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Preliminary studies on anti-inflammatory and analgesic activities of *Salpichroa rhomboidea* Miers extract

M. Luigi*, M. Paola[1], B. Giancarlo[1], T. Bruno[2]

Department of Drug Science, University "G.d'Annunzio" of Chieti-Pescara, Via dei Vestini, 66013 Chieti, Italy
1. Department of Pharmacology "Giorgio Segre", University of Siena, Via delle Scotte 6, 53100 Siena, Italy.
2. Institute of Botany, University of Urbino, Via Bramante 28, 61023 Urbino, Italy.

Abstract

Objective: To study the anti-inflammatory and analgesic activity of a fraction obtained from the ethanolic extract of *Salpichroa rhomboidea* Miers aerial parts. Material and methods: Wild samples of *Salpichroa rhomboidea* Miers plants were collected at the flowering stage, powdered and then extracted with ethanol. The alcohol extract was partitioned by liquid-liquid chromatography and the fractions obtained evaporated to dryness. The residues were suspended and tested for anti-inflammatory activity in carrageenin rat paw oedema test, and for analgesic activity in Randall and Selitto test and in Tail-flick test. 24 h after the treatment, the gastric mucosa of each rat was observed macroscopically. Result: Results show interesting peripheral analgesic activity and good central analgesic activity but a lack of anti-inflammatory activity for *Salpichroa* extract. In rats treated with the tested compounds hyperaemia and ulcers were absent. Conclusion: This preliminary study shows the potential of *Salpichroa* extract, due to marked analgesic activity and the absence of gastric ulcerogenic activity.

Key words: *Salpichroa rhomboidea*, anti-inflammatory, analgesic, pharmacological activity.

1. Introduction

Salpichroa rhomboidea Miers is endemic in South America, especially in Argentina and Paraguay, where its growth is abundant and spontaneous. The popular name is "*huevos de gallo*" (Cock's eggs), because the edible fruit is a big white berry looking-like an egg.

We could not find any phytochemical study about this species, therefore we referred to a general research on plants of the same genus. The main compounds found in this genus are alkaloids and withanolides, which were generally considered responsible for the pharmacological activity. Only the Argentinian work group of Valeiro [1-3] studied the chemical composition of the genus *Salpichroa* and confirmed the presence of withanolides in *Salpichroa origanifolia*. In this work, we test the anti-

Corresponding author:
E-mail-lmenghini@unich.it

inflammatory and analgesic activities of a fraction from ethanolic extract.

2. Materials and methods

2.1 Plant material

Samples of *Salpichroa rhomboidea* Miers plants were collected at the flowering stage in a park near Perugia, Central Italy, at 400 metres a.s.l.. The plant material was authenticated at Department of Plant Biology and Agro-ambiental Biotechnologies. Faculty of Farmacy, University of Perugia, Italy, where is deposited a voucher specimen (No. A2418).

2.2 Extraction and purification

The plants were dried in an oven for 48 h at 40°C, then powdered. A sample of 145 g was extracted for two days in a percolator with an appropriate quantity of ethanol. The alcohol extract was then evaporated to dryness at 35°C under reduced pressure. The dry residue, according to the method used by Valeiro [2] in *S. origanifolia*, was partitioned by liquid-liquid chromatography with hexane-methanol-water (10-3-1).

The aqueous-methanolic phase was separated and concentrated *in vacuo,* and the resulting aqueous phase was extracted with chloroform. The chloroformic extract was dried on anhydrous sodium sulphate and evaporated to dryness. The residue (1.3g) was suspended before the test.

2.3 Pharmacological studies

The compound was preliminarily tested to evaluate its anti-inflammatory and/or analgesic activities.

Sprague-Dawley male rats (Harlan Italy s.r.l., Correzzana, Milan, Italy) 180-200 g body weight were used in these studies and were divided randomly into groups. At least five animals per group were used. The dried fraction, suspended in the solvent (a 1.6% methylcellulose solution with 1.6% tween 80), was administered orally in a dosage equivalent to 2.5g of dry herb/Kg. Indomethacin suspended in the same solvent was used as reference drug (10 mg/Kg). Control group was treated with vehicle only.

2.4 Anti-inflammatory activity

The carrageenin rat paw oedema method was used [4]. The compounds were administered orally and after 30 min oedema was produced in the right hind paw by injecting 0.1 ml of a 1% carrageenin suspension into the plantar area. The volume of the paw was measured by a plethysmometer (Ugo Basile, Comerio, Varese, Italy) before treating the animals (basal value) and then 0.5, 1, 2, 3 and 6 h after carrageenin suspension injection.

2.5 Analgesic activity

2.5.1 Randal and Selitto test [5]

Analgesic activity was measured in terms of the increase in mechanical pressure applied to the rat paw by using an analgesimeter (Ugo Basile, Comerio, Varese, Italy). The rats were dosed orally with the suspension to be tested and after 30 min 0.1 ml of a 1% carrageenin suspension was injected into the plantar tissue of the right hind paw. The pain threshold was measured prior to treatment (basal value) and at 1, 2, 3 and 6 h intervals following the carrageenin suspension injection.

2.5.2. Tail-flick test

This experiment was carried out using the technique by D'Amour and Smith [6]. The painful stimulus was applied to the rat tail by a radiant source and the reaction time was determined on a tail-flick unit (Ugo Basile, Comerio, Varese, Italy). The reaction time after the application of the painful stimulus was determined before treatment (basal value) and at 1, 2, 3 and 6 h intervals following the administration of the suspension to be tested.

Table 1.
Anti-inflammatory activity (carrageenin oedema test in the rat)

Compound	Dose	% variation of injected paw volume (± s.e.)				
	mg/Kg p.o.	0.5 h	1 h	2 h	3 h	6 h
Controls	-	33.22±3.67	38.68±5.58	43.31±1.71	56.27±2.56	72.94±5.03
S. rhomboidea	2500 (°)	26.90±4.01	34.46±3.38	47.16±4.03	53.10±3.98	69.09±6.91
Indomethacin	10	25.02±2.23	26.98±3.10	28.01±3.23*	31.10± 4.26*	46.00± 3.01*

* p =< 0.05; (°) Dose is expressed as mg of dry herb/Kg

Table 2.
Analgesic activity (Randall and Selitto test in the rat)

Compound	Dose	Pain threshold (g) (± s.e.)				
	mg/Kg p.o.	Basal	1 h	2 h	3 h	6 h
Controls	-	141.67± 3.07	136.67± 3.33	135.00± 5.12	134.00± 4.00	130.87± 4.15
S. rhomboidea	2500 (°)	148.50± 4.08	175.00± 9.72*	170.00±10.81*	185.00±7.66**	190.00±7.86**
Indomethacin	10	144.67± 4.23	170.00± 4.00**	190.00± 4.08**	210.00±3.98**	163.83± 7.50*

*p =< 0.05 ** p =< 0.001; (°) Dose is expressed as mg of dry herb/Kg

2.6 Statistical analysis

All data are presented as mean ± s.e. (see Tables 1-3) and were analysed with Student's *t* - test. Values of $p < 0.05$ were considered significant. The statistical comparisons between basal values of control and treated groups were evaluated in the same way and were not-significant.

2.7 Gastric ulcerogenic action

24 h after the treatment, the rats were sacrificed and autopsied. The gastric mucosa of each rat was observed macroscopically as reported elsewhere [7].

3. Results

3.1 Anti-inflammatory activity

The results reported in Table 1 are expressed as % variation of oedema volume versus basal volume value of each group. It is clear that *S.rhomboidea* lacks anti-inflammatory effect. It is evident a lack of anti-inflammatory activity for *Salpichroa* extract. The percentage values of oedema variation are closer to the control than to the reference substance.

3.2 Analgesic activity

3.2.1 Randal and Selitto test

The results of the peripheral analgesic activity test, reported in Table 2, are very interesting. In fact the pain threshold values obtained with *Salpichroa* extract are comparable to or better than the reference substance values. The difference between the extract and control group is always significant up to 6th h after carrageenin injection.

3.2.2 Tail-flick test

The extract (as reported in Table 3) shows a good central analgesic activity, with longer reaction time values than the reference substance ones. Also in this case the pharmacological activity is protracted in time.

Table 3.
Analgesic activity (Tail-flick test in the rat)

Compound	Dose	Reaction time in sec (± s .e.)				
	mg/Kg p.o.	Basal	1 h	2 h	3 h	6 h
Controls	-	7.43± 0.21	6.53± 0.35	6.58± 0.30	6.94±0.24	7.35±0.28
Salpichroa	2500 (°)	7.62± 0.30	8.40± 0.60*	8.52± 0.28*	8.65± 0.58*	11.00± 1.28*
Indomethacin	10	7.03± 0.37	8.33± 0.62*	8.50± 0.37*	8.84± 0.40*	9.93± 1.00*

* p =< 0.05 ; (°) Dose is expressed as mg of dry herb/Kg

3.3 Gastric ulcerogenic action

All the animals treated with indomethacin showed hyperaemia and ulcers, whereas these lesions were absent in the rats treated with the tested compounds.

4. Discussion

The fraction obtained by sequential extraction from ethanolic extract of *S. rhomboidea* showed a pharmacological profile which is clearly good to analgesic activity, however it is not considered interesting for anti-inflammatory activity. At the tested dose oedema growth inhibition by the extract is not significant. The extract shows better peripheral and central analgesic activity. Compared to Indomethacin, it shows increased values of resistance to the painful stimulus. The continuity of the extract activity is remarkable, unlike Indomethacin which shows the highest value at the third hour, the pain threshold in rats treated with the extract continue to increase throughout the observation time.

This preliminary study shows the potential of *Salpichroa*, due to marked pharmacological activity and the absence of gastric ulcerogenic activity. Further chemical investigation are required to detect and quantify the presence of the active compounds responsible for the extract pharmacological activity.

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