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Antiulcer activity of methanolic extract and fractions of *Picralima nitida* seeds(Apocynacaea) in rats

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ABSTRACT

Objective: To investigated the antisecrectory activities of the methanol extract, chloroform fraction and methanol fraction of *Picralima nitida* seeds. **Methods:** The methanol extract of *Picralima nitida* seeds was fractionated into chloroform fraction and methanol fraction. They were evaluated for antiulcer activity and gastric emptying time in rats using aspirin-pylorus-ligation model. **Results:** Oral administration of the methanol extract, chloroform fraction and methanol fraction at 1 000 mg/kg reduced gastric ulcer by 56.4%, 40.0% and 56.3%, respectively; and the fractions of the extract significantly ($P<0.05$) reduced gastric emptying time when compared to the control. Gastric acidity was significantly decreased when compared with saline group, 40.25 mEq/L in methanol extract, 50.0 mEq/L in chloroform fraction 51.25 mEq/L in methanol fration but had no significant effect on the gastric secretion volume. **Conclusions:** These findings showed that methanol extract, chloroform fraction and methanol fraction of the seeds of *Picralima* possessed potent antiulcer properties and some antisecretory properties.

1. Introduction

Gastric and duodenal ulcers affect a large proportion of the world population and are induced by several factors, including stress, smoking, nutritional deficiencies, and ingestion of nonsteroidal anti-inflammatory drugs[1,2]. It remains one of the most common gastrointestinal diseases that results in loss of work and high-cost of medical services[3]. Natural products form the bases of many standard drugs used in modern medicine, and are enjoying a revival in popularity in the developed economies around the world. *Picralima nitida (P.nitida)* is a shrub plant that is distributed in tropical Africa including Nigeria. It is reported to have various traditional medicinal uses ranging from use for pains and fever (roots), malaria and pneumonia, aphrodisiacs and stomach ache (seeds)[4,5], anti-tussive, skin infections and antidiabetics (leaves). Published works have shown its potent antioxidant[6], analgesic and anitinflammatory[7] and hypoglycemic activities[8].

This present study investigated the the antiulcer activity of the methanolic extract, methanolic and chloroform fractions of *P. nitida* seeds using the aspirin-pylorus-ligation model.

2. Materials and methods

2.1. Animals

Albino rats (150-250 g) bred in the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used for the experiment. They were maintained under standard conditions and were fed with pellets (Guinea Feeds Plc, Nigeria) and allowed unrestricted access to water. The animals were fasted for 24 hours (but given water *ad libitum*) before the experiments. Twenty rats for the different models (forty rats in total) were used. They were divided into five groups of four animals each.

2.2. Preparation of extracts and fractions

The seeds of the plants were collected in mid-May from the forests of Obukpa, Nsukka Local Govermnent Area, Enugu State. They were identified at the Bioresources Development and Conservation Programme (BDCP) Centre,

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Nsukka, Enugu. Sample specimens were deposited in the herbarium of the Department of Pharmacognosy, University of Nigeria, for future references.

The seeds were dried for over one week, milled to fine particle size, and immersed in analytical grade methanol. After intermittent shaking for 48 hours, the mixture was filtered with a large-sized filter paper. The filtrate was subjected to a rotary evaporator and the marc (extract) collected and allowed to air dry for some days.

Part of the extract was then subjected to column fractionation using silica gel as adsorbent and analytical chloroform and methanol were used as mobile phase solvents independently. The different fractions were collected after complete elution.

2.3. Phytochemical screening

Using procedures outlined by Trease and Evans[9], phytochemical constituents were identified and quantified.

2.4. Experimental models

2.4.1. Aspirin-pylorus ligation method

Rats were divided into five groups of four each and received treatments per oral as follows: first group received 5 mL/kg normal saline, second group 100 mg/kg whole methanolic extract, third group received 100 mg/kg of chloroform fraction, fourth group received 100 mg/kg methanolic fraction and fifth group 50 mg/kg cimetidine. Thirty minutes later, all the rats were given 200 mg/kg aspirin suspended in 1% carboxymethylcellulose once daily and continued for 4 days. On the 5th day, food was withdrawn but water was allowed and rats were fasted for 24 hours. On the sixth day, pylorus ligation was done under ketamine[10]. After 4 hours of pylorus ligation, animals were sacrificed; stomachs were removed and cut open along the greater curvature. Gastric juice was collected into test tubes for biochemical analysis. Ulcer wounds were counted using a×10 magnifying lens and scored as described in Ganguly and Bhatnagar[11]. The total ulcer indices were divided by the magnification of 10.

2.4.2. Measurement of gastric emptying by phenol red concentration in rat stomach

Twenty rats (fasted for 12 hours) were divided into five groups of four rats each and received treatments as described above. After thirty minutes, phenol red solution (prepared by dissolving 50 mg of phenol in 100 mL of methylcellulose) was administered at 1 mL/100g by oral route. Animals were sacrificed after 1 hour of phenol red administration and stomach clamped at both the esophageal and pyloric region. The stomach was gently removed and the viscera and its content subjected to analysis of phenol red concentration. Estimation of phenol red concentration involved homogenization (in 5 mL 0.1N NaOH) and centrifugation (at 2 000 rpm for 15 minutes) of the visceral content. The supernatant was then subjected to a colorimetric assay at absorbance of 560 nm.

2.4.3. Measurement of gastric acidity

One mL of the total centrifuged gastric contents from each pylorus-ligated rat was analysed for hydrogen ion concentration by titrating against a 0.01N solution of NaOH. The experiment was done in triplicate.

2.5. Statistical analysis

Results are displayed as mean±SEM and differences in mean values of gastric emptying time were tested using unpaired Student's *t*-test. For test of statistical significance, ANOVA followed by Tukey's post hoc test were employed and $P< 0.05$ was used.

3. Results

The phytochemical tests for the chloroform and methanol fractions showed abundant presence of glycosides and tannins. The chloroform fraction showed trace amounts of saponins, flavonoids and terpenoids while the methanol fraction showed abundance of saponins and alkaloids.

Antisecretory parameters showed a decrease in total acidity at 40.25 mEq/L with the whole methanolic extract, 50.0 mEq/L with chloroform fraction and methanolic fraction reduced acidity to 51.25 (Table 1). These reductions in total acidity were statistically significant compared with saline group. Reduction in pepsin activity by the methanolic extract and fractions were also statistically significant (Table 1).

Table 2 displayed results of effects of treatments on mean ulcer indices and phenol red concentration. All reductions were statistically significant compared with saline control group.

Table 1

Effects of methanolic extract, chloroform fraction and methanolic fraction of *Picralima nitida* seed on various acid secretory paramaters.

Treatment	Dose	Volume (mL)	Total acidity (mEq/L)
NS	5 mL/kg	1.40±0.14	72.00±6.75
ME	100 mg/kg	1.30±0.08	40.25±5.86*
CF	100 mg/kg	1.40±0.05	50.00±5.58*
MF	100 mg/kg	1.70±0.22	51.25±3.20*
Cimetidine	50 mg/kg	1.10±0.02	20.00±0.25*

*Significance at $P<0.05$. NS= normal saline, ME=Methanolic extract, CF=Chlorofrom fraction, MF=methanol fraction.

Table 2

Effects of methanolic extract, chloroform fraction and methanolic fraction of *Picralima nitida* seed on ulcer index.

Treatment	Dose	UI	Inhibition (%)	Phenol red conctent (mL)
NS	5 mL/kg	5.50±0.03	–	12.00±0.56
ME	100 mg/kg	2.40±0.01*	56.36	23.00±0.63
CF	100 mg/kg	3.30±0.01*	40.00	21.00±0.74
MF	100 mg/kg	2.40±0.02*	56.36	28.00±0.79
Cimetidine	50 mg/kg	1.60±0.01*	70.90	30.00±0.88

*Significance at $P<0.05$. UI=ulcer indices, NS= normal saline, ME=Methanolic extract, CF= Chloroform fraction, MF=Methanolic fraction.

4. Discussion

This study has shown that the fractions of P*icralima nitida* seeds produced significant reduction of ulcer index, total acidity, pepsin activity and increase in mucoprotective parameters such as phenol red content. Although in most cases the etiology of ulcers is unknown, it is generally accepted that they result from an imbalance between factors such as acid and pepsin production and the maintenance of mucosal integrity through endogenous defense mechanisms[12]. The aspirin ligation ulcer model causes an increase in acid secretion which in turn increases gastric volume and total acidity, while all these factors cause ulcer wounds[13]. *Picralima* seed extract and fractions produced significant reduction in total acidity but did not significantly reduce the volume of gastric content. This corroborates the theory of an anti-secretory effect of the fraction and extracts. Though this effect was lower than that of cimetidine, reduction by the methanolic extract was more pronounced than the fractions. This protection could reflect the inhibition of gastric acid secretion or an increase in the release of protective substances by the mucosa such as prostaglandins that protect against acid mucosal injury[14]. These effects are also producible by histaminergic antagonist like cimetidine which possesses antisecretory effect in blocking the release of acid (HCl) and pepsin in the stomach. The phytochemical screening showed presence of flavonoids which may have produced the antiulcer activity seen in this experiment.

The fractions and extract exhibited potent antiulcer activity by significant decreases in ulcer indices. Reduction of ulcers in aspirin ligation model usually signifies a protective action and possibly an antisecretory effect. Non steriodal antiinflammatory agents (NSAIDS) such as aspirin suppress prostaglandin synthesis and further increase the susceptibility of the mucosal linings to injury and ulceration[15]. Reduction of mean ulcer index and acid content by methanol extract, chloroform fraction and methanol fraction in the presence of high acidity strongly suggests an antisecretory activity.

The gastric emptying time is reduced by significantly higher concentrations of phenol red in all the treatment, which could be a proposition of gastroprotection as a possible mechanism of action of the extract and fractions. This action is believed to result from an activation of cholecystokinin, which slows the gastric emptying time. This reduction in gastric emptying time is a key role in the mucoprotective effect of prostaglandins[16].

This study proved that the methanolic extract, methanolic and chloroform fractions of seeds of *P. nitida* using the aspirin-pylorus-ligation model possessed some antisecretory properties and slowed the gastric emptying time. The exact mechanism(s) of action of the antiulcer activity of the methanolic extract, methanolic fractions and chloroform fractions have not be fully investigated and there is need for further study to find out specific mechanisms of action of these extract and fractions.

Conflict of interest statement

We declare that we have no conflict of interest.

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