Evaluation of the anti-inflammatory, antispasmodic and healing effects of walnut leaves *Juglans regia* L. aqueous extract

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Abstract

As a part of the valorisation of medicinal plants of the Algerian flora, we have undertaken a study of plant extract (*Juglans regia* L.) growing in the Blida region, Algeria.

The main objective of this work is to evaluate the in vivo pharmacological effects, namely the anti-inflammatory, antispasmodic and healing effects of common walnuts's leaves aqueous extracts and ointment. The Carrageenan-induced paw edema test is used for screening of anti-inflammatory activity on 4 batches of mice (control batch, treated batch with Diclofenac® , treated batches with the aqueous extract at 5 and 10%), the analgesic effect was conducted using Acetic acid-induced endogenous spasm test, and the healing effect was conducted on rabbits. The anti-inflammatory test performed on laboratory mice, revealed a remarkable anti-inflammatory effect of the aqueous extract comparing with chosen standard pharmaceutical molecule (Diclofenac®), with a reduction percentage of 25 and 31.72 % for the aqueous extract and Diclofenac® respectively. The results for the analgesic effect was revealed to be significant comparing with those obtained with the reference product Spasfon. Finally, the healing effect was performed on rabbits, revealing high healing power comparing with those obtained with pharmaceutical healing paste 'MADICASSOL" used as a reference. The ointment prepared using walnut leaves has shown more effectiveness with delay of healing less than a week compared to the ointment of reference which took almost 10 days for total healing effect. The obtained results confirm some information obtained during the ethnobotanical survey in a previous study; and that *Juglans regia* L. is endowed with an anti-inflammatory, antispasmodic and healing properties.

Keywords: Anti-inflammatory, antispasmodic, *Juglans regia* L., healing effect.

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1. Introduction

Inflammation, pain issues or healing issues represent the biggest problem of health care, leading to a high use of drugs in order to feel relief, which, due to their continuous use and administration may result in serious side effects including renal problems, gastrointestinal irritation and ulceration (Bertolini et al., 2001).

Therefore, researches meet the increasing need of discovering potent molecules that are devoid of these side effects as alternatives. This has prompted scientific investigation of medicinal plants used in traditional and folk medicine for treating fever, pains, rheumatoid arthritis and healing wounds (Basu and Hazra, 2006 ; Nworu et al., 2013).

Those health-promoting effects are linked to the secondary metabolites that each plant produces, including compounds like essential oils, phenolic compounds, terpenoids, alkaloids, steroidal compounds, glycosides, terpenes, and tannins, which may be responsible for various therapeutics effects. These plant constituents possess numerous favorable physiological properties, such as antioxidant, antiinflammatory and anti-atherosclerotic (Paudel et al , 2016; Jun et al 2016).

For the common walnut, *Juglans regia* L., its different parts are known to containing potent chemical constituents, used since antiquity to treat diverse ailments for instance diarrhea, hyperglycaemia, cancer, infectious disease, anorexia, eczema, asthma, helminthiasis, arthritis, sinusitis, stomach-ache, skin disorders, among others (Kunwar et al., 2005 ; Tagarelli et al., 2010).

The leaves of walnut are popular in complementary and alternative therapy as antimicrobial, depurative, keratolytic, antidiarrheal, carminative, anthelmintic, astringent, tonic, hypoglycaemic and for the treatment of sinusitis, cold and stomachache (Warrier et al., 1994 ; Gîrzu et al., 1998), to alleviate fever and to reduce rheumatismal pain (Fujita et al., 1995 ; Qamar et al., 2011) They are used in the treatment of colitis diabetes, cardiac disease and inflammatory conditions, skin diseases , toothache and to promote hair growth and healing wounds (Kunwar et al., 2005 ; Jaradat, 2005 ; Kaileh et al., 2007 ; Tagarelli et al., 2010 ; Rahimipanah et al., 2010 ;Torres-González et al., 2011), as well as to improve vascular and prostate health in elderly males **(**Spaccarotella et al., 2008).The list of illnesses treated with walnut parts goes on, also including the treatment of gastric, liver and lung cancer (Liu et al., 2004).The ethnobotanical study, carried out in Algeria Blida region, revealed the importance

of *Juglans regia* and its use as a natural remedy against pain, the healing of chapped feet, eczema, back pain, antirhumatismal and also hypertension, anticholesterolemia, hypoglycemia and for other type of relief (Bennacer and Cherif , 2017).

In this perspective, this study aims the confirmation of information collected in previous ethnobotanical study, by evaluating the pharmacological effects, namely anti-inflammatory, analgesic and wound healing properties of *Juglans regia* leaves grown in Algeria.

2. Material and methods

2.1. Collection and preparation of the aqueous extract of *Juglans regia'* leaves

Healthy, free of pest attack adult leaves of *Juglans regia* were collected from 30 years old trees before fruiting in May 2014 ,Bab-Khewikha region (Wilaya of Blida, Center of Algeria). The botanical identification of the species was validated by Mrs Bradai. M.S. Professor of Botany, Department of Agronomy, Blida 1 University, Algeria.

The freshly harvested leaves were dried out of light and moisture for two to three weeks. Once dried, the leaves were crushed using a house mill.

The aqueous extract used in the study is prepared according to the method reported by Bruneton (1999), where 20 g of powder are left infused for 15 minutes in 200 ml of boiling distilled water. The infused was filtered to obtain the aqueous extract.

2.2. Experimental animals

To carry out the experimental part, rabbits and mice, provided from the pet shop of the Pharmaco-Toxicology Laboratory of the Anitibiotical Complex Saidal (Medea, Algeria), were used.

Forty SWISS albino mice weighing each 20 to 24 g males were divided into 8 batches. Each batch consists of five mice, and four batches were used for the anti-inflammatory and the remaining four to the antispasmodic activity. Three CALIFORNIAN rabbits males, each weighing 2300 to 2600 g, divided into 3 batches (one rabbit per batch) were used for healing activity. The research protocols were in accordance with the ethical rules and recommendation of the National institutes of health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.3 Carrageenan-induced paw edema test for screening of anti-inflammatory activity

The anti-inflammatory activity was carried out according to the method of Levy (1969) cited by Berkan and his collaborators (1991).

This study makes it possible to compare the reaction of plantar edema after administration of equal doses of the anti-inflammatory product to be tested and the corresponding reference product (Diclofenac ®) (Colot, 1972).

Briefly, the protocol works as the injection of carrageenan 1% under the plantar fascia of the mouse paw causes an anti-inflammatory reaction, which can be reduced by an anti-inflammatory product (aqueous extract of the plant). In order to undoubtedly demonstrate the anti-inflammatory effect, the mice are divided into 4 batches of 5 mice each, namely three treated batches and one control batch, the mice were fasted for 18 hours before the experimentation. The force-feeding (at time T0) was carried out using a gastric tube.

At time, zero (T0) all the batches are treated as followed:

Batch E1: The mice are force-fed with 0.5 ml of an anti-inflammatory agent (Diclofenac ®); 1 tablet of 75 mg in 750 ml of physiological water.

Batch E2: Mice force-fed with 0.5 mL at a dose of 10% aqueous extract corresponding to 0.1 g /mL.

Batch E3: Mice force-fed with 0.5 mL at a dose of 5% aqueous extract corresponding to 0.05g /mL.

Batch control: Mice force-fed with 0.5 mL of physiological water at 0.9%.

At time zero(T0) + 30 minutes, the 1% carrageenan solution is injected under the plantar fascia of the left hind legs in a volume of 0.025 mL to all the animals tested.

At time zero (T0) + 4 hours, after having sacrificed the animals having been subjected to a high concentration of diethyl ether, we cut the hind legs at the level of the joint and we weighed them with an analytical balance.

Expression of results

- Calculate the arithmetic means of the weights of the left paw and the right paw for each batch.

- Calculate the percentage increase in paw weights (% of edema) by the following formula:

$$\%\ of\ edema = \frac{average\ left\ leg\ weights - average\ right\ leg\ weights}{average\ right\ leg\ weights} * 100$$

- Calculate the percentage reduction in edema in the treated mice compared to the controls.

$$\%\ \text{reduction of edema} = \frac{edema\ of\ batch\ E1 - edema\ of\ batch\ E2\ or\ E\#}{\text{edema of batch control}} * 100$$

The results are expressed as the average weight of the left hind legs ± standard deviation, the statistical significance is determined by means of the one-way analysis of variance test (ANOVA) followed by the NKT test for peer comparison. P<0.05 is considered to be a significant difference; the statistical study is carried out using XLSTAT 2009.

2.3. Acetic acid-induced endogenous spasm test for screening of analgesic activity

To carry out this test we used the protocol validated by the laboratory of pharmacotoxicology of the Antibiotical Saidal pharmaceutical industries. The injection of 1% acetic acid intraperitoneally causes a painful reaction in the mice. This pain manifests as spasms in the form of torsional movements of the abdomen with stretching of the hind legs. We divided the 20 mice into 4 batches, as follows:

• Control batch: the mice receive 0.5 mL of distilled water (physiological water at 0.9%)

• Batch E1 and E2: Mice force-fed with 0.5 mL at a dose of 10 and 5% of aqueous extract "infusion" corresponding to 0.1 and 0.05g / mL respectively.

• Batch E3: mice force-fed with 0.5 mL by the reference drug Spasfon® (Phloroglucinol) at a dose of 0.025 mg / mL

After 30 min of the treatment, we inject the mice with 0.1 mL of 1% acetic acid. 5 minutes later, we count the number of spasms for 10 minutes.

• The calculation of the percentage of decrease in spasms is done by the following formula

$$Percentage\ (\%) = \frac{\text{Spasm number of batch E3} - \text{Spasm number of batch E1 or E2}}{\text{Spasm number of batch E3}}$$

2.4. Evaluation of the wound healing property

The evaluation of the healing property of *Juglans regia* leaves is carried out according to the protocol reported by Pourrat (1993), by comparing the bleeding in rabbits and to another reference ointment.

2.4.1. Formulation of the traditional healing ointment

The ointment was prepared according to the protocol mentioned by Le Hair (2001) with a concentration of 20 % and 100 g of weight, the following constituents are weighed:

- 20 g of dry, fine and sterile leaf powder (sterilized following exposure to UV in a laminar flow hood for 30 min);
- 30 g of Vaseline oil;
- 50 g of Vaseline.

The ointment was prepared in a porcelain mortar at room temperature in the laboratory, under rigorous aseptic conditions (under laminar flow hoods). Vaseline is heated in a water bath at 36 °C to facilitate mixing and to be able to dissolve the powder more easily there (Le Hair, 2001). Mixing of the mixture must be carried out until complete cooling to avoid separation of the constituents (Fonteneau et al., 1999). The ointment was packaged in dermal ointment packaging tubes, the tubes are then closed in order to avoid any external contamination.

2.4.2. Physico-chemical and microbiological control of the ointment

In order to confirm the good quality of the formulation, a physico-chemical and microbiological control were carried out according to method mentioned by European pharmacopoeia (1997).

- **PH measurement**

10g of ointment was triturated with distilled water, and pH measured by a pH meter.

- **Homogeneity of the ointment**

It was Verified macroscopically by spreading the ointment in a thin layer on a flat surface (laboratory bench) using a spatula (Le Hair, 2001).

- **Microbiological control of the ointment**

The protocol followed is that listed by the European Pharmacopoeia in 1997. Briefly

10g of ointment were weighed and introduced into a 100 ml bottle of pH 7 buffer solution, then heated in a water bath at 45 °C until an emulsion is obtained. The latter was placed in Petri dishes containing different culture media.

The results are expressed in colony forming unit (CFU), the product is declared compliant if the results are below the limits mentioned in the table 1.

Table 1. Limits of the microbiogical control of the ointment.

Parameters	Limit
Total viable aerobic germs	≤ 5.102 UFC/Ml
Yeast and mold	≤ 5.102 UFC/mL
Enterobacteria and Gram (-)	≤ 10 UFC/mL
Staphylococcus aureus	absence
Pseudomonas aeruginosa	absence
Escherichia coli	Absence

2.4.3. Pharmacological test of the ointment

The test was carried out on 4 Albino rabbits of Californian race, that had their hair removed using a pair of scissors and a razor; after depilation and disinfection of the skin with a cotton pad soaked in alcohol, parallel scarifications and deep to the bleeding limits are carried out using a scalpel. The application of the leaf ointment is carried out on the wound caused on the right side in the first and second rabbit, while the application of the reference ointment (Madecassol) is carried out on the wounds caused in the third rabbit while the fourth rabbit is left without any treatment (control).

At the aim of monitoring the progress of the healing process, a macroscopic examination is carried out, a rating scale has been set taking into account four parameters:

- The depth of the wound
- The appearance or not of edema
- The presence or absence of a bud
- The thickness of the crust.

Each of these four parameters is qualified by a numerical value from 0 to 4, defined in the table 2.

Table 2. Healing effect rating scale

	Depth	**Bud**	**Edema**	**Crust thickness**
0	Deep zero	Absence of bud	No edema	No crust
1	Slightly dug	Little bud	Very light	Start of crust
2	A little deep	Big bud	Visible edema	thickening crust
3	Fairly deep	Massive budding	moderate edema	Thick crust
4	Very deep	Excessive budding	severe edema	Very thick crust

3. Results

3.1. Evaluation of the anti-inflammatory effect

For each batch, we calculated the percentage increase in edema of the inflamed paw relative to the weight of the healthy paw according to the formula of Levy (1969). The results of the means, reduction of inflammation and of the statistical study obtained are compiled in the table 3 and figure 1.

Table 3. Percentages of edema and edema reduction for the four batches.

Treatments / Batches	% of edema	% reduction of edema

Control	35.84%	0
E1 (Diclofenac®)	24.45%	31.72%
E2 (Aqueous extract 10%)	27.94%	22.04%
E3 (Aqueous extract 5%)	26.88%	25%

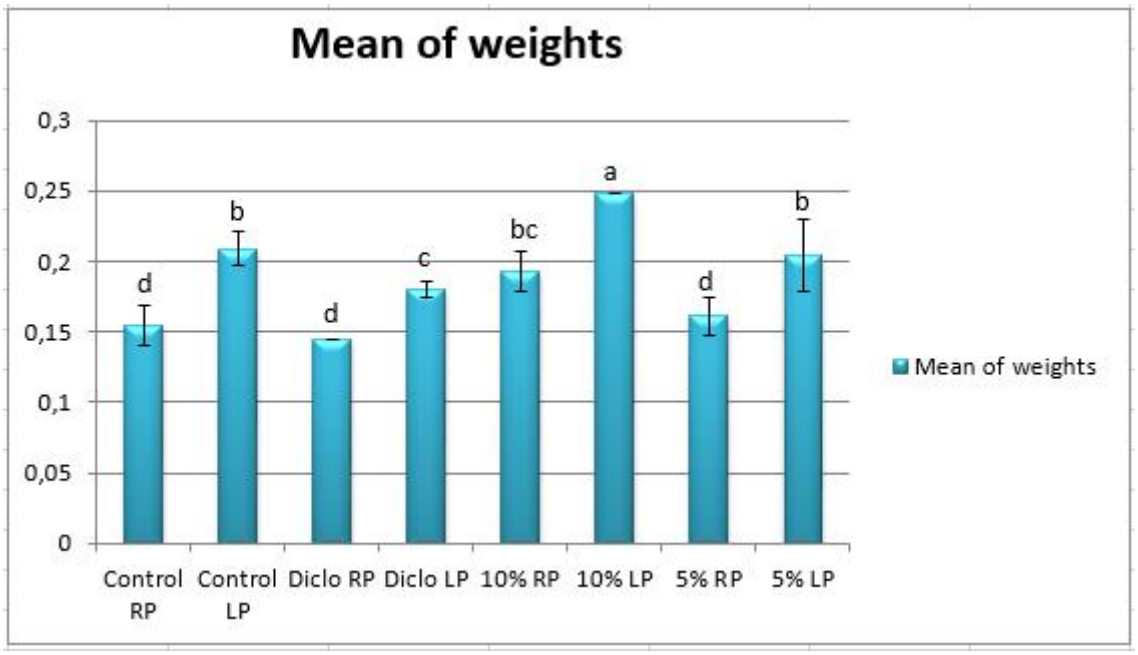

Figure 1. Variation in the weight of the right and left hind legs for each batch (Significant difference P<0.05 confidence interval is 95%).

Results for the anti-inflammatory effect of aqueous extracts of walnut leaves are presented in figure 1. In all treatments, and as expected, left legs are heavier, due to presence of edema. Four hours after the injection of the carrageenan, the edema shows a significant increase (P<0.01) of 35.84 % in the control group, but also a significant increase (P<0.05) of 24.45, 27.94 and 26.88% in the batches treated, respectively (Diclofenac®, E10 %, E5 %).

Figure 1 shows a significant difference (P<0.05) between the inflamed legs (left hind legs), which received carrageenan and the healthy legs (right hind legs) whose average weight of the four lots is respectively (T) 0.154 ± 0.01, (Diclofenac®) 0.145 ± 0.005, (E3 10%) 0.193 ± 0.01, and (E4 5%) 0.161 ± 0.01. Thus, a significant difference is observed (P<0.05).

However, no significant difference (P>0.05) is observed in the control group which received only physiological water, on the other hand, there is a significant reduction (P>0.01) of 31.72, 22.04, 25% for the batch treated with Diclofenac®, with a dose of 10% and 5% of the aqueous extract, respectively.

We can deduce that the aqueous extract has a more or less important anti-inflammatory effect at a dose of 5%, however, at a dose of 10% it has a weak it is probably that the extract is more efficient at low doses, but less intense anti-inflammatory effect than that which has been observed in the batch treated with Declofenac®.

3.2. Evaluation of the analgesic effect

The analgesic activity of the aqueous extract (infusion) of *Juglans regia* L. was evaluated by counting the spasms or abdominal contractions induced in mice by intra-peritoneal injection of acetic acid 1%. The results of this study are recorded in the figure 2 that illustrates the percentage of protection of the aqueous extract compared with the reference drug Spasfon® and the control batch.

After 10 minutes of the injection of acetic acid into the control batch of mice, an average of 24.2 abdominal cramps was recorded. The administration of the aqueous extract of the leaves of *Juglans regia* L. at 10 and 5 % reduced the average abdominal cramps up to 9.6 and 9.8, with a protection percentage of 60.33 and 59.50 % respectively. These percentages are somewhat close to that of the Spasfon® reference solution which has a protection rate of 83.47 %, with a mean contraction of 4.

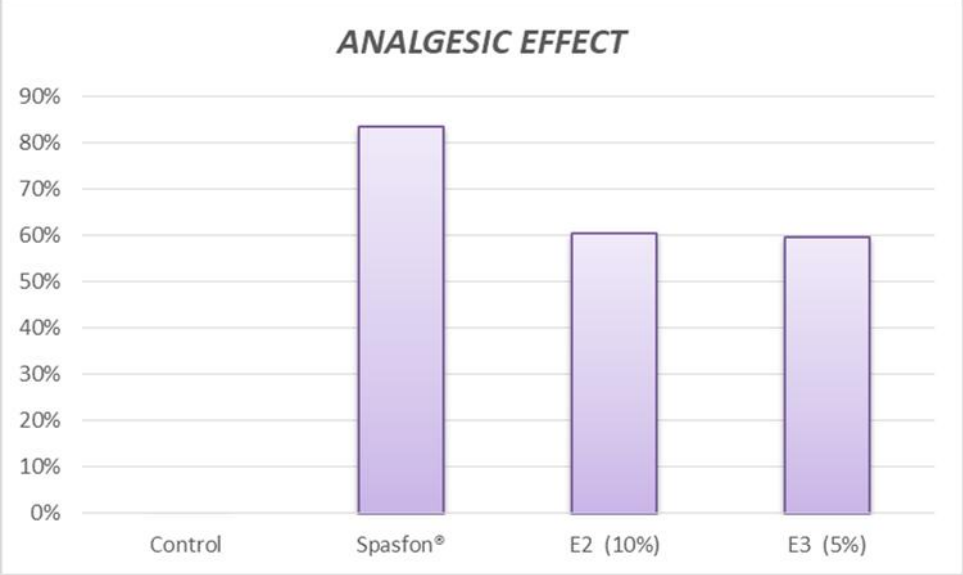

Figure 2. Protection percentage of the aqueous extract compared to the reference drug Spasfon®. E2 (10 %): aqueous extract 10 %, E3(5 %):aqueous extract 5 % and the negative control.

3.3. Evaluation of the healing effect

3.3.1. Results of the control of the traditional ointment

- **Physico-chemical control results**

The homogeneity of the ointment was verified macroscopically by spreading it in a thin layer on a flat surface, and by observation no aggregates, lumps were observed, and a good distribution of the ointment was found, being considered as perfectly homogeneous.

The pH measurement of the ointment was of 6.16, a value close to neutral. Accordingly, it can be stated that the ointment does not cause irritation on the skin (European Pharmacopoeia, 1997)

- **Microbiological control results**

After 24 H, 48 H and 5 days of incubation for bacteria, yeast and molds , Petri dishes are germs free.

The microbiological control of the ointment did not reveal any bacterial nor fungal contamination, the ointment is therefore free from all microbial contamination, hence it is in compliance with the standards of the European Pharmacopoeia (European Pharmacopoeia, 1997)

- **Results of the preliminary healing test**

Generally, the observation shows the absence of edema for the area treated by the ointment based on the leaves of *Juglans regia* L. and by the reference ointment Madecassol which means that these two treatments do not favor the inflammatory process. However, on the control wound, a slight edema appeared from the first day and fades to disappear on the 4th day.

The bud is relatively unmarked, appearing from the first day in treated wounds and faded quickly and disappear on the 4th day. In the control wound, the bud appeared only around the 3rd day and persists until the 6th day.

In treated rabbits, the formation of a light crust after 24 hours of treatment was observed, which thickens during the first days, disappearing on the 5th day for the area treated with the ointment from the leaves of common walnut. For the assay with Madecassol, it only disappeared on the 7th day for the areas treated by Madecassol. After the 3rd day, it was less thick, increasing its thickness and persisting until the 11th day for the control wound.

The scarifications are much deeper for the untreated area (control) than for those treated, after the first day. The depth of the wound treated with the leaf ointment is less marked compared to those treated with Madecassol. The two treatments gave good healing with only slightly different healing times, which were 7 days for the ointment from the leaves of *Juglans regia* and 9 days for Madecassol. Natural scarring is not complete in the control assay until after the 14th day (Fig. 3 and Fig. 4).

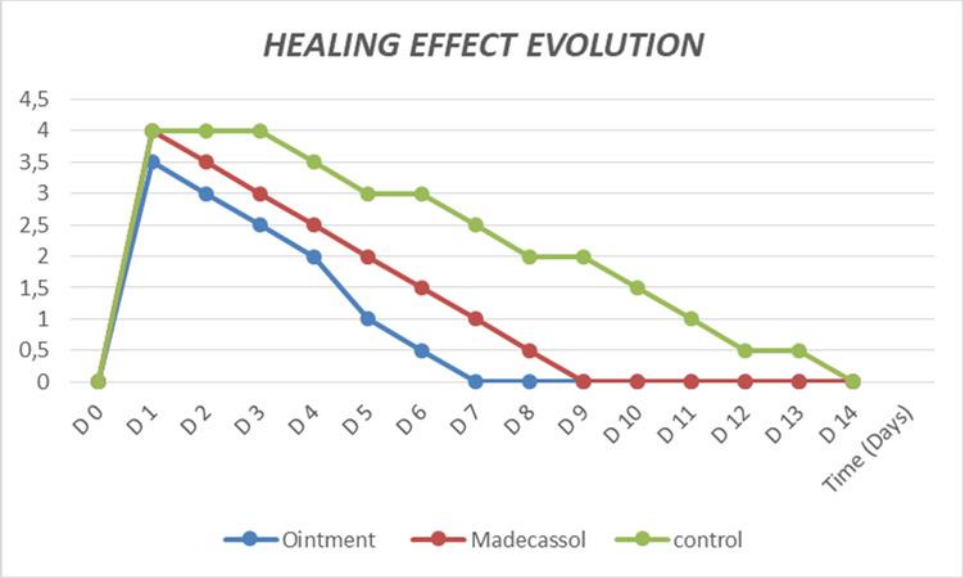

Figure 3. Daily evolution of the depth of the wound expressing the healing effect.

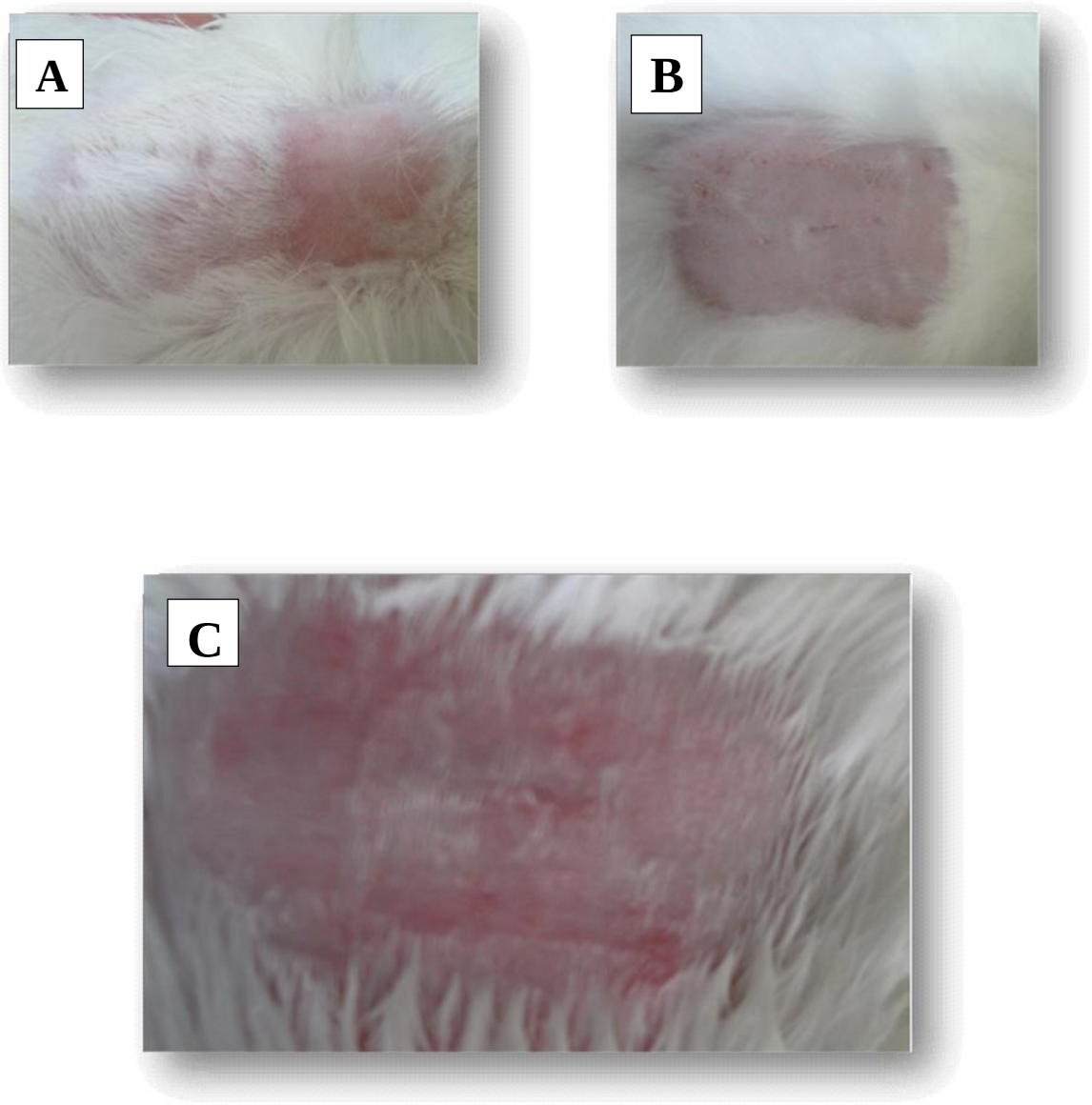

Figure 4. Healing of treated wounds (9th day of the treatment).

A. Wound treated with *Juglans regia*'s leaves ointment, B. Control wound, C. Wound treated with

Madecassol reference ointment.

4. Discussion

The present study investigated the anti-inflammatory, analgesic and healing effect of the aqueous extract of *Juglans regia* leaves. The different parts of this plant are used to treat

pain and to heal injuries from the Algerian population, especially the inhabitants of Blida region **(**Bennacer et Cherif, 2017), and so do different populations all over the world (Tene et al., 2007 ; Idolo et al., 2010 ; Sher et al., 2011). These folkloric claims prompted the present study in which anti-inflammatory, antispasmodic and healing activities of *Juglans regia* L extract were evaluated and found of great effectiveness.

The results of the anti-inflammatory activity showed that the aqueous extract has a significant effect. Our results are in agreement with those obtained by Hosseinzadeh and his collaborators (Hosseinzadeh et al., 2011) on the leaves of the Iranian walnut and in another study reported by Abdulalsalam and his collaborators (2017). These authors show that the aqueous extract of *Juglans regia* has an important anti-inflammatory effectiveness by inhibiting protein denaturation, linked to the cause of inflammation (Oyedepo and Femurewas, 1995). Other authors, Eswayah et al. (2019), have confirmed the anti-inflammatory power of the species *Juglans regia*, with recorded potential to protect cell membrane tests on human blood.

The analgesic properties of the aqueous extract of the leaves of *Juglans regia* showed to be positive and had a remarkable effect in comparison with a reference drug (Spasfon®), which its main active principal is phloroglucinol (belongs to the family of musculotropic antispasmodics). The percentage of protection is estimated for the aqueous extract with 59.50 % and 60.33 % for both concentration 5 and 10% respectively while the highest rate of protection was achieved with the reference compound with a rate equal to 83.47 %.

The results obtained in this study are in agreement with those found by Hosseinzadeh et al. (2011) and Nael and Mohammed (2011). Similarly, Mokhtari and his collaborators (2008) reported that the administration of walnut leaf extract has an analgesic effect ; so that the administration of this extract in the acute phase of pain could be resulted in a significant reduction of pain. The administration of walnut leaf extract, together with morphine, causes analgesia in the acute phase (Hosseinzadeh al. 2011; Nael and Mohammed, 2011 ; Mokhtari et al., 2008).

This analgesic effect might be linked to the presence of flavonoids such as quercetin, that have analgesic effect through adrenergic pathways and by reducing the sensitivity of the central system and eventually reduce pain (Mokhtari et al., 2008; Kaur et al., 2003 ; Delaviz et al., 2017) . it is possible that walnut leaves are associated with analgesic effects due to having flavonoid compounds such as quercetin (Oliveira et al., 2008).

Both anti-inflammatory and analgesic effects were proven to be important using *Juglans regia*'s leaves extracts. Methanol leaf extract produced statistically significant inhibition of edema induced by carrageenan at nearly all doses (250-1000 mg/kg) when compared to the control groups. The effect was dose-dependent. The highest activity was at 1000 mg/kg, and inhibited 77% of inflammation. The same activity (73 %) ($P>0.05$) was found for Diclofenac at 100 mg /kg (Nabavi et al., 2011). The ethanolic extracts of *Juglans regia* leaves exhibited potent anti-inflammatory activity (as potent as indomethacin) against carrageenan-induced hind paw edema model in mice without inducing any gastric damage (Erdemoglu et al., 2003) which goes in the line with our results.

The aqueous extract of the leaves of common walnut has proven to have great potential for wound healing, as results showed total healing after 7 days of treatment in comparison with a pharmaceutical product Madecassol which took 9 days for total healing.

The results of the healing activity are similar to those found on the leaves of a plant of the same genus *Juglans nigra* L. (Logeeswari and Shubashini, 2012) and also with those found using extracts from the fruit of *Juglans regia* (Afaf et al., 2018). The phytochemical screening of *Juglans regia* L. has previously shown the presence of saponins which, according to Bruneton (1999), have a healing activity. This can be the reason behind the use of walnut leaves in folk medicine, as previously reported in Italy (Idolo et al., 2010), but also the one conducted in the region of Blida (Bennacer et Cherif, 2017) that reported the same information about healing feet cracks and injury.

The absence of edema is justified by the anti-inflammatory activity which the plant possesses and which has already been mentioned previously in our ethnobotanical study (Bennacer et Cherif, 2017).

5. Conclusion

The results from the present study show that *Juglans regia* has good anti-inflammatory, analgesic and healing properties and this could explain the vast use and benefit of this plant in folk medicine. Furthermore, the study also provided experimental evidence for the effectiveness of *Juglans regia* in folk medicine for treating inflammation-associated diseases such as rheumatism or pain aches such as headaches, but also for healing wounds like in the case of damaged feet. Therefore, *Juglans regia* should be further investigated as potential source of anti-inflammatory, analgesic and healing compounds for pharmacological purposes.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

Abdulalsalam H, Naksheen M, Ardalan S, Ahmed H (2017) Evaluation of anti-inflammatory effect by using iron nanoparticles prepared by *Juglans regia* water extract , Current Research in Microbiology and Biotechnology Vol. 5, No. 4: 1151-1156

Afaf Al-Nadaf H, Nesrin Seder J, Abu Rayyan W (2018) Wound healing antimicrobial and anti-oxidant activity for Jordanian *Juglans Regia* L. unripe fruits Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS) ISSN: 2349-2759 Jordan JIPBS, Vol 5 (3), 26-34.

Baba Aissa F, (1991) .Les plantes médicinales en Algérie. Ed. Bouchène et Ad. Diwan, Alger, p 97.

Basu S, Hazra B E (2006) Evaluation of nitric oxide scavenging activity, in vitro and ex vivo, of selected medicinal plants traditionally used in inflammatory diseases, Phytother. Res.(20) 896–900.

Bennacer A, Cherif S H (2017) Contribution to the Ethnobotanical, Phytochemical, Antimicrobial and Antioxidant Study of the Leaves' Aqueous Extract of the Common Walnut *Juglans regia* L. Inter J of Pharmaco Phyto Ethno . 7:41-52.

Berkan T, Ostunes I, lermiolu F, Ozer A (1991) Anti-inflammatory analgesic and antipyretic effets of an aqueous extract of nine plant. Journal of pharmacology .1991. pp : 375.

Bertolini A, Ottani A, Sandrini M (2001) Dual acting anti- inflammatory drugs: A reappraisal, Pharmacol. Res. .44: 437–450.

Bruneton J, (1999) Pharmacognosie: élément de phytochimie et pharmacognosie .3eme édition ,TEC et DOC ,Paris, pp 405-446.

Chamouleau A (1979) .Les usages externes de la phytothérapie.Ed. Maloine, Paris, ISBN. 2-224-00526-1, p 53.

10. Colot M (1972) .Notions techniques de pharmacologie générale.Edition Masson France : pp123-126.

11. Delaviz H , Mohammadi J , Ghalamfarsa G (2017) A review study on phytochemistry and pharmacology applications of *Juglans regia* plant. Pharmacognosy reviews.vol. 11, no 22, p. 145.

Erdemoglu N, Küpeli E, Yeşilada E (2003) Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine. J. Ethnopharmacol. 89: 123-129.

Eswayah A, Labyad N , Aleanizy F,Belaid A, Alqahtani F, Alfassam H (2019) Antimicrobial and Anti-inflammatory properties of *Juglans regia* Leaves. Orien j of chemi.35: 1756-1759.

Fonteneau J, Orecchioni M, Pain J (1999) .Galénique préparateur en pharmacie "Edition centre régional de documentation ,Pédagogie d'Aquitaine,204p.

Fujita T, Sezik E, Tabata M, Yesilada E, Honda G, Takeda Y, Tanaka T, Takaishi Y (1995) Traditional medicine in Turkey VII. Folkmedicine in Middle and West Black Sea regions. Econ. Bot. 49: 406-422.

Gîrzu M, Carnat A, Privat AM, Fialip J, Carnat AP, Lamaison JL (1998) Sedative effect of walnut leaf extract and juglone, an isolated constituent. Pharm Biol. 1998; 36(4): 280–286.

Hosseinzadeh H, Zarei H, Taghiabadi E (2011) Antinociceptive, antiInflammatory and acute toxicity effects of *Juglans regia* L. Leaves in mice. Iran Red Crescent Med. J. 13:27-33.

Idolo M, Motti R, Mazzoleni S (2010) Ethnobotanical and phytochemical knowledge in a long-history protected area the Abruzzo,Lazio and Molise National park(Italian Apennines) » Journal of ethnopharmacology 127,Italy. pp379-395.

Jaradat NA (2005) Medical plants utilized in Palestinian folk medicine for treatment of diabetes mellitus and cardiac diseases. J Al-Aqsa Unv. 2005; 19: 1–28.

Jun MY, Karki R, Paudel KR, Sharma BR, Adhikari D, Kim DW (2016) Alkaloid rich fraction from Nelumbo nucifera targets VSMC proliferation and migration to suppress restenosis in balloon-injured rat carotid artery. Atherosclerosis. 248: 179–189.

Kaileh MB, Berghea WV, Boonec E, Essawib T, Haegemana G (2007) Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity. J Ethnopharmacol. 113(3): 510–516.

Kaur K, Michael H, Arora S, Härkönen PL, Kumar S (2003) Studies on correlation of antimutagenic and antiproliferative activities of *Juglans regia* L.. J. Environ. Pathol. Toxicol. On col. 22: 59-67.

Kunwar RM, Adhikari N (2005) Ethnomedicine of Dolpa district, Nepal: the plants, their vernacular names and uses. J Ecol App. 8(1): 43–49. 18.

Levy L, (1969) Carrageenan paw edema in the mouse. Life Sciences Volume 8, Issue 11, Part 1, 1 June .601-606.

Le Hair A (2001) Pharmacie galénique : Bonnes pratiques de fabrication de médicament » Edition Masson,402p.

Liu L, Li W, Koike K, Zhang S, Nikaido T (2004)New alphatetralonyl glucidose from the fruit of *Juglans mandshurica*. Chem Pharm Bull (Tokyo). 52(5):566-569.

Logeeswari K , Shubashini K.S . Wound healing Medicinal plants» International journal of chemical environmental and pharmaceutical research .2012.Vol 3,India,pp199-218.

Mokhtari M, Shariati M, Sadeghi N (2008) . Effect of alcohol extract from leave *Juglans regia* on antinociceptive induced by morphine in formalin test. Medical Science Journal of Islamic Azad Univesity-Tehran Medical Branch. 18(2), 85-90.

Nabavi SF, Ebrahimzadeh MA, Nabavi SM, Mahmoudi M, Rad SK (2011) Biological activities of *Juglans regia* flowers. Revista Brasileira de Farmacognosia . 21(3):465-470.

Nael AT, Mohammed AA-W (2011) Utility and importance of walnut, *Juglans regia* Linn: a review. Afr J Microbiol Res. 5(32): 5796–5805.

Nworu C S, Nawke H C,Akah P A,Okoye F, Esimone C (2013) Extracts of Ficus exasperata leaf inhibit topical and systemic inflammation in rodents and suppress LPS-induced expression of mediators of inflammation in macrophages, Journal of Immunotoxicology. (10) 302-310.

Oliveira I V, Sousa A, Ferreira ICFR, Bento A, stevinhol LE, Pereira JA (2008) Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. Food Chem. Toxicol. 46:2326-2331.

Oyedepo O and Femurewas A J (1995) Anti-protease and membrane stabilizing activities of extracts of Fagra santhoxiloides, Olax subscorpioides and Tetrapluera tetraptera. Int. J. Pharm. 33: 65-9.

Paudel K R, Lee UW, Kim DW , Chungtaejeon A (2016) Korean fermented tea, prevents the risk of atherosclerosis in rats fed a high-fat atherogenic diet. J Integr Med. 2016 14(2): 134–142.

Pharmacopée européenne , (1997) . Direction de la qualité du medicament du conseil d'Europe (DEQM) . troisième addendum de la troisième edition, Série des traités européens N°50, Strasbourg,France.

Pousset J L , (2004) Plantes médicinales d'Afrique .Edition Secum, Paris, p.7-8.

Pourrat A , (1993) Cicatrisation des plaies chez le lapin et le rat. Edition J.Pharm,Belgique.

Qamar W, Sultana S (2011) Polyphenols from *Juglans regia* L.(Walnut) kernel modulate cigarette smoke extract induced acute inflammation, oxidative stress and lung injury in Wistar rats. Hum.Exp. Toxicol. 30:499-506

Rahimipanah M, Hamedi M, Mirzapour M (2010) Antioxidant activity and phenolic contents of Persian walnut (*Juglans regia* L.) green husk extract. Afr. J. Food Sci. Technol. 1:105-111.

Sher Z, Ud din khan Z, Frrukh H (2011) Ethnobotanical studies of some plants of Chagharzai valley discrit buner, Pakistan. Pakstan Journal of ethnobotanical studies 43, Pakistan.pp1445-1452.

Spaccarotella KJ1, Kris-Etherton PM, Stone WL, Bagshaw DM, Fishell VK, West SG, Lawrence FR, Hartman TJ (2008)The effect of walnut intake on factors related to prostate and vascular health in older men. Nutr J. Vol 7(13): 1–10.

Tagarelli G, Tagarelli A, Piro A (2010) Folk medicine used to heal malaria in Calabria (southern Italy). J Ethnobiol Ethnomed. pp; 6: 27.

Tene V ,Malagon O ,Vita Finzi P, Vidari G , Armijos C , Zaragoza T (2007) An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador.Journal of ethnopharmacology 111,Ecuador. pp63-81.

Torres-González L, Muñoz-Espinosa LE, Rivas-Estilla AM, Trujillo-Murillo K, Salazar-Aranda R, Waksman De Torres N, Cordero-Pérez P (2011) Protective effect of four Mexican plants against CCl4-induced damage on the Huh7 human hepatoma cell line. Ann Hematol.vol : 10(1): 73–79.

Warrier PK, Nambiar VPK, Ramankutty C (1994) Indian medicinal plants: a compendium of 500 species. 3rd ed. Telangana: Orient Black Swan. 59–64.

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