CYTOPROTECTIVE AND ANTIOXIDANT PROPERTIES OF THE STEM BARK AQUEOUS EXTRACT OF ENANTIA CHLORANTHA

OLIVER (ANNONACEAE) IN RATS

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ABSTRACT

Enantia chlorantha is a plant used in African traditional medicine to treat many diseases including gastric ulcers. In this study, we evaluated the anti-ulcerogenic effects of the aqueous extract of the stem bark of this plant using different methods of gastric lesion induction in experimental Wistar rats (140-190 g): 1. HCl/Ethanol, 2. HCl/Ethanol/Indomethacin, 3. indomethacin, 4. absolute ethanol, 5. cold water immersion. 6. Pylorus ligation. Phytochemical screening of the aqueous extract of Enantia chlorantha revealed the presence of phytoconstituents such as tannins, alkaloids, saponins, flavonoids. The extract at different doses (250 and 500 mg/kg) significantly reduced the ulcer index in all models used (67.87 % (p <0.001) with HCl/ethanol; 63.39 % (p <0.001) with HCl/ethanol/indomethacin; 78 % (p <0.001) with absolute ethanol; 65.22 % (p <0.05) with pylorus ligation), at the highest dose of 500 mg/kg. Apart from pylorus ligation where the mucus production increased but not in a significant manner, the reduction of lesion formation in the other models was accompanied by a very significant increase in gastric mucus production. The pylorus ligation technique revealed that the extract has no antisecretory activity. The
oxidative stress parameter assay indicates a significant drop of MDA concentration in the extract-treated groups. The results show that the extract can offer cytoprotection to the gastric mucosa by a mechanism which involves its antioxidant potential particularly by reducing the level of MDA.

KEYWORDS: *Enantia chlorantha*, gastric ulcer, cytoprotection, antioxidant.

INTRODUCTION

Gastric or duodenal ulcer is a loss of mucous membrane substance which in depth surpasses the muscular mucous membrane.[1] Gastric and duodenal ulcers may be due to an imbalance between aggressive factors (acid and pepsin) and protective factors (bicarbonate, mucus) with a predominance of the first on the second. The development of these ulcers is favored by tobacco, alcohol, stress, bad eating habits and non steroidal anti inflammatory drugs (NSAIDs). In the West, it is a frequent disease which touches 8 to 10 persons out of 100 residents.[2] The introduction of endoscopy in Africa at the beginning of the 1980s helped to reveal the high degree of prevalence of the disease in the pathology of the black African.[3] The prevalence of gastric ulcers in Cameroon is about 31.65 %.[4]

The objective of medical treatment of gastric ulcers is to relieve the patient, accelerate scar formation and prevent recurrence and complications. The treatment employs antisecretory agents, antacids and antibiotics drugs. However, these different treatment regimens, despite their effectiveness, possess numerous side effects. For example, H2-receptor antagonists (e.g. cimetidine) may cause gynecomastia in men and galactorrhea in women,[5] while proton-pump inhibitors (e.g. omeprazole and lanzoprazol) can cause nausea, abdominal pain, constipation and diarrhea.[6, 7] In addition, in developing countries, due to the typical poverty of the populations, insufficiency of modern health infrastructures and the prohibitive cost of modern techniques of treatment, about 80 % of the population relies on medicinal plants in diverse forms (decoctions, macerations, infusions and powders) to ensure their health.[8] In order to rationalize the use of medicinal plants, the World Health Organization (WHO) has prescribed minimum criteria to be respected for the valorization of phytomedicines.

The antiulcerogenic effects of many plant extracts have been evaluated all over the world. One of these plants is *Enantia chlorantha* Oliver (*Annonacée*), an ornamental tree which may
grow up to 30 m high, widely distributed along the coasts of West and Central Africa.\textsuperscript{[9]} Enantia chlorantha has many therapeutic properties. The bark infusion of the plant is used to treat cough and wounds.\textsuperscript{[10]} The sap is used against diarrhea.\textsuperscript{[11]} The extract is also used to treat jaundice and infection of the urinary tract\textsuperscript{[12]} and malaria.\textsuperscript{[13]} The antipyrogen, anti-viral and anti-bacterial activity of this plant has also been investigated.\textsuperscript{[14, 15, 16]} The plant is commonly used in Cameroon in form of a decoction for gastric ulcer therapy, and it has been demonstrated that a protoberberine alkaloid extracted from Enantia chlorantha has antiulcerogenic effects on gastric ulcers.\textsuperscript{[17]} In the present study, we have evaluated the cytoprotective and antioxidant actions of the decoction of E. chlorantha against various ulcerogens. The possible modes of action of the extract are discussed in relation to the pathogenic mechanisms of action of the various necrotizing agents used.

**MATERIALS AND METHODS**

**Preparation of plant extract**

The stem bark of Enantia chlorantha was harvested in Ambam, South region of Cameroon in July 2011. The plant was identified in comparison with the existing specimen N° 25918/SRFCAM held at the Cameroon National Herbarium. The fresh stem-bark of Enantia chlorantha was cut up, dried and ground to a powder. A mixture (10 % (w/v)) of the powder and distilled water was boiled for 20 minutes and then cooled to room temperature. After filtration of the decoction obtained through Wattman filter paper number 3, the filtrate was evaporated at 40°C using a Raven convection air oven (Jencons PLS, UK). The yellowish dried solid obtained (4.53 % yield) was stored at 4°C and used later for our pharmacological tests.

**Phytochemical Tests**

Phytochemical tests for the major metabolites of the extract were performed. The aqueous extract of Enantia chlorantha was screened for the presence of biologically active compounds such as tannins, alkaloids, saponins, flavonoids, anthocyanins, phenols, quinones, coumarins, sterols, triterpenoids, glycosides, proteins. Based on the intensity of coloration, the lather or the precipitate formed during the test, secondary metabolite proportions were characterized as present (++) or weakly present (+) when the test result was positive, and absent (−) when the test result was negative.
Animals
Male Wistar albino rats (140-190 g) that were raised in the animal house of the Faculty of Science, University of Yaoundé I were used. The animals were fed a standard laboratory diet and given fresh water *ad libitum*. The authorization for the use of laboratory animals in this study was obtained from the Cameroun National Ethics committee (Reg. No FWA-IRB00001954).

Induction of gastric ulcers

HCl/ethanol-induced gastric lesions in rats
The rats were deprived of food for 36 h prior to experimentation but all the animals had free access to tap water. The HCl/ethanol solution was used to induce ulcers in the gastric mucosa according to the method of.[18] The animals received the plant extract by oral route, 1 h before they were given the necrotizing solution. Positive control rats received sucralfate in place of the extract. They were killed using ether, the abdomen of each opened and the stomachs removed. The ulcers produced in the glandular region of each stomach were measured and scored as earlier described[19] and the ulcer index (UI), percentage of inhibition (% I) and percentage of ulcerated surface (%US) were calculated.

HCl/ethanol-induced lesions in rats pre-treated with indomethacin
Indomethacin (Allphamed PHARBIL Arzneimittel GmbH Hildebrandstrasse 12 D-37081 Gottingen, Germany) was given to the rats (20 mg/kg) by intra peritoneal route at the end of the 24 h fast. This was followed 1 h later by the HCl/ethanol ulcer procedure as described above.

Absolute ethanol-induced gastric lesions
The method described previously for the HCl/ethanol method was used, the only difference being that 1 ml of absolute ethanol was used as the necrotizing solution.

Indomethacin-induced gastric lesions
The animals were deprived of food for 36 hours. The vehicle and the extract (250 and 500 mg/kg) were given to them 3 times at 12-hour intervals. Indomethacin (50 mg/kg) was given to the rats by oral route, 1 h after the animals received the last administration of the plant extract and vehicle. They were sacrificed another hour later and the ulcers produced in the
glandular region of the stomachs were measured and expressed according to the score described by. Petechial lesions were counted and then every five lesions were taken as 1 mm of ulcer. Blood and gastric tissue samples were taken, prepared and preserved frozen for the measurement of different oxidative stress parameters.

**Cold stress-induced gastric lesions**

Stress-induced gastric ulcers were provoked in rats using a slight modification of the method earlier described by. The animals were deprived of food for 36 hours (but not water deprivation). Test rats were given the extract (250 and 500 mg/kg) by oral route while control rats received the vehicle or cimetidine 3 times at 12-hour intervals. One hour later, after the last administration of vehicle or extract, the rats were placed in small individual wire cages and immersed in cold water (20 ± 1°C), up to the level of the xiphoid. Three hours later blood samples were taken and the animals were sacrificed using ether and the stomachs removed. The same protocol used with indomethacin model for the assessment of lesion formation was performed. Blood and gastric tissue samples were also taken, prepared and preserved frozen for the measurement of different oxidative stress parameters.

**Pylorus ligated gastric secretion and ulceration in rats**

The method of was used to study the ability of the extract to reduce gastric acid secretion as well as prevent gastric ulceration resulting from auto digestion by stomach secretions. The test rats received the extract, while the controls received distilled water (1ml) or Cimetidine. One hour later, laparotomy was performed under ether anesthesia, the pylorus of each rat was ligatured, and the abdominal incisions stitched up. The gastric juice produced during six subsequent hours was collected from each rat, the volume measured and 1 ml aliquots kept for gastric acid measurement. The ulcers produced in the glandular region of the stomachs were measured and ulcer index, % of inhibition, and % of ulcerated surface were determined.

**Measurement of mucus production**

The mucus covering of each stomach was gently scraped using a glass slide and the mucus weighed carefully using a sensitive digital electronic balance.
Measurement of gastric acidity

One ml of centrifuged gastric contents from each rat was assayed for hydrogen ion concentration by pH-metric titration against 0.1 N NaOH using a digital pH meter. Gastric acidity was expressed as mEq/L.

Measurement of pepsin activity in gastric juice

Peptic activity of gastric juice obtained from pylorus ligated animals was determined by subjecting a solution of albumin (50 mg/mL) to the digestive action of the gastric juice. 50 μL of the albumin solution were incubated with 50 μL of gastric juice at 25°C for 10 min. The quantity of hydrolyzed protein was estimated using the Biuret method.

Measurement of in vivo antioxidant capacity

Blood and gastric tissue samples were taken and prepared for the measurement of different oxidative stress parameters: Cellular glutathione (GSH) was measured based on the reaction between 2,2-dithio-5,5-dibenzoic acid and the thiol (SH) groups of glutathione to yield a complex whose absorbance was read at 412 nm.[23] The glutathione concentration was calculated using the molar extinction coefficient ε= 1.36 10^4 M^{-1} cm^{-1}. Superoxide dismutase (SOD) activity was measured using a standard method[24] and expressed in U/mg of protein, while catalase was determined[25] and expressed as mM of H_2O_2/min/mg of protein, and tissue protein was measured using the Biuret method of protein assay. Lipid peroxidation was assessed by measuring the levels of malondialdehyde (MDA).[26] Quantification of MDA was done using an extinction coefficient of ε = 1.56 10^5 M^{-1} cm^{-1}.

Statistical analysis

The data were analyzed using one way analysis of variance (ANOVA) followed by the student-Newman-keuls test. P values less than 0.05 were considered significant. Values in tables are given as arithmetic means ± standard error of the mean (S.E.M).

RESULTS

Phytochemical screening

The preliminary phytochemical screening carried out on the aqueous extract of E. chlorantha revealed the presence of many phytoconstituents. These included tannins, saponinins, anthocyanins, acids, glycosides (++), alkaloids, ketones, flavonoids, sugars, coumarins,
amino acids and proteins (+). Phenols, quinines, oils, sterols, triterpenoids and resins (-) were absent.

**Anti-ulcer activity**

*E. chlorantha* aqueous extract produced significant cytoprotection. Lesion index scores decreased from 6.63 for the negative control to 2.41 and 2.13, respectively, for the 250 and 500 mg/kg doses, corresponding to a percentage protection of 67.87 % at 500 mg/kg dose (Table 1). Significant increases in mucus production (P<0.001) were obtained as the dose of the extract was increased from 250 to 500 mg/kg compared with the controls.

**Table 1: Effect of *Enantia chlorantha* extract on HCl/ethanol-induced gastric lesions in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>% ulcerated surface</th>
<th>Ulcer index</th>
<th>Inhibition (%)</th>
<th>Mucus production (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>4.30</td>
<td>6.63 ± 0.58</td>
<td>_</td>
<td>125.55 ± 12.75</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>250</td>
<td>5</td>
<td>0.41</td>
<td>2.41 ± 0.05***</td>
<td>63.65</td>
<td>218.51 ± 17.80***</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>500</td>
<td>5</td>
<td>0.34</td>
<td>2.13 ± 0.13***</td>
<td>67.87</td>
<td>239.38 ± 12.21***</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>100</td>
<td>5</td>
<td>0.48</td>
<td>2.77 ± 0.14***</td>
<td>44.34</td>
<td>157.79 ± 17.59**</td>
</tr>
</tbody>
</table>

Statistically different relative to control; **p<0.01; ***p<0.001; N, number of rats. The values are expressed as mean±SEM.

Table 2 shows a significant reduction in ulcer index, 1.49 for the maximal dose compared with 4.07 for the vehicle control. Although the mucus production in all the groups dropped compared with that of the HCl/ethanol-induced gastric lesion, the cytoprotection was accompanied by a significant increase in mucus production, from 89.27 mg in the vehicle control to 139.61 mg and 163 mg with extract doses. Lesion inhibition by sucralfate was poor (37. 84%) although it significantly raised mucus production following pre-treatment with indomethacin.
Table 2: Effect of *Enantia chlorantha* extract on HCl/ethanol-induced gastric lesions in rats pre-treated with indomethacin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses (mg/kg)</th>
<th>N</th>
<th>% ulcerated surface</th>
<th>Ulcer index</th>
<th>Inhibition (%)</th>
<th>Mucus production (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>1.43</td>
<td>4.07 ± 0.39</td>
<td>-</td>
<td>89.27 ± 6.21</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>250</td>
<td>5</td>
<td>0.40</td>
<td>2.34 ± 0.21**</td>
<td>42.51</td>
<td>139.61 ± 9.50**</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>500</td>
<td>5</td>
<td>0.19</td>
<td>1.49 ± 0.25***</td>
<td>63.39</td>
<td>163.25 ± 11.66***</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>100</td>
<td>5</td>
<td>0.50</td>
<td>2.53 ± 0.46**</td>
<td>37.84</td>
<td>151.15 ± 9.09***</td>
</tr>
</tbody>
</table>

Statistically different relative to control; **p<0.01; ***p<0.001; N, number of rats.

The values are expressed as mean ± SEM.

Although the ulcer index dropped from 2.09 ± 0.10 in the vehicle control group to 1.28 ± 0.19 with the maximal extract dose, the degree of gastric protection against indomethacin was poor (14.83% and 38.76%, respectively for 250 and 500 mg/kg extract dose). Mucus production dropped very significantly with cimetidine and was poor with extract doses compared to the values obtained with HCl/ethanol and HCl/ethanol-indomethacin pretreatment even though extract-treated animals had higher values compared with the negative control, and higher and very significant values compared with cimetidine (Table 3).

Table 3: Effect of *Enantia chlorantha* extracts on indomethacin -induced gastric lesions in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses mg/kg</th>
<th>N</th>
<th>% ulcerated surface</th>
<th>Ulcer index</th>
<th>Inhibition (%)</th>
<th>Mucus production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>0.26</td>
<td>2.09 ± 0.10</td>
<td>-</td>
<td>115.40 ± 7.12</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>250</td>
<td>5</td>
<td>0.20</td>
<td>1.78 ± 0.21</td>
<td>14.83</td>
<td>127.97 ± 7.14</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>500</td>
<td>5</td>
<td>0.13</td>
<td>1.28 ± 0.19*</td>
<td>38.76</td>
<td>142.47 ± 7.34*</td>
</tr>
<tr>
<td>Cimétidine</td>
<td>100</td>
<td>5</td>
<td>0.06</td>
<td>0.80 ± 0.20**</td>
<td>61.72</td>
<td>83.96 ± 5.81**</td>
</tr>
</tbody>
</table>

Statistically different relative to control; *p<0.05; **p<0.01; ***p<0.001; N, number of rats.

The values are expressed as mean ± SEM

Table 4 shows that the extract significantly prevented gastric lesions induced by absolute ethanol, with 78 % protection at the maximal dose, (ulcer index 1.12 ± 0.36, compared with 5.09 ± 0.51 for the negative control). This high cytoprotection was accompanied by a slightly
significant increase in mucus production in extract treated groups in comparison with the negative control group.

Table 4: Effect of *Enantia chlorantha* extracts on absolute ethanol-induced gastric lesions in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>N</th>
<th>% ulcerated surface</th>
<th>Ulcer index (mg)</th>
<th>Inhibition (%)</th>
<th>Mucus production (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>2.09</td>
<td>5.09 ± 0.51</td>
<td>-</td>
<td>115.23 ± 6.61</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>250</td>
<td>5</td>
<td>0.46</td>
<td>2.49 ± 0.06***</td>
<td>51</td>
<td>139.56 ± 4.84**</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>500</td>
<td>5</td>
<td>0.12</td>
<td>1.12 ± 0.36***</td>
<td>78</td>
<td>141.49 ± 3.92**</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>100</td>
<td>5</td>
<td>0.09</td>
<td>0.76 ± 0.47***</td>
<td>85.06</td>
<td>150.33 ± 4.32***</td>
</tr>
</tbody>
</table>

Statistically different relative to control; *p<0.05; **p<0.01; ***p<0.001; N, number of rats.
The values are expressed as mean ± SEM

Table 5 shows the effects of subjecting the rats to a combination of restraint and cold stress. *Enantia chlorantha* extract prevented the formation of gastric lesions due to cold/restraint stress. Ulcer index scores reduced from 1.58 to 1.00 with the 500 mg/kg dose. Cimetidine (100 mg/kg), prevented lesion formation by 49.37 per cent. The production of mucus increased significantly (p<0.001).

Table 5: Effect of *Enantia chlorantha* extract on cold/restraint stress-induced gastric lesions in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses mg/kg</th>
<th>N</th>
<th>% ulcerated surface</th>
<th>Ulcer index (mg)</th>
<th>Inhibition (%)</th>
<th>Mucus production (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>0.20</td>
<td>1.58 ± 0.12</td>
<td>-</td>
<td>30.28 ± 3.26</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>250</td>
<td>5</td>
<td>0.10</td>
<td>1.35 ± 0.09</td>
<td>14.57</td>
<td>45.21 ± 4.32**</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>500</td>
<td>5</td>
<td>0.07</td>
<td>1.00 ± 0.28</td>
<td>36.71</td>
<td>63.05 ± 6.21***</td>
</tr>
<tr>
<td>Cimétidine</td>
<td>100</td>
<td>5</td>
<td>0.06</td>
<td>0.80 ± 0.20*</td>
<td>49.37</td>
<td>77.49 ± 5.74***</td>
</tr>
</tbody>
</table>

Statistically different relative to control; *p<0.05; **p<0.01; ***p<0.010; N, number of rats.
The values are expressed as mean ± SEM

Tables 6 and 7 show the results obtained using the pylorus ligation ulcer induction method. *E. chlorantha* aqueous extract protected the stomach against lesions with a protection
percentage of 65.22 at the 500 mg/kg dose. The cytoprotection was accompanied by a slight increase in mucus protection, from 30.76 ± 3.70 to 38.99 ± 6.53 mg and 46.48 ± 4.12 mg, respectively, for the 250 and 500 extract doses (Table 6). In comparison with the negative control, the volume of gastric juice (6.66 ± 0.61 mL), which considerably reduced with cimetidine, did not change significantly with extract administration. Gastric acidity significantly (p<0.001) dropped from 126.80 mEq/l in the negative control to 100.00 mEq/l and 66.90 mEq/L, respectively, for the 250 mg/kg dose of extract and cimetidine. The gastric acidity remained unchanged when extract dose was raised to 500 mg/kg (Table 7).

Table 6: Effect of *Enantia chlorantha* extract on pylorus-ligated gastric ulceration in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses mg/kg</th>
<th>N</th>
<th>% ulcerated</th>
<th>Ulcer index</th>
<th>Inhibition (%)</th>
<th>Mucus production (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>0.47</td>
<td>2.30 ± 0.13</td>
<td>-</td>
<td>30.76 ± 3.70</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>250</td>
<td>5</td>
<td>0.26</td>
<td>1.90 ± 0.33</td>
<td>17.39</td>
<td>38.99 ± 6.53</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>500</td>
<td>5</td>
<td>0.06</td>
<td>0.80 ± 0.37*</td>
<td>65.22</td>
<td>46.48 ± 4.12</td>
</tr>
<tr>
<td>Cimétidine</td>
<td>50</td>
<td>5</td>
<td>0.14</td>
<td>1.43 ± 0.37</td>
<td>37.83</td>
<td>34.91 ± 4.70</td>
</tr>
</tbody>
</table>

Statistically different relative to control; *p<0.05; N, number of rats.

The values are expressed as mean ± SEM

Table 7: Effect of *Enantia chlorantha* extract on gastric secretion in pylorus-ligated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>Gastric contents (ml)</th>
<th>Gastric acidity (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>5</td>
<td>6.66 ± 0.61</td>
<td>126.80 ± 2.11</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>250</td>
<td>5</td>
<td>5.54 ± 1.22</td>
<td>100.00 ± 3.02***</td>
</tr>
<tr>
<td><em>E. chlorantha</em></td>
<td>500</td>
<td>5</td>
<td>5.60 ± 0.64</td>
<td>108.10 ± 3.99**</td>
</tr>
<tr>
<td>Cimétidine</td>
<td>50</td>
<td>5</td>
<td>3.54 ± 0.38</td>
<td>66.90 ± 5.72***</td>
</tr>
</tbody>
</table>

Statistically different relative to control; **p<0.01; ***p<0.001; N, number of rats.

The values are expressed as mean ± SEM

Table 8 shows that in cold restraint stress, antioxidant enzyme concentrations in extract-treated groups, compared with the negative control did not considerably vary for GSH and Catalase, while the concentration of SOD slightly increased with the 500 mg/kg dose. On the other hand, MDA significantly dropped in extract-treated groups compared with the negative control.
Table 8: Effect of *E. chlorantha* aqueous extract on oxidative stress parameters in stomach tissues of rats subjected to cold restraint stress.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>N</th>
<th>MDA (µmol/mg protein)</th>
<th>GSH (µmol/mg protein)</th>
<th>Catalase (mM H$_2$O$_2$/min/mg protein)</th>
<th>SOD (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td>5</td>
<td>1.73 ± 0.10</td>
<td>2.19 ± 0.08</td>
<td>37.67 ± 1.20</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>Negative control</td>
<td>5</td>
<td>2.07 ± 0.18</td>
<td>0.84 ± 0.08</td>
<td>37.18 ± 0.60</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td><em>E. chlorantha</em> 250 mg/kg</td>
<td>5</td>
<td>1.83 ± 0.10</td>
<td>0.57 ± 0.02*</td>
<td>35.25 ± 1.49</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td><em>E. chlorantha</em> 500 mg/kg</td>
<td>5</td>
<td>1.11 ± 0.10***</td>
<td>0.73 ± 0.06</td>
<td>35.15 ± 37.28</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td>Cimetidine (100mg/kg)</td>
<td>5</td>
<td>1.89 ± 0.14</td>
<td>0.49 ± 0.05**</td>
<td>37.28 ± 1.67</td>
<td>0.60 ± 0.05*</td>
</tr>
</tbody>
</table>

Statistically significant relative to the control, *(P<0.05); ***(P<0.001). N, number of rats. The values are expressed as mean ± SEM. SOD, superoxide dismutase; GSH, reduced glutathione; MDA, malondialdehyde (values obtained from blood sample assay).

The concentration of MDA significantly dropped (p<0.001) in extract-treated groups compared with the negative control. GSH and Catalase slightly increased 3.90 and 33.91 for the maximal dose in comparison with the negative control 3.74 and 31.49 respectively for GSH and catalase (table 9).

Table 9: Effect of *E. chlorantha* aqueous extract on oxidative stress parameters in stomach tissues of rats subjected to indomethacin treatment.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>N</th>
<th>MDA (µmol/mg protein)</th>
<th>GSH (µmol/mg protein)</th>
<th>Catalase (mM H$_2$O$_2$/min/mg protein)</th>
<th>SOD (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td>5</td>
<td>2.26 ± 0.19</td>
<td>5.76 ± 0.39</td>
<td>37.67 ± 1.20</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>Negative control</td>
<td>5</td>
<td>2.40 ± 0.08</td>
<td>3.74 ± 0.50</td>
<td>31.49 ± 0.63</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td><em>E. chlorantha</em> 250 mg/kg</td>
<td>5</td>
<td>1.29 ± 0.09***</td>
<td>2.23 ± 0.48</td>
<td>35.75 ± 0.97</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td><em>E. chlorantha</em> 500 mg/kg</td>
<td>5</td>
<td>0.84 ± 0.07***</td>
<td>3.90 ± 0.89</td>
<td>33.91 ± 1.57</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td>Cimetidine (100 mg/kg)</td>
<td>5</td>
<td>1.92 ± 0.13*</td>
<td>0.88 ± 0.07</td>
<td>28.47 ± 2.18</td>
<td>0.30 ± 0.02</td>
</tr>
</tbody>
</table>

Statistically significant relative to the control, *(P<0.05); ***(P<0.001). N, number of rats. The values are expressed as mean ± SEM. SOD, superoxide dismutase; GSH, reduced glutathione; MDA, malondialdehyde.
DISCUSSION

Antiulcer studies

Previous work revealed the prophylactic and healing properties of an anti-ulcer alkaloid (7, 8-dihydro-8-hydroxypalmatine) from *E. chlorantha*. In this study, we evaluated the effects of the aqueous extract of *E. chlorantha* stem bark, in order to suggest a therapeutic dose for the widely used decoction of the plant for peptic ulcer management in Cameroon. The results show that the aqueous extract of *E. chlorantha* significantly prevented the formation of gastric ulcers induced by HCl/ethanol solution by reducing the ulcer index and contributed to a significant production of gastric mucus. The HCl/ethanol solution directly irritates the stomach mucosa, reduces mucosal resistance and erodes the mucosal barrier. Products that have gastric protective effects against similar gastric irritant substances are said to possess cytoprotective potential.[27] Generally, pretreatment with indomethacin increases the irritant effect of HCl/ethanol on the mucosal barrier. This was evident from the percentage protection which dropped from 63.65, 67.87 and 44.34% to 42.51, 63.39 and 37.84 %, respectively for the 250, 500 mg/kg extract doses and cimetidine, following indomethacin pre-treatment. Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) which reduces prostaglandin secretion, as well as gastric mucosal blood flow.[27] Suppression of prostaglandin synthesis leads to a marked alteration in the microcirculation of the stomach and intestine. This is highly critical to the early events in the pathogenesis of gastric ulceration. The reduced microcirculation can also have a negative impact on the secretion of bicarbonate and mucus by the gastric and duodenal epithelium and on the proliferation of epithelial cells.[28] Thus, pre-treatment with indomethacin led to a 30, 36 and 45% drop in mucus production for the negative control, 250 and 500 mg/kg doses of extract. In spite of this, *E. chlorantha* extract in both models significantly increased mucus production. However, mucus production considerably decreased when indomethacin was administered alone by oral route, even though the values obtained with the extract were slightly higher than for the negative control. When cytoprotection against HCl/ethanol is significantly reduced by pre-treatment with indomethacin, the cytoprotection is interpreted to be mediated by endogenous prostaglandins. Absolute ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the hemorrhage and necrotic aspects of tissue injury[29], hence the elongated thick red band lesions observed...
abundantly in the glandular gastric portion of the control group. The extract significantly protected the gastric mucosa against ulcers induced with absolute ethanol, by 51% and 78%, respectively for the 250 and 500 mg/kg doses. Although the mucus production was lower than that obtained with HCl/ethanol method, it was significantly higher (p<0.01) compared with the negative control. Similar results were obtained with 50 and 100 mg/kg doses of the alkaloid (7, 8-dihydro-8-hydroxypalmatine) obtained from E. chlorantha bark.[17] The increase in mucus production in all the models tested is indicative of a possible mode of action of the extract since cytoprotection by the mucus is mainly a direct mucosal protective action similar to prostaglandins. Prostaglandins play a central role in gastric epithelial defense/repair. Every component of mucosal defense is, to some extent, prostaglandin-dependent and compounds and extracts which improve prostaglandin generation possess the cytoprotective activity.[28]

Water immersion stress is one of the best models of stress to induce gastric ulcers in rats. The model provides both emotional stress as well as physiological stress to the animals.[31] The aqueous extract of E. chlorantha considerably prevented the formation of stress-induced ulcers. This was accompanied by a highly significant (p<0.001) increase in mucus production. Oxidative stress resulting from the increased production of oxygen-derived free radicals (e.g. superoxide anion, hydrogen peroxide and hydroxyl radicals), has been known to take part in the pathogenesis of various diseases including gastric ulcer.[32] Extensive damage to the gastric mucosa by indomethacin or stress leads to increased neutrophil infiltration into the ulcerated gastric tissue. These neutrophiles, which are a major source of inflammatory mediators, inhibit gastric ulcer healing by mediating lipid peroxidation through the release of highly cytotoxic and tissue-damaging reactive oxygen species such as superoxide, hydrogen peroxide, myeloperoxidase-derived oxidants, MDA.[33] In this study, we observed that the levels of MDA dropped very significantly in extract-treated groups compared with the negative control group in both cold restraint stress and indomethacin models.

Antioxidants help to protect cells from damage elicited by oxidative stress while enhancing the body’s defense systems against degenerative diseases.[34] Phytochemical screening of the aqueous extract of E. chlorantha stem bark revealed that it contains flavonoids, polyphenolic compounds, saponins and tannins, phytochemical compounds with well-known anti-oxidant properties. [34] The gastro protective effect of E. chlorantha could be attributed to the
presence of anti-oxidant phytochemicals which reduced lipid peroxidation and serum MDA levels. These phytochemicals inhibit gastric mucosal injury by scavenging the indomethacin or stress-generated oxygen metabolites.\textsuperscript{[32]}

Gastric acid plays a major role in the pathogenesis of gastric and duodenal ulcers\textsuperscript{[35]} Gastric acid secretion is mediated by the enzyme H\textsuperscript{+}, K\textsuperscript{+}-ATPase or by the proton pump localized on the luminal membrane of parietal cells.\textsuperscript{[36]} In the pyloric ligation-induced ulcer model, ulceration is caused by the accumulation of acidic gastric juice in the stomach.\textsuperscript{[28]} The resulting hyperacidity leads to auto digestion of the mucosa by a combination of the accumulated HCl and pepsin.\textsuperscript{[17]} \textit{E. chlorantha} extract (500 mg/kg) significantly reduced the pylorus ligated ulcer index by 65\% and produced a slight increase in mucus production compared with the negative control. On the other hand, although gastric acidity dropped significantly in extract-treated groups compared with the negative control, the acid levels remained high in all groups (100 -126 mEq/L). Gastric acid levels ranging between 50 and 63 mEq/L have previously been shown to induce serious ulceration of the gastric mucosa in rats.\textsuperscript{[19]} Similar results were observed with the protoberberine alkaloid obtained from \textit{E. chlorantha} where ulcer index scores decreased while gastric acidity remained high in the control and test groups (50 and 100 mg/kg).\textsuperscript{[17]}

**CONCLUSION**

These results suggest that the aqueous extract of \textit{E. chlorantha} has no antisecretory activity. The cytoprotective action of the extract is possibly due to a combination of its ability to increase gastric mucus secretion by a mechanism similar to endogenous prostaglandins, and the gastric protective effects that are linked to the presence of anti-oxidant phytochemicals.

**REFERENCES**


