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Anti-Inflammatory Activity Evaluation of the Extract and Topical Pre-Formulation of *Morus nigra* Fruits

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Natural products are often a source for bioactive compounds which have great potential for developing novel therapeutic agents. In this sense, the present study aimed to formulate and evaluate a gel containing *Morus nigra* leaf extract and to evaluate its anti-inflammatory (in-vivo) and antioxidant (in-vitro) activity. The anti-inflammatory activity was evaluated using Carrageenan-induced paw oedema. The antioxidant activity is evaluated by the lipid peroxidation inhibition and DPPH radical scavenging method. The results indicated that the extract (oral and topical route) and the blackberry gel (topical route) produced a significant anti-inflammatory activity ($p < 0.05$)

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compared to the untreated control, and similar to the reference drugs (ibuprofen and diclofenac). Regarding antioxidant activity, the *Morus nigra* extract also showed a significant activity in the tested models. Based on the above observations and results, mora gel formulation was found to be a promising anti-inflammatory formulation and further extensive in-vitro and in-vivo studies are warranted to evaluate its safety and biological potency and which may be useful for further clinical applications.

Keywords: Anti-inflammatory activity; mora gel; Morus nigra; blackberry; preformulation.

1. INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed medications for the treatment of inflammatory diseases. In addition, the long-term use of NSAIDs is associated with serious side effects, particularly on the gastric mucosa, renal and cardiovascular systems. Therefore, the search for new drug candidates, especially with local action, is of great importance. The alternative to these drugs are traditional medicines and natural products and their development into drugs for the treatment of inflammatory diseases.

Plant biodiversity offers a wide variety of medicinal plants that could be used as supplements or alternatives to different pathologies. Having medicines obtained from natural sources for different pathologies is a challenge for researchers, however, it is necessary to prove and scientifically endorse the ancestral use that has been given to a certain plant [1].

“Due to their content of healthy phytochemicals, the intake of fruits and vegetables is considered highly protective for human health. There are epidemiological studies that have highlighted the association between the consumption of foods with a high content of phytochemicals, mainly flavonols, phenolic acids and anthocyanin’s, and the prevention of degenerative diseases such as cardiovascular diseases, ageing, cancer and other degenerative disorders” [2,3]. “In addition, these compounds exert an effective capacity as anti-inflammatory agents, by blocking both two essential signalling pathways which serve the important function in the generation of various proinflammatory mediators” [4].

“Morus nigra (BlackBerry) belongs to the genus *Morus* of the *Moraceae* family. Mulberry is widely distributed in all regions from the tropic to the subarctic and from sea level to altitudes as high as 400m. It is native to Southwest Asia, where it has been cultivated for so long that its precise natural range is unknown. The edible fruit dark purple, almost black when ripe, 2-3 cm long, a group composed of several small drupes, has a rich and intense flavour” [5]. “Blackberries are a good source of vitamins and minerals, especially as they contain a large amount of anthocyanin’s” [6]. “In addition to its use as food, mulberry fruits, leaves, and bark have been widely used in traditional medicine in Turkey” [7]. “The berries are used as an anti-inflammatory and to stop bleeding, the bark for toothaches and the leaves as an antidote to snake bites. In Europe, mulberry leaves have recently been used to stimulate insulin production in diabetes” [7]. Regarding the scientific background of the species under study, Yiğit et al. (2008) and Gholam et al. (2004) determined the antioxidant activity of the blackberry fruit and its relationship with the content of polyphenols and flavonoids [5,8].

“Anthocyanins are a group of red, violet, violet, and blue water-soluble polyphenolic pigments widely distributed in berries that can act as antioxidants or free radical scavengers, thus preventing oxidative stress” [9]. “These powerful antioxidants may be responsible for its medicinal properties” [10]. “Many extracts from plants of this family have been proven to possess anti-inflammatory activities in many animal models. The ethanolic extract from the leaves of *Morus indica* showed anti-inflammatory activity on carrageenan induced edema in rats and cotton-pellet granuloma models and antinociceptive properties of the methylene chloride extract of *M. nigra* leaves in mice” [11].

The objectives of this work were a) to evaluate the antioxidant activity and content of polyphenols and flavonoids of the fruits of *M. nigra*, b) to study the oral and topical anti-inflammatory activity of the *Morus nigra* extract and c) to obtain a semi-solid pharmaceutical form (gel). of the fruit of *Morus nigra* and to evaluate its Potential topical anti-inflammatory activity.

2. MATERIALS AND METHODS

2.1 Plant Material

We worked with fruits of *Morus nigra*, a species of the *Moraceae* family. Collected in Yerba Buena, province of Tucumán, Argentina, between the months of August and September 2016 at 516 metres above sea level. The plant has been authenticated in the Botanical Laboratory of Bioquimica, Quimica y Farmacia Universidad Nacional de Tucumán (UNT). Voucher specimens have been deposited in the Herbarium of Cátedra de Farmacoquímica (Facultad de Bioquímica, Química y Farmacia, UNT, Argentina) under number 2016/015.

2.2 Preparation of the *M. nigra* Extract

The extract of *Morus nigra* fruits was obtained by expression of the ripe fruits, the juice obtained was reduced by heating in an oven with an alcoholic environment. The dry extract obtained (Yield: 7.91%) was kept at -8 ° C until use.

2.2.1 Determination of total phenol and flavonoid content

Total phenolic content was estimated by the Folin–Ciocalteu method [12]. Two hundred microlitres of diluted sample were added to 1 ml of 1:10 diluted Folin–Ciocalteu reagent. 4 minutes later, 800 µl of saturated sodium carbonate (7.5 %) was added and after 30 min of incubation at room temperature, the absorbance at 765 nm was measured. Gallic acid (0-10 mg/l) was used for the standard calibration curve. The results were expressed as mg gallic acid equivalent (GAE)/100g dry weight of vegetable material, and calculated as mean value ± SD (n = 6).

Total flavonoids content was determined by the colorimetric [13]. The mixture included 0.5 mL of water caltrop extracts and 0.5 mL of 2% aluminium chloride (AlCl3) ethanol solution. After reaction for 1 hr at room temperature, the absorbance was measured at 430 nm. Quercetin (0-10 mg/l) was used for the standard calibration curve. Total flavonoids contents were calculated as mg quercetin equivalent (mg QE /100g dry weight of vegetable material and calculated as mean value ± SD (n = 6).

2.3 Preparation of Pharmaceutical Gel Base Containing Mora Fruit Extract

Carbopol 934P (10 g) and glycerine (5 ml) were measured. Distilled water and preservative (0.15 g) were then added. The mixture was gradually stirred by means of a stirrer until a cloudy and lump-free dispersion was obtained. It was then allowed to stand to remove air bubbles. Then triethanolamine (q.s.) was added to neutralise the carbopol 934P solution and form the gel [13].

The aqueous extract of *M. nigra* was resuspended in propylene glycol (0.5 ml). This mixture is added to the base gel (25 g) until completely incorporated and homogeneous, stirring manually in a mortar. Finally, solbac is added as a preservative [14].

2.4 Characterization of Mora Gel

The quality controls were based on the experimental study and quantitative determination of the characterization parameters of semi-solid formulations, the main objective being the determination of the adequacy of the physical quality of the formula. The parameters analysed were the following: Organoleptic properties (Corg), Extensibility (ε), PH (ph) and Centrifugation (Cf).

2.4.1 Organoleptic properties (Corg.)

In this case it has been divided into 5 attributes measured by 5 qualitative tests, and it has been considered that all have the same weight in the final radius of the parameter (1/5), because none was considered more prevalent over the rest (based on practical experience in our laboratory) [15].

In the absence of a quantifiable value of each qualitative test, the assignment of a value between 0 and 2 has been directly established in order to contribute to obtaining the radius final value of the organoleptic characteristics properties, and to facilitate the quantification of this radius.

Visual inspection of the sample and overall appearance is performed by placing a sample amount on a glass plate. The radius final value for organoleptic properties parameter is determined by adding together the experimental values (eV) of each of the five following properties: homogeneity, colour, flow, absence of air and texture [16].

2.4.1.1 Homogeneity (for the sample on the glass plate): limit value (eV) = 2

The criteria for maximum homogeneity is no physical discontinuities (oil, water) or no clumps visible in the sample with the naked eye and under a microscope. If small discontinuities appear (only visible under a microscope, not with the naked eye), a value of 1 is assigned. If some discontinuities are visible with the naked eye, a value of 0 is assigned.

2.4.1.2 Colour: limit value (eV) = 2

The colour is checked with the naked eye. If colour is uniform throughout the sample: 2. If there are non-uniform parts, but they are almost imperceptible: 1. If different shades of colour are visible: 0.

2.4.1.3 Flow through a tube or cannula: limit value (eV) = 2

The sample's flow through a cannula with a diameter of 4.80 mm using manual force is studied and its dispersion is observed. If it passes smoothly: 2. If it flows with some difficulty and force is required: 1. If it does not flow or excessive force is required: 0.

2.4.1.4 Absence of air: limit value (eV) = 2

The absence of air is checked with the naked eye. If there is absence of air with the naked eye and under a microscope: 2. If there is presence of air (only visible under a microscope, not with the naked eye): 1. If there is presence of air with the naked eye: 0.

2.4.1.5 Texture (on glass): limit value (eV) = 2

If the texture is as expected and it can be spread properly: 2. If the texture is not as expected and it is difficult to spread: 0. The value of the radius is calculated by adding together the organoleptic valuations with the following formula: So the maximum value of the radio will be 10 and the minimum value 0.

2.4.2 pH measurement

For pH verification, the pH meter was used, with a previously calibrated apparatus with standardised solutions with pH = 7 and pH = 4, the data were subjected to analysis of variance.

2.4.3 Extensibility (ε) or spreadability analysis

Determination of spreadability was evaluated by placing on a slide 20 mg of each of the formulations to be tested on which another slide of known weight is applied, waiting one minute and recording the diameter of the circle formed on a graph paper (Fig. 1). The experiment continues with the same procedure, always at one minute intervals, using increasing weights (2 gr, 5 gr and 10 gr) [16,17]. The radii are calculated and with them the corresponding surfaces. Said determinations are made in triplicate for each sample, calculating the mean value based on the weight on the slide.

2.4.4 Centrifugation (Cf)

Approximately 5 g of sample is weighed in a centrifuge tube at a speed of 5000 rpm for 15 minutes. If phase separation occurs, the test is repeated, reducing the speed to 1000 rpm. The results for this parameter are determined using the values obtained after centrifugation. The values fluctuate between 0 -10, and were assigned as follows [16]:

With no phase separation at 5000 rpm/15 min: 10
With no phase separation at 1000 rpm/15 min: 5
With phase separation at 5000 rpm/15 min: 0

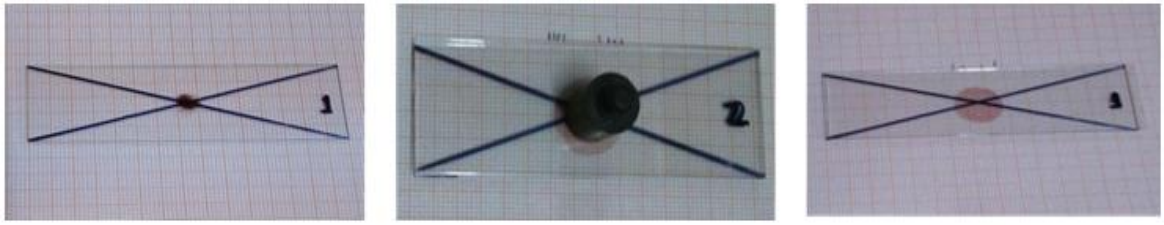

Fig. 1. Evaluation of the extensibility of the mora gel

2.5 Stability Analysis of Mora Gel

The stability of the semi-solid formulation was evaluated under standard conditions and under stress conditions (high and low temperatures). Finished product: base gel (without blackberry extract) as control, and blackberry gel, under room temperature conditions [16].

- At 24 hours: the parameters were evaluated 24 hours after manufacturing.
- At 30 days: Finished product controls at 30 days after manufacturing.

Stress conditions: the mora gel, is someted to different temperature changes (internal laboratory conditions for stress assays).

- Condition 1: 30 days at 30 °C (The sample is submitted to the Accelerated condition)
- Condition 2: 30 days at 8°C (The sample is submitted to a low temperature).

2.6 Biological Activities Evaluation

2.6.1 Antioxidant activity

2.6.1.1 DPPH scavenging activity

The antioxidant activity was assessed by the measurement of the scavenging ability of extracts towards the stable free radical 1,1 diphenyl -2-picrylhydrazyl (DPPH). After dissolving the samples in ethanol, aliquots of 0.75 ml of extracts of *Morus nigra* fruits, the mixtures were vigorously shaken and left to stand at room temperature and were added to 0.25 ml of an ethanol solution of DPPH (300 µM) [18].

Absorbance at 517 nm was measured versus ethanol as a blank. Quercetin, a natural antioxidant and butylated hydroxyl toluene (BHT), a synthetic antioxidant, were used as reference solutions (n=6). Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the equation:

AA % = (Abs control – Abs simple/ Abs control) x 100

2.6.1.2 β-carotene bleaching method

The antioxidant activity of extracts of *Morus nigra* were evaluated using the β–carotene–linoleate model system, as described by [19]. Two milligrams of β–carotene were dissolved in 10 ml chloroform and 1 ml of β–carotene solution was mixed with 20 mg of purified linoleic acid and 200 mg of Tween 20 as an emulsifier. These emulsions were transferred into different test tubes containing 0.2 ml of *Morus nigra* fruits extract (0.5 and 15 mg/ml) and 0.2 mg/ml of the reference antioxidants (Quercetin and BHT). Absorbance at 470 nm, which was regarded as to, was measured immediately against a blank consisting of the emulsion without β–carotene. The caped tubes were placed in a water bath at 50°C and the absorbance was measured every 20 min up to 120 min. All samples were assayed in triplicate. The antioxidant activity (AA) was measured in terms of successful bleaching of β–carotene by using the equation:

$$AA = [1- (A_0 – At / A_{00} – At_0)] \times 100$$

Where A_0 and A_{00} were the absorbance values before incubation for test sample and control, respectively. At and At_0 were the respective absorbances of the test sample and the control after incubation for 120 min. The results were expressed as % of the prevention of bleaching of β–carotene.

2.6.2 Anti-inflammatory activity study

2.6.2.1 Animals

Male Wistar rats (weighing 190–240 g) used for this study were obtained from the Bioterio de la Facultad de Bioquímica, Química y Farmacia, Instituto de Biología (INSIBIO), Universidad Nacional de Tucumán. The rats were first left for 7 days to acclimate to laboratory conditions. All animals were kept under normal laboratory conditions of humidity, temperature (25 ± 1°C) and light (12hs dark/light cycle), and allowed free access to food and water ad libitum. The studies were conducted in accordance with the internationally accepted principles for laboratory animal use and care (EEC Directive of 1986; 86/609/EEC). All the experimental protocols were approved by the institutional committee for the care of laboratory animals of the National University of Tucumán (CICUAL).

2.6.2.2 Carrageenan-induced hind paw edema in rats

Paw edema was induced in rats by carrageenan injection 0.1 ml of 1.5 % (w/v) into the sub plantar region of the right hind paw of the rats according to the method described by [20]. All rats (six per group) were given free access to food and water after the sub plantar injections. Control group rats received saline solution [0.9 % (w/v) NaCl] (2 ml/kg) and the reference group received 100 mg/Kg ibuprofen, orally and diclofenac gel 2% topical administration (200 µl / kg). The test groups of rats were treated orally with 500 and 1000 mg / kg of the extracts of *M. nigra* and, by topical administration 200 µl / kg of extract and mora gel 4%, 30 min before the carrageenan injection. The paw volume was measured before administering carrageenan (Vo) and 1, 2, 3, 4 and 6 hs after (Vt). Inflammation was calculated as the increase in volume (ml) of the paw after treatment subtracted from the basal volume. Results were expressed as percentage of inhibition of oedema, calculated according to the following formula, [(Vt−Vo)/Vo] × 100 [21].

2.7 Statistical Analysis

Data obtained from animal experiments were expressed as the mean and standard error of the mean (mean±S.E.M.). Statistical differences between the treated and the control groups were evaluated by ANOVA and Dunnett's tests. The criterion for statistical significance was $p < 0.05$.

3. RESULTS

3.1 Characterization of Mora Gel

The mora gel obtained presented a consistent, shiny, dark purple appearance due to the colour of the extract, without apparent loss of its structure. Table 1 shows the results obtained from the characterization and the stability of the blackberry gel.

The results expressed in Table 1 indicate that the organoleptic characteristics obtained are acceptable for the natural product. The colour is characteristic since the extract used for this preparation is dark purple. The smoothness to the touch is good and no lumps are observed.

The values of pH, extensibility and centrifugation do not vary significantly in the recent preparation, in the gel at 30 days and under stress conditions, being within the limit values. This suggests that the designed gel would be suitable for topical use.

3.2 Quantification of Total Polyphenols and Flavonoids

The results of the experiments showed that the total amount of flavonoids was 9.200 ± 0.715 mg QE/ 100 g m.v. The content of polyphenols determined for the extract of blackberry was 15.950 ± 2.530 mg GAE / 100 g m.v.

3.3 Antioxidant Activity

The antioxidant activity of the *M. nigra* extract was examined in comparison with the activity of known antioxidants such as BHT and quercetin by the following two in vitro tests; DPPH radical inhibition and β-carotene bleaching method. In the case of the DPPH method, the *Morus nigra* extract showed a significant free radical scavenging activity, compared to the negative control (ethanol 96°). To get an idea of the antioxidant power of the extract compared to the reference antioxidants, the inhibitory or bleaching concentration 50 (IC50) is calculated, obtained by plotting the percentage of bleaching activity as a function of concentration. The blackberry extract was able to reduce the stable free radical DPPH with an IC50 of 4.29 mg/ml. The antioxidant activity of BHT and Quercetin was significantly higher, with considerably lower IC 50 values (2.00 and 80.00 µg/ml, respectively) (Fig. 2).

Fig. 3 shows the decrease in absorbance of β-carotene emulsion in the presence of the extracts and the reference antioxidants (BHT and Quercetin, 1 mg/ml). The addition of 80 and 160 mg/ml of *Morus nigra* extract were significatively effective in inhibiting the oxidation of linoleic acid and subsequent bleaching of β- carotene, in comparison with the control ($p < 0.05$). The percentages of activity were 75.30 % and 85.00 % respectively, compared to positive controls BHT (95.00 %) and Quercetin (93.00 %). The results indicated that the extracts of mora were an effective antioxidant in a β-carotene linoleic acid model system.

Table 1. Characterization of the blackberry gel compared to the base gel (without the addition of the blackberry extract) under standard conditions (SC) and stability test under stress situation (SS) or accelerated conditions

Parameters	Limit value	Experimental value				
		Base gel (SC)	blackberry gel (SC)		blackberry gel (Stress conditions)	
			24 Hs	30 days	30 °C	8 °C
Organoleptic properties	0 - 10	8.0 (±1.0)	7.0 (±1.5)	8.0 (±2.0)	7.0 (±1.0)	8.0 (±1.5)
PH	5 a 7	5.0 (±0.5)	5.0 (±1.0)	5.0 (±1.0)	6.0(0.5)	5.5 (±0.5)
Extensibility (5 g)	100	68 (±3.0)	50 (±10)	55 (±15)	60 (±10)	55 (±5.0)
Extensibility (10 g)	200	136 (±12)	132 (±18)	140 (±10)	150 (±5.0)	138 (±15)
Extensibility (15 g)	250	176 (±16)	160 (±20)	170 (±15)	185 (±10)	180 (±12)
Centrifugation	0 a 10	5.0 (±1.5)	5.0 (±1.0)	5.0 (±0.5)	5.0 (±1.0)	5.0 (±2.0)

Values are expressed in mean±S.E.M. (n = 3)
There were no significant differences between the groups (p > 0.05)

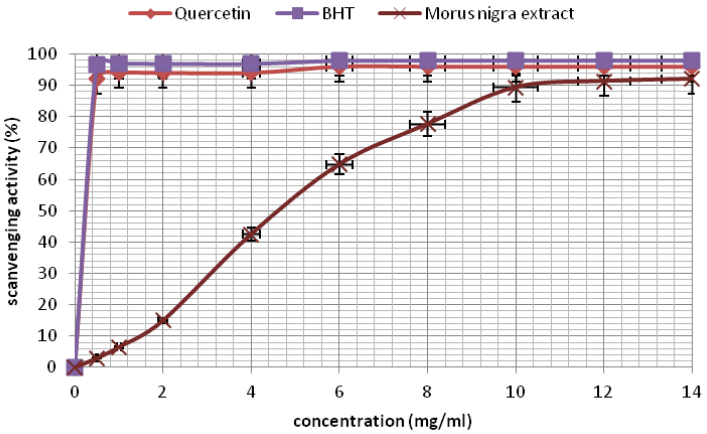

Fig. 2. DPPH radical scavenging activity of the extract of *Morus nigra*. Quercetin and BHT were used as reference antioxidants
Values represent the mean ± S.E.M. (n=6)

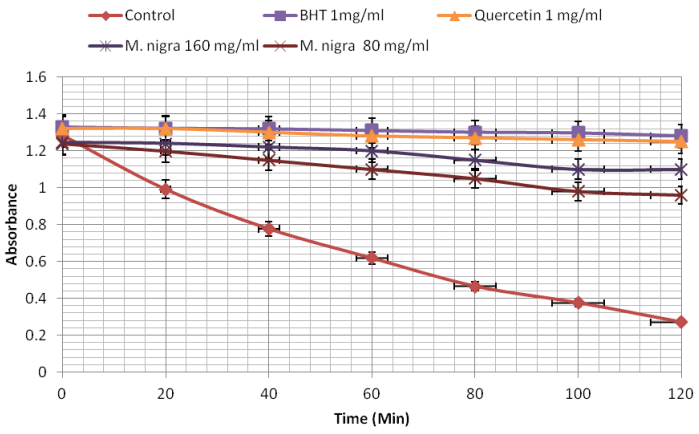

Fig. 3. Antioxidant activities of *Morus nigra* measured by b-carotene bleaching method, Quercetin and BHT were used as reference antioxidants
Values represent the mean ± S.E.M. (n=6)

Table 2. Effect of extract and mora gel 4% of *Morus nigra* fruits on edema carrageenan-induced rat paw

Group (n=6)	Dose (mg/kg)	Paw edema vol in ml (Mean ± S.E.)						
		Administration	0 H[a]	1 H[a]	2 H[a]	3 H[a]	4 H[a]	6 H[a]
Control	Sol. Fis.	oral	1.70 ± 0.10	2.30 ± 0.05	2.40 ± 0.10	2.45 ± 0.05	2.20 ± 0.10	2.10 ± 0.20
Ibuprofen	100	oral	1.70 ± 0.05	1.75 ± 0.15 (*)	1.80 ± 0.10 (*)	1.85 ± 0.10 (*)	1.85 ± 0.15 (*)	1.75 ± 0.05 (*)
Aqueous Extract	500	oral	1.65 ± 0.05	2.15 ± 0.15	2.30 ± 0.05	2.05 ± 0.10 (*)	1.90 ± 0.15	1.85 ± 0.05
Aqueous Extract	1000	oral	1.60 ± 0.10	1.90 ± 0.15	1.90 ± 0.10 (*)	1.95 ± 0.15 (*)	1.95 ± 0.15	1.90 ± 0.10
control	base gel	topical	1.70 ± 0.10	2.35 ± 0.05	2.30 ± 0.10	2.35 ± 0.05	2.15 ± 0.15	2.10 ± 0.15
Diclofenac gel 2%	100 µl/kg	topical	1.60 ± 0.05	1.70 ± 0.10 (*)	1.80 ± 0.05 (*)	1.75 ± 0.15 (*)	1.80 ± 0.10 (*)	1.90 ± 0.15
Aqueous Extract	500 µl/kg	topical	1.60 ± 0.05	1.95 ± 0.15	1.85 ± 0.05 (*)	1.90 ± 0.10 (*)	1.85 ± 0.10 (*)	1.90 ± 0.10
Mora gel 4%	200 µl/kg	topical	1.65 ± 0.05	1.85 ± 0.10 (*)	1.75 ± 0.10 (*)	1.75 ± 0.15 (*)	1.80 ± 0.20 (*)	1.85 ± 0.10 (*)

s.s.: saline solution

Values are expressed in mean±S.E.M. (n = 6).

[a] Time after carrageenan injection (h).

** Statistically significant from the control group: p < 0.05.*

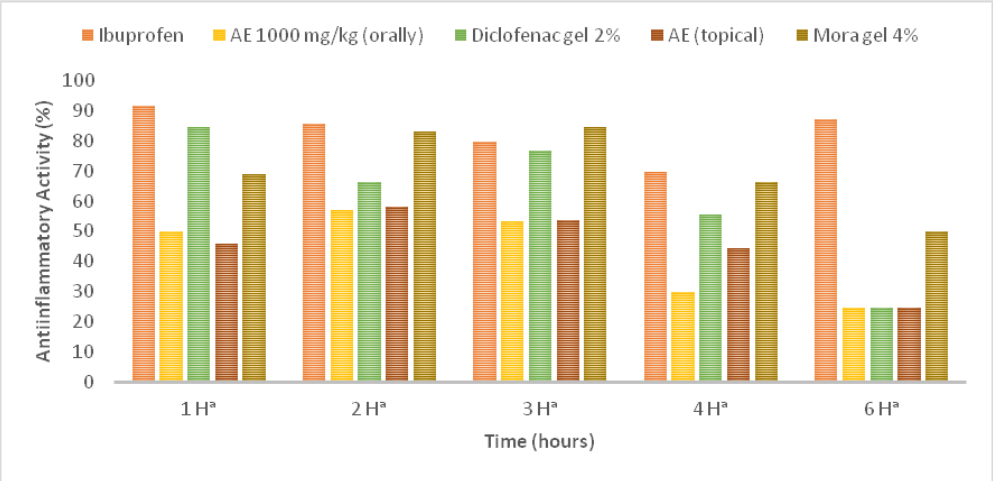

Fig. 4. Anti-inflammatory Activity percentage of extract and mora gel 4% of *Morus nigra* fruits on edema carrageenan-induced rat paw

3.4 Anti-inflammatory Activity

3.4.1 Carrageenan-induced rat paw edema

In the carrageenan-induced edema test, the average right back paw volumes by the extracts and standard drug are shown in Table 2. For the control group, the injection of the phlogistic agent caused localised edema starting at 1.0 h after injection. The swelling increased progressively to a maximum volume of 2.45 ± 0.05 ml at 3.0 h after the carrageenan injection.

Pre-treated rats with the gel 4% of *M. nigra* (topically), had significant reduction of the edema 1.0 h post-dosing. This behaviour is similar to the standards, ibuprofen (100 mg / kg, oral administered) and diclofenac (2% topical administration).

The *Morus nigra* extract, obtained by expression of the fruits, showed a significant activity in both routes of administration (oral and topical) 2 hours after the administration of carrageenan, lower than the activity shown for the *M. nigra* gel (Fig. 4).

4. DISCUSSION

“Inflammation is defined as a set of physiological defence mechanisms taking place in the body. However, inflammation is also considered an initial event of major chronic diseases such as cardiovascular, autoimmune, eye, age-related, neurodegenerative diseases, and cancers” [22]. Despite the great development in the field of synthetic drugs in recent years, they have been found to have some or the other side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents.

“Inflammation, as the most important response of a damaged tissue which is observed in many inflammatory disorders, is characterised by redness, edema, heat, and pain at the site of injury” [23]. “During the inflammatory process, the sensitivity of nociceptors is heightened and the threshold of pain is decreased” [24].

“It is well known that carrageenan induced paw edema is characterised by biphasic events with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play roles, while in the second phase (3 – 4 h after carrageenan injection). Kinin and prostaglandins are involved” [25]. Our results revealed that topic administration of mora gel inhibited the oedema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation. Our results confirmed that topical administration of *M. nigra* showed superior antiedematous activity compared to systemic administration in the same experimental model of inflammation.

These findings are in agreement with previous studies of *M. nigra*. Chen et al. [26] reported that

"the total flavonoid extract of fruits can dose-dependently inhibit xylene-induced ear edema (edema rate 60.1% at a concentration of 200 mg/20 mL/kg) and carrageenan-induced paw edema (edema rate 9.5% at a concentration of 100 mg/20 mL/kg; 8.6% at a concentration of 200 mg/20 mL/kg) in mice. Levels of pro-inflammatory cytokines including IL-1β, TNF-α, NO, and interferon-gamma (IFN-γ) were also significantly decreased after the treatment of *M. nigra* fruit extract in mice with xylene-induced inflammation. In addition, *M. nigra* fruits extract significantly reduced levels of NO in LPS-stimulated RAW 264.7 cells without showing the cytotoxicity effect at the concentration of 50 to 100 µg/mL".

"Several studies have shown that an intraplantar injection of carrageenan leads to an increase in the expression and release of several cytokines, for example, TNF-α and IL-1β, which in turn cause the release of more proinflammatory mediators, including IL- 6, kinins, leukotrienes, arachidonic acid metabolites, and reactive oxygen species" [27,28]. "The antioxidant activity demonstrated in this work could contribute to the neutralisation and protection of membranes from the action of these reactive oxygen species. Many researchers are interested in the antioxidant activity of naturally-occurring ingredients because phenolic compounds and flavonoids, the largest phytochemical molecules from natural resources, possess a variety of biological properties including antioxidant activity" [29]. "It has also been widely reported that mulberries are rich in anthocyanin constituents having remarkable antioxidant activities and other health benefits such as anti-inflammatory, antimicrobial, anti-obesity, antidiabetic, anti-hyperlipidemic, antihypertensive, cardioprotective (reduced risk of coronary heart disease and stroke), and anticancer effects" [30,31]. "Numerous researches have proven antioxidant properties of *M. nigra* with different in vitro methods, including DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and β-carotene bleaching assay" [32].

5. CONCLUSION

From the results obtained, it was concluded that the extract applied orally and topically and the blackberry gel significantly reduced the edema induced by carrageenan. The extract also showed significant antioxidant activity in the models tested. These findings, added to the excellent organoleptic, pH, extensibility and stability characteristics of the preform designed for topical use. They suggest that *M. nigra* can be used as a promising resource to control and prevent various inflammatory processes, both local and systemic. Since most of the research is carried out in vitro and in animal models, in-depth pharmacotechnical and toxicological studies are required in order to advance in clinical trials to establish the efficacy and safety of *M. nigra* in humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The studies were conducted in accordance with the internationally accepted principles for laboratory animal use and care (EEC Directive of 1986; 86/609/EEC). All the experimental protocols were approved by the institutional committee for the care of laboratory animals of the National University of Tucumán (CICUAL).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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