August 30, 2020

PharmacologyOnLine

Archives • 2020 • vol.2 • 1-12

COMPARATIVE GASTROPROTECTIVE ACTIVITY OF *Malva pseudolavatera* Webb & Berthel and *Malva sylvestris L*. GROWN IN ECUADOR.

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Abstract

Species of the genus Malva (*Malva pseudolavatera* and *Malva sylvestris*), introduced in Ecuador, are widely used traditionally. However, unlike the species *M. sylvestris*, for *M. pseudolavatera* there is no information on its composition and properties. The species were collected in the province of Chimborazo Ecuador. Aqueous decoction extracts were made which were lyophilized to obtain dry extracts. Gastroprotective activity was studied in two models of ulcers induced by ethanol and NSAIDs (ASA). Male mice with average weights of 30 ± 2 g were used, which were divided into 13 groups of 5 mice, for each of the models. The treatments of aqueous extracts of *M. pseudolavatera* and *M. sylvestris* were administered orally, for seven days, in doses of 1000, 500, 250, 125 and 75 mg / Kg of mouse weight respectively. The degree of gastric protection was compared between the two species studied and it was evidenced that at the dose of 1000 mg / kg, *M. pseudolavatera* presented greater gastric protection than *M. sylvestris*, which was met at lower doses for the two models used. The inhibition percentages of gastric ulcers showed that the positive control treated with Sucralfate reached 87.5% inhibition, while the groups treated with the extracts of *M. pseudolavatera* and *M. sylvestris* at doses of 1000 mg / Kg reached 77 and 72%. inhibition respectively in the induction model alcohol and 84 and 78% inhibition respectively in the induction model NSAID.

Keywords: *Gastroprotective activity, Malva pseudolavatera; Malva sylvestris, Ethanol-induced ulcer, NSAID-induced ulcer*

Introduction

Different species of the Malva genus grow in Ecuador, with M. pseudolavatera being one of the most traditionally used, however, for this species there are no reports in the literature on its composition and properties. For this reason, in this work a comparative study of the species M. pseudolavatera and M. sylvestris is carried out, for which there is abundant information on their chemical composition, medicinal properties and biological activity.

The leaves, roots and flowers of M. sylvestris are traditionally used for the effects: digestive, healing, expectorant, anti-inflammatory, laxative, demulcent, emollient, antidiarrheal, hepatic and normoglycemic. In addition, the flowers are edible [1,2].

As chemical components phenolic acids, flavonoids, tannins, anthraquinone derivatives, and fatty acids are reported [3,4]. Mucilage, small amounts of tannins, anthocyanosides, coumarins and flavonoids, polyphenols, vitamins, sesquiterpenes, diterpenes, and monoterpenes [5-8] are reported for flowers and leaves.

The aim of this work is to: Evaluate the cytoprotective activity on the gastric mucosa of aqueous extracts of the leaves of Malva pseudolavatera and Malva sylvestris,using two models of ulcer induction (Ethanol and non-steroidal anti-inflammatory drugs NSAIDs).

The results of this research allow us to consider the phytotherapeutic potentialities of two species of the Malva genus (Malva pseudolavatera; Malva sylvestris) as sources of plant material for the possible future development of gastroprotective phytomedicines, thus providing scientific data for traditional Ecuadorian herbal medicine.

Methods

Collection, drying and processing of the extracts.

The leaves of M. sylvestris and M. pseudolavatera were collected in the month of November 2019 in the city of Riobamba, province of Chimborazo, at 2750 masl, with coordinates: 1 ° 40'15.5''S 78 ° 38'49.6 ''OR.

A sample of each species was deposited in the GUAY herbarium of the Faculty of Natural Sciences of the University of Guayaquil, Ecuador, where the herbarium numbers were assigned, 13118 M. sylvestris and 13119 M. pseudolavatera. The species were genetically characterized by Sarmiento-Tomalá [9].

The leaves were washed with potable water and dried in a Mettler Toledo oven at 40 ° C, at constant weight, they were crushed in a Pulvex mill with blades at a particle size of 2 mm.

With the dried plant, aqueous extracts were prepared by decoction from 500g in 1L of water. The extracts were filtered hot, stored refrigerated, and lyophilized in Labconco tube laboratory equipment. USES.

Constitution of the test groups and initial weighing of the biomodels.

For each model of ulcer induction, 65 male CD-1 mice, 30 ± 2 g, were selected. of weight, which were acquired at the National Institute for Research and Public Health (INSPI) in the city of Guayaquil, Ecuador.

The biomodels placed in their respective plastic cages, with food, water ad libitum, had an acclimatization period of 10 days, at a temperature between 23 to 25 ° C, relative humidity of 40%, photoperiods of 12 hours and change of bed according to hygienic need.

The initial weight of c / mouse was recorded, prior to the formation of 13 treatment groups (5 u / group), they were marked in the tail for their respective identification: 3 control groups (normal, positive and negative), 5 groups treatment with M. pseudolavatera and M. sylvestris respectively.

The extracts to be administered were prepared by dissolving the calculated amount of the lyophilizate in 3 ml of water, to achieve the required dose, and 0.5 ml was administered to each animal per experimental group.

Induction of gastric ulcer. Ethanol Model

The treatments of aqueous extracts of M. sylvestris and M. pseudolavatera were administered for seven days, orally in doses of 1000, 500, 250, 125, 75 mg / Kg respectively.

On the 6th day after the administration of the treatments and extracts, the food was removed to leave the animals fasting. On the 7th day, 30 minutes after the administration of the treatments and extracts; 0.2 mL of absolute ethanol was administered for a single time as an ulcer inducing agent, 1 hour later the gastrectomy was performed.

The positive control group was administered the gastric mucosa cytoprotective drug (Sucralfate). Treatments are specified in Table 1 [10].

Administration of treatments: AINES model

The treatments of the aqueous extracts of M. sylvestris and M. pseudolavatera were administered for seven days, orally in doses of 1000, 500, 250, 125, 75 mg / Kg of weight of the mice respectively. The mucosal protective drug (Sucralfate) was administered to the positive control group. After 30 minutes post-administration of the treatments, ASA (ulcer inducing agent), 300 mg / Kg, was dosed for seven days. The treatments are specified in Table 2.

Acetyl Salicylic Acid (ASA) was suspended in water to obtain a concentration equivalent to 300 mg / Kg of weight. A volume of 0.25 ml of this solution was administered to each biomodel [11].

Gastrectomy

At the end of the treatments, the mice were sacrificed in compliance with all the ethical precepts involved in using animals for experimental purposes. The principles of the 3 Rs for animal experimentation were followed: Reduce, Refine and Replace. All the animals used received care and attention according to the established international regulations, following Bioethics and Biosafety Standards established by The World Medical Association [12]. Once sacrificed, they were placed on the work table and dissection was performed along the greater curvature and the stomachs were removed. These were washed with 0.9% sodium chloride solution and then placed in watch glass for macroscopic evaluation, with a Zeizz Lumar.V12 Germany model stereoscope.

Macroscopic analysis

The Marhuenda scale [13] was used to establish the degree of injury present in the tissue of the stomach mucosa according to its size and number (Table 3).

The results of the macroscopic analysis were reported as a percentage of inhibition of gastric ulcers; This value was obtained by comparing the score according to the control group scale with the score of the standard group (2). It was calculated with the following formula:

$$\% \text{ Inhibición} = \frac{\text{P. media del grupo control- P. media del grupo patrón}}{\text{P. media del grupo control}} \times 100$$

Microscopic analysis

For histological evaluation, parts of the EtOH and NSAID-induced gastric ulcer tissues obtained from each animal were fixed in 10% formaldehyde for 48 h, subsequently dehydrated in increasing degrees alcohol, clarified in xylene and included in paraffin. Sections (5 µm thick) were taken on the Carl Zeiss microtome, Germany and stained with hematoxylin and eosin. Histological analyzes of these gastric sections were carried out using a NOVEL microscope (10X lens) with a coupled camera model HDCE-50B.

Results

Macroscopic evaluation. Ethanol model.

The evaluation of the degree of ulceration was carried out using the Marhuenda scale. With the macroscopic analysis, different lesions were observed in the stomach mucosa of the biomodels; the results are expressed in figure 1 and table 4

The analysis of the percentages obtained in the calculation of gastric ulcer inhibition is presented in Figure 2.

Macroscopic evaluation. AINES model. Assessment of the degree of ulceration by the Marhuenda scale.

With the macroscopic analysis, it was possible to observe the different lesions in the stomach mucosa of the biomodels, the results are expressed in Figure 3 and Table 5.

The analysis of the percentages obtained in the calculation of inhibition of gastric ulcers is presented in Figure 4.

Histopathological evaluation of ulcerated gastric tissues.

Figures 5 and 6 show the photomicrographs of the histological sections of the stomachs of the treated rats induced and treated with the Malva extracts.

Discussion

Analysis of antiulcer activity. Ethanol model.

In the macroscopic analysis of the degree of ulceration in the ethanol model, different lesions were observed in the stomach mucosa of the biomodels (Table 4). The negative control group presented hemorrhagic ulcers caused by Ethanol, which, according to the Marhuenda scale, represented a score of 7.8, out of a maximum of 8.

In the positive control group, the evaluation of ulcers was 1.0, which confirms that Sucralfate is

effective in protecting the gastric and duodenal mucosa by preventing the production of ulcers. Sucralfate promotes the secretion of mucus and bicarbonate, forming a protective barrier in the mucosa, it also facilitates the release of prostaglandins, decreasing the volume of gastric acid [14].

Alcohol solubilizes gastric mucus, decreases the transmucosal action potential, increases the flow of sodium and hydrogen ions, stimulating the secretion of histamine and pepsin that result in gastric ulceration characterized by multiple hemorrhagic streaks along the glandular part of the stomach [15].

The degree of gastric protection was compared between the two species studied and it was evidenced that at the dose of 1000 mg / Kg, M. pseudolavatera reached a score of 1.2 on the Marhuenda scale compared to 2.2 for M. sylvestris; The degrees of ulceration obtained in lower doses were also compared, verifying that M. pseudolavatera had greater antiulcer activity.

The percentages obtained in the gastric ulcer inhibition calculation (Figure 2), showed that the positive control treated with Sucralfate reached 87.5% inhibition, while the groups treated with the extracts of M. pseudolavatera and M. sylvestris at doses of 1000 mg / Kg reached 77 and 72% inhibition, respectively.

At doses lower than 500, 250, 125, 75 mg / Kg, the extracts of M. pseudolavatera and M. sylvestris presented different percentages of inhibition, demonstrating the antiulcer efficacy of both species. Similar results were reported for Scutia buxifolia by Boligon et al [16].

Histopathological evaluation in gastric tissues with ethanol-induced damage (Fig. 5) shows a dense epithelial ulceration with a maximum presence of inflammatory infiltrates composed of lymphocytes, neutrophils and plasma cells that show epithelial mucosal damage and edema in the negative control group (Fig. 5B).

The submucosa also shows the presence of mild inflammatory infiltrates in the histological study of group B animals. Inflammatory infiltrates obstruct the microvasculature, causing a local decrease in mucosal blood flow and a marked release of factors that damage tissues, such as proteolytic enzymes and leukotrienes, which increase vascular tone, exacerbate tissue ischemia, stimulate the production of reactive oxygen species (EROS), and eventually promote the destruction of vascular tissue. intestinal matrix, leading to a severe degree of tissue necrosis, particularly in the presence of a low luminal pH [17, 18].

The histopathological study in these stomach tissues with pretreatment of Sucralfate (Fig. 5 C) and Malva extracts at the maximum dose (Fig. 5 D, E) showed a reduction in the infiltration of leukocytes, neutrophils and plasma cells and these results corroborate with the result of the ulcer index presented in figure 2. The mode of action of the gastroprotective activity of the Malva species studied may be due to different factors, among which are mentioned: increased activity of the enzymes endogenous antioxidants (CAT and SOD) or for their antisecretory property. Mojzis et al., Have reported that the polyhydroxyphenolic substances present in plants exhibit powerful antioxidant properties, antisecretory activity, stimulating the formation of PGE2, forming an impermeable protective layer on the mucosa, which is why it is thought that they exert this activity [19-22].

Analysis of antiulcer activity. AINES model.

The negative control group presented hemorrhagic ulcers, the degree of ulceration was assessed by the Marhuenda Scale, obtaining a score of 7.4 out of a maximum of 8.

The positive control group presented ulcer formation, its evaluation was 0.8, corroborating the efficacy of Sucralfate in protecting the gastric mucosa. The comparison of the degree of gastric protection between the species under study allowed to verify that at the dose of 1000 mg / Kg, M. pseudolavatera reached a score of 1.2 on the Marhuenda scale compared with 2.2 for Malva sylvestris, in the remaining doses the values of M. pseudolavatera on the Marhuenda scale were lower, which was demonstrated in the calculation of the inhibition of gastric ulcers.

For this model, the positive control treated with sucralfate reached 89.3% inhibition, while the groups treated with extracts of M. pseudolavatera and M. sylvestris at doses of 1000 mg / Kg reached 84 and 78% inhibition, respectively.

At lower doses, the extracts of both species also showed inhibition (figure 4).

Histopathological analysis

The gastric mucosa obtained from the control group presented normal mucosa, submucosa, muscular and serous (Fig 6A). The administration of the ulcerogenic agent AAS presented formation of ulcers with distorted gastric glands and damaged mucous epithelium and cell debris (Fig 6B); however, the co-administration of sucralfate with aspirin protected against these changes (Fig. 6 C), as did the extracts of M. pseudolavatera and M. sylvestris (Fig. 6D and E).

One of the mechanisms by which ASA damages the gastric mucosa is the increased production of nitrous oxide (NO) due to the overexpression of iNOS [23]. NO is a mediator not only of the defense of the gastrointestinal mucosa [24] but also of its damage [25].

Different concentrations of NO have been shown to have completely opposite effects in the same tissue [26]. In general, low concentrations of inducing agents produce low amounts of NO and vice versa [25-28].

The results obtained in this investigation are the first reports for M. pseudolavatera Webb & Berthel.

In general, the development of these preclinical studies provide scientific data of interest that help to demonstrate the use of extracts from the leaves of these two Malva species as sources of plant material for the possible development of waste-protective phyto-products, and thus endorse their employment for traditional herbal medicine in Ecuador.

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Table 1. Treatments used in the evaluation of aqueous extracts of Malvas. Ethanol Model

GROUPS	TREATMENT (1st to 7th day)		TREATMENT 7th day (last)
	Active substance / drug	**Dose mg/kg**	Ulcer inducer (10 ml / Kg) +
1 (normal)	Saline solution	----------	active substance / drug
2 (negative)	Saline solution	----------	Saline solution
3 (positive)	Sucralfate	50 mg/kg	Ethanol (0.2 ml) + Saline solution
4	Aqueous extract M. pseudolavatera	1000 mg/kg	Ethanol + Sucralfate
5	Aqueous extract M. pseudolavatera	500 mg/kg	Absolute alcohol (0.2 ml) + 0.5 ml Malva pseudolavatera extract
6	aqueous extract M. pseudolavatera	250 mg/kg	Ethanol (0.2 mL) +0.5 mL extract M pseudolavatera
7	aqueous extract M. pseudolavatera	125 mg/kg	Ethanol (0.2 mL) +0.5 mL extract M. pseudolavatera
8	Aqueous extract M. pseudolavatera	75 mg/kg	Ethanol (0.2 mL) +0.5 mL extract M. pseudolavatera
9	Aqueous extract of M. sylvestris	1000 mg/kg	Ethanol (0.2 mL) + 0.5 mL extract M. pseudolavatera
10	Aqueous extract of M. sylvestris	500 mg/kg	Ethanol (0.2 mL) + 0.5 mL M. sylvestris extract.
11	Aqueous extract of M. sylvestris	250 mg/kg	Ethanol (0.2 mL) + 0.5 mL M. sylvestris extract.
12	Aqueous extract of M. sylvestris	125 mg/kg	Ethanol (0.2mL) + 0.5 mL M. sylvestris extract.
13	Aqueous extract of M. sylvestris	75 mg/kg	Ethanol (0.2 mL) + 0.5 mL M. sylvestris extract.

Table 2. Tratamientos empleados en la en la evaluación de extractos acuosos de Malvas. Model AAS.

GROUPS	TREATMENT (1st to 7th day)		Induction of Ulcers 1st to 7th day
	Active substance / drug	DOSE mg/kg	Ulcer inducer + Active substance / drug
1	(Normal) Saline solution	----------	0.5 mL saline
2	(Negative) Saline solution	----------	ASA (300 mg / kg) + 0.5 mL saline solution
3	(Positive) Sucralfate	50 mg/Kg	ASA (300 mg / kg) + Sucralfate
4	Aqueous extract M. pseudolavatera	1000 mg/Kg	ASA (300 mg / Kg) + 0.5 mL extract M. pseudolavatera
5	Aqueous extract M. pseudolavatera	500 mg/Kg	ASA (300 mg / Kg) + 0.5 mL extract M. pseudolavatera
6	aqueous extract M. pseudolavatera	250 mg/Kg	ASA (300 mg / Kg) + 0.5 mL extract M. pseudolavatera
7	aqueous extract M. pseudolavatera	125 mg/Kg	ASA (300 mg / Kg) + 0.5 mL extract M. pseudolavatera
8	Aqueous extract M. pseudolavatera	75 mg/Kg	ASA (300 mg / Kg) + 0.5 mL extract M. pseudolavatera
9	Aqueous extract M. sylvestris	1000 mg/Kg	ASA (300 mg / Kg) + 0.5 mL M. sylvestris extract
10	Aqueous extract M. sylvestris	500 mg/Kg	ASA (300 mg / Kg) + 0.5 mL M. sylvestris extract
11	Aqueous extract M. sylvestris	250 mg/Kg	ASA (300 mg / Kg) + 0.5 mL M. sylvestris extract
12	Aqueous extract M. sylvestris	125 mg/Kg	ASA (300 mg / Kg) + 0.5 mL M. sylvestris extract
13	Aqueous extract M. sylvestris	75 mg/Kg	ASA (300 mg / Kg) + 0.5 mL M. sylvestris extract

Table 3. Marhuenda scale.

SCORE	CHARACTERISTICS
0	No injury
1	Fine and scattered hemorrhagic ulcers less than 2 mm in length.
2	A fine hemorrhagic ulcer less than 2 mm in length
3	More than one grade 2 ulcer
4	An ulcer less than 5 mm in length and less than 2 mm in diameter
5	One to three grade 4 ulcers
6	No injury
7	Fine and scattered hemorrhagic ulcers less than 2 mm in length.
8	A fine hemorrhagic ulcer less than 2 mm in length

Table 4. Macroscopic evaluation, evaluation of gastric ulcers by the Marhuenda scale. Ethanol Model

Group Number	Treatment Groups	Assessment: Marhuenda Scale	Level of significance < 0.05
A	Normal	0.0	E
B	Negativo (alcohol)	7.8±0.45	A
C	Positivo (Sucralfato)	1.0±0.7	DE
D	M. P 1000 mg/kg	1.2±0.4	DE
E	M. P 500 mg/kg	1.8±0.4	BCD
F	M. P 250 mg/kg	2.0±1.2	BCD
G	M. P 125 mg/kg	2.2±0.8	BCD
H	M. P 75 mg/kg	2.4±0.9	BC
I	M. S 1000 mg/kg	2.2±0.8	BCD
J	M. S 500 mg/kg	2.2±0.8	BCD
K	M. S 250 mg/kg	2.4±1.5	BC
L	M. S 125 mg/kg	2.8±1.8	B
M	M. S 75 mg/kg	3.0±1.2	B
Legend: M.P = M. pseudolavatera. M.S = M. sylvestris. Different letters indicate significant differences between groups for p <0.05 n = 5			

Table 5. Macroscopic evaluation, evaluation of gastric ulcers using the Marhuenda scale. AINES model

Group Number	Treatment Groups	Assessment: Marhuenda Scale	Level of significance < 0.05
A	Normal	0.0	F
B	Negativo (AAS)	7.4±0,54	A
C	Positivo (Sucralfato) + (AAS)	0.8±0.4	EF
D	M. P 1000 mg/kg + (AAS)	1.2+ 0.4	EF
E	M. P 500 mg/kg + (AAS)	1.4±0.9	EFD
F	M. P 250 mg/kg + (AAS)	1.4±0.54	EFD
G	M. P 125 mg/kg + (AAS)	1.6±0.5	CDE
H	M. P 75 mg/kg + (AAS)	2.0±1.0	CD
I	M.S 1000 mg/kg+ (AAS)	1.6±0.5	CDE
J	M.S 500 mg/kg + (AAS)	1.6±0.5	CDE
K	M.S 250 mg/kg + (AAS)	2.0±0.7	BCD
L	M.S 125 mg/kg + (AAS)	2.2±0.8	BC
M	M.S 75 mg/kg + (AAS)	2.6±0.5	B
Legend: M.P = M. pseudolavatera. M.S = M. sylvestris. Different letters indicate significant differences between groups for p <0.05 n = 5			

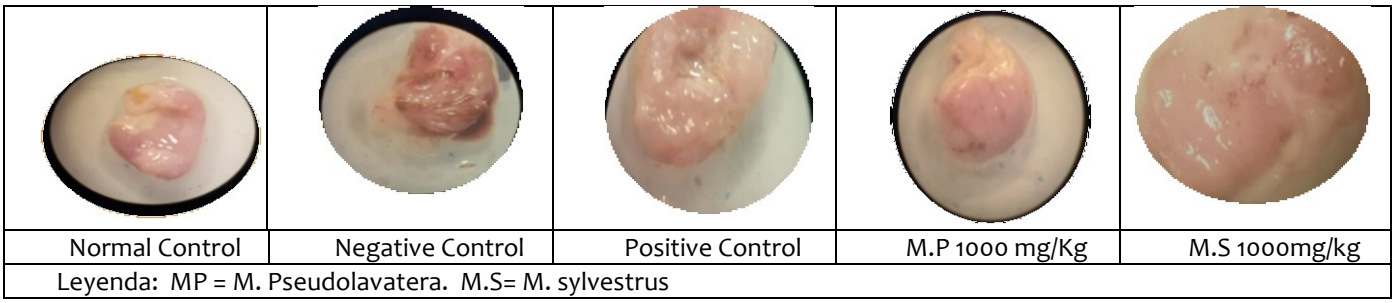

Figure 1. Macroscopic observation of the stomachs of the mice under study. Ethanol Model

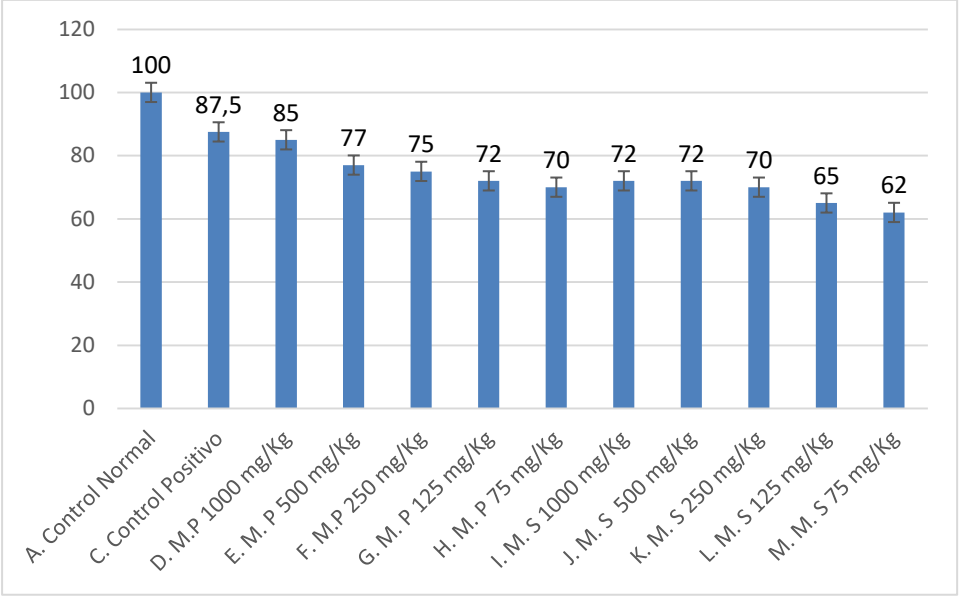

Figure 2. Percentage of inhibition of gastric ulcers: Ethanol model
Legend M.P Malva pseudolavatera; M. S M. Sylvestris

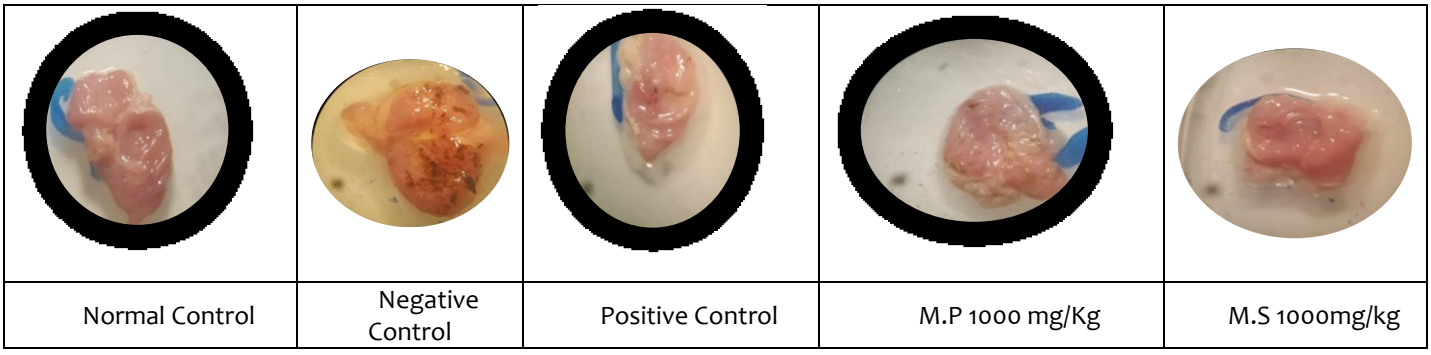

Figure 3. Macroscopic observation of the stomachs of the mice under study. Model AINES

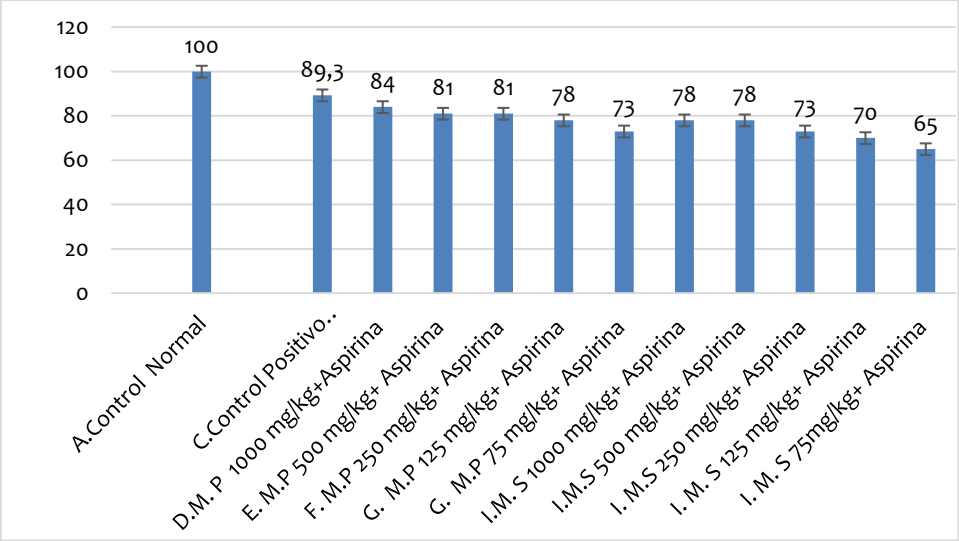

Figure 4. Percentage of inhibition of gastric ulcers: NSAID model
Legend M.P Malva pseudolavatera; M. S M. sylvestris

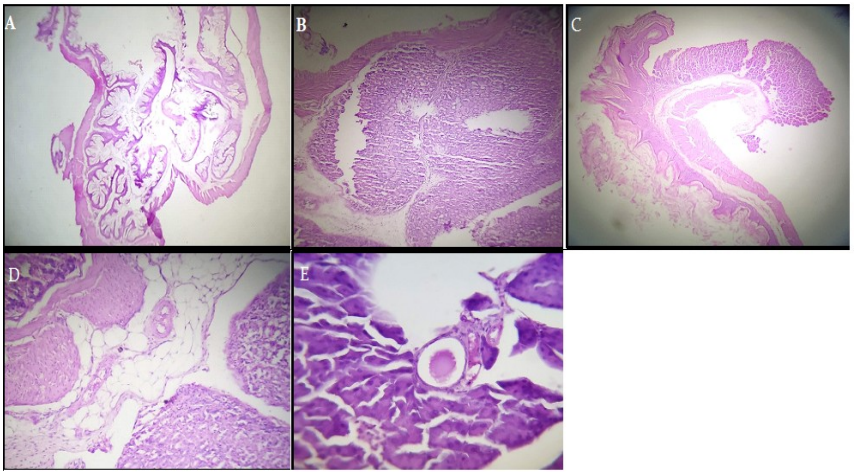

Figure 5. Photomicrograph of the stomachs of rats subjected to gastric ulcer induction by Ethanol. (A) Normal control (B) Negative control (C) Positive control (D) M. pseudolavatera extract 1000 mg / Kg, (D) M. sylvestris extract 1000 mg / Kg.

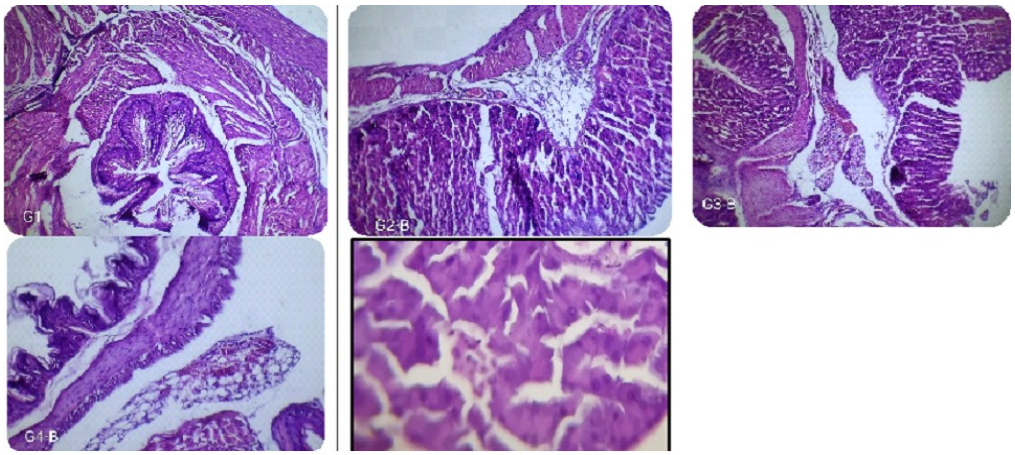

Figure 6. Photomicrograph of the stomachs of rats subjected to gastric ulcer induction by NSAIDs. (A) Normal control (B) Negative control (C) Positive control (D) M. pseudolavatera extract 1000 mg / Kg, (D) M. sylvestris extract 1000 mg / Kg.

ISSN: 1827-8620