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Argentine stingless bee honey: bioactive compounds and health-promoting properties

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ABSTRACT: Despite all the advantages of consuming meliponid honey, meliponiculture has not been sufficiently promoted in Argentina yet, and published studies on these species of bees are very scarce. *Tetragonisca fiebrigi* honey (**TfH**) or Yateí honey has recently been incorporated into the Argentine food code. This study assesses antioxidant, anti-inflammatory and analgesic properties and acute toxicity by oral administration of **TfH** in rats. In addition, we present the melissopalynological analysis and physicochemical characterization. High-performance liquid chromatography identifies and quantifies sugars and phenolic compounds. The *T. fiebrigi* honey analyzed exhibited $ABTS^{\bullet+}$ and DPPH radical scavenging effect (IC_{50}= 98.28 mg/ml and IC_{50}=337.83 mg/ml, respectively). A significant reduction in hind paw edema (44.44%) was observed in rats pretreated with **TfH** honey (1000 mg/kg b.w.) 3.0 h after dosing and significantly reduced transudative and granuloma weights at all doses tested (27.34%, 35.53% and 47.53% granuloma inhibition). The **TfH** honey oral administration produced analgesic responses in the three models used (acetic acid, formalin, tail immersion). Ferulic, ellagic, coumaric, gallic, cinnamic acids and the flavonoids quercetin and hesperetin were identified and quantified. Fructose (40.9%), glucose (29.02%) and sucrose (1.06%) were the main sugars. **TfH** honey administration did not produce lethal effects or clinical signs of disease in the acute toxicity study. The results showed that *T. fiebrigi* honey could be a good source of bioactive natural compounds with therapeutic and nutritional value.

1. INTRODUCTION

Honey is a sweet and slimy substance produced by different honey bees, including stringed and stingless bees. Carbohydrates, water, amino acids, vitamins and minerals are the main constituents of stingless bees honey (Chuttong et al., 2016) . Additionally, because honey is high in polyphenols and flavonoids with pharmacological properties, it has been used as a medicine throughout human history (Jones, 2001) . Apitherapy is a branch of alternative medicine that uses honey and other bee products to treat a variety of diseases Bogdanov et al. (2013); Cherbuliez and R (2003) .

The native stingless bees belong to the Apoidea superfamily, where the large Apidae subfamily, which includes the Meliponini tribe (bees without stings), is found. Only 11 are exploited in agriculture, the list being headed by *Tetragonisca fiebrigi* (Schwarz) followed by *Scaptotrigona aff* postica, *S. jujuyensis* and *Tetragonisca aff.* Angustula. The honey from *T. fiebrigi* (Schwarz) was recently incorporated into the "Argentine Food Code" (article 783 bis chapter X sugary foods) among approved foods for human consumption. The cultural, medicinal and nutritional importance that different ethnic groups attribute to these bees is enhanced when their ecological and economic importance is added.

In a preliminary review of the literature, very few studies were found that validate the medicinal use of stingless honey (Kujawska et al., 2012; Zamudio & Hilgert, 2011; Zamudio et al., 2010) . In addition, despite this product's long consumption tradition, there is little information regarding the composition and nutritional properties of stingless bee honey produced in our country. The works detail typical parameters of honey with particular reference to honey from Brazil, Guatemala and Venezuela (Laurino et al., 2006; Vit et al., 1994, 2009), but the reported data of Argentine honey are

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scarce.

To our knowledge, the results of this work constitute the first report on the pharmacological properties of *T. fiebrigi* honey or Yateí honey (TfH). Its antioxidant capacity was evaluated *in vitro* and its anti-inflammatory and analgesic properties by its oral administration in rats. Wistar rats were employed as an experimental model because their genome is known, it is easy to care for 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 toxicology, microbiology, virology, pharmacology tests, etc.

In addition, the melisopalinological analysis, physicochemical characterization, and sugar and phenolic compounds quantified by HPLC were presented to correlate these data with the pharmacological properties observed. The physicochemical characterization provides essential information to establish quality standards that favor stingless bee honey's normalization, use, and value. Finally, a single high dose evaluated the acute toxicity of the *T. fiebrigi* honey. Acute toxicity is generally defined as adverse effects (lethal or sublethal) induced in the organisms under test, occurring immediately or after a short period of exposure or administering a single dose of a substance or substance.

2. MATERIALS AND METHODS

2.1. Reagents and standards

The chemicals employed in the investigation were of analytical grade. Sigma–Aldrich provided the reagents Folin–Ciocalteu, potassium persulfate, Trolox, ascorbic acid, gallic acid, DPPH radical, $ABTS^{\cdot+}$ (Argentine, Buenos Aires). Sintorgan SA provided ethyl acetate, methanol, ethanol, and DMSO (Argentina). Sigma-Aldrich Chemical Co. provided carrageenan, NaCl, NH_4OH and phenol red (St. Louis, MO, USA). The local pharmacy provided bromhexine hydrochloride, ibuprofen and codeine syrups (Laboratory Boehringer Ingelheim, Germany; Laboratory Roemmers S.A.I.C.F. and Laboratory Andromaco, Argentina) and meprednisone oral solution (Laboratory Biotenk S.A., Argentina).

2.2. Honey samples

During december-january 2015-2017, samples of *Tetragonisca fiebrigi* honeys (TfH) were collected from beehive meliponaries at INTA's Estación Experimental Agropecuaria Famaillá in Tucumán, Argentina. A sterile syringe was utilised to collect the honey, while a sterile spatula extracted the pollen. A total of 12 samples of honey were gathered from 6 beehives. The honey and pollen were collected and stored in sterile tubes at 4–8°C until they were analysed.

2.3. Melissopalynological analysis

The pollen types discovered in each sample were identified using pollen catalogues and reference to the Reference Palinoteca collection of the Palinology Laboratory, Faculty of Agricultural Sciences, UNJu (PALJUA). The classification was based on the following criteria: dominant pollen type (PD, greater than 45%), secondary pollen type (SP, 16-45%), significant minor pollen (IMP, 3-15%), and minor pollen (MP, less than 3%). Each honey sample was diluted to ten grammes with warm water and ethanol 95 %, centrifuged, and dried with anhydrous acetic acid. They were then centrifuged again after acetolyzed with acetic anhydride and sulfuric acid (9: 1). The pollen grains were mounted on slides containing glycerinated gelatin and then analyzed and identified using optical microscopy. At least 300 pollen grains were used to define frequency classifications for each honey sample.

2.4. Physicochemical analysis

The physicochemical properties of stingless bee honey (colour, free acidity, pH, moisture, and electrical conductivity) were determined using the official techniques of analysis of the AOAC and the International Honey Commission and the regulations of the Argentine Food Code (CAA).

2.4.1 Total phenolic content

Folin – Ciocalteu technique was employed to determine the total phenolic content of honey samples Singleton et al. (1999) . Each assessment was conducted in triplicate, and the results were represented as milligrams of gallic acid equivalent (GAE) per gram of honey. The detailed procedures were given in the supplementary information (Appendix A).

2.5. HPLC analysis

The major constituents of the honey samples were identified by comparing their retention times with commercial standards. Quantification was accomplished by developing calibration curves for each constituent.

2.5.1 Sugars

The sugar profile was measured using a HPLC system (Waters 1525) linked to a refractive index detector (Waters 2414). The chemicals were separated using a Polyamine II column (250 mm X 4.6mm, -YMC HPLC Column). The mobile phase was acetonitrile/water HPLC 8:2 (v / v) with a 1 ml / min run flow rate. The system was kept at a constant temperature of 35 °C. The results were expressed as a gram of sugar per 100 grams of honey.

2.5.2 Phenolic compounds

The phenolic components in stingless bee honey were identified and quantified using HPLC (Waters 1525) linked to a diode array detector (Waters 2998). The compounds were separated using a C18 column (250 mm X 4.6mm, -YMC HPLC Column). The mobile phase was a mixture of methanol and acetic acid HPLC 95:5 (v / v) at a 1 ml/min flow rate. The results were expressed as a gram of phenolic compounds per 100 grams of honey.

2.6. Pharmacological activities

2.6.1 *In vitro pharmacological evaluation*

The antioxidant activities of the stingless bee honey (**TfH**) were evaluated through DPPH radical scavenging activity (Reynoso et al., 2013) and ABTS·+ scavenging assay (Re et al., 1999) . The detailed procedures were given in the supplementary information (Appendix A).

2.6.2 *In vivo pharmacological evaluation*

The carrageenan-induced hind paw edema (Winter et al., 1962) and the effect of TfH honey on granuloma development using Cotton pellet-induced granuloma formation test (Reynoso et al., 2016) in Wistar rats were assayed. Further, the stingless bee honey (**TfH**) was evaluated through various in vivo assays, antinociceptive assays (Gorzalczany et al., 2011), acetic acid-induced writhing method (Koster et al., 1959) and Tail immersion test Wen et al. (2014) . The detailed procedures were given in the supplementary information Appendix A.

2.7. Acute toxicity

Following the *in vitro* and in vivo pharmacological assays, the safety assessment of stingless bee honey (**TfH**) was evaluated through acute toxicity assay following the protocol of (OECD 420, 2001). The detailed procedures were given in the supplementary information (Appendix A).

2.8. Statistical analysis

The mean and standard deviation of two or more experiments are used for each experiment. We utilised Student's test or ANOVA with the Tukey test for paired data to find statistically significant differences between vehicles. Statistical significance was set at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

The use of honey has a very long history. Melponiculture is a growing activity in Latin America, particularly in Argentina. Since ancient times, Stingless bee honey has been a resource used for nutritional and medicinal purposes and has differential characteristics compared to honey produced by Apis mellifera. The honey of *Tetragonisca fiebrigi* Schwarz, commonly known as Yateí, Rubita or Mestizo, has recently been included in the Argentine Food Code (CAA), ensuring the implementation of good manufacturing practices at the level of primary production. According to its botanical origin and other characteristics such as bee species, location, time of year, storage mode, and even harvesting equipment and conditions, honey's chemical composition, taste, and aroma vary significantly from batch to batch (Costa et al., 2017) . The habitat of the stingless bee in Famaillá, Tucumán, is characterized by the presence of eucalyptus (Eucalyptus grandis, Eucalyptus camaldulensis), pine (Pinus taeda), ash (Fraxinus sp.), Ibirapitá (Peltophorum dubium), lemon, orange, grapefruit (Citrus sp.), Espinillo or Aromo (Acacia sp.), Sugar cane (Saccharum officinarum), medlar (Eriobotrya japonica) of the Rosaceae family and plantations of strawberry (Fragaria ananassa). Melisopalinology is based on identifying the pollen grains present in honey, taking into account their morphological characters. Twenty-two pollen species have been identified in the honey harvested along different years, revealing a general habit in this species (Table 1). All *T. fiebrigi* honey analyzed were classified as multiflora.

Table 1
Frequency of pollen grains classes in multi-floral honey in **TfH**

Pollen identification (botanical genus)	(%*)
Eucalyptus sp.	>45%
Salix	16–45%
Schinus sp.	3–15%
Apiacea type	3–15%
Citrus sp.	3–15%
Asteraceae	<3%
Rapistrum rugosum	<3%
Ligustrum sp.	<3%
Solanum sp.	<3%
Baccharis sp.	<3%
Prosopis sp.	<3%
Ziziphus mistol	<3%
Monocotyledoneae	<3%
Peltophorum dubium	<3%
Apium type	<3%
Amaranthaceae	<3%
Apocynaceae	<3%
Acanthaceae	<3%
Malpighiaceae	<3%
Undetermined	

Classification according to%: Dominant pollen type (DP, >45%), secondary pollen type (SP, 16–45%), important minor pollen (IMP, 3–15%) and minor pollen (MP, <3%).

The physicochemical parameters of the honey produced by *T. fiebrigi* studied are presented in Table 2. Colour measurement is a norm for honey insofar as it can be correlated to a certain extent with its botanical or geographical origin (Bertoncelj et al., 2007) . However, its importance is of a purely commercial order, and it is not necessarily related to its organoleptic or nutritional quality. Honey is generally marketed according to the Pfund scale (Bogdanov et al., 2004) . The **TfH** in this work presented average color values of 70 mm Pfund, classified as light amber. The darker a honey looks, the higher its minerals and phenolic compounds content (Solayman et al., 2016) . However, exposure to light, heat and storage time, and enzymatic reactions can also affect this parameter (Almeida-Muradian et al., 2013) .

Although organic acids represent less than 0.5% of the total composition of honey (White, 1979) , they are correlated with different physicochemical, sensory, and melisopalynological parameters that reference them as markers of botanical and geographic origin (Luque et al., 2002) . Organic acids influence the aroma and flavor of honey (White, 1979) by helping to preserve it against deterioration caused by microorganisms and

Table 2
Physicochemicalproperties and antioxidant activity (DPPH radical and ABTS·$^{+}$) of *Tetragonisca fiebrigi* honeys

Parameters		Values
Colour	Optical density (nm)	0.857
	Pfund scale (mm)	75 ± 13
pH		4.80 ± 0.12
Free acidity (mEq/ kg honey)		51.74 ± 3.02
Moisture (%)		24.03 ± 1.29
Electrical conductivity (mS/cm)		2.39 ± 3.56
Fructose*		40.90 ± 3.01
Glucose*		29.02 ± 2.8
Sucrose*		1.06 ± 0.3
Maltose*		0.34 ± 0.1
Trehalose*		0.57 ± 0.2
Arabinose*		ND
Phenolic total (mg GAE/ 100 g of honey)		0.98 ± 0.07
Gallic acid*		0.06 ± 0.04
Coumaric acid*		0.05 ± 0.09
Ferulic acid*		0.38 ± 0.04
Elagic acid*		0.21 ± 0.6
Cinnamic acid*		0.03 ± 0.01
Quercetine*		0.13 ± 0.05
Hesperetine*		0.06 ± 0.1
DPPH radical (mg QEAC/ 100 g of honey)		20.8 ± 0.5
ABTS ·$^{+}$ (μmol TEAC/ 100 g of honey)		58.54 ± 0.05

*g/ 100 g of honey; All results are expressed as means of triplicate ± standard deviation.
ND, not detected.

giving it antioxidant and antibacterial properties. So far, 19 organic acids have been identified in honey (Crane, 1990) . Many authors report that free acidity increases over time due to fermentation processes since the sugars in honey and alcoholic compounds are transformed into acids by the presence of yeasts (Bath and Singh, 2000). The **TfH** analyzed in this work presented 51.74 mEq/kg free acidity values. Our results coincide with those reported by other authors (F.C. Biluca et al., 2016; Sousa et al., 2016) . However, a study of honey from Thailand of three species (Homotrigona fimbriata, Tetrigona apicalis and T. melanoleuca) displayed an remarkable total acidity (440.0 to 592.0 mEq/kg) (Chuttong et al., 2016) .

On the other hand, the mean pH of TfH was 4.80 ±0.12. In general, the pH of stingless honey ranged from 3.15 to 6.64. Sousa et al. (2016b) reported pH ranges from 3.1 to 5.3. F.C. Biluca et al. (2016) found pH values of 3.3 to 6.6, and Chuttong et al. (2016) detected pH values from 3.1 to 3.9. Honey's pH has aided in determining its geographical origin (Acqyuarone et al., 2007) . However, mineral content (Vanhanen et al., 2011) and compounds added to nectar by bees' mouths, such as enzymes and proteins, can alter pH values (Rodriguez et al., 2005; Santos et al., 2013) .

High levels of humidity are necessary for Meliponina honey since they modify characteristics such as flavour and viscosity and promote their natural fermentation. **TfH** recorded a moisture level of 24.03g/ 100g, above the limits for A. mellifera honey. Our findings corroborate those of other authors (Almeida-Muradian et al., 2013; Souza et al., 2006; Vit et al., 1998) . The extraction of nectar from understory flowers and water-rich ripe fruits can result in high water content in stingless honey (Sierra et al., 2015) .

The concentration of minerals, salts, organic acids, and proteins directly influences honey's electrical conductivity (Solayman et al., 2016) . This parameter complements the others used to determine honey's floral origin (Acqyuarone et al., 2007) . Electrical conductivity in stingless bees ranges from 0.102 ms/cm to 8.770 ms/cm (Nordina et al., 2018) . **TfH** used in this work presented an average electrical conductivity of 2.39 mS/cm (Table 2). These values were higher than those obtained by Alves et al. (2011) for Melipona subnitida, M. scutellaris and M. mexicana and (Sousa et al., 2016) for M. subnida and M. scutellaris. This fact may be due to higher mineral content in the **TfH** analyzed.

Stingless honey includes fewer reducing sugars than Apis mellifera honey standards (F.C. Biluca et al., 2016, 2014; Chuttong et al., 2016; Sousa et al., 2016) . After fructose (30.87%), glucose was the most prevalent type of sugar in the **TfH** examined (29.02 %), followed by sucrose (1.06 %). Maltose (0.34 %) and trehalose (0.57 %) were also discovered. The sugar profile of stingless honey varies according to the main flora and vegetation of the region (Se et al., 2018) . The ratio of fructose to glucose (F / G) determines the time required for the honey to crystallize. The F / G ratio of **TfH** examined in this article was 1.41, which agrees with the relationship established by Sousa et al. (2016) in honey samples generated by stingless bees in Brazil (1.1 to 1.5). Honey with a ratio higher than 1.3 can remain liquid for a long period. Fructose is more soluble in water than glucose, so the water content of honey has a direct effect on sweetness since fructose is sweeter than glucose (Escudero et al., 2014) .

Tenore et al. (2012) revealed that places with a hot and humid environment and high solar exposure (climatic characteristics of northwestern Argentina) had a significant effect on the polyphenol content of plants. This would directly influence their metabolism and the composition of the nectar taken by bees for honey production. The total phenolic content of *T. fiebrigi* honey samples was 1.58 mg gallic acid equivalent (GAE) / 100 g honey (Table 2). However, because the colorimetric test measures total polyphenols and react with any reducing substance, the colour response can occur with any oxidisable phenolic hydroxy group and a variety of non-phenolic substances (sugars) found in honey (Suarez et al., 2010).

Although some compounds were present only in small amounts, making identification and quantification difficult, seven chromatographic peaks were detected in the analyzed **TfH** samples. Phenolic acids represented approximately 77.55% of the total phenolic content in all honey samples. Ferulic acid (38%) and ellagic acid (21%) were abundant antioxidants. Gallic and coumaric acids were also detected to a lesser extent. The flavonoids found were quercetin (13%) and hesperetin (6%). Quercetin is one of the most abundant flavonoids in natural foods and honey (Socha et al., 2011), while hesperetin

is a phenolic compound constitutive of citrus nectar (Escriche et al., 2011). These compounds can be considered possible markers of honey's origin (Figure 1).

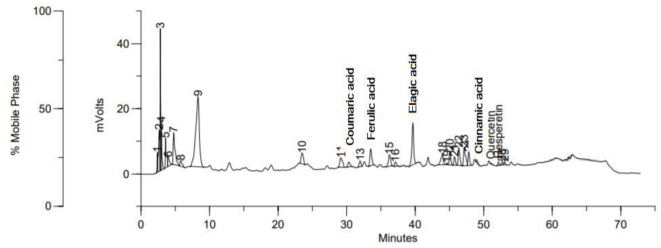

Figure 1. Profile of phenolic compounds in *T. fiebrigi* honey

Biological evaluation

The two distinct methods utilized to test the antioxidant capabilities of *T. fiebrigi* honey provided insight into the extracts' activity at various phases of the oxidation reaction (Prior et al., 2005). The **TfH** samples examined here shown a greater ability to inhibit the $ABTS^{\bullet+}$ (IC_{50} = 98.28 mg / ml) than they did against DPPH radicals (IC_{50} = 337.83 mg / ml). These findings may be explained by the $ABTS^{\bullet+}$ assay's particular action, which detects hydrophilic and lipophilic substances (Kuskoski et al., 2005; Meda et al., 2005), whereas DPPH radical can be dissolved only in an organic media. In general, the structures of the phenolic compounds found in various honey samples are partly responsible for the antioxidant effects of honey, as these compounds are capable of donating hydrogen ions or electrons to free radicals (Gai et al., 2014). Nevertheless, other factors are involved as enzymes (glucose oxidase, catalase), ascorbic acid, carotenoids, acids organic, Maillard reaction products, amino acids and proteins (Pérez et al., 2007; Vela et al., 2007) . Additionally, we could hypothesize that the honey's antioxidant capabilities are mostly due to its phenolic and total flavonoid concentration. These findings corroborate with F. Biluca et al. (2017); Silva et al. (2013); Sousa et al. (2016) findings and would support the hypothesis that the botanical source affects the antioxidant capabilities of honey (Lianda et al., 2012; Piljac-Žegarac et al., 2009) .

Anti-inflammatory medicines, both steroidal and non-steroidal, are the most frequently utilised in inflammatory illnesses. However, both have significant detrimental consequences, resulting in their use being restricted to specific groups of the population. The carrageenan test was chosen because of its sensitivity in identifying anti-inflammatory drugs administered orally, particularly during the acute phase of inflammation (Rosa et al., 1971). The simultaneous release of histamine, serotonin, and kinin mediators and their effect on vascular permeability constitutes the first phase (0-3 h after injection). By contrast, the second phase is characterised by a large generation of prostaglandins, free radicals originating from oxygen, and inducible cyclooxygenase (Panthong et al., 2004) . Pre-treatment with **TfH** honey resulted in a considerable reduction in edema (44.44 %) 3.0 h after dosing at 1000 mg/kg body weight, comparable to normal ibuprofen at a 100 mg/kg bw, p.o. dose. However, ibuprofen's impact lasts longer than **TfH** at all levels studied (Table 3).

The cotton pellet granuloma method has been widely used to assess chronic inflammation's transudative, exudative, and proliferative phases (Swingle & Shideman, 1972). The quantity of fluid absorbed by the pellet significantly affects the granuloma's wet weight, but the dry weight is highly correlated with the amount of granulomatous tissue generated. Steroid medications, such as meprednisone, successfully shrink granulomas (Purnima et al., 2010). Ibuprofen, meprednisone, and **TfH** at doses of 250, 500, and 1000 mg/kg/day considerably decreased transudative and granuloma weights, as demonstrated by their inhibition of granulomas of 48.43%, 62.23%, 27.34%, 35.53% and 47.53 %, respectively.

Our findings indicate that the anti-inflammatory activity of **TfH** may be mediated by reducing the manufacture of pro-inflammatory mediators such as prostaglandins, histamine, or serotonin, as an oral treatment of **TfH** demonstrated considerable anti-inflammatory activity in both models studied (Table 4). Ahmad et al. (2012); Borsato et al. (2014); Hussein et al. (2013) all obtained similar results.

This work demonstrated that **TfH** exhibited a significant antinociceptive effect in three standard models of nociception in rats (Figure 2). All are valuable procedures for detecting potential antinociceptive chemicals. Additionally, the formalin test can be used to identify the ability of new compounds to alter peripheral or central nociceptive pathways, owing to their biphasic nociceptive features, referred to as the early and late phases caused by formalin administration (Amaral et al., 2007) . At all dosages (250, 500, and 1000 mg/kg, b.w., p.o.), the *T. fiebrigi* honey significantly reduced ($p < 0.05$) the time spent by mice licking the injected paws, both in the early and late stages, compared to controls. The effect appears to be greater in the late period than in the early phase. The acute phase is immediately detected following formalin injection and lasts for 5 minutes (0 to 5 minutes) due to formalin's direct impact on nociceptors (neurogenic pain). The late phase is a sustained response induced by inflammatory processes triggered by the release of inflammatory mediators (inflammatory pain).

Additionally, it was observed that the antinociceptive impact was dose-dependent in both stages. Positive controls included morphine (1 mg/kg p.v., p.o.) and ibuprofen (100 mg/kg p.o.). However, the latter was effective only in the second phase. Opioids, which operate centrally, inhibit both stages, but NSAIDs, which act peripherally, inhibit just the late phase. According to the findings, **TfH** has an antinociceptive effect centrally and peripherally and extra anti-inflammatory activity (Vontagu et al., 2004) .

The acetic acid-induced abdominal constriction test described by Daud et al. (2006) was selected as a typical model of inflammatory pain studies. Algesia is caused by the rapid release of numerous mediators into the peritoneal fluid, including histamine, serotonin, bradykinin, cytokines, and eicosanoids. These mediators increase vascular permeability and eventually trigger peritoneal nociceptors localized to the

Table 3
Effect of *T. fiebrigi* honey on edema carrageenan-induced rat paw edema

Group (n=6)	Doses (mg/kg)	Paw edema vol in mL (Mean ± S.E.) 0 H	1 H	2 H	3 H	4 H	5 H	6 H
Control	Sol. Fis.	1.48 ± 0.06a	1.80 ± 0.10a	1.90 ± 0.13a	2.00 ± 0.13a	2.00 ± 0.18a	2.15 ± 0.13a	2.20 ± 0.12a
Ibuprofen	100	1.40 ± 0.05a	1.45 ± 0.15b	1.47 ± 0.10b	1.50 ± 0.10b	1.50 ± 0.15b	1.50 ± 0.05b	1.55 ± 0.15b
TfH	250	1.50 ± 0.09a	1.71 ± 0.10a,c	1.73 ± 0.26a,c	1.82 ± 0.28a	2.08 ± 0.16a	2.10 ± 0.36a	1.99 ± 0.41a
	500	1.48 ± 0.11a	1.60 ± 0.07b,c	1.60 ± 0.18c	1.72 ± 0.14a	1.90 ± 0.15a	1.96 ± 0.20 a	1.95 ± 0.17a
	1000	1.48 ± 0.04a	1.50 ± 0.07b	1.52 ± 0.12c	1.56 ± 0.07b	1.75 ± 0.11a	1.90 ± 0.19a	2.00 ± 0.14a

TfH: *Tetragonisca fiebrigi* honey
ss (saline solution).
[a] Time after carrageenan injection (h). The values followed by the same letter are not significantly different. The significance level was P < 0.05 (one-way ANOVA, followed by Tukey's test).

Table 4
Effects of *T. fiebrigi* honey on cotton pellet-induced granuloma formation in rats.

Cotton pellet-induced granuloma formation					
Groups (n=6)	Dose (mg/kg/d)	Transudative weight (mg)	Granuloma weight (mg)	Granuloma inhibition (%)	dry Thymus weight (mg/100 g BW)
Control	ss	575.90 ± 28.80a	147.75 ± 12.75 a	–	30.49 ± 2.06 a
Ibuprofen	100	178.90 ± 15.50 b	76.20 ± 2.80 b	48.43	33. 24 ± 5.40 a
Meprednisone	5	165.10 ± 14.85 b	55.80 ± 9.00 c	62.23	22.61 ± 3.95 b
TfH	250	437.40 ± 26.60 c	107.35 ± 14.95 d	27.34	28.50 ± 4.50 a,b
	500	341.95 ± 7.60 d	95.25 ± 7.45 e	35.53	29.50 ± 7.35 a,b
	1000	287.67 ± 12.27 e	77.53 ± 5.83 b	47.53	31.15 ± 2.50 a,b

TfH: *Tetragonisca fiebrigi* honey
ss (saline solution).
Values are expressed as mean ± S.E.M. 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

Table 5
Effect of *T. fiebrigi* honey (**TfH**) on pain with the tail immersion

		Interval following treatment (h) 0.0	0.5	1.0	1.5	2.0
Treatment	Dose (mg/kg)	Reaction time (seg)				
Control	SW	2.05±0.15	2.25±0.10	2.40±0.10	2.40±0.11	2.15±0.05
Ibuprofen	400	2.10±0.13	2.35±0.06(*)	2.35±0.18	2.35±0.09	2.29±0.05
Morphine	5	2.10±0.10	3.85±0.15(*)	4.20±0.22(*)	4.00±0.20(*)	3.40±0.17(*)
	250	2.10±0.56	2.44±0.20(*)	2.64±0.31(*)	2.80±0.05	2.18±0.15(*)
TfH	500	2.15±1.11	2.57±0.85(*)	3.37±1.52(*)	3.40±0.50(*)	3.15±0.25(*)
	1000	2.10±0.96	3.43±0.51(*)	4.13±0.25(*)	3.52±1.54(*)	3.34±1.47(*)

Values represent the mean ± SEM in seconds (n=6). * The asterisks denote significance levels compared with the control group, p < 0.05 (one-way ANOVA, followed by Tukey's test).
SW (sterile water).

peritoneum (Deraedt et al., 1980) . **TfH** at a dose of 1000 mg/kg body weight significantly decreased the number of contortion episodes in rats, demonstrating that it inhibits acetic acid-induced visceral nociception.

Opioid agents exert analgesic effects through supraspinal and spinal receptors (Wen et al., 2014) . The tail immersion test assesses a possible central action of *T. fiebrigi* honey (**TfH**). A significant reduction of the painful sensation due to tail immersion in warm water was observed after oral administration of **TfH** at doses of 250, 500, 1000 mg/kg of body weight (Table 5). The inhibitory effects of **TfH** became pronounced at 90 min (80.85%) after the dose of 1000 mg/kg of body weight. Ibuprofen did not affect this test (Figure 3).

Our results show the **TfH** of molecules with potential antinociceptive and anti-inflammatory activities. Oral administration of the different doses of **TfH** did not cause deaths or toxic symptoms in any of the treated animals. No behavioural alterations were observed during the evaluation period (48 h). Bodyweight or food and water intake between the control and treated groups did not significantly differ.

4. CONCLUSION

T. fiebrigi honey (**TfH**) has recently been incorporated into the "Argentine Food Code". According to our knowledge, the results of this work constitute the first report on the therapeutic properties of **TfH**, evaluated in vivo. The results

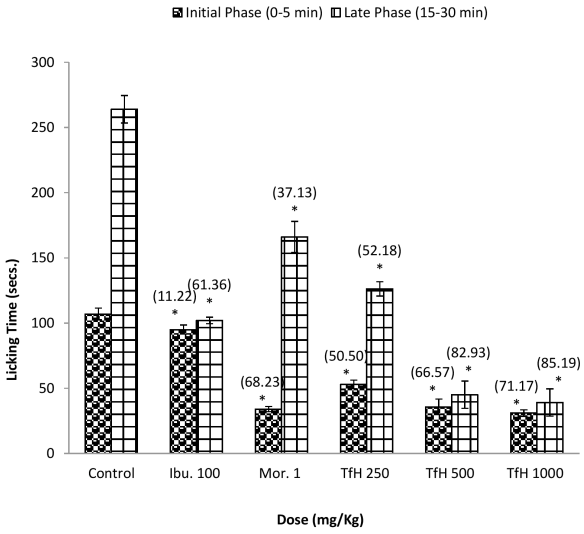

Figure 2. Effect of *Tetragonisca fiebrigi* honey on the nociceptive response of the formalin test in the first and second phases. Control, ibuprofen (Ibu 100 mg/kg b.w.), morphine (Mor 1 mg/kg b.w.), *T. fiebrigi* honey (TfH 250-500-1000 mg/kg b.w.). Values represent the mean ± SEM (n=6)* The asterisks denote significance levels compared with the control group, $p < 0.05$ (one-way ANOVA, followed by Tukey's test). Values in parentheses are the percentage of inhibition.

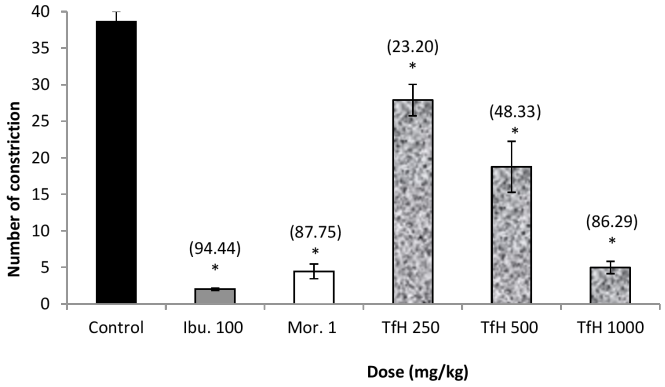

Figure 3. Oral dosing has little effect on acetic acid-induced writhing in rats. The intensity of the nociception behavior was evaluated by counting the total number of writhes occurring 20 minutes after the stimulus injection. Oral administration of ibuprofen (Ibu 100 mg/kg b.w.), morphine (Mor 1 mg/kg b.w.), and *T. fiebrigi* honey (TfH 250-500-1000 mg/kg b.w.) was used to treat rats. The proportion of inhibition is indicated in parentheses. * The asterisks indicate significant differences from the control group, $p < 0.05$ (one-way ANOVA followed by Tukey's test). The values represent the mean ± SEM (n=6).

showed that these honey have a significant antioxidant activity (ABTS$^{\cdot+}$ and DPPH radical assays) and antiinflammatory and antinociceptive potential, which may be helpful in the chronic inflammation process. We were able to identify and quantify phenolic compounds of **TfH**, describing for the first time relevant quantities of some phenolic acids and flavonoids which could be responsible for the pharmacological properties studied. Our results add value to **TfH,** which, due to its unique characteristics depending on botanical and geographic origin, becomes a healthy food that can provide nutrients and new perspectives for applying honey and its products like food and in specific medical treatments complementary to classical medicine.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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A. APPENDIX. SUPPLEMENTARY INFORMATION

Supplementary information to this article can be found online at https://doi.org/10.53365/nrfhh/144727.

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ETHICAL APPROVAL

The Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) approved the protocol for anti-inflammatory, antinociceptive, and acute toxicity testing on 07/14/2019 (No. CICUAL 012/2018, dated 07/14/2019). The copy of CICUAL approval opinion and protocol 012-2018 was given in the online supplementary file (Appendix A).

AUTHOR CONTRIBUTIONS

VMS, IYB, NRV - Research concept and design; VMS, IYB, GPG, LMM, CMR - Collection and/or assembly of data; VMS, IYB, GPG, LMM, CMR - Data analysis and interpretation; NRV - Writing the article; VMS, IYB, GPG, LMM, CMR, NRV - Critical revision of the article. All authors approved the final version of the article for submission.

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