

## GASTRIC HEALING PROCESS WITH RAW EXTRACT OF *EUPHORBIA TIRUCALLI* L.: STUDY IN RATS

*Cicatrização gástrica com uso do extrato da Euphorbia tirucalli L.: Estudo em ratos*

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**ABSTRACT - Background** - The use of plants in the prevention and treatment of disease is age-old practice. The aveloz (*Euphorbia tirucalli*) is a plant native of Africa and has been associated with antimicrobial, antiulcers, anticarcinogenic, antiviral, healing, anti-helminthic, antisiphilitic effects. **Aim** - To analyze the effect of the crude extract of *Euphorbia tirucalli* L. in the stomach healing process of mice. **Methods** - Sixteen Swiss mice, adult females were subjected to 1 cm longitudinal incision in the gastric body and sutured with 6-0 polypropylene stitches. After the procedure, the animals were randomly divided into two groups of eight animals. These were redistributed into four subgroups: Aveloz (GA7) and Control (CG7) with programmed death for 7th day postoperatively and Aveloz (GA14) and Control (GC14) with programmed death for 14 days postoperatively. The group GA used 1 ml of hydroalcoholic solution of the crude extract of *Euphorbia tirucalli* at 30 mg/ml orally by gavage route and the CG, 0.9% saline solution at the same volume and route. After death, the inventory of the abdominal cavity was conducted and the stomach removal was performed, fixing in formalin and sent for microscopic analysis. In the comparative analysis between the two groups were evaluated the macroscopic and microscopic parameters of healing. **Results** - There were no signs of peritonitis, fistulas or hematomas in the animals. There were adhesions of the stomach, especially with the liver and omentum in the animals at 7 and 14 days postoperatively in both groups. The analysis of histological parameters showed no statistically significant difference between groups in any of the parameters evaluated. **Conclusion** - The evaluation of the use of the crude extract of *Euphorbia tirucalli* L. on stomach wound healing in mice showed equivalence in comparison to the control group.

**HEADINGS** - Phytotherapy. Stomach. Mouse. Aveloz, *Euphorbia*.

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**RESUMO - Racional** - A utilização de plantas na prevenção e no tratamento de doenças é prática milenar. O aveloz (*Euphorbia tirucalli*) é uma planta originária da África e tem sido relacionada com efeitos antimicrobiano, antiulceroso, anticarcinogênico, antivirais, cicatrizante, antihelmíntico e antisifilítico. **Objetivo** - Avaliar o uso do extrato bruto de *Euphorbia tirucalli* no processo de cicatrização de estômago de camundongo. **Métodos** - Dezesseis camundongos da espécie Swiss, adultos, fêmeas foram submetidos à incisão longitudinal de 1 cm no corpo gástrico e síntese em plano único com pontos separados de polipropileno 6-0. Após o procedimento os animais foram distribuídos aleatoriamente em dois grupos de oito. Eles foram redistribuídos em quatro subgrupos: Aveloz (GA7) e Controle (GC7) com morte programada para 7º dia de pós-operatório e Aveloz (GA14) e Controle (GC14) com morte programada para 14º dia de pós-operatório. No seguimento o grupo GA utilizou-se 1 mL de solução hidroalcoólica do extrato bruto de *Euphorbia tirucalli* L. na concentração de 30 mg/ml por via oral através de gavagem e no GC, soro fisiológico 0,9%, no mesmo volume e via. Após a morte, foi realizado o inventário da cavidade abdominal e procedeu-se a retirada do estômago, fixação no formol e enviado para a análise microscópica. Na análise comparativa entre os dois grupos foram avaliados parâmetros macroscópicos e microscópicos da cicatrização. **Resultados** - Não foram detectados sinais de peritonite, fístulas ou hematomas no animal. Houve aderências do estômago, principalmente, com o fígado e omento, nos animais dos 7º e 14º dias do período pós-operatório nos dois grupos. A análise dos parâmetros histológicos não demonstrou diferença estatisticamente significativa em nenhum dos parâmetros avaliados. **Conclusão** - A avaliação do uso do extrato bruto de *Euphorbia tirucalli* L. em cicatrização de lesões em estômago de camundongos mostrou equivalência em comparação ao grupo controle.

**DESCRIPTORES** - Fitoterapia. Estômago. Rato. Aveloz. *Euphorbia*.

## INTRODUCTION

Brazil is one of the countries with the highest environmental diversity. Nevertheless, the research related to herbal medicines are still below the potential and benefits<sup>1</sup>. Nowadays, a good number of studies are being done related to different areas.

*Euphorbia* species have been described and used in popular medicine as antimicrobial, antiulcers, anticarcinogenic, antiviral, healing, anti-helminthic, antisiphilitic<sup>2-5</sup> and is known in Brazil as "aveloz". Some authors have been studied its healing benefits, and this property is explained by the action of phytoconstituents of this plant, that increases collagen on extracellular matrix, as tannins and flavonoids<sup>6,7</sup>.

The objective of this study was to evaluate the use of the crude extract of *E tirucalli* in the healing process of stomach in mice, emphasizing the macroscopic and histological features.

## METHODS

The study was approved by the Ethics Committee in Research at the Federal University of Maranhão (UFMA) under the protocol nº 00386/2004. It followed the guidelines for animal research as set out by the Brazilian College of Animal Experimentation (COBEA).

The sample was made-up of 16 male Swiss mice (*Swiss Webster*, Rodentia Mammalia) supplied by the UFMA vivarium. There were aged between 40 and 60 days (46 days mean age). The weight was between 24 g and 30 g (25.3g mean weight). The animals were kept in groups of four per standard cage for the species at the Research Laboratory of the Physiology Department where the light-dark cycle was 12 h and humidity was the same as a normal environment without any artificial regulation. The temperature was constant at 20±2 °C. The animals were fed on standard commercial food and had free access to water throughout the experiment.

Each animal was subjected to anesthesia with 2% xylazine (5 mg/kg IM Kensol® and 5% ketamine hydrochloride 22 mg/kg, IM Vetanarcol®) combined in the same syringe.

The animals were divided randomly into two groups of eight mice, one Control Group (CG) and one Group Aveloz (GA). Each group was further divided in two subgroups CG7 and GC14 and subgroups GA7 and GA14, according to the days of death, scheduled for 7 and 14 days postoperatively, respectively.

The surgery followed methodization for animals of both groups. After anesthetized each mouse was positioned supine and immobilized and prepared the surgical field with aseptic technique.

Longitudinal median laparotomy was performed from 1 cm below the xiphoid process, extending over the tail in 3 cm, the dieresis of the skin and subcutaneous tissue was carried out with a scalpel blade nº 15 and while that of aponeurosis, muscle plan and peritoneum were performed with scissors; the abdominal cavity was examined, the identification and exteriorization of the stomach was performed and a longitudinal incision of 1.0 cm in length to its longest axis in the anterior wall of the gastric body was carried out, equidistant from the smaller and larger curvatures involving all plans and lesion suture with four stitches separate using blue polypropylene thread (Prolene® Ethicon) 6-0 75 cm long with non-traumatic circular needle of 1.3 cm. Later, the peritoneum synthesis, aponeurotic muscle plan and skin was performed continuously with black nylon monofilament (mononylon® Ethicon) 5-0 45 cm long with cutting circular needle of 2.5 cm. At this time, analgesia was carried out with dipyrone 25 mg/kg/IM.

After recovery from anesthesia, each animal in the subgroups Control (CG7 and GC14) received 0.9% saline solution by gavage daily. In animals of subgroups Aveloz (GA7 and GA14) were administered 100mg/kg crude extract of *Euphorbia tirucalli* at 100mg/mL by the morning obeying the days previously established for observation. The animals were placed in their cages with free access to food and water, put in the same preoperatively conditions of temperature and brightness. The clinical parameters were evaluated daily.

At the scheduled date of death, the animals received a lethal dose of 2% xylazine (20mg/kg/IM) and 5% ketamine hydrochloride (30mg/kg/IM), both applied combined in the same syringe.

After death, the inspection of abdominal scar was carried out, the presence or absence of hematoma; surgical site infection and wound dehiscence were evaluated. Subsequently, the opening of the abdominal wall by two transverse incisions and one longitudinal connecting the past ones was performed, being distant 1cm to the left of the median scar resulting from the previous surgery. The presence of perigastric adhesions was evaluated by graduating them according to Nair score, but also aspects of the gastric mucosa (8).

The stomach removal was performed. It was fixed in 10% formaldehyde within individual vials and sent to histopathology. The pathologist was unaware of the group corresponding to each vial.

A tetraocular microscope was used in which the histological field of each blade of injury of the gastric mucosa was evaluated using the objective lens of 5, 10 and 40x and ocular of 10x magnification. The histological criteria included: acute inflammation, nonspecific chronic inflammation, fibroblast proliferation, collagen diffusion and wound

epithelialization, classified as mild, moderate and intense according to the gradation of intensity in crosses (x to + + +)

The program Biostat 5.0 (Microsoft) through the the Student t test and Mann-Whitney test were used for data analysis at p <0.05.

## RESULTS

In clinical evaluation, all animals developed similarly, with good dietary compliance and absence of secretions, bulging, retraction, dehiscence or fistula in the wound.

The analysis of the abdominal cavity of the animals under study showed that 25% of CG7 subgroup showed adhesions with omentum and liver, while 50% of GA7 subgroup showed the same change. In subgroups whose death was carried out on the 14th day of observation, such changes mentioned above accounted for 50% in the Control subgroup and 75% of animals in the subgroup Aveloz. There was no indication of peritonitis or other collections during the study.

Regarding the histological evaluation, the main aspect of this study, the results are detailed in Tables 1-3. Adding to the remaining histological evaluation, it can be observed the absence of hemorrhage, glandular atrophy and intestinal metaplasia in all parts analyzed.

Applying statistical methods on the classifying variants obtained from histopathology, there was difference between both subgroups of the 7th and 14th days of aveloz compared with the control group, without being statistically significant as shown in Table 1-3.

**TABLE 1** - Histological evaluation (chronic gastritis, inflammatory activity, mononuclear and polymorphonuclear cells) of gastrorrhaphy healing parameters in animals treated with crude extract of *Euphorbia tirucalli* and control (0.9% saline solution)

Histological variables /p	Days/Grups			
	7 Days		14 Days	
	Aveloz	Control	Aveloz	Control
Chronic gastrites	+ (1)	+ (1)	+ (2)	+ (1)
	++ (3)	++ (3)	++ (2)	++ (1)
	0,124		0,500	
Inflammatory activity	+ (1)	+ (1)	+ (3)	- (1)
	++ (3)	++ (2)	++ (1)	++ (1)
	0,124		0,500	
Mononuclear cells	++ (4)	+ (1)	+ (3)	+ (1)
	0,193		0,281	
	- (1)	- (1)	+ (4)	- (1)
Polymorphonuclear	+ (1)	+ (1)		+ (1)
	+++ (2)	+++ (2)		+++ (2)
	0,281		0,500	

Legend: Variables were classified as absent (-), discreet (+), moderate (+ +) or severe (+ + +), based on the relative intensity of chronic gastritis, inflammatory activity, mononuclear and polymorphonuclear cells by H&E. The number in parentheses is of animals in each classification.

**TABLE 2** - Histological evaluation (giant cell reaction, fibroblast proliferation, angiogenesis, fibrin-leukocyte crust) of gastrorrhaphy healing parameters in animals treated with crude extract of *Euphorbia tirucalli* and control (0.9% saline solution)

Histological variables /p	Days/Grups			
	7 Days		14 Days	
	Aveloz	Control	Aveloz	Control
Giant cell reaction	- (2)	- (1)	- (1)	- (2)
	+ (2)	+ (3)	+ (3)	+ (2)
	0,500		0,281	
Fibroblast proliferation	- (1)	+ (1)	+ (3)	- (1)
	+ (3)	++ (3)	++ (1)	+ (2)
	0,281		0,156	
Angiogenesis	+ (4)	+ (3)	+ (3)	- (1)
	0,124		0,281	
		++ (1)		+ (2)
Fibrin-leukocyte crust	- (2)	- (2)	- (1)	- (2)
	+ (2)	+ (2)	+ (3)	+ (2)
	0,2819		0,500	

Legend: Variables were classified as absent (-), discreet (+), moderate (+ +) or severe (+ + +), based on the relative intensity of giant cell reaction, fibroblast proliferation, angiogenesis, fibrin-leukocyte crust by H&E. The number in parentheses is of animals in each classification.

**TABLE 3** - Histological evaluation (presence of foveolar hyperplasia, lymphoid follicles and reactive/reparative epithelial changes) of gastrorrhaphy healing parameters in animals treated with crude extract of *Euphorbia tirucalli* and control (0.9% saline solution)

Histological variables /p	Days/Grups			
	7 Days		14 Days	
	Aveloz	Control	Aveloz	Control
Foveolar hyperplasia	- (2)	- (1)	- (1)	- (2)
	+ (2)	+ (3)	+ (3)	+ (2)
	0,500		0,281	
Lymphoid follicles	- (2)	- (4)	- (s)	- (4)
	+ (2)		+ (2)	
	0,124		0,124	
Reactive/reparative epithelial changes	+ (4)	+ (4)	- (1)	+ (4)
	0,281		0,500	
			+ (3)	

Legend: Variables were classified as absent (-), discreet (+), moderate (+ +) or severe (+ + +), based on the relative intensity of presence of foveolar hyperplasia, lymphoid follicles and reactive/reparative epithelial changes by H&E. The number in parentheses is of animals in each classification.

## DISCUSSION

The Aveloz, *Euphorbia tirucalli* L., has been used in folk medicine for various therapeutic purposes, such as rheumatism, anti-herpetic, emetic and purgative<sup>9</sup>.

The effect of extract of *Euphorbia tirucalli* L. in healing has not yet been scientifically proven, despite being used in folk medicine as healing for so long. This leads to the proposition of this study, and the experimental model used here. However, several pharmacological properties have been demonstrated in experimental studies as its anti-bacterial effect, molucicidal, anti-herpetic, anti-mutagenic<sup>10-14</sup>.

The gastrotomy was performed on the body, equidistant from the small and large curvatures in a single plane and sutured with three separate stitches, because this technique is usually used in the literature, easy performing and safe. The choice of 6-0 polypropylene suture was based on its strength and tensile strength in the making the suture and little inflammatory reaction and, for these reasons it is often recommended in similar studies.

Suture of the abdominal wall was measured daily. None of the animals showed infection and dehiscence thereof. Various degrees of adhesions were observed and the rate of adhesion increased post-surgery. These facts have also been recognized by other authors, but they did not interfere with the anastomoses that could be analyzed free of complications<sup>15</sup>.

Regarding the choice of animals, mice were elected by the ease of transportation and handling, their high resistance to infections and greater representation to analyze with other studies of similar goals, once small-size animals are most frequent on this type of study, such as Silva *et al.* who researched the healing properties of *Passiflora edulis* (passion fruit) in rats' gastrotomy and Santos *et al.* who used adult rats to test possible pro-regenerative effects of the extract from *Schinus terebinthifolius* Raddi (aroeira)<sup>15,16</sup>.

The periods of mice death on the 7th and 14th day after surgery were chosen so that it could be studied the gastric healing in their later stages, opposing most of the experiments that used the 3rd and 7th days postoperatively such as Batista *et al.* who evaluated the regenerative action of the aqueous extract of *Orbignya phalerata* (babaçu) in gastric lesions, and Silva *et al.* on morphological and breaking strength study of gastrotomy in rats using the extract of *Passiflora edulis*<sup>16,17</sup>.

The route of administration of the extract by gavage was based on the greater ease in performing the daily administrations to avoid a faster absorption, keep the protective action of hepatic biotransformation (first pass), protective factors that would not exist if the parenteral route was chosen, which is the route chosen by some works such as Silva *et al.* who conducted a gastrotomy morphological study on rats and used intraperitoneally extract of *Passiflora edulis*. The concern in maintaining these elements of defense is necessary due to there being few studies on the toxicity of plant derivatives of the target species<sup>16</sup>.

The histopathology study adopted the H&E as a staining method, which is the standard for this type of experiment, such as studies that have adopted as probable healing derivatives the plants: Aroeira, Babaçu, Pião-roxo and passion fruit<sup>14-17</sup>.

Concerning the results obtained, one can infer that there was a 25% greater incidence of adhesions (gastrohepatic and gastroepiploic) in the subgroups of cases compared with the corresponding control subgroups; however, histological analysis showed no statistically significant difference with respect to the

variables studied. Therefore, the fibrotic process that originated the adhesions cited maintains broad and direct relationship with the favoring the gastrotomy healing, even because this improvement was not noticed.

## CONCLUSION

The evaluation of the use of the crude extract of *Euphorbia tirucalli* L. on stomach wound healing in mice showed equivalence in comparison to the control group.

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