In Vivo Curative and Antacid Effects of Cameroonian Clay (MY41g) on Chronic and "Unhealed" Gastric Ulcers in Rats

J.F. Emakoua1, A.P. Amang2, C. Banenzoue3, C. Mezui4, G.T. Siwe1, P.V. Tan1*, G.E. Enow-Orock5

1Department of Animal Biology & Physiology, Faculty of Science, University of Yaounde I, Yaounde, P.O. Box 812, Cameroon.
2Department of Biological Sciences, Faculty of Science, University of Maroua, Maroua, P.O. Box 814, Cameroon.
3Department of Inorganic Chemistry, Faculty of Science, University of Douala, Douala, P.O. Box 24157 Cameroon.
4Department of Biological Sciences, Higher Teachers’ Training College, University of Yaounde I, Yaounde, P.O. Box 047, Cameroon.
5Department of Biomedical Sciences, Faculty of Health Sciences, University of Buea, Buea, P.O. Box 63, Cameroon.

ARTICLE DETAILS

Article history:
Received 21 July 2020
Accepted 08 August 2020
Available online 13 September 2020

Abstract

This study evaluated the in vivo curative and antacid effects of MY41g clay on chronic and "unhealed" gastric ulcers in rats. Chronic gastric ulcers were induced by injecting 0.05 mL of acetic acid (30%) into the stomach wall. From day 5-14 after induction of ulcers, rats were treated daily with MY41g clay (125 and 250 mg/kg). For "Unhealed" gastric ulcers, from day 5-18 rats received MY41g clay orally concomitantly with indomethacin (1 mg/kg/day) subcutaneously. The ulcer index, percentage of healing, mucus secretion, histological parameters, oxidative stress parameters and gastric acidity were assessed. Treatment with clay solution for 10 days resulted in accelerated spontaneous healing of chronic gastric ulcers (93.69-90.2%). However, indomethacin administration did not induce significant variations in the percentage of healing (89.23-91.66%) in rats. For both ulcer models performed, ulcer healing was accompanied by a significant increase (p<0.001) of mucus secretion at the highest dose. Clay increased concentrations of antioxidant enzymes and decreased gastric acidity and lipid peroxidation. Administration of clay accelerated the spontaneous healing of both induction models. The mode of action of the clay could involve increased gastric mucus production, gastric mucosal re-epithelialization, improved antioxidant status and gastric acid neutralization. MY41g clay can be used as antacids in the ulcer treatment regime.

1. Introduction

The problem of treating gastric ulcers in underdeveloped countries remains a major concern due to poverty, the inadequacy of modern health infrastructures and the very high cost of conventional triple therapy as well as the associated side effects [1]. Thus, most of the affected persons in these countries are using traditional medicine. Traditional medication uses medicinal plants, animal parts and minerals to cope with gastric ulcers [1]. While many studies have shown the anti-ucker properties of medicinal plants, very little has been devoted to mineral sources such as clay. Clay represents different sedimentary rocks with a high mineral content. The structures and properties of clays therefore vary according to their mineral composition and concentration [2]. For example, smectites represent a family of clays containing montmorillonites, bentonites, saponites, nontronites and beidellites [3]. This family of clays is known for its ability to trap water molecules and to fix cations to form a gel that is a good dressing for the digestive tract [4].

A WHO study in 2002 [5] demonstrated the curative effect of clay against Buruli ulcer. Clays are found in pharmacies as drugs for the treatment of certain digestive diseases. The modes of action of some clay-based products have been elucidated: Bedelux (smectite beidellic clay) for the symptomatic treatment of irritable colon syndrome; Gelox (smectite clay) for the symptomatic treatment of painful manifestations during oesophageal-gastro-duodenal disorders; Smecta (smectite clay) for the treatment of acute and chronic diarrhoea, symptomatic treatment of pain related to oesophageal-gastro-duodenal and colic disorders; Kaologeais (kaolinite clay) for the symptomatic treatment of digestive functional disorders accompanied by anxiety symptoms [6].

Cameroon has large clayey deposits, particularly kaolinite and halloysite. Cameroonian clays are consumed by geophagia; as antibiotics for wounds, as detoxifiers, as antiadiarrhetics, as antiemetics in pregnant women and as antacids against gastric ulcers [7]. The valorization of these clays in the pharmacological field could open up other ways of using these resources. Preliminary in vitro work carried out by Banenzoue et al. [8] on clays from the West region of Cameroon showed that the Mayouom clay sample (MY41g) when combined with 2% calcium carbonate, had maximal antacid capacity at an inclusion rate of 2.5 g. The central role of gastric acid hypersecretion in the etiology of peptic ulcers is well known [9], and the control of gastric acidity is a cornerstone for promoting ulcer healing [10]. Hence our interest in studying the antacid and ulcer healing effects in vivo of the MY41g clay sample on chronic gastric ulcers in rats. In some cases, ulcer healing may be delayed as in elderly patients routinely using non-steroidal anti-inflammatory drugs to relieve the pain induced by other age-related conditions. For these reasons, the aim of our study was to evaluate the antacid and curative effects in vivo of the MY41g clay sample of Cameroonian origin on both simple chronic and "unhealed" gastric ulcers. Simple chronic gastric ulcers were induced in experimental animals using glacial acetic acid, and "unhealed" chronic ulcers were produced by associating a non-steroidal anti-inflammatory drug – indomethacin.

2. Experimental Methods

2.1 Material

2.1.1 Geologic Material

The MY41g clay and limestone used in this experiment were obtained, respectively, from the Mayouom clay deposit in the Noun Division, West Region of Cameroon, and the Figuil limestone deposit in the Mayo Louti Division, North Region of Cameroon [11]. After harvesting, they were crushed in a mortar into a fine powder and passed through a sieve. Only the particles that passed through the one nanometer sieve pore diameter were used in this study.

*Corresponding Author: verdzekoumso@yahoo.com (P.V. Tan)
2.1.2 Experimental Animals

The animals used were male albino rats of the Wistar strain (Rattus norvegicus), aged 12 to 14 weeks and with body weights between 150 g and 200 g. The rats were raised in the Animal house of the Animal Physiology Laboratory, Department of Animal Biology and Physiology of the University of Yaoundé I. They were kept at room temperature under natural day/night cycles, fed with a standard laboratory diet (supplied by SPC Ltd, Bafoussam, Cameroon) and given tap water ad libitum. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroonian National Ethics Committee (registration number FWA-IRB00001954), which permits, among other procedures, the use of ether anesthesia for animal research. Otherwise, the use, handling, and care of animals were done in adherence to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (ETS-123), with particular attention to Part III, articles 7, 8, and 9 [12].

2.2 Methods

2.2.1 Preparation of Clay Solution

2.2.2 Induction of Gastric Ulcers

2.2.2.1 Induction of Simple Chronic Acetic Acid Ulcers

The induction of chronic gastric ulcers was performed according to the method described by Pillai and Santhakumari [13]. After 24 hours of non-hydric fasting, 30 rats were divided into 6 groups of 5 animals each. Under ether anesthesia, an abdominal incision was made. A volume of 0.05 ml of glacial acetic acid (30%) was injected into the stomach wall at the small curvature. After cleaning the stomach with cotton soaked in NaCl solution (9%), a suture was performed to close the incision. An antibiotic (Betadine) was applied to the incision to prevent infection of the wound.

Three days after ulcer induction, group 1 rats were fasted for 24 hours, the incisions re-opened and the pylorus of each rat was ligated according to the method described by Hara and Oka [14]. These rats were sacrificed 6 hours later under anesthesia, and the stomachs were opened in order to establish the degree of ulceration prior to the onset of treatment. From the 5th day after injection with acetic acid, groups 2, 3, 4, and 5 were treated daily by gavage for 10 days as follows: group 2 (longitudinal control) received 1 mL/200 g distilled water; group 3 and 4 rats received MY41g clay solution at 125 and 250 mg/kg, respectively; group 5 rats received 50 mg/kg sucralfate. On the 9th day of treatment, the animals were sacrificed for 24 hours. The next day, 30 minutes after the last dose of treatment, the incisions were re-opened, the pylorus of each rat ligated, and the abdomens re-sutured. The rats were sacrificed 6 hours later under anesthesia, and then underwent the same protocol as the animals sacrificed 4 days after ulcer induction.

2.2.2.2 Induction of “Unhealed” Gastric Ulcers

The method described by Pillai and Santhakumari in 1984 was used and supplemented by that of Wang [15] with some modifications: From the 5th day after induction of chronic gastric ulcers, rats in groups 2, 3, 4, and 5 were given indomethacin (1 mg/kg/day) subcutaneously 30 minutes before each clay treatment; the treatment lasted for 14 days.

2.2.3 Measurement of Mucus Production

The mucus on the glandular part of the stomach of each rat was gently scraped off using a microscope slide [16], and weighed using a sensitive electronic balance.

2.2.4 Measurement of Gastric Acidity

The gastric juice collected from each rat was centrifuged at 4000 rpm for 10 minutes to remove residual debris. 1 mL of this centrifuged juice was used to determine the hydrogen ion concentration by pH-meter titration against a 0.1 N NaOH solution using a digital pH meter. The acid concentration was expressed in meq/L [17].

2.2.5 Preparation of Histological Sections

Sections of stomach walls were made perpendicular to the surface of each ulcer crater. Sections of the normal stomach were also made for comparison. The haematoxylin-eosin (H&E) staining technique was used according to the standard histological procedure described by Bayedet-Vincent [18] and the sections were observed microscopically.

2.2.6 Measurement of In Vivo Antioxidant Capacity

Oxidative stress parameters were measured on supernatant of crushed stomach samples after centrifuging at 5700 rpm for 10 min. Total protein was determined using the Biuret method [19]. Cellular glutathione (GSH) was measured on the basis of the reaction between 2,2'-dithio-bis(5,5'-dibenzoic acid) and the thiol (SH) groups of glutathione to give a complex whose absorption was read at 412 nm [20]. The glutathione concentration was calculated using the molar extinction coefficient ε = 1.36 104 M-1 cm-1. Superoxide dismutase (SOD) activity was measured using a standard method [21], while catalase was determined and expressed in mU of H2O2/min/mg protein [22]. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels in gastric tissue samples [23]. The quantification of the MDA was performed using an extinction coefficient of ε = 1.56 x 105 M-1 cm-1.

2.2.7 Statistical Analysis

Significant differences between the means of the treatment groups were determined by the analysis of variance (one-way ANOVA) followed by the Tukey multiple comparison test. Values of p < 0.05 were considered significant. The results were expressed as arithmetic means ± standard error of the mean (S.E.M.).

3. Results and Discussion

Fig. 1 show macroscopic photographs of the stomachs of the rats from different treatment groups after induction of simple chronic gastric ulcers. Fig. 1(a) shows the stomach of a normal rat without ulcer. The stomachs of rats sacrificed 4 days after ulcer induction had deep and wide ulcer craters with raised edges and scabrous interior, representing an ulceration surface of 72.00 mm2 (Fig. 1(b)). The treatment of the ulcers with distilled water for 10 days (longitudinal control) resulted in a reduction of the ulcerated areas to 20.75 mm2, representing an auto-healing rate of 71.18% (Fig. 1(c)).

Treatment of ulcerated rats with MY41g at 125 and 250 mg/kg for 10 days resulted in a significant decrease (p<0.01) and p<0.001, respectively in the ulcerated areas (11.75 mm2 and 7.00 mm2, respectively) (Figs. 1(d) and (e)) compared to the longitudinal control; corresponding to a healing rate of 83.69 and 90.20%, respectively. This healing was accompanied by a significant increase (p<0.001) in mucus secretion from 92.50 mg in the longitudinal control to 160.30 mg in animals treated with MY41g clay at the 250 mg/kg dose. For rats treated with sucralfate, the significant decrease (p<0.001) in the ulcerated area (0.50 mm2, Fig. 1(f) healing rate, 99.30%) was also accompanied by a significant increase (p<0.001) in mucus production (152.3 mg) compared to the longitudinal control (20.75 mg) (Table 1). Treatment with the MY41g clay solution caused a significant decrease (p<0.001) in gastric acidity at 125 and 250 mg/kg doses compared to the cross-sectional control.

Indeed, gastric pH and acidity increased from 2.41 ± 0.23 in the cross-sectional controls to 5.45 ± 0.66 and 5.83 ± 0.32 in rats treated with 125 and 250 mg/kg MY41g clay, respectively (p<0.01). Corresponding values for gastric acidity dropped progressively from 73.75 mg eq/L in the cross-sectional controls to 35.25 and 25.44 mg eq/L for clay-treated groups (p<0.001). Both doses of clay treatment were more efficient than Sucralfate in reducing gastric acidity (Table 2).

![Stomachs of rats with simple acetic acid-induced chronic gastric ulcers. (a): Normal rat, (b): Group 1 rats sacrificed 4 days after induction of chronic gastric ulcers to confirm ulcer formation; (c): Longitudinal control/group 2 rats that received daily distilled water (1 mL/200 g) for 10 days from the 5th day after induction of chronic gastric ulcers; (d) and (e): group 3 and 4 rats treated with MY41g clay solution; (f): group 5 ulcerated rats given 50 mg/kg sucralfate (reference drug) for 10 days from the 5th day after induction of chronic gastric ulcers.](https://doi.org/10.30799/jpmr.048.20050103)
Table 1 Effects of MY41g clay on simple acetic acid-induced chronic gastric ulcers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>Ulcer index (IU)</th>
<th>% ulcerated area</th>
<th>% Healing</th>
<th>Mucus production (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>-</td>
<td>5</td>
<td>72.00 ± 0.81</td>
<td>10.66</td>
<td></td>
<td>54.25 ± 0.62</td>
</tr>
<tr>
<td>Control 2</td>
<td>-</td>
<td>5</td>
<td>20.75 ± 1.10***</td>
<td>3.07</td>
<td></td>
<td>92.50 ± 3.79***</td>
</tr>
<tr>
<td>MY41g</td>
<td>125</td>
<td>5</td>
<td>11.75 ± 1.181###</td>
<td>1.74</td>
<td></td>
<td>27.75 ± 3.816###</td>
</tr>
<tr>
<td>MY41g</td>
<td>250</td>
<td>5</td>
<td>7.00 ± 1.29###</td>
<td>0.10</td>
<td></td>
<td>160.3 ± 119.2###</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>50</td>
<td>5</td>
<td>0.50 ± 0.28###</td>
<td>0.07</td>
<td></td>
<td>152.3 ± 9.132###</td>
</tr>
</tbody>
</table>

Control 1 (4 day ulcerated rats); Control 2 (spontaneous healing); N = number of rats; the values in the table represent averages ± SEM; (x-y) ± self-healing: *p < 0.05; **p < 0.01; and ***p < 0.001: Statistically significant compared to Control 1; #p<0.05; ##p <0.01 and ###p <0.001: Statistically significant compared to Control 2.

Table 2 Effects of MY41g clay on gastric pH in rats with simple chronic gastric ulcers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>Gastric pH</th>
<th>Gastric acidity (meq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>-</td>
<td>5</td>
<td>2.41 ± 0.23</td>
<td>73.75 ± 3.45</td>
</tr>
<tr>
<td>Control 2</td>
<td>-</td>
<td>5</td>
<td>4.45 ± 0.38**</td>
<td>42.50 ± 1.07***</td>
</tr>
<tr>
<td>MY41g</td>
<td>125</td>
<td>5</td>
<td>5.45 ± 0.66***</td>
<td>35.25 ± 0.51***</td>
</tr>
<tr>
<td>MY41g</td>
<td>250</td>
<td>5</td>
<td>5.83 ± 0.32***</td>
<td>25.44 ± 1.78***</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>5</td>
<td>5</td>
<td>3.09 ± 0.31*</td>
<td>53.75 ± 0.71**</td>
</tr>
</tbody>
</table>

Control 1 (4 day ulcerated rats); Control 2 (spontaneous healing); N = number of rats; the values in the table represent averages ± SEM; *p < 0.05; **p < 0.01 and ***p < 0.001: Statistically significant compared to Control 1; #p<0.05; ##p <0.01 and ###p <0.001: Statistically significant compared to Control 2.

Table 3 Effects of MY41g clay on tissue oxidative stress parameters in rats with simple chronic gastric ulcers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>SOD (U/mg protein)</th>
<th>Catalase (μmol H₂O₂/min/mg protein)</th>
<th>GSH (mmol/g protein)</th>
<th>Malondialdehyde (μmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Rats</td>
<td>-</td>
<td>5</td>
<td>2.48 ± 0.17</td>
<td>5.53 ± 1.25</td>
<td>1.68 ± 0.32</td>
<td>3.55 ± 0.50</td>
</tr>
<tr>
<td>Control 1</td>
<td>-</td>
<td>5</td>
<td>1.50 ± 0.15</td>
<td>8.35 ± 0.65</td>
<td>3.23 ± 0.03</td>
<td>9.60 ± 0.33</td>
</tr>
<tr>
<td>Control 2</td>
<td>-</td>
<td>5</td>
<td>1.58 ± 0.11</td>
<td>8.50 ± 0.29</td>
<td>2.85 ± 0.20</td>
<td>8.92 ± 0.51</td>
</tr>
<tr>
<td>MY41g</td>
<td>125</td>
<td>5</td>
<td>3.19 ± 0.42***</td>
<td>5.12 ± 0.23***</td>
<td>4.43 ± 0.22*</td>
<td>6.18 ± 3.34***</td>
</tr>
<tr>
<td>MY41g</td>
<td>250</td>
<td>5</td>
<td>5.55 ± 0.43***</td>
<td>8.21 ± 0.50</td>
<td>3.44 ± 0.21</td>
<td>7.63 ± 0.24*</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>5</td>
<td>5</td>
<td>1.67 ± 0.17</td>
<td>8.45 ± 1.55</td>
<td>2.43 ± 0.23</td>
<td>7.85 ± 0.15</td>
</tr>
</tbody>
</table>

Control 1 (4 day ulcerated rats); Control 2 (spontaneous healing); N = number of rats; the values in the table represent averages ± ESM; *p < 0.05; **p < 0.01 and ***p < 0.001: Statistically significant compared to Control 1; #p<0.05; ##p <0.01 and ###p <0.001: Statistically significant compared to Control 2.

Fig. 2 shows the histological sections of the stomachs of the rats with simple chronic gastric ulcers induced with acetic acid. In comparison with the normal gastric mucosa in non-ulcerated rats (Fig. 2a) day 4 of ulceration created deep ulcer crater with superficial loss of substance, glandular destruction, fibrosis, sclerosis and edema (Fig. 2b). Rats in the spontaneous healing group show an ulcerated area invaded by inflammatory cells, an onset of glandular recovery with regression of edema but with persistence of fibrosis (Fig. 2c). Rats treated with 125 and 250 mg/kg MY41g clay had glandular proliferation, with near total recovery of the gastric glands and with disappearance of fibrosis and edema (Figs. 2d and e). Sucralfate treatment resulted in marked healing but with slight persistence of the destroyed mucusa (Fig. 2f).

Fig. 3 shows the macroscopic aspect of the stomachs of rats subjected to the unhealed gastric ulcers (chronic ulcers + indomethacin administration for 2 weeks). Figs. 3a and b shows the stomachs of a normal rat and a group 1 rat (day 4 after ulceration) similar to those described above. Administration of indomethacin to ulcerated rats for 14 days further widened and deepen the ulcer wound, giving an ulcer index of 37.50 ± 1.44 and healing % of 47.91 (Fig. 3d). Photo 3c shows the stomach of ulcerated rat in the simple longitudinal control group given water for 14 days without indomethacin. A reduction in crater depth with mucus deposition on the surface of the ulcer and hemorrhagic ulcer boundaries: with 3.62% ulcerated surface and self-healing of 65.97% (Table 4). Figs. 3e and f represent the stomachs of ulcerated rats treated with MY41g clay at 125 and 250 mg/kg, respectively; ulcer craters were reduced to 1.14 and 0.88% of the total glandular area respectively, with a lower mucus deposition (71.25 and 72.25 mg, respectively) compared to the spontaneous healing in ulcerated rats without indomethacin (142 mg).

The ulcer indices decreased from 24.50 and 37.50 mm² in spontaneous healing in ulcerated rats without and with indomethacin, respectively, to 7.75 and 6.00 mm² in rats treated with MY41g clay at 125 and 250 mg/kg, respectively (Table 4). Administration of MY41g clay at 125 and 250 mg/kg caused in a significant decrease (p < 0.001) in gastric acidity compared to control 3 (spontaneous healing in ulcerated rats with indomethacin) (MDA: 14.1 ± 3.25 mmol/g protein) activity, reduced glutathione (GSH: 2.14 ± 0.89 mmol/g protein) and catalase (CAT: 8.25 ± 1.93 mmol H₂O₂/min/g protein) compared to control 1 (spontaneous healing in ulcerated rats with indomethacin) (MDA: 14.1 ± 3.25 mmol/g protein; SOD: 1.90 U/mg protein; GSH: 2.14 ± 0.89 mmol/g protein; and Catalase: 4.82 ± 0.92 mmol H₂O₂/min/g protein) in rats at 125 and 250 mg/kg; and an significant increase (p < 0.001) in MDA levels of 5.23 ± 1.49 mmol/g protein, respectively, in rats at 125 and 250 mg/kg, and a significant increase (p < 0.001) in GSH levels of 10.91 ± 1.52 mmol/g protein, respectively, and Catalase (p < 0.001) of 12.46 ± 0.22 mmol H₂O₂/min/g protein compared to control 3. These same parameters (MDA, GSH, Catalase and SOD) varied in a high resemblance to human ulcers. In addition, the second experiment evaluated firstly on acetaminophen-induced gastric ulcers, in replacement of the CaCO₃ microspheres, the clay solution on chronic and “unhealed” gastric ulcers demonstrated by Banenouze et al. [7], sparked the interest that led us to conduct an in vivo study of the curative and anti-inflammatory effects of MY41g clay (MY41g) on chronic and “unhealed” gastric ulcers in rats, J. Pharm. Med. Res. 5(1) (2020) 93–99.

**Table 4** Effects of MY41g clay on “unhealed gastric ulcers”

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>Ulcer index (IU)</th>
<th>% ulcerated area</th>
<th>% Healing</th>
<th>Mucus production (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>-</td>
<td>5</td>
<td>72.00 ± 0.81</td>
<td>10.66</td>
<td>37.25</td>
<td>52.75 ± 0.64</td>
</tr>
<tr>
<td>Control 2</td>
<td>1</td>
<td>5</td>
<td>72.00 ± 0.81</td>
<td>10.66</td>
<td>37.25</td>
<td>52.75 ± 0.64</td>
</tr>
<tr>
<td>Control 3</td>
<td>2</td>
<td>5</td>
<td>72.00 ± 0.81</td>
<td>10.66</td>
<td>37.25</td>
<td>52.75 ± 0.64</td>
</tr>
</tbody>
</table>

**Control 1 (4-day ulcerated rats); Control 2: (spontaneous healing in ulcerated rats without indomethacin) Control 3: (spontaneous healing in ulcerated rats given indomethacin).** N = number of rats; the values in the table represent averages ± SSM; *p < 0.05; **p < 0.01; and ***p < 0.001: Statistically significant compared to Control 1; p < 0.05; *p < 0.01 and **p < 0.001: Statistically significant compared to Control 2; p < 0.05; *p < 0.01 and **p < 0.001: Statistically significant compared to Control 3.

**Table 5** Effects of MY41g clay on gastric pH in rats subjected to “unhealed gastric ulcers”

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Gastric acidity (meq/L)</th>
<th>Gastric pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>1.87 ± 0.23</td>
<td>3.73 ± 0.34</td>
</tr>
<tr>
<td>Control 2</td>
<td>2.14 ± 0.21</td>
<td>3.95 ± 0.75</td>
</tr>
<tr>
<td>Control 3</td>
<td>2.41 ± 0.29</td>
<td>4.02 ± 0.46</td>
</tr>
</tbody>
</table>

**Control 1 (4-day ulcerated rats); Control 2: (spontaneous healing in ulcerated rats without indomethacin) Control 3: (spontaneous healing in ulcerated rats given indomethacin).** N = number of rats; the values in the table represent averages ± SSM; *p < 0.05; **p < 0.01; and ***p < 0.001: Statistically significant compared to Control 1; p < 0.05; *p < 0.01 and **p < 0.001: Statistically significant compared to Control 2; p < 0.05; *p < 0.01 and **p < 0.001: Statistically significant compared to Control 3.

**Table 6** Effects of MY41g clay on tissue oxidative stress parameters in rats with “unhealed gastric ulcers”

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>SOD (U/mg protein)</th>
<th>Catalase (µM H₂O₂/min/mg protein)</th>
<th>GSH (µmol/g protein)</th>
<th>Malondialdehyde (µmol/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>-</td>
<td>5</td>
<td>1.50 ± 0.15</td>
<td>9.53 ± 1.25</td>
<td>6.00 ± 0.11</td>
<td>14.1 ± 3.32</td>
</tr>
<tr>
<td>Control 2</td>
<td>2</td>
<td>5</td>
<td>2.00 ± 0.37</td>
<td>8.25 ± 0.08</td>
<td>2.14 ± 0.01</td>
<td>13.20 ± 1.56</td>
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<tr>
<td>Control 3</td>
<td>2</td>
<td>5</td>
<td>2.48 ± 0.17</td>
<td>10.6 ± 0.19</td>
<td>3.18 ± 0.23</td>
<td>11.90 ± 1.52</td>
</tr>
<tr>
<td>MY41g + Indo</td>
<td>2</td>
<td>5</td>
<td>2.48 ± 0.17</td>
<td>10.6 ± 0.19</td>
<td>3.18 ± 0.23</td>
<td>11.90 ± 1.52</td>
</tr>
<tr>
<td>MY41g + Indo</td>
<td>2</td>
<td>5</td>
<td>2.48 ± 0.17</td>
<td>10.6 ± 0.19</td>
<td>3.18 ± 0.23</td>
<td>11.90 ± 1.52</td>
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<tr>
<td>MY41g + Indo</td>
<td>2</td>
<td>5</td>
<td>2.48 ± 0.17</td>
<td>10.6 ± 0.19</td>
<td>3.18 ± 0.23</td>
<td>11.90 ± 1.52</td>
</tr>
</tbody>
</table>

**Control 1 (4-day ulcerated rats); Control 2: (spontaneous healing in ulcerated rats without indomethacin) Control 3: (spontaneous healing in ulcerated rats given indomethacin).** N = number of rats; the values in the table represent averages ± SSM; *p < 0.05; **p < 0.01; and ***p < 0.001: Statistically significant compared to Control 1; p < 0.05; *p < 0.01 and **p < 0.001: Statistically significant compared to Control 2; p < 0.05; *p < 0.01 and **p < 0.001: Statistically significant compared to Control 3.

The histological sections of “unhealed gastric ulcers” are shown in Fig. 4. The histological sections of the stomachs of rats in the normal and control 1 (4-day ulcerated rats) groups (Figs 4(a) and (b)) are the same as those described above. In control 2 (spontaneous healing in ulcerated rats without indomethacin), the section (Figs 4(c) and (d)) shows signs of self-healing, and the onset of mucosal regeneration can be seen. MY41g clay (125 mg/kg and 250 mg/kg) resulted in progressive muscular restoration with decreased inflammatory zone compared to controls 1, 2 and 3 (spontaneous healing in ulcerated rats with indomethacin) (Figs 4(e) and (f), respectively). Histological section of rats treated with sulcrate shows normalization of the mucosa, but inflammation can still be perceived by the presence of lymphocyte infiltration zone (Fig. 4(g)).

**Fig. 4** Histological presentation of “unhealed gastric ulcers”, 4(a): normal rat (shows normal muscle and mucosa); 4(b): control 1 (shows destruction of the mucosa with lymphocyte infiltration); 4(c) and 4(d): controls 1 and 2 (shows signs of self-healing, with an onset of mucosal regeneration but with more extensive mucosal destruction in the control 2); 4(e) and 4(f): 125 and 250 mg/kg of MY41g clay; (progressive restoration of the mucosa with reduction of the inflammatory zone); (g): 50 mg/kg sulcrate (shows normalization of the mucosa); D: destruction; E: edema; H.E: Hematoxylin-Eosin; L: Leukocyte infiltration; La: Lymph-gutumen; Mu: mucosa; Mc: Muscle layer.

The injection of acetic acid into the stomach walls of rats produces ulcers (gastric ulcers) that are histologically similar to human ulcers [24]. The development of these ulcers is due to the action of acetic acid, which corrodes the layers of the gastric wall and causes the acidity of the gastric juice to increase by gastric obstruction [25, 26]. These ulcers are also induced by the stress caused by the laparotomy performed during ulcer induction. Through physiological and psychological factors (decreased flow, increased hyperactivity), stress promotes a significant accumulation of acid and peptic acid in the stomach lumen. The result is tissue necrosis that causes the release of arachidonic acid metabolites; this attracts leukocytes (neutrophils and macrophages), resulting in the transformation of the mucosa into a series of coordinated cellular events that manifest themselves in angiogenesis, collagen deposition, and increased cohesive strength of the epithelium of the gastric mucosa [54], thus leading to the accelerated spontaneous healing process. Inflammation of the stomach also plays an important role in delaying the healing of gastric ulcers. Severe fibrosis, persisting beyond the healing process; chemical protection against oxidative stress is well known [50]. All these phenomena would explain the significant damage observed in the ulcerated animals concomitantly treated with indomethacin.

Macroscopically, chronic treatment at doses of 125 and 250 mg/kg resulted in a significant reduction in the ulcer index. The anti-inflammatory properties of this clay could be an effective weapon in the fight against ulcerogenic agents, by reducing the production of pro-inflammatory factors and would therefore explain the decrease in the percentage ulcerated surface. In addition, we observed that kaolinites, smectites and attapulgite clays stimulate coagulation factors in vitro [51]. Tarnawski et al. showed that re-epithelialization of the ulcerated mucosa is an essential process for the healing of gastrointestinal ulcers, and without this re-epithelialization of an epithelial barrier, the mucosa would be vulnerable to infections and lesions of mechanical or chemical origin [52]. Significant promotion of mucous production allows clays to solve the major problem of indomethacin which is to inhibit prostaglandin and production and would thus contribute to accelerate the spontaneous healing of these ulcer. Gwozinski et al. showed that the binding of clay crystals to mucous and viscous acetic acid induces the increase (p < 0.001) in mucus production. The importance of reinforcement of the gastric epithelium by increasing mucus production is well known [29]. Indeed, mucus is a glycoprotein that intervenes in the protective barrier of the gastric mucosa, forming an insoluble gel that adheres to the surface of the mucosa and prevents its destruction by aggressive substances such as hydrochloric acid. The work of Kuisu et al. [31-33] have shown that during the healing process, the aqueous extracts of Oxicum suave, Eremomastax, speciosa and Enantia chlorantha, respectively, increase mucus secretion, thus offering protection of the ulcer crater against gastric acid secretion and consequently hastening the healing process. In addition, Leonard et al. [34] showed that kaolinite and smectite clays are also effective in increasing mucus production by increasing the thickness of the gastric mucosa. MY41g clay could act in a similar way to accelerate the spontaneous healing process. Inflammation in general is characterized by intense neutrophil infiltration associated with vascular dilatation. This is followed by the proliferation stage, which marks the beginning of indomethacin, collagen synthesis and epithelialization. The subsequent remodeling phase consists of the formation of new collagen and an increase in the cohesive strength of the newly formed tissues [35, 36]. Healing is a normal physiological process that takes place through a series of coordinated cellular events that culminates in the restoration of the anatomical and functional integrity of tissues [37]. Ulcer healing is a complex process that depends on the regeneration of the structure of the glandular mucosa and the migration of epithelial cells to the ulcer crater in order to cover it [38]. In this study, clay caused a decrease in the ulcerated area, with repair of the glandular epithelium. It is therefore obvious that MY41g clay promotes the healing of chronic ulcers by acting on one or more cellular and molecular processes involved in the healing process. The anti-inflammatory, healing and covering properties of MY41g clay are likely due to the mineralogical presence of kaolinite, whose content of copper (105 ppm) will stimulate immunity and have an anti-inflammatory effect [39]. Mahraoui et al. [40] showed that aluminum silicates can prevent cell disjunction induced by inflammatory cytokines. In fact, studies have shown that SiO2 (47.7%) and Al2O3 (35.5%) represent 80% of the minerals contained in the MY41g sample [41]. Thus, the mineralogical composition of MY41g clay could be exploited as an ideal healing and protective dressing for the mucous membranes of the digestive tract [42, 43]. It is known that, non-steroideal anti-inflammatory drugs have a negative impact on ulcer healing. This fact has been verified by Wang et al., Amagase et al. and Amang et al. [16, 44, 45], who concluded that repeated administration of indomethacin for 14 days significantly delays spontaneous healing of gastric ulcers induced with acetic acid, creating “unhealed gastric ulcers”. This was confirmed in the present study when the degree of self-healing reduced from 65.9% in ulcerated rats given no NSN to 47.91% in ulcerated rats given indomethacin. Indeed, the pathogenesis of gastric lesions associated with the administration of indomethacin is related to the non-selective and irreversible inhibition of cyclooxygenases 1 and 2 [46]. This inhibition prevents the synthesis of prostaglandins (PGs) and thromboxanes (TXs) which have protective functions in the gastric mucosa. Indeed, PGE2 via these IP receptors is responsible for relaxation of vascular smooth muscles of the gastric microcirculation, by increasing the production of intracellular cAMP. Prostaglandins (PGE2) and prostacyclins (PGI2) are two powerful vasodilators that, once bound to the EP2 and IP receptors, control almost all aspects of defense (the spontaneous mucus secretion, the release of arachidonic acid secreted on parietal cells by decreasing intracellular cAMP concentration and indirectly by inhibiting histamine release from the gastric mucosa) [47]. In addition, PGE2 has been shown to be a potent inhibitor of the release of tumor necrosis factor (mast cells and macrophages), platelet activation factor (macrophages), leukotrienes, interleukins-8 (neutrophils) which are all pro-inflammatory mediators [48]. In addition, indomethacin will cause the expression of intercellular adhesion molecules (ICAM-1) responsible for neutrophil adhesion [49]. They pile up in the microcirculation, causing a local decrease in the blood flow of the mucous membrane which increases vascular tone, exacerbates tissue ischemia, stimulates the production of reactive oxygen species, and thus lead to a severe degree of necrosis, particularly in the presence of a low luminal pH [45]. Indomethacin also reduces the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) [49]. The role of these enzymes in the body’s defense against oxidative stress is well known [40]. All these phenomena would explain the significant damage observed in the ulcerated animals concomitantly treated with indomethacin.


https://doi.org/10.30799/jpmr.048.2005103
rats without and with indomethacin groups. The clay solution could therefore neutralize the H+ ions in the stomach lumen, and therefore increase the pH of the solution. Leonard et al. showed that clay captures pepsin and can therefore totally inhibit mucosal damage, hemorrhagic lesions and ulcerations usually created by excessive pathological secretion of pepsin [34]. Thus, this capacity of the clay solution to buffer the acidity could allow it to accelerate the spontaneous healing process.

Reactive oxygen species (ROS) are known to be involved in the genesis of gastric lesions [52]. Lipid peroxidation resulting from oxidative stress is a mechanism by which oxygenated free radicals cause tissue damage [56]. Oxidative stress thus causes cytotoxicity and inhibition of wound healing [57], while antioxidants help cells to protect them from damage due to oxidative stress [50].

In this study, concomitant administration of MY41g clay with indomethacin significantly prevented the increase in MDA levels, reverting them back to above the normal values. The significant reduction in MDA levels accompanied by a significant increase in GSH levels and catalase activity suggests a reduction in oxidative stress characterized by a decrease in lipid peroxidation and an increase in antioxidant capacities. The ability of MY41g clay to prevent the delayed healing of "unhealed gastric ulcers" may also be related to its antioxidant activity. Similar results have been observed by Aman et al. and Kuissu et al. [45, 33] with the aqueous extracts of Eremomastax speciosa and Enantia chlorantha respectively, on "unhealed gastric ulcers" [12].

4. Conclusion

Acknowledgments
The authors would like to thank the University of Yaoundé I and the institute of medical research and study of medicinal plants (IMMP) of Cameroon for the setting up of the technical platform during the realization of this work.

References

https://doi.org/10.13079/juem.048.20051013


