an erosion of mucus layer, necrosis, and congestion in the blood vessels of ulcer untreated group by action of indomethacin. This is comparable to previous result of Adewoye and Salami[29] whereby NSAIDs inhibited the mucus layer and prostaglandin when evaluating the anti-ulcer mechanism of magnesium and *Vernonia condensata* Baker, family Compositae, respectively. Parietal and mucosal cells are present in abundance with mild hemorrhagic lesion in cimetidine and *C. pilosa* treated groups. This showed that both plants possess gastroprotective properties comparable with the standard drug.

Biochemical assays

Effect of methanol extracts of C. pilosa and S. jollyanum on Nitric Oxide (NO) Level

The NO levels of all treated groups were increased significantly relative to the ulcer untreated. The NO level of *C. pilosa* 100 mgkg^{-1} *b.w.* was significantly increased in comparison with all the treated groups (Figure 8).

Nitric oxide prevents gastric secretion from enterochromaffin-like cells by blocking histamine release.[30] The moderated nitric oxide levels observed in *C. pilosa* and *S. jollyanum* treated groups might be another mechanism by which the plants enhanced gastroprotection by possibly causing blood movement increase and supply of nutrients and oxygen to the site of gastric injury.

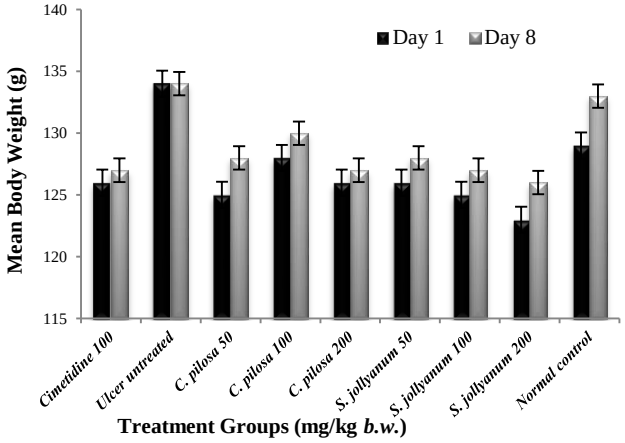

Figure 7: Effect of *C. pilosa* and *S. jollyanum* methanol extracts on body weights Each vertical bars represents Mean ± SEM of five rats per group

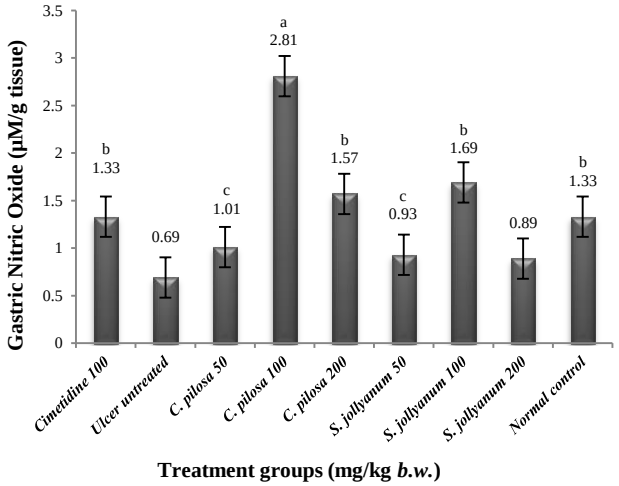

Figure 8: Effect of *C. pilosa* and *S. jollyanum* methanol extracts on gastric Nitric Oxide [a] $p < 0.05$ in comparison with normal control, [b] $p < 0.05$ in comparison with ulcer untreated, [c] p in comparison with cimetidine

Effect of C. pilosa and S. jollyanum methanol extracts on Total Gastric Protein, Catalase (CAT), and Superoxide Dismutase (SOD)

The total gastric protein increased significantly in the *C. pilosa* 50 and 100 mgkg^{-1} *b.w.* (0.26 ± 0.00 U/mg, 0.27 ± 0.00 U/mg) groups in comparison with the ulcer untreated (0.23 ± 0.00 U/mg) group. A reduction in CAT was detected in the *C. pilosa* groups (33.10 ± 1.33 mmol/min/mg, 31.48 ± 0.83 mmol/min/mg, 38.79 ± 2.04 mmol/min/mg), *S. jollyanum* treated groups (31.23 ± 0.51 mmol/min/mg, 30.79 ± 1.08 mmol/min/mg, 37.41 ± 2.47 mmol/min/mg), and ulcer untreated group (36.79 ± 3.91 mmol/min/mg) compared with normal control (45.04 ± 5.15 mmol/min/mg). The SOD in groups treated with *S. jollyanum, C. pilosa* and cimetidine significantly increased relative to the ulcer untreated group (Table 2).

The increased total gastric protein observed in the extracts treated groups could have assisted in repair of worn out tissues at the ulcer site. Evidence has shown that oxygen dependent free radicals actively contribute to the formation of several diseases including PUD with antioxidants that are identified as actively involved in averting gastric mucosal injury.[31] This could be a reason for increasing antioxidant activity in the extracts treated groups. The observed increase in SOD and CAT activity of *C. pilosa* and *S. jollyanum* treated groups could have contributed to gastroprotection activity.

Table 2: Effect of *C. pilosa* and *S. jollyanum* methanolic extracts on total gastric protein, catalase, and superoxide dismutase

Groups	Total Gastric Protein (U/mg)	Catalase (CAT) (mmol/ min/ mg)	Superoxide Dismutase (SOD) U/mg
A – Cimetidine	0.25 [c] ± 0.00	37.85 ± 3.18	31.24 ± 0.25 [b]
B – Ulcer untreated	0.23 ± 0.00	36.79 ± 3.91	25.10 ± 2.12
C – *C. pilosa* 50 mg/kg *b.w.*	0.26 [b] ± 0.00	33.10 ± 1.33	34.33 ± 0.12 [b]
D – *C. pilosa* 100 mg/kg *b.w.*	0.27 [b] ± 0.00	31.48 ± 0.83	32.96 ± 0.91 [b]
E – *C. pilosa* 200 mg/kg *b.w.*	0.24 ± 0.01	38.79 ± 2.04	32.72 ± 0.10 [b]
F – S. *jollyanum* 50 mg/kg *b.w.*	0.16 ± 0.02	31.23 ± 0.51	45.86 ± 2.12 [a]
G- S. *jollyanum* 100 mg/kg *b.w.*	0.19 ± 0.02	30.79 ± 1.08	44.39 ± 0.90 [a]
H- S. *jollyanum* 200 mg/kg *b.w.*	0.16 ± 0.00	37.41 ± 2.47	47.61 ± 1.36 [a]
I – Normal control	0.23 ± 0.03	45.04 ± 5.15	36.37 ± 0.65 [b]

Effect of C. pilosa and S. jollyanum methanol extracts on Glutathione (GSH)

S. jollyanum 100 mgkg^{-1} *b.w.* exhibited a significant increase in GSH level in comparison with other groups (Figure 9). The increased GSH observed in *C. pilosa* and *S. jollyanum* treated groups might have assisted in the neutralization, reduction, or prevention of some damages caused by free radicals.

Effect of C.pilosa and S. jollyanum methanol extracts on Malondialdehyde (MDA)

The MDA level of *C. pilosa*, *S. jollyanum* and cimetidine groups was significantly decreased compared to the untreated group (Figure 10). Indomethacin could facilitate the inflammatory response by inducing oxidative stress. This is in line with the present study as induction of indomethacin increased the gastric MDA. The estimated MDA rate in the biological system is a vital oxidative stress marker. *Curculigo pilosa* and *S. jollyanum* reduced the MDA values, suggesting that the plant extracts aided in moderating the MDA levels thereby reducing free radicals produced at the ulcer site. The reduction of lipid peroxidation could also have been as a result of the synergistic action of phytochemical constituents present in the plants. The phytochemical constituents present in *C. pilosa* and *S. jollyanum* may be responsible for the observed gastroprotective activity. Tannins have been found to speed up the wound healing process and/or swollen mucous membrane [32] possibly by tanning the membrane surface thereby acting as a defensive coating against aggressive substances. Tannins could have been responsible for mucosal surface coating thereby contributing to the reduction of lipid peroxidation in the extracts treated groups. Flavonoids are diverse class of secondary metabolites with potentially useful health properties.[33] They possess anti-secretory, cytoprotective, and antioxidant activities. They also act as gastroprotective and ulcer healing agents, which can be new alternatives for subduing peptic ulcer diseases related to *Helicobacter pylori*.[33] Flavonoids also possess certain defensive actions against free radicals, ulcers, allergies, inflammation, microbes, viruses, and tumors.[34] They are capable of activating the mucosal defense system by stimulating gastric secretion and scavenge for reactive oxygen species. Flavonoids have been reported to scavenge free radicals and might have been responsible for the reduced oxidative reactions in gastroprotection study. Also, saponins are involved in the stimulation of mucous membrane defensive factors thus exerting its defensive activities in gastric ulceration.[35]

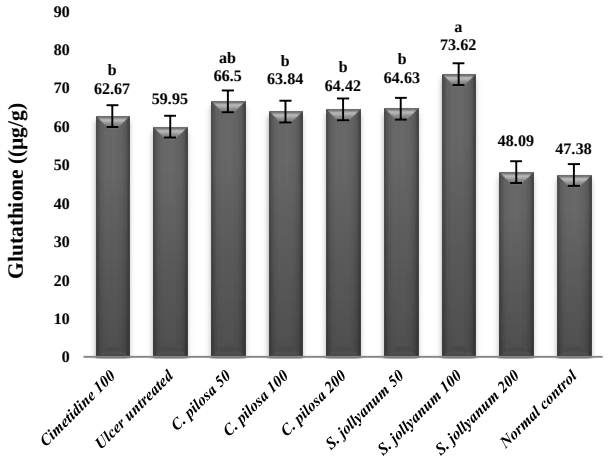

Figure 9: Effect of *C. pilosa* and *S. jollyanum* methanol extracts on glutathione. Each vertical bars represents Mean ± SEM. Values are significant when P < 0.05. Keys of Significance: [a] p < 0.05 in comparison with normal control, [b] p < 0.05 in comparison with ulcer untreated.

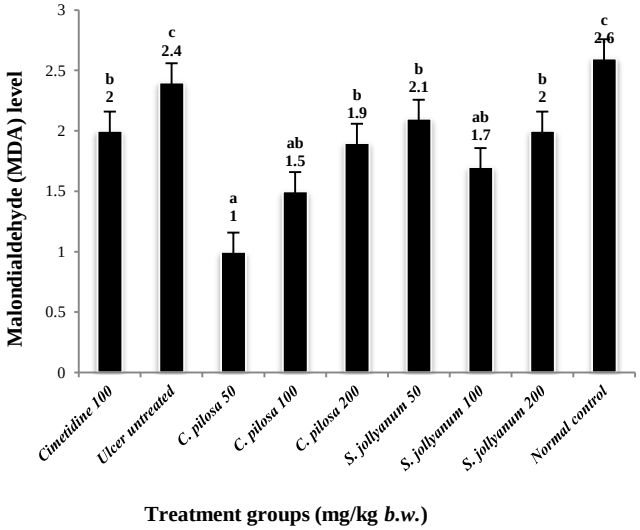

Figure 10: Effect of *C. pilosa* and *S. jollyanum* methanol extracts on lipid peroxidation (MDA) level. Vertical bars represents Mean ±SEM. Values are significant when p<0.05. Keys of Significance. [a] p < 0.05 in comparison with normal control, [b] p < 0.05 in comparison with ulcer untreated, [c] p in comparison with cimetidine.

Conclusion

Curculigo pilosa and *Sphenocentrum jollyanum* methanol extracts demonstrated anti-ulcer activities comparable to cimetidine. The decrease in ulcer index and increased percentage inhibition showed the gastroprotective potential of the plants. Therefore, these medicinal floras could be used for prevention and treatment of gastric ulcers.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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