Document heading   doi:

Homopterocarpin contributes to the restoration of gastric homeostasis by *Pterocarpus erinaceus* following indomethacin intoxication in rats

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ARTICLE INFO

ABSTRACT

Objective: To investigate the restorative effect of *Pterocarpus erinaceus* (*P. erinaceus*) and homopterocarpin, an isoflavonoid isolated from it, on indomethacin−induced disruption in gastric homeostasis in rats. **Methods:** Adult rats were divided into five groups and fasted for 48 h before treatment. Group 1 received olive oil (vehicle), group 2 received 25 mg/kg indomethacin while groups 3−5 received cimetidine (100 mg/kg), homopterocarpin (25 mg/kg) and *P. erinaceus* ethanolic stem bark extract (100 mg/kg) respectively. After 1 h, all the groups except group 2 were administered 25 mg/kg of indomethacin. One hour later, the rats were sacrificed and the ulcer index and other gastroprotective indices were evaluated. **Results:** Indomethacin caused significant injury to the stomach of the rats as reflected in the ulcer indices (9.0±1.4) as compared with that of control (2.0±0.0). Equally, there were significant increases in gastric acid concentration and malondialdehyde level in the stomachs of the ulcerated animals compared with the control. However mucus content, reduced gluthatione level and gastric pH were significantly reduced in the ulcerated animals compared with the control. Pretreatment with either *Pterocarpus* bark extract or homopterocarpin reversed the effects of indomethacin on the evaluated parameters. **Conclusions:** These results indicate that both homopterocarpin and *Pterocarpus* extract offered gastroprotection against indomethacin−induced ulcer by antioxidative *mechanism* and the modulation of gastric homeostasis. The results also suggest that homopterocarpin might be responsible for, or contribute to the antiulcerogenic property of *P. erinaceus*.

1. Introduction

Despite significant medical advances, gastric ulcer is still a common global disease with increasing incidence and prevalence. Currently the treatment drugs include antibiotics to kill *Helicobacter pylori (H. pylori)*, acid blockers (*eg.* cimetidine, ranitidine and famotidine), proton pump inhibitors (*eg.* omeprazole), and tissue lining protecting agents (*eg.* sucralfate and bismuth). These drugs have decreased the morbidity rates but produce many adverse effects such as relapse of the disease, and are often expensive for the poor people.

Plants have an almost limitless ability to synthesize a large number of organic compounds and some of these might offer better protection against ulcer with decreased relapse, while being affordable[1]. *Pterocarpus erinaceus* (*P. erinaceus*) is one of the genus of a family Fabaceae that exhibits a wide ecological range and is found extensively in West Africa. The plant is used traditionally to treat various diseases including stomach troubles. The stem bark has equally been used in traditional medicine to treat inflammatory pathologies such as rheumatism, gastric ulcer and dermatitis[2,3]. Previous studies demonstrated the antimalarial property[4] and the antimicrobial activity of *P. erinaceus*[5]. The need therefore arises to investigate the phytochemical constituents and antiulcerogenic potential of this plant to give scientific basis for its popular use in traditional medicine. The objective of this study was to investigate the protective property of *P. erinaceus* and homopterocarpin, an isoflavonoid obtained from it, on indomethacin−induced ulcer in albino rats.

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2. Materials and methods

2.1. Chemicals and drugs

Alcian blue, 5, 5–dithio–bis (2–nitrobenzoic acid), thiobarbituric acid (TBA) and reduced glutathione (GSH) were obtained from Sigma–Aldrich (St Louis, MO, USA). Indomethacin was obtained from a pharmaceutical store in Akure, Ondo State, Nigeria. Other chemicals were obtained from standard chemical suppliers and were of analytical grades.

### 2.2. Plant material and preparation of crude extract

P. erinaceus stem barks were obtained from a local market in Ondo State, Nigeria. Authentication was done at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. Stem barks of P. erinaceus were air–dried, pulverized and macerated in ethanol (1:3 w/v) for 24 h. The mixture was filtered and the filtrate obtained was air dried. The caked residue was powdered and stored at room temperature until needed.

2.3. Phytochemical screening of Pterocarpus extract

The ethanolic extract of P. erinaceus was screened for phytochemical groups using standard methods[6].

2.4. Isolation of homopterocarpin

Homopterocarpin (3, 9–dimethyoxypterocarpan) (Figure 1) was isolated from P. erinaceus and the chemical structure was established as previously described[7].
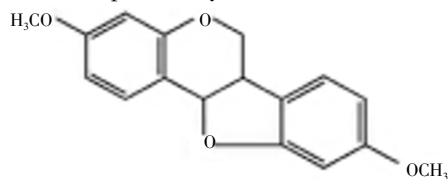
Figure 1. Structure of homopterocarpin.

2.5. Establishment of the optimum dose of homopterocarpin and Pterocarpus erinaceus

In a pilot study, 25, 50, 75 and 100 mg/kg of homopterocarpin and 50, 100, 150 and 200 mg/kg of P. erinaceus ethanoilc extract were assessed for protective effect in ulcerated rats. The lowest ulcer scores and therefore the greatest protection were recorded in groups treated with 25 mg/kg homopterocarpin and 100 mg/kg Pterocarpus extract and this informed the use of these single doses for the present study.

2.6. Animal handling and treatment

Wistar rats weighing 150–200 g obtained from Biochemistry Department, Federal University of Technology, Akure were used for the experiments. The animals were maintained in standard laboratory conditions under natural light/dark cycle and fed with standard rat chow and water ad libitum. Animals were handled and used in accordance with the international guide for the care and use of laboratory animals[8]. Animals were starved for 48 h before the administration of the tested samples. Rats were randomly divided into 5 groups and there were 18 rats in each group. Group 1 (control) received 0.3 mL of olive oil, group 2 was administered 25 mg/kg of indomethacin, group 3 received 100 mg/kg of cimetidine (reference drug) while groups 4 and 5 received 25 mg/kg and 100 mg/kg of homopterocarpin and Pterocarpus extract respectively orally. After 1 h, all the groups except group 2 were administered 25 mg/kg of indomethacin. One hour later, the rats were sacrificed. The stomachs were removed and opened along the greater curvature and the gastric contents were collected in sterile bottles. Ulcer scores were determined as described by Robert[9].

2.7. Determination of the pH of gastric juice

The gastric contents collected were centrifuged at 3 500 × g for 15 min for determination of gastric pH. Acidity in gastric secretion was determined by back titration with 0.01 M NaOH according to the method reported by Campos et al[10]. Phenolphthalein was used as the indicator.

2.8. Estimation of gastric mucus content

Mucus content was evaluated as mucin concentration. Alcian blue binding to gastric wall mucus was determined according to the method reported by Kiliç et al[11]. The stomachs of rats were rinsed with 0.25 M sucrose solution and then incubated in 10 mL aliquots of 0.1% alcian blue solution for 2 h at room temperature. After 2 h, the stomachs were removed, washed with 0.25 M sucrose solution again and separately incubated in 3 mL aliquots of 0.5 M magnesium chloride solution for 2 h at room temperature with shaking at 30 min intervals to elute the alcian blue bound to the mucosa of the stomachs. The stomachs were then removed and 2 mL of each aliquot of magnesium chloride solution containing the alcian blue eluted from each stomach was shaken with 5 mL of diethyl ether. The aqueous phase was separated out, centrifuged at 3 200 × g for 5 min and the absorbance of the supernatant was measured at 605 nm. The amount of alcian blue bound per stomach was determined using a standard calibration curve.

2.9. Evaluation of the extent of in vivo lipid peroxidation

Lipid peroxidation inhibitory activity was evaluated by measuring the formation of malondialdehyde (MDA) according to the method described by Olaleye et al[12]. The stomachs of rats from the various groups were removed, emptied and soaked in phosphate buffered saline. The stomach linings were scraped, weighed and homogenized in phosphate buffered saline (1:4) and then centrifuged at 6 000 rpm for 15 min to obtain a clear supernatant used for the

assay. To 300 μL of homogenates was added 8.1% sodium dodecyl sulphate, 20% acetic acid and 0.8% TBA and the mixture was incubated at 100 ℃ for 1 h and allowed to cool. The absorbance was read at 532 nm

2.10. Evaluation of gastric reduced glutathione level

To 0.2 mL of the supernatants prepared in section 2.9 was added 1.8 mL of distilled water followed by 3 mL of sulphosalicylic acid. The mixture was allowed to stand for 5 min and then filtered. To 1 mL of filtrate was added 4 mL of 0.1 M phosphate buffer (pH 7.4) and then 0.5 mL of Ellman's reagent. Absorbance was read at 412 nm[13].

2.11. Statistical analyses

Values were reported as mean±SEM. The statistical significance of differences between groups was assessed using one–way ANOVA followed by Duncan's multiple range test. Significance was accepted at $P < 0.05$.

3. Results

Flavonoids, terpenoids, tannins and steroids were detected in *P. erinaceous* extract (Table 1). The indomethacin–induced disturbance in gastric homeostatic equilibrium of rats on the one hand and the amelioration afforded by pretreatment with the test substances on the other hand are clearly highlighted by the results obtained. Indomethacin caused a significant degree of ulceration as reflected in the ulcer score of the indomethacin–administered group compared with the control. Groups pretreated with either homopterocarpin or *P. erinaceus* extract showed significantly reduced ulcer scores compared with the indomethacin–administered group and the protection offered was better than that of the reference drug, cimetidine (Figure 2). The indomethacin–administered group also showed increased gastric acid and MDA concentrations (Figures 3 and 4 respectively) but lower pH (Figure 5) and decreased mucus content and GSH concentration (Figures 6 and 7 respectively), compared to the control ($P<0.05$) and the homopterocarpin and *Pterocarpus* extract treated groups ($P<0.05$ except for mucin content).

Table 1
Phytochemical constituents of ethanolic stem–bark extract of *P. erinaceus*.

| Compound group	Present/absent
Saponins	–
Alkaloids	–
Tannins	–
Phlobatannins	+
Anthraquinones	–
Flavonoids	+
Terpenoids	+
Steroids	+

Key: + indicates present; – indicates absent.
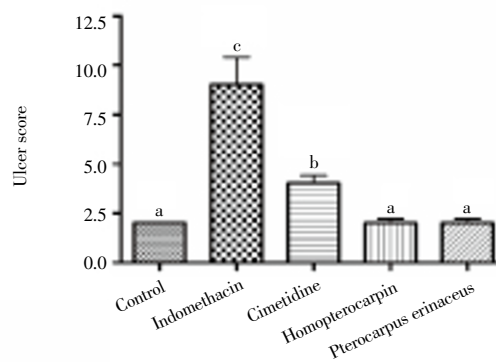
Figure 2. Ulcer scores of all experimental groups. Values with different letters are statistically different ($P<0.05$).
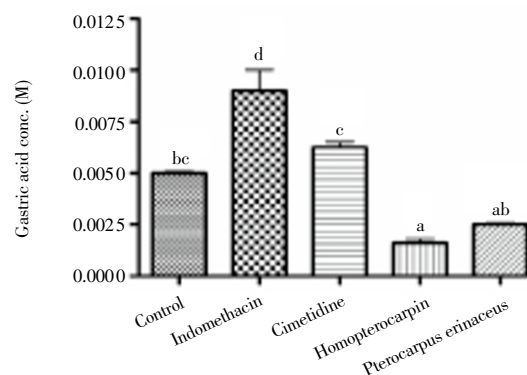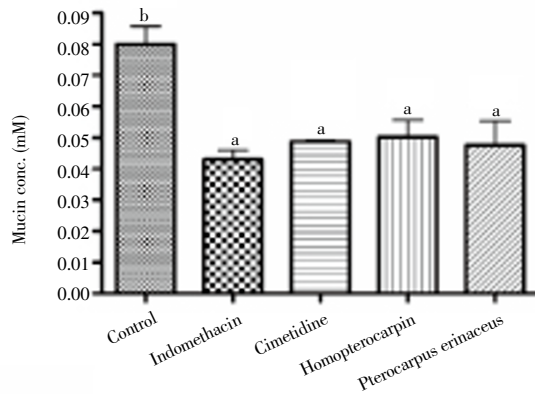

Figure 3. Gastric acid concentration of all experimental groups. Values with different letters are statistically different ($P<0.05$).
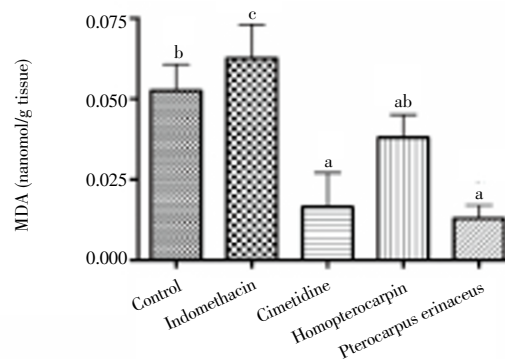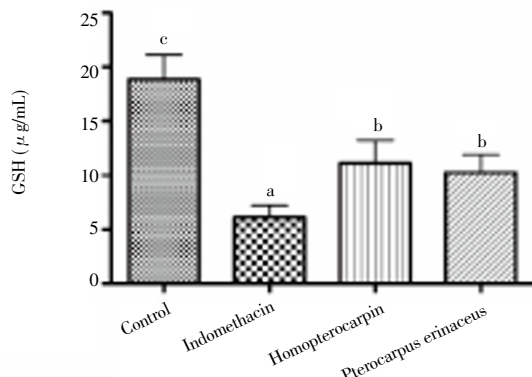

Figure 4. MDA level of control and test groups. Values with different letters are statistically different ($P<0.05$).
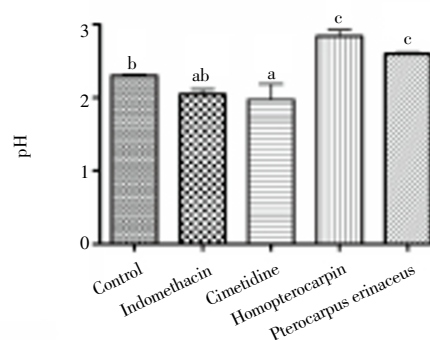
Figure 5. Gastric pH in control and test groups. Values with different letters are statistically different ($P<0.05$).

Figure 6. Effects of *P. erinaceus* and homopterocarpin on mucin concentration of experimental animals. Values with different letters are statistically different (*P*<0.05).

Figure 7. Reduced Glutathione level of experimental groups. Values with different letters are statistically different (*P*<0.05).

The cimetidine treated group showed similar trends to those observed for homopterocarpin and *Pterocarpus* extract treated groups in almost all the assays but generally exhibited less potency in ameliorating indomethacin–induced gastric disturbances. Homopterocarpin pretreatment led to particularly remarkable positive effect on the gastric juice concentration and pH of the animals as compared with cimetidine and Pterocarpus extract.

4. Discussion

The presence of flavonoids, terpenoids, tannins and steroids gives an insight into the therapeutic potentials of the crude plant. This probably explains the earlier reported medicinal uses of *P. erinanceus*[4,5].

Results from this study indicate that both *P. erinaceus* and homopterocarpin possess antiulcerogenic potential. The results are also suggestive that homopterocarpin which is an isoflavonoid may be responsible for, or contribute to the antiulcerogenic activity of *P. erinaceus*. Several researches have reported the antiulcerogenic potentials of flavonoids[14–19]. To the best of our knowledge, the gastroprotective property of homopterocarpin has not been reported.

Indomethacin is an indol derivative, non–steroidal, anti–inflammatory drug with analgesic, and antipyretic effects. It has been suggested that indomethacin induces gastric damage via inhibiting the release of protective factors like cyclooxygenase–1, prostaglandin E_2, bicarbonate and mucus; increasing aggressive factors like gastric acid; and increasing oxidant parameters while decreasing antioxidant parameters[20]. The import of this is that indomethacin creates an imbalance in gastric homeostasis by promoting pro–ulcerogenic factors while inhibiting anti–ulcerogenic ones. This is clearly evident from this study. Indomethacin administration decreased the mucus content and pH but increased the concentration of gastric acid. *P. erinaceus* and homopterocarpin increased gastric mucus content (albeit non–significantly) and the pH. The increase in mucus content may be associated with the increase in pH level in the treated animals and the modulation of these indices contribute in conferring gastroprotection on the animals. Gastric mucus is composed chiefly of mucins, large, heavily glycosylated proteins which lubricate the stomach lining and form a barrier against gastric acid. The contribution of mucins to host defense against *Helicobacter pilori has* been reported[21].

Indomethacin intoxication also increased the MDA level in the animals. Ulcerogens have been reported to induce lipid peroxidation in stomach tissue through excess superoxide anion radicals generated by the reaction of xanthine oxidase with hypoxanthine[22] and the hydroxyl radical generated by the Fenton reaction. Superoxide anion radicals react with superoxide dismutase and are converted to hydrogen peroxide. *P. erinaceus* and homopterocarpin significantly decreased the production of MDA in the stomach of ulcerated animals as compared with indomethacin–induced group. This implies that both *P. erinaceus* and homopterocarpin were able to significantly decrease the production of lipid peroxides in rat stomachs. The ability of *P. erinaceus* and homopterocarpin to decrease lipid peroxidation may be due to the fact that homopterocarpin is an isoflavonoid. Flavonoids and other polyphenols are secondary metabolites with well documented health benefits. One of such is their gastroprotective property which has been attributed to their free radical scavenging and antioxidant activity. They have been reported to promote mucosa formation, diminish acid secretion, and inhibit production of pepsinogen to provide cytoprotection[23]. In addition, *P. erinaceus* and homopterocarpin caused significant increase in the GSH level in the gastric mucosa of the albino rats as compared to the indomethacin group further buttressing the fact of their antioxidant potential. This result is in accord with the earlier findings of Ishige *et al*[24] who reported that flavonoids can alter GSH metabolism and scavenge free radicals.

In conclusion, findings from this study indicate that the cytoprotective and antisecretory properties demonstrated by *P. erinaceus* and homopterocarpin are mediated by antioxidative mechanism and the regulation of gastric dynamics leading to the restoration and maintenance of normal gastric equilibrium.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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