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

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ARTICLE DETAILS

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

**Keywords:**
MY41g Clay  
Chronic Gastric Ulcers  
Unhealed Ulcers  
Antacid Potential

**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 (83.69-90.2%). 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-ulcer 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 beidellite clay) for the symptomatic treatment of irritable colon syndrome; Gelox (smectite clay) for the symptomatic treatment of painful manifestations during oesophageal-gastro-duodenal disorders; Smectal (smectite clay) for the treatment of acute and chronic diarrhoea, symptomatic treatment of pain related to oesophageal-gastro-duodenal and colic disorders; Koologeais (kaolinite clay) for the symptomatic treatment of digestive functional disorders accompanied by anxiety symptoms [6].

Cameron has large clayey deposits, particularly kaolinite and halloysite. Cameroonian clays are consumed by geophagia; as antibiotics for wounds, as detoxifier, as antidiarrehetics, as antiinfectives 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 Mayoumou 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 antacids 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 Geological Material

The MY41g clay and limestone used in this experiment were obtained, respectively, from the Mayoumou clay deposit in the Noun Division, West Region of Cameroon, and the Fguil 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.

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Cite this Article as: J.F. Emakoua, A.P. Amang, C. Banenzoue, C. Mezui, G.T. Siwe, P.V. Tan, G.E. Enow-Orock, In-vivo curative and antacid effects of cameroonian clay (MY41g) on chronic and "unhealed" gastric ulcers in rats, J. Pharm. Med. Res. 5(1) (2020) 93–99.
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 Cameroon 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 Pilili and Santhakumar [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 Okabe [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 rats (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 fasted 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 Pilili and Santhakumar 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-metric 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 Bayeld-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 absorbance 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 mM 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 sclerous interior, representing an ulceration surface of 72.00 mm² (Fig. 1b). The treatment of the ulcer 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. 1c).

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. 1d 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. 1f) 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/L in the cross-sectional controls to 35.25 and 25.44 mg/L for clay-treated groups (p<0.001). Both doses of clay treatment were more efficient than Sucralfate in reducing gastric acidity (Table 2).

https://doi.org/10.13079/jmpm046.20500103

Cite this Article as: J.F. Emakoua, A.P. Amang, C. Banzenouze, C. Menei, G.T. Sise, P.V. Tan, G.E. Enow-Orock, In-vivo curative and antacid effects of Cameroon clay (MY41g) on chronic and "unhealed" gastric ulcers in rats, J. Pharm. Med. Res. 5(1) (2020) 93–99.
Table 1 Effects of MY41g clay on simple acetic acid-induced chronic gastric ulcers

| Treatment     | Dose (mg/kg) | N | Ulcer index (IU) | % ulcerated area | % Healing | Mucus production (mg) |
|---------------|--------------|---|------------------|------------------|-----------|----------------------|
| Control 1     | -            | 5 | 72.00 ± 0.81     | 10.66            |           | 54.25 ± 0.62         |
| Control 2     | -            | 5 | 20.75 ± 1.10     | 3.07             |           | 92.50 ± 3.79*        |
| MY41g         | 125          | 5 | 11.75 ± 1.18**   | 1.74             |           | 27.75 ± 3.816###     |
| MY41g         | 250          | 5 | 7.00 ± 1.29***   | 0.10             |           | 160.3 ± 11.92**###   |
| Sucralfate    | 50           | 5 | 0.50 ± 0.28****  | 0.07             |           | 152.3 ± 9.132###     |

Control 1 (4 day ulcerated rats); Control 2 (spontaneous healing); N = number of rats; the values in the table represent averages ± SEM; (x ± s) - 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

| Treatment     | Dose (mg/kg) | N | Gastric pH (Eq/mL) |
|---------------|--------------|---|--------------------|
| Control 1     | -            | 5 | 73.75 ± 3.45       |
| Control 2     | -            | 5 | 42.50 ± 1.07***    |
| MY41g         | 125          | 5 | 35.25 ± 0.51***    |
| MY41g         | 250          | 5 | 25.44 ± 1.78###**  |
| Sucralfate    | 50           | 5 | 53.75 ± 0.71**     |

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

| Treatment     | Dose (mg/kg) | N | SOD (U/mg protein) | Catalase (U/mg H2O/min/mg protein) | GSH (nmol/g protein) | Malondialdehyde (pmol/mg protein) |
|---------------|--------------|---|--------------------|-----------------------------------|----------------------|----------------------------------|
| Normal Rats   | -            | 5 | 3.19 ± 0.11        | 8.35 ± 0.65                      | 68 ± 0.8            | 3.35 ± 0.5                      |
| Control 1     | -            | 5 | 4.86 ± 0.11        | 4.25 ± 0.11                      | 168 ± 0.8           | 7.35 ± 0.3                      |
| Control 2     | -            | 5 | 1.50 ± 0.11        | 8.50 ± 0.29                      | 2.05 ± 0.2          | 8.92 ± 0.5                      |
| MY41g         | 125          | 5 | 3.19 ± 0.42**      | 5.12 ± 0.23**                    | 4.43 ± 0.22*        | 6.18 ± 3.34**                   |
| MY41g         | 250          | 5 | 5.55 ± 0.43***     | 8.21 ± 0.50                      | 3.44 ± 0.21         | 7.83 ± 0.24*                    |
| Sucralfate    | 50           | 5 | 1.67 ± 0.17        | 8.45 ± 1.55                      | 2.43 ± 0.23         | 8.85 ± 0.15                     |

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.
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 (p < 0.01), with healing rates of 89.23 and 91.66%, respectively (Table 4). Administration of MY41g clay at 125 and 250 mg/kg caused a significant decrease (p < 0.001) in gastric acidity compared to the cross-sectional and longitudinal indomethacin controls. This gastric acidity value decreased from 73.75 in the cross-sectional control to 36.25 and 21.25 in rats treated with MY41g clay at 125 and 250 mg/kg, respectively. The significance of gastric acidity observed previously was same when animals were treated with sulcrate (Table 5).

| Treatment | Dose (mg/kg) | N | Ulcer index (IU) | % ulcerated area | (% Healing) | Mucus production (mg) |
|-----------|--------------|---|------------------|------------------|------------|-----------------------|
| Control 1 | -            | 5 | 72.00 ± 0.81     | 19.66            | -          | 54.25 ± 0.62          |
| Control 2 | -            | 5 | 24.50 ± 2.06     | 3.62             | -          | 142.00 ± 3.93         |
| Control 3 | -            | 5 | 37.50 ± 1.34     | 4.64             | -          | 47.91 ± 1.84          |
| MY41g 125 | -            | 5 | 7.75 ± 0.62      | 1.14             | -          | 82.38 ± 4.93          |
| MY41g 250 | -            | 5 | 6.00 ± 0.81      | 0.88             | -          | 71.25 ± 3.77          |
| Sulcrate 50 | 4.75 ± 0.47 | 5 | 1.43 ± 0.07      | 0.70             | -          | 73.00 ± 3.62          |

Control 1 (4-day ulcerated rats); Control 2: (spontaneous healing in ulcerated rats without indomethacin); Control 3: (spontaneous healing in ulcerated rats given indomethacin).

| Treatment | Dose (mg/kg) | N | Gastric pH | Gastric acidity (meq/L) |
|-----------|--------------|---|------------|-----------------------|
| Control 1 | -            | 5 | 2.41 ± 0.23| 73.35 ± 3.45          |
| Control 2 | -            | 5 | 3.59 ± 0.23| 48.24 ± 1.44          |
| Control 3 | -            | 5 | 2.41 ± 0.21| 82.38 ± 4.93          |
| MY41g 125 | -            | 5 | 5.33 ± 0.38 | 36.25 ± 2.14          |
| MY41g 250 | -            | 5 | 6.14 ± 0.49 | 21.25 ± 1.13          |
| Sulcrate 50 | 5.55 ± 0.20 | 5 | 26.81 ± 0.52 | 20.00 ± 0.30 |

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 ± 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;

The effects of MY41g clay on some parameters of oxidative status are shown in Table 6. Indomethacin ip injection (1 mg/kg) for 14 days induced in rats an increase in malondialdehyde (MDA; 13.20 pmol/mg protein) and a reduction in superoxide dismutase (SOD) activity, reduced glutathione (GSH; 2.14 mmol/g protein) and catalase (CAT; 8.25 mmol H₂O₂/min/mg protein) activity compared to control3 (spontaneous healing in ulcerated rats with indomethacin) (MDA: 14.10 pmol/mg protein; SOD: 19.0 U/mg protein; GSH: 22.0 mmol H₂O₂/min/mg protein; CAT: 6.44 mmol H₂O₂/min/mg protein). Administration of MY41g clay concomitantly with indomethacin for 14 days resulted in a significant decrease in MDA levels (12.46 pmol/mg protein and 10.91 pmol/mg protein) compared to rats at 125 and 250 mg/kg; and a significant increase (p < 0.001) in GSH levels of 5.23 and 4.75 mmol/mg protein, respectively, and CAT (p < 0.001) of 12.89 and 13.63 compared to controls. These same parameters (MDA, GSH, CAT, Malondialdehyde) showed a similar way in animals treated with sulcrate.

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 sections of animals treated with sulcrate shows normalization of the mucosa, but inflammation can still be perceived by the presence of lymphoocyte infiltration zone (Fig 4(g)).

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The in vitro anti-oxidant capacity of MY41g clay demonstrated by Banenzoue et al. [7], sparked the interest that led us to conduct an in vivo study of the curative and antacid effects of the clay solution on chronic and “unhealed” gastric ulcers. In replacement of the CaCO₃ used in the in vitro study, we used a local material (limestone) to improve the buffering capacity of the MY41g clay. In this study, the healing potential of clay was evaluated firstly on acetic acid induced chronic gastric ulcers which bear a high resemblance to human ulcers. In addition, the second experiment simulated the real case of elderly patients who, while treating chronic gastric ulcers, are often required to take NSAIDs for the relief of pain associated with other age-related conditions like arthritis and rheumatism. NSAIDs have been shown to retard the healing of gastric ulcers, leading to the so called “unhealed” or “hard healing” ulcers.
The injection of acetic acid into the stomach walls of rats produces ulceration, erosions, and tumors, similar to human ulcerations and tumors [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 gastric flow, increased gastric acid, and hyperactivity), stress promotes a significant accumulation of acid and pepsin 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 superficial lesions into deeper wounds; and inactivation of growth factors impairs the healing process. The release of tumor necrosis factor (TNEF), interleukin-1, interleukin-6, and interleukin-8 observed in ulcerated rats 4 days after ulcer induction is the result of this pathophysiological mechanism of acetic acid ulcer induction.

In the chronic ulcer induction model with acetic acid, ulcerated rats given distilled water only during the treatment period showed 71.18% self-healing. This healing is justified by the fact that tissue necrosis causes the release of arachidonic acid metabolites, which attracts leukocytes (neutrophils and macrophages). These leukocytes phagocyte necrotic tissue and release pro-inflammatory cytokines and growth factors that activate fibroblasts, endothelial cells and epithelial cells. This activation is at the origin of the formation of the granulation tissue (tissue replacing the damaged area) [28]. However, human patients with chronic gastric ulcers, can’t rely on self-healing and treatment should therefore be combined with the elimination of etiological factors.

The MY41g clay solution, administered once a day for 10 days per os, significantly accelerated the spontaneous healing of chronic gastric ulcers. This acceleration of spontaneous healing was manifested by 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 digestive enzymes, elastase, pepsin and hydrochloric acid. The works of Kaibara et al. [31-33] have shown that during the healing process, the aqueous extracts of Ocimum suave, Eremomastax, spicula 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 MY41g clay accelerates the rate of ulcer healing by increasing the thickness of the gastric mucus. MY41g clay can 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 the complex remodeling that depends on the 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]. Ultrasonic waves are a complex wave that depolarizes the membranes and induces 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) would be vulnerable to infections and lesions of mechanical or chemical origin [52]. Significant promotion of mucus production allows clays to solve the major problem of indomethacin which is to inhibit prostaglandin production and would thus contribute to accelerate the spontaneous healing of these ulcers. Gwozinski et al. showed that the binding of clay to mucus and the induction of mucus acylation is a simple longitudinal control. This would be due to the action of smectites and attapulgite clays stimulatory effect on the synthesis of mucus, mainly through the induction mechanism of "unhealed gastric ulcers" [16]. Although the ulcerated rats given indomethacin showed a significant decrease in the ulcer index (72.00 mm) observed in ulcerated rats 4 days after ulcer induction, the result is the significant reduction in the ulcer index. In addition, kaolinites, smectites and attapulgites clays stimulate coagulation factors (ICAM-1) responsible for neutrophil adhesion [48]. They pile up in the microcirculation, causing a local decrease in the blood flow of the mucous membrane which increases vascular tone, exacerbates platelet aggregation (mast cells and macrophages), platelet activation factor (mast cells), leukocyte adhesion, randomization of ICAM-1 and interleukin-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 [48].

Macromorphologically, clay 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 factors. Mucosal ulcers are caused by pro-inflammatory factors and would therefore explain the decrease in the percentage ulcerated surface. In addition, it was observed that kaolinites, smectites and attapulgites 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 the continuous restoration of an epithelial barrier, the mucosa would be vulnerable to infections and lesions of mechanical or chemical origin [52]. Significant promotion of mucus production allows clays to solve the major problem of indomethacin which is to inhibit prostaglandin production and would thus contribute to accelerate the spontaneous healing of these ulcers. Gwozinski et al. showed that the binding of clay to mucus and the induction of mucus acylation is a simple longitudinal control. This would be due to the action of smectites and attapulgite clays stimulatory effect on the synthesis of mucus, mainly through the induction mechanism of "unhealed gastric ulcers" [16]. Although the ulcerated rats given indomethacin showed a significant decrease in the ulcer index (72.00 mm) observed in ulcerated rats 4 days after ulcer induction, the result is the significant reduction in the ulcer index. In addition, kaolinites, smectites and attapulgites clays stimulate coagulation factors (ICAM-1) responsible for neutrophil adhesion [48]. They pile up in the microcirculation, causing a local decrease in the blood flow of the mucous membrane which increases vascular tone, exacerbates platelet aggregation (mast cells and macrophages), platelet activation factor (mast cells), leukocyte adhesion, randomization of ICAM-1 and interleukin-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 [48].

The effects of MY41g clay, and sucralate were better visualized on the histological sections of the stomach tissue of each group. Ulcerated rats given indomethacin showed more destruction of the mucosa than the simple longitudinal control. This would be due to the action of indomethacin, which would slow down the process of spontaneous healing. Severe fibrosis, persistent neutrophil infiltration, interference with the action of growth factors, slowing of angiogenesis at the base of the ulcer and maturation of granulation tissue are processes involved in the induction mechanism of "unhealed gastric ulcers" [16]. Although the ulcerated rats given indomethacin showed a significant decrease in the ulcer index, the ulcer was also showed with the ulcerated rats without indomethacin a restoration of the mucusular content compared to 14 days ulcerated rats, which would be the result of the self-healing process, put in place by the body. MY41g clay as well as sucralate caused a progressive and improved reconstitution of the mucosa and muscular system. The effectiveness of MY41g clay could be due in large part to the fact that it stimulates the synthesis of mucus, which strengthens the mucus-barrier role. The secretion of gastric acid in the stomach also plays an important role in delaying the healing of gastric ulcers. This acid interferes with gastric mucosal healing processes, resulting in the conversion of superficial lesions into deeper mucosal lesions, and the inactivation of growth factors important for maintaining mucosal integrity and repairing gastric lesions. In 125 ulcer model, studied, there was a significant decrease in gastric acidity in the MY41g clay-treated groups compared to 14 days ulcerated rats and in ulcerated
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 *Entanla chlorantha* respectively, on "unhealed gastric ulcers" [1].

4. Conclusion

Administration of MY41g clay accelerated the spontaneous healing of chronic acetic-induced gastric ulcers and prevented the delay in the healing of chronic gastric ulcers caused by indomethacin. The mode of action of the MY41g clay cool indicated increased gastric mucosal thickness, increased gastric reepithelialization, improved antioxidant status and effective antacid activity. The rich mineralogical composition of MY41g clay can be exploited for the development of an affordable replacement for other antacids in the triple therapy regimen for ulcer treatment.

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.

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Cite this Article as: J.F. Emakoua, A.P. Amang, C. Banenzoue, C. Mezui, G.T. Siwe, P.V. Tan, G.E. Enow-Orock, In-vivo curative and antacid effects of cameroonian clay (MY41g) on chronic and "unhealed" gastric ulcers in rats, J. Pharm. Med. Res. 5(1) (2020) 93–99.

https://doi.org/10.30799/jpmr.048.20050103

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