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PREVENTIVE ANTI-INFLAMMATORY ACTIVITY OF AN AQUEOUS EXTRACT OF *LARREA DIVARICATA CAV*. AND DIGESTIVE AND HEMATOLOGICAL TOXICITY

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Keywords:

Larrea divaricata, anti-inflammatory activity, nordihydroguaiaretic acid, aqueous extract.

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ABSTRACT: *Larrea divaricata* Cav. commonly known as "jarilla hembra" is used in folklore medicine as anti-inflammatory. There was no scientific evidence justifying the medicinal use of the aqueous extract for inflammation diseases. Nowadays, there are two principal types of anti-inflammatory drugs: Steroidal anti-inflammatory drugs (corticosteroids) and non-steroidal anti-inflammatory drugs (NSAIDs), which exert gastrointestinal ulceration and bleeding. In this work the preventive anti-inflammatory activity of *L. divaricata* aqueous extract (AE) in C3H/He mice was determined by carrageenan- induced inflammation and ear edema tests, also its toxicity on gastric tissues and on blood cells was studied. The chromatographic profile showed: nordihydroguaiaretic acid (NDGA) as majority compound. AE exerted anti-inflammatory action without inducing gastric or blood cell toxicities. Also, it could modulate anti-inflammatory and pro-inflammatory cytokines. NDGA was not the compound responsible of the anti-inflammatory action. This work rescues the potentiality of AE as a gastric and blood cells innocuous anti-inflammatory agent and confirms its ethno-pharmacological uses.

INTRODUCTION: *Larrea divaricata* Cav. (Zygophyllaceae) is a plant that grows in South America and is widely distributed in Argentina. It is used in folk medicine for its anti-inflammatory properties[1]. By other way, the aqueous extract of its leaves, possess well documented antitumoral, antimicrobial and immunomodulatory activities [2, 3] and an antioxidant activity demonstrated on peroxidase secretion in rat salivary glands [4].

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The anti-inflammatory activity of a methanol extract of *L. divaricata* was studied before [5], nevertheless, the anti-inflammatory activity of the aqueous extract, the form that is consumed by population, has not been studied yet in a preventive manner. By other way, nordihydroguaiaretic acid (NDGA), which is a known antioxidant compound, has been described before in this plant [6].

It is known that inflammation is a complex biological response of vascular tissues to harmful stimuli [7]. It can be classified as either acute or chronic. In the acute phase, in the early stages of inflammation, neutrophils, monocytes and eosinophils contribute to cytokines production (TNF-α and interleukin-6 (IL-6)), which spread inflammatory events. By other side, in the

inflammatory process some interleukins can have anti-inflammatory action (IL-4, IL-9 IL-10, IL-11, IL-13, and IL-19) but interleukin-10 (IL-10) is the most widely studied of the anti-inflammatory interleukins related with the suppression of pro-inflammatory mediators [8].

It is important to note that, many diseases have etiological origins in inflammatory processes such as cancer, arthritis, atherosclerosis, and ischemic heart disease. The prevention of inflammation and pain is of significant concern particularly for those afflicted with arthritis and others musculoskeletal ailments. By other way, there are two principal types of anti-inflammatory drugs: Steroidal anti-inflammatory drugs (corticosteroids) which reduce inflammation by binding to cortisol receptors and non-steroidal anti-inflammatory drugs (NSAIDs), which decrease pain by counteracting the cyclooxygenase (COX) enzyme. These anti-inflammatory drugs posses many risks in particular gastrointestinal ulceration, bleeding and hepatic toxicity.

It is known that, NSAIDS can decrease platelets as well as red and white blood cells increasing the risk of bleeding, anemia or infection respectively [9] Meanwhile, corticosteroids can increase hemoglobin and red blood cells, possibly by retarding erythrophagocytosis, effect that is shown in Cushing disease. Also, corticosteroid´s treatment results in increased polymorphonuclear leukocytes in blood as a result of increased rate of entrance from bone marrow and a decreased rate of removal from the vascular compartment. In contrast, the lymphocytes, eosinophils, monocytes, and basophils decrease in number after administration of glucocorticoids due to a consequence of redistribution of these cells, although certain lymphocytes also undergo glucocorticoid-induced apoptosis.

The participation of cytokines, principally an increase in serum TNFα, is well demonstrated in NSAIDS –induced gastric injury in rats after the administration of indomethacin [10].

Consequently, it is important the development of novel anti-inflammatory drugs with minimal adverse effects.

Taken into account, the aim of this study was to determine the preventive anti-inflammatory activity of an aqueous extract from the leaves of *L. divaricata* "*in vivo*" and to study the toxicity on digestive tissues and on hematological cells. Also serum cytokines were analyzed to determine their participation in tissues toxicity. Furthermore, NDGA was studied to determine if it could be the compound responsible for the anti-inflammatory action. This work rescues the potentiality of AE as a gastric and blood cells innocuous anti-inflammatory agent and confirms its ethno-pharmacological uses.

MATERIALS AND METHODS:

Plant material and extract:

Leaves of *Larrea divaricata* Cav. were collected in the province of Cordoba, Argentina and identified using morphological, anatomical and histochemical analysis. A voucher specimen (BAFC Nr 38) was deposited in the Museum of Pharmacobotany, School of Pharmacy and Biochemistry, University of Buenos Aires.

An aqueous extract (AE) of the leaves was prepared as follows: An aqueous extract (AE) of the leaves was prepared at 7.5 %. For this, the air-dried leaves were extracted for 10 min with boiling distilled water, then, the extract was filtered and lyophilized. The final yield was 26.6 g % of plant material. AE was aliquoted and stored at -20°C until used.[11]

HPLC analysis:

The HPLC analysis was performed in a Varian Pro Star instrument equipped with a Rheodyne injection valve (20 µl) and Photodiode array detector set at 280 nm A reversed-phase column Phenomenex – C18 (2) Luna (250 mm x 4.6 mm and 5 µ dp) was used. As mobile phases, water and acetic acid (98:2, mobile phase A) and methanol and acetic acid (98:2, mobile phase B) were employed, The gradient was from 15 % B to 40 % B in 30 min; 40 % B to 75 % B in 10 min; 75 % B to 85 % B in 5 min and 100 % B in 5 min. Mobile phase B was kept at 100 % for 10 min before restoring the initial conditions. Mobile phases were delivered with a flow rate of 1.2 ml/min. The chromatographic procedure was performed at room temperature (18-25ºC). Pure standard of NDGA

(Sigma (USA)) was used for identification and quantification by comparing retention times and by plotting peak areas respectively [6].

Total Polyphenol Determination:

The total polyphenols content was determined by spectrophotometry according to the Folin-Ciocalteu method [12] using gallic acid as standard. The lyophilized extract were weighted and dissolved in distilled water. Briefly, 1.0 ml of this sample extract was transferred to separate tubes containing 7.0 ml distilled water, 0.5 ml of Folin–Ciocalteu´s reagent, and 1.5 ml of a 20% sodium carbonate anhydrous solution. The tubes were then allowed to stand at room temperature for 60 min and then the absorbance at 765 nm was measured by employing a UV-vis spectrophotometer. The concentration of polyphenols in samples were derived from a standard curve of gallic acid ranging from 10 to 50 μg/ml (Pearson´s correlation coefficient: r^2 = 0.9996).

Animals and treatment:

Seven week old male C3H/He mice were mainly provided by Dr. Norberto San Juan (Dep. Microbiology. UBA) and maintained on a standard laboratory diet and water *ad libitum*. Animals were housed and cared for at the Animal Resource Facilities, Faculty of Pharmacy and Biochemistry, National University of Buenos Aires, in accordance with institutional guidelines. The studies were approved by the institutional animal research committee from Faculty of Pharmacy and Biochemistry, University of Buenos Aires, (Number: 220612-1) and were conducted in accordance with the internationally accepted principles for laboratory animal use and care Guide for the Care and Use of Laboratory Animals, U.S. National Research Council 1996.

Mice were separated in three groups. One group of 30 animals received PBS 1 % per oral (control animals) during 15 days, 30 animals were treated with a daily dose of AE 50 mg/kg per oral (this dose was selected taking into account previous studies, corresponding to concentration of AE that exerts antioxidant activity and also is related to the dose used as folk medicine for inflammation) [2], 10 mice were treated with a minor dose 10 mg/kg and 10 animals with a major dose 100 mg/kg of AE. A third group of 30 animals were treated with a daily dose of NDGA 0.29 mg/kg (this dose represents, the approximate amount of NDGA found in the dose administered of the crude extract corresponding to 50 mg/kg). After 15 days of treatment the animals were submitted to different inflammation test.

Carrageenan-induced inflammation:

For this test, ten animals with different treatments (AE 10, 50 and 100 mg/kg and NDGA 0.29 mg/kg) and controls (treated with PBS 1 %) were used. Also, indomethacin (SIGMA) reference drug 10 mg/kg, p.o.) was given to five animals. Mice were treated as described above and the inflammation was induced by injecting 500 μg of carrageenan (Sigma, USA) suspended in PBS in the left hind foot pad. Foot-pad swelling was measured every 90 min during 6 h after induction with a caliper. The difference between the initial and subsequent values gave the actual edema which was compared with control.

The inhibition of inflammation was calculated using the following formula: % inhibition = 100 (Vc-Vt/Vc), where Vc represents mean edema in control and Vt mean edema in group treated with standard/extract [13]

Ear edema test:

Ear edema was induced in groups of 10 treated (50 mg/kg AE) and untreated animals (control) as explained above. Five animals were treated with indomethacin (anti-inflammatory reference drug) as was explained before. The right ear of each mouse received a topical application of 2.5 μg of 12-*O*-tetradecanoylphorbol-13 acetate (TPA) (Sigma, USA).

After 4 h, the animals were sacrificed. Disks of 6 mm diameter were removed from each ear and their weight was determined. The percent inhibition of edema was calculated in comparison with control animals as % of inhibition related to control = [average ear weight control animals - average ear weight treated animals/average ear weight control animals] ×100 [13].

Blood cells determination: Blood was collected from cardiac puncture of ten AE (50 mg/kg) and

NDGA (0.29 mg/kg) treated and untreated animals, into coated EDTA and uncoated containers. Some of the studies were made in whole blood (gathered in tubes with EDTA) (RBC, WBC, and platelets). The remaining studies were conducted in serum (obtained from the exudates of the blood gathered in tubes without EDTA). An automated analyzer Coulter LH 750 was used to determine white (WBC) and red (RBC) blood cells, neutrophil, eosinophil, basophil, lymphocytes and monocyte and platelets. Hemoglobin (HGB) was evaluated by cytomethamoglobin methods using Beckman Model for spectrophotometer.

In serum TNF-α, IL-6 and IL-10 were determined by ELISA utilizing commercial kits (Chemicon International, Billerica, USA) used according to the manufacturer´s instructions. Also, TNF-α was measured in serum of mice treated with NDGA. The concentration of cytokines was determined with standard curves and expressed in pg/ml. Alanine transaminase (ALT) activity (IU/L) was determined in serum of extract-treated or untreated mice using a commercial kit (Wiener Lab. Rosario. Argentina).

Histopathological studies:

Immediately after blood extraction, the animals were sacrificed and autopsied. First, a macroscopic observation of the gastric organs (small and large intestine, liver, glandular and no glandular stomach and esophagus) was done in comparison with those of control animals. Then, the dissected organs were maintained in 10 % formaldehyde saline solution for further histopathological studies. The studies described in this section were performed by medical pathologist Dr Alfredo Molinolo, researcher of CONICET (Argentina). The material was examined macroscopically, cut, dehydrated and clarified according to standard techniques and procedures, and colored with hematoxilin-eosin for further observations under microscope [14].

RESULTS:

Total polyphenol quantification and HPLC analysis:

Firstly, the total polyphenol content was determined to standardize the extract. The extract presented a concentration of polyphenols of 11.395 ± 0.57 mg GAE/ g extract. The chromatographic profile of AE showed a peak corresponding to NDGA (0.30g % w/w) with a retention time at 43.2 min (**Fig.1**).

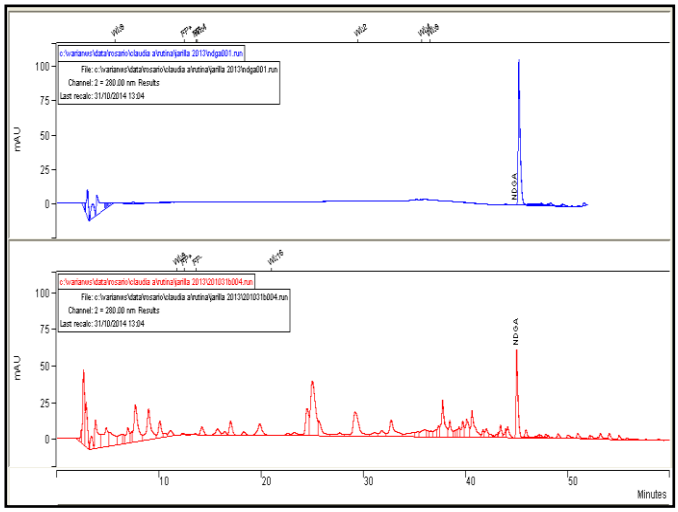


FIG.1: HPLC ANALYSIS OF AE OBTAINED FROM *L. DIVARICATA* CAV. A) Chromatographic profile of NDGA standard. B) Chromatogram of AE.

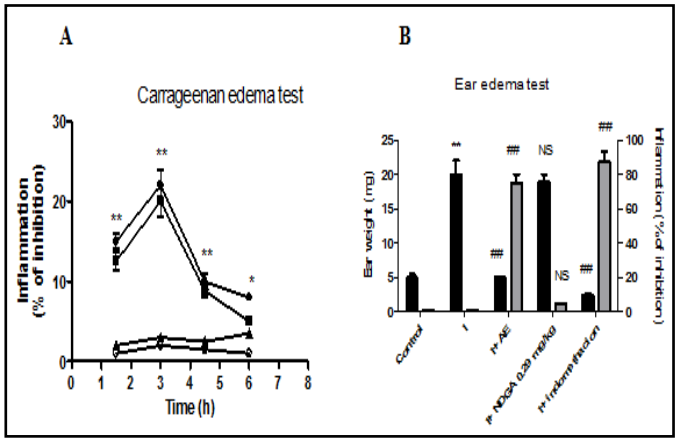


FIG.2: EFFECT OF AE FROM *L. DIVARICATA* ON INDUCED INFLAMMATION. After treatment, inflammation was induced in mice with carrageenan or TPA. Inflammation was determined measuring foot-pad swelling (A) or the ear weight (B) in treated (AE) and untreated (Control) mice. Indomethacin was used as reference anti-inflammatory agent. In A: o AE 10 mg/kg, ■ AE 50 mg/kg, • AE100 mg/kg, ▲ NDGA 0.29 mg/kg. ** $p<0.01$ significantly differences between AE and control in accordance to Student´s T test. In B, Control: control animals without inflammation, I: control animals with inflammation, I+ AE: animals treated with AE 50 mg/kg and submitted to inflammation. I+ NDGA: animals treated with NDGA (0.29 mg/kg) and submitted to inflammation, I+ indomethacin: animals treated with indomethacin and submitted to inflammation, Black column: ear weight and grey column: inflammation, % of inhibition. ** $p<0.01$ significant differences between control and I; ## $p< 0.01$ significant between I and I+ treatments, NS: not significant in accord with Student ´s T test. Results were expressed as mean ± SEM of ten determinations in all case.

Anti-inflammatory activity

First, the effect of AE, in different doses, on carrageenan -induced paw edema was studied. The minimum active dose selected by this model was then studied on ear induced edema. Indomethacin was used as the reference drug in the two tests. It can be seen in **Fig.2A** that, AE 50 and 100 mg/kg exerted a significant anti-inflammatory effect ($p \leq 0.01$) in all measured times in the carrageenan test, but not differences were found among them. By other way, 10 mg/kg of AE did not exert anti-inflammatory activity.

On the other hand **NDGA did not modify TNFα level (Fig.3B)** NDGA, studied at the concentration present in 50 mg/kg of the extract, did not exert anti-inflammatory action. AE 50 mg/kg exerted its maximal activity at three hours. Indomethacin (data not shown in **Fig.2A**) exerted the major activity at 4.5 h (1.5 h: 20± 1 %, 3 h: 30 ± 2 %, 4.5 h: 60 ± 4 %, 6 h: 35 ± 2 % of inhibition). To study specifically the effect of the minimum active dose, 50 mg/kg of extract, was selected to study on arachidonate acid metabolism pathway, so the effect of the extract was studied on TPA ear inflammation model. In **Fig. 2B** it can be seen that, the extract decreased significantly ($p \leq 0.05$) the ear weight and inflammation respect to control ($p \leq 0.05$). **On the other hand** NDGA (0.29 mg/kg) did not exert anti-inflammatory action. The effect exerted by AE was in the order of that exerted by indomethacin.

Toxicity studies:

After AE administration, a toxicity study was done analyzing digestive organs by histological studies and hematological cells by biochemistry determinations respectively. In **Table 1** it can be seen that AE did not modify white and red blood cells, platelets, neutrophils or lymphocytes but decrease significantly blood eosinophils. By other way, hemoglobin was not modified.

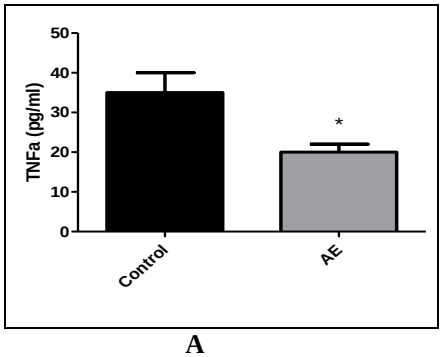
TABLE 1: BIOCHEMICAL BLOOD ANALYZES.

	WBCx10^3	RBCx10^6	HGB	Seg N	EOS	BAS	LYM	MON	PLATx10^3
C	5.8 ± 0.6	7.2±0.6	12.2 ± 1.0	37.5 ± 11	5 ± 0.1	0 ± 0	56.3 ± 8.5	9.0 ± 5.6	727 ± 131.5
AE	4.9 ± 0.8	7.0±0.6	12.3 ± 1.0	26 ±10	2 ± 0.8*	0 ± 0	64.8 ± 7.5	6.8 ± 2.8	88.0± 91.3

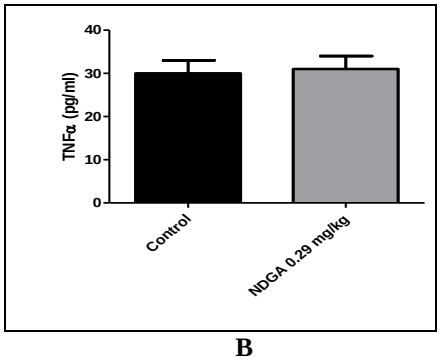
Results are expressed as Mean ± SD of ten determinations. WBC (mm^3): white blood cells; RBC: red blood cells (mm^3), HGB (g/dl): hemoglobin, Ptl (mm^3): Platelets, Bas: basophils (%); Eos: eosinophils (%), seg.N: segmented neutrophils (%), Lym: lymphocytes (%), Mon: monocytes (%).

* P< 0.05 significant differences between EOS from treated and control animals according to Student`s T test.

Then, a histology study was done in order to determine if the treatment with AE (50 mg/kg) during 15 days affected gastric tissues. The histology of the mucosa of gastrointestinal organs of AE treated animals was the same of the control ones, as none of the organs studied presented signals of toxicity (**Table 2 and Fig. inserted**). Also, the extract did not modify alanine transaminase activity (IU/ml), a marker of hepatic functionality (Control: 28 ± 6; AE 50 mg/kg: 20 ± 5; NDGA 0.29 mg/kg: 29 ± 6.8) To complete this study, serum cytokines, from animals treated with AE 50 mg/kg and untreated (control), were determined in order to study if the extract was capable to modify them in relation to digestive tissues toxicity. AE 50 mg/kg decreased significantly TNFα level as well as IL-6 (**Fig. 3A** and **C**) but increased IL-10 level (**Fig.3 D**). By other way, NDGA did not modify TNFα level (**Fig.3 A**).



A



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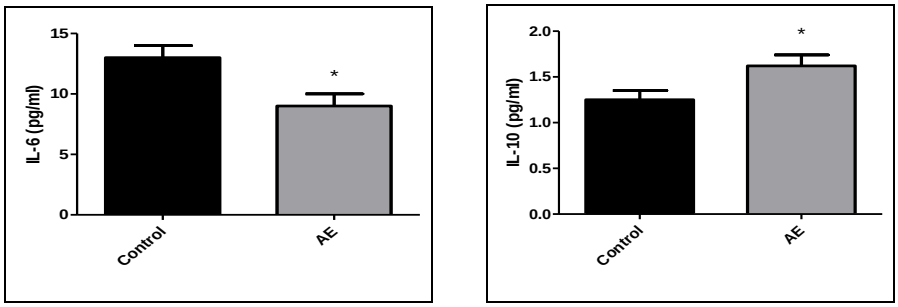


C **D**

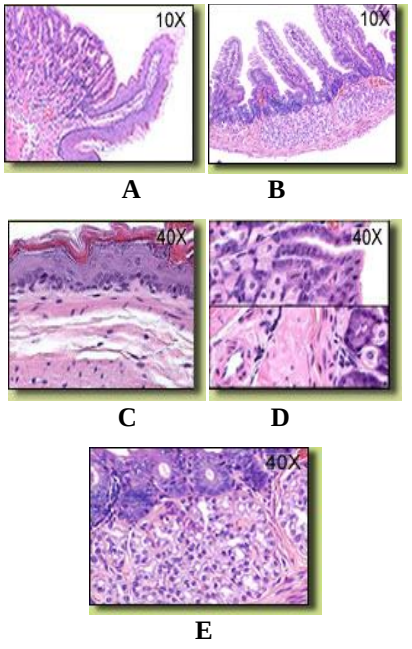
FIG. 3: CYTOKINE DETERMINATION IN SERUM FROM TREATED AND UNTREATED MICE.

Cytokines were determined in serum from mice treated with AE (50 mg/kg) or NDGA (0.29 mg/kg) and untreated (Control). TNF-α in AE treated animals (A), TNF- α in NDGA treated animals (B), IL-6 in AE treated animals (C) and IL-10 in AE treated animals (D).

Results were expressed as mean ± SEM of three experiments made by triplicate. * p< 0.05 significantly differences respect to basal value.* p< 0.05 significantly differences between control and treatment accordingly to Student´s T test.

TABLE 2: HISTOLOGICAL STUDIES OF GASTROINTESTINAL TISSUES.

Animals	Esophagus	Glandular and no glandular stomach	Small and large intestine	Liver
C1	N	N	N	N
C2	N	N	N	N
C3	N	N	N	N
C4	N	N	N	N
C5	N	N	N	N
C6	N	N	N	N
C7	N	N	N	N
C8	N	N	N	N
C9	N	N	N	N
C10	N	N	N	N
AE1	N	N	N	N
AE2	N	N	N	N
AE3	N	N	N	N
AE4	N	N	N	N
AE5	N	N	N	N
AE6	N	N	N	N
AE7	N	N	N	N
AE8	N	N	N	N
AE9	N	N	N	N
AE10	N	N	N	N



C: Control animals **AE:** animals treated with extract**. N:** No significant alterations**.** Ten animals of each group were studied.

Figures inserted: A: Forestomach and glandular stomach of control mice: The glandular mucosa of the glandular stomach separated by the limiting ridge from the stratified squamous epithelium of the forestomach. **B: Small intestine of control mice**: micrographs the villi and crypts formed by the duodenal mucosa are seen. **C**: **Forestomach of AE trteated animals**: micrograph the layers of the stratified squamous epithelium as well as the muscularis mucosae are visible. **D**: **Glandular stomach of AE treated animals**: micrographs depict the various cells present in the fundic glands, showing both the upper parts (with the opening of a gastric pit) and the lower parts (with a fundus (base) of a gland) of these gastric glands. **E: Small intestine of AE treated animals**: micrograph displays the Brunner's glands in detail. The photographs corresponded to a representative animal.

DISCUSSION:

Inflammation is a physiopathological complex response to different stimuli. It can be treated and resolved by acting on different mediators, enzymes, and pathways implicated in the process. This can include the action on the known arachidonic acid metabolism, by inhibiting either certain transcription factors or the production of prostaglandins, by scavenging free radicals, produced during the process, or by acting on the cells implicated in the process, such as macrophages and lymphocytes.

In this work it was demonstrated that the aqueous extract of *L. divaricata* exerted anti-inflammatory action by a preventive action in two inflammatory

models. First it was shown that the extract exerted anti-inflammatory action on the carrageenan model in a dose of 50 mg/kg and 100 mg/kg, no significantly differences were found between them. Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation. It is also used to study non-steroidal anti-inflammatory drugs. It is believed to be triphasic. The first phase (0-1.5h) is mainly mediated by histamine and serotonin; the second phase (1.5 -2.5h) mediated by kinin and the last phase (2.5 h- 6 h) which begins after the kinin phase and is consecutive to the liberation of prostaglandins [15]. The results from this study pointed that *L. divaricata* extract showed significant inhibitory effect on rat paw edema development by inhibiting prostaglandins and kinin as it maximum response was observed in the last phase.

To study specifically the interaction of the extract with the pathway related to prostaglandins and leukotrienes synthesis, the dose of extract selected in the first test, corresponding to 50 mg/kg, was studied on TPA ear induced swelling. It is known that phorbol esters, like TPA induce skin inflammation, and a hyperproliferative response with an infiltration of neutrophils [16]. It is also known that TPA stimulates phospholipase A2 (PLA2) and that consequently a release of arachidonic acid and prostaglandins occurs [16]. The activity of the extract was superior on the ear test than in the carrageenan test. In the ear test the anti-inflammatory effect of AE was similar to that exerted by indomethacin a reference NSAIDs. This last result confirmed, in a preliminary study, that the anti-inflammatory action of the extract could be related to arachidonic acid metabolism.

The anti-inflammatory activity of a methanol extract from *L. divaricata* has been reported before in a model of experimental arthritis, in relation to the elevated free radical scavenging capacity which also contributes to a gastric anti-ulcerogenic or protective effect [5]. In addition, it was shown that a fraction free of NDGA obtained from an aqueous extract decreased nitric oxide level suggesting an anti-inflammatory action in mice [17].

It is known that during anti-inflammatory drug (NSAIDS) treatment blood cells are affected such as platelets, red and white blood cells inducing bleeding, anemia or infections respectively. AE extract did not affect these cells so it did not produce hematological toxicity like many NSAIDS did in short time of treatment. Although, AE did not modify white blood cells such as neutrophils, or lymphocytes it decreased the number of eosinophils. It is known that eosinophils produce interleukin (IL)-3, IL-5 and granulocyte-macrophage colony stimulating factor (GM-CSF) that contributes to their pro-inflammatory functions. If the extract could modulate these cells in basal conditions it could probably do this during inflammatory process but more experiments are needed to confirm this.

Recent evidence indicates that several extracts or products isolated from plant possess anti-eosinophilic activity, for example, aqueous extract of *curcuma domestica*, among others, inhibits gastric erosion inflammation induced by aspirin by decreasing eosinophils [18], By other way chloroform fraction of an ethanol extract of *Ipomoea carnea* flowers showed a significant decrease in circulating neutrophil and eosinophil in blood in a model of toluene diisocyanate-induced inflammation in Wistar rats[19]. In addition, it is demonstrated that NSAIDs causes gastropathies and that patients with rheumatoid arthritis are more susceptible to NSAIDs-induced gastri lesions than other NSAIDs users [20].

The animals treated with AE did not exert any abnormality in gastric organs after 15 days of treatment, suggesting that AE did not affect gastric mucosa as did corticosteroids and NSAIDs. In accordance with these results AE did not modify the activity of the hepatic functionality marker (alanine transaminase). It is known that during digestive toxicity, induced by NSAIDS, some pro-inflammatory cytokines such as TNFα are increased. In basal conditions (animals without inflammation process) AE was capable to decrease TNFα and IL-6 and to increase IL-10 (an anti-inflammatory cytokines).

These results confirmed that AE had not toxicity effects. By other way, if the extract could modulate pro and anti-inflammatory cytokines in basal conditions it could do the same during inflammation process (more experiments are

needed to be done in future to confirm this). In accordance with us some authors showed that several plants regulate the production of pro-inflammatory cytokines in immune system cells [21].

It could be probably that the decrease of eosinophils induced by AE could be related to the increase produced on IL-10 level, as it is known that IL-10 decreases the number of eosinophils in the airways and that intranasal administration of recombinant IL-10 inhibits recruitment of eosinophils in allergic mice [22]. The decrease in eosinophils induced by AE could also be related to the decrease in TNF α level as it was described that, the production of TNF α by basophils is involved in the recruitment of eosinophils from the bloodstream into the skin in a murine irritant contact dermatitis (ICD) as a model[23]. It known that, IL-10 can affect neutrophils recruitment and also decrease TNFα level.

Furthermore, the anti-inflammatory action of AE could be related to its antioxidant activity. It is known that inflammatory events are related to the production of reactive oxygen species at the site of inflammation, so the stimulated polynuclear cells are capable of producing not only cytotoxic but also superoxide anion ($O_2^{\bullet -}$), which can react with other molecules to produce hydroxyl radical ($OH^{\bullet}$), an extremely reactive which it could initiate the lipid peroxidation and in this way promotes inflammation [24]. It is reported that AE possess antioxidant activity on rat's salivary glands [7] and also presents DPPH scavenger activity [25].

By HPLC the majority compound found in AE was NDGA, which is a known antioxidant compound that it was described before in this plant [6].

As NDGA possess low solubility in water the quantity isolated in the aqueous extract was low. Despite the anti-inflammatory properties of NDGA [26], at low concentrations did not exert anti-inflammatory activity in the two models used and moreover did not modify TNF-α level in mice. So, it could be concluded That it was not a principle which participated in the preventive anti-inflammatory activity exerted by AE. In fact, the anti-inflammatory action of AE could be related to The presence of other polyphenols compounds (quantified by Folin Ciocalteu method) principally those with flavonoid structure which have been described before such as O-glycosides of quercitin, myricetin and a C-glycoside of apigenin [26]. It is known that flavonoids exert anti-inflammatory activity and possess free radical scavenger, anti-carcinogenic, and anti-inflammatory activities [27].

CONCLUSIONS: In this work it was demonstrated that an aqueous extract of *Larrea divaricata* exerted an anti-inflammatory activity after an orally treatment in mice without exerting adverse effects on gastrointestinal organs or in hematological cells. Also, AE was capable to modulate eosinophils and both pro and anti-inflammatory cytokines in basal conditions suggesting that it could do the same during inflammation process. Also, it was demonstrated that NDGA was not the compound responsible of the anti-inflammatory activity observed by AE. All this together suggested the potential use of AE as an innocuous anti-inflammatory agent to be used during chronic inflammatory process.

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