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Research Article

PERIPHERAL AND CENTRAL ANTINOCICEPTIVE ACTIVITIES OF THE CRUDE METHANOLIC EXTRACT AND FRACTIONS OF *MOMORDICA BALSAMINA* LINN

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ABSTRACT

The peripheral and central antiniciceptive properties of *M. balsamina* were evaluated in animal pain models. Methanolic extract of the whole plant of *M.balsamina* and its fraction(s) significantly (P>0.05) inhibited nociception in Albino mice and Wister rat. *M. balsamina* was less effective than Aspirin, a known antinociceptive agent. Aqueous fraction of *M. balsamina* was found to possess greater percentage of the active constituents in *M. balsamina,* implying that polar constituents of *M. balsamina* may be responsible for the observed antinociceptive effects. N-hexane fraction had insignificant nociceptive inhibitory effect while, Chloroform, ethylacetate, and N-butanol fractions inhibited nociceptive activity in writhing and hot plate models. Unlike the non-steroidal anti-inflammatory antinociceptives, *M. balsamina* has no ulcerative property; rather it has slight insignificant ulcer protective effects. These findings suggest that the extract of *M. balsamina* contain antinociceptive agents and supports its use in folk medicine for the management of Pain.

Keywords: Antinociceptive, *Momordica balsamina,* Tail flick, Writhing reflex, Hot plate and formalin pain models.

INTRODUCTION

Pain is usually perceived as an unpleasant feeling, but it is important for survival. It is the sense of pain felt that alerts of danger and warns of impending danger. The sense of pain helps us to know when to withdraw from a hot surface or to seek medical attention for broken bones. Pain affects man physically, mentally and socially. It affects the quality of life we live and is one of the determinants of our joy. Potent drugs are needed to alleviate the suffering of patient with pain, especially pain associated with terminal diseases (like AIDS, and cancer).

A number of drugs have been used for the management of pain. Most of these drugs are either not very effective, expensive or have side effects that limit their uses. This development has led to an unending search for potent antinociceptive with fewer side effects. Paracetamol (acetaminophen) is one of the most popular and widely used drugs for the treatment of pain and fever[1].

It is relatively safe, but has no effect on chronic pain and could damage liver cells. Another group of drugs that is widely used for the management of pain are the non-steriodal antiinflammatory drugs (NSAIDS). They inhibit the action of cyclooxygenase (COX-1 or COX-2). Cyclooxygenases catalyze the synthesis of prostaglandin from arachidonic acid[2]. Aspirin, Ibuprofen, Ketoprofen, and Naproxen sodium are common examples of NSAIDs. Common side effects of NSAIDs, include gastrointestinal bleeding, ulcers, and kidney damage. Celebrex, Vioxx, and Bextra are newer types of NSAIDs. They inhibit only COX-2 form of cyclooxygenase.

It is however becoming increasingly evident that NSAIDs exert their antinociceptive effect through a variety of other peripheral and central mechanisms[3,4]. Opiates (codeine, methadone, morphine) are another class of antinociceptives. They are used with caution for the management of chronic pain. Opiates bind to opioid receptors to produce analgesia, but they have serious side effects (for example, addiction).After several trials to relief pain without meaningful relief, or developing serious side effects as a result of the use of analgesics, patients often resort to the use of herbal medicines which they believe are more friendly and safe for consumption.

M. balsamina Linn. (Family- Cucurbitacea) is one of the herbs claimed to have antinociceptive and other pharmacological properties. It is a climber or trailer with stems attaining 4-5mm in length[5]. It is locally known as Balsam apple (English), Garahuni (Hausa), Akbon-ndewe (Igbo) and Ejirin (Yoruba)[5]. Their leaves and young fruits are eaten as vegetable in Cameroun, Sudan and Southern Africa. Leaf extract is used for the management of high fever, excessive uterine bleeding and for the treatment of syphilis[6]. It is also used in the treatment of rheumatism, hepatitis and skin diseases, diabetics, and gastroenteritis[6]. The whole plant is used as a bitter stomachic and an infusion is used as a wash in the management of fevers and yaws[7]. Macerate of the whole plant is also used as a galactogogue and to massage the chest, to relieve intercostals pains[5].

Much has been done on the evaluation of the pharmacological properties of *M. balsamina*. The plant has been reported to have anti-diabetic, anti-microbial, anti-diarrheal and smooth muscle relaxant effects[8]. The present study was therefore designed to find out the central and peripheral antinociceptive effects of *M. balsamina* and the fraction(s) responsible for its antinociceptive activity.

MATERIALS AND METHODS

Collection and identification of plant materials

Momordica balsamina was collected between May and September 2005, identified (authenticated) by a taxonomist (Mr. Kareem) of the Federal College of Forestry and Prof. S.W.H Husseini of the Department of Botany, University of Jos, Jos, Plateau State, Nigeria. Voucher specimen (MBs 00035) was deposited at the School of Forestry Herbarium, Jos, Nigeria.

Preparation of Plant Materials

Collected plant parts were cleaned and taken to the Laboratory where they were dried in the shade. The air dried plant was reduced to powder using a mortar and pestle. The powders were stored in air-tight containers till use.

Extraction

Powdered *M. balsamina* was extracted by maceration in water/ methanol (MEW) mixture (25:75) for 72 hours. The resultant extracts were filtered and dried in the oven at low temperature (40^{o}C). The resultant solid residues were stored in a decicator till use.

Fractionation of *M. balsamina* extract

Approximately 45.0 g of *M. balsamina* extract (extracted with methanol / water, 75:25, mixture) was dissolved in about 200 mL of distilled water. N-hexane was added to it and after vigorous shaking of the mixture, the n-hexane layer was removed, and process was repeated twice. The three n-hexane fractions obtained were combined and evaporated on a rotary evaporator and labeled, Mb.Hex. The remaining aqueous fraction (Mb.Aq) was further fractionated using the same procedure with dietylether, then ethylacetate and lastly with water-saturated n-butanol using the same method. The fractions obtained were then assayed for Peripheral and Central antinociceptive properties using writhing test and tail flick test models respectively.

Animals used

Adult albino mice (weighing 25-30g) and Wistar rats (200-250 g) were housed in groups of five and fed with food (Pfizer, Nigeria) and water *ad libitum*. The animals were exposed to alternating 12 h/12 h day/night cycle (lights on at 07:00 am.) for at least 6 days before the experiments were started. Colony room temperature was maintained at 25^{o}C. All experiments were conducted during the light period of a day/night cycle. The study was approved by the Ethical Committee, Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.

Drugs and chemicals

Acetic acid (BDH chemicals, England), *n*-Hexane, Diethylether, Ethylacetate, Butanol, Aspirin, formalin, (Fluka, Taufkirchen, Germany), *M. balsamina* extract and fractions (*n*-hexane, diethylether, ethylacetate, *n*-butanol, and water fractions), and distilled water were used for this study.

Test for antinociception

Writhing Reflex Test

Mice were divided into four groups of six each. Group one received vehicle (control), group two received standard drug (aspirin) and the remaining groups received graded doses of plant extract or fractions intraperitoneally (ip). Thirty minutes later, 0.1 ml of 1% acetic acid was injected into the abdominal cavity. After 5 minutes lag time, the number of abdominal contractions (writhing movement) was observed for 15 minutes and recorded[9]. Percentage inhibition of writhing movement was then calculated from the values obtained.

Hot plate Test

Rats were kept individually in a glass cylinder (open at both ends) on a hot plate, such that the rats had direct contact with the hot plate maintained at a constant temperature of 55±1^{0} C. Time taken for paw licking and/or jumping were recorded. Rats were divided into four groups. Group one (control) received distilled water, group two received standard drug (aspirin), while groups three and four animals received different doses of extract thirty minutes before placement on the hot plate.

Tail Flick Test

Rats were acclimatized to the environmental condition in the restrainers that were designed for this experiment. Only rats that successfully acclimatized to the restrainer were selected for this experiment. Selected rats were divided into five groups of six rats each. Group one received distilled water, group two received standard test drug (aspirin), while groups 3. 4, and 5 animals received different doses (200, 500 and 1,000 mg /Kg respectively) of the *M. balsamina* extract. Thirty minutes later, each rat's tail was inserted into a water bath, maintained at 55^{o} C. The difference between the immersion time and tail withdrawal time were noted animals in groups three and four received *M. balsamina* (200 and 600 mg/Kg) extract. Rats were placed individually in an open Plexiglas chamber (rectangle-like glass cage 40 by 35 cm) with a mirror angled at 45^{o} positioned behind to allow an unobstructed view of the paws by the observer. Animals were habituated to the observation bowl for 30 min prior to the experimental sessions. Drugs were administered to the different groups. Thirty minutes later, two percent Formalin (0.2 ml) was injected (subcutaneously, s.c) into the plantar surface of the rat hind paw using a 27 gauge needle. After injection, rats were immediately returned to the observation bowl and the formalin-induced behaviours were recorded every 6 min for a period of 60 min[10]. The number of licking and biting of the injected paw was scored and quantified every 6 min for the 60 min observation period. The 6 min interval was chosen based on an earlier report on the time course of the first (0 to 6 min) and second (12 to 60 min) phases of the formalin-induced peripheral and central pain respectively[11]. and recorded. The same procedure was repeated using *M. balsamina* fractions.

Formalin Test

Rats were divided into four groups of six animals each. Group one received distilled water, group two received morphine, while

Statistical analysis

Data were analyzed using SPSS software (ver. 11). Number of bites, licks and paw flicks were subjected to the distribution-free analysis of variance (ANOVA) and student t- test[12].

RESULTS

***LD_{50}* and Percentage Yield**

The intra-peritonial LD_{50} of the whole plant of *M. balsamina* extract was greater than 5,000 g, while the percentage yield was 36.45%. The LD50 revealed that *M. balsamina* is well tolerated when administered intra-peritoneally.

ANTINOCICEPTIVE ACTIVITY

Methanolic extract of the whole plant of *M. balsamina* showed peripheral (writhing reflex test and early phase of formaline test) and central (hot plate test, tail flick test and latter phase of formaline test) antinociceptive activities. Fractions of methanolic extract of the whole plant of *M. balsamina* showed varying degree of antinociceptive activity in peripherally and centrally induced pain.

Writhing Reflex

Methanolic extract of the whole plant and the fractions of *M. balsamina*, effectively reduced the number of writhes induced in mice by 0.1 % acetic acid (Table 1). Effect of water fraction of *M. balsamina* was comparable to that of the crude extract and five times less effective as aspirin.

Hot Plate Test

M. balsamina prolonged the stay time of rats on the hot plate, its effect was less than that of morphine, (Table 2).

Tail Flick Test

Results showed that *M. balsamina* was effective in hot water tail flick test. Its effect was lesser than that of morphine. Morphine, crude extract and fractions of the extract *M. balsamina*

significantly prolonged the stay time of rat`s tail in hot water regulated at 55^{o}C, (Table 3). Water fraction was more potent than other fractions, while *n*-hexane fraction did not show any significant antinociceptive effect (P>0.05) in tail flick test.

Formalin Test

M. balsamina (200 and 600 mg/Kg) effectively inhibited peripherally and centrally induced paw flick, paw bite and paw lick in formalin test (Table 4 and 5). This further shows that *M. balsamina* possesses both central and peripheral antinociceptive properties. The extract and morphine significantly (P<0.05) reduced the number of paw flick and paw licks in the first 6 seconds and subsequent 6 seconds after 12 seconds, up to sixty seconds. No noticeable movement of the rats paw was observed between 6 and 12 seconds in all the groups, (Tables 4 and 5).

Table 2: Antinociceptive effect of methanolic extracts on the hot-plate test

Treatment	Dose (mg/kg)	Latency (S)
Control	----	1.79 ± 0.67
M. balsamina	400	8.55 ± 0.57**
	1,000	11.16 ± 0.51**
Morphine	50	10.11 ± 1.32**
	100	14.28 ±2.39**

*P<0.05, **P<0.005 when compared with the control (ANOVA and student T test) n = number of rats per group = 6

Table 1: Effect of various fractions of *M. balsamina* extracts on acetic acid- induced writhing reflex

Treatment Plant	Dose (mg/kg, i.p)	Number of writhing	Inhibition (%)
Control		49.32 ± 3.23	----
N- Hexane	500	43.62 ± 1.52	11.56
	1,000	40.18 ±1.95	18.53
Chloroform	500	30.20 ± 2.32*	38.77
	1,000	25.25 ± 1.46*	48.80
DEE	500	20.15 ± 3.31*	59.14
	1000	14.36 ± 2.32**	70.88
EA	500	17.20 ± 2.41**	65.13
	1,000	12.42 ±1.99**	74.82
Butanol	500	15.91 ± 1.15**	67.74
	1,000	09.31 ±1.37**	81.23
Water	500	10.32 ± 1.01**	79.08
	1,000	07.29 ±1.00**	85.22
M. balsamina	400	15.00 ± 2.60**	69.90
(crude)	1,000	08.16 ±2.30**	83.45
Aspirin	100	10.03 ±3.01**	79.66
	200	07.51 ±2.53**	84.77

*P<0.05, **P<0.005 when test is compared with the control, n = number of mice per group = 6

Table 3: Effect of *M. balsamina* fractions on hot water tail flick test

Treatment mg /Kg	N	Latency (sec) ± SEM	% Inhibition
Control	5	0.20 ± 0.12	---
DEE			
500	5	0.18 ± 0.09	10
1,000	5	0.22 ± 0.14	10
EA			
500	5	0.17 ± 0.09	15
1,000	5	0.28 ± 0.07	40
Chloroform			
500	5	0.19 ± 0.12	5
1,000	5	0.25 ± 0.13	25
Butanol			
500	5	0.49 ± 0.11*	145
1,000	6	0.82 ± 0.31*	310
Water			
200	6	1.17 ± 0.11*	485
500	6	2.82 ± 0.07*	1310
M.balsamina			
200	6	0.17 ± 0.11	15
500	6	0.82 ± 0.31	409
1,000	6	2.38 ± 0.72	1090

SEM = Standard error mean *P<0.05, when test were compared with the control.

Table 4: Effect of *M. balsamina* on Formalin induced Paw Flick

Treatment	Number of Paw Flick per 6 seconds Time									
Dose mg/Kg	6	12	18	24	30	36	42	48	54	60
Control	111.25±11	0.00±00	73.00±81	73.50±52	59.00±52	56.25±11	71.75±12	65.25±12	69.75±61	65.50±13
M. B 200	42.25±42+	0.00±00	0.00±83+	20.00±51+	27.00±63+	35.00±52+	40.25±63+	36.75±32+	32.50±30+	28.75±23+
600	53.00±80+	0.00±00	16.75±21+	27.25±41+	31.75±10	19.00±43+	29.75±42+	38.75±84+	32.25±43+	39.00±72+
Morphine 50	99.20±30+	0.00±00	19.55±09+	31.15±12+	33.46±11	18.99±09+	25.39±12+	42.58±12+	39.15±14+	44.20±12+

Values are mean SEM, +P<0.05, when test is compared with control, student`s t- test, n = 5 animals per group, *M.B = M. balsamina*

Table 5: Effect of *M. balsamina* on formalin induced Paw lick and bite

Treatment	Number of Paw Flick per 6 seconds Time									
Dose mg/Kg	6	12	18	24	30	36	42	48	54	60
M.B 200	41.25±09+	0.00±0	125.00±20	28.00±06+	39.75±12+	73.75±09+	22.25±04+	26.25±04+	19.00±30+	28.75±20+
600	14.00±50+	0.00±0	0.00±0	8.25±20+	5.00±20+	18.25±40+	50.50±60+	38.25±40+	46.25±40+	0.75±1.20+
Morphine 50	144.30±32+	0.00±0	0.00±0	10.65±05+	3.10±03+	12.14±06+	40.54±15+	30.15±09+	37.35±11+	0.95±03+

Values are mean SEM, +P<0.05, when test are compared with control, student`s t- test, n = 5 rats per group

DISCUSSION

M. balsamina is used in traditional medicine for the management of several disease conditions. It has both medicinal and nutritional values. It is a good source of protein, fibre, carbohydrate and mineral. *Momordica species* serves as source of protein and carbohydrate. *Momordica foecide* and *Momordica involucrata* leaves are consumed as food in Swaziland13. Nutritional values of *M. balsamina* were reported by Hassan and Umar (2006)14. They reported that the leaves of *M. balsamina* contains seventeen amino acids (isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine,valine, alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, proline and serine)

Its constant use in food could also have the advantage of protecting against bacterial infection[10]. Otimenyin *et al* (2008)[10] reported the antimicrobial, anti-diarrhea, anti-spamolytic and blood glucose lowering effects of *M. balsamina.* Sub acute toxicity studies showed that *M. balsamina* is safe for consumption. LD50 was greater than 5000g. This may explain why the consumption of this plant in food has not resulted in any toxicological effects19.

This study revealed two main findings. First, *M.balsamina* inhibited peripherally induced pain. The experimental results obtained from writhing and early phase of formalin test showed that the crude extract and the fractions of crude extract of *M. balsamina* significantly reduced induced pain to varying degrees. Antinociceptive effect of *M. balsamina* was lesser than that of aspirin (Tables 1). According to Turner (1965)20, plants that are effective in writhing test have peripheral antinociceptive activity. Drugs that inhibited the first phase of formalin test possess peripheral antinociceptive activity10. McCall, (1996)21 and Padi, (2006)10 reported that the first (early) phase (occurring between zero to six minutes) is caused predominantly by C-fibre activation due to the peripheral stimulation. Result obtained from this stage of formalin test was in consonance with the results from writhing test. Implying that, the extract and its fractions have inhibitory effects on peripherally induced pain, (Table 1, 4, 5). *N*-hexane fraction had no antinociceptive effect, but diethylether, ethylacetate, *n*-butanol and water factions had varying degrees of antinociceptive effects. Solvents used for fractionation were selected in order of polarity. *N*-Hexane (being the least polar of the solvents) was expected to extract non-polar constituents in the plant extracts. The inactivity of *N*-Hexane fraction showed that the active principle in this plant is likely polar. As the polarity of the solvent increases, the antinociceptive activity of the fractions increased, with water fraction producing the most potent antinociceptive effect.

Secondly, *M. balsamina* inhibited centrally induced pain. Its effect was lesser than the effect of morphine (Tables 4 and 5). The central antinociceptive effect of *M. balsamina* was revealed in its ability to inhibit the second phase of formalin induced pain and prolong stay time of rats on hot plate and rat's tail in warm water in tail flick test. The second (late) phase (occurring between 12 to 60 minutes) in formalin induced pain models is dependent on ongoing stimulation of nociceptors and/or via spinal cord hyperexcitability22,23. The observed inhibition in the later phase of formalin induced pain (which started twelve minutes after administration of formalin) revealed that the extract possesses central antinociceptive properties.

The ability of *M. balsamina* to inhibit both peripheral and centrally induced pain supports its use in folk medicine for the management of pain associated with several types of diseases. Water fraction was shown to have greater antinociceptive activity than the other fractions, this also explain why the decoction is used in folk medicine for the management of pain.

CONCLUSION

M. balsamina, a plant used in folk medicine has been shown scientifically in this study to possess peripheral and central antinociceptive properties. Elucidated properties explain the ith glutamic acid, leucine and aspartic acid being the predominance amino acids. Isoleucine, leucine, valine and aromatic acids were reported to be higher than requirement pattern for children15, while sulphur containing amino acids are the only limiting amino acids for adults. *M. balsamina* leaves also contains valuable minerals, (K , Na, Ca, Mg, P, Fe, Cu, Mn, and Zn) and fibre15. Its leaves are poor sources of plant lipid14, which is in agreement with general observation that leafy vegetables are low lipid containing food, thus advantageous health wise to avoid obesity and related health problems16. Dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon cancer and breast cancer17, 18.
traditional use of this plant for the management of pain associated with several types of diseases.

RECOMMEDATIONS

M. balsamina and its fractions have shown promising peripheral and central antinociceptive properties. Elucidation of the structure of the potent antinociceptive constituents in needed for its acceptance for use in orthodox medicine.

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