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FULL ARTICLE

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Anti-inflammatory, antioxidant effects, and bioaccessibility of Tigzirt propolis

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Abstract

This work aims to assess the anti-inflammatory effects of Tigzirt propolis native to Algeria. We divided 48 male Wistar rats into 8 groups. We orally administered ethyl acetate extract of propolis (EAP), pure polyphenols compounds, or diclofenac 5 days before induction of inflammation by of carrageenan (100 μg/ml, i.p.). We determined the development of paw edema, biological parameters, myeloperoxidase activity, TNF-α, and prostaglandin E2 and measured the oxidative status parameters, as well. Finally, we analyzed the absorption and bioaccessibility of propolis in rats' plasma using GC–MS after orally dosing rats (250 mg/kg). The pretreatment by 200 and 250 mg/kg of propolis significantly reduced the edema rates after the third hour. Propolis can restore the disruption of homeostasis as well as markers of inflammation induced by carrageenan in Wistar rats, and an increase of the enzymatic activities. Furthermore, the inflammation was better resolved in rats that received propolis than in those treated with pure polyphenols.

Practical applications

Propolis is a natural mixture that bees produce by mixing gathered resin and gums to bee saliva and wax. Our research investigated the effect of Tigzirt propolis on the inhibition of biomarkers of inflammation and the development of paw edema. Propolis extract helped to reduce PGE2, TNF-α, myeloperoxidase, and malondialdehyde levels and increase the total antioxidant levels in plasma. Our findings emphasized the use of phenolic extract of propolis in industries such as nutraceuticals for the prevention of inflammatory diseases. It can also protect the body against damage under oxidative stress.

KEYWORDS

antioxidant, bioaccessibility, inflammation, paw edema, propolis

1 | INTRODUCTION

Inflammation is a pathophysiological disorder that contributes to a wide range of diseases. It is the most primitive protective response of the body to harmful stimuli (Ashley et al., 2012). There are many possible causes of inflammation. Still, inflammation shows similar basic pathological changes, such as tissue and cell degeneration, partial response for microvascular leakage of blood components, necrosis, hyperplasia, and repair; redness, swelling, fever, pain and dysfunction are the clinical symptoms of this disease (Santos et al., 2011). Non-steroidal anti-inflammatory drugs (NSAIDs) have a therapeutic effect on acute and chronic inflammation, pain, and fever

Abbreviations: CAT, catalase; CBC, complete blood count; CRP, C-reactive protein; EAP, ethyl acetate dry extract of propolis; EEP, ethanolic dry extract of propolis; GPx, glutathione peroxidase; i.p, intraperitoneal injection; MDA, malondialdehyde; MPO, myeloperoxidase; NIH, National Institutes of Health; PBS, phosphate-buffered saline; PGE2, prostaglandin E2; SOD, superoxide dismutase.

(Eccles, 2006; Sng & Schug, 2009; Zahradnik et al., 2010), through inhibition of cyclooxygenase (COX). However, the use of these drugs causes many side effects such as nephrotoxicity, gastrointestinal lesions, and bleeding (Rathee et al., 2009).

The result of inflammation is the production of free radicals. While the natural antioxidant defenses of the organism system can curb the production of free radicals. When it is too intense or chronic, free radicals become too numerous, overwhelm antioxidant defenses, and generate dangerous chain reactions. However, the use of chemical anti-inflammatory or antioxidant synthetic substances is always accompanied by side effects, whereas the use of phytochemicals is useful without side effects (El Hilah et al., 2015; Moulai-Hacene et al., 2020).

Propolis contains several complex resinous material harvested by *Apis mellifera* honeybees from tree barks, leaf buds, and exudates from a variety of plant sources (Boufadi et al., 2017; Silva et al., 2012). It is used by bees in the assembly and repair of their hives or as a protective barrier against external invaders (heat, humidity, and wind) due to its resinous nature and mechanical properties (Wagh, 2013). The biological activity of propolis depends on its contents in flavonoids, aromatic acid, diterpenic acid, and phenolic compounds (Boufadi et al., 2016; Chaa et al., 2019).

The purpose of this study is to evaluate the anti-inflammatory and antioxidant effects of Tigzirt Propolis, native to Algeria, in animal models together with the development of paw edema after injecting carrageenan.

2 | MATERIALS AND METHODS

2.1 | Materials

Solvents, i.e.CH_3OH, EtOH, ethyl acetate and $CHCl_3$ were obtained from Sigma-Aldrich (St Louis, MO, USA). Reagents, namely carrageenan, hyaluronic acid sodium salt, potassium tetraborate, p-dimethylaminobenzaldehyde,5-thio-2-nitrobenzoic acid (TNB), H_2O_2, catalase, trans-ferulic acid, and quercetin were also purchased from Sigma-Aldrich (St Louis, MO, USA). Kit MPO Rat was obtained from HyclutBiotec, Germany, andProstaglandin E2 Kit from Abcam Discover More, UK.

2.2 | Methods

2.2.1 | Propolis extraction

In a previous work (Boufadi et al., 2014), different types of propolis were provided by beekeepers from several regions of Algeria. In vitro testing of these samples showed that Tigzirt propolis had the highest concentration of antioxidant compounds and, subsequently, the highest antioxidant activity. These results sparked our interest in studying the anti-inflammatory effect of Tigzirt propolis.

We divided 10 g of propolis samples into small parts, crushed then extracted three times by means of ethanol 95% (100 ml) in an ultrasonic water bath for 1.5 hr. We filtered the suspension through a Whatman filter nr 1. The solvent then totally evaporated under reduced pressure at 60°C. The remaining solid particles represent the ethanol dry extract of propolis (EEP). We subsequently suspended EEP samples in 200 ml of water and extracted with 200 ml of chloroform. We discarded the organic layer, whereas we extracted the aqueous phases with 200 ml of ethyl acetate (EtOAc) three times. We collected and evaporated the organic phases of EtOAc in order to obtain ethyl acetate dry extract of propolis (EAP) (Boufadi et al., 2014).

We divided Tigzirt propolis into small parts and extracted it with ethanol 95% (1/10: W/V) in an ultrasonic water bath for 90 min. We performed this extraction operation three times. We carried out the filtration using Whatman filter No. 1 before evaporating the solvent to dryness under reduced pressure at 60°C. We then suspended this filtrate (ethanolic extract propolis EEP) in water and chloroform (1/1/1:V/V/V), extracted the aqueous phase with ethyl acetate (EtOAc) (1/1:V/V) three times. After the evaporation of the organic phase of EtOAc, we obtained the dry extract ethyl acetate of propolis (EAP) (Boufadi et al., 2014).

2.2.2 | Anti-inflammatory activity hyaluronidase assay in vitro

We determined the inhibition rate of hyaluronidase according to the method described by Silva et al. (2012) with a few modifications. We mixed 50 µl of EAP (1, 2.5, 5, 10, 15, 20, and 25 mg/ml) with 50 µl (350 units) of hyaluronidase enzyme and incubated at 37°C for 20 min. Then, we added 1.25 µl of calcium chloride to activate the enzyme. After incubating, the reaction was put medium at 37°C for 20 minutes, then 0.5 mL of hyaluronic acid sodium salt was added. After incubation at 37°C for 40 min, 0.1 ml of potassium tetraborate was added and the mixture was incubated in a boiling water bath for 3 min. We placed the mixture at +6°C to stop the reaction, and added 3ml of p-dimethylaminobenzaldehyde. We performed the incubation at 37°C for 20 min. Finally, we measured the absorbance at 585 nm. We performed all tests three times.

2.2.3 | Experimental groups

We used a total of 48 rats male Wistar albino weighing between 110 and 150 g for this experiment. These animals were provided by Pasteur Institute (Algiers, Algeria). All animal experiments were approved by the local ethical committee for animal care of the institution (University Abdelhamid Ibn Badis, Mostaganem) (rat/mouse 20% maintenance, RN-01-20K12; Carfil Quality). We randomly placed them in metabolic cages for an adaptation period (2 weeks), with a constant light dark cycle at a temperature of 22 ± 2°C and relative humidity of 40%. All animals were provided with food and water.

After the period of acclimatization, we divided the rats into eight groups. First, we, respectively, used Groups1 and 2 (G1 and G2) as negative and positive controls. Second, we, respectively, orally

dozed those in groups 3, 4, and 5 (G3 to G5) by 50, 200, and 250 mg/kg per day of EAP. Those in group 6 (G6) received 50 mg/kg per day of chrysin, whereas we orally dozed group 7 (G7) 50 mg/kg per day of trans-ferulic acid. Third, we orally dozed Group 8 (G8) diclofenac at a concentration of 100 mg kg^{-1} day^{-1}. The treatment lasted 5 days.

2.2.4 | Paw edema

We measured the diameter of paw edema earlier then again each hour for a period of 6 hr using a plethysmometer to determine the anti-inflammatory effect of Tigzirt propolis on edema (Figure 1). On day 5 (G2 to G8), 30 min after completion of treatment, we injected 100 µl of carrageenan at 1% (intradermal injection) on the right hind paw, the control group (G1) received the same volume of vehicle (saline solution) (Hu et al., 2005).

2.2.5 | Carrageenan-induced peritoneal fluid in rat

We elicited induction of acute inflammatory reaction by the injection of carrageenan in the peritoneal cavity of the rats, as done by Paulino et al. (2008). This mainly concerned groups 2 to 8, 30 min after treatment completion (i.e., after 5 days), by intraperitoneal (i.p) injection of 100 µl of carrageenan (1%) (Figure 2). Four hours following the induction of inflammation, we maintained the rats under light anesthesia with chloroform prior to their termination in order to avoid change of biochemical parameters before blood collection. We collected blood samples by cardiac puncture in dry and dry heparinized tubes.

We washed the remaining erythrocytes with physiological saline solution three times, added ice-cold distilled water to lysis them, then centrifuged at 5,000 t/min for 5 min to remove cell debris, and recovered the lysate for the determination of antioxidant enzymes erythrocyte. We stored the peritoneal fluid in sterile phosphate-buffered saline (PBS).

2.2.6 | Determination of biological parameters

We determined the total protein amount by means of the method of Gornall et al. (1949), the albumin concentration through the method of Doumas et al. (1977), the fibrinogen concentration using the kit of Biomérieux (Biomérieux SA, France), erythrocyte

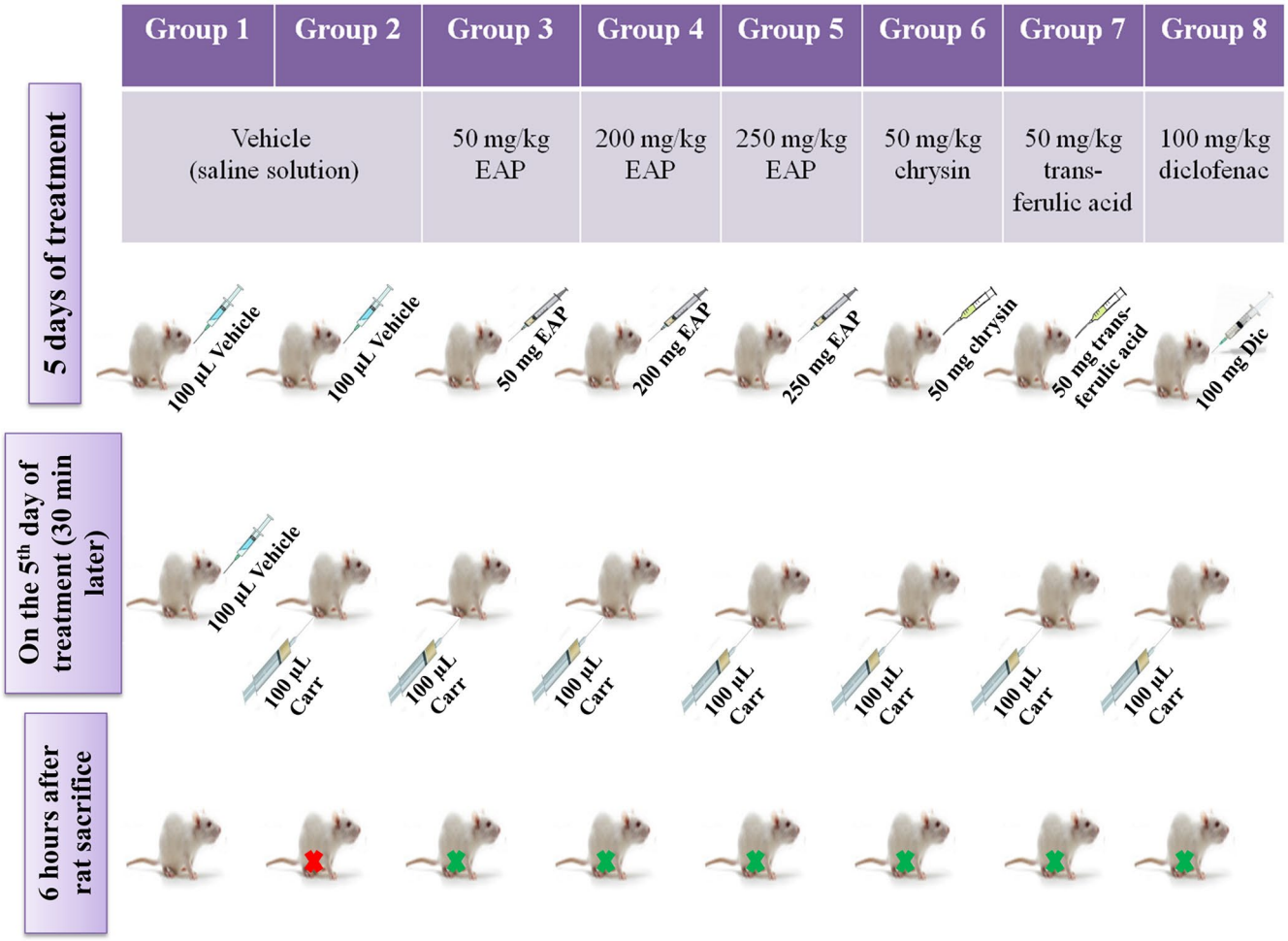

FIGURE 1 Experimental design for carrageenan-induced paw edema in rat

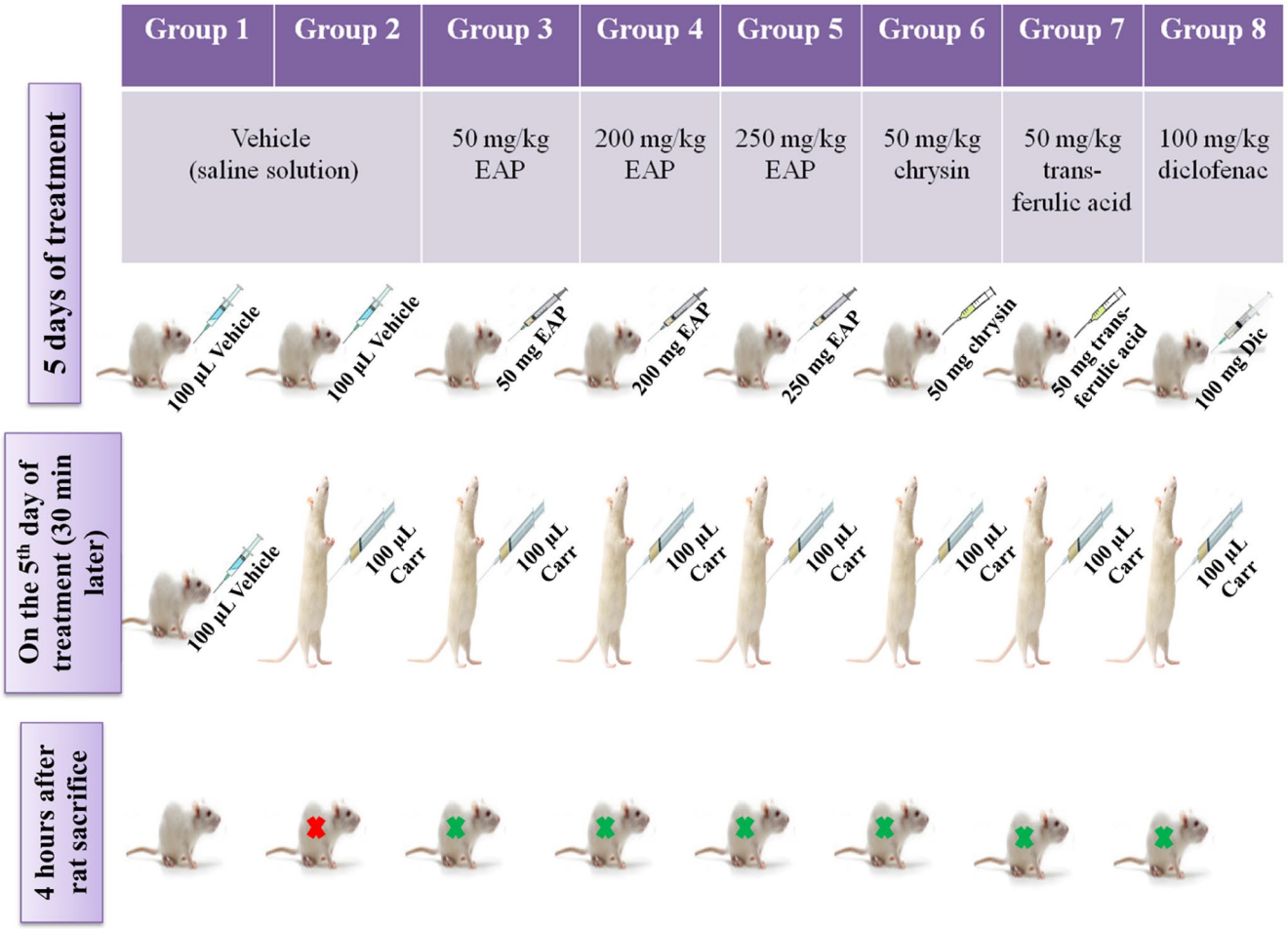

FIGURE 2 Experimental design for carrageenan-induced peritoneal fluid in rat

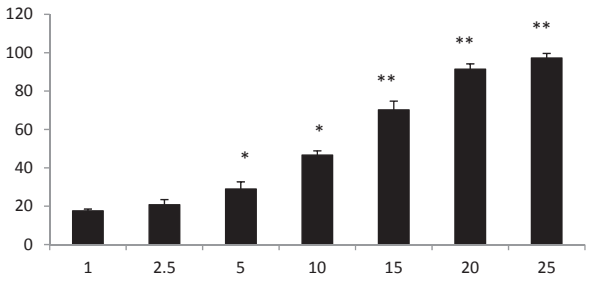

FIGURE 3 Percentage of inhibition of hyaluronidase activity by ethyl acetate extract of propolis from Tigzirt (EAP) for each concentration. The values are expressed as mean ± *SD* ($n = 5$). *Significant difference from the control group ($p < .05$). **Significant difference from the carrageenan inflammation group ($p < .05$)

sedimentation rate by the method of Westergren. Furthermore, we determined CRP (C-reactive protein) by Rat CRP/C-Reactive Protein ELISA Kit (Sigma-Aldrich, Belgium), the transferrin concentration using the kit of Biomérieux (Biomérieux SA, France), whereas Complete blood count (CBC) by a Beckman Coulter device (France).

2.2.7 | Inhibition of Myeloperoxidase (MPO) assay

We mixed 200 µl of the reaction mixture (10 mM phosphate buffer PO43− / 300 mM NaCl pH 7.4, 15 mM taurine) in a 96-well plate in order to quantify the activity of MPO by the principle of oxidation dependent on H_2O_2 of the TMB. We added 20 µl of peritoneal exudate, 50 µl of TNB (1.35 mM), 10 µl of H_2O_2 (100 µM) and 10 µl of catalase (8 U / µl). Then, we measured the absorbance of the solutions at 412 nm using a microplate reader (Boufadi et al., 2014). We determined the quantity of MPO by use of the kit MPO, Rat, ELISA kit (Hyclut. Biotec, Germany).

We determined the quantity of MPO by use of the kit MPO, Rat, ELISA kit (Hyclut. Biotec, Germany).

2.2.8 | TNF-α and prostaglandin E2 (PGE2) quantification

To determine the amount of TNF-α present in the peritoneal fluid, we used an ELISA kit (Thermo Fisher Scientific, USA) together with another ELISA Kit Prostaglandin E2 ELISA Kit (Abcam Discover More, UK) to measure PGE2 in the peritoneal fluid.

2.2.9 | Antioxidant status evaluation

We determined superoxide dismutase in accordance with the protocol of Elstner et al. (1983) and the level of enzymatic activity of catalase in the erythrocyte by the method of Lück (1965) and Aebi (1974).

We measured the activity of *GPx*in erythrocytes in accordance with the method of Paglia and Valentine (1967). Concerning the measurement of malondialdehyde (MDA) we used plasma in accordance with the method described by Yagi (1976).

2.3 | Bioaccessibility of propolis in blood plasma

We used a chromatography (GC–MS) to analyze the plasma of animals treated with 250 mg/kg of propolis (G5) with column DB-5ms (30 m × 0.25 mm × 250 microns) from Agilent, set the carrier gas (helium) at 0.7 ml/min, held the injector at 220°C, operating in split mode and conducted Phenolic acid detection at a temperature of 310°C (Boufadi et al., 2017).

2.4 | Statistics

We repeated each experiment three times in a fully randomized device. We subjected the results to unidirectional analysis of variance ANOVA using SigmaStat® software (SPSS, 3.0, SPSS Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | Hyaluronidase inhibition

Our findings show that the inhibitory activity of hyaluronidase increases with the increase of propolis extract (EAP) concentrations (IC_{50} value of 6.29 ± 0.7 mg/ml). The percentage of inhibition was 97% at a propolis concentration of 25 mg/ml (Figure 3).

3.2 | The effects of carrageenan-induced paw edema in rats

Rats intake of 200 and 250 mg/kg of propolis significantly ($P < .05$) reduced paw edema the third, fourth, fifth, and sixth hours following carrageenan injection. Diclofenac significantly inhibited the paw edema after the induction of carrageenan inflammation as well (Figure 4).

3.3 | Biochemical parameters

The effects of EAP, pure compounds, and diclofenac on the hematological parameters of the rats' inflammation are given in Table 1 and 2. These results show that carrageenan alone (in G2) induced a significant increase ($p < .05$) in white blood cells (WBC) in the cured rats while their red blood cells (RBC) count decreased, whereas rats provided with 200 and 250 mg/kg of EAP (G4 and G5) proved normal total WBC count as well as neutrophil and lymphocyte percentages comparing to their control values.

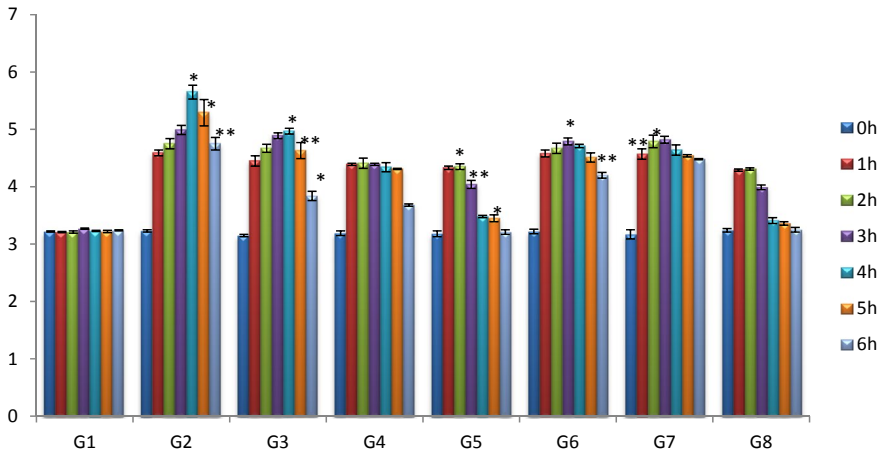

FIGURE 4 Effect of propolis on carrageenan-induced hind paw edema in rats. The values are expressed as mean ± *SD* ($n = 5$). *Significant difference from the control group ($p < .05$). **Significant difference from the carrageenan inflammation group ($p < .05$). G1, Control; G2, 200 µl of carrageenan 1% (*i.p*); G3, G4, and G5, treatment with 50, 200, and 250 mg/Kg of EAP (5 days) then 200 µl of carrageenan 1% (*i.p*); G6 and G7, treatment with 50 mg/Kg of chrysin or trans-ferulic acid (5 days) then 200 µl of carrageenan 1% (*i.p*); G8, treatment with 100 mg/Kg of diclofenac (5 days) then 200 µl of carrageenan 1% (*i.p*)

TABLE 1 Complete blood count (CBC) for the experimental groups of rats

	Experimental groups							
Hematological criteria	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
RBC ($\times 10^6$)	7.98	6.42*	7.57	8.38	8.59	8.3	7.97	7.92
WBC ($\times 10^3$)	4.77	8.55	7.08*	3.71**	3.61	4.42	5.51	6.5**
Neutrophil (%)	35	67*	55**	43	27	37**	41**	33
Eosinophil	1.75	0.25	0.75	1.25	1.5	1.25	1.75	1.5
Basophil	0.5	0.75	0.25	0.25	0.5	0.25*	0.25	0.25
Lymphocyte	57.75	29	41	51.5**	66	58.5*	54	61.25*
Monocyte	5	3	3	4	5	3	3	4
Hb (g/dL)	14.5	12	13.2	14.7	15	14.6	14.4	14.1
Plaquettes ($\times 10^3$)	366	870	620	200	193	340**	456	554

Note: The values are expressed as mean ± *SD* ($n = 5$).

G1, Control; G2, 200 µl of carrageenan 1% (*i.p*); G3, G4, and G5, treatment with 50, 200, and 250 mg/Kg of EAP (5 days) then 200 µl of carrageenan 1% (*i.p*); G6 and G7, treatment with 50 mg/Kg of chrysin or trans-ferulic acid (5 days) then 200 µl of carrageenan 1% (*i.p*); G8, treatment with 100 mg/Kg of diclofenac (5 days) then 200 µl of carrageenan 1% (*i.p*).

*Significant difference from the control group ($p < .05$).

**Significant difference from the carrageenan inflammation group ($p < .05$).

Comparing to the control group (G1), a significant decrease of the total protein (52.69%) is proven in the group that was injected with carrageenan only (G2). Such a decrease is also observed in the group that received 50 mg/kg of Tigzirt propolis (G3). The values measured in the group that had *trans*-ferulic acid (G7) show little decrease in total protein contents. The intake of 250 mg/kg of EAP (G5) gives the same value of total protein as that of negative control group (G1). Moreover, the total protein values measured in groups G6 and G8 are closer to those of the group of rats only injected with carrageenan (G2). The same results are observed for the albumin concentration (Table 2): inflammation by carrageenan injection caused severe decrease of plasma albumin in group G2 ($p < .05$), while little decrease is noticed in groups G3, G7, and G8 when compared to that of control.

Levels of fibrinogens and CRP measured in the positive controls (G2) are significantly higher ($p < .05$) than those in the negative control group (G1) (Table 2). In comparison to the positive controls, groups treated with EAP show a significant lower level ($p < .05$) of fibrinogen and CRP.

Acute-phase protein transferrin in the group with carrageenan-induced inflammation (G2) shows a severe decrease, while it appears more normal in the groups that had EAP (G3 to G5). Transferrin level is higher in the group that was treated with diclofenac (G8) than it is in the positive control group (G2), but not as high as in the groups that had high concentrations of EAP (G4and G5).

3.4 | Myeloperoxidase inhibition assay

Rats administered with 50, 200, and 250 mg/kg of extract of propolis (G3–G5) before the induction of inflammation by carrageenan show, respectively, rates of an inhibition of the MPO in the peritoneal exudate of 51%, 68%, and 86% (Figure 5a). Those cured with diclofenac (100 mg/kg) show the same level of inhibition as that of 250 mg/kg of EAP. However, the assessment of the activity of MPO reflects both the direct inhibition of MPO and the inhibition related to the release of MPO from neutrophils. To investigate whether this inhibition is the result of a decrease of the MPO release or of an inhibition of the MPO activity, we assessed the quantity of MPO in the peritoneal fluid. Figure 5b illustrates the quantity of MPO in ng/ml. The results show that EAP reduced also the release of MPO from neutrophils, as clearly shown in groups G3, G4, and G5. In contrast, no significant reduction of MPO release is noticed in the rats that were treated with diclofenac (G8). Calculation of the values of the MPO activity versus quantity of MPO was done to determine the inhibitory activity of EAP on the activity of MPO. It shows that EAP does not only reduce the release of MPO but also inhibits its activity (Figure 5c). This calculation of the MPO activity versus the quantity of MPO also indicates that diclofenac inhibits MPO rather than reduces its release.

3.5 | Prostaglandin E2 quantification

PGE2 is a pro-inflammatory prostaglandin involved in the cascade of inflammatory actions, such as the pro-inflammatory cytokine LB4.

Measuring the concentration of PGE2 in the peritoneal fluid from the rats which had the Tigzirt propolis extract (G3–G5) and those solely injected with carrageenan (G2) shows that, respectively, the concentrations of 200 and 250 mg/kg of EAP (G4 and G5) significantly ($p < .05$) decreases the levels of PGE2 by 35% and 64%, (Figure 6), whereas PGE2 increases significantly in the group cured solely by carrageenan (G2) with a concentration of 1,255.8 pg/ml, when compared with a concentration of 838.8 pg/ml in the negative

TABLE 2 Biological parameters of the experimental groups of rats

Parameters	Experimental groups							
	G1	G2	G3	G4	G5	G6	G7	G8
Total protein (g/dL)	7.52 ± 0.45	4.6 ± 0.33*	5.71 ± 0.68	6.88 ± 0.47	7.44 ± 0.31	5.22 ± 0.78	6.11 ± 0.24**	6.78 ± 0.59**
Albumin (g/dL)	5.13 ± 0.45	2.97 ± 0.32*	4.07 ± 0.27**	4.71 ± 0.37**	5.23 ± 0.44	5.08 ± 0.19	4.8 ± 0.65	4.17 ± 0.62**
Fibrinogen (g/L)	2.8 ± 0.36	8.3 ± 0.67*	4.38 ± 0.55**	3.54 ± 0.51	2.85 ± 0.22**	4.11 ± 0.34	4.74 ± 0.3	3.73 ± 0.41
Transferrin (g/L)	2.54 ± 0.22	0.75 ± 0.11*	1.2 ± 0.37	2.37 ± 0.12	2.61 ± 0.33	2.00 ± 0.46**	1.88 ± 0.65	1.94 ± 0.38
CRP (mg/L)	4.68 ± 0.58	9.69 ± 0.66*	6.87 ± 0.87**	5.68 ± 0.37	4.54 ± 0.44	5.74 ± 0.19	6.11 ± 0.54*	4.69 ± 0.25**

Note: The values are expressed as mean ± *SD* ($n = 5$).

G1, Control; G2, 200 µl of carrageenan 1% (*i.p*); G3, G4, and G5, treatment with 50, 200, and 250 mg/Kg of EAP (5 days) then 200 µl of carrageenan 1% (*i.p*); G6 and G7, treatment with 50 mg/Kg of chrysin or trans-ferulic acid (5 days) then 200 µl of carrageenan 1% (*i.p*); G8, treatment with 100 mg/Kg of diclofenac (5 days) then 200 µl of carrageenan 1% (*i.p*).

*Significant difference from the control group ($p < .05$).

**Significant difference from the carrageenan inflammation group ($p < .05$).

control group (G1). We also noticed a decrease in PGE2 concentrations in the rats that had diclofenac (G8), 50 mg/kg of EAP (G3), chrysin (G6), and *trans*-ferulic acid (G7) with rates varying between 360.6 and 657.2 pg/ml.

3.6 | TNF-α quantification

As shown in Figure 7, TNF-α levels in the peritoneal fluid significantly increased following carrageenan injection (G2: 5,345 pg/ml), when compared to that in the control group (G1: 1871,66). The intake of propolis at a dose of 50, 200, and 250 mg/kg resulted in the concentration of TNF-α, which is a significant decrease, respectively, 35%, 52%, and 78%, when compared to that of group 2 (the rats that were injected with carrageenan).

3.7 | Antioxidant status evaluation

Antioxidant status represents the dynamic balance between the antioxidant defense system and the production of free radicals by the mechanism of peroxidative degradation of lipids (oxidation of unsaturated lipids). MDA is a compound resulting from the peroxidative lipid degradation. In the rats treated with carrageenan only (G2), plasma MDA levels are found to be elevated (+6.68 mmo/ml), but the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in the erythrocytes are lower (Table 3) when compared to the negative controls (G1). The administration of the 50, 200, and 250 mg/kg of extract of propolis, (respectively, G3, G4 and G5) generated an increase of the enzymatic activities of SOD, CAT, and GPx in relation to the dose administered. Our results show that the doses of 200 and 250 mg/kg of EAP are more effective than those of diclofenac (G8).

3.8 | Bioaccessibility of polyphenols in plasma

After GC–MS cured the rats with 250 mg/kg of propolis (G5), we measured the bioaccessibility of phenolic acids in rat plasma. Many polyphenolic compounds, such as caffeic acid, gallic acid, cinnamic acid, coumaric acid, ferulic acid, and several flavonoids, such as kaempferol, quercetin, epicatechin, chrysin, and vanillin, were present in plasma after 24 hr (Table 4).

4 | DISCUSSION

The composition of Tigzirt propolis native to Algeria was previously assessed by means of LC–MS/MS (Boufadi et al., 2014) and LC/UV (Chaa et al., 2019). Several chemical constituents that possess biological activity were identified. These included tyrosol, caffeic acid, trans-ferulic acid, galangin, kaempferol, chrysin, quercetin, pinocembrin, tectochrysin, and genistein. Because of the high amounts of

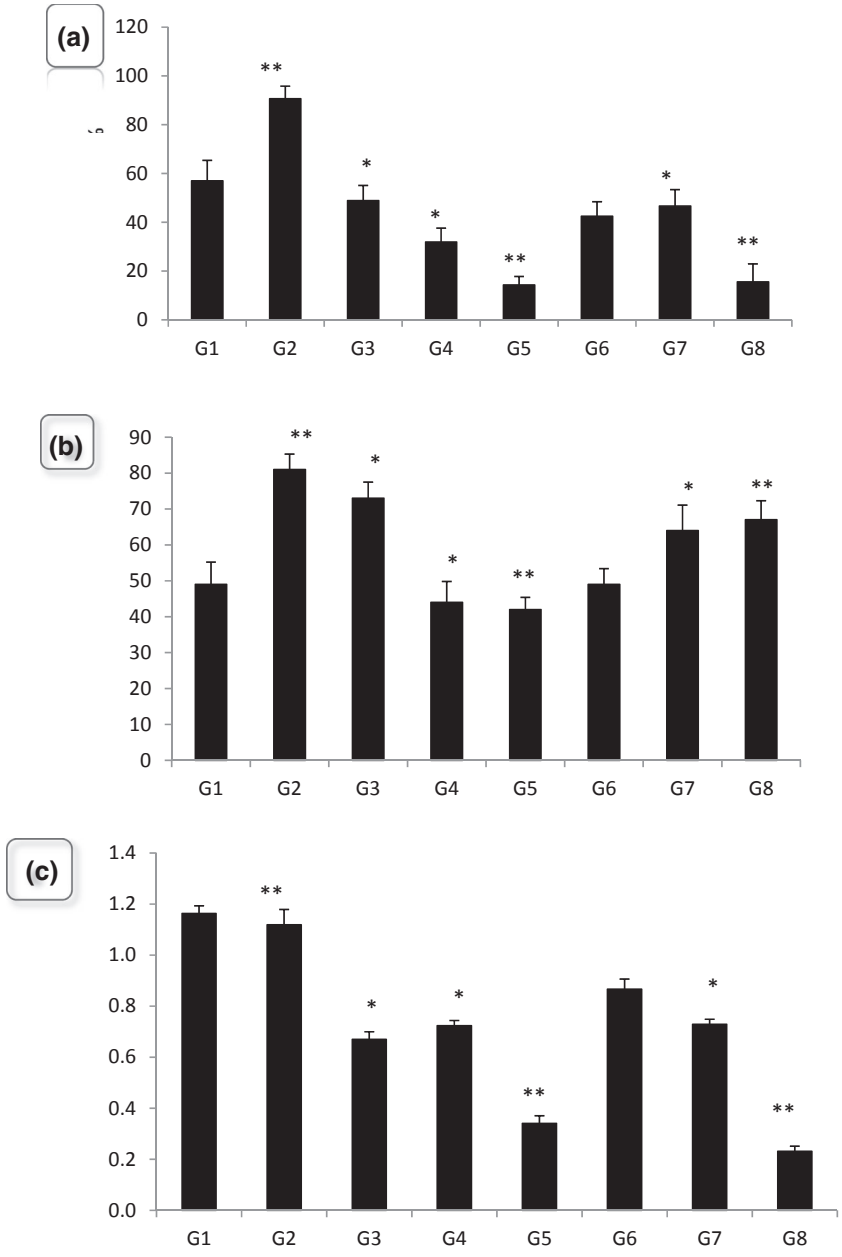

FIGURE 5 Percentage of activity % (a), quantity ng/ml (b), and relation activity/quantity (c) of the myeloperoxidase (MPO) in peritoneal exudates of rats for the tested groups. The values are expressed as mean ± *SD* ($n = 5$). *Significant difference from the control group ($p < .05$). **Significant difference from the carrageenan inflammation group ($p < .05$). G1, Control; G2, 200 µl of carrageenan 1% (*i.p*); G3, G4, and G5, treatment with 50, 200, and 250 mg/Kg of EAP (5 days) then 200 µl of carrageenan 1% (*i.p*); G6 and G7, treatment with 50 mg/Kg of chrysin or trans-ferulic acid (5 days) then 200 µl of carrageenan 1% (*i.p*); G8, treatment with 100 mg/Kg of diclofenac (5 days) then 200 µl of carrageenan 1% (*i.p*)

polyphenols and flavonoids in Tigzirt propolis, its color is dark brown. Abundant plants in that region are crataegus, oak, lavender, eucalyptus, and carob. The climate is Mediterranean and water is available in abundance (Boufadi et al., 2014; Chaa et al., 2019). The extracts from Tigzirt showed high polyphenol concentration (293 mg/g), while the concentration of flavonoid was lower (69 mg/g) (Boufadi et al., 2014).

Hyaluronidase is a family of enzymes that hydrolyze hyaluronan which is a major compound of the extracellular matrix. It causes an increase in granulation tissue, a decrease in the volume of edema, and a regulation of the inflammatory response by causing production of pro- and anti-inflammatory cytokines, growth factors, and mediators of eicosanoids (Monzón et al., 2008).

The findings of this study show that an intake of 250 mg/kg of EAP decreased the development of paw edema for a period of 3 hr after carrageenan injection. These results are consistent with the work of Hu et al. (2005) who showed that propolis from China reduced the paw inflammation after 3 hr of carrageenan injection.

However, Debbache et al. (2014) reported that propolis nigra aqueous fraction of chloroform (200 mg/kg) significantly inhibited onset of edema after 2 hr.

The inflammation process is part of the immune response to a damage or an infection. This process involves a cascade of successive events that result in a migration of the neutrophils to the inflammatory focus (Du et al., 2018; Geng et al., 2014). In some cases, the inflammation process causes damages to the tissues—an issue that explains the use of anti-inflammatory agents. During the later stage of inflammation, some compounds are formed and released to resolve the inflammatory response reducing the severity of inflammation, in order to avoid the deleterious effects (Napimoga et al., 2007, 2012). During the inflammation and in oxidative stress, albumin and total proteins tend to decrease in plasma while other proteins such as CRP increase. In addition, the migration of the neutrophils increase the level of their oxidative enzyme MPO, resulting in oxidative damages to the host tissues.

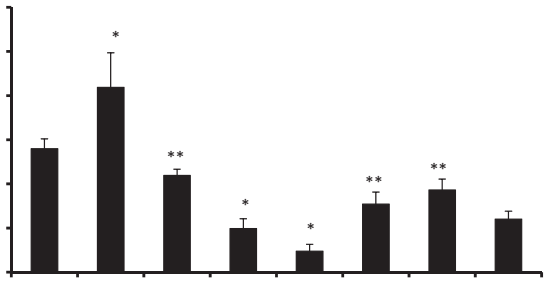

FIGURE 6 Concentrations of prostaglandin E2 (pg/ml) in peritoneal exudates of rats for the tested groups. The values are expressed as mean ± *SD* ($n = 5$). *Significant difference from the control group ($p < .05$). **Significant difference from the carrageenan inflammation group ($p < .05$). G1, Control; G2, 200 µl of carrageenan 1% (*i.p*); G3, G4, and G5, treatment with 50, 200, and 250 mg/Kg of EAP (5 days) then 200 µl of carrageenan 1% (*i.p*); G6 and G7, treatment with 50 mg/Kg of chrysin or trans-ferulic acid (5 days) then 200 µl of carrageenan 1% (*i.p*); G8, treatment with 100 mg/Kg of diclofenac (5 days) then 200 µl of carrageenan 1% (*i.p*)

Administration of carrageenan to create inflammation causes a decrease in the total protein and albumin levels and an increase in the migration of neutrophils. The decrease in total proteins and albumin may be attributed to a deficiency of their synthesis as a result of liver dysfunction due to the injection of carrageenan. An alteration at the receivers of biological membranes and the increase in permeability were due to the lipid peroxidation (Nehru & Anand, 2005). Newairy et al. (2009) have shown that the increase in lipoperoxydantion is a consequence of the decreased activity of superoxide dismutase (SOD) and catalase.

The adminitration of propolis for the rats inhibits the migration of neutrophils and, therefore, also limits the concentration of MPO. In fact, our results indicate that giving propolis extracts decreases the quantity of MPO as well as its activity. These inhibitions can be explained by the decrease of the neutrophils and their inhibitory effect on MPO (Boufadi et al., 2014). Bueno-Silva et al. (2013) have shown the effect of red propolis from Alagoas on inhibition of neutrophil migration into peritoneal cavity of mice upon induction of inflammation by carrageenan.

In the same study, it was shown that the propolis compounds (vestitol and neovestitol) have the same effect as dexamethasone in levels of the inhibition of neutrophil migration (Bueno-Silva et al., 2013). Rosalen et al. (2014) also observed that vestitol and neovestitol decrease migration of leukocytes during the inflammatory reaction.

Araújo et al. (2012) have demonstrated that propolis has an anti-inflammatory effect through several mechanisms, such as, inhibition of cyclooxygenase and prostaglandins E2.

Paulino et al. (2008) reported that an increase in the transport of solutes between plasma and the dialyzate has been observed during peritonitis, which explains cell migration. This disorder is due

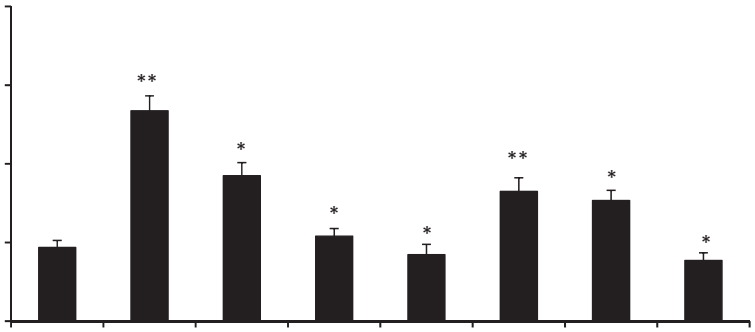

FIGURE 7 Concentrations of TNF-α (pg/ml) in peritoneal exudates of rats for the tested groups. The values are expressed as mean ± *SD* ($n = 5$). *Significant difference from the control group ($p < .05$). **Significant difference from the carrageenan inflammation group ($p < .05$). G1, Control; G2, 200 µl of carrageenan 1% (*i.p*); G3, G4, and G5, treatment with 50, 200, and 250 mg/Kg of EAP (5 days) then 200 µl of carrageenan 1% (*i.p*); G6 and G7, treatment with 50 mg/Kg of chrysin or trans-ferulic acid (5 days) then 200 µl of carrageenan 1% (*i.p*); G8, treatment with 100 mg/Kg of diclofenac (5 days) then 200 µl of carrageenan 1% (*i.p*)

TABLE 3 Anti-oxidant enzyme activity in the plasma of the experimental groups of rats

Parameters	Experimental groups							
	G1	G2	G3	G4	G5	G6	G7	G8
MDA (mmol/L)	2.85 ± 0.42	9.81 ± 1.5*	4.12 ± 1.26	2.05 ± 1.08**	1.31 ± 0.65	2.55 ± 0.57	3.01 ± 0.59**	3.25 ± 0.88
SOD (U/cg Hb)	19.01 ± 1.45	8.77 ± 1.31*	12.27 ± 0.69	19.32 ± 1.41	23.3 ± 1.65	16.54 ± 1.7**	12.28 ± 0.83	13.4 ± 1.29
CAT (U/mg Hb)	79.98 ± 7.52	25.77 ± 3.27*	50.87 ± 5.21	85.64 ± 3.11**	98.08 ± 11.3**	75.43 ± 3.09	66.81 ± 2.71	57.85 ± 2.97**
GPx (U/g Hb)	69.4 ± 5.12	22.36 ± 1.82*	31.57 ± 3.03	48.03 ± 3.09	59.2 ± 5.01	44.85 ± 1.93	36.22 ± 2.36**	47.58 ± 3.54

Note: The values are expressed as mean ± *SD* ($n = 5$).

G1, Control; G2, 200 µl of carrageenan 1% (*i.p*); G3, G4, and G5, treatment with 50, 200, and 250 mg/Kg of EAP (5 days) then 200 µl of carrageenan 1% (*i.p*); G6 and G7, treatment with 50 mg/Kg of chrysin or trans-ferulic acid (5 days) then 200 µl of carrageenan 1% (*i.p*); G8, treatment with 100 mg/Kg of diclofenac (5 days) then 200 µl of carrageenan 1% (*i.p*).

*Significant difference from the control group ($p < .05$).

**Significant difference from the carrageenan inflammation group ($p < .05$).

TABLE 4 Compounds found in plasma for G5 (250 mg/kg of EAP during 5 days). The identification was performed by GC–MS as trimethylsilyl ethers derivatives (% of total ion current)

RT	Compounds	RT	Compounds
	Aliphatic acids		*Esters*
31.27	9-Octadecanoic acid	8.12	3-Methyl-2-butenyl isoferulate
22.45	Hexadecanoic acid	12.39	Ethyl stearate
39.35	Succinic acid	22.82	Cinnamic acid ester
		34.34	Benzyl benzoate
	Aromatic acid		*Sugars*
17.21	Ferulic acid	15.17	Mannose
19.67	Caffeic acid	18.49	Sucrose
27.48	Gallic acid	17.24	Glucose
21.69	benzoic acid	15.47	Fructose
22.39	Propenoic acid	21.32	Sorbose
39.61	Trans-cinnamic acid	19.65	Xylose
32.36	Cinnamic acid	18.02	inositol
42.74	Coumaric acid		
41.33	3,4 dimethoxycinnamic acid		
28.99	2-Propenoic acid		
	Flavonoids		*Terpens and alcohols*
25.31	Kaempferol	8.11	Menthol
28.64	Quercetin	48.39	Pimaric acid
21.38	Bis-methylated quercetin	12.39	Phytol
24.98	Chrysin	18.01	Thymol
31.47	Myricetin	41.32	β-Eudesmol
33.84	Vanillin	28.09	Totarol
29.68	Pinocembrin	38.97	Dehydroabietic acid
21.66	Epicatechin	31.33	Thunbergol
29.44	Luteolin	19.39	Eudesmol
36.85	3,5,7-Trihydroxy flavone	27.52	Bisabolol
		51.17	Chrysophanol
		47.47	Glycerin

to vasodilation of capillaries in the peritoneal membrane caused by the production of neutrophils and prostaglandin E2.

An injection of 10 mg/kg of artepillin C in mice can inhibit the level of prostaglandin E2 and neutrophils during inflammation of the peritoneal cavity by carrageenan (Paulino et al., 2008). Dallegri and Ottonello (1997) observed inhibition of myeloperoxidase MPO in mice treated with artepillin C. Moreover, this observation is also supported by Tan-No et al. (2006), who showed that the anti-inflammatory activity of Chinese propolis reacted positively on the decrease in the level of nitric oxide in edema of the mouse paw induced by carrageenan.

Boufadi et al. (2017) have shown that the Tigzirt propolis compound blend can increase bioavailability through the presence of

the antioxidant pure compounds in the blood of rats after oral intake of 50 mg/kg of propolis during 45 days. Which explains that treatment with propolis from Tigzirt can inhibit the production of prostaglandin E2 and MPO during peritoneal inflammation, reducing the development of paw edema induced by carrageenan in Wistar rats.

5 | CONCLUSION

Our findings demonstrate that propolis extract have anti-inflammatory effects like that of diclofenac. In addition, they indicate that propolis has a stronger anti-inflammatory effect than some of its compounds. This can be explained by an increase of the bioavailability of the compounds when found in propolis comparing to these compounds when they are pure.

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CONFLICT OF INTEREST

We, the authors, acknowledge that there is no conflict of interest associated with respect to this study.

AUTHOR CONTRIBUTION

Mokhtaria Yasmina Boufadi: Conceptualization; Formal analysis; Investigation; Methodology; Validation. **Jalal Soubhye:** Methodology; Validation. **Pierre Van Antwerpen:** Investigation; Methodology; Supervision; Visualization.

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REFERENCES

Aebi, H. (1974). Catalase. In H. U. Bergmayer (Ed.), *Methods of enzymatic analysis* (2nd ed., Vol. *2*, pp. 673–684). Verlag Chemie.

Araujo, M. A. R., Liberio, S. A., Guerra, R. N. M., Ribeiro, M. N. S., & Nascimento, F. R. F. (2012). Mechanisms of action underlying the anti-inflammatory and immunomodulatory effects of propolis: A brief review. *Brazilian Journal of Pharmacognosy*, *22*, 208–219. https://doi.org/10.1590/S0102-695X2011005000167

Ashley, N. T., Weil, Z. M., & Nelson, R. J. (2012). Inflammation: mechanisms, costs, and natural variation. *Annual Review of Ecology, Evolution, and Systematics*, *43*, 385–406.

Boufadi, Y. M., Soubhye, J., Nève, J., Van Antwerpen, P., & Riazi, A. (2016). Antimicrobial effects of six Algerian propolis extracts. *International Journal Food Sciences Technology*, *51*, 2613–2620. https://doi.org/10.1111/ijfs.13247

Boufadi, Y. M., Soubhye, J., Riazi, A., Rousseau, A., Vanhaeverbeek, M., Nève, J., Boudjeltia, K. Z., & Van Antwerpen, P. (2014). Characterization and antioxidant properties of six Algerian propolis extracts: Ethyl acetate extracts inhibit myeloperoxidase activity. *International Journal Molecular Sciences*, *15*, 2327–2345. https://doi.org/10.3390/ijms15022327

Boufadi, Y. M., Van Antwerpen, P., Chikh Alard, I., Nève, J., Djennas, N., Riazi, A., & Soubhye, A. (2017). Antioxidant effects and bioavailability evaluation of propolis extract and its content of pure polyphénols. *Journal Food Biochemestry*, *42*(1), e12434. https://doi.org/10.1111/jfbc.12434

Bueno-Silva, B., Alencar, S. M., Koo, H., Ikegaki, M., Silva, G. V. J., Napimoga, M. H., & Rosalen, P. L. (2013). Anti-inflammatory and antimicrobial evaluation of neovestitol and vestitol isolated from Brazilian red propolis. *Journal of Agricultural and Food Chemistry*, *61*, 4546–4550. https://doi.org/10.1021/jf305468f

Chaa, S., Boufadi, Y. M., Keddari, S., Benchaib, A. H., Soubhye, J., Van Antwerpen, P., & Riazi, A. (2019). Chemical composition of propolis extract and its effects on epirubicin-induced hepatotoxicity in rats. *Revista Brasileira De Farmacognosia*, *29*(3), 294–300. https://doi.org/10.1016/j.bjp.2019.01.005

Dallegri, F., & Ottonello, L. (1997). Tissue injury in neutrophilic inflammation. *Inflammation Research*, *46*, 382–391. https://doi.org/10.1007/s000110050208

Debbache, N., Atmani, D., & Atmani, D. (2014). Chemical analysis and biological activities of *Populus nigra*, flower buds extracts as source of propolis in Algeria. *Industrial Crops and Products*, *53*, 85–92. https://doi.org/10.1016/j.indcrop.2013.12.018

Doumas, B. T., Biggs, H. G., Arends, R. L., & Pinto, P. V. C. (1977). Determination of serum albumin. Standared Methods. *Clinical Chemistry*, *7*, 175–188.

Du, B., Zhu, F., & Xu, B. (2018). An insight into the anti-inflammatory properties of edible and medicinal mush rooms. *Journal of Functional Foods*, *47*, 334–342. https://doi.org/10.1016/j.jff.2018.06.003

Eccles, R. (2006). Efficacy and safety of over-the-counter analgesics in the treatment of common cold and flu. *Journal of Clinical Pharmacy and Therapeutics*, *31*, 309–319. https://doi.org/10.1111/j.1365-2710.2006.00754.x

El Hilah, F., Ben Akka, F., Dahmani, J., Belahbib, N., & Zidane, L. (2015). Étude ethnobotanique des plantes médicinales utilisées dans le traitement des infections du système respiratoire dans le plateau central marocain. *Journal of Animal & Plant Sciences*, *25*(2), 3886–3897.

Elstner, E. F., Youngman, R. J., & Obwald, W. (1983). *Superoxyde dismutase in methods of enzymatic analysis* (3nd ed.). H. B Bergmeyer.

Geng, Y., Zhu, S., Lu, Z., Xu, H. Y., Shi, J. S., & Xu, Z. H. (2014). Anti-inflammatory activity of mycelia extracts from medicinal mush rooms. *International Journal of Medicinal Mushrooms*, *16*, 319–325.

Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*, *177*, 751–766. https://doi.org/10.1016/S0021-9258(18)57021-6

Hu, F., Hepburn, H. R., Li, Y., Chen, M., Radloff, S. E., & Daya, S. (2005). Effects of ethanol and water extracts of propolis (bee glue) on acute inflammatory animal models. *Journal of Ethnopharmacology*, *100*, 276–283. https://doi.org/10.1016/j.jep.2005.02.044

Luck, H. (1965). Catalase. In H. U. Bergmeyer (Ed.), *Methods of enzymatic analysis* (pp. 895–897). Academic Press.

Monzón, M. E., Manzanares, D., Schmid, N., Casalino-Matsuda, S. M., & Forteza, R. M. (2008). Hyaluronidase expression and activity is regulated by pro-inflammatory cytokines in human airway epithelial cells. *American Journal of Respiratory Cell and Molecular Biology*, *39*, 289–295. https://doi.org/10.1165/rcmb.2007-0361OC

Moulai-Hacene, F., Boufadi, M. Y., Keddari, S., & Homrani, A. (2020). Chemical composition and antimicrobial properties of Elettaria cardamomum extract. *Journal ofPharmacognosy*, *12*, 5.

Napimoga, M. H., Cavada, B. S., Alencar, N. M. N., Mota, M. L., Bittencourt, F. S., Alves, J. C., Grespan, R., Goncalves, R. B., Clemente-Napimoga, J. T., de Freitas, A., Parada, C. A., Ferreira, S. H., & Cunha, F. Q. (2007).

Lonchocarpus sericeus lectin decreases leukocyte migration and mechanical hypernociception by inhibiting cytokine and chemokines production. *International Immunopharmacology*, *7*, 824–835. https://doi.org/10.1016/j.intimp.2007.02.001

Napimoga, M. H., da Silva, C. A. T., Carregaro, V., Farnesi-de-Assuncao, T. S., Duarte, P. M., de Melo, N. F. S., & Fraceto, L. F. (2012). Exogenous administration of 15d-PGJ(2)-loaded nanocapsules inhibits bone resorption in a mouse periodontitis model. *The Journal of Immunology*, *189*, 1043–1052.

Nehru, B., & Anand, P. (2005). Oxidative damage following chronic aluminium exposure in adult and pup rat brains. *Journal of Trace Elements in Medicine and Biology*, *19*, 203–208. https://doi.org/10.1016/j.jtemb.2005.09.004

Newairy, A. S. A., Salama, A. F., Hussien, H. M., & Yousef, M. I. (2009). Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. *Food Chemistry Toxicology*, *47*, 1093–1098. https://doi.org/10.1016/j.fct.2009.01.032

Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxydase. *Journal of Laboratory and Clinical Medicine*, *70*, 158–169.

Paulino, N., Abreu, S. R. L., Uto, Y., Koyama, D., Nagasawa, H., Hori, H., Dirsch, V. M., Vollmar, A. M., Scremin, A., & Bretz, W. A. (2008). Anti-inflammatory effects of a bioavailable compound, Artepillin C, in Brazilian propolis. *European Journal of Pharmacology*, *587*, 296–301. https://doi.org/10.1016/j.ejphar.2008.02.067

Rathee, P., Chaudhary, H., Rathee, S., Rathee, D., Kumar, V., & Kohli, K. (2009). Mechanism of Action of Flavonoids as Anti-inflammatory Agents: A Review. *Inflammation & Allergy-Drug Targets*, *8*, 229–235.

Rosalen, P. L., Franchin, M., Bueno-Silva, B., Denny, C., Castanheira, F. V., Napimoga, M. H., Ikegaki, M., Alencar, S. M., & Cunha, T. M. (2014). Vestitol and neovestitol from Brazilian red propolis reduce leukocytes adhesion in the inflammatory process. *Planta Med*, https://doi.org/10.1055/s-0034-1382570

Santos, E. N., Lima, J. C., Noldin, V. F., Cechinel-Filho, V., Rao, V. S. N., Lima, E. F., Schmeda-Hirschmann, G., Sousa, P. T., & Martins, D. T. O. (2011). Anti-inflammatory, antinociceptive, and antipyretic effects of methanol extract of *Cariniana rubra* stem bark in animal models. *Anais Da Academia Brasileira De Ciências*, *83*, 557–566. https://doi.org/10.1590/S0001-37652011005000006

Silva, J. C., Rodrigues, S., Feás, X., & Estevinho, L. M. (2012). Antimicrobial activity, phenolic profile and role in the inflammation of propolis. *Food Chemistry and Toxicology*, *50*, 1790–1795. https://doi.org/10.1016/j.fct.2012.02.097

Sng, B. L., & Schug, S. A. (2009). The role of opioids in managing chronic non-cancer pain. *Annals of the Academy of Medicine Singapore*, *38*, 960–966.

Tan-No, K., Nakajima, T., Shoji, T., Nakagawasai, O., Niijima, F., Ishikawa, M., Endo, Y., Sato, T., Satoh, S., & Tadano, T. (2006). Anti-inflammatory effect of propolis through inhibition of nitric oxide production on carrageenin-induced mouse paw edema. *Biological &/and Pharmaceutical Bulletin*, *29*, 96–99. https://doi.org/10.1248/bpb.29.96

Wagh, V. D. (2013). Propolis: a wonder bees product and its pharmacological potentials. *Advances in Pharmacological and Pharmaceutical Sciences*, https://doi.org/10.1155/2013/308249

Yagi, K. (1976). A simple fluorometric assay for lipoperoxide in blood plasma. *Biochemical Medicine*, *15*, 212–216. https://doi.org/10.1016/0006-2944(76)90049-1

Zahradnik, H. P., Hanjalic-Becka, A., & Groth, K. (2010). Nonsteroidal anti-inflammatory drugs and hormonal contraceptives for pain relief from dysmenorrhea: A review. *Contraception*, *81*, 185–196. https://doi.org/10.1016/j.contraception.2009.09.014

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