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Anti-inflammatory and Antispasmodic Activities of Isorhamnetin Glycosides Isolated from *Opuntia ficus-indica* (L.) Mill. Flowers.

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ABSTRACT

The aims of this work are to define the anti-inflammatory and antispasmodic activities *in vivo* of the methanol extract of *Opuntia ficus-indica* flowers, and compared these activities to those shown by isorhamnetin glycosides extracted from these flowers. The anti-inflammatory and antispasmodic activities of the methanol extract gave a percentage of 53.88 % and 78.39 % respectively for a dose of 500 mg/kg, however the isolated compound, isorhamnetin 3-O-robinobioside, it gave a percentage of 77.51 % and 79.17 % respectively for the same dose, which means that the isorhamnetin 3-O-robinobioside is the responsible product for the biological activities of *Opuntia ficus-indica* flowers.

Keywords: Anti-inflammatory, Antispasmodic, Cactaceae, *Opuntia ficus-indica,* Flowers, Isorhamnetin-3-O-robinobioside.

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INTRODUCTION

Medicinal plants contain multiple components as primary and secondary metabolisms that might be responsible for its therapeutic effects. Flavonoïds are the most widely distributed group of phenolic compounds in plants which are found in seeds, herbs and flowers. They exhibit several biological effects such as anti-inflammatory, antimicrobial, anti-diabetic, antitumor, and antioxidant activities [1]. The main objective of the study of flavonoïds is to define and separate them in order to understand and explain their bioactivity, using many techniques [2], view their significant and desirable effects on health.

Opuntia ficus-indica belonging to Cactaceae family is a desert plant, subsists in a semi-arid climate. Discovered for the first time in Mexico, but now it is prevalent all over the world in Central America, Mediterranean peninsula, southern USA, Asia, and southern Europe. This plant has various therapeutic activities like antioxidant activity conferred by the presence of ascorbic acid or flavonoïds (quercetin, myrcitin, kaempferol and isorhamnetin) [3-4], neuroprotective activity provided by quercetin, (+)-dihydroquercetin, and quercetin 3-methyl ether [5], and antiproliferative activity due to isorhamnetin glycosides [6]. Moreover, fruits and cladodes presented an anti-inflammatory activity attributed to different phytochemicals such as isorhamnetin glycosides [7-9].

Opuntia ficus-indica (L.) Mill. flowers were the subject of a limited number of researches. Recent studies have determined the composition of flavonoïds present in flowers [10-11] such as isorhamnetin, quercitin and kaepmferol glycosides. Other studies have determined the biological activities of these flowers as diuretic [12], antiulcerogenic [13], and for the treatment of an enlarged prostate gland [14]. Furthermore, an *in vitro* study presented the anti-inflammatory and antioxidant activities of cactus pear flower extracts [15]. In traditional medicine, dried flowers are mixed with honey and used to treat asthma and bronchitis, also they are used as infusion against kidney stone pains, for that, in this work, we studied *in vivo* the anti-inflammatory and antispasmodic activities of methanol extract of *Opuntia ficus-indica* (L.) Mill. flowers and compared it to isorhamnetin-3-O-robinobioside, the abundant flavonoïds separated from these flowers.

MATERIALS AND METHODS

Plant materials

Opuntia ficus-indica (L.) Mill. flowers collected from the region of Annaba, Algeria, were dried in shadow then stored until used. A voucher specimen was deposited in the *LSBO Laboratory under the number Ann-2011/01.*

Chemicals

Carrageenan was purchased from Sigma-Aldrich. Acetic acid was purchased from Merck. Diclofenac was obtained from Sandoz and phloroglucinol was obtained from Frater-Razes. All other chemicals used were of analytical grade.

Animals

Healthy male Albinos mice weighting 17-24g, obtained from Pasteur Institute (Algiers, Algeria) were kept in approved plastic cages, with metal mesh lids and bottoms, at a temperature of 24°C, humidity 50% and were exposed to 10h light. Food and water were supplied *ad libitum* throughout the duration of the study. All procedures were performed in accordance with the European Union Guidelines for Animals Experimentation (2007/526/EC)

Preparation of plant extract and isolation of compound

100g of dried flowers were extracted with 1L methanol-water (80: 20, v/v) for 24h at room temperature. After that, the extract was concentrated and the aqueous layer was extracted three times with 500 mL of dichloromethane-propanol (3:2, v/v). Organic layers were condensed in rotavapor to obtain 2,43g of brown residue. The residue was analyzed on thin layer chromatography on silica gel 60 F254 using ethyl acetate-methanol-water (85: 10: 5, v/v/v) as eluant. After that the dried plate was sprayed with 1% ethanolic

solution of aluminum chloride. A yellow fluorescence in long wavelength UV light (360nm) determines the presence of flavonoïds, with one major compound at R_f= 0.3.

The residue was subjected on a silica gel column and the column was eluted with 500 mL of ethyl acetate-methanol-water (85: 10: 5, v/v/v), then 400mL methanol-water (50: 50, v/v). The eluent was collected in fractions of 5mL and analyzed by TLC and the fractions of similar profiles were condensed. The major compound was identified by ultraviolet spectroscopy and nuclear magnetic resonance NMR.

Preparation of extract for pharmacological activities

50g of dried flowers were extracted with methanol-water (80: 20, v/v) for 24h at room temperature. The extract was filtered; the solvent was condensed in rotavapor and finally lyophilized. 5g of brown powder were kept in the refrigerator until used.

Acute toxicity

Ten healthy animals were randomly divided into two groups (n=5). The first group received per os *Opuntia ficus-indica* methanol extract and the second group received isorhamnetin-3-O-robinobioside dissolved respectively in water at a dose of 5000mg/kg. Animals had free access to food and water, and they were observed for symptoms and mortality for 48 h.

Anti-inflammatory activity

Animals were weighed and randomized into six groups (n=5). Carrageenan-induced paw edema in mice by subcutaneously injection of 0.025mL of 1% saline solution of lambda carrageenan in 0,9% in the plantar region of the left posterior paw, 1 hour before administration per os of treatments: methanol extract (2500mg/kg, 5000mg/kg), isorhamnetin-3-O-robinobioside (2500mg/kg, 5000mg/kg) and diclofenac (12,5mg/kg). Only water was administered per os to control group. After 4 hours animals were killed and paws removed by cutting at the tibiotarsal level, weighed and compared with healthy paws [16]. The percentage of edema was calculated using the following formula:

$$\%\ edema = \frac{average\ left\ paws\ weight - average\ right\ paw\ weight}{average\ right\ paw\ weight} \times 100$$

The percentage inhibition of edema was calculated using the following formula:

$$\%\ inhibition = \frac{\%\ edema\ control\ group - \%\ edema\ treated\ group}{\%\ edema\ control\ group} \times 100$$

Antispasmodic activity

Acetic acid induced writhing response in mice by intraperitoneal injection of 0.1mL acetic acid at 1%, 30 min after administration per os of treatment methanol extract (2500mg/kg, 5000mg/kg), isorhamnetin-3-O-robinobioside (2500mg/kg, 5000mg/kg) and phloroglucinol (80mg/kg). The control group received per os only water. The mice were observed and the number of writhing during the following 10 min periods was counted starting 5 min after acetic acid injection. Finally the percentage inhibition in each group (n=5) was calculated using the following formula [17]:

$$\%\ inhibition = \frac{average\ writhing\ of\ control\ group - average\ writhing\ of\ treated\ group}{average\ writhing\ of\ control\ group} \times 100$$

Statistical Analysis

Statistical calculations were carried out with Graph Pad Prism 6. Data were expressed by mean ± SEM and analyzed by one way analysis of variance (ANOVA) followed by Dunnett's and Turkey's tests and results were regarded as significant at $p < 0.05$.

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RESULTS AND DISCUSSION

Spectroscopy identification of isorhamnetin-3-O-robinobioside

Yellow crystalline solid. UV (MeOH) λ_{max} 255, 355 nm. 1H NMR (MeOD, 500 MHz) δ 7.9 ppm (1H, s, H-2'), 7.6 (1H, d, J=8.4, H-6'), 6.9 (1H, d, J=8.4, H-5'), 6.3 (1H, s, H-8), 6.1 (1H, s, H-6), 5.2 (1H, d, J=6.5, H-1''), 4.5 (1H, s, H-1'''), 3.9 (3H, s, O-CH_3), 3.8–3.2 (10H, m, CH-glycoside), 1.1 (3H, d, J=6.2, H-6'''). ^{13}C NMR (MeOD, 500 MHz) δ 179.165 ppm (C, C-4), 165.907 (C, C-7), 162.779 (C, C-5), 158.690 (C, C-9), 158.287 (C, C-4'), 150.733 (C, C-2), 148.160 (C, C-3'), 135.481 (C, C-3), 123.955 (C, C-1'), 122.857 (CH, C-6'), 116.065 (CH, C-5'), 114.529 (CH, C-2'), 105.594 (C, C-10), 104.564 (CH, C-1''), 102.483 (CH, C-1'''), 99.975 (CH, C-6), 94.977 (CH, C-8), 78.107 (CH, C-3''), 77.234 (CH, C-5''), 75.896 (CH, C-2''), 73.815 (CH, C-4''), 72.236 (CH, C-3'''), 72.031 (CH, C-2'''), 71.548 (CH, C-4'''), 69.746 (CH, C-5'''),68.487 (CH_2, C-6''), 56.734 (CH_3, OCH_3), 17.891 (CH_3, C-6'''). These results present the molecule of *isorhamnetin-3-O-robinobioside* (figure 1) by comparison to those reported by literature [10, 18].

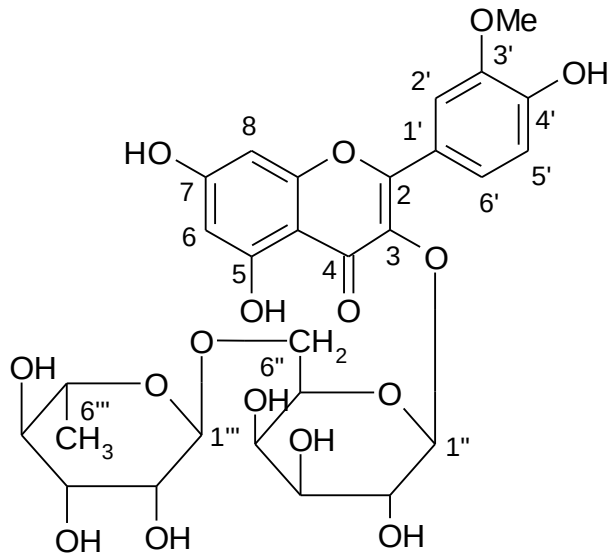

Figure 1: Structure of Isorhamnetin-3-O-robinobioside

Acute toxicity

After 48h no mortality was observed in treating mice administered 5000mg/kg of methanol extract or isorhamnetin-3-O-robinobioside, which means that these extracts present any toxic effect at this dose.

Anti-inflammatory activity

The results presented in table 1, show that methanol extract inhibited carrageenan-induced paw edema with a percentage of 53.88% at a dose of 5000mg/kg and 21.05% at a dose of 2500mg/kg, while isorhamnetin-3-O-robinobioside inhibited paw edema with a percentage of 77.51% at a dose of 5000 mg/kg and 49.68% at a dose of 2500mg/kg. Diclofenac; as reference anti-inflammatory; inhibited paw edema with a percentage of 36.05% at a dose of 12.5mg/kg.

Carrageenan-induced paw edema has been frequently used as an experimental model of acute inflammation. It evokes a potent local acute response with a biphasic profile [19] and is mainly mediated by histamine, serotonin, and prostaglandins. The anti-inflammatory properties of plant extracts are associated with diverse bioactive substances; however, several studies have shown that phenolic compounds play an important role in the prevention of inflammation. Such as quercetin that inhibits prostaglandin, leukotriene and histamine mediators [20]. In this study, the anti-inflammatory activity observed from *Opuntia ficus-indica* (L.) Mill. presented in table 1 was close to isorhamnetin-3-O-robinobioside at a dose of 5000mg/kg and it resembles to the reference drug diclofenac.

Table 1: Effect of *Opuntia ficus-indica* flowers methanol extract and isorhamnetin-3-O-robinobioside on carrageenan-induced edema in mouse

Group	Dose (mg/kg)	Average paw weight (mg)		% Edema	% inhibition
		Left paw	Right paw		
Control	-	239.88 ± 2.04	187.18 ± 7.37	28.15	-
Diclofenac	12.5	193.60 ± 6.40*	164.06 ± 3.38	18.01	36.05
Methanol extract	5000	198.56 ± 10.56*	175.74 ± 8.53	12.99	53.88
	2500	227.14 ± 3.35	185.84 ± 7.88	22.22	21.07
Isorhamnetin-3-O-robinobioside	5000	179.66 ± 3.05*	168.96 ± 2.44	6.33	77.51
	2500	204.84 ± 6.63	179.42 ± 3.51	14.17	49.68

All values represent Mean ± SEM, n=5 in each group.* p<0.0001, when compared with the control group.

Antispasmodic activity

The results presented in table 2, show that methanol extract and isorhamnetin-3-O-robinobioside presented an important inhibition of abdominal twitches at a dose of 5000 mg/kg by 78.39% and 79.17%, respectively. They were closed to phloroglucinol as reference antispasmodic that reduced the twitches of 74.26% at a dose of 80 mg/kg, while at a dose of 2500 mg/kg they presented the low percentage inhibition of 58.35% and 63.65% respectively.

Table 2: Effect of *Opuntia ficus-indica* flowers methanol extract and isorhamnetin-3-O-robinobioside on acetic acid-induced writhing

Group	Dose (mg/kg)	Writhing	% inhibition
Control	-	101.8 ± 1.16	-
Phloroglucinol	80	26.2 ± 0.86	74.26
Methanol extract	5000	22.0 ± 1.00	78.39
	2500	42.4 ± 1.21	58.35
Isorhamnetin-3-O-robinobioside	5000	21.2 ± 1.28	79.17
	2500	37.0 ± 1.58	63.65

All values represent Mean ± SEM, n=5 in each group.

Acetic acid injection provokes a stereotypical behavior in mice and rats, which is characterized by abdominal contractions, movements of the body as a whole, twisting of dorsal abdominal muscles, and a reduction in motor activity and coordination [21]. The administration of antispasmodic agent like phloroglucinol decreased the number of spasms and relieved pain. Also Methanol extract showed an important decrease of spasms at a dose of 5000mg/kg, caused by the presence of isorhamnetin-3-O-robinobioside.

CONCLUSION

In this study, we have determined that the separated flavonoïds which is isorhamnetin-3-O-robinobioside present in the methanol extract of *Opuntia ficus-indica* (L.) Mill. flowers is the responsible product for the anti-inflammatory and antispasmodic activities showed by these flowers.

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