American Journal of Biology and Life Sciences
2015; 3(5): 151-154
Published online August 30, 2015 (http://www.openscienceonline.com/journal/ajbls)

Open Science

Analgesic and Anti-inflammatory Activities of *Melastomastrum capitatum* (Vahl) A. Fern. & R. Fern. (Melastomataceae) Leaf Methanol Extract

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To cite this article

Ukwubile Cletus Anes, Elsie Joseph Agabila. Analgesic and Anti-inflammatory Activities of *Melastomastrum capitatum* (Vahl) A. Fern. & R. Fern (Melastomataceae) Leaf Methanol Extract. *American Journal of Biology and Life Sciences*. Vol. 3, No. 5, 2015, pp. 151-154.

Abstract

Majority of the population around the world especially in Africa had always used medicinal plants as first source of health care to fight various diseases. Most of these medicinal plants may have scientific evidence to be considered in health care delivery. This present study was conducted in order to determine the analgesic and anti-inflammatory activities of leaf methanol extract of *M .capitatum* in *invivo* albino mice model. Acetic acid-induced abdominal constriction or writhing analgesic models in Swiss albino mice (weighing between 15-25g) were used for studying analgesic activity of the leaf extract. Doses 25, 50 and 100 mg/kg body weight of the extract were administered intraperitoneally (i.p). The activity was compared with a standard reference drug ibuprofen B. P (200 mg/kg) and negative control. Results were analyzed by SPSS version 22 using ANOVA and compared at $p \leq 0.05$ significance level. In the acetic acid-induced writhing reflex model, M. capitatum extract and the reference drug significantly (P =0.01 to 0.05) decreased the mean total number of abdominal constriction in the mice in a dose dependent fashion. The percentage inhibition of the abdominal constriction reflex was increased dose dependently from 0% in the negative control group to 78.6% at the highest dose of 100mg/kg. Anti-inflammatory evaluation on the other hand against carrageenan-induced paw oedema in Swiss albino male mice showed also a dose-dependent anti-inflammatory effect with inhibition of 92.00 %when compared to that of the standard drug ibuprofen. The values obtained in both studies were not significantly different from that of the standard drug. The study showed that the *M. capitatum* was effective in pain reduction as well as an anti-inflammatory agent. The study showed that *M. capitatum* leaf extract demonstrated significant analgesic and anti-inflammatory activities than the standard drug mediated through peripheral pain mechanism.

Keywords

Analgesic, Anti-inflammatory, M. Capitatum, Acetic Acid, Carrageenan

1. Introduction

Melastomastrum capitatum is an erect or sub-erect perennial herb or a shrub 40–60(90) cm. high. Branches obtusely 4-gonous and deeply furrowed upwards, cylindrical towards the base, sparsely strigillose, the bristles short, oppressed, longer at the nodes; inter-nodes 4–17 cm. long. Leaf-lamina 5–13(16cm) x 1·5–6·5(7·5cm), ovate to narrowly elliptic, acute at the apex, acute or roundish at the base, membranous to somewhat rigid, dark green above, paler beneath, oppressed-strigose on both faces, the bristles on the upper face prolonged by white raised lines, longitudinally 5–7-nerved, the longitudinal nerves impressed above, prominent beneath, the transverse ones inconspicuous above, conspicuous beneath, very oblique; petiole 0·5–3 cm. long, slender, somewhat compressed, strigose [1].

Flowers are numerous, in dense heads surrounded by the upper leaves and by foliaceous bracts; inner bracts closely sheathing the receptacle, ovate to ovate-lanceolate, scarious, ciliate at margin, glabrous or strigose on the back along the median line. Receptacle 10 x 5 mm., cylindric or ovoid-cylindric, surrounded by long whitish bristles at the very base. Sepals 6–7 x 2·5–3 mm., triangular-subulate, ciliate; intersepalar segments. Petals 20 x 15 mm., widely obovate, light mauve to violet. Long stamens: anthers 7–8 mm. long, lanceolate-subulate, purple; pedoconnective 5–6 mm. long with the 2 anterior appendages 2 mm. long. Short stamens: anthers 6–7 mm. long, lanceolate, yellow; pedoconnective 0·75 mm. long. Fructiferous receptacle 13–15 x6–7 mm.,

urceolate, cream-coloured to greyish. Seeds 0·75 mm. long, indistinctly tuberculate. The plant is found in Zambia, Tanzania; Uganda; W. tropical Africa; Zaire; Nigeria; Sierraleone, Senegal and habituated on fringes of riparian woodland, semi-evergreen rain forests, swamps and fire clearings as well as plateaus [2].

The leaves are used as anti-rheumatic agent, cure stomach aches, purification of blood vessels and blood as well as for alleviating diuresis (leaf-sap) Medicines: sedatives, etc. (leaf-sap) Medicines: pulmonary troubles (leaf-sap) Medicines; "intestines" [3]. These uses were however not scientifically proved. Aside these, there are no other uses which has been documented for the plant in traditional medicine.

This present study was conducted in order to determine the analgesic and anti-inflammatory activities of leaf methanol extract of *M. capitatum* in *in vivo* albino mice model.

2. Materials and Methods

2.1. Collection and Identification of Plant

Fresh leaves of *Melastomastrum capitatum* were collected in the evening hours from Mambila plateau Sarduana L.G.A of Taraba State in June 2015 and was identified by a taxonomist at the Department of Science Laboratory Technology where a voucher specimen number *FEDPOBAL2015MELA001*was deposited for the plant. A plant press was done and deposited in the herbarium of Biology Unit, Science Lab. Tech. Dept., Federal Polytechnic Bali, Taraba State.

2.2. Preparation of Plant Material and Extraction

The leaves of *M. capitatum* were air dried at room temperature at 25^0C for 2 weeks and using electronic blender. The dried and ground powder (585.5g or 0.59kg) was defatted in 900mL pet-ether and then extracted with aqueous methanol (1250mL) in an air tight separately funnel for two (2) days at room temperature. The extract was then filtered using a Whatman filter paper. The filtrate was concentrated in vacuo at room temperature. The methanol extract was further fractionated successively using solvents of increasing polarity from the eluotropic series in this order: carbon tetrachloride (CCl_4), Chloroform ($CHCH_3$), Acetone ($(CH_3)_2CO$), Ethyl acetate ($CH_3COOCH_2CH_3$) Methanol (CH_3OH) [4].

Final weight of the methanol leaf extract was 35.5g.

% yield of extract = 6.0% (*i.e. %Yield= final wt/initial wt x100*).

2.3. Analgesic Studies of Extract in Acetic Acid-Induced Writhing in Mice

The method described by Ukwubile was used [5]. Twenty-five Swiss albino male mice were each weighed and divided into 5 groups of 5 animals each. Animals from group one (negative control) were administered intraperitoneally (i.p) with saline, group two (positive control) were administered with ibuprofen 10mg/kg (i.p), while groups 3, 4 and 5 were given (i.p) 25mg//kg, 50mg/kg and 100mg/kg of the methanol extract respectively. After 30 minutes, the animals were given (i.p) acetic acid 0.6 % v/v, and observed for abdominal contraction by viewing the animals on the abdomen for contraction of abdominal muscle using hand lens for 10 minutes after a stimulation period of 5 minutes. Percentage inhibition of writhing was calculated using:

$$\% \text{ Inhibition} = \frac{\text{MnWc} - \text{MnWt} \quad X \quad 100}{\text{MnWc}}$$

Where, MnWc = mean number of writhing negative control

MnWt = mean number of writhing treated

2.4. Anti-inflammatory Studies in Carrageenan-Induced Paw Oedema in Swiss Albino Mice

Carrageenan was administered to the paw of the animals to manifest oedema, and followed by test drug which was given in the presence of the positive control (ibuprofen 200mg B.P). The animals were also divided into 5 groups of 5 animals per group. Group one is the negative control (normal saline) and group 2 is the positive control (ibuprofen 200mg/kg) given i.p. Groups 3, 4 and 5 were each administered 25mg/kg, 50mg/kg and 100mg/kg of the crude extract. Acute inflammation was produced by sub-planar administration of 0.1ml 1% carrageenan in the right hind paw of the animals in all the groups. Paw volumes were then measured at 0 hour, 1 hour, 2 hours, 3 hours and 4 hours after carrageenan injection, using vernier calipers [5].

3. Results and Discussion

Acetic acid - induced writhing test is commonly used as an experimental animal model for analgesic study. This method is very sensitive, and able to detect analgesia at doses that may appear to be inactive in other analgesic screening procedures [5]. The effect of *M. capitatum m*ethanol extract against writhing caused by acetic acid [table 1] showed decreased production of irritant such as prostaglandins and blocking the pain sensitizing mechanism, induced bybradykinin, interleukin and other analgesic substances. The percentage inhibition obtained with *Melastomastrum capitatum* leaf methanol extract was greater than of the positive control Ibuprofen 200 mg/kg b.w at $p \leq 0.05$ (One-way ANOVA).Analgesic activity of *M. capitatum* was in dose dependent fashion in the mice.

Carrageenan induced rat paw edema is a suitable experimental animal model for evaluating the anti-inflammatory activity of natural products and this is believed to be in three phases [5]; the first phase (1h after carrageenan challenge) involves the release of serotonin and histamine from mast cells, the second phase (2h) is provided bykinins and the third phase (3h) is mediated by prostaglandins, the cycloxygenase products and lipoxygenase products [6]. The

metabolites of arachidonic acid formed through the cycloxygenase and lipoxygenase pathways are two important classes of inflammatory mediators, especially prostaglandin E_2 is known to cause or enhance the cardinal signs of inflammation, similarly, leukotriene B_4(product of lipoxygenase pathway) is a mediator of leukocyte activation in the inflammatory cascade [7-18]. From the results, the methanol extract of *M. capitatum* leaves inhibited Carrageenan induced rat paw oedema at 50 mg/kg and 100 mg/kg, which was significantly different ($P \leq 0.05$) from the control [table 2]. The methanol extract significantly inhibited ($p \leq 0.05$) carrageenan induced rat paw oedema, at the third hour the activity of the crude extract was higher than that elicited by ibuprofen 200mg [fig.1] in a dose-dependent manner, so inhibition of carrageenan induced pawoedema by the crude extract could also be due to its inhibitory activity on the lipoxygenase enzyme. The study showed that methanol extract of *M. capitatum* showed a dose dependent activities in albino mice.

4. Conclusion

This study showed the efficacy of a novel plant *M. capitatum* as an analgesic and anti-inflammatory agent which also justifies the use of this plant as an agent in folk medicine for treating various ailments. Further studies are needed to determine the constituents responsible for the observed activities.

Table 1. *Effect of M. capitatum Leaf methanol extract on Acetic acid-induced writhing in Swiss albino Mice.*

Animal group/dose (mg/kg)	Mean abd.contraction/10 min± SE	% Inhibition
Group I (normal saline)	42±2.6 *	0
Group II (Ibuprofen;200)	20±1.2	52.4
Group III (MCME;25)	12±0.6	71.4
Group IV (MCME;50)	11±0.5	73.8
Group V (MCME;100)	9±0.2*	78.6

MCME (*M. capitatum methanol* extract), abd (abdominal), * Statistical significant difference at p<0.01 (One-way ANOVA).

Table 2. *Effect of M. capitatum methanol extract and ibuprofen in carrageenan-induced paw edema in mice.*

Group	Treatment	Dose(mg/kg)	Paw diameter ±SEM(mm)				% Inhibition
			1h	2h	3h	4h	
1	Normal saline	10	1.64±0.09	2.36±0.08	3.04±0.14	2.24±0.13	0
2	Ibuprofen	200	0.60±0.13	0.46±0.07	0.38±0.10	0.20±0.03	91.00
3	MCME	25	0.80±0.17	1.4±0.16	1.34±0.21	1.22±0.16	45.50
4	MCME	50	0.58±0.15	0.42±0.19	0.34±0.10	0.26±0.15	88.40
5	MCME	100	0.68±0.10	0.38±0.08	0.26±0.12	0.18±0.09	92.00

* MCME = M. capitatum methanol extract, h = hours, p≤0.05 (ANOVA)

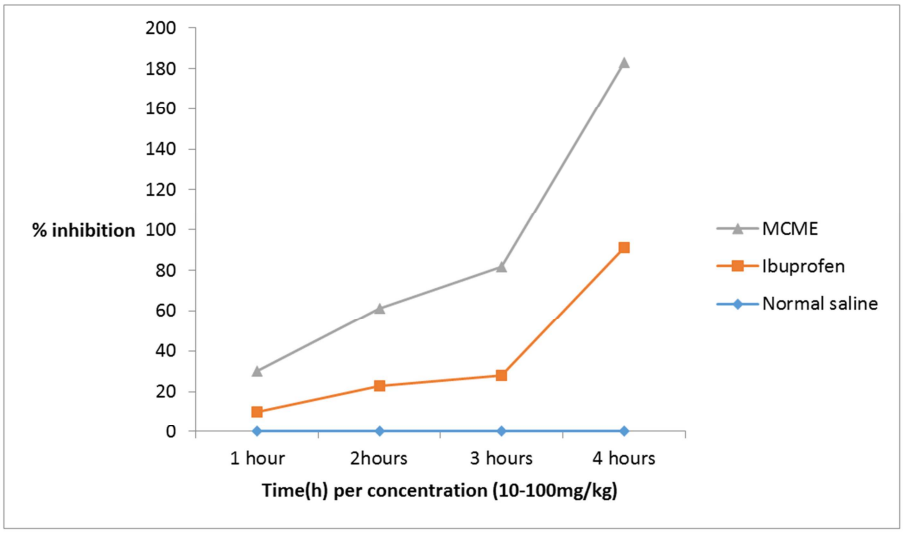

Figure 1. *Anti inflammatory activity of fractions of methanol leaf extract of M. capitatum (MCME).*

Animal Ethics

Animals used in this research was according to animal in research act of the University of Jos Nigeria.

Acknowledgement

The authors are grateful to Mr. BulusAuta of the

Department of Pharmacology Faculty of Pharmaceutical Sciences, University of Jos Plateau State Nigeria, for supplying the mice used in this research work.

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