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IN VITRO ANTACID EFFECTS OF KHAYA GRANDIFOLIOLA (MELIACEAE) AND CURATIVE EFFECTS FORMULATED WITH CLAY (MY41G) ON CHRONIC GASTRIC ULCERS IN RATS

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ABSTRACT

Aim: The current study investigated the *in vitro* antiacide and in vivo curative actions of aqueous extract of *Khaya grandifoliola* + MY41g^c clay in rats. **Materials and Methods:** This study evaluated possible antacid action with the neutralizing effect (*in vitro*) of the extract against pylorus ligation; and curative effects of *Khaya grandifoliola* combined with MY41g^c clay on chronic gastric ulcers in rats. Chronic gastric ulcers were induced by injecting 0.05 mL of acetic acid (30 %) into the stomach wall. From day 5-10 after induction of ulcers, rats were treated daily with the mixture of *K. grandifoliola* + MY41g^c clay (250 + 250 and 250 + 500) mg/kg. The ulcer index, percentage of healing, mucus secretion, histological parameters, and oxidative stress parameters were assessed. *In vitro*, *K. grandifoliola* had antacid and neutralizing effects practically similar of sodium bicarbonate. Curative treatment with *K. grandifoliola* + MY41g^c clay solution for 10 days resulted in accelerated spontaneous healing of chronic gastric ulcers in both formulation (66.26-100 %) and promoted significantly higher levels of catalase and highly decreased levels of MDA in rats. **Conclusion**: *K. grandifoliola* possess neutralizing effects and his formulation with MY41g^c clay has curative actions in rats.

KEYWORDS: Neutralizing effect, chronic gastric ulcers, MY41g^c clay.

INTRODUCTION

Peptic ulcer represents a major health problem. They are one of the most prevalent gastrointestinal disorders, commonly occurring in developed countries. 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. Medicinal plants contain numerous biologically active compounds such as nutrients and phytochemicals which have physiological actions on the human body.[2] The inherent active ingredients are used to cure disease or relieve pain.[3] *Khaya grandifoliola* (Meliaceae) is also called African Mahogany and it is found in many countries. It is used in the form of concoction for the treatment of convulsion, cough, stomach ache, fever, threatened abortion, rheumatism, dermatomycosis and malaria fever in Nigeria.[4,5] The stem bark of this plant has been scientifically evaluated for some activities : antimalaria activity,[6,7] anti-ulcer ant antisecretory property,[8,9,10,11] anti-microbial,[12] anti-inflammatory,[13] anti-anaemic,[14] hypoglycaemic, hypoproteinaemic and hypocholesterolaemic.[15] *Khaya grandifoliola* extract was also reported as possessing the antioxidant activity and hepatoprotective effect.[16] The phytochemical tests showed the presence of limonoids, saponins, tannins, alkaloids, anthraquinones, flavonoids, reducing sugars; phlobatannins, carbohydrates, proteins, magnesium, calcium, sodium, potassium, magnesium, iron and manganese in this plant with lowest toxicity effects.[17, 18] Antacids heal ulcers through elimination of gastric acid by neutralization; however, they do not decrease the volume of gastric secretions.[19] Different antacid drugs vary markedly in their in vivo and *in vitro* potency, and that should be taken into account when antacids are prescribed. 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 for example in the symptomatic treatment of irritable colon syndrome; painful and the treatment of acute and chronic diarrhea (Bedelix, Gelox, Smecta).[20] Cameroonian clays are consumed by geophagia; as antibiotics for wounds, as detoxifyer, as antidiarrhetics, as antiemetics in pregnant women and as antacids against

gastric ulcers.[21] The MY41g^c clay showed had maximal antacid capacity, the control of gastric acidity promoting ulcer healing, accelerated the spontaneous healing of chronic acetic acid-induced gastric ulcers and prevented the delay in the healing of chronic gastric ulcers.[22, 23] In the current study was conducted to evaluate the gastric acid neutralizing capacity of the only *in vitro* and the effet on the healing activity of aqueous extract of stem-bark of *K. grandifoliola* + MY41g^c clay on chronic gastric ulcers induced by glacial acetic acid in rats.

MATERIAL AND METHODS

Experimental Animals

Animals Male Wistar rats (150–220 g) were raised using a standard laboratory diet and tap water in the animal house of the Faculty of Science, University of Yaoundé.[1] Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Reg. No. FWA-IRB00001954).

Mineral Material

The MY41g^c 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.[24] 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.

Vegetal Material

The plant material, fresh stem-bark of *Khaya grandifoliola*, was collected in Mbokam village (Jakiri) in the North-West Region of Cameroon (6° 06' North and 10°39' East). Botanical identification was done at the National Herbarium in Yaoundé by comparison with existing herbarium specimen No. PM 098 /95. The fresh stem-bark of *K. grandifoliola* was cut up, dried and ground to a powder. 1 kg of the dried material was boiled in 5 liters of water for 30 minutes. The extract solution was filtered through Whatman filter paper No. 3. The resulting filtrate was evaporated at 40°C using a ventilated oven (Jencons-PLS, UK) to obtain 66.35 g of a red powder.

Preparation of Clay And Extract Solution

The extract of *Khaya grandifoliola* re-dissolved readily in distilled water which was used as the vehicle with a concentration of 100 mg/mL. 2.4 g of clay powder was mixed with 0.1 g of limestone and 2.5 mL of distilled water. The two solution was homogenized using a magnetic stirrer to obtain a stock solution with a concentration of 100 mg/mL.

Evaluating the antacid and neutralizing effects (*in vitro*) of the extract

The neutralizing effect of the aqueous extract of stem-bark of *K. grandifoliola* on gastric juice was evaluated. Fifteen female rats (180-200g) fasted for 24 hours were allocated. The laparotomia was performed under light ether anesthesia and the pylorus of each rat was tied followed by the closure of the abdominal incisions. The stomachs were removed 6 hours later and the stomach contents collected, centrifuged at 2000 rpm for 10 minutes and divided into four batches of beakers. The method of Shay et al.[25, 26] Ninety milliliters of NaCl solution (0.9 %), 90 mL of sodium bicarbonate, 25 mL of gastric juice and 90 mL of extract were placed in beakers. The pH was measured at 25 ° C immediately after centrifugation. The temperature was raised to 37 ° C using a sea bath and the pH was measured immediately after centrifugation and then 4 hours later. Likewise, 90 mL of NaCl solutions (0.9 %), 90 mL of sodium bicarbonate and 90 mL of extract were again placed in beakers. The contents of each beaker were incubated in 25 mL of gastric juice, and then centrifuged at 2000 rpm for 1 minute. The pH measurement at 25 ° C. and 37 ° C was carried out.

Induction of Simple Chronic Acetic Acid Ulcers

The induction of chronic gastric ulcers was performed according to the method described by Pillai and Santhakumari.[27] 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.[28] These rats were sacrificed 6 hours later under anesthesia, and the rat 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 mixture of aqueous extract of stem-bark of *K. grandifoliola* + MY41g^c clay at (250+250) and (250+500) 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.

Measurement of Mucus Production and Gastric Acidity

The mucus on the glandular part of the stomach of each rat was gently scraped off using a microscope slide and weighed using a sensitive electronic balance.[29]

Measurement of *In Vivo* Antioxidant Capacity

Blood and gastric tissue samples were taken and prepared for the measurement of different oxidative stress parameters: Cellular glutathione (GSH) was measured based on the reaction between 2,2-dithio-5,5-dibenzoic acid and the thiol (SH) groups of glutathione to yield a complex whose absorbance was read at 412 nm.[30] The glutathione concentration was calculated using the molar extinction coefficient ε= 1.36 104 M-1 cm-1. Superoxide dismutase (SOD) concentration was measured using a standard method.[31] and expressed in U/mg of protein, while catalase was determined.[32] and expressed as mM of H2O2/min/mg of protein, and tissue protein was measured using the Biuret method of protein assay. Lipid peroxidation was assessed by measuring the levels of malondialdehyde (MDA) [33]. Quantification of MDA was done using an extinction coefficient of ε = 1.56 105 M-1 cm-1.

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 BayeletVincent.[34] and the sections were observed microscopically.

Statistical analysis

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

RESULTS

***In vitro* antacid effects of aqueous extract of *K. grandifoliola* (Kg).**

pH values of the tested solutions at temperatures ranging from 25°C to 37°C

The pH values of the *K.g* solution at temperatures from 25°C to 37°C ranged from 6.82 to 6.72 respectively. The pH values of water, NaCl, gastric juice, HCl and $NaHCO_3$ solutions at temperatures from 25°C to 37°C ranged from 6.12 to 6.20; 6.47 to 6.62; 3.8 to 3.98; 1.12 to 1.20 and 12.46 to 12.67 respectively. The results indicated that temperature did not affect pH significantly.

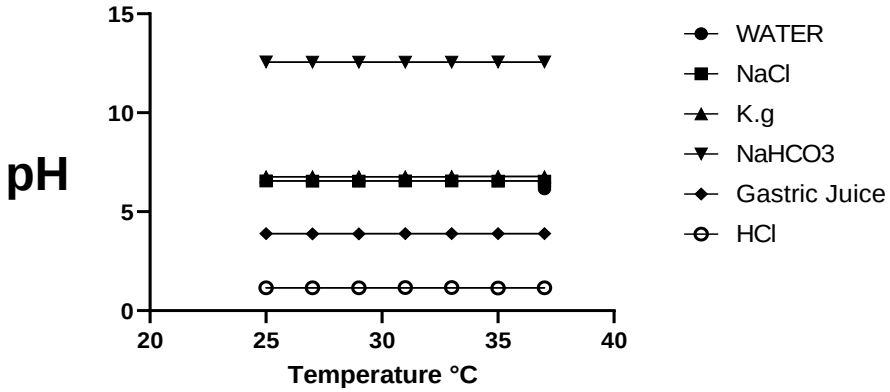

Fig. 1: pH values of the tested solutions at temperatures ranging from

Neutralizing effects on artificial gastric acids

When 45 mL of the test solution was added to 50 mL of the artificial gastric juice (pH 1.2) and biological gastric juice, the pH values of *K.g* solution was found to be 1.45 ± 0.04. The pH values of water, NaCl and $NaHCO_3$ solutions were 0.45 ± 0.03; 0.85 ± 0.03 and 3.50 ± 0.03, respectively. And, the pH of *K.g* and $NaHCO_3$ solutions + gastric juice was 7.11 ± 0.02 and 8.75 ± 0.30 respectively. This result shows that the neutralizing capacity of *K.g* was better than water and NaCl but less than $NaHCO_3$ (Table-1.)

Table-1: Neutralizing effects on artificial and biological gastric acids.

Drug	pH values
1- With artificial gastric juice	
Water	0.45 ± 0.03**
K.g	1.45 ± 0.04
NaCl	0.85 ± 0.03*
$NaHCO_3$	3.50 ± 0.03
2- With biological gastric juice	
K.g + Gastric juice	7.11 ± 0.02
$NaHCO_3$ + Gastric juice	8.75 ± 0.30

Data are presented as mean ± SD (n = 6). K.g (Khaya grandifoliola). The values represent the means ± ESM (number of samples = 5); *p < 0.05 and **p < 0.01: statistically significant relative to NaHCO3.

Duration of consistent neutralization effect on artificial and biological acidity

The durations for consistent neutralizing effects of *K.g* solution were 120 ± 32 min. Those of water, NaCl and $NaHCO_3$ solutions were 87 ± 4, 110 ± 13 and 121 ± 14 min, respectively. The action duration of *K.g*, NaCl and $NaHCO_3$ were not significantly different (Table-2.).

Table-2: Duration of neutralization effect on artificial and biological acidity.

Drug	pH values
1- With artificial gastric juice	
Water	87 ± 40 *##©©
K.g	120 ± 32
NaCl	110 ± 13
$NaHCO_3$	121 ± 14
2- With biological gastric juice	
K.g + Gastric juice	180 ± 27

Data are presented as mean ± SD (n = 5). K.g (Khaya grandifoliola). The values represent the means ± ESM (number of samples = 5); *p < 0.05: statistically significant relative to NaCl. ##p < 0.01: Statistically significant relative to NaHCO3 and ©©p < 0.01: Statistically significant relative to K.g groups.

Curative effects of aqueous extract of MY41g^c+ *K. grandifoliola*

Figure. 1 shows the macromorphological presentation of gastric mucosa of the rats after acetic acid ulcer induction. Figure. 1b (control 2) shows that 4 days after ulcer induction the gastric mucosa presented with well-defined gastric ulcer craters measuring 72 mm^2 on average. Two weeks after ulcer induction, rats that received the vehicle (Figure. 1c) still had ulcer craters but with some degree of autohealing. Treatment with *K. grandifoliola* +M Y41g^c mixture or Sucralfate was associated with increased mucus production and significant reduction of ulceration (Figures. 1d, e and f) compared with the 4-day controls. Stomach sections of the rats sacrificed 4 days after ulcer induction showed obvious loss of substance in the superficial mucosal layers. There was a diffuse mature infiltrate of mononuclear inflammatory cells, with the presence of congestion and edema which are indicative of an acute ulceration. The muscular layers were spared (Figure. 2b). In the negative control rats that were maintained for 10 days after ulcer establishment but without antiulcer treatment, the entire glandular depths of the stomach sections were invaded by inflammatory cells. The superficial layers remained greatly ulcerated, and the muscular layers were attained by the inflammatory process (Figure. 2c). Figures. 2d & 2e show ulcer sections from animals that received the solution of *Kg* + MY41g^c (250+250) and (500+250) mg/kg for 14 days. Macro morphological and histological sections pictures showed good and perfect healing with reestablishment of mucosal loss at (250+500) mg/kg, granulation tissue; they still no showed an invasion of the glandular depths. With Sucralfate, treatment for 10 days (Figure. f) showed the healing process, however the inflammatory process could still be seen as depicted by intra muscular edema and sparse inflammatory cells.

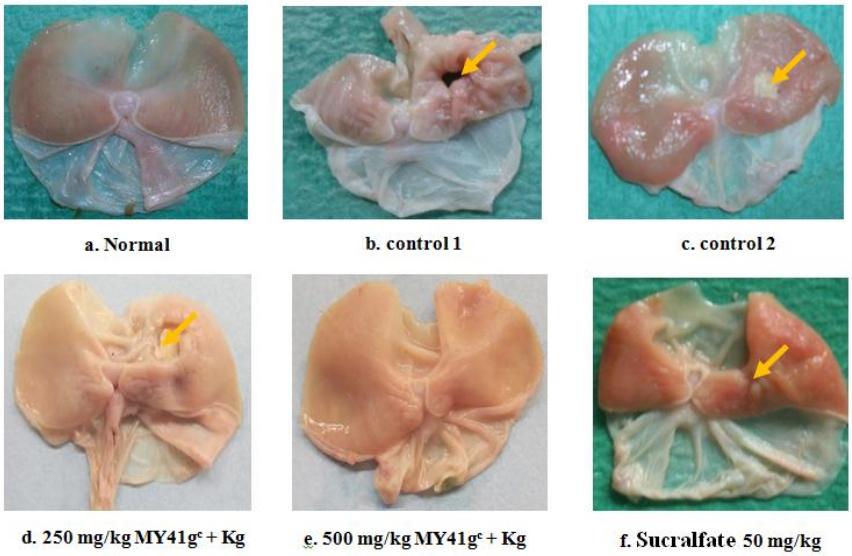

Fig. 1: Stomach of rats presentation of the healing of aqueous extract of *K. grandifoliola* + MY41g^c in acetic acid-induced chronic ulcers.

(a) : Normal rat; **(b)** : control 1 ; **(c)** : control 2 ; **(d)** : 250 mg/kg of **MY41g^c + Kg**; **(e)** : **MY41g^c + Kg (250** +500) mg/kg of; **(f)** : 50 mg/kg Sucralfate ; ⟶ : Chronic ulcer.

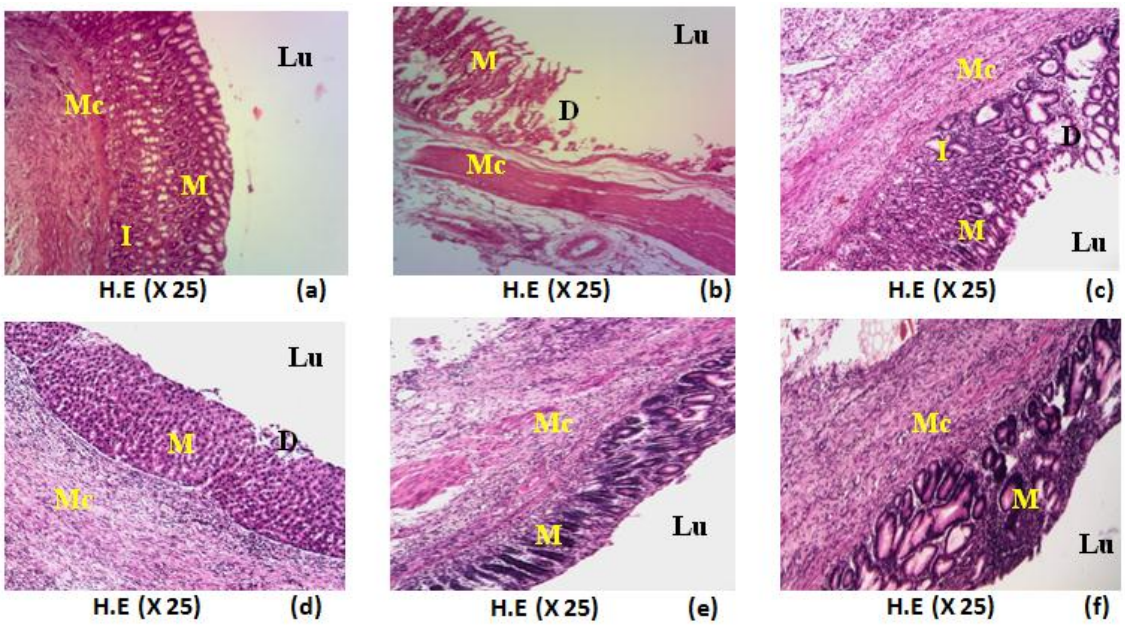

Fig. 2: Histological presentation of the healing of aqueous extract of *K. grandifoliola* + MY41g^{c} in acetic acid-induced chronic ulcers.

Histological presentation of simple chronic ulcers in rats. 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 250 and 500 mg/kg of K.g + 250 MY41g^{c} clay, with glandular proliferation, leukocyte infiltration and partial and total recovery 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.

The treatment of the ulcers with distilled water (longitudinal control or Control 2) for 10 days resulted in a reduction of % ulcerated areas to 10.66 % in the Control 1 or transversal to 3.55 % corresponding at 66.66 % of healing. Table 3 shows an almost similar percentage of healing of acetic acid-induced chronic gastric ulcers (66.66 and 66.26 % respectively) following daily treatment between the mixture of *K.g* + MY41g^{c} (250+250) mg/kg and longitudinal control (control 2) representing an auto-healing or spontaneous healing. A healing rate of 97.59 % was recorded for Sucralfate (50 mg/kg) and 100 % for MY41g^{c}+ Kg (250 + 500) mg/kg. The MY41g^{c}+ Kg (250 mg/kg) solution only promoted significantly ($P < 0.05$) higher levels of mucus production (80.50 and 76.76 respectively).

Table-3: Healing effect of aqueous extract of *K. grandifoliola* + MY41g^{c} clay on chronic acetic acid- induced gastric ulcers in rats.

Treatment	Dose (mg/kg)	N	Ulcer index	% ulcerated Surface	(%) healing	Mucus production (mg)
Normal rats	_	5	_	_	_	58.70 ± 4.88
Control 1	_	5	72.00 ± 0.81	10.66	_	54.25 ± 0.62
Control 2	_	5	24.00 ± 0.70***	3.55	66.66	61.27 ± 0.40
K.g + MY41g^{c}	250 + 250	5	7.00 ± 1.08***$^{###}$	1.03	66.26	80.50 ± 3.20*
K.g + MY41g^{c}	250 + 500	5	0.00 ± 0.00***$^{###}$	00	100	51.25 ± 4.13
Sucralfate	50	5	0.50 ± 0.28***$^{###}$	0.07	97.59	152.33 ± 9.13***$^{###}$

*N: number of rats; I: Indomethacin; Control 1 (4 day ulcerated rats); Control 2 (spontaneous healing). the values in the table represent averages ± ESM; *p < 0.05 et ***p < 0.001: statistically significant relative to Control 1 and $^{###}$p < 0.001: Statistically significant relative to Control 2.*

The mixture of MY41g^{c} + Kg promoted significantly ($P < .001$) higher levels of catalase (10.31 ± 1.09 and 13.03 ± 0.78U/mg protein) and highly ($P < .001$) decreased the level of MDA (1.54 ± 0.17 and 1.39 ± 0.09 mmol/g protein .10^{-6}) content of the stomach tissue obtained at the end of the treatment period compared with the control 1 (0.02 ± 0.00 H_2O_2/min/mg of protein for the catalase and 9.60 ± 0.33 mmol/g protein .10^{-6} for the MDA respectively) (Table-4.).

Table-4: Effect of *K. grandifoliola* + MY41g^c on oxidative parameters in stomach tissues of rats subjected acid acetic -induced gastric lesions.

Treatment	Dose (mg/kg)	N	SOD (U/mg protein)	Catalase (µmol H_2O_2/min/mg of protein)	GSH (mol/g protein. 10^{-3})	MDA (mmol/g protein .10^{-6})
Normal rats	_	5	2.48 ± 0.17	5.53 ± 1.25	1.68 ± 0.32	5.35 ± 0.50
Control 1	_	5	1.50 ± 0.15	0.02 ± 0.00	3.23 ± 0.03	9.60 ± 0.33
Control 2	_	5	1.58 ± 0.11	8.50 ± 0.29***	2.85 ± 0.20	8.92 ± 0.51
K.g+MY41g^c	250 + 250	5	1.88 ± 0.00	10.31 ± 1.09***	2.93 ± 0.15	1.54 ± 0.17***$^{\#\#\#}$
K.g+MY41g^c	250 + 500	5	1.88 ± 0.00	13.03±0.78***$^{\#\#}$	3.76 ± 1.64	1.39 ± 0.09***$^{\#\#\#}$
Sucralfate	50	5	1.67 ± 0.17	8.45 ± 1.55***	2.43 ± 0.23	7.85 ± 0.15

*N: number of rats; The values in the table represent averages ± ESM; MY41g^c = MY41g+ clay. The values in the table represent averages ± ESM; **p < 0.01 and ***p < 0,001: statistically significant relative to Control 1. $^{\#}$p< 0.05; $^{\#\#}$p < 0.01 et $^{\#\#\#}$p < 0,001: statistically significant relative to Control 2.*

DISCUSSION

In recent years, there have been many advances in the understanding of the pathophysiology and treatment of Peptic Ulcers (PU).[35-36] These PU are most often the result of an imbalance between aggressive factors (the most cited being acid and pepsin) and maintenance of the mucosal integrity through an endogenous defense mechanism.[37] For maintenance of the mucosal integrity, different therapeutic agents (with antacids, H_2 receptor antagonists and proton pump inhibitors, antibiotics) including plant extracts, are used to inhibit or neutralize gastric acid secretion or to stimulate the mucosal defense mechanism by increasing the mucosal production of the surface epithelial cells for many years.[38]

Antacids heal ulcers through elimination of gastric acid by neutralization; however, they do not decrease the volume of gastric secretions. Different antacid drugs vary markedly in the *in vivo* and *in vitro* potency, and that should be taken into account when antacids are prescribed. On the other hand, potency is not the only factor that should be considered in the choice of an antacid. Cost, taste, salt content, bowel habit, and side effects are also important.[25]1. In Africa, medicinal plants and geological material, like *K. grandifoliola* and MY41g^c clay are amongst the most attractive sources of new drugs or compounds, and have been shown to give promising results in the treatment of gastric ulcers. Therefore, the present study applied the titration method of Fordtran's model.[39] to explore the antacid effects of the aqueous extract of stem-bark of *K. grandifoliola* and the curative effects of the mixture of MY41g^c + Kg 250 mg/kg and (250+500) mg/kg.

In the previous study, the aqueous extract of *K. grandifoliola* was found to have antacid effects *in vivo*.[40] In this study this extract was found to have antacid effects *in vitro* also. Compared with the water group, all the treatments including *K. grandifoliola*, NaCl and Sodium bicarbonate were shown to possess significant gastric acid neutralizing effects (Table-1.). With regard to the duration for consistent neutralization of gastric acids, the neutralization duration of *K. grandifoliola*, NaCl and Sodium bicarbonate were significantly longer than that of water (Table-2). Also they exhibited significant antacid capacities compared to water. According to these findings, aqueous extract of *K. grandifoliola* is suggested to have antacid effects similar to Sodium bicarbonate. SB should be avoided even though it is a potent neutralizer of acid because it contains significant amounts of sodium and may alter the systemic pH. In addition, antacid drug interactions have been frequently reported and this is a problem worthy of being noticed. The most clinically significant interactions occur with ferrous sulfate, tetracycline and quinolone antibiotics. Other interactions are potentially significant because they involve drugs with narrow therapeutic ranges.[25] Considering the side effects and interactions of antacids, the extract of *K. grandifoliola* possessing fewer side effects should be looked to as an alternative for the treatment of PU.

Acetic acid-induced gastric ulcer by its perforating nature, spreads over a relatively large area that does not heal with time. The application of glacial acetic acid to the gastric serous membrane caused ulcers with well-encircled deep craters [41]. The results of the present study demonstrate that the mixture of *K.g* + MY41g^c (250+250 and 250 + 500) mg/kg accelerates the healing of chronic gastric ulcer in rats (66.26 and 100 % respectively) compared with control 1. The healing effect of the gastric mucosa was highly associated with increase in gastric mucus production (80.5 mg at 250+250 mg/kg and 152.33 mg with Sucralfate respectively) compared with the control (54.25 mg). Activities of antioxidant enzymes like catalase only (10.31 ± 1.09 and 13.03 ± 0.78U/mg protein) was also highly increased, while the levels of MDA decreased (.54 ± 0.17 and 1.39 ± 0.09 mmol/g protein .10^{-6}) compared with the control (0.02 ± 0.00 H_2O_2/min/mg of protein for the catalase and 9.60 ± 0.33 mmol/g protein .10^{-6} for the MDA respectively). The importance of increased mucus strength in protecting the regenerating gastric epithelium is well-known [42]. Healing is a normal physiological process that proceeds through a series of coordinated cellular events, culminating in the restoration of the functional integrity of tissues [43]. It can be observed from figure. 1 and 2 that there was a decrease in areas of gastric mucosal necrosis, with glandular re-

epithelialisation in the mixture *K.g* + MY41g^{c} (250+250) mg/kg and Sucralfate treated groups. In the mixture *K.g* + MY41g^{c} (250+500) mg/kg-treated group, re-epithelialization was almost complete. Cellular proliferation plays an essential role in maintaining the integrity of the gastric mucosa.[44]

The ability of *K. grandifoliola* to improve the antioxidant status and the mucus production enhancing property may be responsible for its healing promoting effects on chronic gastric ulcers. The presence of phytochemicals like flavonoids and polyphenolic compounds, saponins and tannins are also important for the healing-promoting effect through the stimulation of gastric mucus secretion and enhanced re- epithelialization.[39, 41, 45] Also, the recent study demonstrates that the administration of MY41g^{c} clay accelerated the spontaneous healing of chronic acetic acid-induced gastric ulcers by increased gastric mucus thickness and gastric reepithelialization, improved antioxidant status and effective antacid activity. The mode of action of the MY41g^{c} clay cool include: The rich mineralogical composition of MY41g^{c} clay reported to have a other antacids in the triple therapy regimen for ulcer treatment.[46-47] Also the association of MY41g^{c} clay and *K. grandifoliola*, only at the dose of 250+500, have the best synergic effect from promoting healing on chronic gastric ulcers.

CONCLUSION

Administration of the mixture of MY41g^{c} clay+*K. grandifoliola* accelerated the spontaneous healing of chronic acetic acid-induced gastric ulcers. The mode of action of this mixture include: antacid ans neutralizing activity, increased gastric mucus thickness, increased gastric reepithelialization and improved antioxidant status. This composition can be exploited for the ulcer treatment.

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