Available online on 15.11.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Anti-inflammatory and healing effect of leaf-flower mixture extract of *Cytisus triflorus* L

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ABSTRACT

Objective: The present study aims to study the anti-inflammatory and healing effects of the crude leaf and flower mixture extract of *Cytisus triflorus* L., known in Algeria as Igoulli.

Methodology and results: The method consists of studying the anti-inflammatory effect by measuring the diameter of edema of the paw of rats that received carrageenan. In addition, 2 cm diameter circular incision wounds were made in rats to evaluate the healing activity of the crude leaf and flower mixture extract of *Cytisus triflorus* L. at doses of 200 mg/kg and 400 mg/kg. The anti-inflammatory effect of *C. triflorus* showed that E.Br at the dose 400 mg has the higher activity, which induces a significant decrease in the thickness of the rat paw from the second hour, its effects being similar to those of Diclofenac. The percentages of inhibition of edema at 4 h and 6 h are 80.05 and 88.56% for E.Br and Diclofenac respectively. With respect to healing activity, the results show that after 18 days, complete healing was achieved with almost two concentrations of crude *C. triflorus*, tissue remodeling and reoccurrence of hair was observed at level of scars.

Conclusion: The results of the study show that the leaf extract and flower extract of *Cytisus triflorus* L. has healing and anti-inflammatory properties that could justify the use of this plant in traditional medicine against inflammatory diseases.

Keywords: *Cytisus triflorus*, anti-inflammatory, healing activity.

Article Info: Received 07 Sep 2019; Review Completed 09 Oct 2019; Accepted 12 Oct 2019; Available online 15 Nov 2019

Cite this article as:

Madoui S, Mokhnache K, Khither H, Aouchria S, Charef N, Anti-inflammatory and healing effect of leaf-flower mixture extract of *Cytisus triflorus* L, Journal of Drug Delivery and Therapeutics. 2019; 9(6):1-6
http://dx.doi.org/10.22270/jddt.v9i6.3625

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INTRODUCTION

Inflammation is a defense reaction of the body to various aggressions that can be of physical, chemical, biological or infectious origin. The current treatment for inflammation uses steroidal and nonsteroidal anti-inflammatory drugs. Although these molecules are effective, they most often have undesirable effects that can hinder their long-term use[1].

In developing countries, plants with anti-inflammatory activity could be an alternative in anti-inflammatory therapy, because of their better accessibility and less toxicity, compared with conventional anti-inflammatory drugs[2].

Cytisus triflorus L, known in Algeria as Igoulli. This species is distributed in the Mediterranean region, it is widespread in the mountainous borders between Bejaia and Tizi Ouzou. It is used in traditional Algerian medicine to treat abdominal pain, healing wounds and as hemostatic and antifungal. In addition, the leaves are used as "henna" to treat and dye hair[3].

The objective of our study was to determine the anti-inflammatory activity of the crude leaf and flower extract extract of *Cytisus triflorus L.* on acute inflammatory edema of carrageenan-induced rat paw and the healing effect of *Cytisus triflorus L.* to provide a scientific basis for the traditional use of this plant.

MATERIAL AND METHODS

Plant material

The plant *Cytisus triflorus* (**Fig.1**) was harvested in April 2014 from the Chemini region (Bejaia).

The identification was made by Prof. Oudjhih Bachir, Elhadj Lakhdar University, Batna.

The leaf and flower mixture is cleaned, dried in the shade and at room temperature and stored in the dark until use.

Fig.1: photos of *Cytisus triflorus*. (**A**): flowers and (**B**): leaves.

Animals

The *in vivo* study was carried out on male rats, albino Wistar whose weight varies between 200g and 250g, and on mice whose weight is between 20 and 30 g, obtained from the Pasteur Institute of Algiers. These rats and mice are used after a 7 days adaptation period during which they have free access to water and standard feed provided by the Bejaia National Livestock Feed Office (ONAB).

Experimental design

Preparation of the raw extract

The mixture of leaf and flowers of *Cytisus triflorus*, previously cleaned and crushed, is macerated in methanol for seven nights at room temperature[4]. The macerate is filtered on wattman paper, evaporated by a rotavapor (Buchi) in order to remove the organic solvent and then completely dry in an oven (40 °C) for 24 hours. The extract obtained is considered to be the crude extract (E.Br) of the mixture of *Cytisus triflorus*.

Anti-inflammatory activity

Carrageenan injection under the plantar aponeurosis of the posterior paw of the rats leads to the appearance of edema. The intensity of this edema is evaluated by increasing the volume of the paw (compared to the initial volume). The inflammation caused will be decreased in the presence of the extract having anti-inflammatory activity[5]. The edema of the paw induced by λ-carrageenan in rats was evaluated according to the method of Elion Itou et al. (2014)[6]. In this study, groups of 7 rats are formed. The rats used are deprived of food for 12 h with free access to water before the experimentation period. At the time of the experiment, weigh the rats, divide them into five homogeneous batches and determine the diameters of the paws at the initial time T_o. The rats constituting the two test groups are treated orally (gavage) with 2 ml of E. Br from *C. triflorus* at doses of 200 and 400 mg/kg. One hour later, the injection of 0.2 ml of 1% λ-carrageenan was carried out subcutaneously in the aponeurosis of the right hind paw of the rats. The rats in the positive control group are treated with 2 mL of Diclofenac orally one hour before the intraperitoneal injection of carrageenan. The rats in the negative control group are treated with 2 mL of 0.9% NaCl orally one hour before carrageenan injection. The anti-inflammatory effect was assessed by measuring the scarring edema of the paw that received carrageenan 1% at 1 h, 2 h, 3 h, 4 h, 5 h 6h and 12 h using a caliper. The percentage increase (AUG %) of the edema is calculated for each group. It is given by the following formula:

$$\%\ \mathbf{AUG} = \frac{(D\,n - D\,0) * 100}{D\,0}$$

Dn: diameter of the paw the ^{nth} hour after the injection of carrageenan.

D0: diameter of the paw before the injection of carrageenan.

The percent inhibition (% INH) of the edema is calculated for each treated group relative to the control group. It is obtained by the following formula:

$$\%\ \mathbf{INH} = \frac{(\%\ \text{AUG control} - \%\ \text{AUG treated group}) * 100}{\%\ \text{AUG control}}$$

Healing activity

Healing activity evaluation consists in creating wounds on previously anesthetized animals and then in treating them with the ointment preparations to be tested. The protocol followed is that described by Sagliyan and his collaborators (2010)[7]. This study was performed with *C. triflorus* E.Br at 200 and 400 mg/kg, prepared in petroleum at a concentration of 5% (w/w). Petroleum was chosen as an adjuvant because it gives good consistency to preparations intended for topical use, which allows rapid spreading and increases the shelf life of the various preparations. Framycitin cream is used as a standard cream and the control group receives only Vaseline. Rats were anesthetized by intraperitoneal injection of 1mL urithan 2% (W/V) due to 5mg/kg. The hairs of the dorsal part of our rats were removed with a razor and circular wounds of 300 mm^2 were made using a ruler and a scalpel blade. These hinges are then cleaned with cotton before applying the ointment. The animals were put in individual cages with clean bedding to isolate them at the time of application of different products. The wound dressing was done daily due to once a day with a precise amount of the ointment (about 0.4 g). Wound measurements were made every three days until complete healing. The appearance, color and odor of the wounds were noted throughout the duration of the treatment. The percentage of contraction or narrowing of wounds is calculated according to the following formula:

$$\text{Wound contraction (\%)} = \frac{(\text{wound surface D1} - \text{wound surface nD}) * 100}{\text{wound surface D1}}$$

Where, D : first Day, nD : ^{nth} Day

Statistical analysis

The comparison of the mean percentages of increase and inhibition was made with the Student t-test. A significant difference is represented by a $p < 0.05$; n = 7, represents the number of experiments per group.

RESULTS AND DISCUSSION

Anti-inflammatory activity

To demonstrate the anti-inflammatory activity of C. triflorus E.Br at doses of 200 and 400 mg/ kg, an experimental model of acute inflammation of the rat paw induced by carrageenan was selected. Figure 2 shows that injection of carrageenin causes a gradual increase in the volume of edema in rats treated with physiological saline during the six hours of the experiment. Carrageenan is a mucopolysaccharide that

induces a maximum of edema from the third hour following its injection[6]. Indeed, the injection of carrageenan causes the release of several chemical mediators that are responsible for the inflammatory process. This inflammatory response is biphasic whose initial phase, which lasts approximately one hour, is due to the release of histamine and serotonin, the bradykinin is released during the second phase (1h30min - 3 hours), and the biosynthesis prostaglandins occur beyond the third hour[8]. These mediators increase the permeability of the capillaries of the region. As a result, the exudate escapes from the bloodstream to the interstitial space. This exudate is the cause of localized edema, which, in turn, compresses the nerve endings and thus determines a sensation of pain[9].

The anti-inflammatory effect of *C. triflorus* E.Br. at a dose of 400 mg was more active, it induced a significant decrease in the paw thickness of the rats from the second hour, and up to at the sixth hour of the experiment (**Fig.2**), while the anti-inflammatory effect of E.Br at 200 mg / kg is observed from the 3rd hour. The effects of E.Br of *C. triflorus* at 400 mg / kg are similar to those of Diclofenac (a significant reduction is observed from the second hour and continues until the end of the experiment). The percentages of inhibition of edema at 4 h and 6 h are respectively 80.05 and 88.56% for the dose 400 mg / kg and diclofenac, 79.16 and 83.23% for the dose of 200 mg / kg (**Fig.3**).

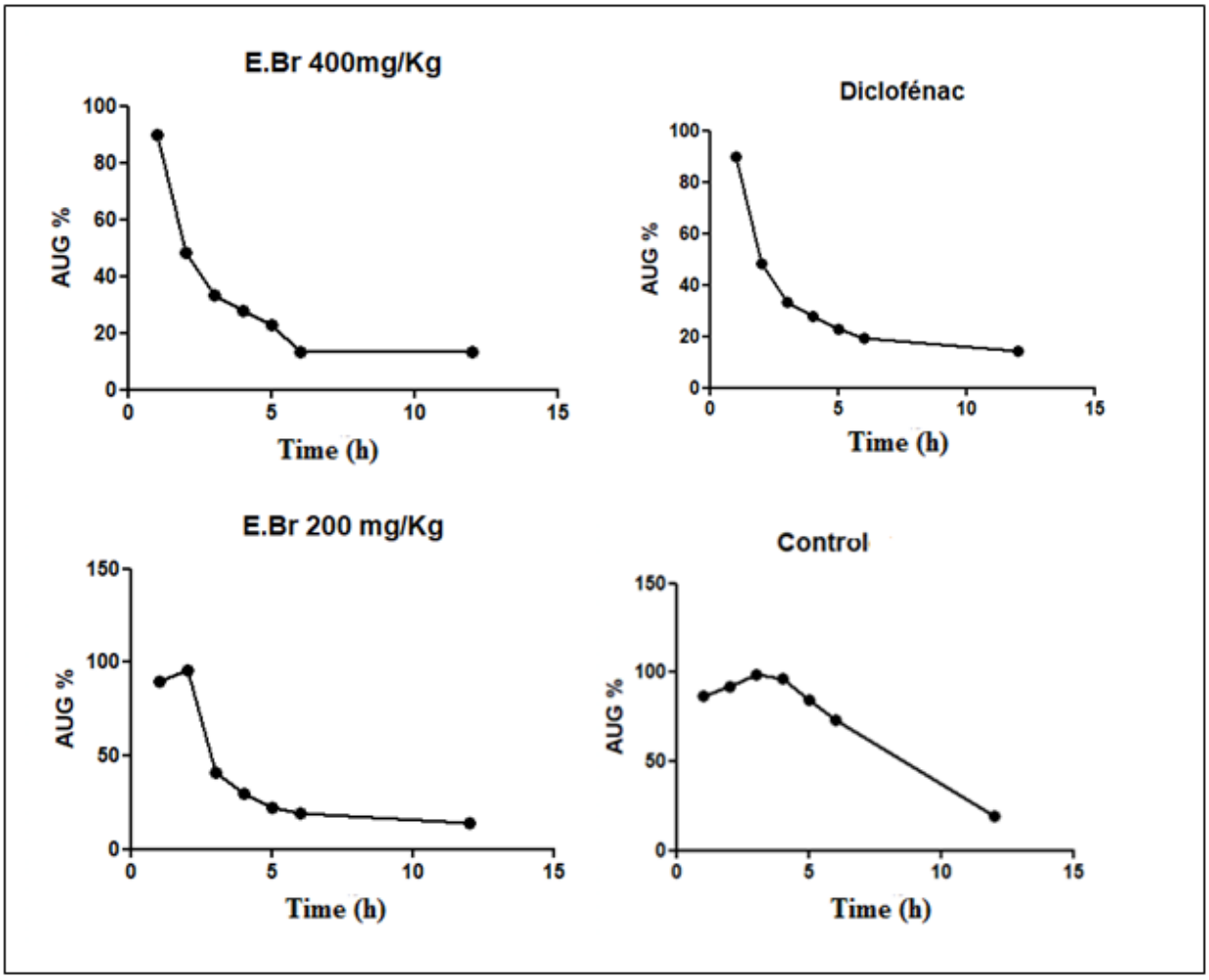

Fig. 2: Percentage increase in paw thickness (AUG%) of rats treated with 200/400 mg / kg crude extract of C. triflorus, Diclofenac and phisiological water for negative control. E.Br: the crude extract

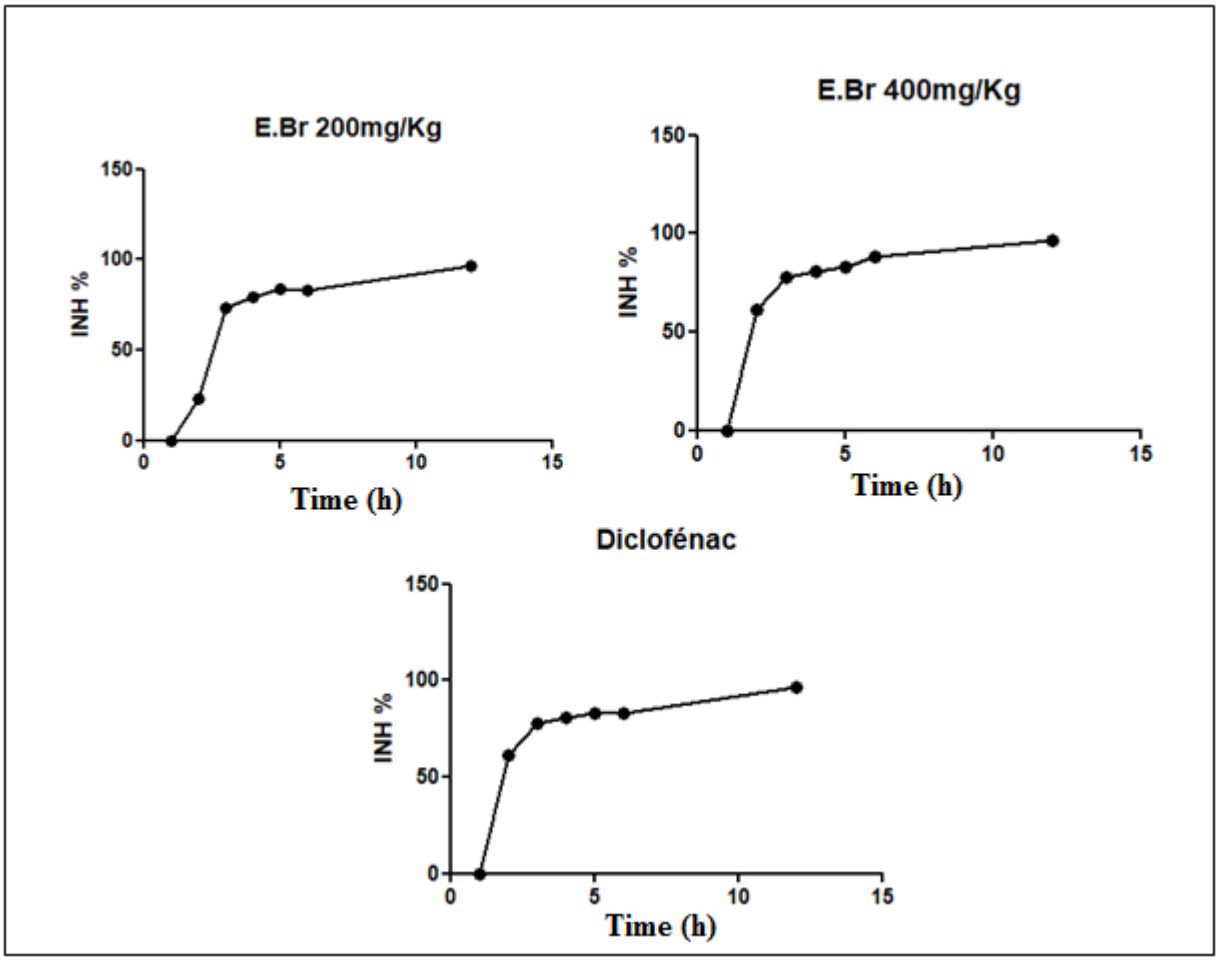

Fig. 3: Percent inhibition of paw thickness (INH%) of rats treated with 200 and 400 mg/kg crude extract of *C. triflorus* and Diclofenac. E.Br: the crude extract.

The richness of E.Br in phenolic and flavonoid constituents (**Table I**) able to prevent the formation of prostaglandins that cause inflammation. In addition, some indicate that the flavonoids contained in plant extracts, possess anti-inflammatory properties capable of modulating the functioning of the immune system by the inhibition of the activity of enzymes that can be responsible for inflammations[10]. In addition, Kim and his collaborators (2014)[11] demonstrated that flavonoids are able to inhibit histamine, flavones and flavonols in glycosylated or free form such as quercetin, kaempferol, myrecetin and have a Cyclooxygenase inhibitory activity.

The anti-inflammatory effects of polyphenols, which can be exerted at the molecular level, are dependent on the specific structure of the polyphenolic compounds. Macrophage functions, including cytokine production, may also be affected by some flavonoids by modulation of inducible cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS). Several experimental studies have reported the immunomodulatory effects of polyphenolic compounds on humoral and cellular immunity[12]. Our results indicate that the methanolic crude extract exhibits remarkable anti-inflammatory activity. This is in perfect concordance with the results of Ait-Kaci Aourahoun et al. (2015)[3]

Healing activity

Evaluation of the in vivo healing activity of the dermal cream was performed on Albino rats. Its purpose is to assess the accelerating potential of the neoformation of dermal tissues. The comparison was made with a group of animals receiving a Framycitin reference cream and a control group treated with petrolatum.

The healing process has gone through several phases; a gradual disappearance of the inflammation (wounds becoming less red), a phase of contraction (the wounds became hard and covered with slightly blackish crusts) and finally the healing phase, the treatment allowed to obtain a complete cure of wounds (**Fig.4**). The wounds were odorless throughout the treatment. The wound areas measured at days 3, 6, 9, 12, 15, 18, and 21 after excision in all ointment-treated ointment-treated groups were lower than those in the control (**Table II**). The crude extract 400 mg showed an effect healing almost 50% after the 6th day of treatment. This effect was better than that of Framecitine (9th day). The effect of the crude extract at 400 mg increased on the 9th and 12th day to reach approximately 65% - 80% healing. After D18, complete healing was achieved with almost two concentrations of crude *C. triflorus* extracts, tissue remodeling, and reoccurrence of hair at the scars (**Fig**. negative control that had healing around 21 days (72%).

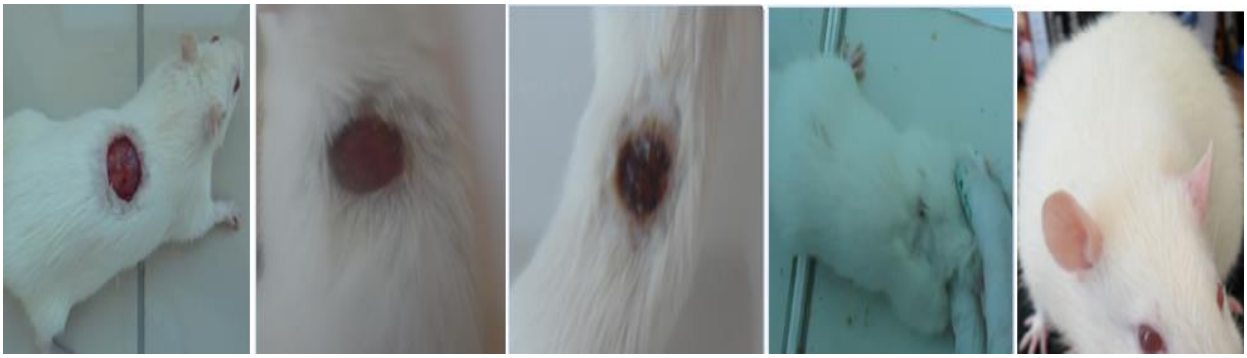

Fig.4: The healing phases. **1**: circular wounds of 300 mm², **2**: a gradual disappearance of inflammation, **3**: a contraction phase, **4**: the healing phase, **5**: complete healing.

Tableau II: Evaluation of the ointments of the crude extract of *C. triflorus* at the concentration 200 and 400 mg on cicatrization by the method of excision of wounds.

Groupe	Wound area in mm² (percentage of wound contraction)						
	D1	D3	D6	D9	D12	D15	D18
Untreated	257.58±0.82 -	236.64±5.79 **(8.23%)**	211.90±5.53 **(17.68%)**	165.84±6.73 **(32.70%)**	107.42±2.52 **(54.41%)**	67.08±2.52 **(64.56%)**	57.08±2.52 **(72.56%)**
Framycitin	244.14±12.92 -	188.29±15.22 **(23.28%)**	173.31±13.16 **(29.41%)**	87.78±7.19 **(53.23%)**	52.33±5.04 **(77.29%)**	36.43±3.76 **(82.17%)**	24.43±3.76 **(90.06%)**
E.Br-400	245.36±9.38 -	174.21±19.11 **(29.00%)**	116.87±19.13 **(42.22%)**	70.90±12.60 **(67.92%)**	36.02±4.36 **(81.14%)**	7.33±1.89 **(89.76%)**	7.33±1.89 **(98.66%)**
E.Br-200	247.59±7.33 -	199.63±20.47 **(20.05%)**	153.68±18.56 **(38.30%)**	83.69±14.64 **(56.65%)**	58.12±12.86 **(76.76%)**	29.94±4.73 **(81.42%)**	29.94±4.73 **(90.20%)**

Data are expressed as mean ± SEM (n = 8). D: day and E.Br: the raw extract.

Healing is characterized by significant changes in the extracellular matrix in which fibronectin, fibrinogen and integrins occur[13]. The topical application of the crude extract induced a decrease in the diameters of wounds, this decrease is important than that of framycitin used as standard. The healing effect of the extract can be attributed to the presence of polyphenols and flavonoids with the ability to accelerate the tissue regeneration process by stimulating the production of collagen and fibronectin [14]. The quality of healing is affected by the 400 mg concentration, a powerful effect on the growth of albino rat hair; they have fully recovered their hair unlike negative control batches that showed no case of hair growth during the 21 days of treatment. It is likely that the effect of promoting hair growth is due to hormonal stimulation of the crude extract of C. triflorus. Estrogen prolongs the anagen phase of hair growth[15]. The work done by Khadri and his collaborators (2018)[16] on the same species, affirm that the methanolic extract is very powerful on the ciccatrization of pleasures.

CONCLUSION

The objective of this work was to adopt scientific bases for the evaluation of certain biological studies attributed to the medicinal plant "Cytisus triflorus", chosen on the basis of its traditional use. In this study, the anti-inflammatory activity of this extract was evaluated in rats, the crude extract of the plant administered orally exerts significant anti-oedematous effects, even during its local healing application justifying their use traditional.

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