

Full Length Research Paper

Evaluation of analgesic and anti-inflammatory activities of n-butanol phase of the leaves extract of *Microtrichia perotitii* DC (Asteraceae)

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The n-butanol phase of the methanolic leaves extract of *Microtrichia perotitii* DC was studied for its analgesic and anti-inflammatory activities. Both percent inhibition obtained from analgesic (59.30% for 100 mg/kg, 33.90% for 50 mg/kg and 69.20% for 25 mg/kg i.p) and anti-inflammatory (values for 25, 50 and 100 mg/kg were found significantly different at $P < 0.05$, $P < 0.01$ and $P < 0.001$) (mean paw diameter in hours) studies has shown that the leaves have very strong activity to prevent pains (writhing) in the central system and motor performance of the mice as well as the growth of oedema in the hind paw of the rats respectively. The analgesic effect is dosage independent while the anti-inflammatory effect is dosage dependent and all these activities could be associated with the type of phytochemicals likely to be present in the leaves of plant.

Key words: *Microtrichia perotitii*, n-butanol phase, analgesic, anti-inflammatory.

INTRODUCTION

Microtrichia perotitii DC belongs to the family Asteraceae or Compositae (Hutchinson, 1973). Locally, the plant is called " mai jankai" because of the yellowish appearance of it's flowers or "sawun keke" because of it's crawling nature by the indigenous Hausa speaking people in communities where the plant is grown (Magaji, 2003). The plant is widely distributed in West Africa. In Nigeria, the plant is found in the North in swampy areas of places such as Zaria province which extends up to Birnin Gwari in the present Kaduna state of Nigeria (Hutchinson and Dalziel, 1963). The plant, *M. perotitii* is a diffused, much branched pubescent annual plant and varies between 1 foot or more in height (Watson and Dallwitz, 1992). The leaves are obovate, cuneately narrowed into petiole, coarsely toothed and 1/3 - 1 inch broad, the flowers are floriate while the fruits are drupes (Daniel, 1887). The Compositae herbal plants were commonly used as

natural remedies by many natives around the world and many of its useful natural properties and compounds have been produced commercially and marketed as natural healthcare products as either natural medicines or supplements (Herbal portal, 2009).

Similar biological studies were conducted on some plants of the Compositae family. These include *Lactuca scariola*, *Artemisia absinthium* and *Tithonia diversifolia* which proved potent in both analgesic and anti-inflammatory studies. Some bioactive compounds isolated from these studies include sesquiterpenes, saponins and alkaloids (Fayyaz et al., 1992; Victor et al., 2003).

Traditionally, the wet or dried leaves and flowers are used for the treatment of toothaches. The leaves are used for the treatment of rashes in children. However, classical Yoruba spiritualists use the herb to dispel evil spirit (Magaji, 2003).

This study was conducted to ascertain the claim by tradomedical practitioners for the use of the plant to treat pain related sicknesses especially in persons with

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toothaches as well to identify the compounds responsible for the efficacy of the plant.

EXPERIMENTAL

Plant collection

Fresh sample of the plant was collected at the swampy areas of Rigasa village, a sub-urban settlement in Kaduna metropolis around April, 2005. The plant was authenticated by Mallam Musa Muhammad at the Herbarium of the Department of Biological Sciences Ahmadu Bello University Zaria and given a herbarium no of 998.

Plant sample preparation

The leaves from the aerial part of *M. perotitii* were removed (plugged) and allowed to dry under shade for about two weeks. The dried leaves were ground into coarse powder using pestle and mortar.

Extraction

300 g of the powdered leaves were extracted with 5 L of methanol. The aliquot was gradually concentrated in vacuo and allowed to dry. A yield of 20 g (6.7%) of a hard cake was obtained (Ahmed et al., 1981). The residue was further extracted by partitioning between, Ether (F1), water (F2), Butanol (F3/F4) and HCl (F5) phases. The F4 portion of n-Butanolic phase was used for the studies from the preliminary investigation carried on it.

Animal

Male and female swiss albino mice (18 - 25 g) and adult Wister rats (180 - 250 g) were used for all pharmacological studies. The animals were kept under standard laboratory conditions and fed with standard feeds (Excel feeds Plc Zaria) and provided with drinking water *ad-libitum*.

Analgesic studies

The acetic acid induced writhing test in mice as described by Koster et al. (1959) was employed. Swiss albino mice were divided into 5 groups of 6 mice each. The first group was given 10 ml/kg of normal saline *i.p* and served as control, groups 2, 3 and 4 received 25, 50 and 100 mg/kg of body weight *i.p* respectively, while the fifth group was given 10 mg of Proxicam per kg body weight *i.p*. Thirty minutes later, mice in all groups were treated with Acetic acid (0.06% 1 ml Acetic acid of 1ml per 100 g *i.p*). Five minutes after Acetic acid injection mice were placed in individual cage and the number of abdominal contractions was counted for each mouse for a period of 10 min. Percentage inhibition of writhing was calculated using the expression:

$$\text{Inhibition (\%)} = \frac{\text{mean number of writhings (control)} - \text{mean no of writhings (test)}}{\text{Mean number of writhing (control)}} \times 100$$

Anti-inflammatory studies: acute inflammation

The Formaldehyde (2.5% v/v) induced inflammation was used as

described by Winter et al. (1962). Therefore, Wister rats were divided into 5 groups of 5 rats each. Thirty minutes before injection of formalin, the groups were treated Intraperitoneally thus: group 1 received 10 mg of ketoprofan per kg (+ve control), group 2 received 1ml normal saline per kg (-ve control), groups 3, 4 and 5 received 25, 50 and 100 mg of n-Butanol extract per kg body weight *i.p* , respectively. The increase in paw diameter was measured using vernier caliper. The difference in weight of the right hind paw and the left hind paw indicates inflammation. Measurement was done immediately before and after 1 - 5 h following formalin injection. The percentage inhibition of the growth of oedema was calculated from the expression:

$$\text{Inhibition (\%)} = \frac{\text{mean control (N/S)} - \text{mean treated}}{\text{Mean control (N/S)}} \times 100$$

RESULTS

Analgesic studies

The result of the analgesic studies is presented as mean \pm SEM and considered significant when compared to students' *t*-test. However, the percentage inhibition obtained for both 25 and 100 mg/kg were higher than the value obtained for Proxicam 10 mg/kg used as standard drug for the studies (Table 1). The percentage inhibition was highest at the lowest dosage (25 mg/kg) and was closely followed by the highest concentration (100 mg/kg) both of which are remarkably higher than the standard drug used (Proxicam 10 mg/kg) (Table 1). The analgesic activity exhibited by the n-butanol phase is thus dosage independent.

Anti-inflammatory studies

The result of anti-inflammatory studies was also presented as mean \pm SEM. In the anti-inflammatory studies, the value obtained showed a significant reduction in the growth of oedema in the hind paw of the rats. The values were higher than that of ketoprofen 10 mg/kg *i.p* which was used as a standard drug in the studies. The values at 25 and 100 mg/kg were similar meaning that the activity is dosage dependent (Table 2).

DISCUSSION

The analgesic properties and anti-inflammatory effect of n-butanol phase of the crude extract of the leaves of *M. perotitii* were investigated in this study. In the analgesic studies acetic acid writhing test was used because of its sensitivity that could provide different grades of noxious stimuli in chemically induced tissue damage. The acetic acid test has an ability to mimic human clinical pain conditions and production of tonic stimulus (Victor et al., 2003). Similarly, the acetic acid induced writhing has

Table 1. Analgesic Activity studies Of the n-butanol phase of crude extract of the leaves of *M. perotitii*.

Treatment (mg/kg)	No of abdominal writhings	% inhibition
N/saline	22.2 ± 2.7	00.00
100	7.7 ± 1.0	59.30
50	12.5 ± 2.3	33.90
25	5.8 ± 1.5	69.20
Proxicam 10	10.3 ± 2.4	45.00

Table 2. Anti-inflammatory activity studies of the n-butanol phase of crude extract of the leaves of *M. perotitii*.

Treatment (mg/kg)	Mean paw diameter (cm) in hours				
	1	2	3	4	5
N/ saline	0.20 ± 0.03	0.16 ± 0.02	0.15 ± 0.01	0.15 ± 0.01	0.13 ± 0.01
25	0.16 ± 0.01	0.16 ± 0.02	0.14 ± 0.01	0.11 ± 0.02 ^a	0.09 ± 0.02 ^a
50	0.18 ± 0.02	0.13 ± 0.02	0.12 ± 0.01 ^a	0.10 ± 0.01 ^b	0.10 ± 0.01 ^a
100	0.15 ± 0.02	0.14 ± 0.01	0.12 ± 0.02 ^a	0.11 ± 0.01 ^a	0.09 ± 0.01 ^a
Ketoprofen (10 ml)	0.13 ± 0.02 ^a	0.12 ± 0.02	0.11 ± 0.01 ^a	0.08 ± 0.01 ^c	0.07 ± 0.01 ^b

Data presented as mean ± SEM, n=5 for all groups. a, b and c are significantly different from control at P < 0.05, P < 0.01 and P < 0.001 respectively in a students' t-test.

been used to evaluate analgesic effects of drugs and the response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathways (Ranjit et al., 2006) The acetic acid induced writhing allows the acid to act via central mechanisms and motor performance of the animal. Therefore, the n-Butanol phase of the crude leaves extract of *M. perotitii* has a significant inhibition in the duration of the writhing in each mouse (Hosseini et al., 2003; Formukong et al., 1988). The intraperitoneal injection of acetic acid produces an abdominal writhing response due to sensitization of chemo-sensitive nociceptors by prostaglandins. Increase level of prostanoids as well as lipoxygenase products have been found in the peritoneal fluid after the injection of the acetic acid. The analgesic effect of any plant extract may therefore be due to either its action on visceral receptors sensitive to acetic acid, to the inhibition of the production of algogenic substances or the inhibition at the central level of the transmission of painful message (Magaji et al., 2008). In this study, the % inhibition at the at the lowest concentration of 25 mg/kg was the most effective as was observed in some members of the family (Hosseini et al., 2003; Chakraborty et al., 2004; Adeolu et al., 2008) (Table 1).

In the Anti-inflammatory studies, the Formaldehyde induced oedema is believed to be a multimediated phenomenon that liberates diversity of mediators which could be in two phases, the first being the release of serotonin and histamine while the second after the one hour is mediated by prostaglandins. The cyclooxygenase products and the continuity between the phases are

provided by kinins (Ageel et al., 2005; Adeolu et al., 2008). In this study, the result obtained showed a significant reduction in the growth of oedema in the hind paw of the rats. There is correlation between reduction in oedema and the concentration of the drug which therefore suggest that the activity is dosage dependent which also was corroborated with the values obtained from Feldene (10 mg/kg *i.p*) used as a standard drug (Hosseini et al., 2003) (Table 2). The extract of some members of the Asteraceae (Compositae) family were found to exhibit active anti-inflammatory activity either in mice or rats. These include, *Achyrocline satureioides*, *Ageratum conyzoides*, *Bidens pilosa*, *Mikana glomerata*, *Vernonia cinerea* etc (Mazumder et al., 2003; Heloina et al., 2005).

Conclusion

The traditional use of *M. perotitii* for the treatment of toothache which most often is associated with swellings at the gums could be ascertain to some certain extent as shown from the results of this study which significantly showed degree of % inhibition of preventing pains (analgesic) as well as swellings of the gum (anti-inflammatory effects). The activity of the leaves could be associated with the type of Phytochemicals such as flavonoids, alkaloids and saponins isolated from some members of the Asteraceae (compositae) family that were found to exhibit analgesic and anti-inflammatory activities.

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