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Phytochemical screening and analgesic activity of *Opuntia ficus indica* cladods extract in Wistar rats

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A b s t r a c t

The purpose of this work is the study of the peripheral and central analgesic activity of the *Opuntia ficus indica* cladods extract. Different concentrations are tested: 1000 mg/kg; 2000 mg/kg and 3000 mg/kg of body weight on rats by three different methods: the tail flick, the hot-plate and the writhing test to the acetic acid. The results obtained show that the aqueous extract of the cladods has an peripheral and central analgesic activity comparable to that of the Aspirin after administration to rats. The phytochemical analysis of the extract reveals the presence of tannins, coumarins, flavonoids, steroids and anthocyanins whose properties can be used to advantage in the treatment of pain. Our results confirm the traditional use of *Opuntia ficus-indica* cladods as an analgesic.

Keywords: Extract, Phytochemical, Pain, Analgesic, Opuntia ficus indica

Introduction

The pain is an unpleasant feeling which originates by stimulation of the nerve endings followed by their psychic integration: it's a Nociceptive pain[1] can be superficial (cutaneous, somatic) conscious or perfectly localized or deep (visceral) involving the autonomic nervous system[1].

The Analgesics minors are of frequent use even in self-medication, while the major analgesics opioids are addictive. For the most part between them, the use of which is subject to strict international control[2].

Medicinal plants are used for a long time in the treatment of inflammatory and painful diseases. However, the modes of operation of herbal resources risk compromising the exercise of traditional herbal medicines[3].

The prickly pear, is a plant of the family of the Cactaceae. Originating from Mexico, this plant is tree and can reach up to 5 m high[4]. It has been consumed for these benefits on the health in particular to relieve headache and pain of the members[5]. The present study allows to show the analgesic effect of the cladods extract by a différents experimentals models of analgesia in rat.

Material and Methods

Plant material

Opuntia ficus indica cladodes used in this study were collected from the el-Khroub region of Constantine (Algeria) in 2011. The annual average temperatures are in the order of 15,15 C. The maximum of temperatures reaches 33,58 C in July, whereas the minimum reaches 2,73 C in January. The pluviometry average is of 600mm.

Preparation of the extract

After the harvest of plant material is performed, each sample was cut into thin slices and dried in a stove at a temperature of 50 C for 72 hours and then ground using a grinder (type Gulatti MFC) that rotates speed of 1000 rpm and equipped with sieve, a square mesh sizes from 0.85 mm

50g of the powder were introduced into an Erlenmeyer flask and macerated in 500 ml of distilled water with mechanical stirring for 24 hours at ambient temperature. Then it was filtered through Whatman filter paper, the filtrate (Aqueous extract) obtained was

evaporated using rotary evaporator[6]. The extract was transferred to a closed container for further use and protection.

Animals

Young albino rats of either sex aged 4-5 weeks, average weight 100-300 g were used for the experiment. The rats were purchased from the Pasteur Institute in Algiers. They were kept in standard environmental condition (at 24 ±1 C temperature and 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase with free access to food and water.

Phytochemical screening

The extract was tested for the presence of bioactive compounds by using following standard methods[7],[8],[9].
Test for reducing sugars (Fehling's test) : Their detection involves treating 1 ml of the extract with 2 ml of distilled water, 20 drops of Fehling's solution and then heated. Appearance of yellow and then brick red precipitate indicates the presence of reducing sugars[9].
Test for Triterpenoids : To extract solution, 10 drops of acetic anhydride was added and mixed well. To this a concentrated sulphuric acid was added from the sides of the test tube. Appearance of greenish blue colour indicates the presence of triterpenoids[8].
Test for flavonoids : Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids[7].
Test for saponins : The extract solution was mixed with 5 ml of water and vigorously shaken. The formation of stable form indicated the presence of saponins[7],[8].
Test for tannins : The diluted ferric chloride solution was added to extract in a test tube and observation made. The appearance of a dark green color or blue-green indicates the presence of tannins[7].
Test for alkaloids : Extract was dissolved in dilute Hydrochloric acid and filtered. Filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids[7].
Test for steroids : Extract was mixed with a chloroform and concentrated sulphuric acid was added. A red colour produced in the lower chloroform layer indicated the presence of steroids[8].

Analgesic activity

Acetic acid induced writhing method

The writhing was induced by an intraperitoneal injection of 1% acetic acid (5 ml/kg of body weight). Five groups of six rats were formed. Each animal of group I received 5ml/kg of body weight of the vehicle (the physiological water), and those from group II received 100 mg/kg of body weight of drug aspirin. Each animal from group III, group IV and group V were orally treated with 1000; 2000 and 3000 mg/kg of body weight doses of extracts (dissolved in water) respectively, one hour before acetic acid injection. The number of writhings occurring between 5 and 20 min after acetic acid injection was recorded. The analgesic effect was expressed as the percentage reduction of writhes in treated rats compared to those in the control [10]. The percentage inhibition was calculated using the formula below:
% inhibition = (A – B/A) x 100, where A is mean for the control group and B is mean for the treated group.

Hot plate method

Experimental animals divided into four groups consisting of six rats in each group for control, positive control sample tests groups respectively, the treatments were given as per Acetic acid induced writhing method. The animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5 C one hour after oral administration of the treatment. Reaction time was recorded when animals licked their fore or hind paws, or jumped [11],[12].

Tail flick method

Groups of six rats each were traited with the same method of Acetic acid induced writhing. Thereafter, the terminal 4 cm of the rats tail were immersed in hot water at 55±1 C. The time of Their responses to thermal pain was recorded using tail flick analgesiometer one hour after administration of the treatment[13].

Statistical analysis

The results of statistical analysis for animal experiment were expressed as mean ± SEM and were evaluated by ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. The $p<0.05$, was considered to be statistically significant.

Results

Phytochemical screening

The results for preliminary phytochemical screening carried out on the Opuntia ficus indica cladods powder were shown in Table 1. From these results, Flavonoids, steroids, Tannins, saponins and reducing sugars were detected. However, they were devoid of alkaloids and Triterpenoids.

Table 1 : Results of phytochemicals tests of the aqueous extract of *Opuntia ficus indica* cladods

Tests	reducing sugars	Triterpenoids	Flavonoids	saponins	Tannins	alkaloids	steroids
Aqueous Extract	+++	-	+++	+	+	-	+++

Very positive Reaction +++ Positive Reaction +Negative Reaction –

Acetic acid induced writhing method

The control group who received the physiological water present, after intraperitoneal injection of acetic acid 1 %, an average of contortions of 95±6.71 over a period of 20 minutes. The latency time of onset of these contortions is 5 minutes

The administration per os of aspirin at a dose of 100 mg/kg, prevented significantly the emergence of contortions related to the administration of the acetic acid (28±2.160 vs 95±6.71) ($p<0.0001$; n= 6). The administration per os of the aqueous extract of O. f.indica cladods prevent in a dose dependent, the emergence of contortions in rats. With the doses 1000, 2000 and 3000 mg/kg (BW) of the aqueous extract, the contortions observed are significantly different from those observed with the control group (67.5 ± 3.5 ; 53.66 ± 2.054 and 31.5 ± 3.5 respectively ($p<0.0001$, n= 6) (Table 2).

Table 2: Study of analgesic activity of the aqueous extract of *O. f.indica* against the pain caused by the acetic acid

Groups	Number of contortions (NC)	Percentage inhibition (PI)
Control	95 ± 15	-
Group1 : 1000mg/Kg	67.5 ± 3,5****	28,947
Group2 : 2000mg/Kg	53,66 ±2,054****	43,515
Group3 : 3000mg/Kg	31,5± 3,5****	66,842
Group4 : Aspirin	28±2,160****	70,526

Hot plate method

The aqueous extract of O. f.indica at doses of 1000, 2000 and 3000 mg/kg and aspirin at a dose of 100 mg/Kg significantly increase the reaction time on the heating plate compared to control group.This time is of 2.19 ± 0.292 sec for the control group while he is of 2.90 ± 0.935 sec; 4.17± 0.676 ; 5.02 ± 0.714 ($P < 0.0001$) respectively for treaties groups and of 5.20 ± 0.736 sec ($P < 0.0001$) for aspirin group (Table 3).

Table 3 : Study of analgesic activity of the aqueous extract of *O. f.indica* against the pain caused by the Hot plate

Groups	The reaction time (sec)
Control	2,19 ± 0,292
Group1 : 1000mg/Kg	2,90 ± 0,935****
Group 2 : 2000mg/Kg	4,17 ± 0,676****
Group 3 : 3000mg/Kg	5,02 ± 0,714****
Group 4 : Aspirin	5,20 ± 0,736****

Tail flick method

For the three doses studied we note that the aqueous extract of O. f. indica significantly increases the reaction time. This effect is more important at doses of 2000 and 3000 mg/kg (BW) and comparable to that of aspirin 100 mg/kg (BW). (Table 4)

Table 4: Study of analgesic activity of the aqueous extract of *O. f.indica* against by tail-flick method

Groups	The reaction time (sec) (sec)
Control	2,32 ± 0,499
Group1 : 1000mg/Kg	4,37 ± 0,798****
Group 2 : 2000mg/Kg	5,22 ± 0,375****
Group 3 : 3000mg/Kg	6,27 ± 0,314****
Group 4 : Aspirin	6,72 ± 0,476****

Discussion

The majority of chemical groups present in the aqueous extract of *O. f.indica*, as the tannins, the saponosides, flavonoids, coumarins and anthocyanosides, are extractable by water. The water seems to be the best solvent to extract the majority of chemical constituents responsible for the different biological activities [2].

According to previous studies, the analgesic activity of extracts would be due to the presence of sterols (campesterol, B sitosterol, lupeol), saponins, phenolic compound or alkaloids. The analgesic effect of these compounds is confirmed in several medicinal plants.

The intraperitoneal administration (IP) of acetic acid in rats has led to serious abdominal contractions. These contractions are due to the production and release of mediators algogenes via the cyclooxygenases (COX) and the biosynthesis of prostaglandins [14], including the PGE2 produced by COX-1. These mediators released awareness nociceptors cholinergic and histamine peritoneal.

The administration of the Aspirin (100mg/kg) in preventive treatment to rats inhibited significantly the algogene action of the acetic acid. This analgesic activity of aspirin is the result of the deletion of the training of mediators of pain in peripheral tissues, because it inhibits the activity of COX-1 and COX-2 [15].

The results obtained show that the aqueous extract of *O.f.indica* presents a significant analgesic effect in reducing the number of abdominal contortions at all doses. This suggests that the extract has compounds that Act by the same mechanism than aspirin.

The Central analgesic activity of the extract has been evaluated by testing its effect on pain induced by a thermal stimulus (the heating plate) and the test of tail-flick in rats. For these two tests, the aqueous extract increase the latency time to the jump of the animal[16].

The results of this study show that aspirin (100 mg/kg) significantly inhibited the pain in both tests. The aspirin acts at the central level and its analgesic power is due to agonist activity of opioidergic receptor, associated with an inhibitory activity of neuronal reuptake of serotonin and noradrenaline. This activity is enhanced by the descending inhibitory control at the spinal level [16].

The aqueous extract of *Opuntia ficus-indica* has significantly inhibited the pain in a dose-dependent induced by the heat. These results suggest that the extract would act via the same mechanisms that the aspirin and would therefore be an inhibitor of central processes of the pain.

It's apparent that the aqueous extract of cladods of *Opuntia ficus-indica* possesses the central and peripheral analgesics properties. Similar results have been obtained by others authors on *opuntia* indicated has analgesic and anti-inflammatory properties [18], [17].

Conclusion

The aqueous cladods extract of opuntia ficus indica demonstrated promissing anti-noceiptive property in all animals models used in this study. Confirming its efficacy in the treatment of pain.

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