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In-vitro Antacid Properties of Cameroonian Clay (MY41g) and its Potential Use in Anti-ulcer Triple Therapy Regimen Formulated with *Eremomastax speciosa* Extract

Joseph Fleurie Emakoua[1], Mesmine Kuissu Teukam Mimosette[2], André Perfusion Amang[3], Mbida Désirée Essama[1], Otto Gustave Lebeau Ndji[4], Christophe Mezui[5], Enow-Orock George Enonchong[6] and Paul Vernyuy Tan[1*]

[1]Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, P.O.Box 812, Yaoundé, Cameroon.
[2]Department of Animal Science, Faculty of Agriculture and Veterinary medicine, University of Buea, P.O.Box 63, Buea, Cameroon.
[3]Department of Biological Sciences, Faculty of Science, University of Maroua, P.O.Box 46, Maroua, Cameroon.
[4]Department of Life Science, Higher Teachers' Training College, University of Ngaoundere, P.O.Box 652, Bertoua, Cameroon.
[5]Department of Biological Sciences, Higher Teachers' Training College, University of Yaoundé I, P.O.Box 047, Yaoundé, Cameroon.
[6]Department of Biomedical Sciences, Faculty of Health Science, University of Buea, P.O. Box 63, Buea, Cameroon.

Authors' contributions

This work was carried out in collaboration between all authors. Author JFE wrote the protocol, collected plant material, performed pharmacological assays, performed the statistical analysis and wrote the first draft of the manuscript. Authors MKTM and APA helped in raw data analysis and provided punctual assistance. Authors MDE, OGLN and CM managed the literature. Authors PVT and EOGE designed and supervised the study. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: verdzekovnso@yahoo.com;*

ABSTRACT

Aims: The antisecretory, antibacterial on Helicobacter, and antacid properties of *Eremomastax speciosa* (*E. speciosa*) and MY41g clay respectively, led us to evaluate the potential use of this clay in the anti-ulcer tri-therapy formulated with *Eremomastax speciosa.*

Place and Duration of Study: Department of Animal Biology & Physiology (Animal Physiology Laboratory), Faculty of Science, University of Yaoundé I, between August 2020 and February 2021.

Materials and Methods: *In vitro* antacid were evaluated by studying: Fordtran's method, and the influence of temperature on the pH values. I*n vivo* activity was studied on chronic gastric ulcers induced by injection of 0.05 ml of acetic acid (30%) into the stomach wall. Rats were treated daily for 10 days after ulcer induction with a combination *of E. speciosa* and MY41g (ESMY) ; (ESMY 100+250 and 200+250 mg/kg). The model of "unhealed" gastric ulcers was also used: from day 5 to day 18 of experimentation, rats were given ESMY orally concomitantly with indomethacin (1 mg/kg/day) subcutaneously. Ulcer index, percentage of healing, mucus secretion, gastric acidity, histological, hematological, and oxidative stress parameters were evaluated.

Results: ESMY showed good neutralizing capacity *in vitro* in Fordtran's method. Treatment with ESMY accelerated the spontaneous healing of chronic gastric ulcers (93.82-96.14%). However, administration of indomethacin did not induce significant variations in the percentage of healing (90.73-94.60%). For both ulcer models performed, ulcer healing was accompanied by a significant (P = 0.001) increase in mucus mass at 200/250 mg/kg. ESMY increased antioxidant activity, decreased gastric acidity, lipid peroxidation, and maintained hematological balance.

Conclusion: In addition to its buffering properties, the healing mechanism of ESMY includes reduced gastric acidity, enhanced mucus production, re-epithelialization of gastric mucosa, improvement of hematological and antioxidant status. ESMY can be used in traditional medicine, as a therapeutic regimen against gastric ulcers.

Keywords: Eremomastax speciosa; MY41g clay; chronic gastric ulcers; unhealed gastric ulcers.

1. INTRODUCTION

Peptic ulcers are wounds that appear on the mucous membrane of the stomach or small intestine, characterized by a loss of deep substance amputating the muscularis and possibly reaching the serosa (perforation) [1]. They generally result from an imbalance between aggression factors (HCl, pepsin, non-steroidal anti-inflammatory drugs, *Helicobacter pylori,* free radicals, ethanol, tobacco, and stress) and defense factors of the gastric mucosa (mucus, surface epithelium, prostaglandins, bicarbonate, NO, and microcirculation) in favor of the former [2]. The incidence of peptic ulcer disease varies according to age, gender, and geographic location and can be associated with serious complications, including hemorrhage, perforation, gastrointestinal obstruction, and malignancies, and represents a global health problem because of its mortality, high economic losses, and morbidity [3]. Several works aimed at treating gastric ulcers have focused on new drug therapies such as antibiotics (Metronidazole, Amoxicillin) to kill *H. pylori*, acid blockers that reduce acid secretion over a prolonged period (Ranitidine, Cimetidine, Famotidine), proton pump inhibitors (Omeprazole) and tissue mucosal protectants (Sucralfate, Bismuth) [4]. Are usually treated with The standard triple therapy regimen recommended for gastric ulcer patients includes an anti-secretory agent, two antibiotics, and an antacid [5]. Despite the reduction of morbidity by these drugs, they produce numerous adverse effects (digestive, disorders, headaches, diarrhoea, drowsiness, fatigue, muscular pain, constipation, mental confusion, abnormalities in serum metabolite concentrations, impaired hepatic function, adverse hematological reactions, and cardiac malfunction for the anti-secretory drugs [6]; the emergence of resistant strains such of *H. pylori* to the antibiotics), high cost for poor populations, relapses of the disease and reduced patient compliance.

Because of these constraints, we propose that a less expensive more simplified regimen including fewer components whose tested activities embody the cardinal requirements for ulcer healing (anti-secretory, anti-biotic, antacid activity, and healing potential) can improve healing and increase patient compliance, with fewer side effects. Banenzoue *et al* have shown that the clay sample My41g combined with 2% calcium carbonate has a real antacid power *in*

vitro [7]. The antacid effects *in vivo* of MY41g clay (250 mg/kg) on both chronic and "unhealed" gastric ulcers in rats [8] have been shown. The antisecretory properties and underlying mechanisms [9] and anti-*H. pylori* actions [10] of *E. speciosa* extract (200 mg/kg) have been demonstrated, and toxicity studies have shown that the dose of 200 mg/kg is non-toxic in acute and subacute intake [11].

In light of the above results, it is possible to formulate a more simplified antiulcer regimen using only two components that respond to the triple therapy requirements (antacid, antisecretory, and antibiotic effects). Thus, in the present experiment, we evaluated the *in vitro* antacid properties of Cameroonian MY41g clay and *E. speciosa* extract against artificial gastric juice and tested the healing actions of the drug combination on simple acetic acid-induced chronic gastric ulcers and “Unhealed" chronic gastric ulcers in rats.

2. MATERIALS AND METHODS

2.1. *In vitro* and *in vivo* Materials

2.1.1 Geological material

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 [12]. 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.

2.1.2 Plant materials

The fresh leaves of *E. speciosa* were collected in April 2015, at 6 a.m., in Yaounde, Center Region of Cameroon. Botanical identification was done at the National Herbarium, Yaounde, by Paul Mezili, by comparison with existing herbarium specimen number HNC/136984.

2.1.3. 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. 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 [13].

2.1.4 Chemicals and reagents

Pepsin, hydrochloric acid, sodium chloride, and sodium bicarbonate were, respectively, from Sigma-Aldrich Chemie Gmbh Switzerland, Labkem AGR ISO France, Riedel-de Haen AG D Seeize 1 the USA and S.D Fine Chemicals Ltd Mumbai India were used in *in vitro* testing.

2.1.5 Instruments

The instruments used in this experiment (*in vitro*) were: a standard pH meter (WW R scientific model 8000), a magnetic stirrer (04644-Series Digital Hot Plate/Stirrer), and a water bath (Bain Précision 180 Series Water Bath) as a temperature regulator.

2.2 *In vitro* and *in vivo* Methods

2.2.1. Preparation of clay solution

2.4 g of clay powder was mixed with 0.1 g of limestone and 50 mL of distilled water. The mixture was homogenized using a magnetic stirrer to obtain a stock solution with a concentration of 50 mg/mL. Limestone (5%) was used as a local material in replacement of calcium cabonate (2%) used in earlier experiments [7].

2.2.2 Preparation of the plant extract

The leaves were chopped and quickly dried under the shade to prevent them from getting moldy and then ground using a mechanical grinder to obtain a fine powder. 400g of the powder was extracted by infusion in 4 liters of boiled water for 15 minutes. After filtration through Whatman filter paper No. 3, the filtrate was evaporated using a Raven ventilation oven (Jencons PLS, UK). The brownish solid obtained (99.7g; 24.9% yield) was stored at 4°C. The extract was dissolved in distilled water which was used as the vehicle.

2.2.3 Preparation of artificial gastric juice

Two grams of NaCl and 3.2 mg of pepsin were dissolved in 500 ml of distilled water. The solution was made up of hydrochloric acid (7.0 ml) and distilled water to make a volume of 1000 ml. The pH of the solution was adjusted to 1.20 [14].

2.2.4 Evaluation of pH of the different solutions

The pH of the solutions (MY41g, and *E. speciosa* + MY41g mixtures (**ESMY**)) was determined at temperatures of 25 and 37 °C. The pH values of the positive ($NaHCO_3$) and negative (H_2O) controls were also determined for comparison [14].

2.2.5 Evaluation of the neutralizing effects of the different solutions on artificial gastric juice

Ninety milliliters (90 ml) of each freshly prepared test and control solution (MY41g, **ESMY** (100+250 and 200+250 mg/kg), and Sodium bicarbonate) were added separately to the artificial gastric juice (100 ml, pH 1.2). The pH values were determined at 25 and 37 ± 2°C (t=0) and after incubation at 25 ± 2°C (t=8h). Five tests were performed for each solution [14].

2.2.6 *In vitro* titration method of Fordtran's model for determination of the neutralization capacity

Ninety milliliters (90 ml) of each freshly prepared sample (test and control) was placed in a 250 ml beaker at 25 °C. Stomach movements were simulated by operating a magnetic stirrer continuously at 30 rpm. The prepared solutions were titrated with artificial gastric juice to the pH 3 endpoint. The volume (V) of artificial gastric juice consumed was measured. The total amount of H^+ (mmol) consumed was measured (0.063096 (mmol/mL) × V (mL)). Six experiments were performed for each freshly prepared test solution. The same test was performed after raising the temperature of the test and control samples to 37 °C [14].

2.2.7 Induction of gastric ulcers

2.2.7.1 Induction of simple chronic acetic acid ulcers

The induction of chronic gastric ulcers was performed according to the method described by Pillai and Santhakumari [15]. 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 lesser curvature. After cleaning the stomach with cotton soaked in NaCl solution (9%), a suture was performed to close the incision, and an antibiotic (Betadine) was applied to the incision to prevent infection of the wound. Three days after ulcer induction, group 1 rats 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 [16]. These rats were sacrificed 6 hours later under anesthesia, and the rats stomachs were opened 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 **ESMY** 100+250 mg/kg and **ESMY** 200+250 mg/kg, respectively; group 5 rats received 50 mg/kg Sucralfate. On the 9th day of treatment, the animals 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 [8].

2.2.7.2 Induction of "Unhealed" gastric ulcers

The method described by Pillai and Santhakumari in 1984 was used and supplemented by that of Wang [17] 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.

2.2.8 Measurement of mucus production

The mucus on the glandular part of the stomach of each rat was gently scraped off using a microscope slide [18], and weighed using a sensitive electronic balance.

2.2.9 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 [19,8].

2.2.10 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. Haematoxylin-eosin (H&E) staining technique was used according to the standard histological procedure described by Bayelet-Vincent [20] and the sections were observed microscopically.

2.2.11 Hematological parameters

Blood samples were collected in sample tubes containing EDTA. Red blood cell (RBC) count, white blood cell (WBC) count, differential white blood cell (lymphocyte, monocyte, granulocyte) count, platelets, hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) were determined using an automated analyzer (Hospitex Diagnostics Hema Screen 18) [8].

2.2.12 Measurement of *in vivo* antioxidant activity

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 [21]. Cellular glutathione (GSH) was measured on the basis of the reaction between 2,2-dithio-5,5dibenzoic acid and the thiol (SH) groups of glutathione to give a complex whose absorbance was read at 412 nm [22]. The glutathione concentration was calculated using the molar extinction coefficient ε = 1.36 104 M-1cm-1. Superoxide dismutase (SOD) activity was measured using a standard method [23], while catalase was determined and expressed in mM of H_2O_2/min/mg protein [24]. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels in gastric tissue samples [25]. The quantification of the MDA was performed using an extinction coefficient of = 1.56. x 105 M-1 cm-1.

2.2.13 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

3.1 Effects of *E. speciosa* Extract and Clay on Artificial Gastric Juice

The initial pH of the different solutions (H_2O, MY41g, **ESMY** and $NaHCO_3$) did not vary significantly between the temperatures of 25 and 37 ± 2°C (Table 1). The addition of artificial gastric juice to the different solutions instantaneously lowered the pH values significantly ($P = 0.001$) compared to the initial pH values both at 25 and 37°C, and the effect was more marked at 37°C. Following incubation of the different solutions with artificial gastric juice for 8 hours at 25°C, the pH values witnessed nonsignificant slight increases compared to time t (0).

3.2 *In vitro* Neutralizing Capacity of *E. speciosa* and MY41g According to Fordtran's Model

Table 2 shows the volume of artificial gastric juice that was necessary to bring the initial pH values of the different solutions (6.66 – 8.97) down to end point pH 3. The volumes of gastric juice consumed by MY41g (4.67 ml), the **ESMY** (44.25 and 58.00) and $NaHCO_3$ (72.50 ml), were significantly ($P = 0.001$) higher compared with distilled water (1.50 ml). The volumes consumed correlated with the calculated amounts of proton (mmol H^+) neutralized in the consumed artificial gastric acid solution.

3.3 Effects of ESMY on Simple Acetic Acid-Induced Chronic Gastric Ulcers

Fig. 1 shows photographs of stomachs of rats with acetic acid-induced chronic gastric ulcers in the different groups. Rats sacrificed 4 days after ulcer induction had deep, wide ulcers with raised edges and a sclerotic interior, representing an ulceration area of 86.33 mm^2 (Fig. 1b). Ulcer treatment for 10 days with distilled water (longitudinal control) reduced ulcerated area to 44.00 mm^2, a self-healing of 49.03%. **ESMY** 200+250 mg/kg and **ESMY** 100+250 mg/kg significantly ($P = 0.001$) reduced ulcer index to 5.33 and 3.33, respectively (Table 3). Sucralfate significantly reduced ulcer index to 15,33. This healing was accompanied by significant ($P = 0.001$) increases in mucus mass in animals treated with the extract + clay mixture.

Table 1. Effects of *E. speciosa* Extract and clay on pH of artificial gastric juice

Solutions	pH at 25 °C	pH at 25 °C (after addition of gastric juice); t(0)	pH at 37 °C	pH at 37 °C (after addition of gastric juice); t(0)	pH at 25°C (after incubation for 8 hours); t(8)
H_2O	6.66 ± 0.52	1.18 ± 0.01	6.25 ± 0.40	0.38 ± 0.02	1.54 ± 0.14
MY41g *(250 mg/kg)*	8.07 ± 0.04	1.40 ± 0.13***	7.76 ± 0.02	0.29 ± 0.02	1.60 ± 0.06
ESMY *100+250 mg/kg*	8.77 ± 0.05	1.20 ± 0.04	8.37 ± 0.03	0.75 ± 0.04	1.77 ± 0.07
ESMY *200+250 mg/kg*	8.97 ± 0.04	1.37 ± 0.12**	8.42 ± 0.03	0.63 ± 0.07	1.44 ± 0.09
$NaHCO_3$	8.84 ± 0.04	2.54 ± 0.12***	8.63 ± 0.03	2.22 ± 0.25	2.57 ± 0.14

**P = 0.05; **P = 0.01; and ***P = 0.001: statistically significant difference from distilled water (H_2O)*
Number of test replicates for each solution = 5

Table 2. Volume (ml) of artificial gastric juice and quantity of protons (H^+) consumedafter titration with the different solutions

Solutions	Dose (mg/kg)	*N*	Volume of gastric juice consumed (ml)	Quantity of protons consumed (mmol)
H_2O	-	*5*	1.50 ± 0.20	0.10 ± 0.01
MY41g	250	*5*	4.67 ± 0.26*	0.29 ± 0.01*
ESMY	100+250	*5*	44.25 ± 2.86***	2.78 ± 0.18***
ESMY	200+250	*5*	58.00 ± 2.85***	3.65 ± 0.17***
$NaHCO_3$	-	*5*	72.50 ± 3.2***	4.57 ± 0.20***

N = number of replicates for each solution
**P = 0.05; **P = 0.01; and ***P = 0.001: statistically significant difference from distilled water*

Table 3. Effects of ESMY on acetic acid-induced chronic gastric ulcers

Treatment	Dose (mg/kg)	N	Ulcer index (IU)	% ulcerated area	% healing	Mucus production (mg)
Control 1	-	5	86.33 **±** 3.28	12.78	-	77.50 **±** 7.04
Control 2	-	5	44.00 **±** 6.11***	6.51	49.03 *(s-h)*	37.33 **±**1.453**
ESMY	100+250		5.33 **±** 0.66***###	0.78	93.82	57.00 **±** 1.155
ESMY	200+250	5	3.33 **±** 0.66***###	0.49	96.14	86.00 **±** 6.245###
Sucralfate	50	5	15.33 **±** 1.20***##	2.27	82.24	73.5 **±** 5.09##

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

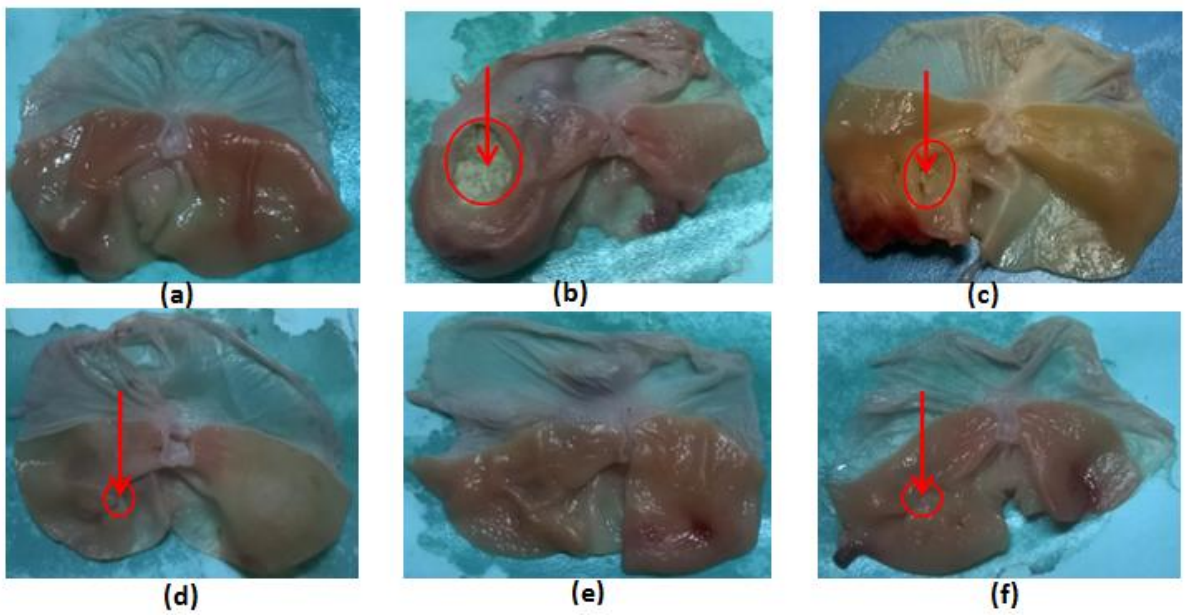

Fig. 1. Stomachs of rats with simple acetic acid-induced chronic gastric ulcers [8]
1(a): Normal rat (untreated, non-ulcerated rats that received distilled water for 10 days);1(b): Group 1 rats sacrificed 4 days after induction of chronic gastric ulcers to confirm ulcer formation;1c:Longitudinal control; 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; 1d and e: groups 3 + 4 rats treated with 200 + 250 and 100 + 250 mg/kg of ESMY;1f: ulcerated rats treated with Sucralfate (50 mg/kg); indication of chronic gastric ulcers

Gastric acidity was high (48.00 – 57.50 mEq/l) for the untreated controls but the extract/clay mixtures significantly (*P* = 0.001) reduced gastric acidity to 20.33 mEq/l (200+250 mg/kg dose). Gastric acidity in Sucralfate-treated rats reduced to 31.50 mEq/l compared with controls (Table 4).

Table 5 shows that ulcer induction reduced red blood cell counts in longitudinal controls (3.63±0.50 x 10^6/µL) but the extract/clay mixtures significantly (*P* = 0.01) increased values back to normal levels (7.09 - 8.98 x 10^6/µL). Hematocrit levels also dropped due to ulcer induction but progressively recovered to above normal levels after extract/clay treatment. White blood cell counts dropped significantly following ulcer induction in all groups (3.74±0.01-6.37±0.53 x 10^3/µL) compared with normal controls (9.90±1.07 x 10^3/µL), and the effect was reflected in the lymphocyte component. Platelet levels also decreased due to ulcer induction but extract/clay treatment and sucralfate caused progressive recovery back to above normal levels.

Tissue oxidative stress parameters in rats with simple chronic gastric ulcers are presented in Table 6. Ulcer induction caused significant decrese in SOD activity (0.33 **±** 0.10) compared with normal rats (2.68 ± 0 .18), but extract/clay treatment reversed values to above normal levels at the highest dose. GSH, Catalase and MDA were not affected by ulcer induction in the controls compared to normal rats and extract/clay and sucralfate treatment maintained above normal levels of these parameters.

The histological sections of the stomachs of rats with simple chronic gastric ulcers are shown in Fig. 2. Rats in the transverse control group had deep ulcers, glandular destruction, fibrosis, sclerosis, and edema (Fig. 2b). Rats in the longitudinal control group show an ulcerated area invaded by inflammatory cells, early glandular overlay with regression of edema (Fig. 2c). In rats treated with **ESMY** 100+250 and **ESMY** 200+250 mg/kg, almost complete recovery of the ulcerated area was observed (Fig. 2d-e). Rats treated with Sucralfate presented stomach tissues with marked scarring and slight persistence of the destroyed mucosa (Fig. 2f).

3.4 Effects of ESMY on "Unhealed" Gastric Ulcers

Fig. 3 shows photographs of the stomachs of the different groups after induction of acetic acid-induced chronic gastric ulcers followed by daily administration of indomethacin for 2 weeks to create "unhealed ulcers". In day 4 ulcerated rats and the ulcerated rats given distilled water or indomethacin for 14 days, ulcer craters remained deep (40.41- 53.21 mm^2), with no visual signs of healing (Fig. 3(b-d)) with a percentage of healing of 38-53% (Table 7). Ulcer index was significantly reduced by Sucralfate (14.33 mm^2) and extract/clay mixtures (8.00 and 4.66 mm^2) compared to the cross-sectional controls (86.33 mm^2). Mucus mass increased significantly (*P* = 0.01 and *P* = 0.001) in these different groups of animals compared with controls (Table 7).

The high gastric acidity in control groups (54,80 - 60,53 mEq/l) was reduced to 43,77 - 46,11 mEq/l in ulcerated rats treated concomitantly with sucralfate and **ESMY** 100+250 mg/kg but the effects were not significant. Only the **ESMY** 200+250 mg/kg dose significantly (*P* = 0.05) decreased in gastric acidity (36,38 mEq/l) compared to the longitudinal control (54.80 mEq/l) (Table 8).

Table 4. Effects of ESMY on pH and gastric acidity in rats with simple chronic gastric ulcers

Treatment	Dose (mg/kg)	*N*	pH of gastric juice	Gastric acidity (mEq/l)
Control 1	_	*5*	3.80 ± 0.11	57.50 ± 4.44
Control 2	_	*5*	3.92 ± 0.23	48.00 ± 5.77
ESMY	100+250	*5*	5.68 ± 0.43*#	34.75 ± 6.25*
ESMY	200+250	*5*	7.24 ± 0.32***###	20.33 ± 4.16***##
Sucralfate	50	*5*	5.98 ± 0.10*#	31.50 ± 0.04*#

Control 1 (4 days ulcerated rats); Control 2 (spontaneous healing)
N = number of rats; the values in the table represent means ± 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*

Table 5. Effects of ESMY on hematological parameters in rats with simple chronic gastric ulcers

	Normal rats	Control 2	ESMY	ESMY	Sucralfate
N	**5**	**5**	**5**	**5**	**5**
Dose (mg/kg)	**-**	**-**	**100 + 250**	**200 + 250**	**50**
Red blood cells (10^6/µL)	7.99±0.34	3.63±0.50$^{\#\#\#}$	7.09±1.00**	8.98±0.19**	2.75±0.51$^{\#\#}$
Hemoglobin (g/dL)	15.17±0.45	12.75±0.80	11.76±1.02	14.84±1.29	15.82±0.41
Hematocrit (%)	42.14±1.31	23.68± 0.88$^{\#\#}$	39.00±1.07	61.71±4.23**$^{\#}$	22.45±3.50*$^{\#\#}$
VGM (fL)	52.75±1.00	74.25±2.57$^{\#}$	54.70±1.80*	68.72±1.01	67.20±4.55
CCMHb (g/dL)	35.91±0.28	91.60±0.5$^{\#\#}$	30.37±1.4**	24.06±2.31***	57.20±1.6*$^{\#}$
TCMHb (pg)	18.92± 0.47	19.20±0.8	16.60±0.11	16.52±0.68*	51.90±0.13**$^{\#\#\#}$
White blood cells (10^3/µL)	9.90±1.07	3.74±0.01$^{\#\#}$	4.58±0.41$^{\#\#}$	4.62±0.98$^{\#\#}$	6.37±0.53*$^{\#}$
Lymphocytes (%)	50.78±0.24	28.63±4.24$^{\#\#}$	43.16±6.81**	26.76±9.48$^{\#\#}$	36.90±3.38$^{\#}$
Monocytes (%)	12.75±0.21	6.66±0.72$^{\#\#}$	40.25±1.15***$^{\#\#\#}$	7.40±0.28$^{\#\#}$	12.33±0.86*
Granulocytes (%)	26.33±1.62	53.63±1.90$^{\#\#}$	14.30±2.03***$^{\#\#}$	66.73±5.19$^{\#\#\#}$	53.15±2.27$^{\#\#}$
Platelets (10^3/µL)	863.4±66.92	540.7±12.71$^{\#}$	286.0±67.82**$^{\#\#\#}$	944.7±82.01**	904.0±12.2**

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 Normal control; VGM: mean globular volume; TCMHb: mean corpuscular hemoglobin concentration; TCMHb: mean corpuscular hemoglobin level*

Table 6. Effects of ESMY on tissue oxidative stress parameters in rats with single chronic gastric ulcers

Treatment	Dose (mg/kg)	N	SOD (U/mg protein)	Catalase (μmol H_2O_2/min/mg protein)	GSH (mmol/g protein)	Malondialdehyde (pmol/mg protein)
Normal rats	_	5	2.68 ± 0.18	7.28 ± 0.14	2.84 ± 0.26	6.42 ± 0.71
Control 1	_	5	0.33 ± 0.10	10.50 ± 0.77	4.08 ± 0.40	16.22 ± 1.29
Control 2	_	5	2.44 ± 0.32**	9.45 ± 1.15	3.26 ± 0.43	6.35 ± 0.64***
ESMY	100+250	5	2.01 ± 0.41**	9.34 ± 0.81	4.64 ± 0.53	8.24 ± 0.47***
ESMY	200+250	5	3.15 ± 0.08***	10.80 ± 1.39	7.11 ±0.69*$^{\#\#}$	14.82 ± 0.94$^{\#\#}$
Sucralfate	50	5	1.63 ± 0.42*	7.69 ± 0.52	4.30 ± 0.58	7.56 ± 0.81***

*Control 1 (4 day ulcerated rats); Control 2 (spontaneous healing); N = number of rats; the values in the table represent means ± 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*

Table 7. Effects of ESMY on "unhealed" gastric ulcers

Treatment	Dose (mg/kg)	N	Ulcer index (IU)	% S.U	% G	Mucus production (mg)
Control 1	_	5	86.33 ± 3.28	12.78	_	77.50 ± 7.04
Control 2	_	5	40.41 ± 5.30***	5.98	53.19(AG)	51.45 ± 2.33**
Control 3	_	5	53.21 ± 6.11***	7.88	38.36 (AG)	37.33 ± 1.45***$^{\#\#}$
ESMY + Indomethacin	100 + 250		8.00 ± 2.30***$^{\#\#\#\varphi\varphi\varphi}$	1.18	90.73	60.75 ± 4.47 $^{\varphi\varphi\varphi}$
ESMY + Indomethacin	200 + 250	5	4.66 ± 0.66***$^{\#\#\#\varphi\varphi\varphi}$	0.69	94.60	92.25 ± 5.54$^{\#\#}$ $^{\varphi\varphi\varphi}$
Sucralfate	50	5	14.33 ± 1.20***$^{\#\#\varphi\varphi\varphi}$	2.12	83.40	87.50 ± 3.09$^{\#\#}$ $^{\varphi\varphi\varphi}$

*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; (s-h) = 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; $^{\varphi}$P = 0,05 ; $^{\varphi\varphi}$P = 0,01 et$^{\varphi\varphi\varphi}$P = 0,001: Statistically significant compared to Control 3*

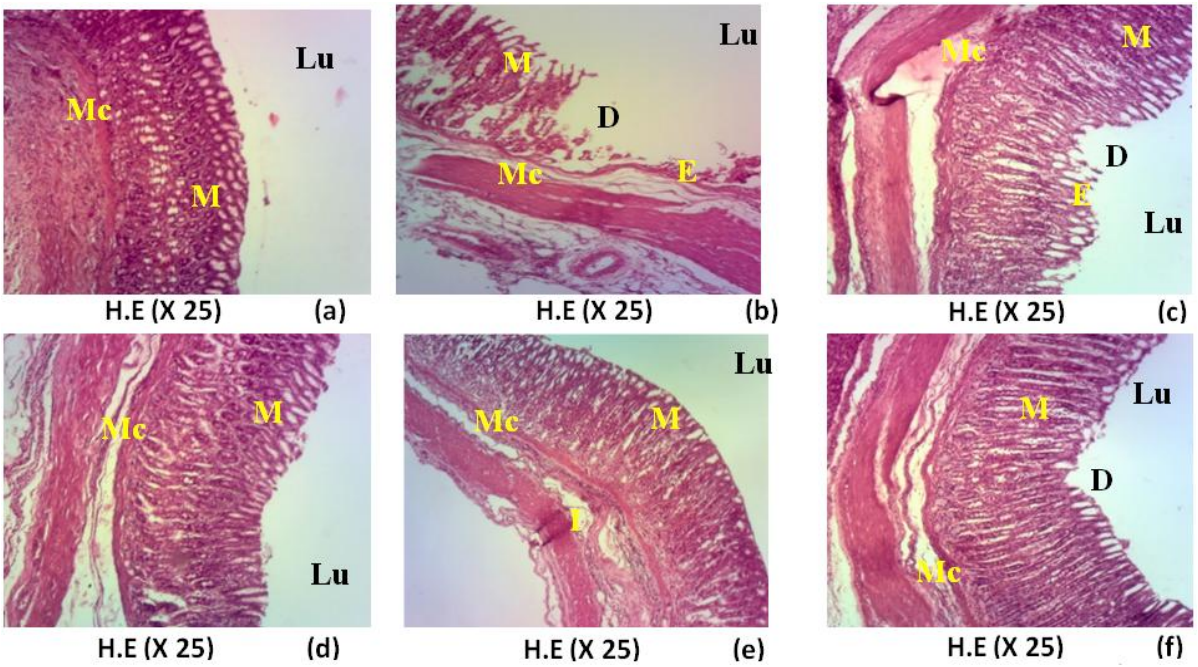

Fig. 2. Histological presentation of simple chronic ulcers in rats [8]

1(a) : normal rat (with normal mucosa and sub mucosa);1(b) : control 1 (with deep ulcers, with superficial loss of substance and glandular destruction down to the sub mucosa);1(c) : Longitudinal control (ulcerated area invaded by inflammatory cells, with onset of glandular recovery;1(d and e): Rats receiving 100 + 250 and 200 + 250mg/kg of ESMY (glandular proliferation with almost complete coverage of the ulcerated area);1(f) : Sucralfate-treated stomach with healing, and a slight persistence of the destroyed mucosa;D: destruction; E: edema; H.E: Hematoxylin-Eosin; I: Leukocyte infiltration; Lu: Gastric lumen; M: mucosa; Mc: Muscle layer

Table 8. Effects of ESMY on gastric pH and acidity in rats subjected to "unhealed" gastric ulcers

Treatment	ESMY dose (mg/kg)	N	Gastric pH	Gastric acidity (mEq/l)
Control 1	_	5	3.80 ± 0.11	57.50 ± 4.449
Control 2	_	5	3.64 ± 0.19	54.80 ± 6.71
Control 3	_	5	3.05 ± 0.11	60.53 ± 7.217
ESMY + Indomethacin	100+250	5	4.00 ± 0.17	46.11 ± 3.98*φ
ESMY + Indomethacin	200+250	5	5.41 ± 0.02**###φφ	36.38 ± 3.125**#φφ
Sucralfate + Indomethacin	50	5	4.37 ± 0.12φ	43.77 ± 5.21*φ

*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;φP = 0,05 ; φφP = 0,01 et φφφP = 0,001: Statistically significant compared to Control 3*

The effects of **ESMY** on some hematological parameters in rats are presented in Table 9 below. Hemoglobin levels (6.24 and 6.84) were significantly (*P* = 0.01) increased in animals treated with both **ESMY** 100+250 / indomethacin and **ESMY** 200+250 / indomethacin compared to the longitudinal control (3.48). Compared to the normal control (7.99), this hemoglobin level decreased significantly (*P* = 0.01) in the longitudinal (3.48) and positive (3.74) controls. White blood cell (lymphocyte and monocyte) levels also increased significantly (*P* = 0.05) in animals treated with the + indomethacin 200 + 250 mg/kg mixture (58.53) compared to longitudinal controls (36.63 and 20.66, respectively). Granulocytes were significantly decreased in the positive control and in the animals treated with the mixture compared to the longitudinal control (52.20). Only the **ESMY** at 100+250 mixture showed a significant (*P* = 0.05)

increase in platelet levels compared to the longitudinal control (510.7). The longitudinal control (510.7), positive control (673.3) and those treated with the mixture (551.33) had significantly lower platelet levels than the normal control (863.40).

The results presented in Table 10 show that SOD increases and MDA decreased significantly (P = 0.01 and P = 0.001, respectively) when animals were given **ESMY** 100+250 and **ESMY** 100+250 mg/kg compared to the cross-sectional control. The GSH level, on the other hand, increases significantly (P = 0.05) after administration of the mixture at the two respective doses, compared to the simple longitudinal control and longitudinal control + indomethacin.

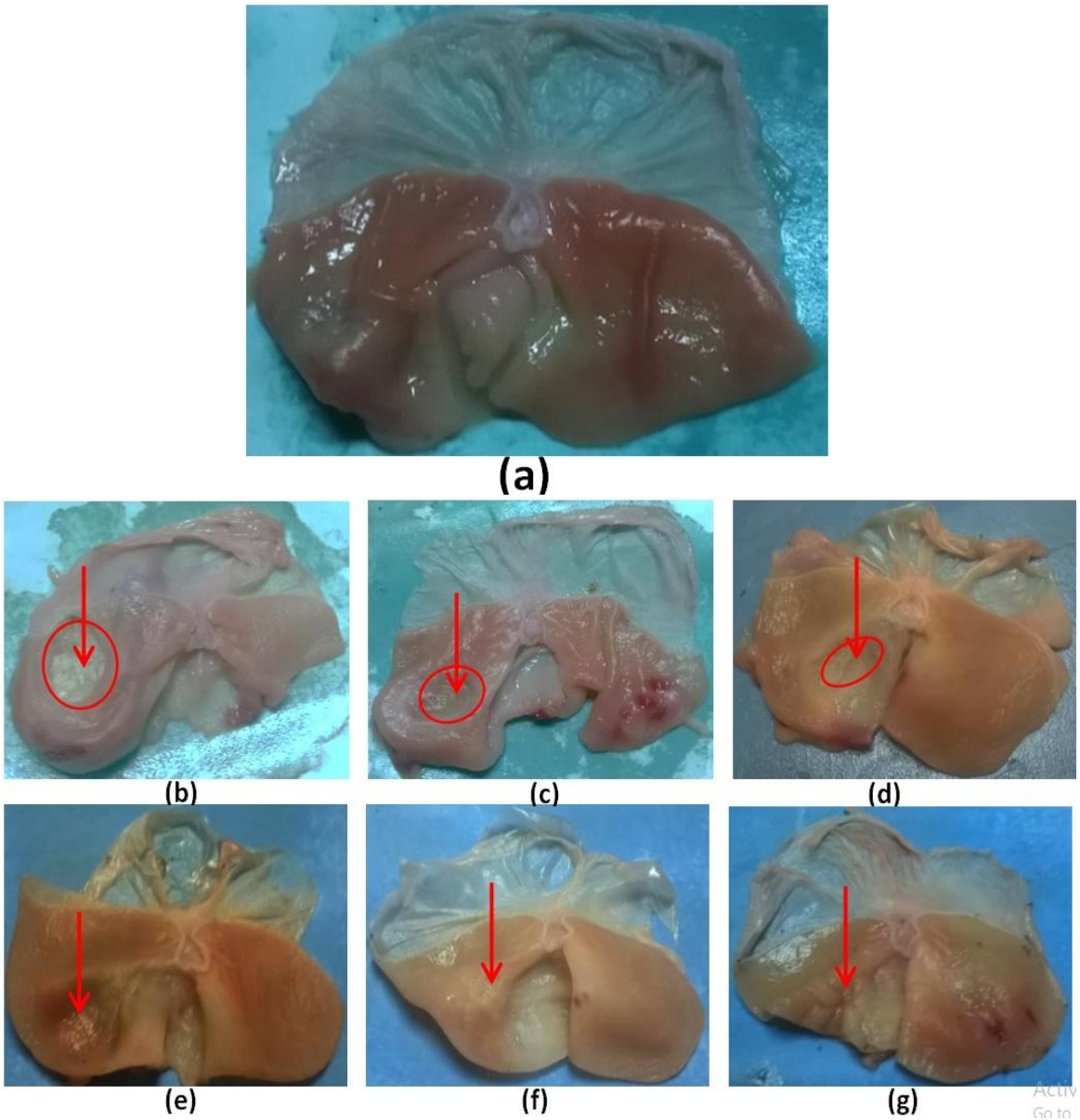

Fig. 3. Stomach of rats with "unhealed" gastric ulcers [8]

1(a):Nonulcerated normal rats given distilled water for 14 days; 1(b): Ulcerated rats sacrificed 4 days after induction of chronic gastric ulcers to confirm ulcer formation; (c): ulcerated rats given distilled water daily (1 ml/200 g) for 14 days from the 5th day after induction of chronic gastric ulcers 1(d): Ulcerated rats that received daily distilled water (1ml/200 g) + indomethacin (1mg/kg) for 14 days from the 5th day after induction of chronic gastric ulcers;(e and f): ulcerated rats that received 100 + 250 and 200 + 250 mg/kg of ESMY + indomethacin (1mg/kg) for 14 days from the 5th day after induction of ulcers;(g) : rats that received Sucralfate daily + indomethacin (1mg/kg) for 14 days from the 5th day after induction of ulcers; Indication of gastric ulcers

Table 9. Effects of ESMY on some hematological parameters in rats with chronic acetic acid-induced gastric ulcers

	Normal	Control 3	ESMY + Indocid	ESMY + Indocid	Sucralfate + Indocid
ESMY *dose (mg/kg)*	-	**-**	***100+250***	***200+250***	50
Red blood cells (10^6/µL)	7.99±0.34	3.48±0.50$^{\#\#}$	6.24±0.407**	6.84±0.36**	3.74±0.32$^{\#\#}$
Hemoglobin (g/dL)	15.17±0.45	13.75±0.80	12.03±0.34	11.54±0.05$^{\#}$	12.63±0.49
Hematocrit (%)	42.14±1.31	34.75± 0.88$^{\#}$	36.27±2.59	37.06±2.4	20.78±1.4*$^{\#\#}$
VGM (fL)	52.75±1.00	53.53±2.57	57.38±4.14	54.56±2.57	52.23±4.24
CCMHb (g/dL)	35.91±0.28	69.53±0.5$^{\#\#}$	31.26±3.33**	31.12±2.18**	44.85±1.14*
TCMHb (pg)	18.92± 0.47	21.41±0.8	17.96±1.14***	17.40±1.56	62.71±1.86***$^{\#\#\#}$
White blood cells (10^3/µL)	9.90±1.07	5.74±0.01$^{\#}$	4.27±0.08$^{\#\#}$	4.12±0.98**$^{\#\#}$	6.04±0.34
Lymphocytes (%)	50.78±0.24	36.63±4.24$^{\#}$	51.86±3.25*	58.53±4.22*	36.83±2.49$^{\#}$
Monocytes (%)	12.75±0.21	20.66±0.72$^{\#\#}$	17.50±1.70	30.50±4.82*$^{\#\#}$	11.80±0.96*
Granulocytes (%)	26.33±1.62	52.20±1.90$^{\#\#}$	32.10±5.01*	33.55±2.31*	40.20±7.21*$^{\#\#}$
Platelets (10^3/µL)	863.40±66.92	510.7±12.71$^{\#\#}$	762.3±54.57*	551.33±72.00$^{\#\#}$	673.3±40.92$^{\#\#}$

Control 3: (spontaneous healing in ulcerated rats given indomethacin); N = number of rats (5); 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 Normal control; VGM: mean globular volume; TCMHb: mean corpuscular hemoglobin concentration; TCMHb: mean corpuscular hemoglobin level; Indocid: indomethacin*

Table 10. Effects of ESMY on tissue oxidative stress parameters in rats with "unhealed" gastric ulcers

Treatment	ESMY dose *(mg/kg)*	N	SOD *(U/mg protein)*	Catalase *(□mol H_2O_2/min/mg protein)*	GSH *(mmol/g protein)*	Malondialdehyde *(pmol/mg protein)*
Normal Rats	_	5	2.68 ± 0.18	7.28 ± 0.14	2.84 ± 0.26	6.42 ± 0.71
Control 1	_	5	0.33 ± 0.10	9.50 ± 1.46	4.08 ±0.40	16.22 ± 1.29
Control 2	_		2.44 ± 0.32**	9.45 ± 1.15(s-h)	3.26 ± 0.43	6.35 ± 0.64***
Control 3	_	5	2.06 ± 0.76**	7.72 ± 1.00(s-h)	3.15 ± 0.13	9.75 ± 0.59**#
ESMY + Indomethacin	100 + 250	5	3.02 ± 0.24***	9.11 ± 1.04	5.30 ± 0.29#φ	7.91 ± 0.61***
ESMY + Indomethacin	200 + 250	5	2.44 ± 0.27**	8.31 ± 0.99	5.14 ± 0.29#φ	10.56 ± 0.27**#
Sucralfate+ Indocid	50	5	2.90 ± 0.09**	10.43 ± 1.34	4.93 ± 0.41	7.09 ± 0.86***

*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 ; (s-h) = 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 ; φP = 0,05 ; φφP = 0,01 et φφφP = 0,001: Statistically significant compared to Control 3 ; Indocid : indomethacin*

Histological sections of the stomachs of rats with "unhealed" gastric ulcers are shown in Fig. 4. Those in the stomachs of the normal and transverse control rats (Fig. 4(a) and (b)) are the same as those described above. In the longitudinal controls, the sections show signs of self-healing, and there is evidence of the beginning of mucosal regeneration (Fig. 4 (c) and (d)). The **ESMY** 100+250 and **ESMY** 200+250 resulted in progressive restoration of the muscularis with a decrease in the inflammatory zone compared to the transverse and longitudinal controls (Fig. 4 (e) and 4 (f), respectively). Histology of rats treated with Sucralfate shows normalization of the mucosa, but inflammation can still be seen by the presence of areas of lymphocyte infiltration (Fig. 4 (g)).

4. DISCUSSION

The aqueous extract of *E. specisa* has already been the subject of several scientific works bringing to light several anti ulcer properties including anti-secretory effects [9] and bactericidal effects on *Helicobacter pylori* [10]. MY41g clay used alone at 250 mg/kg was shown to have antacid effects *in vivo* on chronic and "difficult to heal" gastric ulcers models in rats [8]. On the basis of these results, we aimed to compose an ulcer healing mixture made of the two locally available materials which in combination provide all the requred pharmacological potencies of the standard triple therapy regimen.

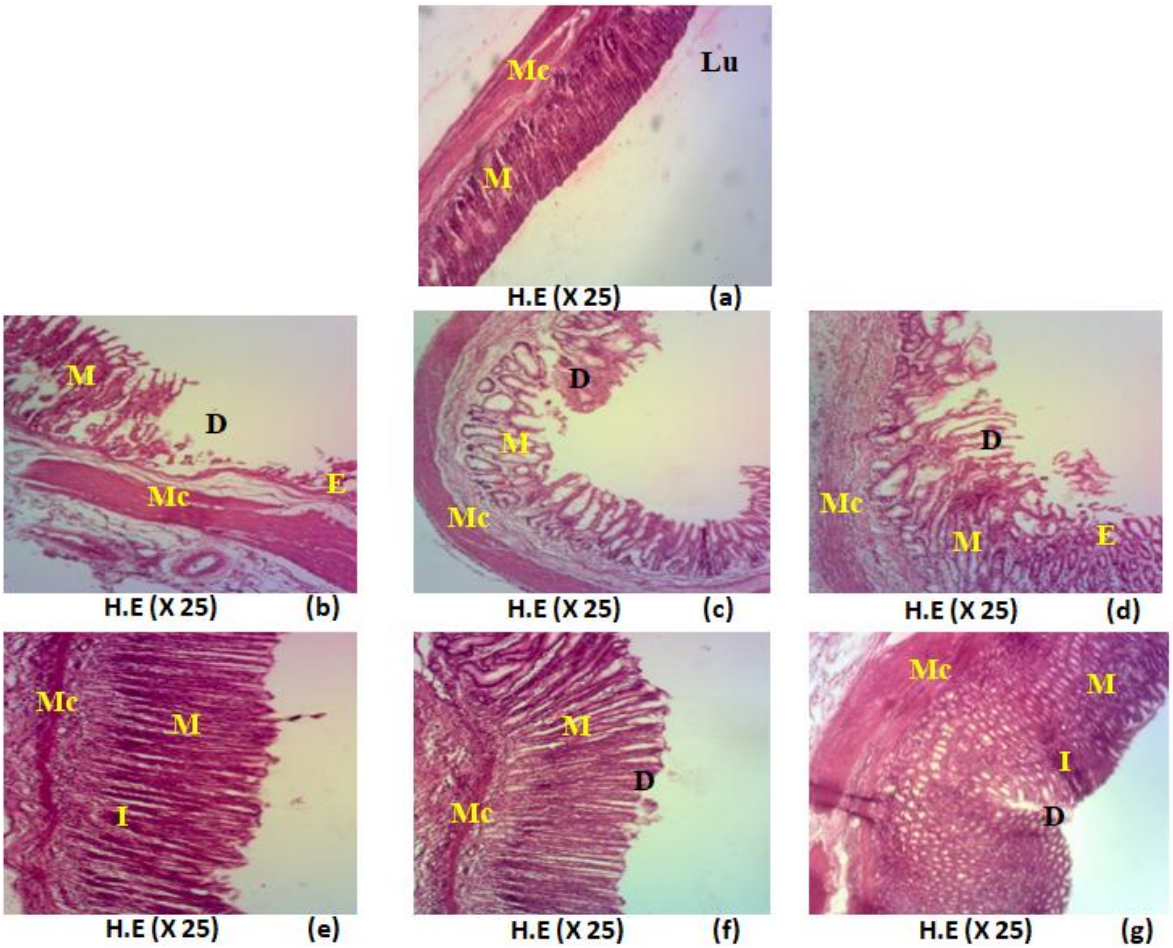

Fig. 4. Effects of ESMY on gastric tissue of rats with "unhealed" gastric ulcers

*4(a): normal rat (shows normal muscle and mucosa); 4(b) : control 1 (shows destruction of the mucosa with lymphocyte infiltration); 4(c and d) : controls 2 and 3 (show signs of self-healing, with an onset of mucosal regeneration but with more extensive mucosal destruction in the control 2); 4(e and f) : **ESMY** 100+250 mg/kg and **ESMY** 200+250 mg/kg; (almost complete restoration of the muscosa with disappearance of the destruction zone); (g) : 50 mg/kg Sucralfate (progressive restoration of the mucosa); D: destruction; E: edema; H.E: HematoxylinEosin; I: Leukocyte infiltration; Lu: Gastric lumen; M: mucosa; Mc: Muscle layer*

Antacids work by neutralizing stomach acid and help heal ulcers, but they do not decrease the volume of gastric secretions [26]. Addition of artificial gastric juice to the various solutions *in vitro* (25 – 37 ^{o}C) provoked immediate significant drop in pH that lasted during incubation for 8 hours due to the presence of HCL, a strong acid which totally dissociates into protons and chloride ions in water. pH remained within the optimum range for pepsin activity (1.2 – 2.57), even with the standard neutralizing agent ($NaHCO_3$). Thus, simple mixing of antacid with artificial gastric juice *in vitro* did not reveal the neutralizing capacity. However, neutralizing capacity was evident when measured by the titration method of Fordtran's model which permits the measurement of the volume of gastric juice as well as the concentration of H^+ ions consumed by the antacid. Thus compared with distilled water (1.5 ml), the volumes of artificial juice required to bring down the pH from the basic zone (pH 6.66 – 8.97) to end point pH 3 increased from 4.67 ml (MY41g alone) to 44-58 ml (extract/clay mixture) and 72 ml for $NaHCO_3$. This correlated with the calculated amounts of H^+ (0.29 – 4.57 mmol) consumed by the solutions, representing their intrinsic chemical neutralization capacity. The observed antacid capacity of the extract/clay mixture results from the presence of elements such as bicarbonate in the limestone, bioflavonoids present in the extract of *E. speciosa* and chemical elements such as Al_2O_3 (amphoteric oxide), MgO (basic oxide) present in the MY41g clay. Indeed, Al_2O_3 and MgO are two molecules capable of reacting with H^+ protons and increasing the pH of the medium [27,28]. This would explain the additive effect observed with the extract/clay mixture.

The *in vivo* test showed that the extract/clay mixture provoked a significant dose-dependent reduction in gastric acidity. This was due to the previously demonstrated antisecretory activity of the extract component [9], in addition to the presence of bicarbonate (HCO_3^-) contained in the limestone component, which combined with Na^+ ions to form alkaline sodium bicarbonate ($NaHC0_3$) [29]. Antacids are usually inorganic salts that dissolve in acidic gastric secretions and release anions that partially neutralize gastric hydrochloric acid. They usually react chemically to buffer or neutralize existing amounts of acid in the stomach, resulting in an increase in the pH value of the stomach contents and thus relieving the symptoms of gastric hyperacidity [30].

Acetic acid injected into the stomach wall of rats produced deep ulcer craters (86.33 mm^2) with close pathologic and therapeutic resemblance to human gastric ulcers [31]. The development of these ulcers is due not only to the diffusion of acetic acid from the injected area to the gastric mucosa, but also to the stress caused by the laparotomy performed during ulcer induction. Stress stimulates the vagus nerve and leads to the secretion of acetylcholine by the post-ganglion neurons of the parasympathetic system, which plays a major role in stimulating the gastrin and histamine and consequently hydrochloric acid and pepsinogen [32].

The degree of self-healing observed in untreated rats is the result of the physiological mechanisms available to the body to defend itself against gastric mucosal inflammation. These include antioxidant defenses that control or limit the deleterious effects of highly reactive activated oxygen species EOA (free radicals*, superoxide anion $O2^-$, hydrogen peroxide H_2O_2, singlet oxygen O^{-*}, hypochlorous acid) [33]. Endogenous antioxidants involved are naturally occurring molecules that can prevent the uncontrolled formation of free radicals and activated oxygen species (AOS) or inhibit their reactions with biological structures [34]. Glutathione and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) protect the gastric mucosa against inflammation by inhibiting the production of reactive oxygen metabolites through the detoxification of the superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2) [35]. In addition, or protein chaperones (HSPs) are involved in restoring protein homeostasis within the cell after stress. During the later, chaperones are massively synthesized. Over expression of HSP70 has an anti-inflammatory effect and a protective effect against gastric damage by inhibiting activation of the NF-KB pathway and COX-2 expression [36]. In addition, there are the sulfhydryl components (NP-SH) which have a gastroprotective effect, their SH group forms a kind of bridge with the mucus and makes it thicker so that the decrease in their concentration makes the mucus more soluble. This type of molecule plays an important role in maintaining the integrity of the gastric mucosa by binding to free radicals generated by ulcerogenic agents, thus functioning as antioxidants, controlling the production and nature of mucus [37,38]. In the present study, the extract/clay mixture improved antioxidant parameters, with a significant increase in GSH and SOD, possibly due to the intervention of polyphenols, tannins and anthocyanins which can reduce the stress that

regulates acid secretion and blood flow [39]. Polyphenols and anthocyanins have been shown to increase the level of GSH and the activity of antioxidant enzymes such as SOD, CAT and GPX at the gastric level [40].

Spontaneous healing of simple chronic and "unhealed" gastric ulcers by **ESMY** was accompanied by significant increase in mucus production as previously reported with *E. speciosa* extract [9]. This effect could be boosted by the Aluminum silicates present in the clay component which that inhibit the corrosive action of pepsin by increasing the thickness of the mucus [41], thus establishing a pH gradient ranging from less than 3 on the luminal side of the mucus layer to more than 7 on the mucous side [42]. The insoluble mucus gel prevents the diffusion of H^+ ions and proteolytic enzymes from the lumen into the gastric mucosa. The protected superficial epithelial cells maintain the integrity of the entire mucosal lining by continuously renewing themselves every 3 to 4 days, while prostaglandins and bicarbonates play an adaptive cytoprotective role in response to aggressive agents by stimulating the secretion of gastric mucus and maintaining mucosal blood flow [43]. In this study, the reduction in ulcerated surface and substantial repair of the glandular epithelium in **ESMY**-treated rats could also be attributed to the presence copper (105 ppm) and phenols, which, respectively, have immune stimulating and anti-inflammatory effects [44], and inhibit the influx of Ca^{2+} with inhibition of nuclease activity. The binding of clay crystals to the mucus improves the rheological characteristics of the mucus (viscosity, hydrophobicity, polymerization of glycoproteins, adhesion to the wall of the digestive tract) and decreases its degradation, while the triterpenoids present in the extract reinforce defense factors through the stimulation of mucus synthesis via the secretion of endogenous prostaglandins [45].

Non-steroidal anti-inflammatory drugs are known to have a negative impact on ulcer healing [46]. In this study, repeated administration of indomethacin for 14 days significantly delayed the spontaneous healing of chronic gastric ulcers. Indomethacin is a highly aggressive NSAID which induces and perpetuates gastric ulceration by inhibiting cyclooxygenase (COX-2), thus preventing the biosynthesis of prostaglandins and consequently the secretion of mucus [47]. The resulting inflammation aggravates the ulceration process by activating phagocytes and leukotrienes that produce free radicals through the action of 5-lipooxygenase on arachidonic acid [48]. The lipid peroxidation produced by these free radicals plays an important role in the aggravation of indomethacin-induced ulcers.

Analysis of blood parameters can provide a high index of the risk of toxicity in humans [49], and the significant increase in red blood cell, lymphocyte and platelet counts in ESMY-treated rats bear testimony to the progressive recovery of the gastric mucosa in these animals, with activation of the immune system and establishment of healing elements for a complete recovery of the gastric mucosa. Earlier studies showed that *E. speciosa* leaves extract did not show signs of hematological toxicity following acute and subacute administration [50], and the consumption of clay for more than a month by anaemic patients has been shown to improve their haemoglobin levels [51].

5. CONCLUSION

The *in vitro* antacid properties of the Cameroonian MY41g clay and its potential use in an anti-ulcer tritherapy formula with *Eremomastax speciosa* leaves extract were tested. The MY41g clay had neutralizing capacity on the artificial gastric juice due mainly to the presence of MgO and Al_2O. The clay/extract mixture had stronger antacid power, and accelerated the spontaneous healing of simple chronic and ‘‘non healing’’ gastric ulcers, and prevented retardation of healing by indomethacin through mechanisms that include increased mucus production, improved re-epithelialisation, and antioxidant status. Results show that the clay/extract mixture can be used to formulate a more simplified antiulcer regimen using only two components that satisfy the requirements of the currently recommended triple therapy regimen, namely, antacid, antisecretory and antibiotic capacity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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.

DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Salducci J, Desjeux A, Chamlian A. Gastric and duodenal ulcer. Hepato-gastroenterol. 2005;290:1-13.
2. Tulassayand Z, Herszényi L. Gastric mucosal defence and cytoprotection. Best Pract Res Clin Gastroenterol. 2010;24(2): 99-108.
3. Brown LF, Wilson DE. Gastroduodenal ulcers: Causes, diagnosis, prevention and treatment. Compr Ther. 1999;25(1):30-38.
4. Wallace JL. Recent advances in gastric ulcer therapeutics. Curr Op Pharmacol. 2005;5:573-577.
5. Delaney B, Moayyedi P, Forman D. *Helicobacter pylori* infection. Clin Evid. 2002;8:453-468.
6. Feldman M, Burton ME. Histamine 2-receptor antagonists. Standard therapy for acid-peptic diseases. N Engl J Med. 1990;323(24):1672-80.
7. Banenzoue C, Signing P, Mbey JA, Njopwouo D. Antacid power and their enhancements in some edible clays consumed by geophagia in Cameroon. J Chem Pharm Res. (2014); 6(10):668-676.
8. Emakoua JF, Amang AP, Banenzoue C, Mezui C, Siwe GT, Tan PV, Enow-Orock GE. *In-vivo* curative and antacid effects of cameroonian clay (MY41g) on chronic and “unhealed" gastric ulcers in rats. J Pharm Med Res. 2020;5(1):93–99.
9. Amang AP, Mezui C, Siwe GT, Emakoua J, Mbah G, Nkwengoua EZ. et al. Healing and antisecretory effects of aqueous extract of *Eremomastax speciosa* (Acanthaceae) on unhealed gastric ulcers. BioMed Res Int. 2017;10:1-11.
10. Siwe GT, Maharjan R, Amang AP, Mezui C, Zondegoumba EN, Akhtar SS, Choudhary MI, Tan PV., *Eremomastax speciosa* (Hochst.) Cufod. (Acanthaceae) leaves aqueous extract eradicates Helicobacter pylori infection in mice. J Pharm Pharmacogn Res. (2020);8(2):135-145.
11. Siwe GT, Enow-Orock GE, Amang AP, Mezui C, Dongmo AB, Tan PV. Acute and Subacute Toxicological Assessment of the Leaf Aqueous Extract of *Eremomastax speciosa* (Acanthaceae) in Wistar Rats. J Adv Méd Pharm Sci. 2015;4(1):1-13.
12. Njoya A, Nkoumbou C, Grosbois C, Njopwouo D, Njoya D, Courtin-Nomade A, Yvon J and Martin F. Genesis of Mayouom kaolin deposit (Western Cameroon). Appl Clay Sci. 2006;32:125-140.
13. Council of Europe. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. European Treaty Series n° 123. Strasbourg 18 III; 1986.
14. Wu T-H, Chen I-C, Chen L-C. Antacid effects of Chinese herbal prescriptions assessed by a modified artificial stomach model. World J Gastroenterol. 2010; 16(35):4455-4459.
15. Pillai NR, Santhakumari G. Effects of nimbidin on acute and chronic gastroduodenal ulcer models in experimental animals. Planta Med. 1984; 50(2):143-46.
16. Hara N, Okabe S. Effect of gefarnate on acute lesions in rats. Folia Pharmacol Japonica. 1985;85:443-448.
17. Tan PV, Nyasse B, Enow-Orock GE, Wafo P, Forcha EA. Prophylactic and healing properties of a new anti-ulcer compound from *Enantia chlorantha* in rats. Phytomed. 2000;7(4):291-296.
18. Wang JY, Yamasaki S, Takeuchi K, Okabe S. Delayed healing of acetic-induced gastric ulcers in rats by indomethacin. Gastroenterol. 1989;96:393-402.
19. Tan PV, Nditafon NG, Yewah MP, Dimo T, Ayafor FJ. *Eremomastax speciosa*: Effects of leaf aqueous extract on ulcer formation and gastric secretion in rats. J Ethnopharmacol. 1996;54(2-3):139-142.
20. Bayelet-Vincent F. Cytology and pathology records technical. Bayer Diagnostics: London; 2002.
21. Henry RJ, Canon DC, Winkelman JW. Clinical chemistry, principles and

Techniques. 2nd Ed. Harper and Row Hagerstown: Maryland; 1974.
22. Ellman GL. Tissue sulfhydryl groups, Arch. Biochem. Biophys. 1959;82(1):70-77.
23. Marhuenda E, Martin MJ, de La Lastra CA. Antiulcerogenic activity of aescine in diff erent experimental models. Phytother Res. 1993;7(1):13-16.
24. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-394.
25. Wilbur KM, Bernheim F, Shapiro OW. Determination of lipid peroxidation. Arch Biochem Biophys. 1949;24:305-310.
26. Maton PN, Burton ME. Antacids revisited: A review of their clinical pharmacology and recommended therapeutic use. Drugs. 1999;57:855–870.
27. Bonnard N, Brondeau M.-T, Jargot D, Nikolova-Pavageau N, Schneider O. Hydrogen chloride, In: Kirk-Othmer-Encyclopedia of chemical technology. 5th edition. Hoboken Wiley-Intersci: Paris, 2005;808-837.
28. Sandhya S, Venkata RK, Vinod KR, Chaitanya R. Assessment of *in vitro* Antacid Activity of Different Root Extracts of *Tephrosia purpurea* (L) Pers by Modified Artificial Stomach Model. As Pac J Trop Biomed. 2012;1487-1492.
29. Brian M, Hand, David F. Treagust Application of a conceptual conflict teaching strategy to enhance student learning of acids and bases. Res Sci Educ. 1988;18:53-63.
30. Oumarou T, Anselme W. Mining in Figuil District (Cameroon), in: Public Health Problems and Environmental Effects. Open edition. Belgeo J: Belgique; 2014.
31. Okabe S, Amagase K. An review of acetic acid ulcer models--the history and state of the art of peptic ulcer research. Biol Pharmacol Bull. 2005;28(8):1321-1341.
32. Lacour B, Belon JP. Physiologie. Edition Elsevier. Masson: France; 2015.
33. Pincemail J, Bonjean K, Cayeux K, Defraigne JO. Physiological mechanisms of antioxidant defense Physiological action of antioxidant defences. Clin Nutr Metab. 2002;16:233-23.
34. Chaudière J, Ferrari-Iliou R, Intracellular antioxidants, Chemical to biochemical mechanisms. F Chem Toxicol. 1999;3:949-962.
35. Derin N, Izgut-uysal VN, Agac A, Aliciguzel Y, Demir N. l-carnitine protects gastric mucosa by decreasing ischemia-reperfusion induced lipid peroxidation. J Physiol Pharmacol. 2004;55(3):595-606.
36. Asai M, Kawashima D, Katagiri K, Takeuchi R, Tohnai, Ohtsuka K. Protective effect of a molecular chaperone inducer, paeoniflorin, on the HCl and Ethanol triggered gastric mucosal injury. Life Sci. 2011;88:350-357.
37. Rozza AL, Hiruma-lima CA, Tanimoto C, Pellizon CH. Morphologic and Pharmacological Investigation in the Epicatechin Gastroprotective Effect. Chem-Biol Interact. 2012;10:70-78.
38. Boligon AA, Freitas RB, Brum TF, Waczuk EP, Klimaczewski CV, de Ávila DS, et al. Antiulcerogenic activity of Scutiabuxifolia on gastric ulcers induced by ethanol in rats. Acta Pharma Sin. 2014;4:358-367.
39. Atmani D, Begoña R-LM, Ruiz-Sanz JI, Lizcano L and Bakkali F. Antioxidant potential, cytotoxic activity and phenolic content of Clematis flammula leaf extracts. J Med Plants Res. 2011;4:589-598.
40. Alvarez-Suarez JM, Dekanski D, Ristic N, Giampieri F, Astolfi P, Battino M. Strawberry polyphenols attenuate ethanol-Induced gastric lesions in rats by activation of antioxidant enzymes and attenuation of MDA increase. Public Library Sci One. 2011;6(10):25-31.
41. Leonard A, Droy-Lefaix MT, Allen A. Pepsin hydrolysis of the adherent mucus barrier and subsequent gastric mucosal damage in the rat: Effect of diosmectite and 16 dimethyl prostaglandin E2. Gastroenterol Clin Biol. 1994;18:609-616.
42. Kamguia H, Guifo F, Fokunang C, Ngameni B, Njinkio-Nono B, Tembe-Fokunang E. Cytoprotective effect of the aqueous extract of dorsteniapsilurus roots on gastric ulcer in male rats of the wistar strain. Health Sci Dis. 2011;12(4):1-11.
43. Dine T, Claerbout JF, Rave M. Treatment of peptic ulcer disease. In : François G. Clinical and therapeutic pharmacy. 3nd edition Elsevier. Masson: Paris; 2008.
44. Pereira TC, Campos MM, Bogo MR. Copper toxicology, Oxidative stress and inflammation using zebrafish as experimental model. J Appl Toxicol. 2016; 36(7):876-85.
45. Gwozdinski K, Jedrzejewska A, Janocka M, Droy-Lefaix MT. Effect of diosmectite on the physico-chemical properties of gastric mucus *in vivo* and *in vitro*. Gastroenterol. 1997;12:1-4.

46. Shawon L, Gautam P. An overview of the current methodologies used for evaluation of gastric and duodenal anti-ulcer agents. Pharmacol. 2012;3(8):249-257.
47. Arun R, Susri R, Chaudhuri BM, Sandip K. Antioxidante activity of ethanol extract ofrhizome of picrorhizakurroa on indomethacin induced gastric ulcer during healing. Indian J Clin Biochem. 2002;2:44-51.
48. Katzung BG. Pharmacologie fondamentale Clinique. 9éme edition. San Francisco: USA; 2004.
49. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G. et al. Concordance of toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol. 2000;32:56-67.
50. Siwe GT, Enow-Orock GE, Amang AP, Mezui C, Dongmo AB and Tan PV. Acute and subacute toxicological assessment of the leaf aqueous extract of *Eremomastax speciosa* (Acanthaceae) in Wistar rats. J Adv Méd Pharm Sci. 2015;4(1):1-13.
51. Hercberg S, Preziosi P, Galan P. Iron deficiency in Europe. Public Health Nutr. 2001;4(2B):537-45.

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