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Analgesic and antiinflammatory activities of the aqueous root extract of Algerian *Bunium incrassatum*

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Abstract: The present study was designed to evaluate the antioxidant, analgesic, and antiinflammatory activities of *Bunium incrassatum* aqueous extract (BIAE) from roots. Tests of radical scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and induced erythrocyte hemolysis using 2,2'-azo bis (2-amidinopropane) dihydrochloride (AAPH) were used for antioxidant activity evaluation. The antiinflammatory activity was tested in Croton oil- and xylene-induced ear edema and carrageenan-induced paw edema. The antinociceptive effect was tested with the pain model induced by formalin and acetic acid-induced writhing response. The results revealed that BIAE exhibited a strong protective effect against AAPH-induced hemolysis of erythrocytes. In contrast, in the DPPH test, BIAE showed moderate activity (IC_{50}: 1.07±0.078 mg/mL) compared to BHT. In the antiinflammatory test, oral administration of BIAE (100, 300, and 600 mg/kg) significantly reduced the edema in the three models used. In antinociceptive experiments, the pretreatment with BIAE produced important analgesic activity. Additionally, the pretreatment of mice with BIAE significantly reduced the paw-licking time in the second phase. The results of this study revealed the antioxidant, analgesic, and antiinflammatory potential of BIAE, and demonstrated the importance of *B. incrassatum* as a source of compounds for therapeutic uses.

Keywords: *Bunium incrassatum*, polyphenols, antioxidant, antiinflammatory, analgesic

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed in the liver due to normal cellular processes. Under physiological conditions, the production of free radicals comprising ROS and RNS is tightly regulated by endogenous antioxidants [1]. The imbalance of ROS production and the antioxidant defense system in living systems causes oxidative stress and results in damage to many biological molecules, including proteins, fatty acids, and deoxyribonucleic acid [2]. Oxidative stress is considered a pathogenic mechanism contributing to aging and the development of different diseases, especially chronic inflammatory disorders such as rheumatism, diabetes, carcinoma, mutagenesis, sarcoma, aging, and circulatory disorders [3].

The inflammatory reaction, which is typically characterized by redness, swelling, heat, and pain, is defined as a non-specific response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation, and acts by removing harmful stimuli and initiating the healing process [4]. Without inflammation, infection will continue developing, wounds will not heal, and the injured organ will continue suppurating. Pain is a sign of tissue lesions due to mechanical, chemical, or physical stimulation. Pain occurs in response to various inflammatory mediators such as histamine, serotonin, bradykinin, and prostaglandins. These mediators activate pain receptors that channel the stimulus to the brain via nerve points with numerous synapses through the spinal cord, bone marrow, and midbrain. These substances, even in small quantities, can cause pain response. Pain results in reduced muscular activities associated with various free radicals as well as ROS that trigger some second messengers involved in the sensitization of dorsal horn neurons that play a fundamentally important role in neuropathic pain [5].

Nonsteroidal antiinflammatory drugs (NSAIDs) are a class of medication used to treat pain, fever, and

other inflammatory processes, but their use causes undesired and sometimes serious side effects [6]. Therefore, finding new and effective antiinflammatory drugs that can reduce pain and inflammation with lower adverse effects is crucial.

Medicinal plants are an abundant source of potential new medicines and often serve as chemical templates for creating new medications. *Bunium incrassatum* (Boiss), Batt and Trab, vernacularly called 'Talghouda', is one among these economically important medicinal plants belonging to the Apiaceae family and is widely distributed in northern Algeria. The genus *Bunium* consists of seven species in Algerian flora, four of which are endemic [7]. *B. incrassatum* saved many Algerians from starvation during the French colonization when people resorted to drying and grinding the harvested tubers of this plant to prepare bread and couscous [8]. The roots of the plant are very nutritious and traditionally used like potatoes. In the indigenous system of medicines, dried and powdered tubers are regarded as astringent and antidiarrheic and have been found to be applicable against inflammatory hemorrhoids. This plant root is also used to treat bronchitis and cough, probably due to its significant antimicrobial activity against various microorganisms [9]. The tubers of *B. incrassatum* are also used to treat thyroid problems [10]. Furthermore, Hajhashemi et al. [11] investigated the analgesic and antiinflammatory effects of *Bunium persicum*, a related species, and found that hydroalcoholic and polyphenolic extracts of the plant showed analgesic and antiinflammatory properties. The aqueous extract of *Solanum torvum* leaves used in traditional medicine possesses both analgesic and antiinflammatory properties [12]. Although these studies focused on related species, they provided evidence that supports the potential analgesic and antiinflammatory activities of the aqueous root extract of Algerian *B. incrassatum*.

The present study aimed to evaluate the *in vitro* antioxidant and *in vivo* analgesic and antiinflammatory activities of the aqueous extract of *B. incrassatum* roots in different experimental mouse models.

MATERIALS AND METHODS

Ethics statement

The mouse strain used for *in vivo* experiments was BALB/c, an inbred homogeneous strain from *Mus musculus domesticus*. Experiments were carried out using adult female mice weighing 25-30 g obtained from the Pasteur Institute of Algeria, Algiers, and housed for seven days before the experiments in plastic cages under standard laboratory conditions (relative humidity 50-70%, 20-22°C temperature, 12:12 h light:dark cycles, with free access to food and water). The animal experiments were according to the guidelines and procedural details in the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, 1985). Permission for experimental use was obtained from the Laboratory of Applied Biochemistry, Ferhat Abbas University of Setif 1. All procedures were performed in compliance with laws and institutional guidelines.

Nomenclature

The medicinal plant used in the present study was *Bunium incrassatum* (Boiss), Batt and Trab, vernacularly called 'Talghouda', which belongs to the Apiaceae family.

Plant material

The roots of *B. incrassatum* were collected from the region of R'mada, Aïn Lahdjar (Setif), Algeria (35° 56' 14" north, 5° 32' 32" east), in January 2022. Roots were identified by Prof. Hocine Laouer (Laboratory for the Valorization of Natural Biological Resources, Ferhat Abbas University, Setif 1, Algeria) and dried under shade at room temperature. The dried material was then pulverized to a powder using an electric grinder and stored at ambient temperature till use.

Preparation of BIAE

The aqueous extract of the plant was prepared according to the methods described in [13]. A total of 100 g of the powdered plant was boiled in 1 L of distilled water for 20 min. The extract was filtered and dried in an oven at 42°C. The crude extract was stored in the dark at -4°C until use.

Determination of polyphenol and flavonoid contents

Total polyphenols in BIAE were determined according to [14]. A volume of 0.1 mL of the aqueous extract or standard was mixed with 0.5 mL of Folin-Ciocalteu reagent (diluted to 1/10). After 4 min, 0.4 mL of 7.5% Na_2CO_3 solution was added. The mixture was shaken and incubated for 90 min at room temperature in the dark, and the absorbance was read at 765 nm. Gallic acid (20-180 µg/mL) was used to calibrate the standard curve, and the total polyphenol concentration was expressed as micrograms of Gallic acid equivalent per milligrams of dried extract (µg GAEq/mg).

The aluminum chloride colorimetric method was used to determine the total flavonoid content. Quercetin served as a standard; 0.5 mL of extract (prepared in distilled water) with adequate concentrations was added to 0.5 mL of $AlCl_3$ solution (2% in methanol). After 10 min of incubation at room temperature in the dark, the absorbance was read at 430 nm, and the concentration of flavonoid was expressed as micrograms of quercetin equivalent per milligrams of dried extract (µg QEq/mg) [14].

Antioxidant activity

DPPH radical scavenging assay

The radical scavenging activity of the BIAE was determined using a 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay according to [15]. A volume of 50 µL of different concentrations of aqueous extract or standard was mixed with 1250 µL of DPPH solution (0.004% in methanol). After 30 min of incubation in the dark at room temperature, the absorbance was read at 517 nm. Butylated hydroxytoluene (BHT) was used as standard. The inhibition percentage of free radical DPPH (I%) was calculated using the following equation:

$$I\% = 100 \times (Ac - Ae) / Ac$$

where A*c* is the absorbance of the control, and A*e* is the absorbance in the presence of BIAE or the standard. The IC_{50} values (the concentration of the sample that could scavenge 50% of DPPH free radical) were calculated.

Antihemolytic activity

The antioxidant activity of BIAE was measured by the inhibition of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidative erythrocyte hemolysis according to the protocol described by [16]. AAPH dissolved in phosphate buffer solution (PBS, pH 7.4) was used to induce the oxidation chain in erythrocytes. The blood used in this test was obtained by decapitation of the mice. The blood was collected in ethylene diamine tetra-acetic acid (EDTA) and centrifuged at 4°C at 1000 ×g for 15 min. The resulting sediment was washed three times with 10 mM phosphate buffered saline (PBS) to obtain a hematocrit of 2%. To assess the antihemolytic activity, an erythrocyte suspension (2%) was incubated with BIAE or vitamin C, followed by incubation with AAPH for 4 h at 37°C. The AAPH-induced oxidative hemolysis was monitored at 630 nm using a 96-well microplate reader (ELX 800, BioTek Instruments, Winooski, VT, USA). Erythrocyte resistance to the AAPH-induced hemolysis is estimated using the half-time of hemolysis (HT_{50}), corresponding to the time required to lyse 50% of initial erythrocytes.

Acute oral toxicity

An acute oral toxicity study was performed according to the Organization for Economic Cooperation and Development (OECD-425) guidelines [17]. Briefly, ten adult female mice fasted overnight with free access to water. The animals were divided into 2 groups of 5 animals each. In the 1st group, a single dose of BIAE at 2 g/kg body weight was given orally to the first mouse that was monitored for 24 h for signs of toxicity or mortality. If the animal survived for 24 h, the four remaining animals were given the same dose as described for the 1st mouse. All mice were closely observed for the first 30 min, intermittently every 30 min for 4 h, and then daily for 14 days for the presence of symptoms of toxicity (behavioral, body weight changes, changes in skin and fur, tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma) or mortality. If the half-lethal dose (DL_{50}) is greater than 2 g/kg, then the same experiment was repeated with the 2nd group of animals to test the 5 g/kg dose of the same extract.

Antiinflammatory activity

Xylene-induced ear edema

The study of the antiinflammatory effects of BIAE on xylene-induced ear edema followed the experimental method described in [18]. Mice were divided into 5 groups, each containing 5 animals. Group 1 (control group) mice were given distilled water. Group 2 (positive control group) mice were fed 50 mg/kg diclofenac. The mice in groups 3, 4, and 5 were treated with 100, 300, and 600 mg/kg of BIAE, respectively. One hour later, 0.02 mL of xylene was applied to the inner surface of the right ear of each group to induce edema. The thickness of the right ear of mice was measured before and 30 min after the induction of inflammation using a digital caliper.

Croton oil-induced ear edema

Detailed procedures were described in a previous study [13]. Briefly, 25 mice were randomly divided into 5 groups (n=5); Group 1 served as a negative control group (treated with the vehicle), Group 2 served as a positive control (treated with diclofenac (50 mg/kg)), and 3 groups were treated with BIAE (100, 300, and 600 mg/kg). The animals in all groups were orally treated 1 h before Croton oil application: edema was induced by topical application of 20 µL of acetone-distilled water (1:1) solution containing 80 µg of Croton oil as an inflammatory agent into the inner surface of the right ear of each mouse. The right ear thickness of the mice was measured with a digital caliper before and 6 h after Croton oil application.

Carrageenan-induced paw edema

Carrageenan-induced paw edema in mice is a well-established model of acute inflammation for screening of antiinflammatory agents. Paw edema was induced in the right hind paw of each mouse by an intraplantar injection of 0.02 mL of 1% (w/v) carrageenan in normal saline. BIAE (100, 300, and 600 mg/kg), diclofenac (50 mg/kg), or the drug-vehicle were orally administered 30 min before the carrageenan injection [19]. The thickness of the paws was measured just before the carrageenan injection and at 1, 2, 3, 4, and 5 h after injection by a digital caliper. The percentage of inhibition of edema (I%) was calculated by the following equation:

$$I\% = 100 \times (D_t - D_0) / D_0$$

where D_0 is the paw thickness before, and D_t is the paw thickness after carrageenan injection at different time intervals.

Analgesic activity

Acetic acid writhing test

The peripheral antinociceptive activity of BIAE was determined by acetic acid-induced abdominal constriction assay in mice [20]. Briefly, the animals were divided into 5 groups, each containing 5 animals (n=5). Group 1 was the negative control and received the vehicle; Group 2 was the positive control and treated with diclofenac (100 mg/kg). Groups 3, 4, and 5 were used as test groups and treated with 100, 300, and 600 mg/kg of BIAE, respectively. The animals fasted overnight before the experiment started with free access to water and then treated by oral gavage. Writhing was induced by an intraperitoneal injection of 0.7% acetic acid (10 mL/kg body weight) 60 min after the treatment of all groups, as stated above. The number of writhes (abdominal constrictions) was counted for each group of mice, starting 5 min after the acetic acid injection and up to 30 min. The number of writhes in each treated group was compared with the negative control.

Formalin-induced pain test

The formalin test was conducted according to the method described by Mustaffa et al. [21]. Mice were injected subcutaneously into the dorsal hind paw with 20 µL of 2.5% formalin prepared in 0.9% saline and transferred immediately to a transparent box for observation. The time (s) spent by animals licking or biting the injected paw was recorded in the early phase (0-5 min) and late phase (15-30 min) after the formalin injection. The animals were administered different doses of BIAE (100, 300, and 600 mg/kg) or diclofenac (100 mg/kg). Control animals received the vehicle. The doses were administered orally to the mice 60 min before the formalin injection. The reaction time of the animals was compared to the control group.

Statistical analysis

The results are expressed as the mean of triplicate±SD *in vitro*, while *in vivo* results are expressed as the mean±SEM. Statistical analysis of difference was performed using ANOVA, followed by Dunnett's test for multiple comparisons, using GraphPad Prism Software (version 8.0). Results were considered statistically significant at P≤0.05.

RESULTS

Total polyphenol and total flavonoid contents

The extractive yield of the decoction BIAE is around 16.68%. The total polyphenol content was 7.7±0.11 µg GAE/mg of dry extract, while the total flavonoid content was 5.36±0.35 µg QE/mg of dry extract.

Antioxidant activity

Scavenging activity of the DPPH radical

Radical scavenging activity was expressed as the mean of the IC_{50} (the concentration of BIAE necessary to decrease the initial concentration of DPPH by 50% under the specified experimental condition). The aqueous extract showed an IC_{50} value of 1.07±0.078 mg/mL. For comparison, a synthetic antioxidant (BHT) was used as a standard, and the antioxidant activity was measured using the same process. The BHT showed an IC_{50} value of 0.087±0.001 mg/mL.

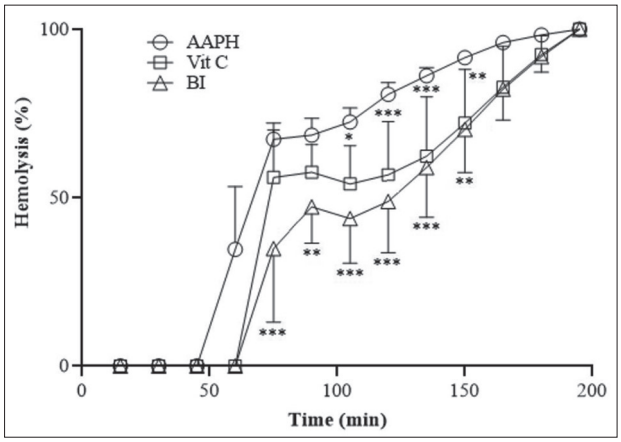

Fig. 1. The kinetic of the action of *B. incrassatum* extract (BI) and ascorbic acid on AAPH-induced hemolysis in RBC. Values were expressed as the mean±SD (n=4). **P<0.01, ***P<0.001 compared to AAPH as the control.

Antihemolytic activity

Fig. 1 shows the effect of BIAE on AAPH-induced hemolysis in mice erythrocytes. The addition of AAPH to the erythrocyte suspension decreased the half-time of hemolysis (HT_{50}=74.13±8.37 min) compared to red blood cells (RBCs) treated with BIAE and vitamin C. The aqueous extract of the plant exhibited a strong protective effect against AAPH-induced oxidative

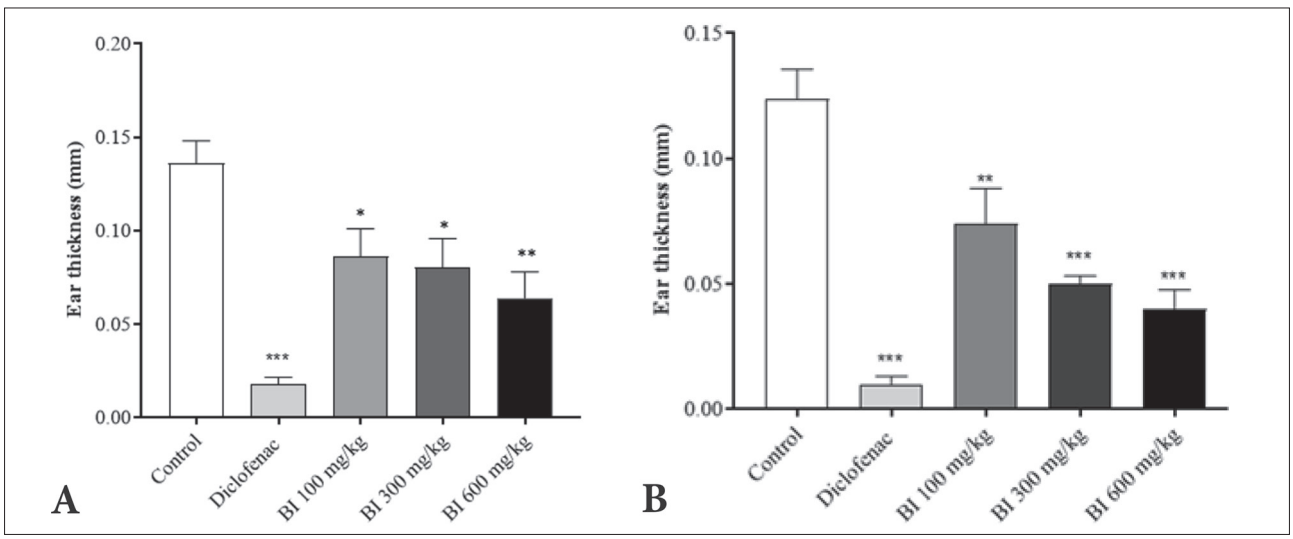

Fig. 2. Effect *B. incrassatum* extract on ear edema in mice. **A** – xylene-induced; **B** – Croton oil-induced. BI – *B. incrassatum*. Data are presented as the mean±SEM (n=5). **P<0.01, ***P<0.001 when compared with the control.

erythrocytes hemolysis. It significantly (P<0.05) increased the half-time of hemolysis (HT_{50}=117.41±19.08 min) compared to AAPH alone. Ascorbic acid at a concentration of 0.1 mg/mL, used as an antioxidant standard, had an HT_{50} of 106.36±26.03 min.

Acute toxicity test

The acute toxicity study of the extract was performed according to OECD guideline 425. The oral administration of BIAE at doses 2 and 5 g/kg in mice did not show any signs of toxicity, and all animals survived until day 14. These results suggest that the DL_{50} of the aqueous extract is higher than 5 g/kg. Based on this, 100, 300, and 600 mg/kg doses of BIAE were selected to evaluate *in vivo* biological activity.

Effects of the *B. incrassatum* extract on xylene-induced ear edema

The effects of BIAE on acute inflammation induced by xylene in mice are shown in Fig. 2A. The results showed that the administration of 100, 300, and 600 mg/kg doses significantly (P<0.05) reduced the ear edema induced by xylene (0.086±0.015 mm, 0.08±0.016 mm, and 0.064±0.014 mm, respectively) compared with the control group (0.136±0.012 mm). Diclofenac (50 mg/kg), the reference drug used, significantly (P<0.001) reduced edema (0.018±0.004 mm) compared to the control group.

Effects of BIAE on Croton oil-induced ear edema

The mice in the control group that received only the croton oil solution developed ear edema after 6 h, characterized by an increase in thickness of 0.124±0.012 mm, as shown in Fig. 2B. The BIAE treatment at doses of 100, 300, and 600 mg/kg produced a dose-dependent suppression of ear edema (0.074±0.014 mm, 0.05±0.003 mm, and 0.04±0.008 mm, respectively), and the highest dose of the extract demonstrated the most significant activity (P<0.001) compared to the control group. In these tests, treatment with the reference drug diclofenac (50 mg/kg) produced a greater reduction of edema (0.01±0.003 mm).

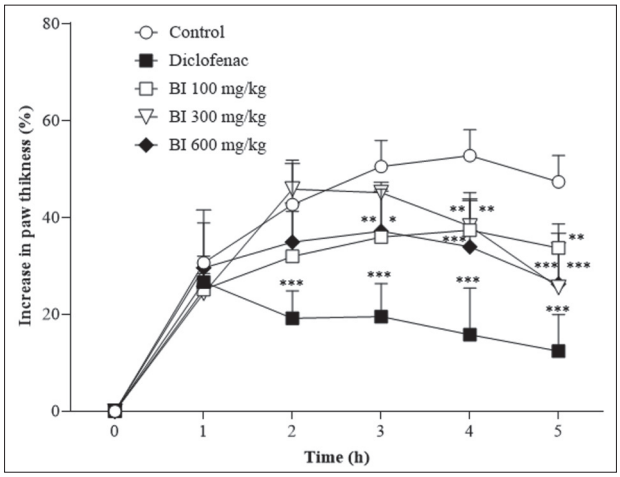

Fig. 3. Effect of *B. incrassatum* extract on carrageenan-induced paw edema in mice. BI – *B. incrassatum*. Data are presented as the mean±SEM (n=5). *P<0.05, **P<0.01 and ***P<0.001 when compared with the control group.

Effects of *B. incrassatum* extract on carrageenan-induced paw edema

Fig. 3 shows the results of the anti-edematous effect of orally administered BIAE on carrageenan-induced paw edema in mice. The subplantar injection of carrageenan into the right hind paw of mice (control) resulted in a time-dependent increase in paw volume (30.65±3.67%, 42.67±4.09%, and 50.51±2.41% in the 1st, 2nd, and 3rd h, respectively). Maximal inflammation was detected at the 4th h (52.77±2.38%), after which a gradual decline was observed. Oral administration of BIAE at a dose of 100 mg/kg significantly (P<0.01) reduced paw thickness from the 3rd to the 5th h (35.98±4.43% and 33.71±2.21%, respectively) compared to the control group. Administration of 300 mg/kg extract showed a significant reduction of edema at the 4th and 5th h. The group treated with the 600 mg/kg dose of the extract showed significant inhibition of edema at the 3rd, 4th, and 5th h (37.16±3.70%, 33.97±4.26%, and 26.27±4.68%, respectively) compared to the control group. Diclofenac caused a significant (P<0.001) reduction in paw edema 2 h after the subplantar injection of carrageenan (19.18±2.53%), the effect persisting until the 5th h (12.43±3.38%).

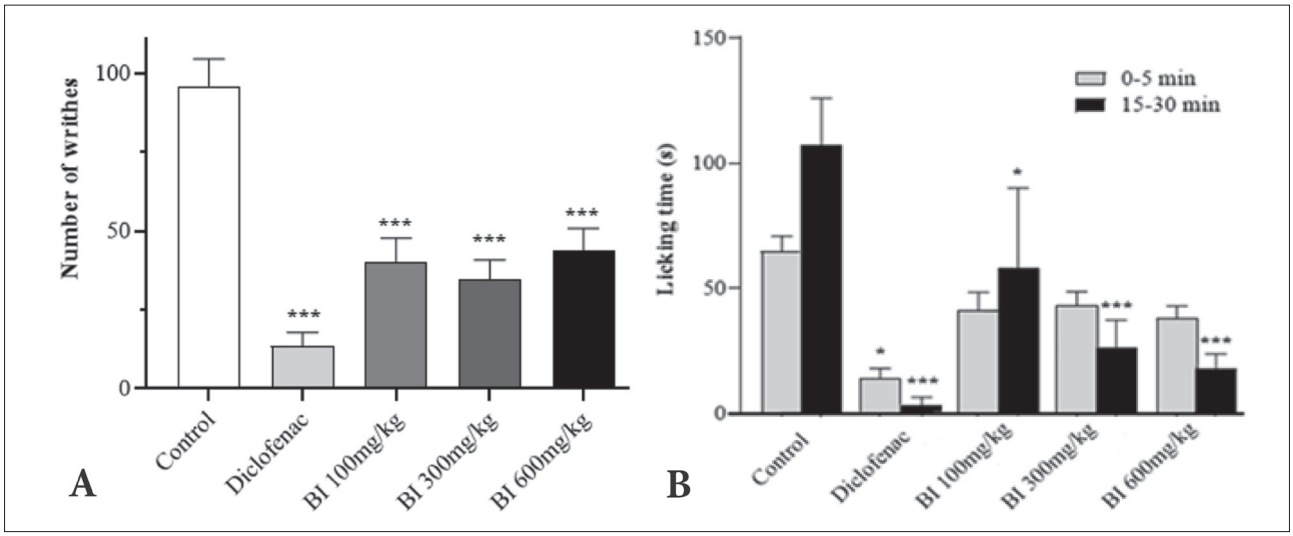

Fig. 4. Effect of *B. incrassatum* extract (BI) on acetic acid-induced writhing in mice (**A**) and on formalin-induced paw-licking in mice (**B**). Data are presented as the mean±SEM (n = 5). *P<0.05 and ***P<0.001 when compared with the control.

Analgesic activity

Effects of *B. incrassatum* extract on acetic acid-induced writhing

The writhing responses in the acetic acid-induced mice were measured to investigate the analgesic effects of BIAE (Fig. 4A). The number of contortions displayed by the control group was 96.00±8.56. The oral administration of BIAE at doses of 100, 300, and 600 mg/kg showed a significant (P<0.001) fall in the number of contortions (40.40±7.33, 34.60±6.18 and 44.00±6.76, respectively) in comparison with the control group. The reference drug, diclofenac, also significantly (P<0.001) reduced the writhing number induced by acetic acid (13.60±4.27).

Effects of BIAE extract on formalin paw test

Fig. 4B shows the results of the paw-licking test in mice. In the negative control group, an average licking time of 64.66±6.14 s and 107.04±18.80 s was observed during the early (0-5 min) and late phase (15-30 min), respectively, of the formalin test. The oral administration of BIAE did not significantly reduce the licking time in the early phase compared with the control group. However, all the extract doses (100, 300, and 600 mg/kg) significantly decreased the formalin-induced licking time in the late phase (with values of 58.20±31.80 s, 26.40±10.91 s and 17.60±5.85 s, respectively). Diclofenac (100 mg/kg) caused a significant reduction in the duration of nociceptive activity in the early and late phases with values of 14.00±3.96 s and 3.20±3.20 s, respectively.

DISCUSSION

Medicinal plants have long been utilized in traditional medicine as sources of therapeutic remedies. They have proven their value as the most important source of biologically active compounds with diverse pharmacological activities [22]. The main bioactive compounds in medicinal plants are polyphenols (especially phenolic acids and flavonoids). These compounds are well known for their biological activity, such as anticancer, antioxidant, antimicrobial, neuroprotective, antidiabetic, cardioprotective, and many other positive effects on the human body [23]. The present study aimed to investigate the pharmacological potential of the roots.

The extract's total phenolic and flavonoid contents were estimated using Folin-Ciocalteu reagent and aluminum chloride colorimetric methods, respectively. In a previous study, the total phenolic and flavonoid contents of BIAE were 06.92±0.00 µg GAE/mg and 06.91±0.01 µg QE/mg of dry extract, respectively [24], which were very similar to the results obtained in our study. However,

Toul et al. [10] reported that the amount of phenolic and flavonoid contents in *B. incrassatum* seeds extract were 101.20±3.64 µg GAE/mg and 46.61±0.91 µg CE/mg of dry extract, respectively, which were higher than that of the present study. Numerous environmental factors clearly influence the amount and composition of phenolic compounds present in plants, such as season, sampling period and geographic origin, precipitations, temperatures, and soil type [25].

Several methodologies are used to evaluate the antioxidant activity of the phenolic compound family. These methods have been classified according to the mechanism of radical deactivation, the physiological relevance of the free radical, or the competitive or direct approach of the reaction [26]. The antioxidant activity of BIAE was evaluated by the DPPH free radical scavenging method and AAPH-induced erythrocyte hemolysis assay. The DPPH radical has been widely used to assess the ability of compounds to operate as free-radical scavengers and hydrogen suppliers. It is a rapid, simple, and inexpensive method for measuring antioxidant properties [27]. The antioxidant effect of polyphenols on DPPH is due to their hydrogen-donating ability. In this study, BIAE showed moderate antioxidant activity. This activity was lower than that reported by [10] (IC_{50}: 0.29±0.02 mg/mL). The presence of high levels of phenolic compounds could explain the high scavenging activity of the plant extracts. Numerous studies of the antioxidant activity of plant extracts have demonstrated a linear correlation between total phenolic and flavonoid content and antioxidant capacity [28].

Erythrocytes are frequently used as a biological model to assess antioxidant potential because they are the first target of free radical attack due to their potential to produce ROS and the hemoglobin redox reactions associated with O_2 transport [29]. In this study, an assessment of the ability of *B. incrassatum* to protect the erythrocytes from oxidative damage caused by AAPH was performed. The results revealed that treatment with BIAE significantly increased the half-time of hemolysis caused by the free radicals. These results demonstrated that BIAE could scavenge AAPH-derived peroxyl radicals, thereby protecting the erythrocyte membrane. This antihemolytic activity should be due to the various phenolic compounds, which can scavenge free radicals and protect the cells against hemolysis induced by oxidative stress [29]. This ability is due to the direct scavenging of free radicals and the breaking of oxidative reactions and/or chelation of prooxidant metal ions stimulating free radical formation [30].

The present research evaluated the antiinflammatory activity using three *in vivo* models: croton oil, xylene, and carrageenan-induced edema. Xylene-induced ear edema in mice is a simple animal model for evaluating potential antiinflammatory agents [31]. The xylene produced fluid accumulation and edema within a few minutes after its application to the skin [32]. It causes the release of proinflammatory mediators from sensory neurons that act on peripheral target cells, such as mast cells and other immune cells, producing neurogenic inflammation characterized by warmth, redness, and edema [33]. The results of the present study indicate that BIAE significantly reduced the thickness of the ear caused by xylene at all doses. The antiinflammatory activity might refer to neurogenic inflammation [34]. The effect of the extract in this model also suggests inhibition of phospholipase A2, which is involved in the pathophysiology of inflammation due to xylene [35,36].

Croton oil-induced ear edema has been widely accepted as a useful pharmacological model to investigate new antiinflammatory drugs. Croton oil contains phorbol esters with 12-O-tetradecanoylphorbol-13-acetate (TPA), the predominant phorbol ester, as an active compound [37]. Topical application of Croton oil induces acute inflammation, and edema formation occurs by activating phospholipase A2 (PLA2), which releases arachidonic acid from the cell membrane, which is in turn metabolized to prostaglandin (PG) by cyclooxygenase and to leukotriene by 5-lipoxygenase [38]. The outcomes of our study in Croton oil-induced ear edema showed significant antiinflammatory activity of BIAE in a dose-dependent manner. These results reveal that the aqueous extract was able to reverse edema formation, suggesting an antiinflammatory activity of the extract.

Carrageenan-induced paw edema is a highly reliable and commonly used model for investigating acute local inflammation physiopathology. The development of carrageenan-induced paw edema is biphasic: the early phase occurs within 1 h upon induction, whereas the

later phase occurs 2-3 h after carrageenan injection [39]. The first phase is associated with the release of mediators, including histamine, serotonin, and bradykinins. The second phase is characterized by the infiltration of leukocytes and is mediated by an eicosanoid like PGE2. Nitric oxide is also involved in this model, which reaches its maximum level at 1 h, and subsequently starts to decline [40]. In the present study, the oral administration of BIAE did not affect the edema in the first phase but significantly decreased paw edema in mice in the second phase. The antiinflammatory activity of BIAE might be due to the inhibition of prostaglandin production [41].

The analgesic effect of BIAE was evaluated using two experimental animal models, the acetic acid-induced writhing response and the formalin test. The acetic acid-induced writhing test is a typical model for measuring inflammatory pain that is widely used for evaluating new agents with peripheral antinociceptive and antiinflammatory properties [42]. The intraperitoneal injection of acetic acid that irritates the serous membranes causes a stereotypical behavior in mice and characteristic abdominal contractions, movements of the body, twisting of the dorsal abdominal muscles, and a reduction in motor activity and coordination [43]. In this model, pain occurs in response to various inflammatory mediators such as PGE2 and PGE2α, histamine, serotonin, bradykinin, tumor necrosis factor (TNF)-α, and cytokines 6 and 8. These mediators also increase vascular capillary permeability, decrease pain threshold, and increase the sensitivity of nerve terminals of nociceptive fibers [44]. In the present study, all doses of BIAE significantly inhibited the acetic acid-induced writhing response in mice. These results suggested that the extract may exert a good analgesic effect against inflammatory pain by inhibiting inflammatory mediators associated with the nociceptive responses, such as PGE2, TNF-α, and interleukin.

The formalin test in mice is a valid and reliable nociception model sensitive to various analgesic drug classes [45]. Two distinct phases are involved in the formalin test: the early phase occurs in the first 5 min after the formalin injection and is caused by non-inflammatory pain resulting from direct stimulation of nociceptors. The late phase occurs between 15 to 30 min after formalin injection [46] due to inflammation with a release of various inflammatory mediators, such as serotonin, histamine, bradykinin, and PGs [42]. In this study, oral pretreatment with BIAE showed a significant inhibitory effect on licking activity only in the second phase.

CONCLUSIONS

This study reports the *in vivo* antiinflammatory and analgesic activities of *Bunium incrassatum* aqueous extract for the first time. The results show that the extract exhibited important *in vitro* antioxidant activity and *in vivo* antiinflammatory and analgesic effects in mice, suggesting the studied plant extract could be an alternative source of natural drugs to treat pain and inflammatory diseases. Further studies are necessary to elucidate the precise mechanisms of action and the phytochemicals responsible for these biological activities.

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Data availability: Data underlying the reported findings have been provided as a raw dataset, which is available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Amraoui%20et%20al_Dataset.pdf

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