

Full Length Research Paper

Antinociceptive potential of the methanolic extract of *Microdermis puberula* (Hook. F. Ex. Planch)

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This study investigated the antinociceptive effects of the methanolic extract of the stem wood of *Microdermis puberula* (Hook. F. Ex. Planch) in Swiss albino mice of either sex with weights ranging from 17 to 25 g. Acetylsalicylic acid and morphine were used as standards for the antinociceptive tests while distilled water was used as control. Acetic acid - induced mouse writhing reflex test and hot plate test were used to determine peripheral and central pain inhibition respectively, using five groups of five animals for each model. The extract was administered in three dosages: 600, 1200 and 2400 mg/kg orally. Acetylsalicylic acid was administered at 100 mg/kg p.o., morphine at 10 mg/kg intraperitoneally and distilled water at 1 ml/kg p.o. Results showed that the extract of *Microdermis puberula*, at 1200 and 2400 mg/kg significantly inhibited peripheral pain, compared with acetylsalicylic acid ($p < 0.05$). The extract of *Microdermis puberula*, at a dose of 2400 mg/kg inhibited pain by increasing the pain threshold in the hot plate test 58.83%. These, therefore, suggest that the methanolic extract of *Microdermis puberula* possesses mild antinociceptive property. The phytochemical analyses of the extract showed the presence of flavonoids, saponins and cardiac glycosides. Results from the LD₅₀ determination showed that a dose of 1412.5 mg/kg i.p. produced 50% death in mice.

Key words: Antinociceptive, *Microdermis puberula*, phytochemical analysis, LD₅₀, acetylsalicylic acid.

INTRODUCTION

In the last decades, there has been a renewed interest in research and utilization of medicinal plants, particularly flora of the tropical rainforest. The revived interest in plant-derived drugs is attributed to the current widespread belief that "green medicine" is cheap, safe, more dependable and accessible than the costly synthetic drugs many of which are associated with intolerable effects (Adeneye and Benebo, 2007). According to the World Health Organization (WHO), about 80% of the world's population depends wholly or partly on plant-derived pharmaceuticals (WHO, 1996). Correspondingly, in most developing countries, including Nigeria, there is a heavy dependence on herbal preparations for the treatment of human and animal diseases despite the

availability of conventional pharmaceuticals.

Microdermis puberula belongs to the family Pandaceae (Hook.f.ex Planch). It is a shrub of about 60 m in height. In Igboland of Southern Nigeria, it is called "Mgbugbo", while in Yorubaland in same southern Nigeria; it is called "Apata". *M. puberula* contains 57.73% DL- methionine, 17.32% crude protein, 9.6% ash, 15.05% crude fibre (Esonu et al., 2004). Previous studies on the roots showed that it has significant hypotensive and vasorelaxing properties (Zamble et al., 2006). The plant has been evaluated as a leaf- meal feed in laying hen diets, improving egg output production and size (Esonu et al., 2004)- The aqueous extracts and alkaloids of *Microdermis* have been shown to stimulate sexual parameters in male rats' sexual behaviour (Zamble et al., 2008).

Whereas much work has been done on this plant as stated above, there is no documented record on its pain relieving activity. The objective of the present study was

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to evaluate the antinociceptive effects of *M. puberula*.

MATERIALS AND METHODS

Drugs

Acetylsalicylic acid, morphine and acetic acid were purchased from Sigma Aldrich, U.S.A. Distilled water of pH 6.62 was obtained from the Department of Biochemistry, Nigerian Institute of Medical Research (NIMR), Yaba and was used as the control test substance.

Animals

Swiss albino mice weighing 17 to 25 g were employed for this study. All the animals were obtained from the Animal House of the College of Medicine, University of Lagos. They were kept in a hygienic, well-ventilated environment and had free access to food and water *ad-libitum*. All animal experiments were conducted in compliance with NIH Guidelines for care and use of laboratory animals (Zimmermann, 1983).

Plant material

The dried stem wood of *M. puberula* was purchased from Mushin market, Lagos. The voucher specimen of the plant was deposited in the herbarium of the Department of Pharmacognosy, College of Medicine, University of Lagos with Number FHI109038, where it was identified and authenticated by the Chief Laboratory Technologist of the Department of Pharmacognosy, Faculty of Pharmacy, College of Medicine, University of Lagos—Mr. T. I. Adeleke.

Preparation of extract

The dried stem wood of *M. puberula* weighing about 100 g was cleaned, air-dried, chopped into smaller bits and dried in the oven at 60°C for at least 5 days. This was then milled into fine powder using the warring blending machine. An 80 g quantity of the powder obtained was extracted with methanol using the Soxhlet extraction apparatus and 83 ml of the extract was obtained. The extract obtained was concentrated to a light brown residue in an oven at 38°C. The yield of the extract was 8.1% w/w (15.2 g)-of dry matter reference to the powdered stem.

Phytochemical screening

The methanolic extract of *M. puberula* stem wood was screened for the presence of its active compounds using different phytochemical analysis procedures (Trease and Evans, 2000).

Chromatography

The methanolic extract of *M. puberula* stem wood was weighed and dissolved in 20 ml distilled water to obtain 200 mg/ml concentration of the herbal preparation. The pH of the resulting solution was determined at 26.8°C ambient temperature. The extract was thereafter subjected to thin layer chromatographic separation.

Acute toxicity study

Fifteen mice were randomly divided into three groups of five mice

each. Mice in Group 1 received 5000 mg/kg of the extract while the mice in Groups 2 and 3 received 10,000 and 15,000 mg/kg respectively, orally. Toxic signs and mortality were observed for 24 h (Jayasekar et al., 1997). No mortality was recorded at the end of this time, for up to 15 g/kg body weight p.o.

The methanolic extract was also administered to another 3 groups of five mice each in graded doses of 1875, 3750 and 7500 mg/kg respectively, intraperitoneally. The highest oral dose was half the value of i.p dose. Signs and symptoms of toxicity and mortality were observed for 24 h. At the end of this period, different degrees of mortality were observed in each of the groups of mice. Probit analysis using SPSS software was used to determine the LD₅₀ of the methanolic extract.

Evaluation of antinociceptive potential

Pharmacological investigation was carried out on the extract of the test plant to evaluate its antinociceptive potential. Two experimental models (acetic acid - induced mice writhing reflex test and Hot-plate test) were employed, using acetylsalicylic acid and morphine as the standard reference drugs and distilled water as the test control.

Rotarod test in mice

Potential central nervous system effects, including sedation or nonspecific motor effects, were evaluated by measuring the ability of mice to maintain balance on an accelerating and rotating rod (rate of rotation was 4 to 40 rpm over a 5-min period) after s.c. administration of normal saline vehicle. Mice were conditioned before the experiment (at least five trials), and animals were selected as those remaining on the Rotarod for 300 s. On the day of experiment, three trials were consecutively performed to establish baseline values. Mice were tested at 30, 60, 90, and 120 min after administration.

Acetic acid induced mouse writhing reflex

The acetic acid induced mouse writhing reflex test was performed based on the method described by Koster et al. (1959). The mice were divided into 5 groups of 5 animals each, A 200 mg/ml concentration of the extract of *M. puberula* at 600, 1200 and 2400 mg/kg and distilled water (1 ml/kg) were administered orally to the mice 30 min before the intraperitoneal injection of 0.6% acetic acid solution (at the dose of 10 ml/kg). The animals were observed for writhing or stretching for 15 min. A reduction in the number of writhings as compared to the control group was considered an evidence of analgesia, which was expressed as the percentage inhibition of writhing.

Hot plate test

The mice were divided into 5 groups of 5 animals. The surface of the hot plate was maintained as 55 ±0.5°C. The mice were individually placed one at a time onto the heated plate and time required for the animal to begin to lick its paw or attempt to escape from the heated surface was taken as the end point for the initial response of the animal to avoid tissue damage. The cut-off time for latency response was 15 s. This was determined before the administration of the different concentrations of the extract (9600, 1200 and 2400 mg/kg) p.o., acetylsalicylic acid (100 mg/kg p.o.), morphine (10 mg/kg i.p) and distilled water (100 ml/kg) at 0, 30, 60, 90 and 120 min intervals, and after the administration of the drug.

Table 1. Effect of *Microdermis puberula* on writhing reflex in mice.

Group	Dose (mg/kg)	Number of Writhes	Percentage Inhibition (%)
Distilled water	-	85.5	-
<i>Microdermis puberula</i> extract	600	60.5	29.23
	1200	46.0	46.20*
	2400	35.2	58.83*
Acetylsalicylic acid	100	15.7	81.60*

n=5, 'n' represents the number of animals in each group. Duration of test =15 min. Values are expressed as percentage inhibition
*P<0.05 is statistically significant.

Data analysis

In the writhing test, the degree of antinociception was expressed as the percentage decrease in the number of writhings and was calculated according to the formula:

$$\text{Percentage inhibition of writhing (\%)} = (C - T)/C \times 100$$

where C is the mean number of writhings in saline-treated mice and T is the number of writhings in drug-treated mice. In the radiant tail-flick test, the data were expressed as percentage antinociception, which was calculated using the equation:

$$\text{Percentage antinociception (\%)} = [(TL - BL)/(7 - BL)] \times 100$$

where TL is test latency and BL is baseline tail-flick latency. All values were expressed as mean \pm SEM. Comparisons of the means of two groups were performed with Student's t-test for non-paired and paired data. Several treatment groups were compared with the control group by using analysis of variance followed by the Duncan test. The differences between means were considered significant when the value of P was < 0.05 and results obtained were recorded as Mean \pm SEM (Standard error of the mean). Statistical comparisons were made using the student's 't' test. P values less than 0.05 (p<0.05) were considered statistically significant.

RESULTS

Antinociceptive tests

Acetic acid induced writhing reflex test

Table 1 shows the effect of the extract of *M. puberula* on mouse writhing reflex as an evaluation of the antinociceptive property of the extract. In this assay, the extract at 600 to 2400 mg/kg p.o produced dose-dependent reduction in the numbers of writhes. The mice given distilled water produced the greatest number of writhes. Acetylsalicylic acid (100 mg/kg) reduced the number of writhes considerably and acted as a good inhibitor of peripheral pain. The extract at 1200 and 2400 mg/kg p.o produced reduction in the number of writhes, comparable with acetylsalicylic acid.

Hot plate test

The antinociceptive effects of the methanolic extract of *M. puberula* are presented in Table 2. The extract (600 to 2400 mg/kg p.o) increased the pain threshold in a dose-related manner. At 600, 1200, and 2400 mg/kg, the extract inhibited pain stimuli by 3.15, 3.5 and 11.90% respectively, comparable with the control group. Acetylsalicylic acid, however, inhibited pain stimuli but could not prolong the reaction time. In contrast, morphine (10 mg/kg i.p) significantly (p <0.05) prolonged the reaction time to pain to almost 10 s and inhibited pain by 61%. The extract at the dose of 2400 mg/kg significantly (p< 0.05) increased the pain threshold, in comparison with the control, and produced antinociceptive effect higher than that of acetylsalicylic acid.

Phytochemical analysis

Previous works have reported the vasorelaxing, hypotensive and antioxidant properties of the root extract of *M. puberula*. These properties could be attributed to the active constituents of *M. puberula*. Phytochemical analyses reported the presence of flavonoids, saponins and cardiac glycosides (Trease and Evans, 2000).

Acute toxicity test

Results obtained from the intraperitoneal administration of graded doses up to 7.5 kg/mg of the extract of *M. puberula* indicated a lethal dose of 1412.5 mg/kg, after 24 h.

DISCUSSION AND CONCLUSION

The rationale behind the antinociceptive evaluation of the methanolic extract of *M. puberula* was to scientifically establish the claim of its effectiveness in peripheral pain management. A dose-dependent antinociceptive effect

Table 2. Effects of *M. puberula* on hot-plate test in mice.

Group	Dose (mg/kg)	Time for licking paw(Pre treatment) (s)	Time for licking paw (Post treatment) (s)	Percentage inhibition (%)
Distilled water	-	1.50±0.21	1.57±0.19	0.52
<i>Microdermis puberula</i> extract	600	1.67±0.22*	2.09±0.25	3.15
	1200	1.96±0.17	2.41±0.34	3.45
	2400	2.01±0.32	3.56±0.56	11.90*
Acetylsalicylic acid	100	1.39±0.17	2.15±0.05	5.58
Morphine	10	1.99±0.31	9.90±0.25	60.79*

n=8, Values are expressed as mean ± SEM, *p<0.05 is statistically significant.

was exerted by the extract on acetic acid induced mouse-writhing reflex. The analgesic action of NSAIDs has been explained by their inhibition of cyclooxygenase, which synthesizes prostaglandins at the peripheral cell-damage sites (Vain, 1971). It is possible that prostanoids released from cyclooxygenase are involved in the processing of acetic acid-induced visceral nociception. The extract (2400 and 1200 mg/kg p.o) produced a slight inhibition of pain stimuli similar to NSAIDs. This suggests that the extract possesses mild antinociceptive activity that may be peripherally mediated.

This effect may be attributed to its ability to prevent some pain mediators such as prostaglandins from sensitizing nociceptors that induce writhes in response to chemical substances injected intraperitoneally.

The hot-plate test is referred to as an acute test of nociception. It is considered selective for centrally acting or opioid-like antinociceptive compounds. Centrally acting antinociceptive drugs elevate pain threshold of animals towards heat. The extract at higher doses elevated pain threshold in hot plate model.

Previous studies have reported that *M. puberula* possessed vasorelaxing, hypotensive and antioxidant activities (Zamble et al., 2006) and these may perhaps account for its resistance to some level of stress and pain.

In conclusion, the extract produced mild antinociceptive actions.

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