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Assessment of the anti-ulcer action of the leaves of calopo (*Calopogonium mucunoides* Desv) in Wistar rats

Osmund C. Enechi, Christian E. Odo* and Chisom Okafor
Pharmacology Research Unit, Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

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ABSTRACT

Background and Aim: The leaves of *Calopogonium mucunoides* is used in South Eastern Nigeria for the management of ulcer. Thus, the phytochemical constituents, acute toxicity and anti-ulcer effect of the ethanol extract of the leaves of *C. mucunoides* using ethanol-induced model of gastric ulceration in rats were evaluated. **Methods :** The anti-ulcer action of the extract was assessed by determining the volume of gastric juice secretion, ulcer index (length of gastric lesions) and concentrations of the free and total acidity with the aid of standard methods. **Results :** The phytochemical analyses of the extract revealed the presence of alkaloids, flavonoids, tannins and terpenoids. The extract at a dose as high as 5000 mg/kg body weight (b.w) was not toxic. The extract at the tested doses (100 and 300 mg/kg b.w) caused significant ($p < 0.05$) dose dependent reductions in the volume of gastric juice secretion, ulcer index and concentrations of the free and total acidity in the treated rats. The anti-ulcer effect exhibited by the extract was comparable with that obtained for the standard anti-ulcer drug [ranitidine (150 mg/kg b.w)]. **Conclusion :** These findings indicate that the ethanol extract of the leaves of *C. mucunoides* has remarkable anti-ulcer effect and therefore, lends believability to its use in folk medicine for the management of ulcer.

Keywords: Acute toxicity, *Calopogonium mucunoides*, Phytochemical constituents and Ranitidine

1. INTRODUCTION

Ulcers are deep lesions penetrating through the entire thickness of the gastrointestinal tract (GIT) mucosa and muscularis mucosa. Peptic ulcer has unquestionably been the disease of the twentieth century. There are different types of ulcers one of which is gastric ulcer. Gastric ulcer is due to damage to the lining of the stomach. It is believed that gastric ulcers develop due to imbalance between aggressive factors [*Helicobacter pyroli*, non-steroidal anti-inflammatory drugs (NSAIDs), gastric acid] and protective factors (mucin, bicarbonate, prostaglandins) leading to the interruption of the mucosal integrity. Various factors are implicated which play a pivotal role in the pathogenesis of ulcerations. These factors include: sedentary lifestyle, alcohol intake, intake of spicy food, certain drugs and various bacterial infections. In herbal medicine, procedure and techniques for the treatment or prevention of digestive disorders have been developed. Numerous plant herbs are used to treat gastro-intestinal disorders in traditional medicine. In recent times, there has been renewed interest in identifying new anti-ulcer drugs from natural sources.[1]

Calopogonium mucunoides Desv (Fig. 1) (Fabaceae) is a vigorous and hairy annual or short–lived perennial trailing legume. It can reach several metres and form a dense, tangled mass of foliage, 30-50 cm deep. The root system is dense and shallow, at most, 50 cm deep. The stems are succulent, covered with long brown hairs. They are creeping in the lower parts, sometimes, rooting at the nodes that come in contact with the soil. The upper part of the stem is twining. The leaves are up to 16 cm long and trifoliate. The hairy leaflets are 4-10 cm long x 2-5 cm broad and ovate to elliptical. The inflorescence is a slender hairy raceme that may be up to 20 cm long and that bears 2 to 12 blue or purple small flowers. The Fruits are 3-8 seeded hairy pods,

Fig. 1: *Calopogonium mucunoides* Desv

***Corresponding author.**
Christian E. Odo
Pharmacology Research Unit,
Department of Biochemistry,
University of Nigeria,
Nsukka, Nigeria

2-4 cm long.[2] Like many other legume forages, the nutritive potential of *C. mucunoides* lies in its protein content. However, a rather wide range of protein content has been reported (5 - 24 %) depending on the part of the plant eaten and its stage of maturity.[3] The leaves are used as anti-scorbutic, anti-diarrhoea and for strengthening the system.[4] The leaves of *C. mucunoides* are used in South Eastern Nigeria for the management of ulcer and therefore, the objective of this study was to evaluate their anti-ulcer potential in Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant
The leaves of *Calopogonium mucunoides* were gathered from the Botanical Garden of the University of Nigeria, Nsukka, Enugu State, Nigeria. The leaves were identified by Prof. (Mrs.) May Nwosu of the Department of Botany, University of Nigeria, Nsukka where the voucher specimens were deposited in the herbarium.

2.2 Preparation of the Extract
The leaves of *Calopogonium mucunoides* were washed with distilled water and spread on a clean mat. The leaves were shade-dried for 3 weeks and homogenised into fine particles using an electric blender. A known weight (500 g) of the ground leaves was macerated in 2500 ml of 96% ethanol for 24 hours at room temperature. The mixture was filtered and the filtrate passed through a rotary evaporator to reduce the ethanol content. Thereafter, the filtrate was further concentrated using a vacuum at a controlled temperature (40 - 45°C) and stored in a refrigerator until used.

2.3 Animals
Adult male Wistar rats of between 7 and 12 weeks old with average weight of 120 ± 20 g and albino mice weighing 30 ± 5 g were obtained from the Animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatised for one week under a standard environmental condition with a 12 hour light and dark cycle and maintained on a regular feed and water *ad libitum*. There was adherence to the Principles of Laboratory Animal Care.

2.4 Chemicals and Reagents
The chemicals used in this study were of analytical grade and included: 96% ethanol (BDH Chemicals Ltd., Poole, England), 80% ethanol (BDH Chemicals Ltd., Poole, England), ranitidine [standard anti-ulcer drug (Sigma-Aldrich, Inc., St. Louis, USA)], 0.1 N NaOH (sodium hydroxide), thymol blue indicator, dilute tetraoxosulphate (vi) acid, 2% (v/v) hydrochloric acid, 1% (w/v) picric acid, methyl orange, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Fehling's solution, 5% (w/v) ferric chloride solution, aluminium chloride solution, lead sub acetate solution, ammonium solution and distilled water.

2.5 Acute Toxicity Study
The acute toxicity and lethality (LD_{50}) of the extract was determined using mice according to slightly modified method of[5].

2.6 Phytochemical Analyses
Qualitative phytochemical analyses were carried out on the ethanol extract according to the procedures outlined by[6,7].

2.7 Ulcer Studies
Evaluation of the volume of gastric juice secretion, ulcer index and concentrations of the free and total acidity were according to the methods described by[8,9].

2.8 Statistical Analysis
The data obtained were subjected to one - way Analysis of Variance (ANOVA). Significant differences were observed at $p < 0.05$. The results were expressed as means of five replicates ± standard errors of the means (SEM). This analysis was done using the computer software known as Statistical Package for Social Sciences (SPSS), version 18.

3. RESULTS

3.1 The Acute Toxicity and Lethality (LD_{50}) of the Ethanol Extract of the Leaves of *Calopogonium mucunoides*
The result of this study shows that there was neither lethality nor any sign of toxicity in the four groups of three mice each that received 10, 100, 1000 mg/kg body weight of the ethanol extract of the leaves of *C. mucunoides* and 5 ml/kg body weight of normal saline respectively at the end of the first phase of the study. At the end of the second phase of the study, there was not death or obvious sign of toxicity in the groups of mice that received 1900, 2600 and 5000 mg/kg body weight of the extract.

3.2 Qualitative phytochemical Composition of the Ethanol Extract of the Leaves of *Calopogonium mucunoides*
The qualitative phytochemical analyses showed the presence of tannins and flavonoids in very high concentration as shown in **Table 1**. Saponins and alkaloids were found to be present in moderately high concentration. Glycosides and cardiac glycosides were found to be present in low concentration. Resins and acidic compounds were not detected in the extract.

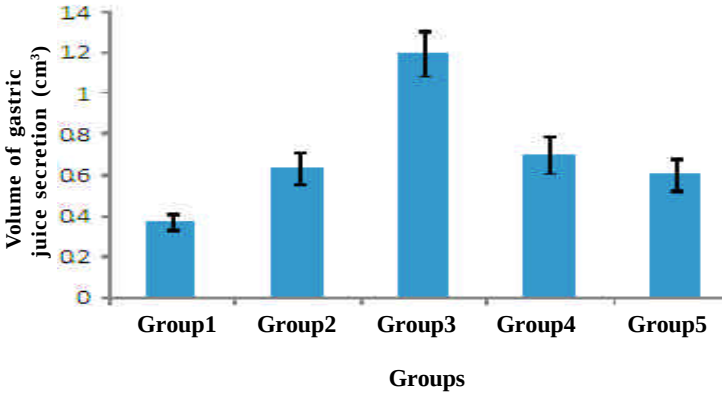
Table 1: Qualitative phytochemical constituents of the ethanol extract of the leaves of *C. mucunoides*

Phytochemical constituents	Inference
Saponins	++
Tannins	+++
Alkaloids	++
Flavonoids	+++
Glycosides	+
Resins	ND
Cardiac glycosides	+
Acidic compounds	ND

ND = not detected ; + = present in low concentration;++ = present in moderately high concentration;+++ = present in very high concentration

3.3 Effect of the Ethanol Extract of the Leaves of *Calopogonium mucunoides* on the Volume of Gastric Juice Secretion
As shown in Fig. 2, the volume of the gastric juice secretion of the rats in group 3 (ulcer-untreated control group) was significantly ($p < 0.05$) higher than that of the rats in group 1 (group treated with distilled water only). The extract in a similar manner as the standard anti-ulcer agent [ranitidine (150 mg/kg body weight), significantly ($p < 0.05$) and dose-dependently reduced the volume of gastric juice

secretions in the rats of groups 4 and 5 when compared to the value obtained for rats in group 3.

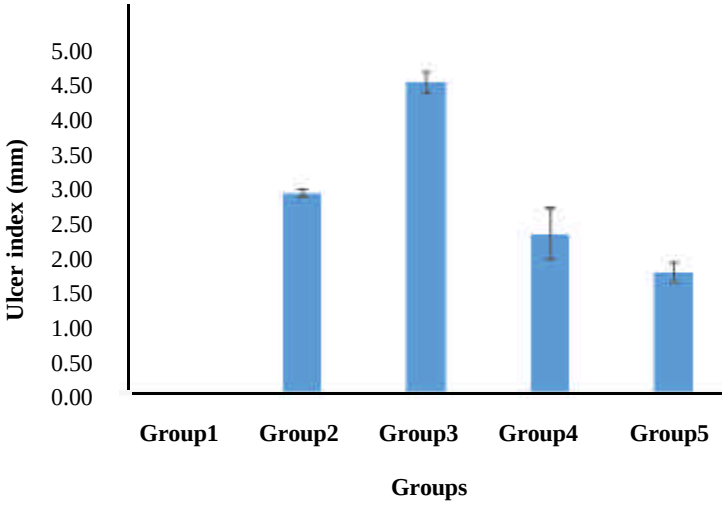


Group 1: 2 ml/kg body weight (b.w) of distilled water only; Group 2: 150 mg/kg b.w of ranitidine + 1 ml/kg b.w of 80% ethanol; Group 3: 2 ml/kg b.w of distilled water + 1 ml/kg b.w of 80% ethanol; Group 4: 100 mg/kg b.w of the of extract + 1 ml/kg b.w of 80% ethanol ; Group 5: 300 mg/kg b.w of the of extract + 1 ml/kg b.w of 80% ethanol

Fig. 2: Effect of the ethanol extract of the leaves of *Calopogonium mucunoides* on the volume of gastric juice secretion

3.4 Effect of the Ethanol Extract of the Leaves of *Calopogonium mucunoides* on Ulcer Index

Fig. 3 shows that there were significant ($p < 0.05$) and dose-related decreases in the ulcer indices of rats fed 100 and 300 mg/kg body weight of the extract (groups 4 and 5 respectively) when compared to the value obtained for rats in the ulcer-untreated control group (group 3). The effect of the extract was comparable to that obtained with the standard anti-ulcer drug [ranitidine (150 mg/kg body weight)] in rats of group 2.

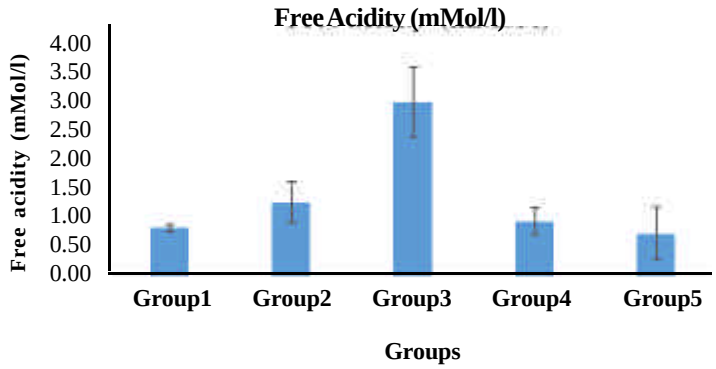


Group 1: 2 ml/kg body weight (b.w) of distilled water only; Group 2: 150 mg/kg b.w of ranitidine + 1 ml/kg b.w of 80% ethanol; Group 3: 2 ml/kg b.w of distilled water + 1 ml/kg b.w of 80% ethanol; Group 4: 100 mg/kg b.w of the of extract + 1 ml/kg b.w of 80% ethanol;Group 5: 300 mg/kg b.w of the of extract + 1 ml/kg b.w of 80% ethanol

Fig. 3: Effect of the ethanol extract of the leaves of *Calopogonium mucunoides* on ulcer index

3.5 Effect of the Ethanol Extract of the Leaves of *Calopogonium mucunoides* on Free Acidity

There was significant ($p < 0.05$) increase in the concentration of free acidity of rats in group 3 (ulcer-untreated control group) when compared to the value obtained for rats in group 1 (group treated with distilled water only). The extract in a like fashion as the standard anti-ulcer agent [ranitidine (150 mg/kg body weight)], significantly ($p < 0.05$) and dose-dependently caused reductions in the concentrations of free acidity in the rats of groups 4 and 5 when compared to the value obtained for rats in group 3 (Fig. 4).

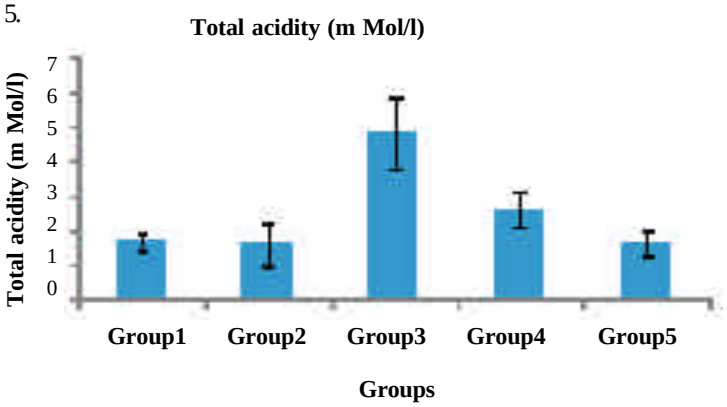


Group 1: 2 ml/kg body weight (b.w) of distilled water only; Group 2: 150 mg/kg b.w of ranitidine + 1 ml/kg b.w of 80% ethanol ; Group 3: 2 ml/kg b.w of distilled water + 1 ml/kg b.w of 80% ethanol; Group 4: 100 mg/kg b.w of the of extract + 1 ml/kg b.w of 80% ethanol; Group 5: 300 mg/kg b.w of the of extract + 1 ml/kg b.w of 80% ethanol

Fig. 4: Effect of the ethanol extract of the leaves of *Calopogonium mucunoides* on the concentration of free acidity

3.6 Effect of the Ethanol Extract of the Leaves of *Calopogonium mucunoides* on Total Acidity

The concentration of total acidity in the rats of group 3 (ulcer-untreated control group) was significantly ($p < 0.05$) higher than that of group 1 rats. The concentrations of total acidity in the rats of groups 4 and 5 were significantly ($p < 0.05$) and dose-relatedly lower than the value obtained for rats in group 3. The extract-caused effect was comparable to that obtained with the standard anti-ulcer drug [ranitidine (150 mg/kg body weight)] in group 2 rats as shown in Fig. 5.



Group 1: 2 ml/kg body weight (b.w) of distilled water only
Group 2: 150 mg/kg b.w of ranitidine + 1 ml/kg b.w of 80% ethanol
Group 3: 2 ml/kg b.w of distilled water + 1 ml/kg b.w of 80% ethanol
Group 4: 100 mg/kg b.w of the of extract + 1 ml/kg b.w of 80% ethanol
Group 5: 300 mg/kg b.w of the of extract + 1 ml/kg b.w of 80% ethanol

Fig. 5: Effect of the ethanol extract of the leaves of *Calopogonium mucunoides* on the concentration of total acidity

4. DISCUSSION

In this study, the phytochemical constituents, acute toxicity and anti-ulcer effect of the ethanol extract of the leaves of *Calopogonium mucunoides* using ethanol-induced model of gastric ulceration in rats were investigated with a view to validating their potential in the folkloric management of ulcer.

Acute toxicity test on the ethanol extract of the leaves of *C. mucunoides* using mice showed that the extract at a dose as high 5000 mg/kg body weight had no mortal effect on the mice which indicates that the extract has low toxicity at high doses.

The anti-ulcerative effect exerted by the ethanol extract of the leaves of *C. mucunoides* might be due to the phytochemicals present in the extract as shown in the present study. It is likely that flavonoids and tannins, acting dually or in combination with other phytochemicals caused the observed effect of the extract. Flavonoids and tannins are among the cytoprotective active compounds for which anti-ulcerogenic property has been extensively confirmed. While flavonoids are suggested to be able to stimulate the secretions of mucus, bicarbonate and prostaglandins and counteract the deteriorating effects of reactive oxidants in the gastro-intestinal lumen, tannins are known to "tan" the outermost layer of the mucosa and render it less permeable and more resistant to chemical and mechanical injuries.[10,11]

Evaluation of the effect of the extract on ulcer experimentally induced with ethanol showed that it exhibited gastric cytoprotective effect as it remarkably decreased the volume of gastric juice secretion, ulcer index and concentrations of the free and total acidity in the treated rats. The significantly ($p < 0.05$) elevated increases in the volume of gastric juice secretion, ulcer index and concentrations of the free and total acidity in the rats of the ulcer-untreated control group (group 3) in each of the ulcer study imply that the ethanol, induced ulcer in all the ethanol-treated rats. Ethanol-induced gastric damage has been associated with depletion of gastric mucus, back diffusion of acid, increased gastric mucosal permeability, increasing leak of hydrogen ion from the lumen, decrease in the transmucosal electrical potential difference, changes in the mucosal blood flow, destruction of microvascular and nonvascular types of cells, mast cell degranulation, neutrophil-mediated mucosal injury (release of oxygen free radicals, proteases and lysosomal enzymes, digestion of proteins and lipid peroxidation in cell membrane) and depletion of certain oxygen free radical scavengers.[12] Thus, anticipatedly, the extract might have exerted its anti-ulcerative action by interfering with any of the aforementioned pathologic processes. Similar findings had been reported by[13].

In conclusion, oral administration of the ethanol extract of the leaves of *Calopogonium mucunoides* to Wistar rats caused a substantial gastric cytoprotection against the ethanol-induced ulcer in the rats and therefore, makes the plant a possible future candidate for refined anti-ulcer drug(s).

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