Tropical Journal of Pharmaceutical Research March 2022; 21 (3): 589-596
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v21i3.19

Original Research Article

Hypoglycemic, analgesic and anti-inflammatory effects of *Pulicaria incisa* (Lam.) DC extracts in animal models

Djamila Sahar[1]*, Somia Saad[1,2], Saida Ouafi[1]
[1]University of Sciences and Technology Houari Boumediene, Faculty of Biological Sciences, Research Laboratory on Arid Zones (LRZA), BP n° 32 El-Alia, Bab Ezzouar 16111, Algiers, [2]Scientific and Technical Research Centre on Arid Regions (CRSTRA), Bioresources Division, Biskra, Algeria

For correspondence: **Email: sahar.djamila@gmail.com*

Sent for review: 11 August 2021 *Revised accepted: 1 March 2022*

Abstract

Purpose: *To investigate acute toxicity, hypoglycaemic, analgesic and anti-inflammatory activities of Pulicaria incisa (Lam.) DC.*
Methods: *Acute toxicity analysis was assessed by adopting the OECD - 423 Guidelines. The hypoglycemic effect of P. incisa infusion was investigated utilizing Oral Glucose Tolerance Test (OGTT) on orally glucose-induced hyperglycemic rats. The analgesic effect was investigated using the writhing test model, and anti-inflammatory activity was ascertained by carrageenan-induced paw oedema method.*
Results: *The results showed that half-maximal lethal dose (LD_{50}) values in mice were higher than 14 g/kg. The results of OGTT showed that the plant infusion produced a significant ($p < 0.05$) reduction in the glucose level in the blood post oral glucose administration. Treatments with diethyl ether, n-butanol and free aglycone extracts at 400 mg/kg significantly reduced ($p < 0.05$) the abdominal cramps by 82.45, 74.36 and 65.35, % respectively. Diethyl ether extract also showed higher inflammatory inhibition (67 %) than standard (54 %). As predicted, these extracts were less effective against pain and acute inflammation at 800 mg/kg. The HPLC profile demonstrated the existence of phenolic acids, flavones and C-glycosides.*
Conclusion: *The findings show that P. incisa has promising hypoglycemic, analgesic and anti-inflammatory effects, which confirms its folkloric medicinal use in Algeria.*

Keywords: *Acute toxicity, Biological activities, Glucose level, HPLC, Pulicaria incisa*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Diabetes, inflammation and pain are three major health problems that face the world's population. However, available treatments including non-steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs or hypoglycemic drugs have benefits, but also could cause adverse effects like liver damage, allergic and respiratory problems [1]. Thus, research geared towards innovative medication is crucial and important.

Herbal medicines are frequently used and trusted by the population. So, this alternative medicine might be a good source to find a harmless and powerful new medication with no side effects.

Algeria has an important source of medicinal plants due to its great biodiversity. In the south of the country, local populations particularly the Touareg still rely on traditional healers to treat different ailments [2]. *Pulicaria incisa* (Lam.) DC. belongs to the Asteraceae family. It is popularly known in Algeria as “Ameyou” or “neougde”. This Saharo-Sindian herb is an annually grown plant characterized by erect stems and sessile leaves. In Algerian traditional medicine, it is utilized for cold, neuralgia, respiratory problems, sinusitis and inflammation, and some consider its infusion good for diabetes [3]. *Pulicaria incisa* is reported to have many anti-spasmodic and antioxidant properties, and this plant appears to be helpful for treating hypocholesterolemic and cardiovascular diseases. Phytochemical analysis indicates that alkaloids, flavonoids, chalcones (pulichalconoid B and pulichalconoid C), tannins, ellagic acids, saponins, sterols, terpenes, cardiac glycosides, essential oils and fatty acids were all present [4].

This study aims to evaluate the acute toxicity by determining the medium lethal dose (LD_{50}), as well as to examine the hypoglycemic, analgesic and anti-inflammatory activities of *P. incisa*. Analysis with HPLC was carried out to find out the principal compounds responsible for these properties.

EXPERIMENTAL

Plant material and collection

Pulicaria incisa (Lam.) DC aerial parts were collected in March 2012 from the Izerzzi area, which is located in the Hoggar region (Algerian Sahara). The plant was identified by following the morphological description of Quezel and Santa [5], and authenticated by botanists of the research station on the protection of the arid regions of Tamanrasset. A voucher specimen (N°2-2012/Tam; PAM/LRZA/USTHB) of this plant was retained in the Laboratory of Research on Arid Zones (LRZA), Algiers, Algeria.

Animals

Healthy *Wistar* rats weighing 160 - 177 g and *albino* mice weighing 20 ± 2 g provided by the Research and Development Center of Saidal (CRD-Saidal, Algeria) were utilized in the various experiments. The animals were housed in laboratory setting to acclimatize to a 20 ± 5 °C temperature, a humidity of 75 % and with 12 h light/dark cycles. Animals were given unrestricted access to drink water and feed. All experimental procedures involving laboratory animals were approved by the University Animal Experimentation Ethics Committee (approval ref 162/2011/8), and following the guidelines prescribed in Guide for the Care and Use of Laboratory Animals.

Standards and reagents

Hydrochloric acid (HCl), diethyl ether, methanol, n-butanol and acetic acid were obtained from Sigma Aldrich (Steinheim, Germany). Acetylsalicylic acid (ASA), diclofenac sodium, carrageenan, metformin, glucose and saline water (NaCl, 0.9 %) were purchased from Saidal (Algeria). The phenolic compounds used as standards were obtained from Sigma Aldrich (Steinheim, Germany), Fluka Chemie, or Gmbh (Buchs, Switzerland). Analytical grade chemicals and reagents were used throughout this experiment.

Infusion preparation

The plant samples were left to dry at room temperature (24 ± 2 °C) and an electric blender was used to grind the samples to fine powder. *P. incisa* infusion (IPI) was prepared by dissolving the fine powder of this plant in hot water. The filtrate was used for acute toxicity tests on animals at different doses.

Extraction method

Several extraction methods were carried out to release the maximum polyphenols from *P. incisa* aerial parts. Flavonoid aglycones and glycosides extraction was executed following the method of Saad *et al* [6]. One gram of *P. incisa* powder was mixed with 80 mL hydrochloric acid (2 N) for 40 min at 40 °C. Then, diethyl ether was added to liberate phenol acids and flavones-flavonols (Di-EthEx). Out of the mixture left, anthocyanidins and C-glycosides were extracted by n-butanol solvent (n-ButEx).

Free aglycones extraction was evaluated using the method of Saad *et al* [6]. This extraction was prepared using cold maceration of 1 g of *P. incisa* powder in 100 mL of di-ethyl ether for 30 min (FAgEx). All the extracts Di-EthEx, n-ButEx and FAgEx were then dried with evaporation and kept at -4 °C for subsequent analysis.

Acute toxicity test

Acute oral toxicity of the IPI was evaluated in line with the Organization for Economic Co-operation and Development (OECD-423) guidelines, adopted on 17th December, 2001 with moderate modifications. Sixty mice were divided into five treated groups and one control group (5 males

and 5 females for each group). Different doses of IPI (2, 4, 8, 12 and 14 g/kg) were orally administered, while mice in the control group were administered with normal saline (0.9 %). Animals were placed under observation for 4 h for any indication of toxicity, then once daily for 14 days to record mortality.

Glucose tolerance test

To determine the effects of the aerial parts of *P. incisa* (Lam.) DC and the infusion extract on blood glucose level, the OGTT was assessed following the protocol of Adedapo *et al* [7]. First, healthy and normal rats had free access to water only, and were randomly shared into four groups with six rats each. Then, these animals were orally administered with 0.9 % saline (as a control), metformin (hypoglycemic standard drug at 500 mg/kg) and IPI at doses of 400 and 800 mg/kg, respectively. Thirty minutes after the administration of these treatments, rats were orally gavaged with 40 % glucose (4 g/kg). Blood glucose levels were monitored before treatments and glucose administration (fasting glucose blood which represents the baseline of glucose level), and at 30, 60, 90 and 120 min after glucose loading. Blood samples were retrieved from the tip of the tail of all rats using the test strip of the blood glucometer (Accu Check Active, Roche, Germany).

Anti-inflammatory activity

In order to determine the anti-inflammatory activity of *P. incisa*, a carrageenan-induced paw oedema experiment was assessed as described by Saad *et al* [8]. Mice were appropriated into 8 groups, each containing 6 mice. Groups 1 and 2 were gavaged with saline (0.9 %) and diclofenac (5 mg/kg) respectively. Groups 3 and 4: Di-EthEx of *P. incisa* at doses of 400 and 800 mg/kg, respectively; Groups 5 and 6: n-ButEx of *P. incisa* at 400 and 800 mg/kg, respectively; Groups 7 and 8: FAgEx of *P. incisa* at 400 and 800 mg/kg, respectively. The three extracts of *P. incisa* and diclofenac were dissolved in 0.9 % NaCl and administered using oral route. Then, these animals were subjected to sub-plantar injection of 0.05 mL of carrageenan suspension (1 % in NaCl 0.9 %) into the left hind paw to produce an acute inflammation 1 h after the administration of all solutions. An upsurge in the weight of the left hind paw was accepted as a symptom of paw oedema. In this test, the paw weight was measured 3 h after the carrageenan solution was injected into the paw. Edema (E) was determined as shown in Eq 1.

E (%) = {(M(LPW) - M(RPW)/M(RPW)}100 .. .(1)

where M(LPW) is the mean weight of the left paw per group and M(RPW) is the mean weight of the right paw per group. The inhibition of the oedema between the treated and control groups was considered as an indicator of anti-inflammatory response, and it was measured following the formula in Eq 2.

$$\% \text{ inhibition} = \frac{OC-OT}{OC} \times 100 \quad \ldots\ldots (2)$$

where OC if the oedema percentage of control group, and OT is the oedema percentage of test group.

Analgesic activity

Analgesic activity was conducted using the acetic acid-induced writhing method in mice following the method of Koster *et al* [9]. Eight groups of animals (6 mice for each) were constituted and were orally treated by diethyl ether (Di-EthEx), n-butanol (n-ButEx) or by free aglycone (FAgEx) *P. incisa* extracts at doses of 400 and 800 mg/kg for each. The control and reference groups received saline (0.9 %) and acetylsalicylic acid (ASA), 200 mg/kg, respectively. Thirty minutes after the oral administration of extracts, glacial acetic acid (0.6 %, 10 mL/kg) was injected intraperitoneally to induce pain that manifests in abdominal constrictions or writhes. Five minutes after the intraperitoneal administration of the acetic acid solution, the animals were kept in clear plastic cages, and then the number of writhes was counted for 10 min, and a reduction in the number of writhes was taken as an analgesic response. The writhing inhibition (H) percentage was calculated from Eq 3.

H (%) = {(1-Wt/Wc)}100 …… (3)

where Wc is the average writhing response of the control group and Wt is the average writhing response of the treated group.

High-performance liquid chromatography (HPLC) analysis

Phenolic compounds in *P. incisa* extracts were identified using HPLC system (Agilent series 1100), coupled with UV Detector and bars of Diodes Surveyor (DAD), equipped with a quaternary rapid separation pump and Hypersil BDS-C18 column (250 × 4.6 mm, 5 µm). The column temperature was fixed at 30 °C. For each extract, 20 µL was injected and the flow rate was 1 mL/min. The samples were analyzed using solvent system B (acetonitrile) in solvent system A (0.2 % of acetic acid in water) in a linear gradient for 30 min. The mobile phase started with 95 % of acetic acid and ended with 100 %

acetonitrile. Phenolic acids were detected at 260 nm, while flavonoid compounds were ditected at 365 nm.

Statistical analysis

The statistical analysis was carried out using the Statistica software 6.0 (StatSoft, TIBCO Software Dell). The data are presented as mean ± SD. Significance between control and treated groups was tested by one-way ANOVA followed by Tukey's t-test. The $P < 0.05$ level was considered to be significant.

RESULTS

Acute oral toxicity

The behaviour of each mouse was observed daily for all 14 days of the experiment. Any changes in their general behaviour were recorded as an indicator of toxicity. Results from the acute toxicity study showed that the oral administration of the IPI given in a single dose up to 14 g/kg to mice did not make any changes in behaviour and body weight. There was no mortality recorded in any of the groups, even at the dose of 14 g/kg in both sexes of mice throughout the 14-day observation period.

Oral glucose tolerance

Blood glucose levels of control, standard and IPI estimated at different times (0, 30, 60, 90 and 120 min) are shown in Figure 1. The results showed that the blood glucose levels were increased in all groups when compared to basal glucose levels, 30 min after the administration of a high dose of glucose (4 g/kg). However, this increase in the blood glucose level is gradually corrected and suppressed for 2 h in rats treated with IPI at different doses (400 and 800 mg/kg), compared with those of the control group ($p < 0.05$). In addition, metformin (500 mg/kg) produced a significant ($p < 0.05$) reduction in the level of blood glucose after oral glucose administration. At the end of the experiment, the blood glucose level of rats treated with the infusion extract of the studied plant and metformin remained significantly below the basal levels when compared to control rats.

Anti-inflammatory activity

Anti-inflammatory activity results of *P. incisa* are shown in Table 1 and Figure 2. These results revealed that the injection of carrageenan generated a gradual increase in paw oedema of mice in the control group by 52 % after 3 h. Administration of diclofenac as standard treatment (5 mg/kg) significantly inhibits carrageenan-induced acute inflammation by 54 % at 3 h following the carrageenan injection. As shown in Figure 2, the administration of *P. incisa* extracts inhibited inflammatory response induced by carrageenan in mice in a dose-related manner. However, Di-EthEx, n-ButEx and FAgEx at the dose of 400 mg/kg were more efficient against acute inflammation than at the dose of 800 mg/kg in comparison with control. Moreover, Di-EthEx at the dose of 400 mg/kg presented the highest inhibition value by 67 %, whereas the lowest value of inhibition was recorded with FAgEx at doses 400 and 800 mg/kg by 19 and 4 %, respectively.

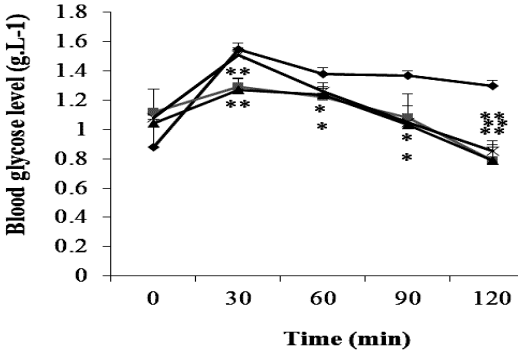

Figure 1: Effect of oral administration of the infusion extract of *Pulicaria incisa* on blood glucose level in normal rats during OGTT. Values presented are expressed as Mean ± SD, n = 6, IPI: infusion of *Pulicaria incisa*. (-♦-) Control 10 mL/kg; (-■-) metformin 500 mg/kg; (-▲-) IPI 400 mg/kg; (✕) IPI 800 mg/kg

Analgesic activity

Results of the analgesic study are presented in Table 2, indicating the number of writhes (cramps) and the inhibition percentage due to the acetic acid administration recorded for 10 min. The animals in the control group that did not receive any treatment presented the highest number of cramps (72.16 ± 3.6), expressing the pain caused by the acetic acid induction. The oral administrations of Di-EthEx, n-ButEx and FAgEx of *P. incisa* significantly ($P < 0.001$) inhibited the number of writhes generated using acetic acid by 82.45, 74.36 and 65.35 % respectively at 400 mg/kg. Whereas at 800 mg/kg, these extracts showed lower efficiency in inhibiting writhes with 61.89, 63.51 and 43.65 % respectively. The ASA at 200 mg/kg decreased significantly ($P < 0.001$) the number of writhes at 81.51 %.

Table 1: Oedema percentage on the carrageenan-induced paw oedema in mice

Group	Dose (mg/kg)	M(LPW)	M(RPW)	Edema (%)
Control	-	0.2014 ± 0.03	0.1322 ± 0.01	52
Diclofenac	5	0.1630 ± 0.01***	0.1311 ± 0.008^{ns}	24
Di-EthEx	400	0.1614 ± 0.01***	0.1380 ± 0.009^{ns}	17
	800	0.1726 ± 0.01*	0.1277 ± 0.01^{ns}	35
n-ButEx	400	0.173 ± 0.02^{ns}	0.1307 ± 0.01^{ns}	33
	800	0.1917 ± 0.02$^{\$}$	0.1301 ± 0.01^{ns}	10
FAgEx	400	0.1776 ± 0.01^{ns}	0.1251 ± 0.01^{ns}	42
	800	0.2015 ± 0.01$^{\$}$	0.1344 ± 0.006^{ns}	50

Values are presented as mean ± SD, n = 6. M(LPW) is the mean weight of the left paw and M(RPW) is the mean weight of the right paw. ns: not significant, *p < 0.05, ***p < 0.001: as compared to control was considered significant, $P: significant compared to diclofenac

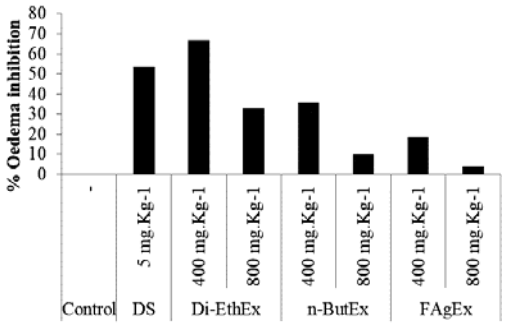

Figure 2: Inhibitory effect of diclofenac sodium and *Pulicaria incisa* extracts on carrageenan-induced paw oedema. DS: Diclofenac sodium, Di-EthEx: diethyl ether extract, n-ButEx: n-butanol extract, FAgEx: free aglycone extract

Table 2: Analgesic activity of *Pulicaria incisa* polyphenolic extracts against acetic acid-induced writhing test in mice

Group	Dose (mg/kg)	Number of writhes	Inhibition (%)
Control	-	72.16 ± 3.6	0
ASA	200	13.3 ± 1.63***	81.52
Di-EthEx	400	12.66 ± 1.36***	82.45
	800	27.5 ± 1.04***	61.89
n-ButEx	400	18.5 ± 3.27***	74.36
	800	26.33 ± 3.44***	63.51
FAgEx	400	26.5 ± 3.08***	65.35
	800	49.66 ± 3.98***	43.65

Values are expressed as mean ± SD, n = 6, *** p < 0.001: significant compared to the control (one-way ANOVA followed by Tukey's test), ASA: acetylsalicylic acid, Di-EthEx: diethyl ether extract, n-ButEx: n-butanol extract, FAgEx: free aglycone extract

HPLC results

The chromatograms of Di-EthEx and n-ButEx are shown in Figure 3A, Figure 3B and Figure 4, respectively. The Di-EthEx HPLC chromatogram at 260 nm revealed the presence of six phenolic acids; cafeic, anisic, 3,4,5 trimethoxy benzoic, trans-cinnamic, trans-dimethoxycinnamic and protocatechuic acids. In addition, two flavonols (quercetin and isorhamnetin), two flavones (luteolin and apigenin) and vanillin were determined at 365 nm. Furthermore, the results indicated that n-ButEx showed the presence of three C-glycosides namely naringenin-6-C-glucoside, naringenin-di-C-glucoside and apigenin-8-C-glucoside.

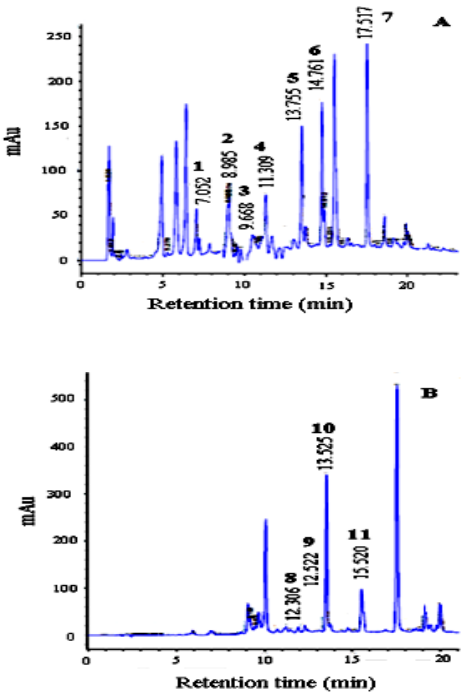

Figure 3: HPLC chromatogram of diethyl ether extract of *Pulicaria incisa*. (A) HPLC profile of phenolic acids at 260 nm; (B) HPLC profile of flavonoids at 365 nm (***Peaks***: 1: cafeic acid, 2: vanillin, 3: anisic acid, 4: 3,4,5 trimethoxy benzoic acid, 5: trans-cinnamic acid, 6: trans-dimethoxycinnamic acid, 7: protocatechuic acid, 8: luteolin, 9: quercetin, 10: apigenin, 11: isorhamnetin)

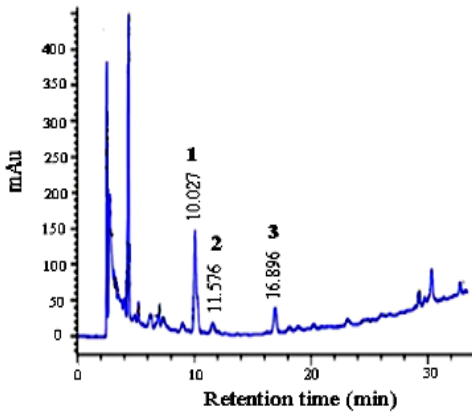

Figure 4: HPLC chromatogram of n-butanol extract of *Pulicaria incisa* aerial parts at 365 nm (**Peaks**; 1: naringenin-6-C-glucoside, 2: naringenin-di-C-glucoside, 3: apigenin-8-C-glucoside)

DISCUSSION

The use of herbal medicine has increased worldwide. Although these remedies have revealed a promising efficacy, many of them are still untested and their use need to be clarified. Moreover, the safety of these therapies is a major issue that requires recognition and comprehension in order to protect public health [10]. Therefore, acute toxicity studies were assessed to identify the dose level that causes mortality. This test consists of a single administration of the test substances to healthy animals by oral route, and observing them for up to 14 days. Data obtained from this study showed that the infusion of *P. incisa* has no toxic effects in both sexes of mice at all tested doses. Moreover, the recording of mortality which was the main criterion in assessing this toxicity showed no death during the 14 days of the experiment. Thus, LD_{50} values of the orally administered infusion was greater than 14 g/kg. According to the classification of Hodge and Sterner [11], *P. incisa* is safe and can be used for efficacy studies. These findings justify the use of *P. incisa* by the local Touareg population of Tamanrasset, and also explains why this plant is frequently used as a decoction by the Bedouins of North Sinai (Egypt) and as tea in Saudi Arabia.

To determine pre-diabetes and diabetes, an oral glucose tolerance test (OGTT) is generally used. The experimental evaluation of the hypoglycemic effect of *P. incisa* using OGTT has shown that the single oral administration of IPI at two different doses to normal rats suppressed the postprandial increase in blood glucose level following oral administration of a heavy glucose dose. Taking into consideration the increasing concern in developing novel, effective and safe anti-inflammatory and analgesic remedies as alternatives to NSAIDs, an attempt has also been made to authenticate the use of *P. incisa* as anti-inflammatory and analgesic agents. Carrageenan-induced paw oedema is a common model that is generally used for studying anti-inflammatory effects. In this test, carrageenan was injected to generate experimental arthritis. However, this injection is non-antigenic, and produces no systemic side effects [12]. In the third hour after carrageenan injection, the results revealed that oral administration of *P. incisa* extracts significantly inhibited the oedema formation, which suggests that these extracts exhibited anti-inflammatory effects on acute inflammation. Similarly, diclofenac, used as a standard drug, significantly inhibited the carrageenan-induced oedema. According to some studies, the response to carrageenan-induced oedema is biphasic, where histamine, serotonin and kinins are released in the first phase; while prostaglandins and lysosomal enzymes are liberated in the second phase [13].

The analgesic effect of the studied plant was ascertained using the acetic acid-induced writhing test in mice. The acetic acid injection is widely performed to induce peripheral pain in mice. The reduction in the frequency of writhings is considered an indication of analgesic activity of any compound [14]. The tested extracts of *P. incisa* demonstrated high analgesic activity by decreasing or inhibiting writhing in mice induced by acetic acid. Di-EthEx of *P. incisa* at the dose of 400 mg/kg was the most potent as an anti-inflammatory and analgesic even more than the standard drugs, diclofenac and ASA. The data revealed that the extracts of *P. incisa* was more potent as an anti-inflammatory and analgesic at low dose (400 mg/kg) than at high dose (800 mg/kg). Thus, the inhibitory effects of *P. incisa* extracts against inflammation and pain was inversely dose-dependent. Furthermore, it is known that as with all NSAIDs, diclofenac and ASA aids the suppression of inflammation and pain via the inhibition of prostaglandin synthesis, and also the inhibition of cyclooxygenases in arachidonic acid pathways [15]. It can therefore be suggested that the possible mechanism by which *P. incisa* extracts relieves pain and inflammation is by the inhibition of cyclooxygenase enzyme COX.

The effects of *P. incisa* extracts can be explained by the existence of compounds or agents with hypoglycemic, peripheral analgesic and anti-inflammatory potentials. Thus, in an attempt to identify these phytochemical compounds, HPLC analysis was performed. The results revealed that *P. incisa* aerial parts contain flavone

aglycones (luteolin and apigenin), flavonol aglycones (quercetin and isorhamnetin), flavone glycosides (naringenin-6-C-glucoside, naringenin-di-C-glucoside and apigenin-8-C-glucoside) and phenolic acids (3,4,5 trimethoxybenzoic, protocatechuic, trans-cinnamic, trans-dimethoxycinnamic, caffeic and anisic acids).

Previous studies have revealed that phenolic acids and flavonoids are responsible for a wide variety of pharmacological activities. The hypoglycemic, analgesic and anti-inflammatory effects of *P. incisa* extracts could be correlated with its richness in polyphenolic constituents especially flavonoids and phenolic acids. Indeed, many studies reported that flavonoids have antidiabetic properties because they stimulate glucose uptake in peripheral tissues [16]. The antinociceptive and the anti-inflammatory effect of these compounds are also reported by many researchers.

According to Sharma *et al* [17], quercitrin significantly decreased the number of writhes in mice. Moreover, naringenin is a strong anti-inflammatory drug that aids effective pain relief, and can be utilized for pain management therapy. Furthermore, 3,4,5-trimethoxybenzoic acid, quercetin and apigenin are known to be powerful inhibitors of the production of pro-inflammatory mediators of pain; cytokines and cyclooxygenases. In a study by Elmann *et al* [18], the inflammatory effect of the infusion of *P. incisa* in microglial cells reported the existence of chlorogenic and ferulic acids, which are known to be relevant to neurodegenerative diseases.

CONCLUSION

The findings reveal that the extracts of *P. incisa* are non-toxic to mice and promote glucose tolerance. The oral administration of *P. incisa* extracts has promising anti-inflammatory and analgesic activities. Flavone aglycones and flavone glycosides as flavonoids of *P. incisa* aerial parts are responsible for these activities. Thus, these findings confirm its use in traditional Saharan medicine to treat pain by the local population of Touareg. The method of isolation of the identified constituents, as well as the mechanisms of action responsible for these effects, are yet to be investigated.

DECLARATIONS

Acknowledgement

This study was supported by the General Direction of Scientific Research and Technological Development (DGRSDT) with the Ministry of Higher Education and Scientific Research. The authors would like to thank greatly Mrs Zhor BETTACH from the Central Laboratory of Scientific Police, Algiers-Algeria for her help in HPLC analysis.

Conflict of interest

No conflict of interest is associated with this work

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Conceptualization: Djamila Sahar; Visualization and Supervision: Saida Ouafi; Methodology and formal analysis: Djamila Sahar and Somia Saad. All authors read and approved the final manuscript.

Open Access

REFERENCES

1. *Perini R, Fiorucci S, Wallace JL. Mechanisms of nonsteroidal anti-inflammatory drug-induced gastrointestinal injury and repair: A window of opportunity for cyclooxygenase-inhibiting nitric oxide donors. Can J Gastroenterol 2004; 18(4): 229-236. https://doi.org/10.1155/2004/890585*
2. *Benarba B. Medicinal plants used by traditional healers from south-west Algeria: An ethnobotanical study. J Intercult Ethnopharmacol 2016; 5(4): 320. https://doi.org/10.5455/jice.20160814115725*
3. *Benchelah A, Bouziane H, Maka M. Fleurs du Sahara, arbres et arbustes, voyage au coeur de leurs usages avec les Touaregs du Tassili. Phytotherapie 2004; 2(6): 191-197. https://doi.org/10.1007/s10298-004-0052-z*
4. *Chaib F, Allali H, Bennaceur M, Flamini G. Chemical composition and antimicrobial activity of essential oils from the aerial parts of Asteriscus graveolens (Forssk.) less. and Pulicaria incisa: two Asteraceae herbs growing wild in the Hoggar. Chem Biodivers 2017; 14(8): e1700092. https://doi.org/10.1002/cbdv.201700092*

5. Quezel P, Santa S. Nouvelle flore de l'Algérie et des régions désertiques méridionales. CNRS (Tome II), Paris 1963. 565 p.
6. Saad S, Ouafi S, Meguellati H, Djemouai N. Chemical diversity analysis and biological activities evaluation of different polyphenolic extracts of Marrubium deserti De Noé from Algeria. J Ethnopharmacol 2021; 281(114494): 1-15. https://doi.org/10.1016/j.jep.2021.114494.
7. Adedapo A, Ofuegbe S, Adeyemi A. The anti-diabetic activities of the methanol leaf extract of Phyllanthus amarus in some laboratory animals. Asian J Med Sci 2013; 4(3): 23-34. https://doi.org/10.3126/ajms.v4i3.6992.
8. Saad S, Ouafi S, Chabane D. Anti-inflammatory and acute toxicity evaluation of aqueous infusion extract obtained from aerial parts of Marrubium deserti de Noé growing in Algeria. Afr J Tradit Complement Med 2016; 13(1): 71-75. https://doi.org/10.4314/ajtcam.v13i1.10.
9. Koster R, Anderson M, De Beer EJ. Acetic acid for analgesic screening. Fed Proce 1959; 18: 412-415.
10. Roy S, Ukil B, Lyndem L. Acute and sub-acute toxicity studies on the effect of Senna alata in Swiss albino mice. Cogent Biol 2016; 2(1): 1-12. https://doi.org/10.1080/23312025.2016.1272166.
11. Hodge HC, Sterner JH. Determination of substances acute toxicity by DL50. Am Ind Hyg Assoc 1943; 10: 93-98.
12. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962; 111: 544-547. https://doi.org/10.3181/00379727-111-27849.
13. Morris CJ. Carrageenan-induced paw edema in the rat and mouse. Methods Mol Biol 2003; 225: 115-121. https://doi.org/10.1385/1-59259-374-7:115.
14. Gawade S. Acetic acid induced painful endogenous infliction in writhing test on mice. J Pharmacol Pharmacother 2012; 3(4): 348. https://doi.org/10.4103/0976-500x.103699.
15. Gan TJ. Diclofenac: An update on its mechanism of action and safety profile. Curr Med Res Opin 2010; 26(7): 1715-1731. https://doi.org/10.1185/03007995.2010.486301.
16. Granados S, Balcázar N, Guillén A, Echeverri F. Evaluation of the hypoglycemic effects of flavonoids and extracts from Jatropha gossypifolia L. Molecules 2015; 20(4): 6181-6193. https://doi.org/10.3390/molecules20046181.
17. Sharma BR, Park CM, Choi JW, Rhyu DY. Anti-nociceptive and anti-inflammatory effects of the methanolic extract of Opuntia humifusa stem. Avicenna J Phytomed 2017; 7(4): 366-375. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5580874/
18. Elmann A, Beit-Yannai A, Telerman E, Ofir R, Mordechay S, Erlank H, Borochov-Neori H. Pulicaria incisa infusion attenuates inflammatory responses of brain microglial cells. J Funct Foods 2016; 25: 110-122. https://doi.org/10.1016/j.jff.2016.05.012.