Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Antinociceptive and anti-inflammatory activities of crude extracts of *Ipomoea involucrata* leaves in mice and rats

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ARTICLE INFO

Article history:
Received 25 October 2010
Received in revised form 27 November 2010
Accepted 15 January 2011
Available online 20 February 2011

Keywords:
Ipomoea involucrata
Antinociceptive
Anti-inflammatory activity

ABSTRACT

Objective: To investigate antinociceptive and anti-inflammatory activities of crude extract from *Ipomoea involucrata* leaves (Convolvulaceae) in mice and rats. **Methods:** The antinociceptive activity was tested using acetic acid-induced abdominal writhing test in mice. The anti-inflammatory activity was evaluated using egg albumin -induced oedema of rat paw. **Results:** Phytochemical screening showed the presence of alkaloids, flavonoids, saponins, terpenoids and tannin. At the doses of 25-100 mg/kg, *Ipomoea involucrata* exhibited dose-dependent and significant increase in pain threshold in acetic acid -induced writhing test of mice ($P<0.05$, student t-test) The administration of *Ipomoea involucrata* leaf extract (25-100 mg/kg) showed dose-dependent decreases in paw volume of egg albumin induced oedema in rats and a significant higher anti-inflammatory activity compared to the standard control (Aspirin). **Conclusions:** These results support the claims on the traditional use of the of *Ipomoea involucrata* leaves in the treatment of toothache, rheumatic pains and other inflammatory conditions. Studies on the isolation and structural elucidation of the active principle are still needed being carried out.

1. Introduction

Inflammation is the characteristic response of mammalian tissue to injury. It is the succession of changes which occur in a living tissue when it is injured, provided that the injury is not of such a degree as to destroy its structure and vitality at once. According to its duration, inflammation could be described as acute, sub acute or chronic.

In Africa herbal drugs are well established in many cultures and still play a major role in the healthcare delivery system. *Ipomoea involucrata* (Convolvulaceae) is commonly known as morning glory weed or railway creeper. In Nigeria, it is called duman-kwaadi in Hausa, alukerese or atewagba in Yoruba and Eriri ala or fifilori in Igbo.

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The plant is an annual or perennial twinning hairy herb, rooting at nodes[1,2]. It is a creeper found in varying habitat ranging from forest, dry to aquatic. It is distributed throughout tropical Africa and northern South Africa. It has stems up to 4 m long. A vigorous twiner, sometimes covering surrounding vegetation. Stems, leaves, peduncles and large bracts hairy. Leaves broadly heart-shaped with a deeply cordate base, up to 13 cm long, hairy on both surfaces, often more densely so below. Flowers funnel-shaped, whitish to pink, often darker in the throat, in heads arising from a distinct boat-shaped bract. Capsule spherical, hairless The cytotoxic effect of *Ipomoea involucrata* has been investigated[3]. The leaf extract showed antidiarrhoeic effect[2]. Traditionally, *Ipomoea involucrata* is used in the treatment of pile, headache, toothache and rheumatism.

This study therefore evaluated the antinociceptive and anti-inflammatory activities of *Ipomoea involucrata* leaves in mice and rats. The purpose was to establish scientific basis for the use of *Ipomoea involucrata* in the treatment of

toothache, rheumatism and other inflammatory conditions since no such study has been documented to the best of our knowledge.

2. Materials and methods

2.1. Plant materials

Fresh leaves of *Ipomoea involucrata* were collected in December 2007, from a local garden in Amike, Orlu LGA in lmo state Nigeria. The plant was authenticated by H.D Onyeachusim of Botany department, University of Port Harcourt, Nigeria, where voucher specimen was maintained .The leaves were air- dried at room temperature for 10 days. The dried leaves were pulverized to fine powder and stored in air tight container.

2.2. Extraction of plant material

The dried powdered leaves (500 g) were extracted with ethanol by maceration for 5 days. The resulting mixture was filtered and re-extracted twice. The filtrates were mixed together and dried in a rotary evaporator. This gave a dried crude extract with 21.60% yield. This was stored in air tight container at temperature of 4 ℃ for further use.

2.3. Animals

Mice (25-35 g) and rats (100-200 g) were obtained from the animal house of Department of Biochemistry and Animal Science of University Port Harcourt, Nigeria. The animals were allowed 7 days for acclimatization and were fed on compressed growers mash and water *ad libitum*. They were starved for 24 hours prior to experiment but were granted access to water.

2.4. Phytochemical screening

The powdered drug and crude extract were screened for bioactive constituents using standard procedures to identify different compounds by characteristic colour changes as described by Sofowora[1].

2.5. Acetic acid- induced writhing test in mice.

The method of Aliyu[4] was adopted in this study with little modification. Swiss albino mice grouped into five groups of six animals per group. These animals (group 1-3) were administered 25, 50 and 100 mg/kg of *Ipomoea involucrata* extract. Group 4 animals were given 5 mL/kg normal saline and group 5 were given 2 mg/kg aspirin. After 30 minutes, 0.6%v/v acetic acid solution (10 mg/kg i.p) was administered to all the groups. The number of writhing that occurred between 5-15 minutes after the administration of acetic acid was counted. The percentage of writhing was calculated and compared to the standard.

$$\text{Percentage inhibition of writhing (\%)} = \frac{\text{Mean number (control)} \times \text{Mean number (test)} \times 100}{\text{Number (control)}}$$

2.6. Anti-inflammatory activity

The method of Akah and Nnambie[5] was adopted with little modification. Inflammation of the hind paw was induced by injection of 0.1 mL of fresh egg albumin into the sub plantar surface of the right hind paw of rats one hour before the experiment. Different doses of the extract (25-100 mg/kg) and standard were administered to the animals. Group 1 was given 100 mg/kg aspirin, group 2 was given 5 mL/kg normal saline and group 3-5 were given 25-100 mg/kg of extract.

Following induction of inflammation, the measurement of the foot up to the tibia- tarsal articulation was done by displacement technique (fluid displacement method) at 30 minutes intervals using phlethysiomometer. Then the percentage reduction of edema was recorded up to two and half hours.

2.7. Statistics

Results were expressed as mean values ± standard error of mean (SEM). Statistical analysis was done using the student *t*-test, where $P<0.05$ was considered significant.

3. Results

The phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, saponins, tannins and carbohydrate in the *Ipomoea involucrata* leaf extract.

The LD_{50} of the ethanol extract of Ipomoea involucrata leaf in male albino rats was found to be 760 mg/kg intraperitoneally, using Lorke's method .

The Table 1, 2 and 3 show the mean paw volume, percentage inflammation and percentage inhibition of inflammation respectively.

Phytochemical screening showed the presence of alkaloids, flavonoids, saponins, terpenoids and tannin. At the doses of 25-100 mg/kg, *Ipomoea involucrata* exhibited dose-dependent and significant increase in pain threshold in acetic acid -induced writhing test of mice ($P<0.05$, student *t*-test) The administration of *Ipomoea involucrata* leaf extract (25-100 mg/kg) showed dose-dependent decreases in paw volume of egg albumin induced oedema in rats and a significant higher anti- inflammatory activity compared to the standard control (Aspirin).

Table 1
Mean volume of paw oedema (mL).

Group	Mean volume of paw oedema time (min)					
	Dose	30	60	90	120	150
Control(N/S)	5 mL/kg	1.90±0.05	1.90±0.04	1.80±0.05	1.90±0.06	1.16±0.05
Aspirin	2 mg/kg	1.60±0.04	1.40±0.03	1.20±0.02	1.00±0.01	0.90±0.01
Ipomoea involucrata	25 mg/kg	1.70±0.06	1.70±0.05	1.50±0.03*	0.90±0.04**	0.86±0.02
Ipomoea involucrata	50 mg/kg	1.60±0.06	1.50±0.03	1.10±0.02*	0.88±0.01**	0.89±0.01
Ipomoea involucrata	100 mg/kg	1.50±0.04	1.20±0.02	1.00±0.01*	0.89±0.01**	0.89±0.01

*$P < 0.05$, ** $P < 0.001$.

Table 2
Percentage inflammation (%).

Group	Percentage inflammation Time (min)					
	Dose	30	60	90	120	150
Control(N/S)	5 mL/kg	70.64	52.29	37.38	47.22	20.00
Aspirin	2 mg/kg	80.73	89.9	64.65	22.22	11.43
Ipomoea involucrata	25 mg/kg	70.63	69.72	60.55	13.89	17.14
Ipomoea involucrata	50 mg/kg	56.88	29.35	12.12	2.80	2.90
Ipomoea involucrata	100 mg/kg	70.64	52.29	37.38	47.22	20.00

Table 3
The percentage inhibition of inflammation (%).

Group	Percentage inhibition of inflammation Time (min)					
	Dose	30	60	90	120	150
Aspirin	2 mg/kg	29.36	47.71	62.63	52.78	80.00
Ipomoea involucrata	25 mg/kg	19.27	10.10	35.36	77.78	88.57
Ipomoea involucrata	50 mg/kg	29.26	30.27	39.44	86.11	82.86
Ipomoea involucrata	100 mg/kg	43.12	70.65	87.88	97.20	97.10

Table 4
The antinociceptive effects of lpomoea involucrata leaf extract on acetic acid-induced writhing in mice.

Group	Dose	Mean number of writhing	Percentage inhibition (%)
Control (N/S)	5 mL/kg	62.10±2.9	0.00
Aspirin	100 mg/kg	28.71±1.70**	53.77
Ipomoea involucrata	25 mg/kg	38.15±1.90*	38.57
Ipomoea involucrata	50 mg/kg	20.20±1.20**	67.47
Ipomoea involucrata	100 mg/kg	12.40±0.80**	80.03

*$P<0.05$, **$P<0.001$.

4. Discussion

The results of preliminary phytochemical screening showed the presence of flavonoids, tannins, terpenoids, saponins and alkaloids in *Ipomoea involucrata* leaves. Alkaloids and flavonoids are well known for their ability to ability to inhibit pain perception[6].

Flavonoids as anti-oxidants[7,8] also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation[6,9,10-13].

These constituents have been reported to exert potent analgesic and anti-inflammatory properties[2,11,15]. It has also been established that flavonoids directly inhibit prostaglandins[16] which could cause pain and inflammation. Therefore the antinociceptive and anti- Egg albumin-induced inflammation model is a significant predictive test for anti-inflammatory activity[9-15]. These results are an indication that *Ipomoea involucrata* can be effective in acute inflammatory disorders.

In acetic acid-induced abdominal writhing which is the visceral pain model[9,10-13,17], the results show that *Ipomoea involucrata* extract produced significant analgesic activity at all doses with $P<0.001$ (student t- test). The results also show that the extract is more potent than aspirin at similar or higher doses. The analgesic effect of *Ipomoea involucrata* can be attributed in part, to its anti-inflammatory effect as,

in visceral pain model. The precursor releases arachidonic acid through cycloxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism[14,15].

This therefore, implies that the inhibition of acute inflammation by these extracts leads to their inhibitory effect on pain development process[10-13,17].

Inflammatory effects of the extract of *Ipomoea involucrata* leaves could play a part in ameliorating the symptoms associated with toothache, rheumatism and inflammatory diseases of mankind.

However, the exact mechanism by which the extract of *Ipomoea involucrata* leaf exhibits its antinociceptive and anti- inflammatory properties is not yet clear. But it may involve the peripheral pain fibers since the antinociceptic was comparable to that of aspirin. What ever be the case, further studies are being carried out for structural elucidation of active principle and determination of the mechanism of the action of the *Ipomoea involucrata* leaf extract

Conflict of interest statement

We declare that we have no conflict of interest.

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