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Full Length Research Paper

Anti-inflammatory activity of *Wigandia urens* and *Acalypha alopecuroides*

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The anti-inflammatory activity of the chloroform, methanol and aqueous extracts of *Wigandia urens* and *Acalypha alopecuroides* were investigated on carrageenan-induced paw edema at doses of 400 mg/kg. The three extracts of *W. urens*, and the aqueous extract of *A. alopecuroides* caused significant inhibition of the edema (58.1±6.5% and 63.5±5.4%, respectively). Indomethacin was used as positive control (8 mg/kg), and inhibited edema by 66.3±5.2%. The methanol extract of *W. urens* and the aqueous extract of *A. alopecuroides*, at doses of 200 mg/kg, inhibited pellet implantation-induced granuloma formation by 69.4±6.5 and 70.6±6.6%, respectively. These levels of inhibition are higher than those exhibited by naproxen at doses of 50 mg/kg (46.1±7.1%). Both extracts showed activity on adjuvant-induced arthritis in rats, with the best effect being observed after 96 h (82.2±4.6 and 80.6±7.3%, respectively).

Key words: *Wigandia urens, Acalypha alopecuroides,* anti-inflammatory activity, plant extracts.

INTRODUCTION

Inflammation is a basic response to many injuries and is characterized by redness, warmth, swelling and pain. The compounds normally used to treat these symptoms may induce side-effects, which has lead to the search for natural compounds that could be useful in treating these kinds of disorders.

Wigandia urens (Ruiz and Pav.) HBK (Hidrofilaceae), commonly known as “suelda con suelda”, is a perennial that grows to a height of 3.0-3.6 m (González-Vazquez-Yañez et al., 2001). This plant is frequently employed in Mexican folk Medicine for the treatment of rheumatic and muscular pain, as well as inflammatory problems. Previous studies have shown that *W. urens* contains flavornoids (Wollenweber et al., 1996) and phenolic derivatives (Cao et al., 2003).

Acalypha alopecuroides (Jacq.), Euphorbiaceae, commonly known as “Hierba del Cáncer”, is a plant that grows up to 70 cm and has light green flowers. It is used in folk medicine for the treatment of asthma, infection, diarrhea and inflammatory problems (Argueta, 1994; Del Rosario et al., 1988). It has been found that *A. alopecuroides* inhibited the growth of some enterobacteria.

Here, the anti-inflammatory activity of three extracts of the aerial parts of *W. urens* and *A. alopecuroides* is reported in various animal models.

MATERIALS AND METHODS

Animals

The male Wistar rats (150-250 g) used for this study were housed at 20°C under a 12 h light/12 h dark cycle. The animals were fed (Purina) and had access to water *ad libitum*.

All the experiments were performed according to the current guidelines for the care of laboratory animals and the ethical guidelines for the use and care of laboratory animals NOM-062-ZOO-1999.

Plant material

The aerial parts of *W. urens* were collected in Zirandaro, Guerrero State, Mexico in May, 2003. *A. alopecuroides* was collected around Fortín de las Flores, Veracruz State, México in September, 2003. Both plants were identified by MS Aurora Chimal of the Department

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of El Hombre y su Ambiente, Universidad Autónoma Metropolitana-Xochimilco, where voucher specimens (MSP72 and MSP73 respectively) were deposited in the herbarium.

Preparation of the extracts

The shade-dried aerial parts were reduced to a powdery form and 100 g of the powdered sample was refluxed for 4 h with 700 mL of chloroform, methanol or water. The resulting extracts were then filtered. The chloroform and methanol were removed under reduced pressure (yield 5.3 and 7.6%, for *W. urens* and for *A. alopecuroides* 4.7 and 6.8%, respectively), and the water was removed by lyophilization (yield of *W. urens* 10.5 and 10.3% for *A. alopecuroides*).

A preliminary screening of the methanol extract of *W. urens* showed a positive $FeCl_3$ reaction for phenolic compounds (Domínguez, 1973), a positive Tortelli-Jaffe and Tschugaeff test for terpenes, a positive boric acid and citric acid test for flavonoids and positive silicotungstic acid and Dragendorff reactions for alkaloids (Domínguez, 1973). The aqueous extract of *A. alopecuroides* show-ed a positive $FeCl_3$ reaction for phenolic compounds, positive Lieberman-Burchard test for terpenes and show positive reaction for saponins (Domínguez, 1973; Harborne, 1998).

Anti-inflammatory activity

The anti-inflammatory activity of the extracts of *W. urens* and *A. alopecuroides* was investigated using the following animal models.

Carrageenan-induced paw edema in rats

Pedal inflammation in male Wistar rats was produced according to the method described by Winter et al. (Winter et al., 1962). Edema in the left hind paw was induced by injection of 0.1 mL of a 1% carrageenan solution into the sub-plantar region. The paw volume of each rat was measured at 1.5, 3.0 and 5.0 h after carrageenan injection with a plethysmometer (Ugo Basile). The drug test groups were treated orally with 50, 100, 200 or 400 mg/kg of the methanol extracts or 200 mg/kg of the chloroform or aqueous extract 1 h before carrageenan injection. The animals in the control group received (p.o.) the vehicle (5% Tween 80). Another group of rats was administered indomethacin (8 mg/kg) as a standard reference. The edema inhibition of each group was calculated according to Olajide et al. (Olajide et al., 2000).

$$\% = \frac{(C_t - C_0)\quad control - (C_t - C_0)\quad treated}{(C_t - C_0)\quad control} \times 100$$

where C_t is the displacement volume at time t after carrageenan administration, and C_o is the displacement volume before carrageenan administration.

Cotton pellet-induced granuloma

Rats were anesthetized and a 3.0 mg cotton pellet was placed subcutaneously through a skin incision at the sternum level (Winter and Porter, 1957). Once daily, beginning 30 min postoperatively, groups of 6 rats were dosed p.o. with 1 mL of water and 25 mg/kg of naproxen (as a positive control), the methanol extract of *W. urens* or the aqueous extract of *A. alopecuroides* (200 mg/kg) in a 5% Tween 80 solution. The administration was continued for 5 days. On the sixth day, the animals were sacrificed with ether, and the pellet with the accompanying granuloma tissue (diameter 6 mm) and a plug of the same size on the opposite side of the granuloma were excised, dried for 24 h at 60°C and weighed. The difference between the weight of the granuloma and the plug without the cotton pellet weight was considered in the determination of the amount of granulomatous tissue produced.

Adjuvant-induced arthritis

The method used to induce arthritis was described by Mizushima et al. (1972). Briefly, 0.1 mL of Freund's complete adjuvant was injected at the base of the tail of groups of Wistar rats. Six days after adjuvant inoculation, 0.1 mL of 1% carrageenan solution was injected into the sub-plantar region of the left hind paw, and 1 h after that, the group was treated orally with 200 mg/kg of the methanol extract of *W. urens* or the aqueous extract of *A. alopecuroides*. At the same time, the control group received p.o. vehicle (5% Tween 80), while the positive control was treated with 8 mg/kg of indomethacin. The degree of pedal edema was determined by measuring the left hind paw volume with a plethysmometer (Ugo Basile). These measurements were made before the adjuvant injection and were repeated again 6 days later at 3 and 5 h (acute phase), and continued for 24 to 96 h after carrageenan injection. The edema volume is expressed as the difference found in the left hind paw compared with the right hind paw. The inhibition of edema was calculated for each animal group in comparison with the control group.

Statistical analysis

Values are expressed as mean ± S.E.M. Treated groups were compared with the controls to statistically assess differences ($p < 0.05$ was deemed significant) via ANOVA followed by Dunnett's multiple comparison test.

RESULTS

Table 1 shows the inhibitory effect of the chloroform, methanol and aqueous extracts of *A. alopecuroides* and *W. urens* on carrageenan-induced paw edema. Both the chloroform and methanol extracts of *A. alopecuroides* were inactive in this model. However, the edema was markedly inhibited by oral pre-treatment at doses of 400 mg/kg of the methanol or aqueous extracts of *W. urens* and the aqueous extract of *A. alopecuroides* (Table 1).

The methanol extract of *W. urens* and the aqueous extract of *A. alopecuroides* were also tested at doses of 50, 100, 200 and 400 mg/kg (Table 2). The methanol extract of *W. urens* showed the strongest activity at doses of 400 mg/kg (53.6%) after 90 min of administration of the flogistic agent. However, the anti-inflammatory activity with different doses of the methanol extract remained for 180 and 300 min after administration, and the effect was similar to that observed with Indomethacin at doses of 8 mg/kg (63.4 and 73.3%, respectively). The aqueous extract of *A. alopecuroides* had no effect at doses of 50 mg/kg, and the greatest effect was observed at a dose of 400 mg/kg after 90 min of administration (63.6%). This effect was similar at 180 and 300 min (68.1 and 72.2%, respectively).

Table 1. Effect of chloroform, methanol and aqueous extracts of *W. urens* and *A. alopecuroides* at doses of 400 mg/Kg on paw edema of rats induced with carrageenan.

Treatment	Solvent	90 min	180 min	300 min
A. alopecuroides	$CHCl_3$	N.E.	N.E.	N.E.
	MeOH	N.E.	N.E.	34.5±5.6
	H_2O	49.7±6.8*	66.2±5.8*	58.1±6.5*
W. urens	$CHCl_3$	30.5±6.7	39.4±4.7	43.6±5.7*
	MeOH	41.9±5.3*	47.1±4.2*	52.4±5.1*
	H_2O	49.1±5.2*	60.7±4.6*	63.5±5.4*
Indomethacin	8 mg/kg	N.E.	63.4±4.6*	66.3±5.2*

Results are the mean of at least 5 determinations ± standard error of increase in paw volume. Values indicate percent reduction in paw volume compared with the control group treated only with carrageenan. n =7, Dunnett's test * $p < 0.05$. N.E. = No effect.

Table 2. Effect of aqueous extract of *A. alopecuroides* and methanol extract of *W. urens* at doses of 50, 100, 200 and 400 mg/Kg on paw edema of rats induced with carrageenan.

Treatment	Dose (mg/kg)	90 min	180 min	300 min
A. alopecuroides	50	N.E.	N.E.	N.E.
	100	39.6±7.5	53.4±6.3*	54.5±5.6*
	200	49.7±6.8*	66.2±5.8*	58.1±6.5*
	400	53.6±6.7*	68.1±7.7*	72.2±7.1*
W. urens	50	30.5±6.7	39.4±4.7	43.6±5.7*
	100	41.9±5.3*	47.1±4.2*	52.4±5.1*
	200	49.1±5.2*	60.7±4.6*	63.5±5.4*
	400	53.6±3.1*	66.4±3.8*	69.6±3.3*
Indomethacin	8	N.E.	63.4±4.6*	66.3±5.2*

Results are the mean of at least 5 determinations ± standard error of increase in paw volume. Values indicate percent reduction in paw volume compared with the control group treated only with carrageenan. n=7, Dunnett's test * $p < 0.05$. N.E. = No effect.

The methanol extract of *W.* urens at doses of 200 mg/kg was found to reduce 69.4 ± 6.5% of the weight of cotton pellet-induced granuloma, and the aqueous extract of *A. alopecuroides* reduced the edema by 70.6 ± 6.6%. Both extracts demonstrated inhibition of edema that was higher than that obtained when naproxen was administered (46.1±7.1%) at doses of 25 mg/kg (Table 3).

After administration of the methanol extract of *W. urens* (200 mg/kg), arthritic swelling was inhibited by 31.8 and 47.2% after 5 and 24 h, respectively. The effect was increased after 72 (76.1%) and 96 h (80.6%) (Table 4).

After 24 h of oral administration of the aqueous extract of *A. alopecuroides* at doses of 200 mg/kg, the inhibition was 20.3%, and this effect was increased after 72 h (77.5%), with the greatest inhibition being observed at 96 h (82.2%). Both extracts exhibited an important anti-arthritic effect in the chronic phase compared to indomethacin, with an inhibition of 46.9 and 75.5% after 5 and 24 h, respectively. The inhibitory effect diminished after 48 h (58.1%) and 92 h (37.9 %). The results are significant ($p < 0.05$) compared to the 5% Tween 80-treated animals.

DISCUSSION

In the present study, the anti-inflammatory effects of the methanol extract of *W. urens* and aqueous extract of *A. alopecuroides* on acute and chronic inflammatory processes has been established. It is well known that edema originated by the action of carrageenan in the rat paw involves different phases as leukocytes migrate to the injured tissues (Carvalho et al., 1999), alter the neutrophil membrane and produce highly reactive oxygen species, such as superoxide. Neutrophils plays an essential role in acute phlogosis (Galati et al., 2005). The early phase (1-2 h) of this model is mainly mediated by histamine and serotonin release (Olajide et al., 2000). The other phase is due to the release of kinins, prostaglandins, proteases and lysosomes (Alexandre-Moreira et al., 1999). The anti-inflammatory effect of the methanol extract of *W. urens* and the aqueous extract of *A. alope-*

Table 3. Effect of the methanol extract of *W. urens* and aqueous extract of *A. alopecuroides* (consecutive for 5 days) on the weight of granuloma in rats.

Treatment (dose)	Cotton pellet (mg)	Inhibition (%)
Control (1 mL)	103 ± 18.4	
Naproxen (25 mg/kg)	55.6 ± 8.9*	46.1 ± 7.1
Aqueous extract of *A. alopecuroides* (200 mg/kg)	36.3 ± 6.7*	70.6 ± 6.6
Methanol extract of *W. urens* (200 mg/kg)	37.7 ± 6.8*	69.4 ± 6.5

Results are expressed as percentage of inhibition and mean of eight determinations ± SE. *$p < 0.005$ for comparison of extract- and naproxen-treated groups with negative control.

Table 4. Effect of the aqueous extract of *A. alopecuroides* and methanol extract of *W. urens* on rat treatment with Freund's adjuvant and paw edema induced with carragenan.

Time (h)	Inhibition (%)			
	Control	*A. alopecuroides* (200 mg/kg)	*W. urens* (200 mg/kg)	Indomethacin (4 mg/kg)
3	0	N.E.	N.E.	35.0±6.4
5	0	N.E.	31.8±6.5	46.9±9.6*
24	0	20.3±2.2	47.2±7.6*	75.5±4.6*
48	0	44.9±7.6*	55.7±8.2*	58.1±4.6*
72	0	77.5±9.3*	76.1±8.9*	56.0±8.8*
96	0	82.2±4.6	80.6±7.3*	37.9±8.2

Results are the mean of at least 5 determinations ± standard error of increase in paw volume. Values indicate percent reduction in paw volume compared with the control group treated only with carrageenan. n=7, Dunnett's test * p< 0.05. N.E. = No effect.

curoides in rats with carrageenan-induced edema was significant at doses of 200 and 400 mg/kg for the study time (5 h) (Table 2).

Cotton pellet-induced granuloma is the typical model used to evaluate the transudative and proliferative components of the chronic inflammatory reaction (Ismail et al., 1997). In this case, the dry weight of the pellets correlates well with amount of granulomatous tissue (Swinglen and Shideman, 1972). The methanol extract of *W. urens*, the aqueous extract of *A. alopecuroides* (200 mg/kg) and naproxen (25 mg/kg) are effective at lowering the moist weight of the cotton pellet, and the effect of the extracts was higher than that demonstrated by naproxen (Table 3). This effect correlates with the ability of both extracts in reducing the proliferative phase of granuloma tissue formation (Ojalde et al., 2000).

The methanol extract of *W. urens* and the aqueous extract of *A. alopecuroides* also exhibited anti-arthritic effects in the chronic phase (Table 4). This extract decreased the edema induced 48 h after carrageenan treatment (6 days after adjuvant inoculation). The effect lasted up to 72 h and was higher than that observed with indomethacin at these time points. Based upon the results obtained, it is concluded that the methanol extract of *W. urens* and the aqueous extract of *A. alopecuroides* have activity in both the acute and chronic phases of inflammation, thereby supporting the use of *W. urens* and *A. alopecuroides* in various ailments in Mexican folk medicine. Detailed studies are in progress to identify the structures of the active principle(s) present in the methanol extract of *W. urens* and aqueous extract of *A. alopecuroides*.

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