

ANTINOCICEPTIVE, ANTI-INFLAMMATORY AND ANTIPYRETIC EFFECTS OF A FLAVONOIDAL MIXTURE LEAF SURFACE OF RHUS RETINORRHAEA

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Abstract

Antinociceptive, anti-inflammatory and antipyretic effects of a flavonoidal mixture collected from the leaf surface of *Rhus retinorrhaea* Steud. ex. A. Rich. (FMRR) (Family: *Anacardiaceae*) was studied on mice and rats. Antinociceptive activity was determined using both chemical and thermal methods of nociception in mice. In chemical method acetic acid writhing test and in thermal methods hot plate and tail flick tests were performed. Anti-inflammatory tests were conducted on rats using carrageenan-induced hind paw edema and cotton pellet granuloma. A dose-dependent algesia and hyperpyrexia was recorded in mice by flavonoid mixture. The flavonoid mixture suspension at doses of 100 and 200 mg/kg body weight produced a significant inhibition of carrageenan-induced paw edema and cotton pellet granuloma in rats. The acute toxicity tests showed no mortality or morbidity in mice up to a 3 g/kg bwdose.

Rezumat

Lucrarea prezintă rezultatele unor cercetări experimentale, efectuate pe animale de laborator (șoareci și șobolani) privind efectele antinociceptiv, antiinflamator și antipiretic ale unor amestecuri de flavonoide extrase din frunzele de *Rhus retinorrhaea*. Efectul analgezic a fost investigat cu ajutorul testului stimulului chimic (contorsiuni induse cu acid acetic), precum și prin teste de stimulare termică: testul plăcii fierbinți și testul de retracție a cozii sub influența căldurii. Efectul antiinflamator a fost cercetat utilizând ca model experimental inflamația labei animalului de experiență indusă cu caraagen.

Keywords: anti-inflammatory; antinociceptive; flavonoidal mixture; *Rhus retinorrhaea* leaves

Introduction

The leaves of *Rhus retinorrhaea* Steud. Ex. A. Rich. (*Anacardiaceae*), are used in Saudi Arabian traditional medicine in order to control and manage various ailments including inflammatory and pain conditions. The leaves are also used for the treatment of diarrhea, dysentery,

fever bleeding disorders, heavy menstrual periods and wound healing [21, 22]. Previous reports from our laboratory showed a significant anti-inflammatory activity of an ethanolic extract of *Rhus retinorrhaea* [21].

Phytochemical investigation of *Rhus retinorrhaea* reported the presence of free and combined flavonoids, tannins, volatile oil, sterols and triterpenes [21]. The present study was prompted by the previous chemical and preliminary pharmacological findings of the total alcoholic extract of the plant pharmacological studies of *Rhus retinorrhaea*. In addition to the well established medicinal benefits of numerous flavonoids such as antioxidant [5], anticancer [16], antiplatelet aggregating [17], anti-inflammatory [10], we decided to explore the possible analgesic, anti-inflammatory and antipyretic effects of isolated flavonoidal mixture from the leaves of *Rhus retinorrhaea* (FMRR) on experimental animals.

Materials and methods

Preparation of the flavonoid mixture

Rhus retinorrhaea was collected from the southern region of Saudi Arabia. The leaves (400 g) were immersed in methylene chloride for 5-10 minutes to dissolve the flavonoids accumulated on the leaf surface as a fine powder, then filtered. Removal of the solvent from the filtered extract under reduced pressure left a brownish yellow powder (weighing 32 g). Analysis of this yellow substance by thin layer chromatography, including, comparison with flavonoids previously isolated and identified from the leaves of *Rhus retinorrhaea* (free flavonoids from *Rhus retinorrhaea*) confirmed the flavonoid nature of the collected substance which was designated as FMRR.

Animals

The experiments were performed on 48 male Wistar rats (180–200g) and 138 Swiss albino mice (25±5 g) of either sex, procured from Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The animals were housed in large polypropylene cages in a temperature-controlled room (22±2°C) and provided standard pelleted food and drinking water ad libitum. The study was approved by the Institutional Animal Ethical Committee of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Neuropharmacological screening: Neuropharmacological experiments were carried out on mice, according to the scheme of Irwin [16]. FMRR in doses of 100 and 200 mg/kg b.w. were administered

intraperitoneally, the animals were observed for excitation, tremors, twitches, motor activity, pinna, corneal reflexes, and respiratory changes.

Acute toxicity studies: Six groups of mice were used consisting on 10 mice each (5 male and 5 female). One group served as control. The FMRR was suspended in distilled water and administered by oral route to five groups after overnight fast. The doses studied were 0.25, 0.5, 1, 2 and 3 g/kg body weight, and animals were observed for seven consecutive days to register mortality or other toxic signs.

Anti-nociceptive activity: The analgesic activity was measured against chemical and thermal stimuli.

Chemical method

Inhibition of acetic acid-induced writhing in mice: The test was carried out using the technique of Siegmund et al. [26], as modified by Koster et al. [15]. Animals were divided into four groups of six animals each. Group 1 was injected intraperitoneally with 0.2 ml of 3% acetic acid solution only. Group 2 and 3 were treated with the suspension of the flavonoid mixture in doses of 100 and respectively 200 mg/kg b.w. orally, and group 4 was treated with indomethacin (4 mg/kg b.w., p.o.), as a positive control, after an overnight fast. One hour after the treatment, the mice from groups 2, 3 and 4, were injected intraperitoneally with 0.2 ml of 3% acetic acid solution in order to induce the characteristic writhings. The number of writhings occurring between 5 and 15 min after the acetic acid injection was recorded.

Thermal methods

Hot-plate test: the test was performed in order to measure the latency of the response as described by Eddy and Leimback [9] with minor modifications. The temperature of the hot-plate was maintained at $56\pm 1^{\circ}\text{C}$. The mice were placed in a 24 cm diameter glass cylinder on the heated surface and the time between placement and licking of the paws or jumping was recorded as response latency. Animals were divided into three groups of six animals each. The control group was orally treated with distilled water, and indomethacin was used as positive control (4 mg/kg b.w., orally). Mice were selected 24 h before the experiment on the basis of their reactivity to the test. The animals were tested at 15, 30, 60, and 120 minutes after oral administration of the FMRR suspension (100 and 200 mg/kg b.w.) and indomethacin administration. The cutoff time was 30 s.

Tail-flick test: animals were divided into three groups of six animals each. The basal reaction time of each mouse was determined using tail-withdrawal response when one-third of the tail was introduced under the heating lamp. The reaction time was evaluated at 15, 30, 60, and 120 minutes after the oral administration of the FMRR suspension (100 and 200 mg/kg b.w.) and indomethacin 4 mg/ kg b.w. (positive control).

Carrageenan-induced paw edema in rats: Pedal inflammation in male albino rats (8 to 10 weeks old), weighing 180–200 g was induced according to the method described by Winter et al. [27]. Animals were divided into four groups of six animals each, as follows: Group 1- an injection of 0.05 ml of 1% carrageenan sodium salt (BDH) solution was made into the right hind foot of each rat under the plantar aponeurosis. Group 2 and 3- rats were treated orally with 100 and 200 mg/kg b.w. of the FMRR suspended in distilled water, 1 h before the carrageenan injection. Group 4- at the same time, the control group was given an aqueous solution of phenylbutazone (100 mg/kg b.w. p.o.), 1 h before the carrageenan injection. The measurements of foot volume were performed by the displacement technique using a plethysmometer (Apelex, France) immediately after and +2 and +3h after the injection of carrageenan. The inhibitory activity was calculated according to the following formula:

$$\text{Percent inhibition} = \left(1 - \frac{a - x}{b - y} \right)$$

where 'b' is the mean paw volume of control rats after carrageenan injection and 'y' before the injection; whereas 'x' is the mean paw volume of treated rats before injection and 'a' is the mean paw volume after carrageenan injection.

Cotton pellet granuloma in rats: the method of Goldstein et al. [11] was used with a few modifications. A sterilized cotton pellet weighing 30 mg was introduced s.c. in the groin region of rats. Animals were divided into four groups of six animals each. Animals in the control group received normal saline. They were treated orally with 100 and 200 mg/kg body weight of the FMRR suspension once daily for four consecutive days. Phenylbutazone 100 mg/kg b.w. (used as standard drug) was administered in another test group. On the fifth day, the animals were sacrificed with ether, the pellets were removed, freed from extraneous tissue and dried overnight at 60°C and weighed.

Antipyretic activity in mice: hyperpyrexia was induced in mice by s.c. injection of 20 ml/kg b.w. of a 20% aqueous suspension of brewer's yeast in the back below the nape of the neck [18]. Animals were divided into three

groups of six animals each. The animals were then fasted for the duration of the experiment (approximately 24 h), having free access to water. Control temperatures were taken 24 h after the yeast injection to determine the pyretic response to yeast. Temperatures taken 1 h prior to drug administration in febrile animals served as a pre-drug control. FMRR suspension (100 and 200 mg/kg b.w.) was given 24 h after the yeast injection and the temperatures were recorded at 60, 90, and 120 minutes after its administration.

Statistical analysis: values were expressed as mean $\pm$ S.E.M. Statistical significance was determined by Student's t-test. Values of $p < 0.05$ were considered significant.

Results

The suspension of FMRR, on acute toxicity and neuropharmacological studies, at various dose levels did not show any mortality or any morbid symptoms or deleterious effects, except at the highest dose where some signs of depression and hypothermia were shown. The antinociceptive activity of the flavonoid mixture of *Rhus retinorrhaea* was evaluated using both chemical and thermal methods of nociception in mice. The flavonoid mixture suspension of *R. retinorrhaea* significantly reduced the number of mouse abdominal constrictions induced by acetic acid solution at higher (200 mg/kg b.w.) dose (Table I). Tables II and III indicate that the FMRR suspension significantly produced analgesic activity on hot-plate and tail flick parameters undertaken. The suspension has also shown a dose-dependent decrease in yeast-induced hyperthermia in mice (Table IV). The FMRR suspension was found to suppress carrageenan-induced rat paw edema significantly at higher doses. The suspension also reduced granulation formation at the 200 mg/kg b.w. dose (Table V), although, the smaller (100 mg/kg b.w.) dose has shown the ability to reduce the inflammation and granuloma, but this effect was not statistically significant.

Table I
Effect of FMRR in mice on acetic acid induced writhing

Treatment (n = 6)	Dose (mg/kg b.w., orally)	Number writhings (mean $\pm$ S.E.) / 10 min	% Inhibition
Control (only 3% acetic acid solution)	0.2 ml 3% acetic acid solution	36.00 $\pm$ 6.32	—
FMRR + acetic acid	100	23.33 $\pm$ 4.41	35.19
FMRR + acetic acid	200	13.50 $\pm$ 2.16**	62.5
Indomethacin + acetic acid	4	10.16 $\pm$ 3.06**	71.77

* $p < 0.01$ Student's t-test.

Table II

Effect of FMRR on hot plate test on mice

Treatment (n = 6)	Dose (mg/Kg b.w. orally)	Pre- drug	Reaction time in seconds			
			Post-drug			
			After			
			15 min	30 min	60 min	120 min
FMRR	100	6.33± 1.03	7.83± 0.75	9.00± 1.41	10.16± 1.16*	8.33± 1.50
FMRR	200	5.83± 0.75	9.5± 1.22*	10.33± 1.21**	9.66± 1.50*	9.00± 1.41
Indomethacin	4	5.00± 0.89	7.83± 1.16	10.33± 1.21**	10.83± 1.16**	10.5± 1.04***

*p<0.05, **p < 0.01, ***p< 0.001 Student's t-test

Table III

Effect of FMRR on tail flick test on mice

Treatment (n = 6)	Dose (mg/Kg b.w. orally)	Pre- drug	Reaction time in seconds			
			Post-drug			
			After			
			15 min	30 min	60 min	120 min
FMRR	100	3.86± 1.05	7.90± 0.85*	7.48± 0.82*	7.61± 0.99*	7.28± 0.38*
FMRR	200	4.00± 1.18	4.5± 1.02	6.55± 0.66**	8.56± 1.21*	7.68± 1.05*
Indomethacin	4	3.66± 1.23	5.63± 1.09	8.20± 1.28*	8.36± 0.92**	8.96± 0.60**

*p<0.05, **p < 0.01 Student's t-test

Table IV

Effect of FMRR on yeast induced hyperpyrexia on mice

Treatment (n = 6)	Dose (mg/Kg b.w. orally)	Pre- drug	Rectal Temperature (°C)			
			Post-drug			
			After			
			15 min	30 min	60 min	120 min
FMRR	100	38.06± 0.33	37.98± 0.65	36.80± 0.73	36.18± 0.32**	36.33± 0.40**
FMRR	200	38.53± 0.42	38.21± 0.48*	37.21± 0.50**	36.41± 0.60*	36.13± 0.33***
Indomethacin	4	39.06± 0.27	37.08± 0.65*	37.1± 0.36**	36.36± 0.40***	36.1± 0.14***

*p<0.05, **p < 0.01 Student's t-test. ***p < 0.001 Student's t-test

Table V

Effect of suspension of flavonoidal mixture of *Rhus retinorrhaea* on carrageenan-induced and cotton pellet granuloma in rats

Treatment (n = 6)	Dose (mg/Kg b.w. orally)	Carrageenan induced edema in right hind paw		Cotton pellet induced granuloma	
		Mean increase in paw volume ml $\pm$ S.E. (3h)	% Inhibition	Mean increase in weight of pellets mg $\pm$ S.E.	% Inhibition
Control (only carrageenan)	0.05 ml of 1%	1.07 $\pm$ 0.18	—	70.66 $\pm$ 9.00	—
FMRR + carageenan	100	0.61 $\pm$ 0.13	42.99	54.83 $\pm$ 10.45	22.40
FMRR + carageenan	200	0.41 $\pm$ 0.11**	61.68	37.00 $\pm$ 8.09*	47.63
Phenylbutazone + carrageenan	100	0.32 $\pm$ 0.06**	70.09	34.16 $\pm$ 5.91**	51.65

*p<0.05 Student's t-test. **p < 0.01 Student's t-test

Discussion

The present results indicate that the suspension of flavonoidal mixture of *R. retinorrhaea* (FMRR) exerted a marked antinociceptive activity. The FMRR suspension also showed anti-inflammatory activity in the used models. The ability of the FMRR suspension to inhibit the abdominal constriction induced by acetic acid in mice is due to the fact that acetic acid causes an increase in peritoneal fluid levels of prostaglandins (PGE2 and PGF2 α), partially involving peritoneal receptors [6], and inflammatory pain by inducing capillary permeability [1]. It is a very sensitive method for screening the antinociceptive effects of compounds [19]. The possible mechanism involved in the analgesic effect of the flavonoid mixture is the inhibition of cyclo-oxygenase [13]. The thermal induced nociception indicates narcotic involvement, as thermal nociceptive tests are more sensitive to opoid μ receptors and non-thermal tests are sensitive to opoid K receptors [3]. It is also possible that the FMRR exhibited its analgesic activity probably by inhibiting the synthesis and release of prostaglandins [25].

Carrageenan-induced paw inflammation has been accepted as a useful phlogistic tool for investigating systemic inflammatory agents [8]. It has been reported that various mediators are released by carrageenan in the rat paw. The initial phase is attributed to the release of histamine and 5-HT.

A second phase is mediated by kinins and finally in a third phase, the mediator is suspected to be prostaglandin [7, 12]. On the other hand, the suspension also caused a significant anti-inflammatory activity in the cotton pellet-induced granuloma in rats. This property of the FMRR reflected its efficacy to inhibit the proliferative phase of the inflammation process, i.e. increase in the inflammation process, increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation [2, 23]. The other possibility of the present anti-inflammatory activity of the flavonoidal mixture may be the reduction in the total leukocytes and monocytes percentage and/or circulating phagocytes [24]. The present study demonstrates the efficacy of the FMRR as an analgesic, antipyretic and anti-inflammatory agent that scientifically justifies the use of this plant as a non-specific agent in Arab traditional medicine [4].

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