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Research Article

IN VITRO AND *IN VIVO* ANTI-INFLAMMATORY POTENTIAL OF *EUCALYPTUS GLOBULUS* ESSENTIAL OIL

Hamza Belkhodja[1*], Boumediene Meddah[1], Khadidja Sidelarbi[1], Djilali Bouhadi[1], Bouchra Medjadel[2], Amel Brakna[2]

[1] *Laboratory of Bioconversion, Microbiology Engineering and Health Safety, Department of Biology, University of Mustapha Stambouli, Mascara, Algeria.*
[2] *Department of Biology, University of Mustapha Stambouli, Mascara, Algeria.*

**Corresponding Author:*
E-mail: hamzabelkhodja@yahoo.fr

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ABSTRACT. This work was conducted as part of evaluation of the in vitro and in vivo anti-inflammatory activities of the essential oil extracted from a plant that belongs to the family of Myrtaceae: *Eucalyptus globulus*. The *E. globulus* essential oil was extracted by hydro-distillation. First, the in vitro anti-inflammatory activity of *E. globulus* essential oil was evaluated using the protein denaturation inhibition method compared to the reference drug Diclofenac. Then, the anti-inflammatory effect of *E. globulus* essential oil was studied in vivo using the 1% dextran edema model in the Wistar rat. The extraction of the essential oil gave a yield of 0,41 ± 0,01%. From the results obtained, it was noted that the *E. globulus* essential oil (250 µg/ml) and the drug Diclofenac (250 µg/ml) are capable of inhibiting denaturation of proteins with very high percentages. Thus, the assessment of the in vivo anti-inflammatory effect indicated that the treatment with the *E. globulus* essential oil showed a reduction of inflammatory reaction with different degrees of inhibition. The excellent anti-inflammatory potential of the essential oil of *E. globulus* may provide supports in the treatment of pathologies such as painful and inflammatory disorder. This shows that the essential oil of *E. globulus* would exert several beneficial effects by virtue of its phytochemicals and pharmacological potentials and could be harnessed in drug formulation.

Keywords: *Diclofenac, essential oil, Eucalyptus globulus, extraction, inflammation*

INTRODUCTION

Plants form the basis of traditional medicine because of their therapeutic potential [1]. According to the World Health Organization, almost 80% of the population depends on traditional medicine for primary health care [2]. Algeria has a considerable collection of natural species representing a phytogenetic heritage of very great importance [3]. The therapeutic power is related to many bioactive molecules such as flavonoids, tannins, coumarins, alkaloids and essential oils [4,5,6]. Currently, essential oils offer significant benefits owing to their medicinal properties and applications in other fields including cosmetics, aromatherapy and food [7].

Free radicals and reactive oxygen species are produced through physiological and biochemical processes in the human body. Overproduction of such free radicals might lead to oxidative stress which can damage biomolecules in the body (e.g., lipids, proteins and DNA), that can initiate diseases such as atherosclerosis, cancer and inflammatory diseases [8]. Inflammation is defined as a complex biological response of vascular tissue

to stimuli caused by injury, environmental agents, infection, and cellular modifications [38]. In addition, there is a relationship between the excessive production of free radicals in the body and the evolution of the inflammatory process. A number of reactive oxygen/nitrogen species can initiate intracellular signaling cascade that enhances proinflammatory gene expression. Thus, inflammation and oxidative stress are closely related pathophysiological events that are tightly linked with one another [9]. For the treatment of inflammation, relief of this pain and stability of joint function, Non-Steroidal Anti-Inflammatory Drugs and classic drugs are available. However, their annoying side effects (peptic ulcers, acute renal failure and even heart complications) prompt people to consider the use of more natural alternatives: medicinal plants [39].

Eucalyptus globulus, known as blue gum, holds an important place in pharmacology. The *E. globulus* extracts are well known examples of tree that used as anti-inflammatory, antibacterial and antioxidant agents [10]. The Eucalyptus essential oils are widely used in the world considered them as safe and non-toxic, even the Council of Europe has approved the use of *Eucalyptus* oils as flavoring agent in foods. Consequently, a growing interest has been given to their use in the scientific research field and industry as a natural food additive, drugs and cosmetics. [11]. This study evaluated the anti-inflammatory potential of *E. globulus* essential oil *in vitro* and *in vivo.*

MATERIALS AND METHODS

Drugs and Chemicals

The following drugs were used: Diclofenac (Hikma Pharmaceuticals, Jordan), ibuprofen (Pharmalliance, Algeria). All other chemicals and reagents as Sodium chloride (NaCl), cyclohexane, Bovine Serum Albumin (BSA), Hydrochloric acid (HCl), dextran, phosphate buffered saline (PBS) were of analytical grade and purchased from Sigma Aldrich.

Animals

The male albino Wistar rats "*Rattus norvegicus* " were used with an average body weight of 200 ± 10 g, provided by the laboratory of the University of Mustapha Stambouli, Mascara. The rats were divided into three groups (one for control and two for experiment) and were kept at a temperature of 20 ± 1 °C with a natural photoperiodic cycle. They were fed with food pellets and water ad-libitum. All experiments were approved by the Animal Ethics Committee of the Mustapha Stambouli University in which the procedures used during these studies were in accordance with the European directive concerning Animal Testing (Directive 2010/63/EU) (decision N°: L276/33) [12].

Essential oil extraction

The leaves and flowers of the species *E. globulus* was collected in Mascara region (35.401898°N, 0.125638°E) during February 2019. The species was identified by botanists of the Department of Biology of the University of Mustapha Stambouli, Mascara. Essential oil was extracted by hydro-distillation in a Clevenger apparatus. A quantity of 100 g of *E. globulus* leaves and flowers was boiled in distilled water for 2 hours. When the temperature stabilizes, the distillate was collected. Sodium chloride (5 g) was added to the distillate to facilitate the separation between the organic and aqueous

phase. Then, the mixture was placed in a separating funnel and distillate was washed with cyclohexane for three times. After agitation, the organic phase was taken to undergo rotary evaporation (R-300, 20-95°C, Germany) to remove the cyclohexane and obtain the essential oil. The essential oil was stored at +4 °C after the calculation of yield [13].

Anti-inflammatory activity

Inhibition of albumin denaturation

The *in vitro* anti-inflammatory activity of essential oil of *E. globulus* was carried out according to the method of Deshpande et al. [14] by monitoring the inhibition of protein denaturation. The method consisted of preparing 0.5 mL of reaction mixture consisting of 0.45 mL bovine serum albumin (5% aqueous solution) and 0.05 mL of essential oil of *E. globulus* (250 µg/ml). The standard mixture of Diclofenac (ss) (0.5 ml) was prepared in the same condition (0.45 mL bovine serum albumin 5% and 0.05 ml of the standard solution of diclofenac with a concentration of 250 µg/ml). pH was calibrated at 6.3 using 1N HCl. After preparation mixtures were incubated at 37 °C for 20 min subsequently heating at 57 °C for 30 min. After cooling the samples, 2.5 mL phosphate buffered saline (pH 6.3) was added to each test tube. Moreover, 0.05 mL distilled water was used in place of essential oil in control test tube whilst product control did not contain bovine serum albumin. The absorbance was measured by the UV-Visible spectrophotometer (Shimadzu UV-1280) at 416 nm and the inhibition percentage of protein denaturation was calculated as follows:

$$\text{Inhibition percentage (\%)} = 100 - (ATS - Apc) \times 100 / ATc$$

(Eqn. 1) [14].

Inflammatory paw edema in rats

Anti-inflammatory effect was evaluated by the edema injection method [15]. To achieve this type of inflammation, 09 rats were divided into three groups: the first group was used for the control treated with saline (0.9% NaCl). The second treated with essential oil of *E. globulus* (250 µl/kg) and the last group was used in the standard treated with ibuprofen (200 mg/kg). The different treatments were administered by oral gavage one hour before the dextran injection. After one hour of the treatment, the rats received 0.1 ml of a 1% dextran solution in the sub-plantar region of the right paw. The paw thickness was measured before and 1, 2 and 3 hours after the injection using a caliper. Then, the inhibition percentage was calculated according to the following formula:

$$\text{Inhibition percentage (\%)} = DC\text{-}DT \times 100 / DC$$

(Eqn. 2) [15].

DC: Paw thickness of control, DT: Paw thickness of treated group

Statistical analysis

The values were expressed as mean ± standard deviation (Mean ± SD). The results were analyzed by ANOVA single factor for multiple comparisons. The P values less than 0.05 ($p < 0.05$) were considered statistically significant.

RESULTS AND DISCUSSION

Extraction yield

The essential oil obtained from the aerial parts of the *Eucalyptus globulus* plant was 0.41 ± 0.14%. It was almost equal to that of the Kabyle region with a value of 0.48% according to the work of Taleb-Toudert [16]. Otherwise, the results obtained were much lower than those reported by Pereira et al. [17] and Mulyaningsiha et al. [18], which were 1.57% and 0.71%, respectively. It was also lower than that of the Tbessa region according to the results of Aouidet [19] where it was estimated at 1.40%. Indeed, Emara and Shalaby [20] observed modifications of conformation of the secretory channels of the essential oil of *E. globulus* through the seasons. In addition, the extraction yield was influenced by many factors such as temperature, relative humidity as well as the apparatus used, the operating pressure and the duration of distillation [21,22,23].

Anti-inflammatory activity

Inhibition of albumin denaturation

Protein denaturation can be induced by the application of external stress: a compound such as an acid or a base, a concentrated inorganic salt, an organic solvent or with heat. It is a process in which proteins lost their tertiary and secondary structures as well as their biological functions; which can lead to the production of autoantigens [24]. It was expressed by the inhibition percentage.

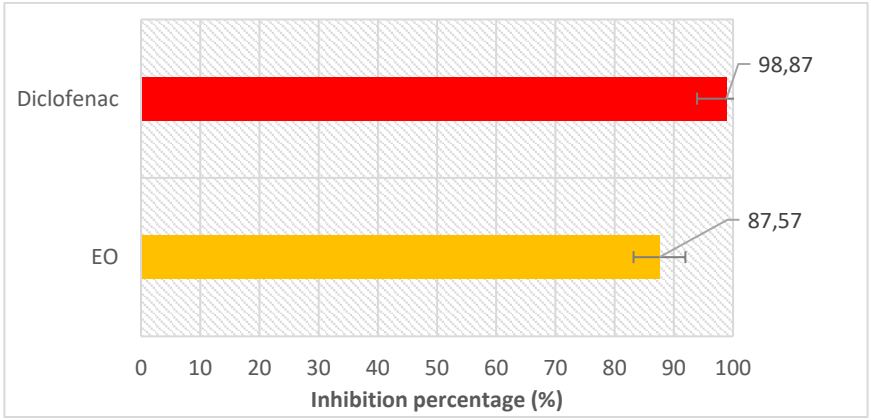

Fig. 1. *Inhibition percentage of BSA denaturation.*

From the figure, it was noted that the essential oil of *E. globulus* (250 µg/ml) and diclofenac were able to inhibit the protein denaturation. This was in perfect agreement with the results of Habibur Rahman et al. [25] who showed the inhibitory power of the essential oil of *E. globulus*. They noted that the essential oil of *E. globulus* at a concentration of 100, 250 and 500 µg/ml showed inhibition of 16.98%, 58.49%, 66.03% of protein denaturation.

The mechanism of denaturation may involve alteration of the electrostatic, hydrogen, hydrophobic or disulfide bond [26]. Therefore, the bioactive molecules in the composition of the essential oil of *E. globulus* can participate in the protection of these different types of structural bonds. Studies have claimed that the presence of limonene,

linalyl acetate, β-trans-caryophyllene, 1,8-cineole, p-cymene, thymol in essential oil of *E. globulus* with predominant percentages showed a strong inhibition of the denaturation of macromolecules [27,28].

Inflammatory paw edema in rats

Administration of a 1% dextran solution in the sub-plantar region caused swelling in the rat's foot. Thus, the assessment of anti-inflammatory effect was based on monitoring edema by measuring the thickness of the treated rat's paw (Fig. 2).

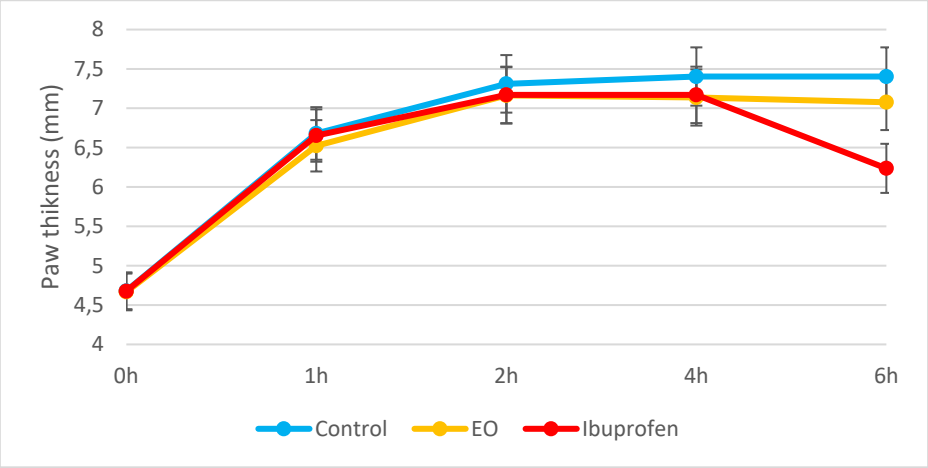

Fig. 2. *Evolution of the foot thickness of control and treated rats.*

After the administration of saline solution, dextran caused a significant increase ($p<0.05$) in the thickness of the rat's paw at 1, 2 and 4h. While, the *E. globulus* essential oil (250 µl / kg) prevented this increase after 2 hours of treatment. Regarding the drug ibuprofen, the results showed a value of 7.17 mm after 2 and 4 hours of treatment (Table1).

Table 1. *Evolution of the paw thickness (mm) of control and treated rats.*

	Control	*E. globulus*	Ibuprofen
0h	4,68 ± 0,05	4,66 ± 0,06	4,67 ± 0,03
1h	6,68 ± 0,09	6,52 ± 0,22	6,65 ± 0,28
2h	7,31 ± 0,09	7,16 ± 0,25	7,17 ± 0,13
4h	7,40 ± 0,07	7,13 ± 0,08	7,17 ± 0,25
6h	7,40 ± 0,003	7,07 ± 0,10	6,23 ± 0,03

After 6 hours of treatment with essential oil of *E. globulus* and the drug Ibuprofen, it was recorded a reduction in the thickness of the paw which reached 7.07 ± 0.1 and 6.23 ± 0.09 mm respectively compared to the group treated with saline solution. The results

revealed that essential oil of *E. globulus* appeared effective in reducing the responses induced by dextran.

The evaluation of the inhibition percentage showed that the essential oil of *E. globulus* has an anti-inflammatory potential for the inhibition of edema which was significantly superior to those obtained with ibuprofen (Fig. 3). *E. globulus* essential oil showed anti-inflammatory activity to different degrees in the model of acute dextran inflammation in rats.

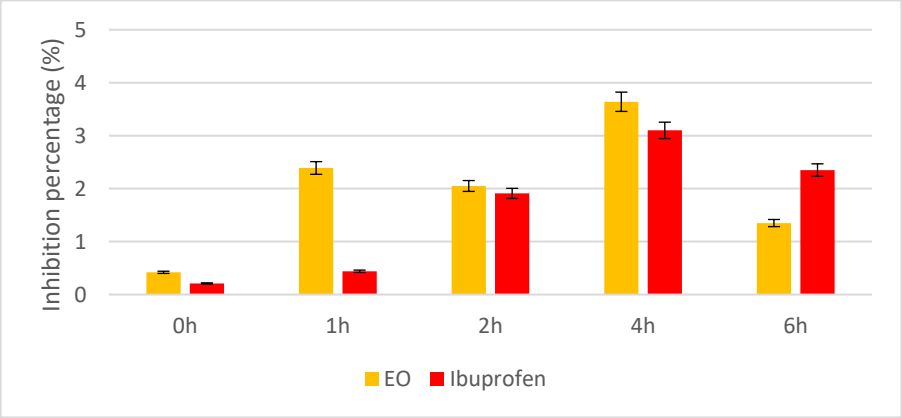

Fig. 3. *Inhibition percentage of swelling in the paw.*

In the experimental conditions, dextran caused edema as a result of local inflammation in the rat's foot. The cause of this inflammatory reaction was tissue damage. This tissue damage induced the synthesis of histamine, prostaglandins, leukotrienes [29], PAF (platelet activating factor), cytokines, NO (nitric oxide) and TNF (tumor necrosis factor) [30]. The injection of the dextran solution 1% caused a biphasic response. The early phase of inflammation (during the first 2 hours) was mainly mediated by chemical mediators such as histamine and serotonin and was characterized by the stimulation of C fibers and the release of substance P and bradykinin. The second late phase (3-4 hours after the injection of dextran) was due to local inflammatory pain [31]. It was associated with the activation of leukotrienes, prostaglandins and several cytokines and the release of proteases and lysosomes. The vascular response reached its maximum level in this phase [32]. The continuity between the two phases was ensured by kinins [33,34].

The anti-inflammatory activity of essential oil of *E. globulus* can be mediated by inhibition of the last phase by restricting the production of several cytokines, bradykinins, leukotrienes and prostaglandins. Therefore, it quickly controlled both phases of inflammation. Studies confirmed the relationship between the presence of limonene, δ-3-carene and alpha-pinene, 1,8-Cineole (selective COX-2 inhibitors) and the significant inhibitory potential on PGE2 production [35]. Thus, 1,8-cineole (a terpene oxide) presented in many essential oils in particular in the essential oil of *E. globulus* inhibited leukotrienes (LTB4) and prostaglandins (PGE2) [36].

The anti-inflammatory potential of 1,8-cineole presented in *Eucalyptus* essential oil was studied using the colitis model induced by Tri-Nitro-Benzene-Sulfonic Acid (TNBS) in rats, which was one of the most common experimental models used to screen for drugs active against inflammatory disease. The treated rats with 1,8-cineole showed a significant reduction in inflammatory damage scores [37].

CONCLUSION

From the results obtained, it was noted that the *E. globulus* essential oil was able to inhibit protein denaturation. Thus, the in vivo study showed that *E. globulus* essential oil can present an anti-inflammatory potential in the edema injection method with different degrees of inhibition. It was suggested to introduce this essential oil in different scientific research fields and industry as a natural alternative and drugs due to the anti-inflammatory activities of its bioactive compounds.

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Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: H.B., B.M., B.M. and A.B., Design: H.B., B.M., Data Collection or Processing: H.B., B.M. and A.B., Analysis or Interpretation: H.B., K.S., and D.B., Literature Search: H.B., B.M. and A.B., Writing: H.B., K.S., and D.B.

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Ethical Statement. This study does not present any ethical concerns.

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