Evaluation of analgesic and anti-inflammatory activities of *Warionia saharae* essential oil

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Abstract. *Warionia saharae* Benth & Coss (W.s) (Asteraceae) is a monospecific genus endemic to Algeria and Morocco. Its leaves are used in their traditional medicine, such as gastrointestinal and inflammatory diseases; for instance, rheumatoid arthritis treatment. In this work, our team investigated the anti-inflammatory and analgesic effects of essential oil extracted from the dried upper parts of *Warionia saharae* based on different standard experimental test models. The analgesic activity was assessed by central and peripheral models, such as “hot plate” and “writhing” tests on Swiss albino mice. The hot plate test used latency measurements to assess acute cutaneous pain sensitivity, as a result; the latency of the hind-paw pain response was by licking and either shaking or jumping, those occurrences were recorded. Writhing test as a chemical method used to induce pain of peripheral origin in mice by injecting acetic acid intraperitoneally (IP). This results in characteristic stretching behavior of the animals (cramps and contortions). The evaluation of the analgesic activity, shows that the essential oil of this plant induces a decrease in the number of abdominal cramps in the contortion test and a maximum inhibition of pain. As for the anti-inflammatory effect, it was studied by the “paw edema” test, a phlogogenic agent (formaldehyde) was used to stimulate inflammation in the paws of mice. Anti-inflammatory properties can be observed by inhibiting this edema compared to the standard drug Diclofenac. In conclusion, *Warionia saharae* essential oil (75 mg/kg) showed a strong anti-inflammatory and analgesic activities which supports the conventional use of this plant in traditional medicine.

Keywords: analgesic effects, anti-inflammatory effects, essential oils, medicinal plants, Swiss Albinos mice, *Warionia saharae*.

INTRODUCTION

Painful sensation manifests in different forms (burning, stinging, cramp, gravity, stretching, etc.) of varying intensity and extension (Larousse Médical, 2006). Inflammation is localized reaction of a tissue, resulting from an aggression (Larousse Médical, 2006; Panda *et al.*, 2009). It manifests itself in four main signs: redness, heat,

tumefaction (swelling) and pain. When tissue undergoes aggression, the specialized cells, the mast cells, release bradykinin, prostaglandins, histamine, serotonin and adenosine triphosphate), which stimulate vasodilatation in the affected part causing redness and heat. The overloaded capillaries release fluid which seeps into the

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tissues causing swelling and painful sensation. This sensation is caused by stimulation of local nerve endings (Beaulieu, 2005; Larousse Médical, 2006; Ohishia *et al.*, 2016). The inflammatory mechanism was used to eliminate the pathogen and restore damage to the tissue (Ohishia *et al.*, 2016; Sherwood & Toliver-Kinsky, 2004).

Many plants and herbs such as ginger (*Zingiber officinale* “Zingibéracées”), curcuma (*Curcuma aromatica* syn. *C. domestica* “Zingibéracées”) and liquorice (*Glycyrrhiza glabra* “Fabacées”) have shown to exhibit potent anti-inflammatory effects (Larousse Médical, 2001). *Warionia sabarae* (W.s) has also been used in traditional medicine, as an anti-inflammatory and in the treatment of gastrointestinal, jaundice (Amezouar *et al.*, 2012; Bellakhdar, 1997; Hilmi *et al.*, 2003), and in some areas, this plant was used against brucellosis (Yakoubi *et al.*, 2015). Dried leaf decoctions were used against arthritis and epilepsy (Bellakhdar *et al.*, 1986; Katinas *et al.*, 2008). In this context, our laboratory began to inspect the anti-inflammatory and analgesic activities of this species after performing a bibliographic search and an ethno-pharmaceutical investigation survey carried out by our team in the region (Yakoubi *et al.*, 2015).

MATERIALS AND METHODS

Animal materials

Permission was obtained from the Committee of use of animal experiments (Approval # 1943-19). Swiss Albino mice weighing 23-43 g of either sex were obtained from Pasteur animal house (Algiers). Animals accommodated under normal laboratory conditions, were kept under a 12/12 hours of dark / light cycle at 25 ± 1 °C (Lalitha *et al.*, 2010; Panda *et al.*, 2009) and supplemented with mouse feeding; with free access to water.

Before the experiment, the mice were fasted for 16 h with free access to water (Riahi *et al.*, 2011; Sanogo *et al.*, 2006). We used prizes of 6 mice range for each experience. It is important to reduce fear, discomfort, pain or distress caused by experimental procedures as long as animal use continues. (Zimmermann, 1983).

Plant materials

Aerial parts of W.s were collected from Bechar

region and voucher specimens were deposited at herbarium of chemistry laboratory, Bechar University. We relied on A.N.N (National Agency Nature protection- Bechar, Algérie) to identify and classify plant.

The air-dried aerial parts of W.s were subjected to hydro-distillation. Once obtained, the extracted essential oil (E.O) was stored in airtight containers before further analysis.

Acute toxicity studies

The animals were divided into 3 groups of 6 animals each. The extract was administered at doses of 50, 75, 100 and 200 mg/kg of animal body weight; transmitted by intraperitoneal (IP) injection. The observation period of the symptoms of intoxication and lethality lasted for 48 hours (Lalitha *et al.*, 2010; Soro *et al.*, 2015).

Analgesic activity

The “writhing” and “hot plate” tests were the most used; they were classified among the five most common nociception tests (Bars *et al.*, 2001).

Acetic acid-induced writhing in mice

To assess analgesic activity and preferentially assess the potential oceanic effects of essential oils as analgesic substances, we used writhing method (Silva, 2003).

All the treatments were administrated by IP injection. Approximately 30 min after each administration; the writhing was provoked by the IP injection of acetic acid (0.6%, 10ml/kg) (Makambila-Koubemba *et al.*, 1997; Siegmund *et al.*, 1957). We waited 5 min before the observation, then counted the number of convulsions the mouse exerted for a period of 30 minutes.

Mice were grouped into 4 groups of 6 animals each: Group 1 received physiological water which served as control group. Groups 2 and 3 received ASPEGIC® at dose 250 and 500 mg/kg respectively; served as the standard group. Acetylsalicylic acid (ASPEGIC®) is a well-known peripheral analgesic drug (Siegmund *et al.*, 1957; Silva *et al.*, 2003) and it was used as a positive control in the present investigation. Group 4 received essential oil of W.s (75 mg/kg).

The calculation formula is as follows (Diatta *et al.*, 2014; Riahi *et al.*, 2011):

$$\text{Inhibition (\%)} = \left(\frac{Mc - Mt}{Mc} \right) \cdot 100$$

Inhibition (%): Percentage inhibition of pain

Mc: Number of writhing in control

Mt: Number of writhing in test (treatment)

Hot plate test

Hot plate test assesses the central analgesic effects of an essential oil (MacDonald *et al.*, 1946; Silva *et al.*, 2003).

The latency period of response to a thermal stimulus applied to the paw, is determined by the time it takes the mouse to lick its paw, or jump from the hot plate surface; these reflexive behaviors involve both cerebral and spinal mediated circuit (Ankier *et al.*, 1974; Bars *et al.*, 1992; Mulder *et al.*, 2004).

The method as described by Turner *et al.* (1965), every mouse (23-43 g) was placed softly on a hot plate maintained at $55^{\circ}\text{C} \pm 1$, the appropriate temperature for this test (Menéndez *et al.*, 2002; Tjolsen *et al.*, 1991); 15 min before (Silva *et al.*, 2003), without exposing the animals to tissue damage (Eddy *et al.*, 1953; Tjolsen *et al.*, 1991). The surface of the hotplate is cleaned with 70% ethanol between the testing of each mouse (IACUC). Each experimental group was performed with at least six animals: Group 1 received physiological water which served as control group. Group 2 received ASPEGIC® (500 mg/kg), served as the standard group. Group 3 received essential oil extracted from *W.s* (75 mg/kg). All treatments were given by IP injection. At 0, 15, 30, and 45 min post-treatment, we recorded each reaction time of the controlled and treated animals (MacDonald *et al.*, 1946; Silva *et al.*, 2003). The percentage of inhibition was obtained by:

$$\text{Inhibition (\%)} = \left(\frac{\text{MRTa} - \text{MRTb}}{\text{MRTb}} \right) \cdot 100$$

Inhibition (%): Percentage inhibition of thermal pain

MRTa: Means reaction time after administration

MRTb: Means reaction time before administration

Anti-inflammatory activity

Inflammatory paw edema

Induction of acute inflammation in all groups was obtained by the injection of a phlogogenic agent such as 1% formalin solution (0.025 ml/kg), under the plantar aponeurosis of the hind paw of the mice. This solution induces an inflammatory reaction, which can be reduced by anti-inflammatory substances (Sen *et al.*, 1991; Winter *et al.*, 1962). The evolution of the inflammatory response and the measurement of the edema volumes are monitored by the plethysmometer (Model LE 7500, Bioseb, Barcelona, Spain) (Riahi *et al.*, 2011).

Paw volume was measured 30 min prior to formalin injection; and at 0, 30, 60, 120, and 180 min after the injection (Panda *et al.*, 2009).

The albino mice were divided into 3 groups of 6 animals each; all of treatments were administered by IP injection, 30 min before the injection formaldehyde 1%: Group 1 received physiological water which served as control group. Group 2 received Diclofenac (25 mg/kg, 10 µl/g), served as the standard group. Group 3 received essential oil of *W.s* (75 mg/kg).

The volume of edema at a given time was recorded and translated into percent inhibition. The anti-inflammatory activity was evaluated by calculating the mean percentages of inhibition of edema using the formula (Ndiaye *et al.*, 2006; Panda *et al.*, 2009):

$$\text{Inhibition (\%)} = \left(\frac{Vc - Vt}{Vc} \right) \cdot 100$$

Inhibition (%): Percentage inhibition of edema

Vc: Edema volume in control

Vt: Edema volume in test (treatment)

Statistical analysis

Statistical analysis was performed, using GraphPad Prism 7 (Student's *t*-test) software. All values were expressed as Mean $\pm$ SEM. Values of $p \leq 0.05$ were considered significant.

RESULTS

Acute toxicity studies

The essential oil of W.s administered to the mice at doses ranging from 50 to 200 mg/kg by the intraperitoneal (IP) route carried no mortality

during (2 h, 24 h, and 48 h) (Table 1). However, the dose of 200 mg/kg presented signs of toxicity (hypoactivity, drowsiness and tachycardia).

We then chose 75 mg/kg as the working dose for the subsequent tests as there were no physiological changes observed at 48 h post-treatment.

Table 1. Results of acute toxicity test (short term result 48 h).

Injected product	Doses (mg/kg)	Symptoms			Mortality
		Hypoactivity	Drowsiness	Tachycardia	
Essential oil W.s	50	-	-	-	0
	75	-	-	-	0
	100	+	+	+	0
	200	++	++	++	0

-: No signs

+: Low signs

Analgesic activity

Acetic acid-induced writhing in mice

We have performed screening of analgesic activity of essential oil. This activity was demonstrated by the abdominal cramp test.

In the group of mice treated with ASPEGIC® at dose 500 mg/kg, the analgesic activity showed significant effect when compared with ASPEGIC® at dose of 250 mg/kg as well as standard drug (Figure 1).

Regarding our extract it was very effective against pain in which it completely suppressed writhing, which means a perfect elimination of nociception (Figure 1).

After injection of acetic acid into the control group of mice, 75±2 abdominal cramps are recorded for 30 min. In the presence of ASPEGIC® at 250, 500 mg/kg and essential oil of W.s at 75 mg/kg, the number of abdominal cramps decreases in the same time interval. It passes respectively to; 60±3, 26±5, and 0 cramps.

Moreover, the W.s essential oil totally suppressed pain in mice with a percentage of inhibition of 100%, except some symptoms such as licking of the hind paw. While the percentage of inhibition of ASPEGIC® was 63.51% for dose 500 mg/kg, 250 mg/kg of ASPEGIC® only resulted in 20.94% inhibition (Figure 2), which is originally, classified as peripheral analgesics. This implies that essential oil possesses a strong

peripheral analgesic activity, or probably contains one or more molecules belonging to the class of peripheral analgesics which can certainly be compared to the effect of morphine against pain caused by acetic acid, with maximum inhibition of 98.37% (Ramirez *et al.*, 2010) at 100% according to the results of Makambila-Koubemba *et al.*, (1997).

Hot plate test

After evaluating the hot plate test, we found that when administering the essential oil, the reaction time was prolonged at several times after 30 min of treatment.

The reaction time in control and treated animals was recorded at 15, 30, 45, and 60 min after the treatment; the jump out was made after 5 to 7±0.02 min (after each deposit) for the mice that have received the essential oil of W.s (Figure 3); also to 6±0.01 min for the mice that have received ASPEGIC® drug, whilst in the control group; reacted very quickly they took 3.0 to 4±0.05 min to jump out of retention cylinder.

Our results confirmed that the essential oil of W.s significantly increased the reaction time about 75%, greater than ASPEGIC® drug which reached 42% of inhibition (Figure 4). Therefore, our essential oil showed an important central analgesic activity.

Figure 1. Analgesic activity of W.s-E.O by writhing test. n= 6. Values are expressed as mean $\pm$ SEM. **** P < 0.0001, when compared with control group.

Figure 2. Percentage of inhibition of abdominal cramps induced by acetic acid. n=6. Data are expressed as mean $\pm$ SEM. **** P value < 0.0001 as compared to the E.O group.

Figure 3. Analgesic activity of W.s-E.O, by hot plate method. $n=6$. Significant different among means $*P < 0.05$ when compared with control group.

Figure 4. Percentage of increases the reaction time (hot plate test). Data are expressed as mean $\pm$ SEM. Significantly different ($*P < 0.05$). $n=6$.

Anti-inflammatory activity **Inflammatory paw edema**

The evaluation of anti-inflammatory efficacy is based on the paw edema process caused by the IP injection of formaldehyde. The graph of figure 5 showed that the animals treated with E.O demonstrated a significant decrease in the edema volume, better than the group treated with Diclofenac especially after 60 min of treatment

(Figure 5). Inhibition is also remarkable compared to the negative control. We obtained a reduction of paw volume by 68% after 3 hours for mice that received E.O treatment (Figure 6). Meanwhile, the standard drug only reduced the paw edema by 48%. Thus, W.s essential oil has a stronger anti-inflammatory activity as compared to the standard drug; Diclofenac treated animals. Percentage of inhibition of edema is presented in Figure 6.

Figure 5. Anti-inflammatory activity of W.s-E.O by paw edema method. Asterisk (*) indicates a significant difference between treatments ($p < 0.05$), $n = 6$.

Figure 6. Percentage of inhibition of paw volume. $n = 6$. Data are expressed as mean $\pm$ SEM. Significant difference between treatments ($p < 0.05$).

DISCUSSION

This study provides an evidence that the essential oil of *Warionia sabarae* possesses excellent analgesic and anti-inflammatory effects. These

activities can be due to the terpenes and sesquiterpenes present in the essential oil (Franchomme *et al.*, 2001; Kaloustian *et al.*, 2012) particularly monoterpenes which have secretolytic properties against several inflammatory mediators (Juergens *et al.*, 1998b). Our essential oil may also

contain terpenols and sesquiterpenols (Yakoubi *et al.*, 2015), which have been shown to have anti-inflammatory and analgesic effects (Franchomme *et al.*, 2001; Kaloustian *et al.*, 2012). Also, the terpenic and no-terpenic esters show a powerful anti-inflammatory activity (actives on primary phase of inflammation) (Franchomme *et al.*, 2001).

Inhibition of the synthesis of prostaglandin (PG) and reduction in the release of chemical mediators that activate, sensitize, or awaken pain nociceptors has been generally recognized as the mechanism behind the pharmacological actions of both the therapeutic and adverse effects of medications since the 1970s (Chan *et al.*, 2016; Hamza *et al.*, 2009).

Both acetic acid and hot plate tests cause tissue damage and, in turn, release a number of chemical mediators such as bradykinin, histamine, serotonin, acetylcholine, and prostaglandins. This results in pain by sensitizing a nociceptor (Beaulieu *et al.*, 2005; Collier *et al.*, 1968; Le Bars *et al.*, 2001; Raj *et al.*, 1996). The results reveal that the essential oil of W.s has an antinociceptive effect (analgesic effect).

Our research focused on the anti-inflammatory effect of W.s essential oil, whichever was based on formalin-induced mouse paw edema. This inflammatory response comprises three distinct phases: a first phase involving histamine and 5-hydroxytryptamine, which promote vasodilation, plasma transudation and edema; a second phase that uses kinins as mediators increases vascular permeability and a third phase whose mediator is supposed to be prostaglandin (Attal *et al.*, 2000; Essaqui *et al.*, 2007; Lindsey *et al.*, 1999; Raj *et al.*, 1996; Sanogo *et al.*, 2006). A positive effect could be explained by the inhibition of the actions or synthesis of pro-inflammatory substances. The findings also show that W.s essential oils greatly inhibited histamine-induced vascular permeability.

The main component of the *Warionia sabarae* essential oil, β -eudesmol (Essaqui *et al.*, 2007; Sellam *et al.*, 2012; Yakoubi *et al.*, 2015; Znini *et al.*, 2013), has several pharmacological activities, among them an anti-inflammatory activity. A thorough study has explained the mechanism of β -eudesmol against inflammations (Kim *et al.*, 2018; Seo *et al.*, 2011). β -eudesmol inhibited the production and expression of interleukin-6 (IL-6)

and p38 MAPK and nuclear factor NF- κ B. In addition, it also suppressed receptor-interacting protein 2 (RIP2) and caspase-1 activation induced by phorbol 12-myristate 13-acetate calcium ionophore A23187 (PMACI) (Koonrungsomboon *et al.*, 2014; Seo *et al.*, 2011). Another study has proved that in high concentrations of β -eudesmol could block neuromuscular transmission (Kimura *et al.*, 1991; Chiou *et al.*, 1992).

CONCLUSION

Our data showed that W.s essential oil have both central and peripheral analgesic effects, as well as anti-inflammatory effects. With a highest peripheral anti-nociceptive activity., Hence, these results support the traditional use of the plant against inflammation. However, additional research is required to clarify the molecular and cellular dynamics of these effects and determination of the active compounds in the essential oil of *Warionia sabarae*.

CONFLICT OF INTERESTS

All authors have completed the ICMJE uniform disclosure form and declare no support from any organization for the submitted work.

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