THERAPEUTIC POTENTIAL OF ORGANIC UNIFLORAL ATAMISQUI HONEY (*ATAMISQUEA EMARGINATA*): *IN VITRO* AND *IN VIVO* BIOLOGICAL EVALUATION

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ABSTRACT

The present study was carried out to investigate the medicinal properties and safety of organic unifloral atamisqui honey (*Atamisquea emarginata*). We evaluated the antioxidant antinociceptive and anti-inflammatory activities of honeys with a previous quantitative analysis of some phytoconstituents such as total phenolic compounds and flavonoids. Safety was evaluated with the acute toxicity test. The unifloral atamisqui honey (UAH) presented an important antioxidant activity with values higher than 90% (from 400 μg / ml) in both methods (DPPH and β-carotene bleaching) and similar to the positive patterns (BHT and quercetin). In addition, UAH exhibited a significant anti-inflammatory activity in the acute and chronic inflammation models. The anti-inflammatory effect was greater in animals treated with both doses 14 days prior to the trial (84.13% reduction in edema and 42.63 % reduction the weight of exudate). UAH has antinociceptive activity at both central and peripheral levels. Acute toxicity results suggest that single oral doses of 2000 and 5000 mg/kg b.w. are safe to use in rats. Our data indicate that UAH is a natural source of bioactive substances with promising beneficial properties for human health.

KEYWORDS: *Atamisquea emarginata*, unifloral honey, anti-inflammatory effects, antinociceptive activity, antioxidant activity.

1. INTRODUCTION

Honey has been an important food for humans since the Stone Age. It is very rich in nutrients, and has constituents with a wide variety of biological properties beneficial to health. Beekeeping is a sustainable life system for the ecosystem, ensuring habitat continuity and biological diversity.[1] Honey is defined as a sweet, non-fermented substance produced by bees (*Apis mellifera*) that collect and process the nectar from flowers or the secretions of certain plant species. Bees transform and combine this substance with other specific ones that are finally stored and matured in the honeycomb. In particular, atamisqui honey is a highly appreciated product in Argentina and the world, not only for its exquisite aroma and flavor, but also for its medicinal and culinary properties. It is obtained from the small flowers of Atamisqui (*Atamisquea emarginata* or *Capparis atamisquea*), a native shrub of northwestern Argentina. The Northwest of Argentina is characterized at a productive level, since it is a region dedicated to primary production, with great potential for organic and / or sustainable production thanks to the vast natural areas that are still conserved in the Gran eco-region American Chaco. In this context, there are small producer communities and peasant families that are committed to the practice of organic beekeeping and the provision of differentiated products of high quality. Price depends on quality and is also related to floral origin.

The botanical origin of honey is one of its main quality parameters, and it has been reported that its composition and both medicinal and organoleptic properties depend on the floral source used, seasonal and environmental factors, as well as processing.[2,3] Honey has been used to treat burns, gastrointestinal disorders, asthma, infections, and some chronic wounds. The antioxidant and anti-inflammatory properties of honey are thoroughly documented. Phenolic acids and flavonoids would be the main cause for the antioxidant activity of honey. They prevent auto-oxidation reactions and have a free radical scavenging effect by different mechanisms.[4-7]

Hussein et al. 2012 reported that the Malaysian Gelam honey reduced the activities of cyclooxygenase-1 and cyclooxygenase-2, thus showing anti-inflammatory effects. Furthermore, the ingestion of diluted natural honey has produced reductions in the concentrations of prostaglandins such as PGE2 (prostaglandin E2), PGF2α (prostaglandin F2a) and thromboxane B2 in plasma from

normal individuals.[9] Interestingly, in an inflammatory model of colitis, honey was as effective as the prednisolone treatment.[10] Honey has an anti-inflammatory action free of major side effects. Furthermore, there are also reports of the analgesic effects of unifloral and multifloral honeys.[11,12] To our knowledge, there are no studies to date of the pharmacological properties of unifloral atamisqui honey (UAH).

The purpose of this work is to evaluate the antioxidant, anti-inflammatory and antinociceptive activities of the unifloral atamisqui honey *in vivo*. We use the Wistar rat as an experimental model because its genome is known, it is easy to care and maintain due to its size, it has high reproductive efficiency and a short generation time, and its maintenance requires low costs. It is excellent for use in toxicology, microbiology, virology, pharmacology tests, etc. In addition, we compared the antioxidant capacity with other honeys marketed in the region and with a product derived from native fruits, as well as the effect of a pre-treatment with unifloral atamisqui honey in the pain and inflammation-induced models to differentiate atamisqui honey for its high potential for apitherapy for future studies and its use as functional food.

2. MATERIALS AND METHODS

2.1 Samples
We work with unifloral atamisqui honey (UAH) samples (n = 8). The samples were provided by COOPSOL limited work cooperative that currently has 1300 beehives under bio certification, located in the province of Santiago del Estero and Chaco, Argentina during the period of 2018–2019. Honeys are reserved in sterile vials at 4 ° C until analysis. Arrope of chañar, multifloral and unifloral lemon honeys were obtained commercially.

2.2 Melissopalynological analysis of honey samples
The origins of each honey sample were confirmed by microscopic pollen analysis. The preparation of honey samples followed the standardized method described by Louveaux et al., 1978. Unifloral atamisqui honey samples were classified according to their botanical origin using the method described by Von Der Ohe et al., 2004. The following terms were used for frequency classes: predominant pollen (>45%), secondary pollen (16-45%), important minor pollen (3-15%) and minor pollen (<3%).

2.3 Quantification of total phenol content and total flavonoids
Total phenolic content of honey samples was estimated by the Folin–Ciocalteu method. [15] The concentration of total phenolics is expressed as mg of gallic acid equivalents (GAE) per 100 g of honey, and calculated as mean value ± SD (n = 3). This gives a blue-color complex whose maximum absorbance can be read at 760nm.

Flavonoid content was estimated using an aluminum chloride method based on the procedure described by Woisky and Salatino (1998). The amount of total flavonoid was measured with a spectrophotometric method at 420 nm and calculated as milligrams of quercetin equivalents per 100 grams of honey (QE 100 g-1 honey) from a calibration curve (5 to 30 μM quercetin). Values are reported as the mean ± SD of 3 independent determinations.

2.4 Biological activities evaluation
2.4.1 Antioxidant activity
Stock honey solution was prepared by diluting 15 g of unifloral atamisqui honey (UAH) in 25 mL of distilled water. [17] In addition, a typical regional product from northwest Argentina (arrope de chañar) and other samples of honey (unifloral lemon honey and multifloral honey both purchased commercially) were used to assess the antioxidant power of UAH.

2.4.1.1 DPPH scavenging activity
The antioxidant activity was assessed by the measurement of the scavenging ability of honeys towards the stable free radical 1,1 biphenyl -2-picrylhydrazyl (DPPH) as described Pandey et al., 2005 with some modification. Different concentrations of honey solutions were made (0.1-15 mg/ml) and the absorbance at 517 nm was measured versus ethanol as a blank. Quercetin (1.50 μg/ml-10 mg/ml), a natural antioxidant and butylated hydroxytoluene (BHT) (1.56 μg/ml-10 mg/ml), a synthetic antioxidant one, were used as reference solutions (n = 6). DPPH degradation was evaluated against a control (0.25 ml of DPPH solution and 0.75 ml of 96% ethanol). Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the equation: AA % =(Abs control − Abs sample/ Abs control) x 100.

2.4.1.2 β-carotene bleaching method
The antioxidant activity of samples of UAH was evaluated using β–carotene–linoleate model system, as described by Sun & Ho (2005). Aliquots (4.8 mL) of this emulsion (β–carotene - linoleic acid - Tween 20) were transferred into different test tubes containing 0.2 ml of samples of UAH (0.5 and 15 mg/ml) and 0.2 mg/ml of the reference antioxidants (Quercetin and BHT) and mixed well. The 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. Ethanol was the negative control. All samples were assayed in triplicate. The antioxidant activity (AA) was measured in terms of successful bleaching of β–carotene by using the equation:

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

A_0 and A_{00} were the absorbance values before incubation for test sample and control, respectively. At and At_0 were the respective absorbance 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.[20]

2.6 Anti-inflammatory activity study

These essays aim to assess the anti-inflammatory effect of UAH with two models or treatment schemes: before (preventive or pre-treatment P-UAH) and during (treatment UAH) the experimentally induced inflammatory process.

2.5 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, Universidad Nacional de Tucumán. All animals were kept under normal laboratory conditions of humidity, temperature ($25 \pm 1°C$) 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 and Use of Laboratory Animals CICUAL), approval number: No. CICUAL 012/2018, dated 14/07/2019.

2.6.1 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 Winter et al (1962). 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. Two groups of rats were treated orally with 500 and 1000 mg/kg of the UAH, 30 min prior to injection of carrageenan. The other two groups of rats were pretreated daily with 500 and 1000 mg/kg v.o of the unifloral atamisqui honey (P-UHA), two weeks before to injection of carrageenan. 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 of the basal volume. Results were expressed as percentage of inhibition of edema, calculated according to the following formula.[22] $[(Vt−Vo)/Vo] \times 100$

2.6.2 Cotton pellet-induced granuloma formation

Male rats weighing 180–200 g were randomly divided into seven groups of six rats each. Two sterilized cotton pellets (20 mg) were implanted subcutaneously, one on each side of the abdomen in all groups, under light ether anesthesia. The animals will be distributed in 5 groups (n= 6) where they will receive for seven consecutive days orally: water, Ibuprofen 100 mg / kg, meprednisone 5 mg / kg, unifloral atamisqui honey (UAH) in doses of 500 and 1000 mg / kg, a sixth group, called group pre-treatment (P-UAH) with unifloral atamisqui honey will

receive a preventive doses of 500 and 1000 mg / kg pc 14 days before the experiment. At the end of the treatments detailed above, each rat was anesthetized. The rat was then sacrificed and the implanted pellets as well as the thymus were dissected out and determined for their wet and dry weights (dried at 60 1C for 18 h). The granuloma and transudative weights and the percent inhibition of granuloma formulation of the test compounds were calculated.[23]

2.7 Formalin-induced nociception

The formalin test was carried similar to that described by Gorzalczany et al., (2011). Rats (six per group) were injected with 20 µl of 2.5% formalin solution (equivalent to 0.92% formaldehyde) made up in saline, into the sub-plantar region of the right hind paw 30 min after treatment with sterile water (control, p.o.), unifloral atamisqui honey (500 and 1000 mg/kg b.w.) and reference drugs ibuprofen syrup (100 mg/kg b.w.) and morphine syrup (1 mg/kg b.w.). Licking time of the injected paw, was recorded as nociceptive response at 0–5 min (neurogenic phase) and 15–30 min (inflammatory phase) after formalin injection.

2.8 Acute oral toxicity study

The animals were divided into five groups, with six animals each. They were treated orally with a single dose of the unifloral atamisqui honey of 2000 and 5000 mg/kg. The control group received distilled water as a single dose, orally, in 10 ml/kg volume. The general behaviour of the rats (changes in skin, hair, eyes, mucous membranes, and respiratory, circulatory, autonomic, and central nervous systems, abnormal behaviour, motor activity, tremors, convulsion, salivation, diarrhea, lethargy, or sleep) was monitored continuously during the first 24 h (0.25, 0.5, 1, 2, 4, and 12 h) and daily until day 14 after dosing. The body weight of the rats was measured on days 1, 7, and 14. [25] At the end of the evaluations, the death of the animals is induced humanely. Euthanasia is performed using a) injection of chemical anesthetics (e.g., pentobarbital 120-210 mg/kg); or b) inhalant anesthetics e.g., CO_2, or isoflurane from a vaporizer, and their organs were excised and examined macroscopically.

2.9 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 Tukey's test. The criterion for statistical significance was $p < 0.05$.

3. RESULTS

3.1 Melissopalinological analyses

Pollen analysis was used to specifically identify and confirm the botanical origin of unifloral honeys. The pollen content of honey samples is depicted in Table 1 with the scientific names and pollen frequency. All honeys were classified as "unifloral of atamisqui",

meaning that the honey must derive at least 51% of the constituent nectar or 45% of contaminant pollen from a single floral source. The highest dominant pollen was measured in *Atamisquea* (0.69-0.82 %).

3.2 Quantification of total phenol and flavonoids content

The average total phenol content of multifloral honey, lemon unifloral honey and UAH was 291 ± 3.20, 95 ± 0.15 and 770 ± 1.35 mg GAE/100 g dry weights respectively. While total flavonoid content was 19.37 ± 0.67, 7 ± 0.21, 97.37 ± 4.47 mg QE / 100 g dry weights respectively.

3.3 DPPH radical scavenging activity

Figure 1 shows that the scavenging effects of samples on DPPH radical were in the following order: QUER = BHT > UAH > Multifloral honey > lemon unifloral honey> Arrope. The effective concentration 50 (IC 50), defined as the concentration at which the DPPH radicals were scavenged by 50 %, was 0.44 ± 0.12 mg/ml for UAH, 0.56 ± 0.18 mg/ml for multifloral honey, 1.00 ± 0.15 mg/ml for lemon unifloral honey and 1.82 ± 0.13 mg/ml for chañar arrope. Though the antioxidant potential of extracts was found to be low ($P < 0.05$) in comparison with BHT and quercetin (0.002 ± 0.001 mg/ml and 0.080 ± 0.010 mg/ml respectively), the study revealed that unifloral atamisqui honey has a prominent antioxidant activity, 75 ± 5.25 % at a concentration of 2 mg/ml).

3.4 Antioxidant activity determined by the β-carotene bleaching method

The antioxidant potential of UAH was also evaluated by the β-carotene bleaching method. Figure 2 shows the decrease in absorbance of the β-carotene emulsion in presence of 0.5 and 1 mg/ml of UAH, 1 mg/ml of the commercial reference antioxidants (BHT and Quercetin) and 1 mg/ml of other honeys and regional edible products (multifloral honey, lemon unifloral honey and arrope of chañar). The addition of both concentrations tested of UAH was significantly effective in inhibiting the oxidation of linoleic acid and subsequent bleaching of β- carotene, in comparison with the control (saline solution, $p < 0.05$), which contained no antioxidant component. The percentages of activity were as follows: UAH (73.62 ± 4.65 and 87.69 ± 3.18 %, at 0.5 y 1 mg/ml respectively), multifloral honey (43.70 ± 4.65 %), lemon unifloral honey (50.29 ± 3.55 %), arrope of chañar (22.14 ± 5.25 %), BHT (95.00 ± 2.37 %) and Quercetin (93.00 ± 2.01 %). The results indicated that the unifloral atamisqui honey was an effective antioxidant in a β-carotene linoleic acid model system.

3.5 Carrageenan-induced rat paw edema

In the carrageenan induced edema test, the size of the edema was measured using a caliber, the average figures for the extracts and standard drug are shown in Table 2. In the control group, the injection of the phlogistic agent caused localized edema starting at 1.0 h after injection. The swelling increased progressively to a maximum volume of 2.35±0.18 ml (84 %) at 4.0 h after the carrageenan injection. Rats treated with the UAH and P-UAH showed a significant reduction of the edema 3.0 h post dosing of 1000 mg/kg b.w. (74.60, and 84.13 respectively). This behaviour is similar to that of the standard ibuprofen (100%) dose of 100 mg/kg b.w., p.o.

3.6 Cotton pellet-induced granuloma formation

Ibuprofen and meprednisone (100 and 5 mg/kg/d), UAH and P-UAH significantly reduced transudative and granuloma weights. The percentages of granuloma inhibition were 45.56%, 57.10%, 23.40% and 42.63% respectively at 1000 mg/kg/d (Table 3). The anti-inflammatory effect was greater in animals treated with both doses of unifloral atamisqui honey (P-UAH) 14 days prior to the trial. Only the group treated with meprednisone revealed a significant decrease in dry thymus weight.

3.7 Formalin-induced pain

Overall, UAH and PUAH showed a significant ($P < 0.05$) antinociceptive activity in both phases of the formalin-induced paw licking test (Figure 3) with the dose of 1000 mg/kg b.w. Morphine was used as positive control (1 mg/kg b.w., p.o.) and the response time of the animals decreased significantly when compared to negative control in both phases, while the other positive control, ibuprofen (100 mg/kg b.w., p.o.), was effective only in the second phase.

3.8 Acute toxicity

No deaths or toxic symptoms were observed in any of the animals after oral administration of the different doses of UAH. There were no changes in body weight or food and water intake between the control and the treated groups. The treated rats did not present any behavioral alterations during the assessment period (48 h). These results suggest that single oral doses of 2000 and 5000 mg/kg b.w. are safe in rats.

Table 1. Pollen percent of unifloral honeys.

Sample Code	Pollen frequency (%)				
	Atamisquea emarginata	*Ziziphus mistol*	*Prosopis sp.*	*Acacia sp.*	Others
MHA1	78	2	11	9	-
MHA2	73	16	14	17	-
MHA3	79	6	13	-	2
MHA4	80	8	12	-	-
MHA5	82	9	9	-	-
MHA6	69	9	2	20	-
MHA7	72	6	12	10	-
MHA8	74	6	13	7	-

Table 2: Effect of unifloral atamisqui honey on carrageenan induced rat paw edema.

Group (n=6)	Dose (mg/kg)	Paw edema vol in ml (Mean ± S.E.)						
		0 H[a]	1 H[a]	2 H[a]	3 H[a]	4 H[a]	5 H[a]	6 H[a]
Control	s.s.	1,47 ± 0.06a	1.78 ± 0.10a	2.07 ± 0.13a	2.10 ± 0.13a	2.35 ± 0.18a	2.24 ± 0.13a	2.07 ± 0.12a
Ibuprofen	100	1.40 ± 0.05a	1.45 ± 0.15b	1.40 ± 0.10b	1.40 ± 0.10b	1.45 ± 0.15b	1.45 ± 0.05b	1.55 ± 0.15b
UAH	500	1.45 ± 0,15a	1.65 ± 0.07b	1.78 ± 0.20c	1.66 ± 0.16b	1.79 ± 0.30b	1.76 ± 0.20c	1.79 ± 0.17c
	1000	1.44 ± 0.10a	1.60 ± 0.05b	1.65 ± 0.05c	1.60 ± 0.13b	1.61 ± 0.22b	1.68 ± 0.19b,c	1.66 ± 0.14b,c
P-UAH	500	1.43 ± 0.15a	1.63 ± 0.06b	1.63 ± 0.12c	1.67 ± 0.26b	1.80 ± 0.16b	1.70 ± 0.15b,c	1.73 ± 0.30c
	1000	1.45 ± 0.10a	1.65 ± 0.13b	1.65 ± 0.15b,c	1.55 ± 0.10b	1.60 ± 0.10b	1.62 ± 0.32b,c	1.65 ± 0.20b,c

UAH: unifloral atamisqui honey, P-UAH: pretreatment with unifloral atamisqui honey,
ss (saline solution). Values are expressed in mean ± SEM (n = 6). [a]Time after carrageenan injection (h). The values followed by the same letter are not significantly different. The significance level $P < 0.05$ (one-way ANOVA, followed by Tukey's test).

Table 3: Effects of unifloral atamisqui honey, on transudative weight, granuloma weight, andpercentage of granuloma inhibition on cotton pellet-induced granuloma formation in rats.

Groups (n=6)	Dose (mg/kg/d)	Cotton pellet-induced granuloma formation				
		Transudative weight (mg)	Transudative inhibition (%)	Granuloma weight (mg)	Granuloma inhibition (%)	dry Thymus weight (mg/100 g BW)
Control	ss	594.35 ± 25.60a	--	156.70 ± 3.30a	--	130.49 ± 2.06a
Ibuprofen	100	178.90 ± 15.50b	69.89	76.20 ± 2.80b	45.56	123.24 ± 5.40a
Meprednisone	5	165.10 ± 14.85b	72.22	55.80 ± 9.00c	57.10	82.61 ± 3.95b
UAH	500	487.57 ± 39.53c	17.96	129.76 ± 4.76d	17.19	128.20 ± 10.50a
	1000	459.67 ± 22.77c	22.66	120.03 ± 5.04d	23.40	114.45 ± 15.05a
P-UAH	500	342.53 ± 10.53d	42.36	94.87 ± 4.86e	39.71	110.59 ± 16.21a
	1000	302.75 ± 17.60d	49.06	89.90 ± 8.90e	42.63	133.81 ± 3.80a

UAH: unifloral atamisqui honey, P-UAH: pretreatment with unifloral atamisqui honey, ss (saline solution). Values are expressed as mean ± S.E.M. (n=6). TrW: Transudative weight, GrW: Granuloma weight, GI: Granuloma inhibition, BW: Body weight, TW: Thymus weight. The values followed by the same letter are not significantly different. The significance level $P < 0.05$ (one-way ANOVA, followed by Tukey's test).
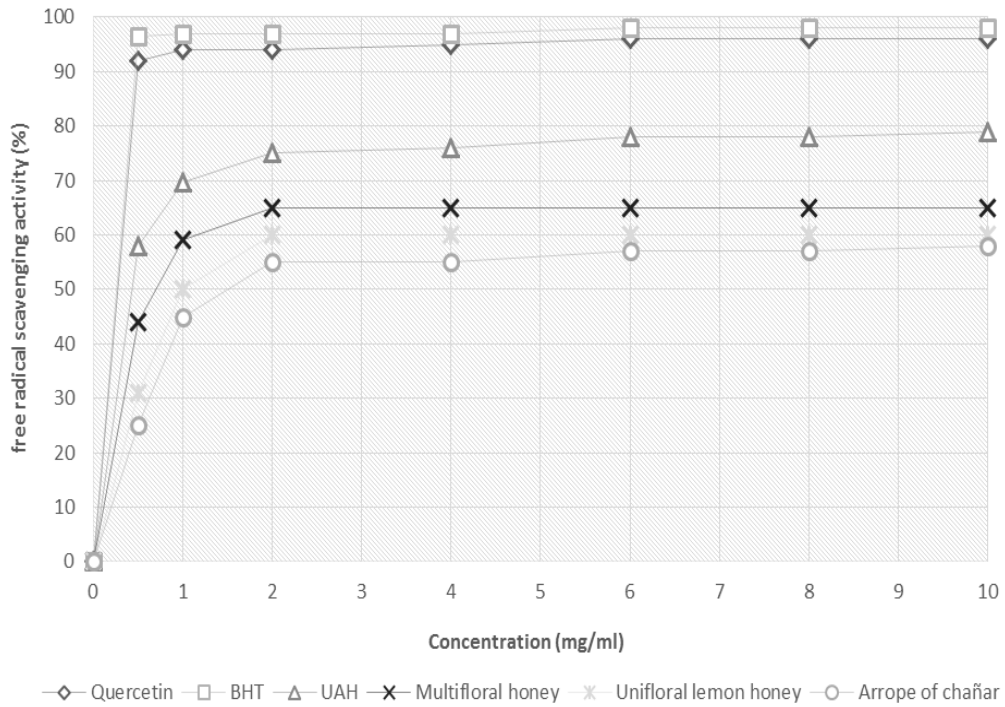
Figure 1: DPPH radical scavenging activity of unifloral atamisqui honey (UAH). Quercetin and BHT were used as reference antioxidant. Values represent the mean ± S.E.M. (n=6).
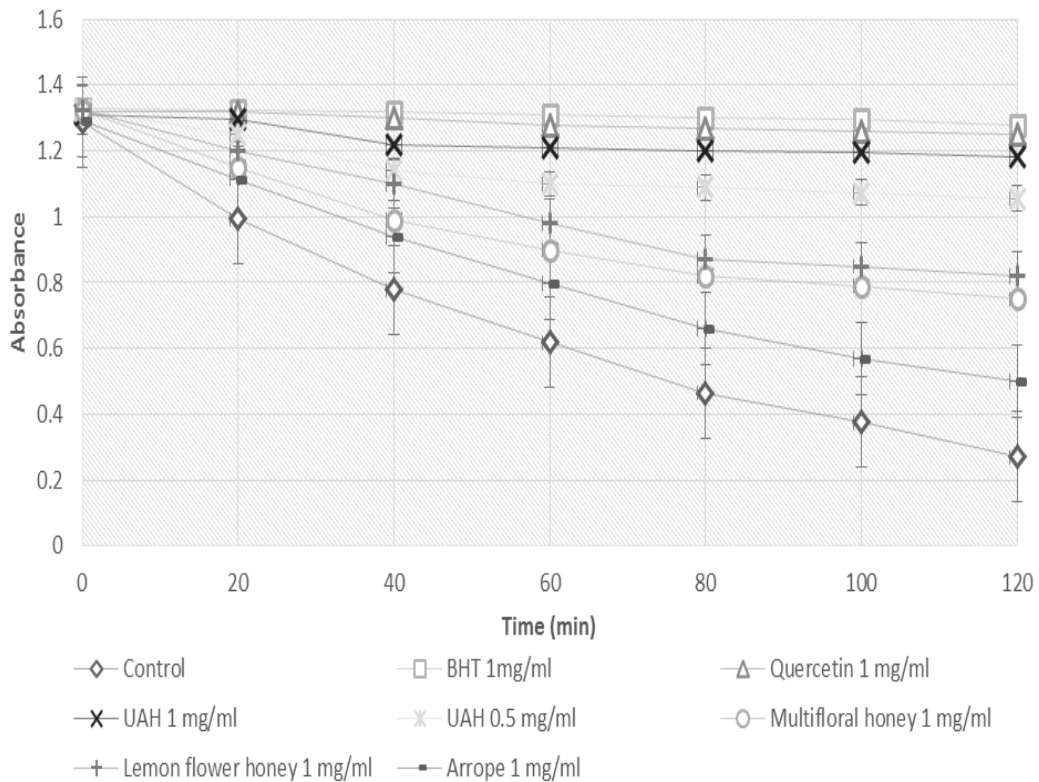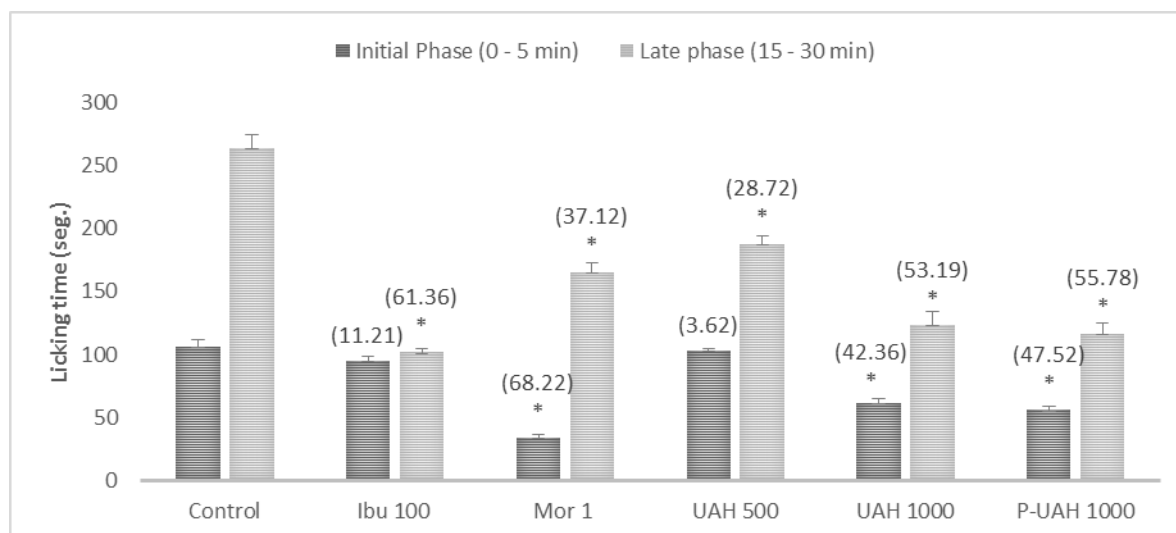

Figure 2: Antioxidant activities of method of unifloral atamisqui honey (UAH), measured by b-carotene bleaching Quercetin and BHT were used as reference antioxidant. Values represent the mean ± S.E.M. (n=6).

Figure 3: Effect of UAH and P-UAH on nociceptive response in first and second phase of formalin test. Control, Ibuprofen (Ibu 100 mg/kg BW), Morphine (Mor 1 mg/kg BW), UAH (500-1000 mg/kg BW), PUAH (500-1000 mg/kg BW). Values in parentheses are percentage of inhibition. Values represent the mean ± SEM (n=6) p<0.05. * The asterisks denote the significance levels compared with the control group (one-way ANOVA, followed by Tukey's test.

4. DISCUSSION

Honey has a privileged place in the world of food since it is natural nourishment without additives and with important nutritional, therapeutic attributes and attractive sensory characteristics. Its healing properties have been known since ancient times and are highly effective in transmitting active ingredients from plants to man, which makes the action and therapeutic fields of use of this substance very broad. The properties of honey depend largely on the floral source. Generally, unifloral honeys have higher economic value than multifloral honeys because their sensorial properties (aroma, color and taste) and nutritive characteristics are more consistent over time.[26] Argentina produces a limited number of unifloral honeys of native plant origin. All our honey samples were classified as "atamisqui unifloral". In these samples, the relative percentages of atamisqui pollen grains varied from 53% to 82%. *Capparis atamisquea* or *Atamisquea emarginata*, also called "Atamisqui", is a woody tree or shrub 2 to 3 m tall, with stiff, alternate, cylindrical and smooth branches. It is common in the native forest of the Great American Chaco eco-region. Its presence indicates that the forest is very well preserved, has quality and is in prime condition. Through beekeeping, peasant families that produce organic unifloral atamisqui honey, promote the displacement of unsustainable activities such as charcoal production and contribute to improving the social, economic and environmental reality of their families and communities.

The antioxidant and antiradical properties of honey are mainly attributed to the presence of phenolic compounds.[27] They were also detected in the samples of UAH, multifloral honey and lemon flower honey analyzed. Phenolic compounds are related to the sensory quality of foods of plant origin, both fresh and processed.

Among the phenolic compounds are flavonoids, natural plant pigments that protect the organism from the damage produced by oxidizing agents. Their content normally reaches levels of 0.5% in pollen, 10% in propolis and almost 6000 µg kg-1 in honey.[28] Most of the flavonoids identified in honey and propolis are groups of flavanones and flavonols.[29]

The UAH samples had the highest content of phenolic compounds (770 ± 1.35 mg GAE / 100 g dry weight) and flavonoids (97.37 ± 4.47 mg QE / 100 g dry weight). They were responsible for the antioxidant activity observed in the samples.

The antioxidant activity of unifloral atamisqui honey (UAH), multifloral honey and lemon flower honey are summarized in figures 1 and 2. In the present study, two standard spectrophotometric methods were used to assess the antioxidant capacity of unifloral atamisqui honey: the DPPH test that determines the ability of free radical scavenging and the whitening of beta carotene that measures the potential to inhibit lipid peroxidation. Among all the test samples, the unifloral atamisqui honey (UAH) was found to be the most potent antioxidant (IC50 0.44 ± 0.12 mg/ml 201µg/mL), followed by the multifloral honey (IC50 values 0.56 ± 0.18 mg/ml 226µg/mL). Arrope of Chañar showed a poor radical scavenging activity (IC50 values 1.82 ± 0.13 mg/ml. BHT and Quercetin were taken as reference antioxidants (IC50 0,080 and 0.002 ± 0.001 mg/ml, respectively). The lipid peroxidation inhibition method showed similar results and the unifloral atamisqui honey (UAH) and multifloral honey presented the highest activity.

The low antioxidant activity found in the arrope of chañar may be explained as the result of a lower content

of phenolic compounds such as flavonoids.[20] Furthermore, it is important to bear in mind that the antioxidant activity of phenolic compounds is of interest from a technological and nutritional point of view since they can act as natural antioxidants in food. They would thus favor the production of food products with a high phenolic content that would reduce the use of synthetic antioxidant additives and help to obtain healthier foods that may even be classified as functional.

On the other hand, phenolic compounds have been attributed pharmacological and medical activities related to disease prevention and / or health improvement. They include vasodilatory, anticarcinogenic, anti-inflammatory, immune response, antiallergic, antiviral and estrogenic effects. Additionally, phenolic compounds are inhibitors of phospholipase A2, cycloxygenase, 1ipoxygenase, glutathione reductase and xanthine oxidase that are directly related to their antioxidant and free radical scavenging power.[30-35]

The anti-inflammatory activity correlates positively with the radical-scavenging activity and total phenolic content. [36] Inflammation is a complex biological response of the body against infections, irritations or other injuries, cell damage and vascularized tissues and is critical for both innate and adaptive immunity. Inflammatory diseases are generally treated with steroidal and non-steroidal anti-inflammatory drugs. However, both have significant negative side effects that reduce their use in certain segments of the population.[37]

The anti-inflammatory effect of UAH samples was investigated in the present study. The carrageenan test was selected because of its sensitivity in detecting orally active antiinflammatory agents particularly in the acute phase of inflammation.[38] The acute inflammation is produced when water and plasma increase in tissues during arachidonic acid metabolism via cyclooxygenase and lipoxygenase enzyme pathways.[39] It has two phases; the first begins inmediately after injection and lasts one hour. It is characterized by the release of histamine, serotonin and kinins on the vascular permeability. The second begins after one hour and lasts three hours. It is correlated with a large production of prostaglandins, oxygen derived free radicals and the production of inducible cyclooxygenase.[40] Oral administration of UAH and PUAH suppressed the oedematous response after 1 h and this effect continued up to 6 h. The effect observed was similar to that of Ibuprofen. Based on these reports, the inhibitory effect of UAH on carrageenin-induced inflammation in rats may be due to inhibition of inflammation mediators. Our results are similar to those obtained by Kassim et al., 2010.

However, in the cotton pellet granuloma assay only PUAH at doses of 500 mg / kg and 1000 mg / kg, elicited a significant inhibitory activity on the wet weight of granuloma (> 40%). Previous consumption of UAH could be the result of the decrease in plasma concentrations of PGE2, PGF2a, and thromboxane B2. Chronic inflammation arises when the acute response is insufficient to eliminate proinflammatory agents and there is a proliferation of fibroblasts and infiltration of neutrophils and exudation.[41,42] The cotton pellet granuloma method has been widely used to evaluate the transudative, exudative and a proliferative phase of chronic inflammation. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma, whereas the dry weight correlates well with the amount of granulomatous tissue formed.

These results suggest that the anti-inflammatory activity may be mediated by the inhibition of prostaglandin biosynthesis. Similar results were obtained by Kassim et al., 2010, who demonstrated in vivo the inhibitory activity of PGE2 of Gelam honeys. Most of the NSAIDs like ibuprofen only slightly inhibit granuloma formation whereas the steroidal drug exhibits a profound reduction of the granuloma.

Pain is a common symptom of injuries and inflammatory related conditions. The perception of pain, commonly known as nociception, depends on integrated receptors and molecular pathways. Inflammatory mediators are involved in the genesis, persistence, and severity of pain. [43] The formalin test represents a model of persistent pain. This test can also be used to determine the ability of new compounds to affect peripheral or central nociceptive pathways due to their biphasic nociceptive characteristics, known as the early and late phases that result from formalin administration.[44] The early phase, classified as a neurogenic pain, is an acute response observed immediately after the formalin injection and persists for 5 min (0–5 min) as a result of a direct action on nociceptors. The late phase, classified as an inflammatory pain, is a tonic response resulting from the inflammatory processes generated by the release of inflammatory mediators. Based on the results obtained, the UAH and P-UAH proved to have central and peripheral antinociceptive and antiinflammatory activities [45]. Drugs with central antinociceptive activity showed activity in the two phases, while peripherally acting drugs (NSAIDs) inhibited only the late phase.

Our studies provide additional evidence of the safety of UAH at higher doses than those that produce a measurable antiinflammatory and anti-nociceptive effect in animal models. The unifloral atamisqui honey did not produce any mortality or alter the behavioral patterns of the rats during the acute toxicity testing.

CONCLUSION

The selectivity of the bee for its floral sources defines the presence of the chemical compounds responsible for the biological properties of its honey. The Native forest is any forest that has been established without the intervention of man. It is a renewable natural resource

that must be conserved and its use must tend to sustainability. According to our knowledge, the results of this work constitute the first report on the therapeutic properties of organic unifloral atamisqui honey. UAH possesses anti-oxidant, anti-inflammatory and analgesic capacities, which may be useful in the chronic inflammation process. The mechanism of action of UAH is not thoroughly known but the therapeutic effects observed can be associated with the phenolic composition of organic unifloral atamisqui honey. Our results add value to UAH which, due to its unique characteristics depending on botanical and geographic origin, becomes a healthy food that can provide not only nutrients but also beneficial properties for health.

Declaration of interest
The authors declare that they have no conflict of interest related to the publication of this manuscript.

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