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Gross and Histo-Morphological Study of Anti-Ulcerogenic Effects of *Cissampelos owariensis* (P. Beauv.) Methanolic Extract in Wistar Rats

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Abstract

Cissampelos owariensis is a tropical medicinal plant widely applied for diverse therapeutic uses such as the treatment of circulatory, reproductive and gastrointestinal conditions. In this study, the gastroprotective activity of methanolic leaf extracts of *C. owariensis* against prolonged exposure to acidic gastric acid was assessed in male Wistar rats. This study involved 25 male Wistar rats (180-200 g) divided into five groups (n=5) A-E. Groups A and B were used as normal and test controls given distilled water while groups C-E were respectively administered with methanolic extracts of *C. owariensis* at dosage of 100 mg/kg, 300 mg/kg and 500 mg/kg respectively. The mode of administration was oral and study period was 21 days. Afterward, gastric mucosal injury was induced in groups B-E animals via pyloric ligation method. Macroscopic and microscopic examinations of gastric tissues were done to ascertain the degrees of gastric mucosal protection or erosion using gross photographic and histological staining techniques. The gross appearance of internal aspect of gastric tissues showed mildly eroded mucosal surface in treated groups B-D but intense erosion was observed in test control group E. Consequently, the ulcer index moderately increased in treated groups but significantly increased ($p<0.01$) in test control group E. Similarly for histological results, the treated groups B-D showed mild or focal mucosal surface erosion compared to intense erosion observed in test control group E. This study thereby indicated that prior treatment with methanolic extracts of *C. owariensis* can stimulate anti-ulcer effects against gastric mucosal offensive factor such as acidic gastric secretions. This anti-ulcerogenic effect of methanolic extracts of *C. owariensis* can be associated with the anti-oxidant properties of its constituent phytochemical compounds.

Keywords: *Cissampelos owariensis*; Anti-ulcer effects; Wistar rats

Introduction

Medicinal plants refer to plants that have parts such as seeds, leaves, barks, roots, stem, fruits or the whole plant with constituent phytochemicals that can be applied for therapeutic purposes in order to ameliorate pains and cure diseases. These plants represent important components of natural plant biodiversity especially in many African countries where they constitute vital source of health care for many individuals due to the relative accessibility, affordability, efficacy and tolerability of medicinal plant products [1-3]. One of such medicinal plants is *Cissampelos owariensis* P. Beauvais ex DC. (also called lungwort) which belongs to the Menispermaceae family that comprises of about 70 genera and 450 species. *C. owariensis* is a twiner plant found in tropical regions especially in the wild of some sub-Saharan African countries [4]. Different parts of the plant (especially leaf and root) are used by traditional medical practitioners for various therapeutic applications. Extracts derived from *C. owariensis* have been applied in the treatment of metrorrhagia, wounds, snake bites, circulatory and reproductive diseases, amnesia and psychosis [5,6] prevention of miscarriage and treatment of sterility [7-9] and treatment of various gastrointestinal conditions such as dysentery, diarrhoea, enteritis, colic and intestinal worms [10,11]. They also exhibited diverse biological activities such as insecticidal activity, antimicrobial and antiviral activity, antibacterial and antifungal activity [12,13]. The previous study had reported potent anti-oxidant activity of methanol extract of *C. owariensis* which was especially linked to the flavonoid component of its phytochemicals [14]. These phytochemical flavonoids are plant-derived phenols that exhibit free radical scavenging, anti-allergic and anti-inflammatory activity and help to prevent diseases including gastric ulcers [15-17]. However, there remains a dearth of documented pharmacological studies on various therapeutic uses of *C. owariensis*. Hence, the need for this present study in

which, the methanolic leaf extracts of *C. owariensis* was investigated to assess its anti-ulcerogenic effects on gross and histomorphology of gastric tissues of experimental animals following exposure to the erosive effect of acidic gastric secretions.

Methods

Plant material

Fresh whole *C. owariensis* plant was harvested from Okada community, Ovia North-East Local Government Area, Edo State, Nigeria. The plant was verified at the Department of Biological Sciences, Igbinedion University, Okada, Edo State, Nigeria.

Method of extraction

The leaves of the plant were detached, dried and pulverized into powdered form using mechanical grinder. 700 g of powdered leaves were infused in 5 litres of methanol for 72 hours. Thereafter, the preparation was filtered, filtrate evaporated using rotary evaporator (regulated at 400°C), and the residue obtained cooled (at room temperature), weighed and used as methanolic extracts for the study.

Experimental animals

This study involved 25 adult male Wistar rats weighing between 170 g-200 g. The animals were divided into five groups control groups A and E and test groups B-D. Each group comprises of five animals (i.e. n=5). Group A animals were given distilled water (5 mls/kg body weight) and they represented normal control animals that were not treated and not induced by pyloric ligation. Group B animals were given 100 mg/kg methanolic extracts of *C. owariensis*. Group C animals were given 300 mg/kg methanolic extracts of *C. owariensis*. Group D animals were given 500 mg/kg methanolic extracts of *C. owariensis*. Group E animals were given distilled water (5 mL/kg body weight) and they represented test control animals that were not treated but induced by pyloric ligation.

Period and mode of study

The treatment period of this study was 28 consecutive days and all treatments were done orally using a flexible orogastric gavage.

Induction of gastric mucosal injury using pyloric ligation method

The animals were fasted for 24 hours in separate cages but allow free access to water. Animals were anesthetized by intraperitoneal injection of Ketamine/Xylazine (50 mg/kg at 1:1). A small midline incision was made on the abdomen of animals to access the pyloric part of the stomach. The pyloric end of stomach was gently pulled up, ligated and gently returned into the abdominal cavity and the abdomen was closed. After an observatory period of 5 hours, the animals were sacrificed and stomach tissue harvested and prepared for macroscopic and microscopic examination [18].

Ethical approval

This study was duly approved by the Research and Ethics committee of the University and all procedures employed in this study conformed to standard guidelines for experimental animal handling.

Macroscopic examination

After the study period, harvested stomach tissue of experimental animals were cut open along the greater curvature and internal aspect of the stomach tissues was photographed, using a 20 Mega-Pixel digital camera, to document the gross appearance of the gastric mucosa and mucosal erosion after the pyloric-ligation method of inducing gastric mucosal injury. The ulcer index of gastric mucosa of experimental animals was calculated as the ratio of total lesion length to a number of animals per group [19].

Tissue processing

The stomach tissue of experimental animals were fixed in 10% neutral buffered formalin, dehydrated using ascending grades of alcohol (two changes each of 70%, 90% and absolute alcohol for 30 minutes each), cleared in xylene for 30 minutes and embedded in molten paraffin and allowed to cool to form tissue blocks.

Sectioning

Blocks of processed gastric tissue were cut into sections at 5 µ thickness by using rotary microtome. The tissue sections were mounted on microscope slides and prepared for histological staining and histomorphological study.

Histological staining using hematoxylin and eosin staining technique

Tissue sections were dewaxed in xylene for 15 minutes, hydrated by treating with decreasing grades of alcohol (i.e. absolute alcohol, 90% alcohol and 70% alcohol in succession) for 3minutes each, sections stained in Haematoxylin for 10 minutes, washed in running tap water for 3 minutes and differentiated in 1% acid alcohol (prepared as 1% HCl in 70% alcohol) for 1 minute. Then, sections were blued by washing in alkaline running tap water (Scoh's tap water) for 10-20 minutes, rinsed in water and stained in 1% aqueous Eosin for 3minutes. Sections were rinsed in water (to wash off excess stain), dehydrated using increasing grades of alcohol (i.e. 70%, 90% and absolute alcohol in succession) for 2minutes each, cleared in xylene for 2 minutes and mounted in distrene polystyrene xylene (DPX) [20]. After staining, the tissue sections were allowed to dry and made ready for microscopic examination.

Results

Macroscopic results

The gross appearance of the internal aspect of gastric tissues of experimental animals **(Figure 1)** showed normal gastric mucosa in the normal control group A, varying degrees of mucosal surface protection in treated groups B-D and prominent mucosal surface erosion in test control group E after exposure to the acidic gastric secretions during the pyloric-ligation method.

Ulcer index of the gastric mucosa of experimental animals

The ulcer index of gastric mucosa showed slightly significant increase in treated groups B-D due to the gastroprotective activity of the plant extracts against the erosive effects of gastric acid exposure. However, a spike in ulcer index was observed in test control group E due to exposure to acidic gastric secretions without prior treatment with the plant extracts.

Microscopic results

This histological examination of the gastric tissues of experimental animals **(Figure 2)** showed normal histological architecture of the gastric mucosa of treated animals in normal control group A, moderate mucosal surface erosion in treated groups B and C, mild mucosal surface erosion in treated group D and intense mucosal surface erosion in test control group E following exposure to acidic gastric secretions during pyloric-ligation method.

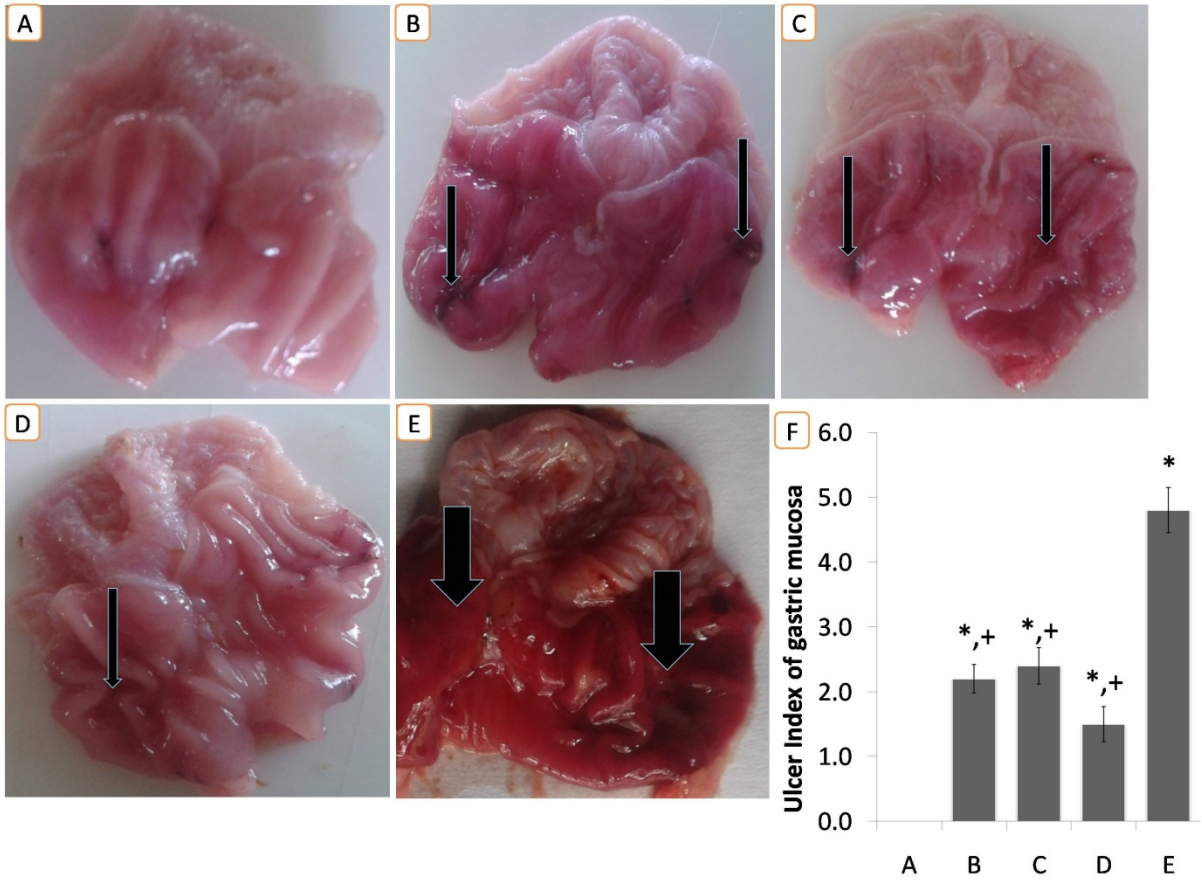

Figure 1: A-E: Macroscopic appearances of the internal aspect of gastric tissues of experimental animals. Group A animals represented normal control animals, group B, C and D animals were given 100 mg/kg, 300 mg/kg and 500 mg/kg methanolic extracts of *C. owariensis* respectively and group E animals represented test control animals. F: Ulcer index of gastric mucosa of experimental animals in groups A-E (*+p<0.05 was considered significant compared to Groups A and E respectively).

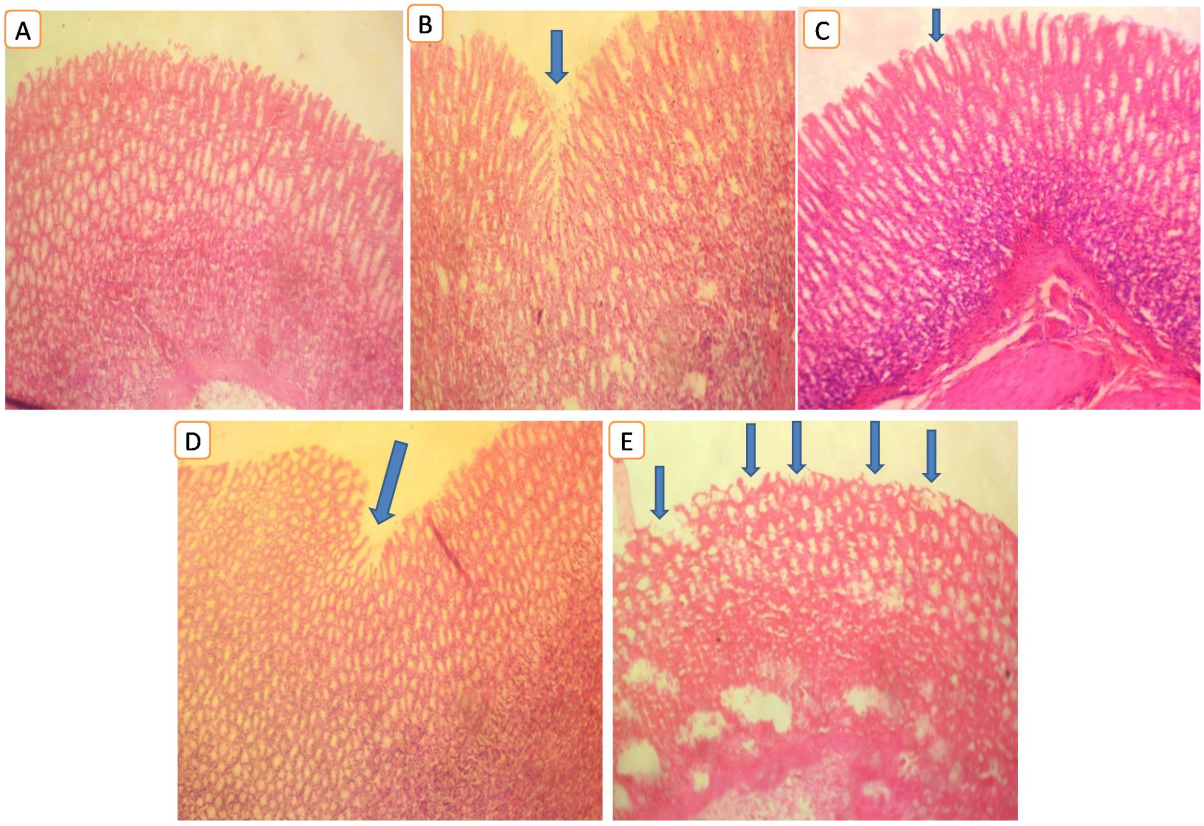

Figure 2: Histological appearances of the gastric tissue of experimental animals (groups A - E) (H and E X100). Group A animals represented normal control animals, group B, C and D animals were given 100 mg/kg, 300 mg/kg and 500 mg/kg methanolic extracts of *C. owariensis* respectively and group E animals represented test control animals. Arrows indicated mucosal surface erosion.

Discussion

The gastric mucosa is constantly vulnerable due to exposure to different substances which may alter the structural integrity of the mucosa causing injury and ulceration especially when gastric mucosal defensive factors are over-whelmed. These defensive factors (such as mucin, bicarbonate, nitric oxide, prostaglandins, and others) help to mitigate against erosive effect of offensive factors (such as gastric acid, pepsin, stress, Helicobacter pylori, and others) thereby preventing mucosal injury and ulcerations [21,22]. Hence, potent anti-ulcerogenic or gastroprotective agents or drugs function by counteracting aggressive factors or stimulating the protective factors [23]. In this study, the pre-treatment of methanolic extracts of *C. owariensis* conferred on the gastric mucosa of experiment animals certain degrees of protection against the erosive effect of acidic gastric secretion exposure during the pyloric-ligation method. According to the result of the macroscopic and microscopic examinations of gastric mucosa of experimental animals **(Figure 1)**, the treated groups B-D compared relatively with the mucosal morphology of the normal control group A. The treated groups B-D also showed very insignificant mucosal surface erosion when compared to the intensely eroded mucosal surface of test control group E. The observed anti-ulcer effects of methanolic extracts of *C. owariensis* in this study may be linked to its constituent bio-active phytochemical compounds. Generally, the constituent phytochemical compounds especially its high-value, low-volume secondary metabolites confer on medicinal plants and or preparations the medicinal properties applied in ameliorating pains and treatment of diseases, hence, called active principles or bioactive components [24-26]. Previous studies have posited that flavonoids derived from medicinal plants exhibit significant anti-oxidant effect and protect against toxic effects of reactive oxygen species (ROS) due to their hydroxyl groups [27,28]. These phytochemical flavonoids have been described to exhibit gastroprotective functions due to by their ability to promote gastric mucus synthesis (by mucous cells), reduce gastric acid secretion (by parietal cells) and down-regulate pepsinogen synthesis (by chief cells) [29-31]. From the result of this study, the gastric mucosa of experimental animals with prior exposure to methanolic leaf extracts of *C. owariensis* exhibited some degrees of gastric mucosal protection against erosive effects of offensive factors (acidic gastric secretions). This is more prominent in higher dosage level of extracts which invariably contain more flavonoids compounds. Hence, the gastroprotective activity of methanolic leaf extracts of *C. owariensis,* like other pharmacological activities of the plant extracts, can be described as a function of its constituent phytochemical compounds. Further studies may be necessary to describe the

cellular and sub-cellular mechanisms of anti-ulcer effects of methanolic leaf extracts of *C. owariensis.*

Conclusion

This study affirmed the anti-ulcerogenic activity of methanolic leaf extracts of *Cissampelos owariensis* (P. Beauv.) and such activity can be associated with therapeutic properties of phytochemical constituents of the plant extract.

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