ANTl-ULCEROGENIC ACTIVITY OF THE METHANOL ROOT BARK EXTRACT OF COCHLOSPERMUM PLANCHONII (HOOK f)

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Abstract

Cochlospermum planchonii (Hook f) is a common medicinal plant used in Nigeria traditional medicine for treatment of different ailments including ulcers. The anti ulcer activity of the root bark methanol extract of Cochlospermum planchonii was evaluated using different [ethanol, acetylsalicylic acid (aspirin), cold/restraint stress and pyloric ligation/histamine – induced ulcers and acid production] ulcerogenic models in rats at the doses of 250, 500, and 1000 mg/kg body weight using cimetidine (100 mg/kg) as a standard reference drug. The different doses of the extract and the reference drug significantly (p < 0.01) decreased all the ulcer parameters in a dose dependent manner in all the models used. The total number of ulcers were significantly (p < 0.05) decreased. The ulcer index was significantly (p < 0.004) reduced by the extract. Similarly, the percentage ulcer preventive index was also increased from 0% in the negative control up to 93.2% at the dose of 1000 mg/kg, while the percentage ulcer severity was dose dependently reduced by the extract. Furthermore, the extract significantly (p < 0.02) decreased free gastric HCl and total gastric acid. In conclusion, Cochlospermum planchonii methanolic root bark extract showed significant antiulcer activity in this study which may be as a result of its cytoprotective, antioxidant or antiserective properties.

Keywords: Anti-ulcerogenic, Cochlospermum planchonii, Cimetidine, Cytoprotective, antisecretory, Flavonoids

Introduction

Ulcers can be defined as conglomerate of heterogeneous disorders which manifest as breaks in the lining of gastrointestinal mucosa that might result in erosions and damage of the stomach wall that may become perforated (Wallace, 2008). They develop when the normal gastric equilibrium between aggressive (acid and pepsin production and Helicobacter pylori) and protective mechanism (mucus and bicarbonate secretion, increased blood flow, cell turn over, impermeability to H+ etc) are disturbed by enhanced aggressive factors or reduced defensive factors or both (Barbastefano et al., 2007). Ulcers have also resulted from inhibition in the synthesis of mucus, bicarbonate and prostaglandins (Khan et al., 2011).

The condition kills few but troubles many (Laurence et al., 1997). Ritter et al. (1995) estimated that up to one million of the UK population suffers from peptic ulceration yearly. In the United States, one year point prevalence is estimated at 1.8%, while life time is approximately 10%. Peptic ulcer disease affects approximately 4.5 million people annually and prevalence has shifted from predominance in males to similar occurrence for both sexes, while age trends for ulcer reveal decline in younger men and increasing rates in older women (Tri and George, 2007).

Symptoms of ulcer vary depending on the location of the ulcer and the age of the persons or animals. The most common symptom observed in man is epigastric pain. They cause burning, gnawing pain in the stomach and the upper middle of the abdomen that is relieved by eating or taking antacids (Smith et al., 1972).

Many drugs are used for treating ulcers but their successes are limited by several adverse effects (Muralidharan and Srikanth, 2009). Treatments of gastric disorders with medicinal plants are quite common in traditional medicine (Schmeda-Hirschman and Yesilada, 2005). Medicinal plants have been documented to have advantage in toxicity considerations based on their long term use and one might expect bioactive compounds obtained from such plants to have low animal and human toxicity (Fabricant and Farnsworth, 2001).

Cochlospermum planchonii belongs to the family Bixaceae and is a shrub or small tree of 2- 2.5 m high. The habitat is savannah and in Nigeria it is usually found in Kogi and Benue States, where it grows in the wild (Burkill, 1985). The folkloric uses include the use for the treatment of diabetes mellitus, infertility and pre-menstrual pain with different parts of the plant and the treatment of stomach pains (suspected to be ulcer) with the plant’s root bark by the Igede people of Benue state, Nigeria (Igoli et al., 2005). C. planchonii have been reported to posses anti diarrhoeal activity (Ezeja and Anaga, 2010), antimalaria activity (Benoit –Vical, 2003), anti- trypanocidal activity (Atawodi, 2005) and hepatoprotective activity (Roseline et. al., 1995). Acute toxicity study of the root bark extract of the plant by Anaga and Oparah (2009) showed that the extract was safe even at very high doses and highly tolerated in rats. The medicinal use of another species of the plant (Cochlosperm tinctorium) in Mali for antiulcer, radical scavenging and immunomodulatory purposes have been reported (Nergard et. al., 2005)
Literature review shows little or no scientific report on the antiulcer activities of *C. planchonii*. The main objective of this study is to establish the pharmacological basis for the folkloric use of *C. planchonii* root bark for the treatment of stomach pains suspected to be ulcers among the Igede people in Nigeria.

**Materials and Methods**

**Collection, identification and extraction of plant material**

The plant material was collected from the premises of University of Agriculture, Markudi, Benue State, Nigeria and was identified as *Cochlospermum planchonii* (Hook f.) by Mr. A. Ozioko, a botanist with Bioresources Development and Conservation Programme (B.D.C.P.) Aku Road, Nsukka, Enugu State. A voucher specimen number (UNVPP/ 2009/3001/75) was deposited in the herbarium of the Department of Botany, University of Nigeria, Nsukka.

The root barks were chopped into small pieces, dried under room temperature, pulverised into a coarse powder and extracted by cold maceration method in 80% methanol for 48 h. The extract was filtered and later concentrated in vacuo using rotary evaporator at 40°C and 210 milibar and designated as *Cochlospermum planchonii* extract (CPE). The percentage yield (w/w) of the extract was calculated.

**Experimental animals**

Mature albino Wistar rats of both sexes bred in the laboratory animal unit of Faculty of Veterinary Medicine, University of Nigeria Nsukka were used for the experiments. They were housed in stainless steel cages and fed with standard commercial pelleted feed (Vital feed®, Nigeria). Ethical conditions governing the conducts of experiments with life animals were strictly observed as stipulated by Ward and Elsea (1997) and the experimental protocol was approved by the Faculty of Veterinary Medicine’s Ethical Committee.

**Evaluation of methanolic root bark extract of *C. planchonii* for antiulcer activities**

**Ethanol-induced gastric ulceration**

The method of Morimoto *et al.* (1991) was adopted for this study. Twenty five mature rats of both sexes were randomly divided into five groups of five rats each. They were fasted for 24 h and clean drinking water provided *ad libitum*. Group 1 rats (negative control) received 10 ml/kg of distilled water. Group 2 rats (positive control) received 100 mg/kg body weight of cimetidine. Groups 3, 4 and 5 rats were treated with *C. planchonii* extract at the doses of 250, 500 and 1000 mg/kg respectively all by gastric gavage.

One hour later, 1 ml of absolute ethanol was administered to each of the rats. Animals were sacrificed 2 h later by cervical dislocation.

**Acetyl salicylic acid (aspirin)-induced gastric ulcerations**

The method of Williamson *et al.* (1986) was adopted for this study. 25 rats were randomly divided into five groups of five rats each and treated as above. After 1 h, acetyl salicylic acid (200 mg/kg) was administered to each of the rats. The animals were sacrificed 4 h later by cervical dislocation.

**Cold stress / restraint gastric ulcerations**

This was studied using the method of File and Pearce (1981). Twenty five rats were randomly grouped into five groups of five rats per group, fasted for 12 h, and were treated as in the ethanol-induced gastric ulceration experiment above. The rats were put in cages in a slanting position to restrain them. Then, the cages were put in a deep freezer at the temperature of −4°C for 2 h after which the rats were removed from the freezer and sacrificed by cervical dislocation.

**Stomach processing and assessment of ulcers**

After each of the above experiments, the abdomen of each of the rats was cut open. The stomach was isolated and cut open along the greater curvature and examined for ulcer lesions with the aid of a magnifying glass (x10). The ulcer lesions were scored and the total number of ulcers and the ulcer index were obtained for each rat according to the method of Main and Whittle (1975). The percent ulcer severity and ulcer inhibition were calculated by the method of Hano *et al.*, (1976).

**Effect of the methanolic root extract of *C. planchonii* on pyloric ligation /histamine- induced gastric ulcers and acid production**

The method of Shay *et al.* (1954) as modified by Okabe *et al.* (1976) was used in this experiment. Five groups of 5 rats each were fasted for 24 h. Thirty minutes before surgery the animals were treated as in the ethanol-induced gastric ulcer experiment above.
Surgery

Surgery was performed on the rats and the pylorus of each of the rats was ligated. Histamine (100 mg/kg) was administered to each of the rats intraperitoneally post operation. At 1 and 3 h following histamine administration each group of the rats received a repeat of the treatment it had before histamine administration by the same route. 6 h after histamine administration, each rat was sacrificed by cervical dislocation. The stomachs were carefully removed after ligating the cardiac end. The contents of the stomach were collected. The gastric volumes were noted. The contents were centrifuged at 10,000 r.p.m for 5 minutes and 2 ml of the supernatants each were used for titration to determine the gastric free hydrochloric acid (HCl) and gastric total acid using 0.1N sodium hydroxide with thymol blue as an indicator. Other ulcer parameters were assessed using the methods mentioned earlier.

Bioassay-guided fractionation of the methanolic root extract of *C. planchonii*

Fractionation was done using column chromatography according to the methods of Abbot and Andrew (1970) and Harbourne (1991), where silica gel (GF254) was used as the stationary phase while gradient solvent system of the combination of hexane, chloroform, ethyl acetate and methanol were used as the mobile phase and thin layer chromatographic technique (Stahl, 1969) using precoated (silica gel GF254) aluminium plates in a thin layer chromatographic (TLC) tank. Later, the fractions were pooled based on their Rf values and eluting patterns. The pooled fractions were concentrated using rotary evaporator.

Evaluation of the fractions of *C. planchonii* on Acetylsalicylic acid (Aspirin)-induced gastric ulceration (in vivo)

The different fractions designated as the methanolic extract of *Cochlospermum planchonii* fractions (MECPFr) obtained from the extract were evaluated for antulcer activity using the method of Williamson *et al.* (1986) as described earlier.

Identification of the chemical family present in the most active fraction of MECPFr

Phytochemical analysis was performed on the most active fraction of *C. plachonii* using the methods of Trease and Evans (1996) to identify the chemical family present in it.

Data analysis

The results of the experiments were presented as mean ± S.E.M and were subjected to statistical analysis using One-way analysis of variance (ANOVA) and Mann-Whitney test. The differences between the means were tested using post hoc Dunnett and values of $p < 0.05$ were considered statistically significant. The statistical analysis was done using SPSS version 17 for windows.

Results

Extract

The percentage yield was 7.62% w/w dry matter. The extract was ox-blood in colour.

Effect of *C. planchonii* on ethanol-induced gastric ulceration in rats

The result of the effect of *C. planchonii* extract on ethanol induced gastric ulceration in rats is presented in Table 1. The result showed that CPE at the doses tested just like cimetidine significantly ($p < 0.0001$) reduced the number of ulcers and decreased the mean ulcer index ($p < 0.004$) in a dose dependent manner when compared to the negative control. The extract also caused a dose dependent decrease in percent ulcer severity and increase in ulcer preventive index.

Effect of *C. planchonii* acetylsalicylic acid (ASA)-induced gastric ulcerations in rats

The result of the effects of *C. planchonii* on acetylsalicylic acid-induced gastric ulceration in rats is presented in Table 2. The result showed that CPE at all the doses used significantly ($p <0.0001$) decreased the mean number of ulcers and the ulcer index respectively in a dose dependent manner when compared to the negative group. Similarly, the ulcer preventive index was increased by CPE in a dose dependent manner with CPE at the dose of 1000 mg/kg causing up to 93% inhibition.

Table 1: Effect of *C. planchonii* on ethanol-induced gastric ulceration in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean no. of ulcers ± S. E. M.</th>
<th>Mean U. I. ± S.E. M.</th>
<th>% severity</th>
<th>Preventive index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled water (10ml/kg)</td>
<td>8.0±1.05</td>
<td>1.48±0.29</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Cimetidine (100mg/kg)</td>
<td>3.8±0.66*</td>
<td>0.48±0.19**</td>
<td>32.0</td>
<td>68.0</td>
</tr>
<tr>
<td>3</td>
<td>CPE (250mg/kg)</td>
<td>8.0±0.45</td>
<td>0.62±0.15**</td>
<td>81.3</td>
<td>18.7</td>
</tr>
<tr>
<td>4</td>
<td>CPE (500mg/kg)</td>
<td>3.2±0.86*</td>
<td>0.44±0.14**</td>
<td>29.3</td>
<td>70.7</td>
</tr>
<tr>
<td>5</td>
<td>CPE (1000mg/kg)</td>
<td>1.0±0.77*</td>
<td>0.10±0.78**</td>
<td>6.7</td>
<td>93.3</td>
</tr>
</tbody>
</table>

*P < 0.0001,  **P < 0.004 when compared to the negative control group*
Table 2: Effect of *C. planchonii* on acetylsalicylic acid (aspirin)-induced gastric ulceration in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean no. of ulcers ± S.E.M.</th>
<th>Mean U. I. ± S.E.M.</th>
<th>% severity</th>
<th>Preventive index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled water (10ml/kg)</td>
<td>14.4±1.04</td>
<td>2.02±0.40</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Cimetidine (100mg/kg)</td>
<td>4.2±0.26*</td>
<td>0.48±0.12**</td>
<td>33.3</td>
<td>73.7</td>
</tr>
<tr>
<td>3</td>
<td>CPE (250mg/kg)</td>
<td>8.6±1.30*</td>
<td>1.12±0.16**</td>
<td>54.4</td>
<td>45.6</td>
</tr>
<tr>
<td>4</td>
<td>CPE (500 mg/kg)</td>
<td>6.0±1.43*</td>
<td>0.38±0.18**</td>
<td>18.4</td>
<td>81.6</td>
</tr>
<tr>
<td>5</td>
<td>CPE (1000mg/kg)</td>
<td>3.2±0.11*</td>
<td>0.14±0.09**</td>
<td>6.8</td>
<td>93.2</td>
</tr>
</tbody>
</table>

* P < 0.0001, **P < 0.05 when compared to the negative control group

**Effect of *C. planchonii* on cold/restraint stress-induced gastric ulceration in rats**

The effect of CPE on cold/restraint stress-induced gastric ulcers in rats is presented in Table 3. The result showed that the extract and the reference drug significantly (p <0.05) decreased the total number of ulcers, the ulcer index and the ulcer severity in a dose dependent manner. The percent ulcer preventive index was also significantly increased with the increase in the dose of the extract from zero percent in group 1 to 84.1% in group 5.

**Effect of *C. planchonii* on pyloric ligation/Histamine-induced gastric ulceration/acid production**

Table 4 shows the result of CPE on pyloric ligation/histamine-induced gastric ulceration/acid production. The result showed that the reference drug and the extract at the doses of 250, 500 and 1000 mg/kg significantly (p < 0.001, P < 0.02, P < 0.018, P < 0.02) decreased the mean number of ulcers, ulcer index, gastric free HCl and total gastric acid respectively in a dose dependent manner when compared with the negative control group. Also, the percent preventive index was increased while percent ulcer severity decreased dose dependently.

**Table 3: Effect of the methanolic root extract of *C. planchonii* on cold/restraint stress-induced gastric ulceration in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean no. of ulcers ± S.E.M.</th>
<th>Mean U. I. ± S.E.M.</th>
<th>% severity</th>
<th>Preventive index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled water (10ml/kg)</td>
<td>9.6±0.81</td>
<td>1.18±0.12</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Cimetidine (100mg/kg)</td>
<td>4.6±0.12*</td>
<td>0.64±0.15*</td>
<td>54.2</td>
<td>45.8</td>
</tr>
<tr>
<td>3</td>
<td>CPE Extract (250mg/kg)</td>
<td>3.2±0.07*</td>
<td>0.44±0.14*</td>
<td>39.0</td>
<td>61.0</td>
</tr>
<tr>
<td>4</td>
<td>CPE Extract (500mg/kg)</td>
<td>3.4±0.66*</td>
<td>0.46±0.29*</td>
<td>37.3</td>
<td>62.7</td>
</tr>
<tr>
<td>5</td>
<td>CPE Extract (1000mg/kg)</td>
<td>2.0±0.09*</td>
<td>0.40±0.19*</td>
<td>15.9</td>
<td>84.1</td>
</tr>
</tbody>
</table>

* P value < 0.05 when compared with negative control

**Table 4: Effect of *C. Planchonii* on pyloric ligation/Histamine-induced gastric ulceration and acid production**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of ulcers ±S.E.M</th>
<th>Mean ulcer index ±S.E.M</th>
<th>Free HCl mmol /L</th>
<th>Total acid mmol /L</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled water (10ml/kg)</td>
<td>17.6±2.42</td>
<td>3.04±0.44</td>
<td>49.2±8.20</td>
<td>132.6±8.20</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Cimetidine (100mg/kg)</td>
<td>5.0±1.05***</td>
<td>0.58±0.25*</td>
<td>36.2±5.1***</td>
<td>86.0±16.0*</td>
<td>81</td>
</tr>
<tr>
<td>3</td>
<td>CPE Extract (250mg/kg)</td>
<td>11.2±0.83***</td>
<td>1.7±0.83*</td>
<td>47.8±4.18</td>
<td>111.6±7.05</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>CPE Extract (500mg/kg)</td>
<td>6.2±1.86***</td>
<td>0.74±0.16*</td>
<td>38.8±6.08**</td>
<td>96.8±19.78</td>
<td>76</td>
</tr>
<tr>
<td>5</td>
<td>CPE Extract (1000mg/kg)</td>
<td>4.4±0.25***</td>
<td>0.48±0.22*</td>
<td>30.4±2.16**</td>
<td>65.2±8.97*</td>
<td>84</td>
</tr>
</tbody>
</table>

*P < 0.18, ** P < 0.02, *** P < 0.001 when compared to the negative group

**Bioassay-guided fractionation of the methanolic root extract of *C. planchonii***

Two hundred and sixty one (261) 10ml aliquots were collected. They were pooled and concentrated into ten (10) fractions designated as methanolic extract of *Cochlospermum planchonii* fractions (MECPFr.) 1-10.

**Evaluation of the fractions of *C. planchonii* on Actylsalicylic acid (Aspirin)-induced gastric ulceration (in vivo)**

The result of the antiulcer activities of *C. planchonii* fractions using aspirin induced gastric ulceration in rats is presented in Fig 1. The result showed that of all the fractions, MECPFr 4 treated group had mean ulcer index of 0.30 which was the lowest and ulcer preventive index of 92.0% which was the highest. This was better than the reference drug (cimetidine, 100mg/kg) treated group that had a mean ulcer index of 0.81 with 75.0% ulcer preventive index, thus confirming MECPFr 4 as the most active fraction of *C. planchonii*. 
The anti ulcerogenic effect of the methanolic root bark extract of *C. planchonii* was evaluated using different [ethanol-, acetylsalicylic acid (aspirin)-, cold/restraint stress and pyloric ligation/histamine-induced ulcers and acid production] ulcerogenic models. The administration of absolute ethanol produced ulcer lesions in the glandular part of the rats’ stomach. Ethanol, a cytodestructive agent, induces ulcers by causing capillary necrosis (Al-Howiriny et al., 2003), generation of free radicals (Coskun et al., 2004), direct toxic action of ethanol, reduction of secretion of bicarbonate, depletion of gastric mucus, and generation of reactive oxygen species (ROS) with impairment of antioxidant properties (Mahuenda et al., 1993; Kweicen et al., 2002).

*C. planchonii* extract showed significant inhibitory effect against ethanol-induced gastric ulcers by decreasing the ulcer parameters and also increasing the percent ulcer inhibition in a dose dependent manner which was comparable to the reference drug cimetidine (Table 1).

It may be possible that *C. planchonii* methanol bark extract possess the ability to mobilise endogenous prostaglandins, prevent capillary necrosis, increase bicarbonate and gastric mucus secretion or may have antioxidant properties which enabled it to protect the rats’ gastric mucosa against ethanol challenge.

Acetylsalicylic acid (Aspirin) produced ulcer lesions in the glandular part of the rats’ stomach. Non-steroidal anti-inflammatory drugs (NSAID), such as Aspirin, produces ulceration by direct injuries to stomach walls (Susan and Mays, 2005). NSAID also induce gastroduodenal ulceration via its ability to suppress prostaglandin synthesis (Wallace, 2001).

In this study, CPE was significantly effective in protecting gastric mucosa against aspirin-induced ulcerations at all the dose levels studied when compared to the negative group. CPE may have achieved the protection against aspirin induced ulcerations in the rats by its cytoprotective property or by enhancing the production or action of prostaglandins.

Exposure of the rats to cold/restraint stress caused the production of gastric ulcerations in the glandular part of the stomach that was significantly reduced in the extract treated groups in a dose dependent manner. Cold / restraint stress-induced ulcer model causes gastric mucosal damage in the glandular part of the stomach that is constant as to frequency of occurrence, type and location (Ogle and Cho, 1995), and the decrease in prostaglandin mucosal biosynthesis seems to be a major determinant in the pathogenesis of stress ulcers. This may be due to luminal acid back diffusion and mucosal H⁺ permeability (Takeuchi and Okabe, 1992).

CPE inhibited stress-induced gastric lesions significantly. The mechanism by which CPE protected the rats’ stomach against cold/restraint stress-induced ulcers may be by prevention of the above effects or probably might be due to the neutralising effect of the extract on gastric acid as suggested by Gurbuz and Erdem, (2007).

Pyloric ligation is an important procedure in determining the possible changes in ulcer parameters relative to the gastric content (de Barros et al., 2008). In pyloric ligation, the digestive effects of accumulated gastric juice and interference with gastric blood circulation are responsible for induction of ulceration (Brodie, 1996). This is further exacerbated by the administration of histamine (Garrison and Rall, 1990). H₂-receptor antagonists inhibit histamine, gastrin and acetylcholine-stimulated acid secretion with reduction of volume of gastric juice in pyloric ligated rats (Rang et al., 2003). Since CPE not only inhibited the pylorus ligated ulcer in the stomach, reduced the volume of free HCl and total acid, but also decreased the volume of gastric juice and other ulcer parameters just like the reference drug cimetidine, it is therefore most likely that the extract acted through the same mechanism as H₂- receptor antagonists.
Ulcer index which expresses the degree and severity of ulcers was minimally decreased by MECPFr 4. It also maximally increased the percentage ulcer preventive index which confirmed its choice as the most active fraction of *C. planchonii*.

Preliminary phytochemical analysis of *C. planchonii* showed the presence of carbohydrates, tannins, glycosides and flavonoids (Anaga and Oparah, 2009), while analysis of MECPFr 4 in this study showed that it contains flavonoids.

Flavonoids have been reported to have pronounced anti-ulcerogenic activity (Alarcon de Lastra et al., 1992). It is therefore understandable that MECPFr 4 which is the active fraction in *C. planchonii* extract and contains flavonoids showed a very potent antiulcer activity. This also suggests that the antiulcer activity of the extract may be due to its phytochemical constituent, flavonoids.

In conclusion, *Cochlospermum planchonii* in this study demonstrated significant antiulcer activity which justifies its use in folkloric medicine in Nigeria for the treatment of ulcers. Its effects may be mediated through one or a combination of cytoprotection, antisecretory, antioxidant action on mucosal prostaglandin, H₂ receptor antagonistic mechanisms, among others, and/or due to its phytochemical constituents.

However, more work is required to isolate and characterise the bioactive compound responsible for the anti ulcer activity and to determine the exact mechanism of action.

References