Horticulture and Forestry Society of Transylvania

Layachi R *et al.* (2023)
Notulae Scientia Biologicae
Volume 15, Issue 1, Article number 11351
DOI:10.15835/nsb15111351
Research Article

Notulae Scientia Biologicae

Assessment of analgesic properties of alcohol and aqueous extracts of *Opuntia ficus-indica* flowers

Rajaa LAYACHI[1a*], Aziz MOUHADDACH[1b], Lamiae AMALLAH[1], Mohammed BENAZIZ[2], Fatima BOUAZZA[1], Souad BENAICH[3], Rachida HASSIKOU[1], Souad SKALLI[1]

[1]*Mohammed V University in Rabat, Faculty of Sciences, Plant and Microbial Biotechnologies, Biodiversity, and Environment Center, Morocco; r.layachi@um5r.ac.ma (*corresponding author); mouhaddach@gmail.com; l.amallah@hotmail.com; fatima.leader2020@gmail.com; r.hassikou@um5r.ac.ma; s.skalli@um5r.ac.ma*

[2]*Sultan Moulay Slimane University, Higher School of Technology, PB170, 54000, Khenifra, Morocco; m.benaziz@usms.ma*

[3]*Mohammed V University in Rabat, Faculty of Sciences, Physiology and Physiopathology Team, Morocco; s.benaich@um5r.ac.ma*

[a,b]*These authors contributed equally to the work*

Abstract

The cactus (*Opuntia ficus-indica* (L.) Mill.) belongs to the Cactaceae family and it's used in traditional folk medicine in treating a number of diseases and conditions. Due to the remarkable biological activity and to the bioactive (phytochemicals) compounds of *O. ficus-indica*, it becomes the aim of many research studies. The current study aimed to evaluate the analgesic activity of various solvent fractions (aqueous and ethanol) prepared from the *O. ficus-indica* flowers. The centrally analgesic potential was evaluated using tail flick latency in tail immersion and hot plate methods in mice. Morphine was used as a positive control at a dose of 3 mg kg^{-1}, s.c.. Intra-peritoneal administration of the aqueous extract of *O. ficus-indica* flowers at the highest dose did not produce any toxicity symptoms, thus the median lethal dose (LD50) was estimated to be greater than 2,500 mg kg^{-1}. The results of the pain behavior evaluation according to the gender approach of mice showed that the pain tolerance threshold is high in males compared to females. We found that various plant extracts at doses of 300, 500, and 1,000 mg kg^{-1} i.p., displayed significant and dose-dependent protective effects ($p < 0.01$, $p < 0.001$, and $p < 0.0001$) as measured by increased latency time compared to vehicle control. The maximum anti-nociceptive effect was with the ethanol extract (71%) at 60 minutes at a dose of 1,000 mg kg^{-1}, which was equivalent to the effect of morphine (70%). The results suggested that *O. ficus-indica* might possess significant analgesic effects, supporting the use of this plant in traditional medicine.

Keywords: centrally analgesic; flowers; *Opuntia ficus-indica*; morphine; traditional medicine

Introduction

Pain is one of the more complex pathological phenomena, this process involving the immune system, neurobiological processes and humoral systems (Smith *et al.*, 1943). In the normal state, prostaglandins and

Received: 16 Sep 2022. Received in revised form: 28 Jan 2023. Accepted: 07 Mar 2023. Published online: 16 Mar 2023.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

other molecules participate in the genesis and remediation of this anomaly (Sébastien, 2010). The current treatment for pain involves anti-inflammatory medications. Due to their undesirable side effects, which limit their uses, there is more interest in natural compounds extracted from medicinal plants. These plants have analgesic and antipyretic activity and could constitute an alternative therapy because of their accessibility and low toxicity (Queneau and Ostermann, 2004; Bussmann, 2011).

The bibliographic survey reports that *Opuntia ficus-indica* (L.) Mill. (The plant name has been checked with *http://www.theplantlist.org*) is an important source of therapeutic agents, due to their pharmacological properties and their richness in bioactive molecules. *O. ficus-indica* has long been used in traditional medicine in various countries (Park *et al*., 2001; Mouhaddach *et al*., 2017). The data of the ethnobotanical survey carried out by Mouhaddach *et al.* (2017) showed that the various parts of the *O. ficus-indica* plant can be used for the treatment of various health conditions including gastric disorders, kidney pain, back pain, and leg pain. Cladodes (57%) were the most used part of the plant, followed by flowers (21%), seeds (11%) and roots (5%). Several parts of the plant have been used to relieve pain, especially cladodes and flowers.

The *O. ficus-indica* flowers exert a wide range of pharmacological activities, including antioxidant, antimicrobial (Ammar *et al.,* 2012), anti-ulcerogenic and healing activities (Ammar *et al*., 2015; Mouhaddach et *al*., 2017), as well as anti-inflammatory (Ahmed *et al*., 2005; Zou *et al*., 2005). *O. ficus-indica* has traditionally been used to reduce inflammation and previous studies have demonstrated its anti-inflammatory potential (Shirazinia *et al.*, 2019). More recently, the anti-inflammatory and the analgesic effects of cladodes methanol extract have also been investigated by Siddiqui *et al.* (2016) and Mouhaddach *et al.* (2017). Within the available literature, few studies have focused on flowers compared to other parts of the plant (cladodes, fruits, and seeds).

This study is the first report on analgesic effect of aqueous and alcoholic extracts of *O. ficus-indica* flowers via tail flick and hot plate tests.

Materials and Methods

Plant material

O. ficus-indica flowers, spineless variety, were collected from the Rhamna region (Morocco) during the month of June 2015. Then they were dried for one week (45 °C) and ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of aqueous and organic extracts

The flower powder (50 g) mixed with distilled water (500 ml) was decocted at 100 °C for 15 minutes. Then the mixture was filtered, lyophilized (using a PHYWE chrisa lyophilizer) and stored at 4 °C. At the time of its administration the extracts were dissolved in 0.9% saline (NaCl). The organic flower extract was prepared via the Soxhlet method using ethanol as the extraction solvent.

Animals

Adult male and female Swiss mice (20-28 g, 2 months of age) obtained from the animal house, physiology and physiopathology laboratory, Faculty of Sciences, University Mohammed V, Rabat, Morocco, were used throughout these experiments. They were housed in cages at room temperature and 12:12 h light/dark cycle. The animals were provided with pelleted diet and tap water *ad libitum*. All experiments were carried out according to strict adherence to ethical guidelines (Zimmerman, 1983).

Analgesic activity method

Hot plate method

Mice of both genders were selected and divided into five groups designated as group-I, group-II, group-III, group-IV and group-V, consisting of eight mice (4♀ and 4♂) in each group for control, positive control and test sample group respectively. Each group received a particular treatment, control group (0.9% NaCl solution, i.p.), positive control (Morphine 3 mg kg^{-1}, subcutaneous, s.c.) and the test sample (ethanol and aqueous extracts of 300 mg kg^{-1}, i.p., 500 mg kg^{-1}, i.p., and 1,000 mg kg^{-1} i.p.). The animals were positioned on Eddy's hot plate kept at a temperature of 53±0.2 °C. A cut off period of 15 seconds was observed to avoid damage to the paw (Franzotti *et al*., 2000). The latency (reaction) time was recorded when the mice licked their paws or jumped at 0, 30, 60, 90 and 120 minutes after intraperitoneal administration (i.p.) of the samples (Eddy and Leimback, 1953; Toma *et al*., 2003).

Tail flick method

Central analgesic activity was assessed according to the method described by Di Stasi *et al.* (1988) Mice in groups of eight (4♀ and 4♂) were held in position in a suitable restrainer with the tail extended. A 2-3 cm area of the tail was marked and immersed in a water bath thermo-statistically maintained at 53±0.2 °C. The withdrawal time of the tail from the hot water (in seconds) was noted at 0, 30, 60, 90, and 120 minutes as the reaction time or tail flick latency. The maximum cut-off time for immersion was 15 seconds to avoid tail injury. Control mice were treated with vehicle (0.9% NaCl solution, i.p.), while the reference group was given morphine subcutaneously (s.c.) (3 mg kg^{-1}, s.c.). Flowers extracts at doses of 300, 500, and 1,000 mg kg^{-1} were administered by intra-peritoneal injection.

Statistical analysis

All values are expressed as mean ± S.E.M. (standard error of mean). Data were analyzed by one-way ANOVA (analysis of variance) followed by Tukey's multiple comparison tests. A level of $p < 0.05$ was considered statistically significant.

Results

Acute toxicity of the aqueous extract

The mice showed no change in general physical appearance and somatomotor skills during the observation period. No manifestations of tremors, convulsions, salivation, diarrhea, coma, or behaviors were observed. However, manifestations of sleep were observed in treated mice during the first 30 minutes after gavage. No other treatment-related changes or mortality were observed in mice over the 24-hour period following oral administration of a single dose of *O. ficus-indica* flower decoction at a dose of 2,000 mg kg^{-1}. The median lethal dose (LD50) was therefore estimated to be greater than 2,500 mg kg^{-1}.

Analgesic effect

Tail Flick Test

Tables 1 and 2 represent the results obtained via the tail immersion test to test the analgesic effect of aqueous and organic extracts of *O. ficus-indica* flowers.

The results showed that the extracts at doses of 300, 500 and 1,000 mg kg^{-1}, i.p. induce significant analgesic activity, demonstrated by an increase in latency time in seconds (Table 1) compared to the control.

The tail withdrawal latency time of control mice was 2.21±0.43 s. At 30 minutes after administration of the extracts, we found that the latency times of the mice of the batches receiving the two aqueous and ethanol

extracts at doses of 300, 500 and 1,000 mg kg^{-1}, i.p. as well as that of the mice of the batch receiving morphine at 3 mg kg^{-1}, s.c., were significantly higher ($p < 0.001$) compared to the control group.

The latency time of the batch that received the extract dosed at 1,000 mg kg^{-1}, i.p., was significantly higher ($p < 0.05$) than that of the batch that received the extract at 500 mg kg^{-1}, i.p. Similarly, the batch which received the extract at a dose of 500 mg kg^{-1}, i.p., turns out to have a significantly higher latency time ($p < 0.05$) than that of the batch which received the extract at 300 mg kg^{-1}, i.p..

As for the percentage of pain inhibition, it was noted that the percentage of pain inhibition after 60 minutes with morphine at a dose of 3 mg kg^{-1} was 70%. This value was reached at a dose of 1,000 mg kg^{-1} of the aqueous extract. Also, the pain inhibition percentage of the ethanol extract after 60 minutes was slightly higher at 71%.

Table 1. Analgesic effect of aqueous and organic extracts of *O. ficus-indica* flower, latency in tail flick test in mice

Treatments	Doses mg kg^{-1}	Reaction time ± S.E.M.				
		0 min	30 min	60 min	90 min	120 min
Vehicle	0.2	2.21 ± 0.43	2.56 ± 0.54	2.06±0.14	2.56±0.61	2.74±0.33
Morphine	3	2.78 ± 0.78	8.6 ± 0.95***	9.38±1.55***	9.03±1.23***	6.29±0.89**
Ethanol extract	300	3.21 ± 0.54	4.55 ± 0.11*a	6.93±0.69**c	4.53±0.39*c	3.77±0.34c
	500	3.06 ± 0.43	7.04 ± 0.88**b	8.37±0.73***b	6.98±0.35**c	4.74±0.37b
	1,000	2.32 ± 0.47	7.20 ± 0.87***c	8.12±0.97***a	7.10±0.85***c	4.75±0.54b
Aqueous extract	300	2.04 ± 0.22	3.85 ± 0.12*c	5.39±0.15***c	4.90±0.21**c	3.93±0.21b
	500	2.73 ± 0.59	5.63 ± 0.23**b	8.22±0.54***b	6.51±0.30**c	5.51±0.17b
	1,000	2.71 ± 0.61	5.57 ± 0.15**b	8.65±0.27***b	6.57±0.18**c	5.77±0.16*b

Results are presented as mean ± SEM (n=8), analyzed by one-way ANOVA followed by Tukey's multiple comparison test

(*): p < 0.01; (**): p < 0.001; (***): p < 0.0001 for difference in change from vehicle control group,

(a): p < 0.05; (b): p < 0.001; (c): p < 0.001 for difference in change from standard reference group.

Table 2. Pain inhibition percentage for different treatment types in the tail flick test

Treatments	Doses mg kg^{-1}	Inhibition percentage (%)			
		30 min	60 min	90 min	120 min
Morphine	3	68	70	69	56
Ethanol extract	300	29	53	29	14
	500	56	63	56	35
	1,000	68	71	67	51
Aqueous extract	300	47	62	58	48
	500	51	68	58	50
	1,000	68	70	69	56

Hot plate test

Results from hot plate testing of latency time and inhibition percentage are presented in Tables 3 and 4.

Morphine at dose of 3 mg kg^{-1}, the aqueous and ethanol extract at doses of 300, 500 and 1,000 mg kg^{-1}, showed a significant increase in the latency time. The latency time results at different doses showed a significant increase, hence a dose-dependent effect.

The highest pain inhibition percentage (69%) for the aqueous extract was obtained by the highest dose 1,000 mg kg^{-1}, while the percentage for ethanol extract was 65%.

Table 3. Analgesic effect of aqueous and ethanol extracts of *O. ficus-indica* flowers on latency via the hot plate test

Treatments	Doses mg kg^{-1}	Reaction time ± S.E.M.				
		0 min	30 min	60 min	90 min	120 min
Vehicle	0,2	2.48±0.27	2.42±0.63	2.32±0.23	2.53±0.25	2.23±0.72
Morphine	3	2.48±0.86	8.34±0.26***	9.62±1.59***	8.33±1.22***	6.24±0.78**
Aqueous extract	300	2.34±0.25	3.44±0.19c	5.03±0.22**c	4.50±0.13*c	3.60±0.22c
	500	2.22±0.28	3.72±0.22c	6.32±0.27**b	4.97±0.23**c	3.41±0.15c
	1,000	2.43±0.19	4.88±0.15**c	7.92±0.18***b	5.46±0.20**c	3.84±0.18c
Ethanol extract	300	2.78±0.43	3.77±0.38c	6.50±1.16**c	4.64±0.38*c	3.63±0.28b
	500	2.65±0.88	6.88±1.53**b	6.86±0.83**c	5.97±0.93**b	4.05±0.18b
	1,000	2.66±0.85	5.81±0.71*c	7.54±0.43***b	6.18±0.62**c	4.25±0.35b

Results are presented as mean ± SEM (n=8), analyzed by one-way ANOVA followed by Tukey's multiple comparison test
(*): p < 0.01; (**): p < 0.001; (***): p < 0.0001 for difference in change from vehicle control group,
(a): p < 0.05; (b): p < 0.001; (c): p < 0.001 for difference in change from standard reference group.

Table 4. Pain inhibition percentage for different treatment types in the tail flick test

Treatments	Doses (mg kg^{-1})	Inhibition percentage (%)			
		30 min	60 min	90 min	120 min
Morphine	3	70	74	70	60
Aqueous extract	300	32	53	48	35
	500	40	65	55	35
	1,000	50	69	55	37
Ethanol extract	300	26	57	40	23
	500	61	61	56	34
	1,000	54	65	57	37

Pain behavior assessment according to the gender of mice

Pain inhibition results of aqueous extract of *O. ficus-indica* flowers obtained via experimental hot plate model in male and female mice are shown in Figures 1, 2 and 3.

The pain behavior results in male and female mice according to the hot plate test showed a significant difference ($p < 0.05$; $p < 0.01$). Then, this difference was observed for all tested doses. Finally, these results show that the pain tolerance threshold is high in males compared to females (the graphs have been represented with reference to the tail flick test model results).

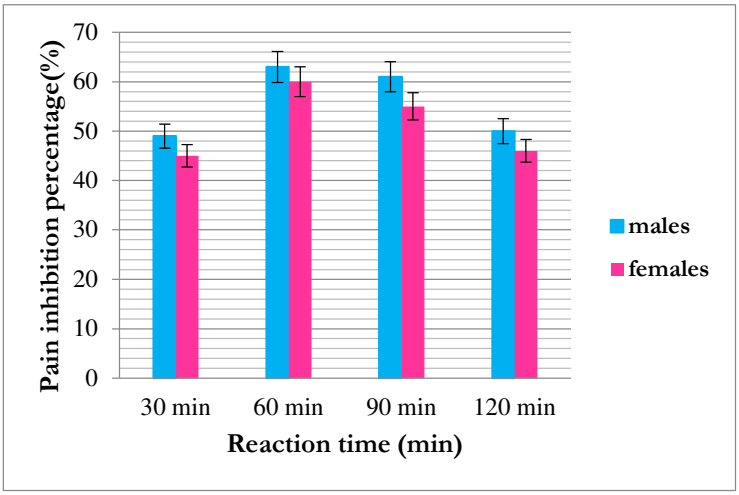

Figure 1. The percentage of pain inhibition according to mice gender after administration of the aqueous extract at 300 mg kg^{-1}

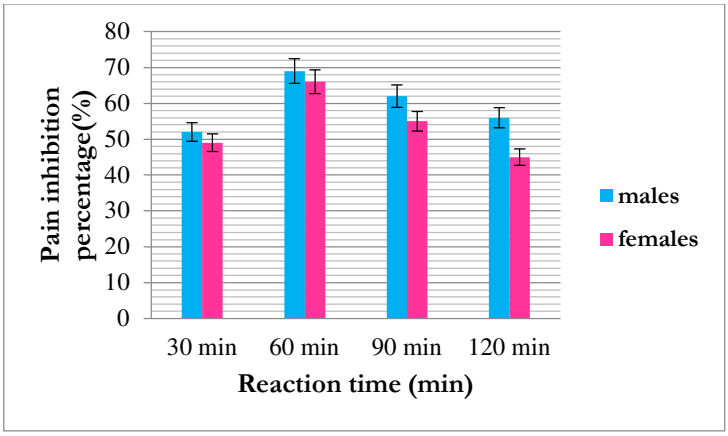

Figure 2. The percentage of pain inhibition according to mice gender after administration of the aqueous extract at 500 mg kg^{-1}

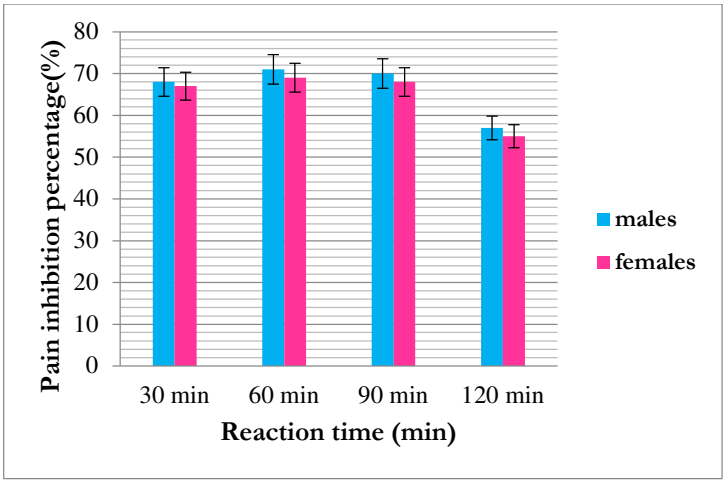

Figure 3. The percentage of pain inhibition according to mice gender after administration of the aqueous extract at 1,000 mg kg^{-1}

Discussion

In the present study, we first examined the toxicity of the aqueous extract and also the anti-nociceptive potential of *O. ficus-indica* flower extracts (aqueous and ethanol) as a central analgesic using tail flick and hot plate test followed by the reaction to the painful behavior according to the gender of the mice.

Firstly, oral administration of the highest dose of 2,000 mg kg^{-1} of *O. ficus-indica* flower extracts was found to produce no symptoms of acute toxicity. Although a slight sedation in the treated mice, no mouse mortality occurred during the 24 hours observation period. The extracts were found to be non-toxic at the highest dose (2,000 mg kg^{-1}), thus, the LD50 was therefore estimated to be greater than 2,500 mg kg^{-1} according to the OCDE decision tree (OCDE, 2001). The highest dose dilutions were designated to assess analgesic activity, namely 300, 500 and 1,000 mg kg^{-1}. The impact of gender on the response to an experimental nociceptive stimulus has been the topic of few studies. Thus, the results obtained within the present work have shown that the pain tolerance threshold is higher in males compared to females. Similarly, Mogil *et al.* (2000) according that female rats are more sensitive to thermal stimuli compared to males. This peculiarity probably results from the dynamic interaction of psychological and physiological factors.

Concerning the analgesic impact study, the anti-nociceptive tests used in the current work were chosen to test the thermal cutaneous stimuli (Tail flick and hot plate tests). Sayyah *et al.* (2004) demonstrated that thermal stimuli are selectively inhibited by central analgesics however not peripheral analgesics in rats. We have used morphine in this study to test our hypothesis.

The obtained results showed a significant difference compared to control for three doses tested, so the two extracts of *O. ficus-indica* flowers would have a central analgesic impact. Therefore, these results justify their significant protective effect and dose-dependent response to the thermo-painful stimulus.

The analgesic effect proven by the tested extracts was approximately comparable to that of morphine. Indeed, at 3 mg kg^{-1} b.w., the pain inhibition of morphine was 70%. In the presence of flower extracts of *O. ficus-indica* at a dose of 1,000 mg kg^{-1}, the inhibition percentage recorded is 70% for the aqueous extract and 71% for the ethanol extract, according to the tail flick test. In fact, the aqueous and ethanol extracts of *O. ficus-indica* flowers caused a reduction of painful behavior in mice. These results showed that *O. ficus-indica* flowers used for pain treatment (Mouhaddach *et al*., 2017) was justified. The obtained results by Chahdoura *et al.* (2017) showed that the aqueous extract of *Opuntia microdasys* flowers at a dose of 100 mg kg^{-1} produced a greater peripheral analgesic effect than that obtained with the positive control, lysine-aspirin (72% and 65.5% protection, respectively).

These results are consistent with a few scientific studies reports that the plant has been used for their analgesic and anti-inflammatory actions. According to Park *et al*. (1998), fruit extract, freeze-dried cladodes and stem extracts had analgesic and anti-inflammatory actions. Considering the results prospected from ethnobotanical survey of *O. ficus-indica*, as well as those reported in the bibliographical review, we assumed that the richness of *Opuntia* in phenolic compounds, particularly in flavonoids, could produce a significant analgesic effect. Indeed, such a conclusion is supported by the phytochemical screening of *O. ficus-indica* which revealed the presence of bioactive molecules, mainly quercetin, nicotiflorin, narcissus, rutin, kaempherol, quercetin 3-O-methylether and 2,3-dihydrokaempferol (Lee *et al.,* 2003; Guevara-Figueroa *et al*., 2010; Ammar *et al*., 2017; Abbas *et al*., 2022). Kuti (2004) reported that quercetin was the dominant flavonol in all analyzed species belonging to four different cactus species, namely *O. ficus-indica*, O. *lindheimeri*, *O. strepthacantha,* and *O. stricta,* and that this molecule has a remarkable analgesic effect.

More recently, Ammar *et al.* (2017) reported that quinic acid was the predominant phenolic acid in the alcohol extract of *Opuntia* flowers, while quercetin-3-O-rutinoside was the major flavonoid identified. It was followed by quercetin-3-O-galactoside, quercetin-3-O-rhamonoside and isorhamnetin-3-O-glucoside. Also, the same study reports that this molecular combination produces a protective and anti-inflammatory effect

according to the model of rat paw edema induced by carrageenan. In addition, fruit phytosterols have been identified as the main active anti-inflammatory component (Park *et al*., 2001; Izuegbuna *et al*., 2019). Therefore, the freeze-dried aqueous extract (100-400 mg kg^{-1}, i.p.) of *Opuntia dillenii* fruits in hot plate assays in mice and rats possessed notable and dose-dependent analgesic activity (Loro *et al*., 1999).

Furthermore, the obtained results in phenolic analysis are in agreement with previous studies reporting the presence of phenolic acids and flavonoids in the methanolic extract of *O. ficus-indica* flowers (Yeddes *et al*., 2014), as well as in the methanol extract of *O. microdasys* flowers (Chahdoura *et al*., 2014). Quinic acid was the major phenolic acid identified in this study, which accounted for 12.4% of the total identified phenolic compounds. This compound is also present in Moroccan *O. ficus-indica* with 3.65% of the total identified phenolic compounds (Benayad *et al*., 2014; Zeghbib *et al.*, 2022). *O. ficus-indica* flowers extract was dominated by flavonol glycoside and the flavonol 3-O-glycosides (quercetin, kaempferol, and isorhamnetin) (De Leo *et al*., 2010; Kolniak-Ostek *et al*., 2020).

These bioactive molecules also showed that certain compounds possessed analgesic activity. Also, flavonoids (kaempherol and isorhamnetin glycosides) are known to inhibit the prostaglandin synthetase enzyme, specifically endoperoxidase (Ramaswamy *et al*., 1985; Benattia *et al*., 219). Bentley (1983) and Negus *et al.* (2006) proved that the pain onset mechanism can be attributed to the release of substances in the intraperitoneal fluid such as prostaglandins (PGE2, PGF2 α), serotonin, histamine, bradykinin which will stimulate nociceptive receptors located in the peritoneum. In addition, the chemical composition of *Opuntia* flowers was characterized by the presence of flavonoids, saponins, tannins, and carbohydrates, contents (Ennouri *et al*., 2014). Thus, flavonoids are 5-lypo-oxygenase inhibitors, which allow inhibition of the inflammatory mediator's effect (Ferreira, 2002). As a result, the presence of flavonoids in the *O. ficus-indica* flowers extract might contribute to the analgesic effect.

Finally, we confirm that *O. ficus-indica* flowers possess a central analgesic activity, justifying their use in traditional medicine (Mouhaddach *et al*., 2017). This effect can be explained by the presence of phenolic compounds and flavonoids inhibiting the pain process. Other more advanced experimental models have made it possible to determine both the analgesic and anti-inflammatory activities of the aqueous extract obtained from *Opuntia microdasys* flowers. These interesting results are used in native anti-cancer regimens and suggest that *Opuntia microdasys* flowers could be explored for their pharmacological and antimutagenic profiles with potential utility of this plant within an herbal medicine or as a nutritional supplement. Furthermore, a more thorough analysis would allow us to discern the precise doses required for both anti-inflammatory and analgesic activities and, on the other hand, to quantify this activity by precisely establishing the action mechanism of mediating molecules in this process. Subsequently, it would be wise to seek a stable galenic formulation that can serve as a basis for this activity to enable design of a medicine to treat pain.

Conclusions

In the present study we demonstrated the analgesic property of the *O. ficus-indica* flowers extracts, using conventional pharmacological models. These findings support its usage within the traditional pharmacopoeia. These properties are most likely related to flavonoids and phenolic compounds highlighted in the bibliography. Intra-peritoneal administration of *O. ficus-indica* flowers aqueous extract did not show any toxicity symptoms at the highest dose, thus the LD50 was estimated to be greater than 2,500 mg kg^{-1}. The pain behavior results according to the gender approach of mice showed that the pain tolerance threshold is high in males compared to females. Further experiments using purified extracts are needed to accurately identify the active compounds responsible for this analgesic effect and to understand their action mode.

Authors' Contributions

MA: Writing-Original Draft, Sampling and analysis; LR: Writing-Original Draft, Methodology; AL: Investigation, Resources; BM: Statistical analysis, Investigation; BF: Validation, Investigation; BS: Formal analysis, Investigation; HR: Supervision, Investigation, Resources; SS: Methodology, Validation.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abbas EY, Ezzat MI, El Hefnawy HM, Abdel-Sattar E (2022). An overview and update on the chemical composition and potential health benefits of *Opuntia ficus-indica* (L.) Miller. Journal of Food Biochemistry 46:e14310. *https://doi.org/10.1111/jfbc.14310*

Ahmed MS, El Tanbouly ND, Islam WT, Sleem AA, El Senousy AS (2005). Anti-inflammatory flavonoids from *Opuntia dillenii* (Ker-Gawl) Haw: flowers growing in Egypt. Phytotherapy Research 19(9):807-809. *https://doi.org/10.1002/ptr.1708.PMID:16220577*

Ammar I, Ben Amira A, Khemakem I, Attia H, Ennouri M (2017). Effect of *Opuntia ficus-indica* flowers maceration on quality and on heat stability of olive oil. Journal of Food Science and Technology 54:1502-1510. *https://doi.org/10.1007/s13197-017-2581-0*

Ammar I, Ennouri M, Attia H (2015). Phenolic content and antioxidant activity of cactus (*Opuntia ficus-indica* L.) flowers are modified according to the extraction method. Industrial Crops and Products 64:97-104. *https://doi.org/10.1016/j.indcrop.2014.11.030*

Ammar I, Ennouri M, Khemakhem B, Yangui T, Attia H (2012). Variation in chemical composition and biological activities of two species of *Opuntia* flowers at four stages of flowering. Industrial Crops and Products 37:34-40. *https://doi.org/10.1016/j.indcrop.2011.11.027*

Benattia KF, Arrar Z, Dergal F, Khabbal Y (2019). Pharmaco-analytical study and phytochemical profile of hydroethanolic extract of Algerian prickly pear (*Opuntia ficus-indica*.L). Current Pharmaceutical Biotechnology 20(9). *https://dx.doi.org/10.2174/1389201020666190620113129*

Benayad Z, Martinez-Villaluenga C, Frias J, Gomez-Cordoves C, Essafi NE (2014). Phenolic composition, antioxidant and anti-inflammatory activities of extracts from Moroccan *Opuntia ficus-indica* flowers obtained by different extraction methods. Industrial Crops and Products 62:412-420. *https://doi.org/10.1016/j.indcrop.2014.08.046*

Bussmann RW, Malca G, Glenn A, Sharon D, Nilsen B, Parris B, Townesmith A (2011). Toxicity of medicinal plants used in traditional medicine in Northern Peru. Journal of Ethnopharmacology 137(1):121-140. *https://doi.org/10.1016/j.jep.2011.04.071*

Chahdoura H, Barreira JCM, Barros L, Santos-Buelga C, Ferreira ICFR, Achour L (2014). Phytochemical characterization and antioxidant activity of *Opuntia microdasys* (Lehm.) Pfeiff flowers in different stages of maturity. Journal of Functional Foods 9:27-37. *https://doi.org/10.1039/c4fo00456f*

Chahdoura H, El Bok S, Refifa T, Adouni K, Khemiss F, Mosbah H, Achour L (2017). Activity of anti-inflammatory, analgesic and antigenotoxic of the aqueous flower extracts of *Opuntia microdasys* Lem. Pfeiff. Journal of Pharmacy and Pharmacology 69(8):1056-1063. *https://doi.org/10.1111/jphp.12734*

De Leo M, Abreu MBD, Pawlowska AM, Cioni PL, Braca A (2010). Profiling the chemical content of *Opuntia ficus-indica* flowers by HPLC–PDA-ESI-MS and GC/EIMS analyses. Phytochemistry Letters 3:48-52. *https://doi.org/10.1016/j.phytol.2009.11.004*

Di Stasi LC, Costa M, Mendaçolli SL, Kirizawa M, Gomes C, Trolin G (1988). Screening in mice of some medicinal plants used for analgesic purposes in the state of Sao Paulo. Journal of Ethnopharmacology 24(2-3):205-211. *https://doi.org/10.1016/0378-8741(88)90153-5*

Eddy NB, Leimback D (1953). Synthetic analgesic II. Dithienylbutenyl and dithienyl butylamines. Journal of Pharmacology and Experimental Therapeutics 107:385-393.

Ennouri M, Ammar I, Khemakhem B, Attia H (2014). Chemical composition and antibacterial activity of *Opuntia ficus-indica F. inermis* (Cactus Pear) flowers. Journal of Medicinal Food 17(8):908-914. *https://doi.org/10.1089/jmf.2013.0089*

Franzotti EM, Santos CVF, Rodrigues HMSL, Mourao RHV, Andrade MR, Antoniolli AR (2000). Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L. (Malva-branca). Journal of Ethnopharmacology 72:273-8.*https://doi.org/10.1016/s0378-8741(00)00205-1*

Guevara-Figueroa T, Jiménez-Islas H, Reyes-Escogido ML, Mortensen AG, Laursen BB, Lin LW, ... Barba de la Rosa AP (2010). Proximate composition, phenolic acids, and flavonoids characterization of commercial and wild nopal (*Opuntia* spp.). Journal of Food Composition and Analysis 23:525-532. *https://doi.org/10.1016/j.jfca.2009.12.003*

Izuegbuna O, Otunola G, Bradley G (2019). Chemical composition, antioxidant, anti-inflammatory, and cytotoxic activities of *Opuntia stricta* cladodes. PLoS One 14(1):e0209682. *https://doi.org/10.1371/journal.pone.0209682*

Kolniak-Ostek J, Kita A, Miedzianka J, Andreu-Coll L, Legua P, Hernandez F (2020). Characterization of bioactive compounds of *Opuntia ficus-indica* (L.) Mill. seeds from Spanish cultivars. Molecules 25(23):5734. *https://doi.org/10.3390/molecules25235734*

Kuti JO (2004). Antioxidant compounds from four *Opuntia* cactus pear fruit varieties. Food Chemistry 85:527-533. *https://doi.org/10.1016/S0308-8146(03)00184-5*

Lee EH, Kim HJ, Song YS, Jin C, Lee KT, Cho J, Lee YS (2003). Constituents of the stems and fruits of *Opuntia ficus-indica* var. *saboten*. Archives of Pharmacal Research 26:1018-1023. *https://doi.org/10.1007/BF02994752*

Loro JF, Rio del I, Pérez-Santana L (1999). Preliminary studies of analgesic and anti- inflammatory properties of *Opuntia dillenii* aqueous extract. Journal of Ethnopharmacology 67:213-218. *https://doi.org/10.1016/s0378-8741(99)00027-6*

Mogil JS, Chesler EJ, Wilson SG, Juraska JM, Sternberg WF (2000). Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype. Neuroscience-Biobehavioral Reviews 24(3):375-389. *https://doi.org/10.1016/s0149-7634(00)00015-4*

Mouhaddach **A**, El-hadi A, Taghzouti K, Bendaou M, Hassikou R (2017). Assessment of *Opuntia ficus-indica in vivo* following ethnobotanical survey: Confirmation of its analgesic activity. Phytothérapie 16 :191-196. *https://doi.org/10.1007/s 10298-017-1109-4*

Organisation de coopération et de développement économiques (OCDE) (2001a/b). Toxicité orale aiguë - Méthode de la dose prédéterminée et Méthode par classe de toxicité aiguë. In Lignes directrice de l'OCDE pour les essais de produits chimiques Vol 1, num. 4, 1-15. OCDE, Paris.

Park EH, Kahng JH, Paek EA (1998). Studies on the pharmacological action of cactus: identification its anti-inflammatory effect. Archives of Pharmacal Research 21(1):30-34.

Park EH, Kahng JH, Lee SH, Shin KH (2001): An anti-inflammatory principle from cactus: identification of its anti-inflammatory effect. Fitoterapia 72 :288-290. *https://doi.org/10.1007/BF03216749*

Queneau P, Ostermann G (2004). Le médecin, le malade et la douleur. Masson, Paris, 488-505.

Ramaswamy S, Pillani NP, Gopalkrishnan V (1985). Analgesic effect of O-(α-hydroxy ethyl) rutoside in mice. Indian Journal of Experimental Biology 23:219-220.

Sayyah M, Hadidi N, Kamalinejad M (2004). Analgesic and anti-inflammatory activity of *Lactuca sativa* seed extract in rats. Journal of Ethnopharmacology 92(2-3):325-329. *https://doi.org/10.1016/j.jep.2004.03.016*

Sébastien F (2010). Analgésiques antipyrétiques. Actualités pharmaceutiques, 49, N° 492.

Shirazinia R, Rahimi VB, Kehkhaie AR, Sahebkar A, Rakhshandeh H, Askari VR (2019). *Opuntia dillenii*: A forgotten plant with promising pharmacological properties. Journal of Pharmacopuncture 22(1):16-27. *https://doi.org/10.3831/KPI.2019.22.002* .

Siddiqui F, Abidi L, Lubna Poh C, Naqvi S, Faizi S, Dar A (2016). Analgesic potential of *Opuntia dillenii* and its compounds opuntiol and opuntioside against pain models in mice. Records of Natural Products 10:6-721.

Smith L, D'Amour C, Marie et D'Amour F (1943). The analgesic properties of certain drugs and drug combinations. Anesthesiology 4.10.1097/00000542-194307000-00036.

Toma W, Gracioso JDS, Hiruma-Lima CA, Andrade FD, Vilegas W, Brito AS (2003). Evaluation of the analgesic and antiedematogenic activities of *Quassia amara* bark extract. Journal of Ethnopharmacology 85(1):19-23. *https://doi.org/10.1016/s0378-8741(02)00334-3*

Valente LM, da Paixão D, Do Nascimento AC, dos Santos PF, Scheinvar LA, Moura MR, da Silva JF (2010). Antiradical activity, nutritional potential and flavonoids of the cladodes of *Opuntia monacantha* (Cac.). Food Chemistry 123(4):1127-1131. *https://doi.org/10.1016/j.foodchem.2010.05.074*

Yeddes N, Chérif JK, Guyot S, Baron A, Trabelsi-Ayadi M (2014). Phenolic profile of tunisian *Opuntia ficus-indica* thornless form flowers via chromatographic and spectral analysis by reversed phase-high performance liquid chromatography-UV-photodiode array and electrospray ionization-mass spectrometer. International Journal of Food Properties 17:741-751. *https://doi.org/10.1080/10942912.2012.665404*

Zeghbib W, Boudjouan F, Vasconcelos V, Lopes G (2022). Phenolic compounds' occurrence in *Opuntia* species and their role in the inflammatory process: a review. Molecules 27:4763. *https://doi.org/10.3390/molecules27154763*

Zimmerman M (1983). Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16(2):109-110. *https://doi.org/10.1016/0304-3959(83)90201-4*

Zou DM, Brewer M, Garcia F, Feugang JM, Wang J, Zang R, Liu H, Zou C (2005). Cactus pear: a natural product in cancer chemoprevention. Nutrition Journal 4 :25-36. *https://doi.org/10.1186/1475-2891-4-25*