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Analgesic and non-ulcerogenic activities of the aqueous extract of the aerial parts of *Eremomastax speciosa* Hochst (Acanthaceae) in mice

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Abstract
To avoid the adverse effects (ulcerogenic activity for exemple) of nonsteroidal anti-inflammatory drugs (NSAIDs) while curing pain, some people use medicinal plants. This study investigated the analgesic and non-ulcerogenic activities of the aqueous extract of dried ground aerial parts of *Eremomastax speciosa* Hochst (Acanthaceae) in mice. The analgesic action was assayed in two experimental models: acetic acid and formalin; and the non-ulcerogenic activity by the observation of the gastric mucosa five hours after administration of different treatments. The aqueous extract of *Eremomastax speciosa* dose-dependently reduced the number of abdominal contractions induced by intraperitoneal injection of acetic acid, with inhibition percentages of 12.13%, 44.74% and 91.40% respectively for doses 100mg/kg, 200mg/kg ($p<0.01$) and 400mg/kg ($p<0.001$). During the second phase of the pain induced by formalin, the aqueous extract of *Eremomastax speciosa* significantly decreased ($p<0.001$) the time of licking and lifting of the leg, with inhibition percentages of 71.08%, 73.26% and 84.61% respectively for doses 100mg/kg, 200mg/kg and 400mg/kg. Unlike indomethacin, the aqueous extract of *Eremomastax speciosa* did not cause gastric ulcer, but at doses 200mg/kg and 400mg/kg it have induced an increase ($p<0.001$) in mucus secretion, with percentages of 65.10% and 65.51% respectively compared to positive control. These results show that aqueous extract of *Eremomastax speciosa* would possess analgesic and non-ulcerogenic properties.

Keywords: *Eremomastax speciosa*, analgesic, ulcerogenic, pain, ulcer

1. Introduction
Pain is an unpleasant sensory and emotional experience, linked to an existing or potential tissue injury [1]. Tissue damage is the immediate pain cause because it induces the release of different chemical mediators such as prostaglandins, bradykinins and a substance P that act on the nociceptors causing this sensation. Depending on time factor there are usually two types of pain: acute pain and chronic pain [1]. According to Jain *et al.* (2002) [2], each individual suffers from pain at least once in his life. Moreover, according to Stucky *et al*. (2001) [3], more than one-third of the world's population is suffering from chronic pain. This prevalence is increasingly high worldwide due to its multiple causes. Pain then affects the entire world population.

There are many medications against pain (such as aspirin, indomethacin, phenylbutazone), but these products are not free of side effects [4]. It has been shown that non-steroidal anti-inflammatory drugs (NSAIDs), widely used in the world, calm pain by inhibiting the synthesis of cyclooxygenase (COX), the enzyme responsible for the synthesis of prostaglandins. Prostaglandins are responsible for the pain sensation on the one hand, and on the other hand responsible for maintaining the gastric mucosal integrity trough mucus production [5]. So these NSAIDs although healing pain have a strong ulcerogenic power as one of the most common side effects. Other adverse effects associated with NSAIDs are bleeding and mucosal damage and other gastrointestinal disorders; all this constitutes a great limit to the use of these analgesic drugs [6, 7].

It is to overcome these limitations of synthesized drugs that preparations of vegetable origin have become an important boon in the health researchs. It is the case of *Eremomastax speciosa* (Acanthaceae), a tropical plant widely used in traditional medicine to treat anemia, urinary tract infections, dysentery, fractures, hemorrhoids, menstrual pain, gonorrhea [8], bacterial infections [9], malaria, kidney pain, diabetes, scabies and nerve pain [10].

Extracts of *Eremomastax speciosa* would have analgesic properties. The aim of this study was to evaluate the analgesic and non-ulcerogenic effects of the aqueous extract of *Eremomastax speciosa* in mice.

2. Materials and methods

2.1 Plant material

Fresh aerial parts of *Eremomastax speciosa* were collected in Yaoundé, Cameroon. Botanic identification was performed at the Cameroon National Herbarium, Yaoundé, Cameroon, by Paul MEZILI in comparison with the voucher No. HNC/136984. The sample was air-dried in the laboratory (room temperature) and then ground to a powder.

2.2 Preparation of extract

The powdered sample (500 g) was soaked in boiling distilled water (4L) for 15 minutes. The mixture was then allowed to cool and after filtration through Wattman filter paper number 3, the filtrate was evaporated using a *Raven convection* air oven (Jencons PLS, UK). The brownish solid obtained (77.7 g (15.5% yield)) was stored at 4°C.

2.3 Animals

Adult male Swiss albino mice *Mus musculus* (25.00 ± 5.00g) obtained from the Animal house, Laboratory of Animal Physiology, Department of Biological Sciences, Higher Teachers' Training College, University of Yaoundé I (Yaoundé-Cameroon) were used for this study. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Reg. No. FWAIRB00001954). The use, handling and care of animals were done in adherence to the European Convention (Strasbourg, 18.III.1986) 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.

2.4 Chemicals

Acetic acid, formalin, indomethacin and the aqueous extract were prepared prior to their use in the biological assays.

2.5 Investigating analgesic properties

2.5.1 Animal allotment and treatment

In each analgesic test, five groups of 6 animals were used. Group 1 served as negative control and received distilled water (0.5ml/30g bw). Group 2 was used as positive control and was treated with indomethacin (50mg/kg *per os*).The 3 last groups received the aqueous extract of *Eremomastax speciosa* at the doses 100; 200 and 400mg/kg *per os*.

2.5.2 Acetic acid-induced writhing test

This test was conducted as previously described by Koster (1959) [11]. One hour after administration of the treatment, acetic acid solution (0.6v/v) was injected intraperitoneally (10ml/kg) to each animal. The number of writhing induced by the acetic acid, consisting of abdominal constrictions and hind limbs stretching were counted for 20 minutes after a latency period of 5 minutes. The percentage of inhibition was calculated as follows:

$$\text{Percentage of inhibition} = 1 - \frac{Nt}{Nc} X\ 100$$

Where Nc is the average number of stretching in the control group, Nt is the average number of stretching in the test group.

2.5.3 Formalin- induced pain

The method was conducted as previously described by Gaertner *et al.*, (1999) [12]. In this procedure, 20µL of 2.5% formalin was injected in the plantar arch of the right hind paw of the rats 1 hour after administration of drugs. These rats were individually placed in transparent cage for observation. The time spent licking the injected paw, was an indicator of the pain sensation following formalin administration, and was recorded in different phases: from 0-5min post injection (first phase) and 15-30min post injection (second phase). These phases represented neurogenic and inflammatory pain response, respectively. The percentage of analgesic activity was calculated as follows:

$$\text{Percentage of inhibition} = 1 - \frac{Tt}{Tc} X\ 100$$

With Tc is the mean time in control group for each phase and Tt the mean time in the test group for each phase.

2.6 Investigating non-ulcerogenic properties

2.6.1 Ulcerogenic test

This test was assayed according to Grewal *et al.* 2014 [13]. Five hours after administration of drugs, mice were sacrificed under ether anaesthesia. The abdomen of each mice was opened and stomach was located and removed after bindings at the levels of the cardia and the pylore. Each stomach was dilated by injection of 1 ml of formalin at 2 % and was open along the greatest curve to examine macroscopically lesions.

2.6.2 Ulcer scores

The ulcers produced in the glandular region of each stomach were measured and scored as described by Martin *et al.* (1993) [14].

Description	Scores
No ulcers	0,0
Dilation of the vessels and small points of ulcers	1,0
Ulcers lower or equal to 4mm length	2,5
Ulcers equal or higher to 5mm length	5

2.6.3 Ulcer index (UI)

The ulcer index (UI) is the average score of ulcers of each treatment ± the standard error on the mean (ESM) and calculated as follows:

$$IU = \sum_1^n Scores \pm ESM$$

2.6.4 Percentage of ulceration

The percentage of ulceration was calculated respectively to the ulcerated surface (US) (mm^2) by the following formula:

$$U.S. = \left(\frac{\text{Total ulcerated surface}}{\text{Total surface of the stomach}}\right) \times 100$$

2.6.5 Determination of the mean area of the mice stomach

The mean area of the stomach of the mouse was determined as follows:

- In a number of 10 mice that received any treatment, the stomach was removed and opened.
- On the glandular part of each stomach was placed a millimetered paper soaked in oil (to make it more translucent) and the number of tiles inscribed was counted. The half-tiles were counted as whole but with only one side between the left and the right, and between the top and the bottom.
- The area of each stomach was calculated assuming that each tile was 1 mm^2 in area and the average of the ten

surfaces was finally calculated; which allowed us to find an average surface area of 204.42 mm^2.

2.6.7 Measurement of mucus production

After estimating the degree of lesion formation, the gastric mucosa of each rat was immediately scraped gently using a glass slide and the mucus obtained was weighed using a precision electronic balance. The same experimenter performed this operation each time.

2.6.8 Statistical analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) followed by the Tukey's post-test for multiple comparisons using Graphpad Prism 05 software and p values less than 0.05 were considered as significant. The results were expressed as mean ± standard error of mean (SEM).

3. Results

3.1 Analgesic properties

3.1.1 Acetic acid-induced writhing test

Aqueous extract of *Eremomastax speciosa* strongly reduced writhing and stretching induced by the *i.p*. administration of acetic acid solution. As can be seen on table 1, extract exhibited significant protection at 200 and 400 mg/kg ($p<0.01$ and $p<0.001$ respectively) with maximum percentage inhibition of constrictions of 91.40% observed at 400mg/kg while indomethacin (50mg/kg) had only 83.76% inhibition.

Table 1: Antinociceptive effect of the aqueous extract of *Eremomastax speciosa* on writhing induced by acetic acid in mice.

Groups	Doses (mg/kg)	Number of writhings within 30mn	Inhibition (%)
Control	/	122,16 ± 16,74	/
Indomethacin	50	19,83 ± 5,90***	83.76
Aqueous extract	100	107,33 ± 4,51	12.13
Aqueous extract	200	67,50 ± 5,98**	44.74
Aqueous extract	400	10,50 ± 2,66***	91.40

Each value represents the mean ± ESM of 6 animals. $^{**}p<0,01$, $^{***}p<0,001$ statistically significant compared to control.

3.2 Formalin- induced pain test

The results of this assay are presented in Table 2. There was a significant ($p<0.001$) reductions in response to nociception during the second phase of the pain at all the doses while at the first phase the reduction was not significant. Maximum percentage inhibition of nociceptive effect was 84.61% in the second phase at 400mg/kg. Indomethacin was significantly active (51.95%, $p<0.001$) only on second phase.

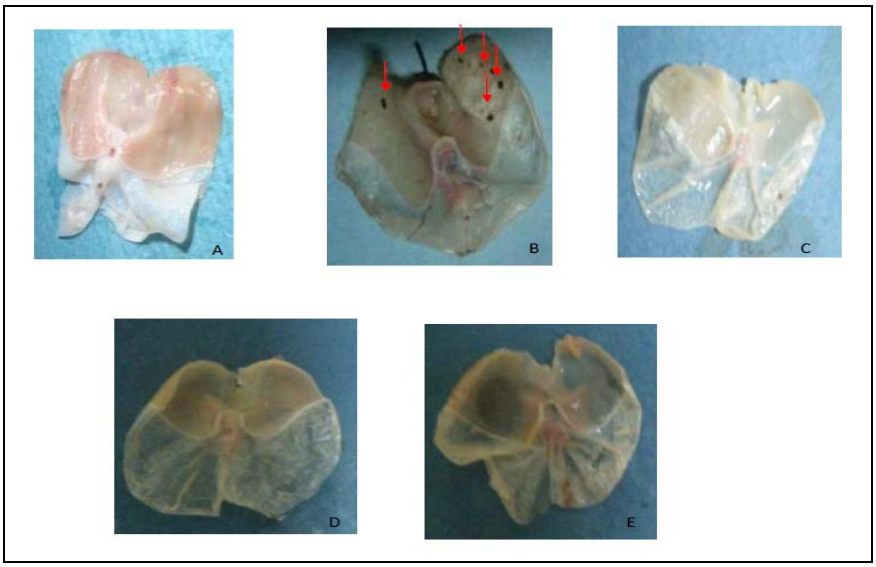
Table 2: Antinociceptive effect of the aqueous extract of *Eremomastax speciosa* on formalin- induced pain in mice

Groups	Doses (mg/kg)	First phase (0-5 mn)	Inhibition (%)	Second phase (15-30mn)	Inhibition (%)
Control	/	267,52± 5,82	/	741,24 ± 33,00	/
Indomethacin	50	259,09 ± 14,77	03.15	356,14 ± 33,01***	51.95
Aqueous extract	100	190,16 ± 19,49	28.91	214,36 ± 81,67***	71.08
Aqueous extract	200	148,92 ± 40,30	44.33	198,16 ± 38,87***	73.26
Aqueous extract	400	143,02 ± 44,21	46.53	114,00 ± 27,10***	84.61

Each value represents the mean ± ESM of 6 animals. $^{***}p< 0,001$ statistically significant compared to control.

3.3 Non-ulcerogenic properties

Figure 1 shows the appearance of the stomach of the mice five hours after the various treatments. It appears that the distilled water as well as the different doses of extract produced no ulceration in opposition to indomethacin, which caused ulcerations with an ulcer index of 2.5 corresponding to an ulcerated surface area of 2.71 mm^2. Moreover, unlike indomethacin, which inhibited mucus synthesis, the aqueous extract of *Eremomastax speciosa* at doses of 200mg/kg and 400mg/kg induced an increase in mucus secretion (Table 3).



(A) Normal control group. (B) Group treated with indomethacin (50 mg/kg) (ulcer control). (C), (D) and (E) Groups treated with aqueous extract of Eremomastax speciosa respectively at 100 mg/kg, 200 mg/kg and 400mg/kg. (→ ulcer indication).

Fig 1: Macroscopic aspect of the gastric mucosa in mice.

Table 3: Non-ulcerogenic effect of the aqueous extract of *Eremomastax speciosa* on the gastric mucosal in mice

Groups	Doses (mg/kg)	Mucus production (mg)	Ulcerated surface (mm^2)	Ulcer index	% of Ulcerated surface
Control	/	12,16 ± 1,32	0,00 ± 0,00	0,00 ± 0,00	00 ± 00
Indomethacin	50	5,00 ± 0,68**	5,54 ± 2,09	2,50 ± 0,60	2,71 ± 1,02
Aqueous extract	100	10,00 ± 1,21	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
Aqueous extract	200	14,33 ± 1,68^{c}	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
Aqueous extract	400	14,50 ± 1,31^{c}	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00

Each value represents the mean ± ESM of 6 animals. $^{**}p<0,01$ statistically significant compared to control and $^{c}p<0,001$ statistically significant compared to positive control (indomethacin).

4. Discussion

The aim of this work was to evaluate the analgesic and non-ulcerogenic properties of the aqueous extract of *Eremomastax speciosa* in mice.

The aqueous extract of *Eremomastax speciosa* significantly decreased the number of abdominal contractions induced by acetic acid (Table 1). Acetic acid-induced writhing test permits to evaluate the peripheral analgesic properties of any substance [15]. Indeed, intraperitoneal injection of acetic acid causes pain by stimulating chemoreceptors (Stai *et al*., 1995) [16] through the activation of ASIC (Acid-Sensing Ionic Channel) channels or by irritating the visceral surface leading to the release of many pain chemical mediators such as histamine, prostaglandins, serotonin, bradykinin [17]. The aqueous extract of *Eremomastax speciosa* would have act by blocking the ASIC channels or by inhibiting the synthesis of prostaglandins and other mediators of pain.

Formalin- induced pain test was performed in order to determine the phase of the pain to which the aqueous extract of *Eremomastax speciosa* acts. Indeed, the injection of formalin beneath the plantar arch of the animal induces pain in two distinct phases: the first phase called the neurogenic or central phase is due to the synthesis of the substance P following the activation of the nociceptors [18], and the so-called peripheral phase is characterized by a tonic nociceptive response exerted by chemical mediators such as prostaglandins, histamine, serotonin, bradykinin [19]. The aqueous extract of *Eremomastax speciosa* significantly inhibited the second phase of formalin-induced pain (Table 2); this indicates that the aqueous extract of *Eremomastax speciosa* would have thus act by inhibiting the synthesis of pain chemical mediators such as prostaglandins, histamine, serotonin, bradykinin via cyclooxygenase (COX) inhibition.

In addition, non-selective nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin or ibuprofen calm the pain by inhibiting cyclooxygenases (COX-1 and COX-2), key enzymes in prostaglandin biosynthesis (PG) [20]. Prostaglandins, by stimulating the production of mucus play an essential role in protecting the stomach against the aggression factors. Inhibition of their secretion reduces the capabilities of the muco-carbon barrier, which exposes the gastric mucosa to ulcerogenic agents. In fact, NSAIDs have a high ulcerogenic potency.

To evaluate the effect of the aqueous extract of *Eremomastax speciosa* on the gastric mucosa, an ulcerogenic test was performed. The aqueous extract of *Eremomastax speciosa* increased the production of mucus (Table 3), which would explain the absence of ulcers in the animals treated with the extract unlike those treated with indomethacin where many points of ulcers were observed (Figure 1). Indeed, COX-1 is an enzyme responsible for the basal synthesis of prostaglandins. This basal synthesis of prostaglandins plays a very precise role in the gastric epithelium, stimulating the secretion of protective mucus from the gastric mucosa, while COX-2 is especially responsible for the synthesis of pain mediators [21]. The aqueous extract of *Eremomastax speciosa* therefore acts by specifically inhibiting the action of COX-2 without inhibiting that of COX-1(Figure 2).

According to Amang *et al* (2014) [22], the aqueous extract of *E. speciosa* contains the following bioactive compounds: tannins, alkaloids, resins, flavonoïds, anthocyanins, phenols, quinones, oils, Sterols, triterpenes, glycosides, amino acids and proteins. Among these bioactive compounds, tannins, flavonoïds, alkaloids, saponins and terpenes have analgesic properties [23].

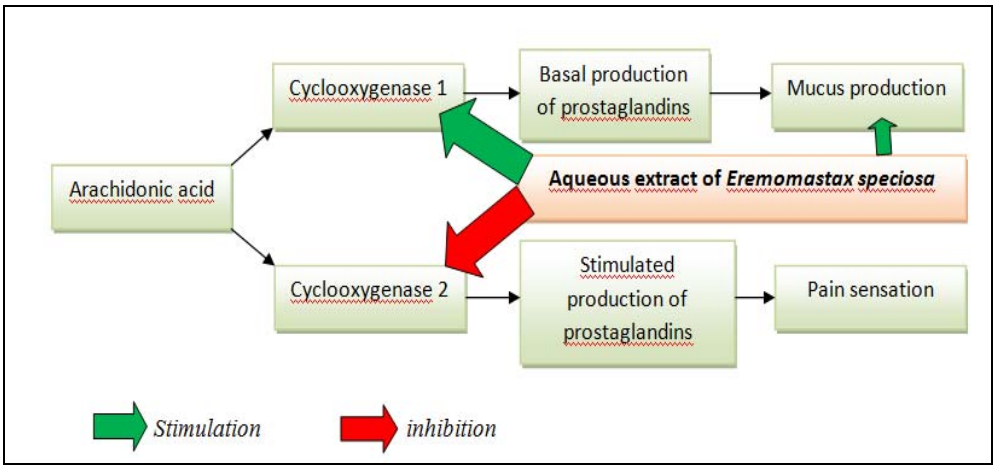


Fig 2: Summary of probable mechanisms of analgesic and non-ulcerogenic activities of aqueous extract of *Eremomastax speciosa*

5. Conclusion

In summary the aqueous extract of *Eremomastax speciosa* showed an analgesic and non-ulcerogenic activity in mice, with an increase in mucus production. Further studies are

needed to determine the chronic effect of that extract on the gastric mucosa.

6. Acknowledgments

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7. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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