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Original Article

EVALUATION OF THE ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOL EXTRACT OF THE ROOT OF *MIMOSA PIGRA* LINN (FABACEAE) IN ALBINO RATS

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ABSTRACT

Objective: *Mimosa pigra* roots are used in traditional medicine in the treatment of fever, headaches and cold. This study investigated the ethanol extract of the root of *Mimosa pigra* for its analgesic and anti-inflammatory activities in albino rats.

Methods: The analgesic activity was evaluated by radiant heat tail flick method while the anti-inflammatory effect was investigated using fresh egg albumin induced paw edema in rats. The plant extract was evaluated at 250 mg/kg and 500 mg/kg. All administrations were done through the oral route.

Results: Preliminary phytochemical screening showed that the extract contains; steroids, tannins, flavonoids, phlobatanins, saponins. The LD_{50} was found to be greater than 5000 mg/kg. The results showed that oral administration of 250 mg/kg of *Mimosa pigra* showed significantly ($P<0.05$) analgesic activity in30, 60 and 150 minutes while 500 mg/kg produced significantly ($P<0.05$) analgesic activity in 30, 60, 120 and 150 minutes. The two tested doses (250 mg/kg and500 mg/kg) were found to produce percentage inhibition of rat paw edema (42.60% and 49%) at 150 minutes compared to the positive control group of 63.20%.

Conclusion: The findings of this study showed that the ethanol extract of this plant possesses significant anti-inflammatory and analgesic activities.

Keywords: Anti-inflammatory, Analgesic, Ethanol, *Mimosa pigra*, Phytochemical.

INTRODUCTION

Plants have important roles as sources of prescription drugs in western medicine and their active constituents also serve as templates for synthetic drug optimization and provide intermediates that are used in the production of semi-synthetic drugs. All over the world, hundreds of higher plants are cultivated for substances useful in medicine and pharmacy [1]. In 2001,the researchers identified 122 compounds used in modern medicine, which were derived from "ethnicmedical" plant sources; 80% of these have had an ethnic medical use identical or related to the current use of the active elements of the plant [2]. Today, scientists are using these renewable resources to produce a new generation of therapeutic solutions. Thus, providing treatments for a lot of diseases such as hypertension, diabetes, cancer, fibrosis, spinal cord injuries, hepatitis, arthritis, inflammation and pains. Inflammation is a protective response and a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [3]. Without inflammation, wounds and infections would never heal. Inflammation is normally closely regulated by the body because progressive destruction of the tissue would compromise the survival of the organism [4].

Pain is the most common reason for physician consultation in the United States [5]. It is a major symptom in many medical conditions, and can significantly interfere with a person's quality of life and general functioning [6]. The roots of Mimosa pigra are sniffed for head colds, fever and headache in traditional medicine. The roots are also used traditionally in the treatment of asthma. In Sumatra, roasted and ground mimosa leaves are made into an infusion, which is drunk to treat a weak heart or weak pulse [7]. Literature revealed that anti-inflammatory and analgesic studies have not been reported for the root of this plant.

Therefore, this study aims to evaluate the anti-inflammatory and analgesic activities of the ethanol extract of the root of *Mimosapigra* Linn.

MATERIALS AND METHODS

Plant material

The root of the plant was collected from Chaza village in Suleja, Niger State by Mallam Mu'azan of National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. The plant was identified and authenticated in the herbarium of the same institute by Jemilat Ibrahim, a staff of the same institute where a voucher specimen (NIPRD/H/6405) has been deposited in the herbarium.

Extraction of plant material

The roots of *Mimosa pigra* Linn were washed under a running tap to remove debris, then sliced into smaller pieces and air-dried under ambient condition. The air-dried root was pulverized by grinding using a mechanical grinder and stored in air tight container. Thereafter, 1 kg of the coarse powder of air-dried root was subjected to solvent extraction by maceration for 72 hours using 96% ethanol (Sigma-Aldrich, Germany). The extract was concentrated using the rotary evaporator and carefully evaporated to dryness over a water bath at a temperature of 40 °C. The percentage yield was then determined. The extract was stored in a refrigerator until use.

Animals used

Albino rats (150-200 g) of both sexes were obtained from the animal house of the Department of Pharmacology, Faculty of Basic Medical sciences, University of Port Harcourt. All animals were housed in cages (5 in each cage) in the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Port Harcourt. The animals were fed with the standard diet and water *ad libitum* and were deprived of food and water overnight prior to the experiment. All the standard ethical requirements for experimental animals were complied with.

Experimental protocols

Phytochemical screening

Phytochemical screening was carried out on the ethanol extract of the *Mimosa pigra* root for the detection of various plant constituents according to the protocol of [8].

Acute toxicological evaluation

A total of eighteen albino rats of either sex weighing 150-200g were used in the determination of the acute toxicity of the root of *Mimosa*

pigra Linn. The animals were randomly allotted into six groups of three animals each and the first three groups were treated with 10 mg/kg, 100 mg/kg and 1,000 mg/kg of *Mimosa pigra* extract respectively, after 14 days of adaptation. The animals were treated via the oral route. They were observed frequently on the day of treatment for 24 hours for signs of toxicity or adverse effects and death. After 24 hours, no animal died, nor showed any sign of adverse effect. Subsequent doses of 1,600 mg/kg, 2,900 mg/kg and 5,000 mg/kg of the plant extract was administered to the other three groups of three animals each and observed as above [9].

Evaluation of analgesic activity

Tail flick test

The animals were divided into four groups of five animals each. The tail flick latency of the rats was assessed with Ugo Basile tail-flick analgesiometer. Group 1 animals was administered with 0.5 ml of distilled water, group 11 animals received 25 mg/kg aspirin while groups 111 and 1V were given 250 mg/kg and 500 mg/kg of the extract respectively. All administrations were done through the oral route by means of a canal. An infra-red (IR) intensity of 50 and cutoff time of the 60s was fixed to avoid tissue damage. The site of application of radiant heat in the tail was maintained at 1.5 cm, measured from the tip of the tail. The initial reading was taken immediately before administration of test and standard drugs and then 30, 60, 90, 120 and 150 minutes after treatment. The withdrawal time of the tail from the radiant heat source (in seconds) was taken as the reaction time or tail flick latency. The difference in tail flick latency or mean increase in latency after drug administration was used to indicate the analgesia produced by the test and standard drugs [10].

Evaluation of anti-inflammatory activity

Egg albumin induced paw edema test

The animals were divided into four groups of five animals of either sex each. Oral route of administration used for treatment. 0.5 ml of distilled water was administered to the negative control group (group A), 10 mg/kg of indomethacin was administered to the positive control group (group B) and groups C and D were treated with 250 mg/kg and 500 mg/kg of plant extract respectively. Thirty minutes after treatments, acute inflammation was induced by intraplantar administration of 0.1 ml of fresh egg albumin in the right hand paw of the animals [11]. Paw volume of rats was measured prior to administration of egg albumin and after at predetermined intervals of 30 minutes for 150 minutes using vernier caliper. The change in paw volume was measured using vernier caliper and anti-inflammatory activity calculated [12]. Percentage inhibition of paw thickness was calculated using the formula:

$$\% \text{ inhibition of paw thickness} = \frac{[tCn - tC_o - (tT_n - tiT_o)]}{(tCn - tC_o)} \times 100$$

Where tC_n = paw thickness of particular time point of control animals,

tC_o= paw thickness before induction;

tT_n = paw thickness of particular time point of treated animals,

tTo = paw thickness before induction.

Statistical analysis

The results were expressed as mean±SEM. Statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by student's t-test. The statistical analysis was done to determine the significance of difference between the control groups and the treated groups. P-values<0.05 was considered to be statistically significant.

Table 1: Phytochemical screening

Chemical constituents	Observation
Alkaloids	-
Tannins	+
Anthraquinones	-
Phlobatannins	+
Flavonoids	+
Triterpenes	+
Saponins	+

+shows the presence of chemical constituent, - shows the absence of chemical constituent

Table 2: Analgesic activity of ethanol extract of *Mimosa pigra* root by tail flick method (mean±Standard Error of Mean)

Groups	Dose Mg/kg	TIME (minute)±SEM					
		0	30	60	90	120	150
Distilled water	0.5 ml	5.26±0.72	4.78±0.84	4.14±1.08	3.64±0.63	4.40±0.68	4.45±0.45
Aspirin	25	6.90±0.84	6.94±1.07*	5.50±0.44*	5.50±0.6*	5.14±0.68	5.10±0.45
Extract	250	5.30±0.84	5.18±1.30*	4.46±0.90*	3.80±0.41	4.32±0.68	4.56±0.32*
Extract	500	6.78±0.80	6.54±0.77*	5.60±0.90*	4.44±0.39	5.56±0.59*	5.18±0.79*

Values are expressed as mean±SEM, n=5

* P<0.05statistically significant values when compared with the control group and the standard drug.

Table 3: Effect of ethanol extract of *Mimosa pigra* root on egg albumin induced rat paw edema. Mean paw size measured in (mm±Standard Error of Mean) with percentage inhibition of rat paw edema

Groups	Dose Mg/kg	Time (minute)±SEM					
		0	30	60	90	120	150
Distilled Water	0.5 ml	3.28±.08	8.48±0.17 (2.76%)	8.46±0.20 (5.50%)	8.41±0.18 (9.30%)	8.36±0.19 (11.60%)	8.28±0.18 (15.20%)
Indomethacin	10	3.46±0.16	7.78±0.40 (16.92%)	7.37±0.32* (24.80%)	7.00±0.36* (31.0%)	6.25±0.28* (45.10%)	5.30±0.31* (63.20%)
Extract	250	3.58±0.13	8.77±0.46 (8.46%)	7.58±0.21* (29.15%)	7.16±0.18* (33.14%)	6.94±0.39 (35.02%)	6.40±0.19* (42.60%)
Extract	500	4.01±0.12	8.03±0.32 (14.42%)	7.19±0.39* (33.59%)	7.00±0.27* (38.60%)	6.50±0.14* (44.10%)	5.50±0.08* (49.00%)

Values are expressed as mean±SEM, n=5,

*P<0.05 statistically significant values when compared with the control group and the standard drug.

RESULTS

Preliminary phyto chemical screening revealed the presence of steroids (triterpenes), tannins, flavonoids, phlobatannins, saponins most of which are phenolic compounds (table 1). The acute toxicity study of the ethanol extract of the root of *Mimosa pigra* showed no adverse effect or mortality even at 5000 mg/kg.

A comparison was made between the control and the treated groups and between the standard and the treated groups. From the results obtained, oral administration of 250 mg/kg of *Mimosa pigra* showed significant (P<0. 05) analgesic activity in 30, 60, and 150 minutes, while oral administration of 500 mg/kg produced significant (P<0 . 05)analgesic activity in 30, 60,120 and 150 minutes when compared with the control group and the standard drug (aspirin) (table 2).

The plant extract at 250 mg/kg significantly (P<0 . 05) inhibited acute inflammation caused by egg albumin induced paw edema at 60, 90 and 150 minutes, while 500 mg/kg of the plant extract showed significant (P<0 . 05) inhibition of rat paw edema at time intervals of 60, 90 120 and 150 minutes when compared with the control group and indomethacin.

The ethanol extract of *Mimosa pigra* inhibited egg albumin induced paw oedema in a dose dependent manner. Oral administration of 250 mg/kg of the extract showed significant (P<0 . 05) percentage inhibition of egg induced rat paw edema at 60, 90 and 150 minutes when compared with the negative control group and indomethacin, while 500 mg/kg dose showed significant (P<0. 05) inhibition at 60, 90, 120 and 150 minutes when compared with the distilled water and the standard drug, but exhibited a greater percentage inhibition than indomethacin at 60 and 90 minutes, respectively as shown in table 3. However, the results showed that the percentage inhibition of paw edema by the two doses of the extract increased with increase in time, even though the 500 mg/kg extract exhibited a higher percentage inhibition when compared to that of 250 mg/kg dose. At 90 minutes, indomethacin (31.0%), 250 mg/kg and 500 mg/kg of the extract showed 33.14% and 38.60% percentage inhibition of edema which could be said to be comparable.

DISCUSSION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic efficacy [13].

The acute toxicity study of the ethanol extract of the root of *Mimosa pigra* showed no adverse effect or mortality even at 5000 mg/kg, thus, it can be considered to be relatively safe [9].

The ethanol extract of *Mimosa pigra* root showed significant analgesic and anti-inflammatory activities.

An analgesic is any member of the group of drugs used to achieve analgesia, relief from pain by acting on the peripheral and central nervous system. The tail flick assay is the method for measuring centrally mediated analgesic activity [10]. *Mimosa pigra* root extract increased tail flick latency towards the radiant heat source in a dose dependent manner. This corresponds to the reports of [14] that the leaf extract of Phlogacanthus thyrsi florus showed significant central analgesic activity (P<0.05). The results obtained in this study also corroborates that of [10] which reported that percentage of inhibition of *Cassia auriculata* extract was found to be statistically significant in comparison to control (aspirin) in the tail flick assay. The analgesic effect of this plant extract may be mediated through inhibition of cyclooxygenase and/or lipo oxygenase (and other inflammatory mediators) [15]

Inflammation is the response of living tissue to injury which involves activation of various enzymes, mediator release, cell migration, tissue breakdown and repair. Egg albumin-induced paw edema in rats is an *in vivo* model of inflammation used to screen agents for anti-inflammatory effect [16].

Chemical mediators such as histamine, serotonin (5-HT), kinins and prostanoids mediate an acute inflammation induced by phlogistic agents such as fresh egg albumin [17]. Mimosa pigra root ethanol extract showed a significant inhibitory effect on rat paw edema in the middle phase and the late phase of egg albumin–induced inflammation. Carrageenan (egg albumin)-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis [18].

Indomethacin acts by inhibiting the cycloxygenase 2 (COX-2) which converts arachidonic acid to prostaglandins, histamines, kinnins which are mediators of inflammation. Thus, the anti-inflammatory activity of the plant may be attributed to its ability to inhibit prostaglandins, histamine, leucotrienes (mediators of inflammation). Since flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase [19].

The findings of this anti-inflammatory study corresponds to the report of [20] that hydro-acetone extracts of *Lannea microcarpa* fruit and leaves inhibited acute inflammation.

The observed analgesic and anti-inflammatory activity of Mimosa pigra may likely be due to the presence of phytochemical constituents such as flavonoids, tannins, saponins and triterpenes. This is because antinociceptive and inflammatory activities of many plants have been attributed to plant constituents such as flavonoids [21], tannins [22] and triterpenes [23].

CONCLUSION

The results obtained from this study have shown that the root extract of *Mimosa pigra* seems to possess both analgesic activity and anti-inflammatory activities which is dose dependent and contains bioactive constituents. Further studies could be done to isolate the active principles and elucidate the mechanism of action of the biologically active constituents.

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CONFLICT OF INTERESTS

The authors hereby declare no conflict of interest

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