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Research Article

Effect of the Aqueous Extract of *Macaranga Barteri* Müll.Arg (Euphorbiaceae) on Acetic Acid Induced Chronic Ulcers in Rats

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ABSTRACT

Macaranga barteri (Euphorbiaceae) is used in Côte d'Ivoire for the treatment of several diseases including gastric ulcer. The present study aims to evaluate the curative potential of an aqueous extract of *Macaranga barteri* leaves (AEMb) in a model of chronic gastric ulcer induced by acetic acid in rats. Chronic gastric ulcers were induced by applying 0.1 ml of acetic acid (30%) to the anterior surface in the region of small curvature of the stomach of rats. Five days after the application of acetic acid, four groups of rats were treated with distilled water (1ml / 100g b.w.), AEMb (250 and 500 mg / kg b.w.) and sucralfate (50 mg / kg b.w.) for 28 days. Then, six rats from each group were sacrificed respectively on the 7th, 14th 21th, 28th days and two weeks after stopping the treatments. Macroscopic analysis of the stomachs, estimated healing percentage of ulcers and histological sections of the stomach were performed. The results obtained showed a healing percentage of ulcerations of 98.22%, 91.47% and 98.07% respectively with sucralfate and AEMb (250 and 500 mg / kg b.w.) after 28 days of treatment. Two weeks after stopping the treatments with sucralfate and AEMb, all the ulcerations in rats were healed. Study of histological sections showed marked reepithelialization of stomach layers after 28 days of treatment and complete recovery two weeks after stopping treatments unlike treatments of sham-operated control rats. In conclusion, AEMb accelerated the healing of chronic gastric ulcers induced by acetic acid.

Keywords: Rats, Stomach Ulcer, Acetic acid, Re-Epithelialization, Plant Leave

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INTRODUCTION

Gastric ulcers are real public health problems that affect a large number of people worldwide and also remains a major cause of morbidity and mortality (Chan and Leung, 2002). In Côte d'Ivoire, epidemiological studies on peptic ulcer disease reported a prevalence of 19.3% (Soro *et al*., 2016). Several molecules of synthetic origin such as H_2 receptor inhibitors, proton pump inhibitors, and antibiotics are used to treat gastric ulcers. However, harmful side effects such as erectile dysfunction, arrhythmias, hyperplasia, gynecomastia and hematopoietic changes are observed during their use (Adinortey *et al.*, 2013; Qaiser *et al.*, 2018). As a result, the exploitation of new effective bioactive molecules devoid of any or less adverse side effects has become one of the relevant objectives of scientists. In this in context, *Macaranga barteri*, a plant belonging to Euphorbiaceae family was chosen for this study. It is traditionally used in the treatment of many pathologies including gastrointestinal ulcers (Oliver-Bever, 1986; Adesegun *et al.*, 2007). Moreover, it has been shown that prior administration of the aqueous extract of the leaves of *Macaranga barteri* (AEMb) at doses between 62.5 and 500 mg / kg b.w. protected gastric mucosa against gastric ulcers experimentally induced with HCl / Ethanol, ibuprofen, stress and pylorus ligation in rats (Ehilé *et al.*, 2018). However, no scientific study of the healing potential of this plant on chronic gastric ulcers has been conducted. The objective of this work was to assess the curative effect of the aqueous extract of the leaves of *Macaranga barteri* on gastric ulcers induced with acetic acid in rats.

MATERIALS AND METHODS

Plant: Fresh leaves of *Macaranga barteri* were harvested in August 2018 from the forest located within the Nangui Abrogoua University (Abidjan, Côte d'Ivoire). The plant was identified at the National Floristic Center (CNF) of the Félix Houphouët Boigny University (Abidjan, Cote d'Ivoire) thanks to samples kept in the national herbarium under voucher number 14735 of April 06, 1979.

Animal: The experiments were carried out on 12 to 13 weeks albino rats (*Rattus norvegicus*) weighing between 185 and 203 g. The animals were maintained under standard laboratory

conditions of a temperature of 20±2°C and a photoperiod of 12h and received Ivograin® (Abidjan, Côte d'Ivoire) pellets and water ad libitum. The different experimental protocols were followed in accordance with the experimental animal protection protocols of the European Council on Legislation 2012/707 / EU **(EU, 2012).**

Chemical substances: The chemicals used were composed of: Ethanol (Sigma, USA), Ulcar® Sucralfate (Sanofi-aventis, France), soduim chloride (Alfa Aesar, USA), trichloroacetic acid (Sigma, USA), betadine® (PubChem, France), formalin (Ubipharm, France).

Preparation of the aqueous extract of the leaves of *Macaranga barteri:* The method used for the preparation of the aqueous extract was the one described by Ehilé *et al.* (2018). It consisted in cutting the leaves of *M. barteri* into small pieces and drying them at room temperature (20-22° C) for one week. These small pieces were pulverized using an electric grinder of the RETSH brand, type SM 100 (Haan, Germany). 100 g of *M. barteri* leaf powder was decocted for 15 min in one liter of distilled water. The aqueous solution obtained was filtered through hydrophilic cotton and Whatman 3 mm filter paper. Half liter of boiling distilled water was added to the plant powder residue and allowed o decoct for 10 min. This mixture was also filtered. The filtrates were mixed and dried in a Selecta (Spain) brand oven at 45°C for 48 hours. The powder obtained was black in color with a yield of 13%.

Induction of acetic acid induced chronic gastric ulcers in rat: The model used to induce chronic gastric ulcer is that described by Okabe and Amagase (2005). In this model, 132 rats were fasted for 24 hours. A laparotomy was performed under ether anesthesia, then gastric ulcer was induced in 126 rats by instilling 0.1 ml of acetic acid 30% via a plastic cylinder of 8 mm diameter on the left side of the stomach serosa for 1 min. After aspiration of acetic acid, the stomach surface of each rat was cleaned using cotton wool soaked in 0.9% NaCl. The other six rats did not receive acetic acid. The abdominal incisions were then stitched up and feeding was resumed in the rats. Every day betadine was applied to the incised area to prevent infections. Four days after the operation, six rats that received acetic acid and the other six that did not receive acetic acid were sacrificed under ether anesthesia. The stomachs were opened in order to determine the degree of ulceration before the start of treatment. The rest of the rats that received acetic acid were divided into four groups of thirty rats each. The rats in group 1 were treated orally with distilled water at a rate of 1 ml / 100 g b.w. per day for twenty-eight days. The rats in group 2 were treated orally with Ulcar (sucralfate) at a dose of 50 mg / kg b.w for twenty-eight days. As for the rats in lots 3 and 4, they were treated orally with AEMb at doses of 250 and 500 mg / kg b.w, respectively, for 28 days. Preliminary studies, acute oral toxicity (LD_{50} > 5000 mg/kg) and preventive anti-gastric ulcer effect of AEMb (Ehile *et al*., 2018) motivated the choice of these doses. On the 7th, 14th, 21st, 28th days of treatment and two weeks after stopping treatment, six rats from each group were sacrificed by overdose of ether.

Measurement of gastric lesions in rat: After the rats were sacrificed, the stomachs were removed and opened along the great curvature. The gastric mucus was collected and weighed and the stomachs were washed and rinsed with NaCl (0.9%). The length (l) and width (L) of ulcers were measured. Ulcer index (UI) was determined based upon the product of the lengh (mm) and they width (mm) of the ulcer (Okabe *et al*., 1971):UI=l×L. Lesions intensity (scores) was determined according to the scale described by Devaraj *et al.*(2007) : 0 = No ulcer, 1 = Superficial erosion of the mucous membranes, 2 = Deep ulcer or transmural necrosis, 3 = perforated or penetrated ulcer.

The percentage curative ratio was calculated using the following formula Zhao *et al*. (2019).

$$\text{Percentage curative (PC)} = \frac{\text{UI control rat} - \text{UI treated rat}}{\text{UI control rat}} \times 100$$

Microscopic analysis: Histological study was made as described by Hould (1984). The fundic part of the stomachs of the rats were removed and fixed in 10% formalin, then dehydrated in ethanol baths of increasing concentrations (30, 50, 70, 95 and 100%). The samples were then embedded in paraffin and thin sections of tissue (5mm) were microtome cut. They were dewaxed in a toluene bath and hydrated in decreasing baths of ethanol (100, 95 and 70%). The sections were stained with hematoxylin and eosin and mounted under slide and coverslip with resin. The slides were read with an Olympus microscope (BX41; Tokyo, Japan).

Statistical analyzes: Statistical analysis was implemented using GraphPad Prism 7.01 software (San Diego, California, USA). The results were expressed as the mean followed by the standard error on the mean (M ± SEM). The one-factor variance analysis test (ANOVA1) was performed to verify the normality of the variables. When significant differences were revealed between the means tested, ANOVA 1 was supplemented by multiple comparisons of the mean values of the different parameters using the Turkey-Kramer test as a post test. The significance threshold was set at $P < 0.05$.

RESULTS

Effect of the aqueous extract of the leaves of *Macaranga barteri* on ulceration index and ulcer score in rat: The administration of 30 % acetic acid in rats induced gastric ulceration. The results indicated an ulceration index of 113 ± 13.5 and a score of 3 ± 00 compared to untreated rats of the control group 1 where no gastric ulceration was observed (Fig 1 and Table 1).

After seven, fourteen, twenty one or twenty eigtht days of treatment of the rats with sucralfate and two AEMb doses (250 et 500 mg/kg b.w.), the ulceration indexes were significantly reduced when compared to those of rats with no treatment while the curative percentage was significanty increased.

Table 1:
Effect of the aqueous extract of the leaves of *Macaranga barteri* on gastric ulcers induced by acetic acid in rats

Duration of treatment	Groups	Score	UI	PC (%)	Mucus weight (mg)
Day 4 (after induction of ulcers)	Control 1(distilled water)	00.00 ± 0.00	00.00 ± 0.00	-	133.85±6.52
	Control 2 (acetic acid)	3.00 ±0.00$^{###}$	113±13.5$^{###}$	00	36.8±2.26$^{###}$
7 days Treatment	Control 2 (acetic acid)	3.00 ±0.00	90.7±3.1	-	43.2±3.94
	Sucralfate (50 mg/kg bw)	2.1 ± 0.25	46.3±0.92***	48.92	91.25±5.41***
	AEMb (250 mg/kg b.w.)	2.5 ± 0.30	58.2±1.4***	35.82	89.25±4.23***
	AEMb (500 mg/kg b.w.)	2.2 ± 0.20	46.7±1.86***	48.54	101.2±5.17***
14 days Treatment	Control 2 (acetic acid)	2.8±0.2	62.11±4.80	-	47.8±7.27
	Sucralfate (50mg/kg b.w)	1.4 ± 0.24	12.8±0.78***	79.38	105.91±3.52***
	AEMb (250 mg/kg b.w.)	1.8± 0.31	23.4±1.39***	62.34	101.76±4.80***
	AEMb (500 mg/kg b.w.)	1.6 ± 0.20	13.20±0.58***	78.74	120.16±6.26***
21 days Treatment	Control 2 (acetic acid)	2.4 ± 0.30	53.41±1.78	-	57.2±6.60
	Sucralfate (50mg/kg pc)	0.6±0.25***	6.05±1.28***	88.26	102.00±6.12***
	AEMb (250 mg/kg b.w.)	1.4 ± 0.22***	11.4±1.18***	78.13	97.44±3.09***
	AEMb (500 mg/kg b.w.)	0.7± 0.24***	6.27±0.88***	87.79	116.80±2.98***
28 days Treatment	Control 2 (acetic acid)	2.00±0.30	44.36±0.66	-	69.3±4.84
	Sucralfate (50mg/kg b.w)	0.2 ±0.20***	0.72±0.45***	98.22	111.52±4.39***
	AEMb (250 mg/kg b.w.)	0.8±0.37***	3.41±2.07***	91.47	109.56±7.22***
	AEMb (500 mg/kg b.w.)	0.4±0.20***	0.77±0.44***	98.07	121.18±5.77***
2 weeks after the end of the treatment	Control 2(acetic acid)	1.09±0.98	27.20±1.93	-	65.21±6.14
	Sucralfate (50mg/kg b.w)	0.00±0.00***	0.00±0.00***	100	106.33±5.90***
	AEMb (250 mg/kg b.w.)	0.00±0.00***	0.00±0.00***	100	111.42±7.09***
	AEMb (500 mg/kg b.w.)	0.00±0.00***	0.00±0.00***	100	117.18±4.52***

$^{###}$ p <0.001: comparison between the values of control group 1 (distilled water) and those of control group 2 (acetic acid)

*** p <0.001: Values in the same column are statistically different from those in control lot 2 (acetic acid) on the same period; n = 6;AEMb: Aqueous Extract of Macaranga barteri; UI: ulceration index; PC: curative percentage

After seven days of treatment, the ulceration index reached 90.7 ± 3.1, while the score of 3 ± 0.00 did not vary in the control 2 group. However, in rats treated with sucralfate (50 mg / kg b.w.) and AEMb (250 and 500 mg / kg b.w.), a significant decrease ($p<0.001$) in ulceration indexes were recorded compared to control 2 group. The indexes were 46.3±0.92 (sucralfate 50 mg / kg b.w.), 58.2±1.4 and 46.7±1.86 respectively for AEMb at 250 and 500 mg / kg b.w. This was equivalent to 48.92% (sucralfate 50 mg / kg b.w.), 35.82% (AEMb 250 mg / kg b.w.) and 48.54% (AEMb 500 mg / kg b.w.) as percentage curative. As for the scores, they diminished in a non-significant way ($p> 0.05$) in the rats treated with sucralfate (50 mg / kg b.w.) and AEMb (250 and 500 mg / kg b.w.) compared to control 2 group. The scores were 2.1 ± 0.25 (sucralfate 50 mg / kg b.w.), 2.5 ± 0.30 and 2.2 ± 0.20 respectively for AEMb at doses of 250 and 500 mg / kg b.w (Table 1)

After fourteen days of treatment, the ulcer index which was 62.11 ± 4.80 (control 2) was significantly ($p <0.001$) reduced and attained 13.2 ± 0.58 in AEMb (500 mg/ kg b.w.) treated rats with a healing percentage of 78.74%. The standard drug (sucralfate) exhibited 79.38% rate of recorvery (Table 1). At the end of the third week of treatment, the ulcer index highly ($p <0.001$) dropped in the rats treated with AEMb with values of 11.4 ± 1.18 (AEMb 250 mg/kg b.w.) and 6.27 ± 0 .88 (AEMb 500 mg/kg b.w.) compared to control 2 rats of which values amounted to 53.41 ± 1.78. Thus, the healing rates were augmented and reached 87.79% with AEMb at 500 mg/kg b.w. Effects of the standard drug varied in the same way like AEMb (Table 1).

The end of the fourth week of treatment disclosed important fall of ulcer indexes in AEMb (250 and 500 mg/kg b.w.) and sucralfate treated rats reaching 3.41 ± 2.07, 0.77 ± 0.44 and 0.72 ± 0.45 respectively compared to control 2 (44.36 ± 0.66). The scores significantly ($p <0.001$) diminished as well varying from 2 ± 0.30 (control 2) to 0.4 ± 0.20 (AEMb 500 mg/kg b.w) and 0.2 ± 0.2 (sucralfate 50 mg/kg b.w.). Thus the healing rate raised to 91.47% and 98.07% respectively for AEMb at 250 and 500 mg/kg b.w. and 98.22% for sucralfate. Two weeks after the end of treatment, no gastric ulceration was observed in rats treated with sucralfate and AEMb (250 and 500 mg / kg b.w.) (Fig 1; table 1).

Effect of the aqueous extract of the leaves of *Macaranga barteri* on mucus production in rat: The contact of 30 % acetic acid with the stomach of the rats, produced 36.8 ± 2.26 mg of mucus (control 2) compared to untreated rats of the control group 1 where 133.85 ± 6.52 mg of mucus were recorded (Table 1).

The treatment of the rats with sucralfate and AEMb (250 et 500 mg/kg b.w.) after seven, fourteen, twenty one or twenty eight days, induced a significant ($p <0.001$) increase in the amount of mucus. Indeed, the quantity of mucus reached important values of 101.76 ± 4.80, 120.16 ± 6.26, 105.91 ± 3.52 mg, respectively for AEMb 250 and 500 mg/kg b.w. and sulcralfate while that of control 2 was 47.8 ± 7.27 mg.

After twenty eight days of treatment, AEMb (250 and 500 mg/kg b.w.) and sulcralfate triggered significant mucus secretion of 109.56±7.22 mg, 121.18±5.77 mg and 111.52±4.39 mg respectively, compared to control 2 (69.3±4.84 mg).

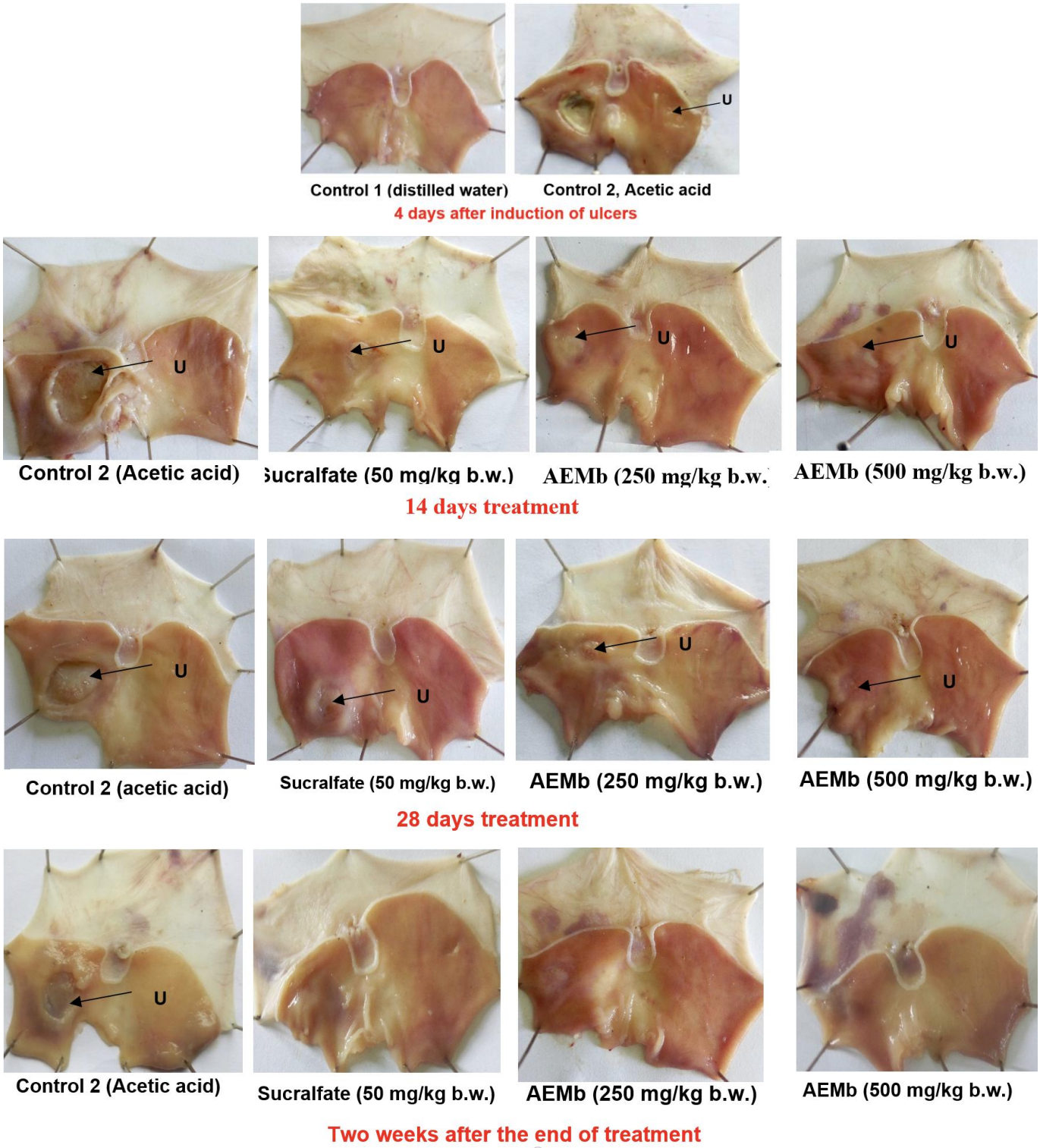

Plate 1:
Photographs of the stomachs of treated rats with sucralfate or EAMb on gastric lesions induced by acetic acid.
AEMb: Aqueous Extract of Macaranga barteri; U: ulceration

Two weeks after the end of treatment, mucus production induced by AEMb and sulcralfate remained nearly constant compared to values of mucus after twenty eight days of treatment but still significantly different to value of control 2.

Histological analyses of gastric wall of ulcerated treated and non-treated rats: The results of histological analyses of the gastric wall in ulcerated rats are shown in Fig 2. The rats in group 1 which did not receive acetic acid showed normal mucosa. Four days after the induction of gastric ulcers, rat which received 30% acetic acid showed a discontinuity in the

mucosa and extensive damage of the submucosa. After fourteen days of treatment, the stomach histology of rats treated with AEMb 250 and 500 mg / kg b.w. and sucralfate 50 mg / kg b.w. revealed a reepithelialization of the gastric mucosa surface, shortened crypts and reorganization of inner tissues.

After twenty-eight days of treatment, rats treated with sucralfate, 50 mg / kg b.w. and AEMb at doses of 250 and 500 mg / kg b.w. showed regeneration of the crypts with reepithelialization of the histological architecture of the stomach compared to the rats of the control 2 group. Two weeks after the end of the treatment, total reepithelialization of the different layers of the stomach was observed in rats treated with sucralfate (50 mg / kg b.w.) and AEMb (250 and 500 mg / kg b.w.) compared to the rats of the control 2 group.

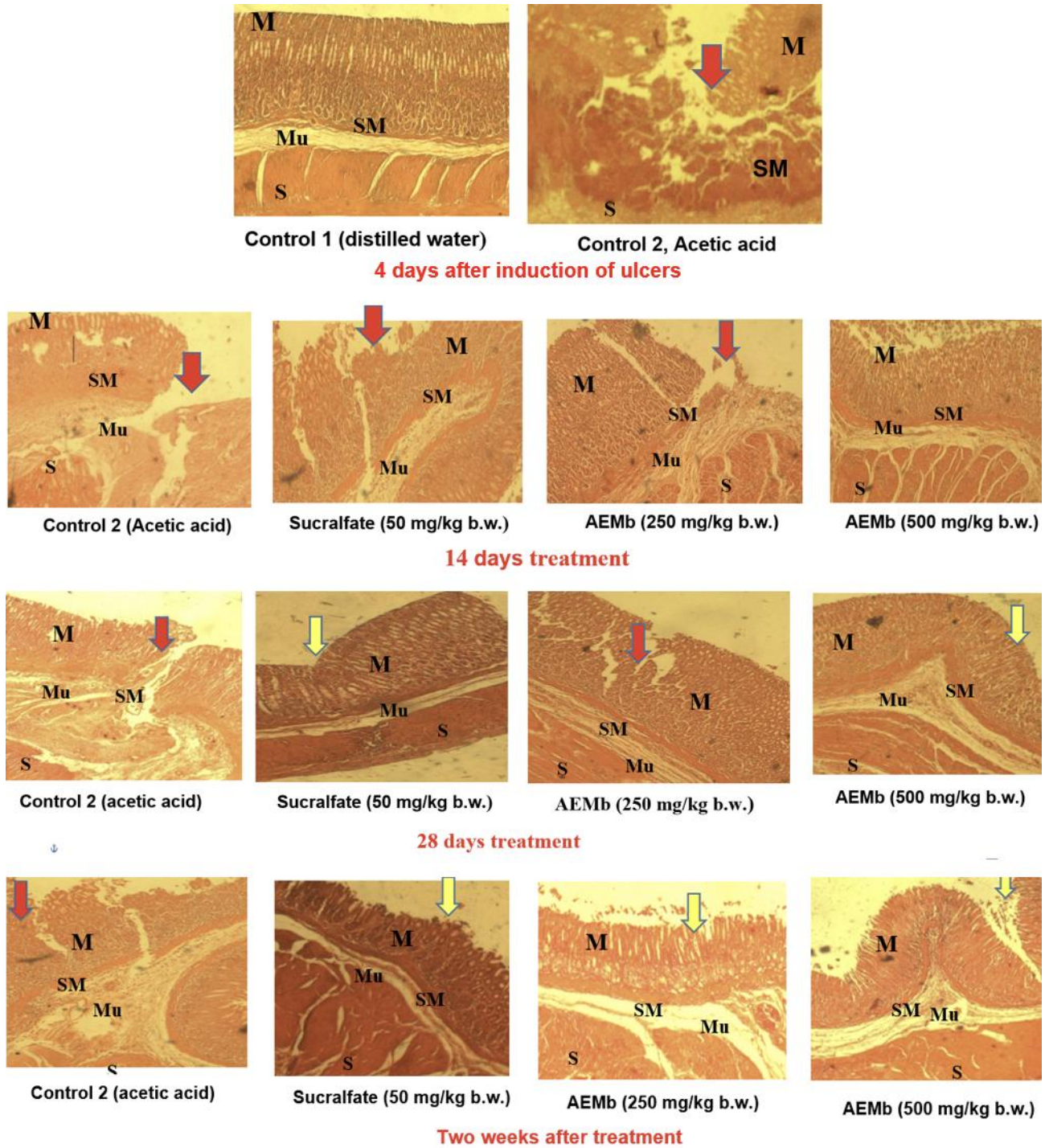

Figure 2

Photomicrographs of the different treatments on the histological sections of gastric ulcers induced by acetic acid in rats

Deformities of crypts and mucosa (red arrow), regeneration of crypts (yellow arrow), M: Mucosa, SM: Submucosa, MU Musculosa, S: Serosa (stain Hematoxylin / Eosin G × 40).

DISCUSSION

The preventive study on several gastric ulcer models in rats undertaken by Ehilé *et al.* (2018) revealed that AEMb had a real antiulcer potential. In order to verify the healing potential of the aqueous extract of the leaves of *M. barteri* on chronic gastric ulcers, the effect of AEMb was evaluated on a chronic gastric ulcer model induced by acetic acid in rats.

The results showed that AEMb significantly reduced gastric mucosal damage induced by acetic acid. This extract, therefore, had a real curative anti-ulcerogenic potential. Indeed, the ulcers observed in the gastric mucosa of rats four days after its induction with 30% acetic acid were mainly due to the corrosive action of acetic acid. This action elicited the backscattering of H^+ ions through the channels of the outer layers to the inner layers of the stomach membrane (Okabe et Amagase, 2005) and led to tissues necrosis. According to Schubert and Peura (2008), gastric necrosis triggers the release of arachidonic acid metabolites. The release of arachidonic acid attracts leukocytes (polymorphonuclear neutrophils and macrophages). Leukocytes induce a transformation of superficial lesions into deeper lesions and an inactivation of growth factors important to the integrity and repair of the mucosa. This explains the high degree of ulceration observed in the stomach mucosa of rats that received acetic acid. However, treatment of rats with AEMb (250 and 500 mg / kg b.w.) resulted in a rapid healing of rats gastric ulcers compared to control rats after the 28 days of treatment. This rapid healing could be due to the phytoconstituents that are flavonoids, saponins, alkaloids, phenols, and tannins contained in the aqueous extract of *M. barteri* as described by Ehilé *et al.*(2018). Moreover, studies showed that flavonoids are endowed with anti-ulcerogenic properties (Kelly *et al.*, 2009; Nwagba *et al*., 2013), decrease the secretion of histamine from mast cells by inhibiting histidine decarboxylase and improve the secretion of mucus and prostaglandins which are involved in the regulation of mucus resulting in a faster healing of ulcers (Borrelli and Izzo, 2000). The presence of flavonoids in this extract could, therefore, explain, at least the anti-ulcerogenic effect of *M. barteri.* Similar results were found by Kim *et al*. (2020) with the aqueous extract of the root of *Polygonum cuspidatum* (Polygonaceae).

Microscopic observations of the stomachs of rats treated with acetic acid showed profound changes in the different layers of the stomach. A rupture of the stomac mucosa with removal of the crypts, and a disorganization of different layers were observed, which is in accordance with the works carried out by Schmassmann (1998) and Zhao *et al*.(2019). Administration of AEMb (250 and 500 mg / kg b.w.) once a day for 28 days after induction of gastric lesions with acetic acid accelerated the healing process favouring reeepithelialization of the different layers. The mechanism by which AEMb works is unknown. However, it can be assumed that AEMb may favour the production of elements involved in the rebuilding of damaged stomach layers. Under physiological conditions, the protection of the gastric mucosa against the action of hydrochloric acid and the digestive action of the proteolytic enzymes of the gastric juice takes place through the mucus (Kangwan *et al.,* 2014). AEMb was shown to restore mucus production. This effect suggests that AEMb could stimuate the synthesis of glandular mucus producing cells. The restoration of mucus production could therefore play a role in the fast reepithelialization induced by AEMb by protecting stomach wall and impeding further acetic acid action (Wallace et Whittle, 1986). These results are similar to those obtained by Amang *et al*. (2020) who showed that the aqueous extract of the leaves of *Eremomastax speciosa* (Acanthaceae) (250 and 500 mg / kg b.w.) promoted reepithelialization of the layers of the gastric mucosa in chronic ulcers induced by acetic acid.

In conclusion, this study revealed that AEMb (250 and 500 mg / kg bw) healed gastric lesions caused by acid acetic. AEMb induced an increase in the amount of mucus produced over weeks of treatment. All the results obtained corroborate the traditional use of AEMb in the treatment of gastric ulcers.

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