Antinociceptive and anti-inflammatory properties of hydroethanolic seed extract of *Monodora myristica* (Annonaceae) in rodents

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ABSTRACT

Background: *Monodora myristica* (Gaertn) Dunal (Annonaceae) is used in traditional medicine for cough, rheumatism, hemorrhoids, diabetes, anemia and headaches.

Objective: This study was carried out to investigate the antinociceptive and anti-inflammatory effects of the hydroethanolic seeds extract of *Monodora myristca* (HMM) in rodents.

Methods: HMM (50-200 mg/kg, p.o.) was administered 1 h before intraperitoneal or intraplantar injection of 0.6%^{v}/v acetic acid or 1% formalin (20 µL), respectively, to evaluate the antinociceptive property. Acute and chronic anti-inflammatory effect was investigated using carrageenan-induced paw and xylene-induced ear oedema, and cotton-pellet induced granuloma tests, respectively.

Results: HMM (50-200 mg/kg) produced dose dependent and significant decrease in mean number of writhes in acetic acid-induced nociception and increased pain threshold to neurogenic and inflammatory pain with 48.59 and 34.2% inhibition, respectively, in the formalin-induced nociception assay. The HMM-induced antinociception was completely blocked by pre-treatment of mice with naloxone, *p*-Chlorophenyalanine (serotonin synthase inhibitor; 100 mg/kg, i.p.) and sulpiride (D_2 receptor antagonist; 50mg/kg) whereas glibenclamide (K_{ATP} sensitive channels blocker; 10 mg/kg) failed to reverse the antinociceptive effect of the extract. In acute inflammatory model, HMM produced time course inhibition of carrageenan-induced paw oedema. In addition, pretreatment of mice with HMM inhibited xylene-induced ear oedema by 60% comparatively similar to the effect of dexamethasone (83.90%). Moreover, in the cotton-pellet granuloma pouch, HMM (200 mg/kg) reduced granuloma formation by 52%.

Conclusion: The hydro-ethanolic seed extract of *M. myristica* possesses antinociceptive effect mediated through interaction with opioidergic, serotonergic and dopaminergic systems and an anti-inflammatory action through inhibition of inflammatory mediator's release. Finally, the study established the scientific basis for its use in the management of pain and inflammatory conditions in traditional medicine.

Keywords: Anti-nociceptive, anti-inflammatory, opioid, serotonergic, dopaminergic, *Monodora myristica*

Propriétés anti-nociceptives et anti-inflammatoires de l'extrait de la graine hydro-éthanoïque de *Monodora myristica* (Annonaceae) chez les rongeurs

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RESUME

Contexte: Le *monodora myristica* (Gaertn) Dunal (Annonaceae) est utilisé en médecine traditionnelle pour la toux, les rhumatismes, les hémorroïdes, le diabète, l'anémie et les maux de tête.

Objectif: Cette étude a été réalisée pour étudier les effets anti-nociceptifs et anti-inflammatoires de l'extrait de la graine hydroéthanolique de *Monodora myristca* (HMM) chez les rongeurs.

Méthodes: Le HMM (de 50 à 200 mg/kg, p.o.) a été administré 1 h avant l'injection intra-péritonéale ou intra-plantaire de 0,6%^{v}/v d'acide acétique ou 1% de formol à (20 pl), respectivement, afin d'évaluer la propriété anti-nociceptive. L'effet anti-inflammatoire aigu et chronique a été étudié en utilisant la patte induite par le carraghénane et l'œdème de l'oreille induit de xylène, et les tests de granulome induit de boulette de coton, respectivement.

Résultats: Le HMM (50-200 mg/kg) a produit une dose dépendante et une diminution significative du nombre moyen de contorsions dans la nociception induite par l'acide acétique et un seuil de douleur accrue à la douleur neurogénique et inflammatoire avec 48,59 et 34,2% d'inhibition, respectivement, dans le dosage de nociception induite de formol. L'anti-nociception induite de HMM a été complètement bloquée par le prétraitement des souris avec la naloxone, le p-chlorophenyalanine (inhibiteur de la synthéase de la sérotonine; 100 mg/kg, i.p.) et le sulpiride (D2 antagoniste du récepteur; 50 mg/kg), tandis que le glibenclamide (bloqueur des canaux sensibles K_{ATP}; 10 mg/kg) n'a pas réussi à inverser l'effet anti-nociceptif de l'extrait. Dans le modèle inflammatoire aigu, le HMM a produit l'inhibition du cours du temps de l'œdème de la patte induite par le carragheen. En outre, le prétraitement des souris avec le HMM a inhibé l'œdème de l'oreille induit par le xylène de 60% comparativement similaire à l'effet de la dexaméthasone (83,90%). En outre, dans le sac de granulome de pastille de coton, le HMM (200 mg/kg) a réduit la formation de granulomes de 52%.

Conclusion: L'extrait de pépin hydro-éthanoïque de *M. myristica* possède un effet anti-nociceptif modéré par l'interaction avec les systèmes opioïdergiques, sérotoninergiques et dopaminergiques et une action anti-inflammatoire par l'inhibition de la libération du médiateur inflammatoire. Enfin, l'étude a établi la base scientifique pour son utilisation dans la gestion des conditions de douleur et inflammatoires en médecine traditionnelle.

Mots-clés: Anti-nociceptif, anti-inflammatoire, opioïde, sérotoninergique, dopaminergique, *Monodora myristica*

INTRODUCTION

Pain and inflammation are part of the body's immune response. They are a common symptom and affect a large number of patients with many types of disease. Because of the significant side effect profiles of steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs), there is a greater interest in natural compounds, such as dietary supplement and herbal remedies, which have been used to reduce pain and inflammation.[1] *Monodora myristica* (Annonaceae) belongs to the custard apple family of flowering plants. It is one of the most important trees of the evergreen forest of West Africa and it is mostly prevalent in the Southern part of Nigeria.[2] It is commonly known as ``*Ehuru*`` or ``*Ehiri*`` (Igbo), ``*Ariwo*`` or ``*Lakosin*`` (Yoruba), ``*Ehinawosin*`` (Ikale), ``*Uyengben*`` (Edo), Jamaica nutmeg, African nutmeg, Calabash nutmeg. The seeds are embedded in a white sweet-smelling pulp and are the most economically important part of the tree. They are aromatic and are used after grinding to a powder as a condiment in food providing a flavour resembling that of nutmeg. They are used as an aromatic and stimulating addition to medicines and to snuff. Ground to a powder they may be taken as a stimulant or stomachic or to relieve constipation. The seeds chewed up are applied to the forehead for headache and for migraine in Gabon; ground up for headaches, rheumatism, rhino-pharyngitis, or loss of voice, to apply on sores, or eaten as an anti-emetic aperative and tonic in Congo.[2,3] The seeds contain 5–9% of a colourless essential oil consisting largely of terpenes and with a pleasant taste and smell and about 35–36% of a reddish-brown fixed oil which is mainly linoleic acid, 46.9%, and oleic acid, 35%.[4,5] Thus, in this study, the anti-nociceptive and anti-inflammatory effects of *M. myristica* were evaluated using scientific models to ascertain the veracity of the folkloric uses. Also, the involvement of the opioidergic, serotonergic, and dopaminergic systems were elucidated.

METHODS

Plant material

The fresh seeds of *Monodora myristica were* purchased from Mushin Herbal market, Mushin, Lagos state. It was identified and authenticated by Mr. T.K. Odewo (a forestry expert, in the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Nigeria), where an Herbarium voucher specimen number LUH 1205 was allotted for reference purpose.

Extract preparation

The seeds of *M. myristica* were air dried and the shells removed. The air dried seeds pulverized into powder and 1043 g of the powdered seeds was soaked in 2 L of 70% ethanol in distilled water for 72 h, after which the preparation was decanted and filtered. The procedure was repeated twice for exhaustive extraction. The filterate obtained was oven dried at 40°C given a percentage yield of 2.19%^{W}/w.

Preliminary phytochemical screening

The preliminary phytochemical screening of the crude leaf extract was carried out employing standard procedures and tests as described by Sofowora.[6]

Drugs and chemicals

Celecoxib (Pfizer manufacturing Deutschland GmbH, Illertissen, Germany), diclofenac (Novartis pharmaceuticals corp.), morphine (Martindale Pharmaceuticals, UK), formalin, carrageenan, xylene, dexamethasone, naloxone chloral hydrate (Sigma Aldrich Chemical, St Loius, MO, USA).

In vitro evaluation of antioxidant activities

1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

The free radical scavenging capacity of HMM were determined using the stable free radical DPPH.[7] *HMM* was mixed with 95% ethanol to prepare a stock solution (5 mg/ml). DPPH solution (0.004%, w/v) was prepared in 95% ethanol. A freshly prepared DPPH solution (0.004%, w/v) was placed in test tubes and HMM was added followed by serial dilution (25 µg to 150 µg) in every test tube so that the final volume was 3 ml. After 10 min, the absorbance was read at 515 nm using a spectrophotometer (HACH 4000 DR UV-visible spectrophotometer, USA). Ascorbic acid (5 mg/ml in distilled water) was used as a reference standard. A control sample of the same volume was prepared without any extract and reference ascorbic acid. A solution of 95% ethanol served as a blank. The percentage (%) scavenging of the DPPH free radical was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}} \quad \text{(Equation 1)}$$

Ascorbic acid was used as the reference standard antioxidant. The effective concentration needed to scavenge DPPH free radical by 50% (IC_{50}) was calculated

by regression analysis of the dose response curve plotted between percentage inhibition versus concentration of the test samples and the standard.

Experimental animals

Male albino mice (18 -25 g) and rats of (90 – 120 g) were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos. The animals were maintained under standard environmental conditions, and had free access to water and standard powdered diet (Livestock Feeds Plc, Lagos, Nigeria). All the animals were acclimatized for one week before the commencement of the investigation. The experimental procedures adopted in this study were in compliance with the ethical standards of the Research Grant and Animal Experimentation Committee of the College of Medicine, University of Lagos, Nigeria and in accordance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (1985).

Acute Toxicity study

The possible acute toxic effect of the extract was determined using the limit test and fixed dose protocol of the Organization of Economic Co-operation and Development (OECD) guidelines for testing of chemicals for oral and intraperitoneal administration, respectively. In limit test for oral acute toxicity,[8] 5 female mice were given HMM (4000 mg/kg, *p.o*.). However, in fixed dose test; 11 mice were given HMM (50 mg/kg, *i.p*., n =1; 200 mg/kg, *i.p*., n =5; and 400 mg/kg, *i.p*., n = 5). Behavioural signs of toxicity and mortality were observed following extract administration; during the first 30 minutes, then the second, fourth, sixth hour and once daily for 14 days for delayed toxicity or mortality.

Evaluation of antinociceptive effect of the extract

Acetic acid-induced mouse writhing test

The acetic acid-induced nociception was carried out using the method of Koster et al.[9] Thirty mice were randomly divided into five groups (n =6); Group I, vehicle (10 ml/kg, p.o., normal saline; control), Group II, diclofenac (20 mg/kg, p.o., standard reference), Group III, IV and V, HMM (50, 100 and 200 mg/kg, p.o.). One hour after vehicle or drug administration, the animals received acetic acid (0.6%$^{v}/_{v}$ in saline, 10 ml/kg, i.p.). The number of abdominal contraction followed by extension of hind limbs (a writhe) was counted for 20 min after injection of acetic acid. A decrease in the mean number of writhes suggest antinociceptive effect in comparison with vehicle treated, control.

Formalin-induced nociception

The formalin test was carried out according to the method of Hunskaar and Hole[10]. Thirty mice were randomly divided into five groups (n =6). Group I, vehicle (10 ml/kg, p.o., normal saline; control), Group II, morphine sulphate (10 mg/kg, *s.c*., standard reference), Group III, IV and V, HMM (50, 100 and 200 mg/kg, p.o., respectively). One hour after extract or vehicle administration and 30 min after subcutaneous injection of morphine, 20µl of 1% formalin in saline was injected into the right hind paw of mice. The duration of paw licking or biting (an index of painful response) was recorded at 0-5 min (early phase, neurogenic pain) and 15-30 min (late phase, inflammatory pain) after formalin injection.

Anti-inflammatory models

Xylene-induced ear edema

Mice were allotted to six groups (n =5). Thirty minutes after oral treatment of mice with normal saline (10 ml/kg), dexamethasone (1 mg/kg, p.o), or HMM (50, 100, and 200 mg/kg), oedema was induced in each mouse by instilling 30 µl of xylene to the inner surface of the right ear. Fifteen minutes after xylene application, the animals were euthanized under ether anesthesia and both ears were cut off, sized, and weighed. The mean of the difference between the right and left ears was determined for each group and percentage inhibition was calculated.[11]

Carrageenan-induced paw edema

The rats used in this assay were divided into five groups of six each. The initial paw circumference of the right hind paw was measured using the cotton thread method of Bamgbose and Noamesi.[12] Then, Group I, received normal saline (vehicle, 10 ml/kg, p.o.), Group II- received diclofenac (20 mg/kg, p.o. reference standard) and Group III, 1V and V- received HMM (50, 100 and 200 mg/kg, p.o.), respectively). One hour after extract or vehicle oral administration, 100 µl of carrageenan 1% $^{w}/_{v}$ in normal saline was injected into the subplantar tissue of the right hind paw to induce oedema. The paw circumference was measured at every 1 h for 6 h.[13] The change in paw circumference (oedema was expressed as the increase in paw circumference (cm)), is the value between the initial (0 h) and values obtained after carrageenan injection (1-6 h), in comparison with vehicle treated control was used to calculate the percentage inhibition of oedema.

$$\%\text{inhibition} = \frac{\text{Increase in paw oedema (control)} - \text{Increase in paw oedema (treatment)} \times 100}{\text{Increase in paw oedema (control)}}$$

Cotton pellet-induced granuloma

The granulomas were developed using the method described by Fabri et al.[14] Rats were divided into five groups (n = 5), as previously described in carrageenan test. After one hour of oral administration, sterile cotton pellets weighing 10 mg were subcutaneously implanted in both axillae of rats under anaesthesia (chloral hydrate (300 mg/kg, i.p.)). The treatments were given daily for 7 consecutive days. On day 8, the rats were euthanized, and the cotton pellets with the surrounding granulomas were removed and dried at 60°C. The weight of granuloma was determined and the percentage inhibition of granuloma was calculated;

$$\%\ \text{inhibition} = \frac{\text{weight of granuloma in control} - \text{weight of granuloma in test} \times 100}{\text{Weight of granuloma in control}}$$

Statistical analysis

The results are expressed as mean±SEM. Statistical significant differences between groups were measured using one or two way ANOVA (whichever is applicable) followed by Tukey post hoc multiple comparison test with the aid of Graphpad prism version VI (Graphpad Inc. CA, USA).

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of HMM showed the presence of alkaloids, flavonoids, tannins, saponins, steroids. The quantitative analysis of the extract indicates that the extract is rich in polyphenolic compounds.

DPPH radical scavenging activity

The values for EC_{50} obtained in this study were 78µg/ml and 63 µg/ml for L-Ascorbic acid and HMM, respectively. (Fig. 1)

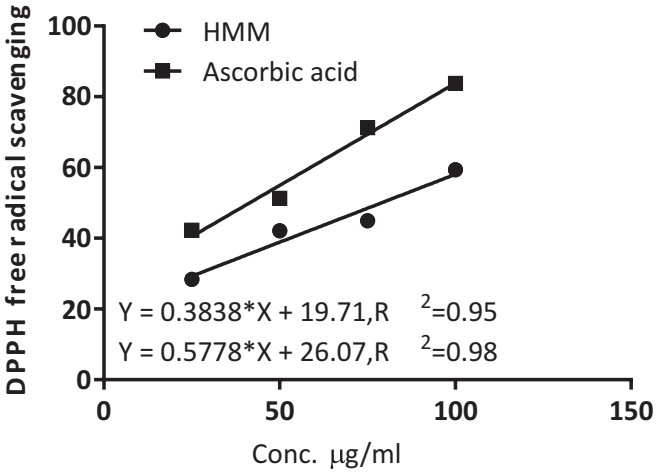


Fig. 1: DPPH free radical scavenging activity of *M. myristica*. HMM- Seed extract of *M. myristica*

Acute toxicity study

There was no mortality recorded after oral administration of HMM up to 4000 mg/kg. The observed toxicity behaviour include tachypnea, diarrhoea and hypoactivity.

Acetic acid induced mouse writhing assay

The mean number of abdominal constrictions after i.p. injection of acetic acid was 121.00 ± 5.47 in control animals. A significant reduction in abdominal constrictions was observed in aspirin treated mice and the mean value being 31.56 ± 0.86 (Fig. 2). HMM elicited a dose proportionate reduction in the number of abdominal constrictions in mice. Nearly 50% inhibition of nociception was observed with 50 mg/kg for HMM and further increase in doses up to 200 mg/kg resulted in a maximum inhibition of nociception 62.80% (Fig. 2).

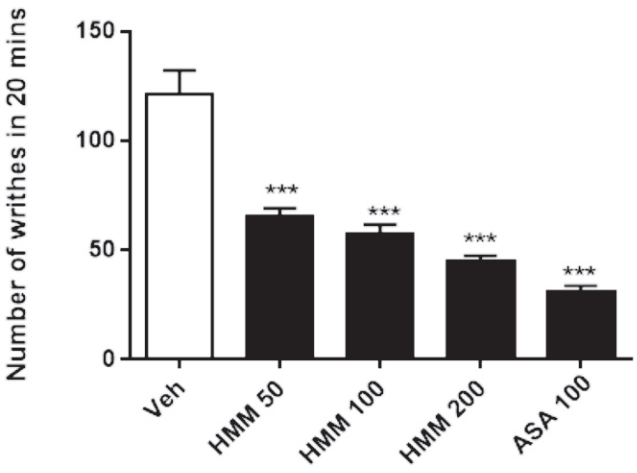


Fig. 2: Effect of HMM against acetic acid induced writhes in mice. Values are expressed as mean ± S.E.M (n = 5). ***P<0.001 versus vehicle-treated, control group.

Formalin-induced nociception

The first phase of formalin induced pain produced nociceptive response of biting and licking of the paw with a duration of 101.8±4.03 seconds in the control group. Significant (P< 0.001) dose dependent inhibition of nociceptive reaction was produced by HMM with peak effect (64.91% inhibition) produced at the highest dose of 200 mg/kg. In the second phase, the duration of nociceptive reaction in the control group was 113.3±18.16 seconds. HMM significantly inhibited the biting and licking response in a dose dependent manner with peak effect (75.19%) produced at the highest dose of 200mg/kg. This effect was less but comparable to that produced by morphine (5 mg/kg) at both phases (Fig. 3).

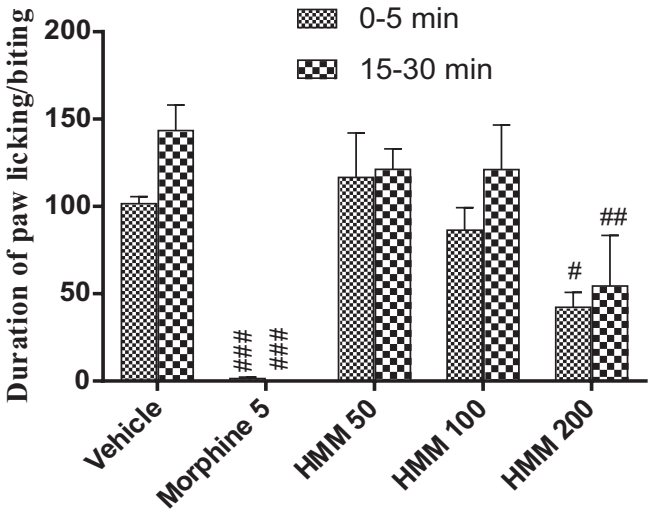
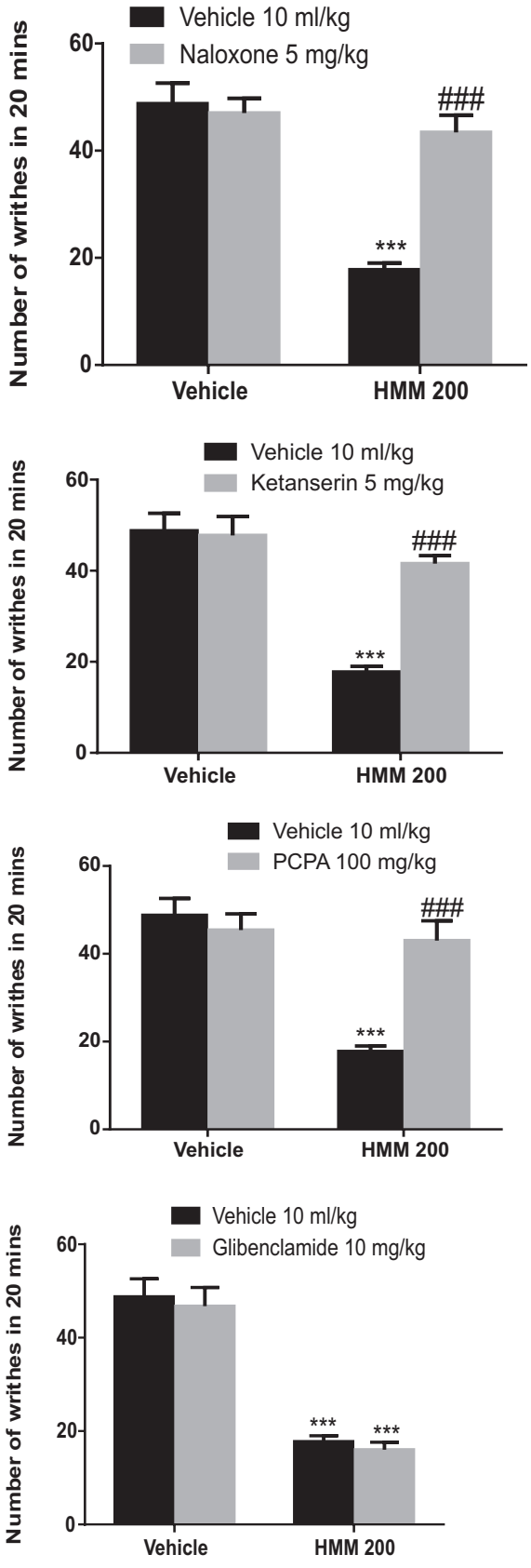


Fig. 3: Effect of HMM against formalin-induced nociception in mice. Values are expressed as mean ± S.E.M (n =5). $^{\#}P<0.05$; $^{\#\#}P<0.01$; $^{\#\#\#}P<0.001$ versus vehicle-treated, control group.

Possible mechanism of action of HMM-induced antinociception in mice

The antinociceptive effect of HMM (200 mg/kg) observed the in mouse writhing test was reversed by pretreatment of mice with naloxone (opioid receptor antagonist) (Fig. 4A), suggesting involvement of opioidergic pathway in its mechanism of antinociceptive activity.

In another series of study, pretreatment of mice with parachlorophenylalanine (100mg/kg, i.p, a serotonin synthase inhibitor) (Fig. 4B), ketanserin (5mg/kg; a $5HT_{2A/2c}$ antagonist) (Fig. 4C), and sulpiride (1 mg/kg; dopamine D_2 receptor antagonist) (Fig. 4D), significantly attenuated the antinociceptive effect of HMM. In contrast, pretreatment of mice with glibenclamide (10mg/kg; an ATP-sensitive K^+ channel blocker) (Fig. 4E), did not modify the antinociceptive response elicited by HMM.



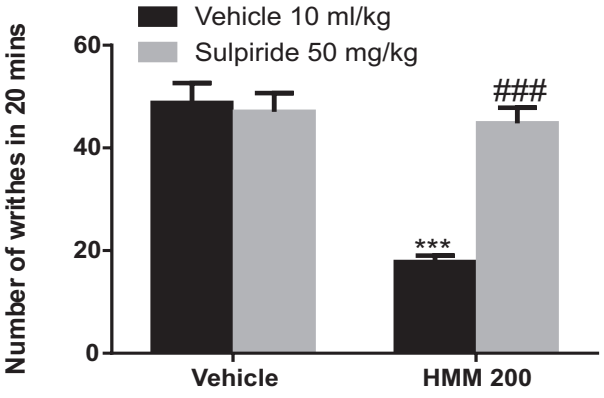


Fig. 4A-E: Effect of pretreatment of mice with (A) naloxone, (B) pCPA, (c) ketanserin, (D) sulpiride and (E) glibenclamide on the antinociceptive profiles of *M. myristica* (HMM). Values are expressed as mean ± S.E.M (n = 5). ***P<0.001 versus vehicle treated; $^{###}$P<0.001 HMM 200mg/kg only treated. Two way ANOVA followed by Tukey post hoc multiple comparison test

Xylene induced ear edema

Animals pretreated with HMM (50,100 and 200 mg/kg) gave a significant dose dependent inhibition of oedema with peak effect observed at 100 mg/kg (77.24%) which compared effectively with the standard drugs diclofenac (50 mg/kg) and dexamethasone (5 mg/kg). (Fig. 5).

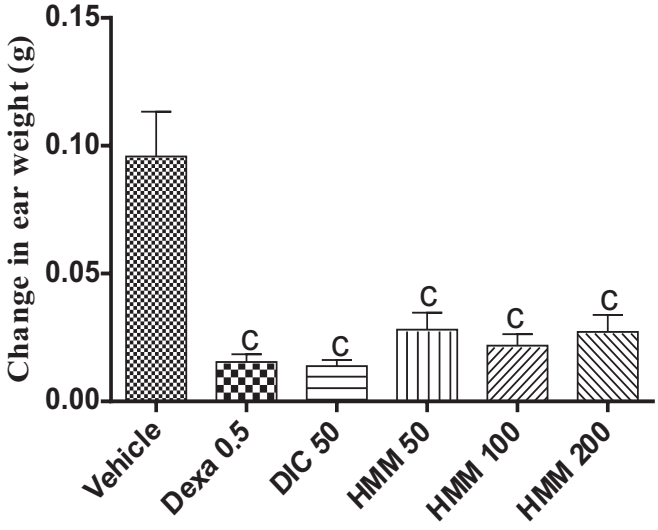


Fig 5: Effect of HMM against xylene-induced ear oedema in mice. Values are expressed as mean ± S.E.M (n =5). ^{c}P<0.001 versus vehicle-treated, control group

Carrageenan-induced paw edema

Pre-treatment of rats with HMM or celecoxib, produced time course and significant inhibition of oedema with peak effect observed 6 h post carrageenan treatment. The highest percentage inhibition observed at 200mg/kg was 60% comparably similar to the effect seen in celecoxib (68%). (Fig. 6).

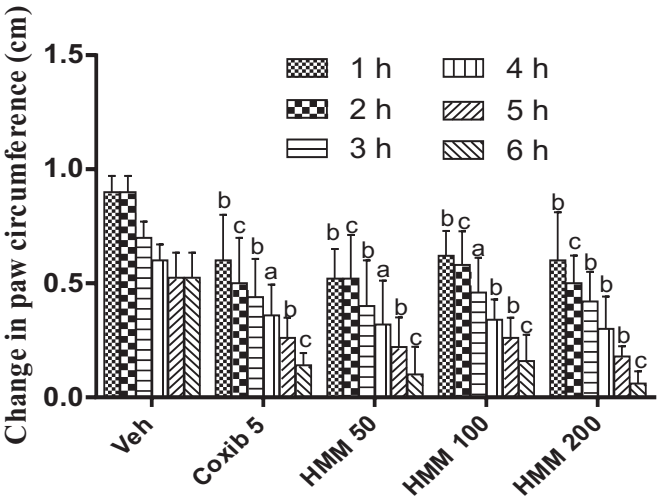
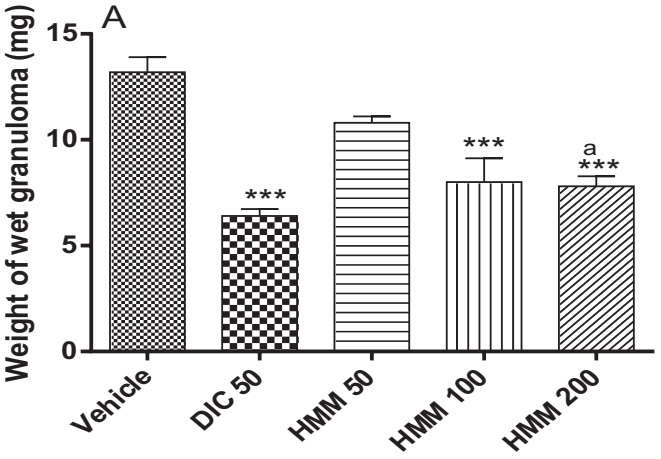


Fig. 6: Time course effect of HMM against carrageenan-induced paw oedema in rats. Values are expressed as mean ± SEM. ^{a}P<0.05; ^{b}P<0.01; ^{c}P<0.001 versus vehicle-treated control group.

Cotton pellet induced granuloma test

Pretreatment of rat with HMM or diclofenac before implantation of cotton pellet significantly reduced granulomatous formation as depicted in the results. Granuloma inhibition was observed as indicated by weight of granuloma. HMM produced a 52% inhibition percentage inhibition observed is 52% compared to 71.13% observed with diclofenac. (Fig. 7).



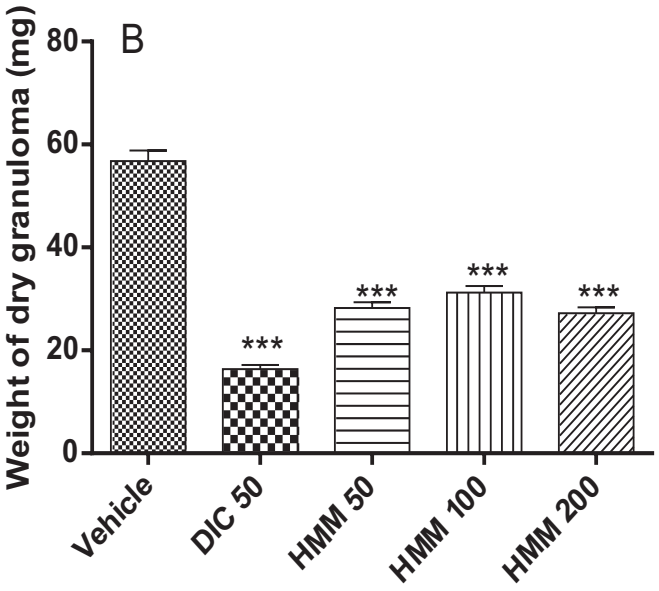


Fig. 6A-B: Effect of HMM on (A) wet granuloma and (B) dry granuloma in cotton pellet-induced granuloma pouch in rats. Values are expressed as mean ± SEM. $^{***}P<0.001$ versus vehicle-treated control group; $^{a}P<0.05$ versus HMM 50 treated group.

DISCUSSION

Findings from this study showed that the seed extract of M. myristica possesses antinociceptive and anti-inflammatory properties. These properties were assessed using different animal models. The antinociceptive activity of M. myristica was investigated using the acetic acid-induced mouse writhing and formalin-induced nociception assays. The acetic acid injection induces tissue damage and causes the release of several endogenous substances such as bradykinin (BKs), prostaglandins (PGs), and serotonin (5-HT) and contributes to the process of inflammation and increased sensitivity of nociceptors.[15] These endogenous substances sensitize peripheral nerve terminal (peripheral sensitization), leading to phenotypic alteration of the sensory neurons and increased excitability of the spinal cord dorsal horn neurons (central sensitization). The results of the present study demonstrate that the seeds extract attenuated the nociceptive responses to chemical stimuli in the acetic acid-induced abdominal constriction evidenced in reduction of abdominal contractions. Diclofenac sodium, the peripheral analgesic drug, also produced a similar antinociceptive action. It has been postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, which stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs and opioids.[16] This test is generally used for the screening of central and peripheral analgesic effects. The centrally acting protective effect of the extract was also corroborated in our study by the formalin test results.

Formalin injection into the mouse right hind paw evokes distinct quantifiable behavior indicative of pain (licking/biting of the injected paw). This test which represents a model of persistent pain can also be used to determine the ability of new compounds to affect peripheral or central nociceptive pathways due to its biphasic nociceptive characteristics, known as the early and late phase, resulting from formalin administration. The early phase classified as the neurogenic pain, is an acute response observed immediately after the administration of formalin which lasts for 0-5 minutes. The late phase, classified as an inflammatory pain is a tonic response resulting from inflammatory processes generated by inflammatory mediators like histamine, serotonin, bradykinin and PGE.[17] Centrally acting drugs (opioids) inhibits both phases while peripherally acting drugs (aspirin) inhibits the late phase only. Findings from this study showed that the hydroethanolic seed extract of M. myristica inhibited both the early and late phases of the formalin test, suggesting its ability to prevent central nociception.

The involvement of different systems in the mechanism of M. myristica-induced antinociception was also elucidated. The opioid system modulates physiological processes by activation of the opioid receptors especially the subtype μ, producing notable effects against nociception[18]. A major advance in understanding pain mechanisms has been the recognition that ongoing activity in nociceptive pathways may lead to profound alterations in the levels of neurotransmitters in primary afferent neurons and to changes in sensitivity to opioid analgesia.[19] In this study, subcutaneous injection of naloxone (a non-selective opioid receptor antagonist) significantly blocked the anti-nociceptive effect of M. myristica seed extract, which suggests the involvement of the opioidergic system in elicitation of the antinociceptive effects of the extract.

Serotonin on the other hand, is an important neurotransmitter in modulating the nociceptive response at many stages in the pain pathway. The descending serotonergic pathways may directly modulate the activity of projection neurons; $5HT_{1A}$, $5HT_{2}$&$_{3}$ are considered to play a major role in nociceptive modulation.[20] Intraperitoneal injection of ketanserin (a $5HT_{2A/2c}$ antagonist) blocked the anti-

nociceptive effect of the extract. The antinociceptive activity of the extract was also blocked by 3 days consecutive pretreatment with pCPA (100 mg/kg, i.p, a serotonin synthesis inhibitor). This is suggestive of the involvement of serotonergic system probably via the $5HT_{2A/2c}$ receptors.

Similarly, dopamine has been suggested to play an important role in the modulation of nociception. The involvement of dopamine in the supraspinal modulation of pain processes has been widely described.[21] Analogous to human research, evidence from animal studies indicates that the supraspinal dopaminergic system is widely involved in pain processing. Thus, the dopaminergic neurons in the nucleus accumbens are related with the suppression of tonic pain. D_2 receptor activation and D_1 blockade diminish neuropathic pain related behavior.[22] In the present study, sulpiride (dopamine D_2 receptor antagonist) blocked the anti-nociceptive effect of M. myristica seed extract suggestive of an interaction with dopaminergic systems in the exertion of its anti-nociceptive effect.

Ion channels play a vital role in pain signal initiation and conduction.[23] K^+ channels play an essential role in setting the resting membrane potential and in controlling the excitability of neurons. Thus, K+ channels represent potentially attractive peripheral targets for the treatment of pain.[24] It has been suggested that K_{ATP} may mediate the analgesic effects of morphine, clonidine and 5-HT_1 agonists because the anti-nociceptive effects of these agents could be reversed by pretreatment with selective K_{ATP} antagonists but not other potassium channels blockers[25]. There is also evidence suggesting that the anti-nociceptive effects of K_{ATP} activators may stimulate mechanisms that produce anti-nociception through opioid receptor activation.[26] The hydro-ethanolic extract of the seed of M. myristica elicits its effect via mechanisms other than K^+ channel blockade as pretreatment with glibenclamide (an ATP-sensitive K^+ channel blocker) failed to block its anti-nociceptive effect.

The xylene ear edema model permits the evaluation of anti-inflammatory steroids and is less sensitive to non-steroidal anti-inflammatory agents.[27]

Histopathologically, severe vasodilation, oedematous changes of skin and infiltration of inflammatory cells are detected as signs of acute inflammation after topical application of xylene. In this model, M. myristica inhibited in a dose dependent manner, the ear edema induced by the phlogistic agent administered. This is an indication that the extract contains anti-inflammatory principles.

Moreover, to evaluate the effect of the extract on acute inflammatory conditions, the carrageenan-induced paw oedema was carried out. It consists of three distinct phases including an initial release of histamine and serotonin; a second phase mediated by kinins; and a third phase involving prostaglandins.[28] In this study, M. myristica showed significant inhibitory effect on rat paw oedema development in the later phase of carrageenan-induced inflammation suggesting the ability of the extract to inhibit prostaglandin release or action based on the fact that prostaglandin is known to mediate the late phase of carrageenan induced inflammation.

The effect of the extract on chronic inflammation was also investigated using the cotton pellet-induced granuloma test. This model is based on foreign body granuloma which is provoked in rats by subcutaneous implantation of pellets indicating the proliferative phase of inflammation.[29] Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which represents the underlying factors in granuloma formation. Hence, the decrease in weight of granuloma suggest the ability of hydroethanolic seed extract of M. myristica to attenuate the proliferative phase of inflammation.

In respect to the identification of the phytochemical constituents of the extract, the preliminary screening revealed the presence of tannins, saponins, flavonoids, alkaloids, cardiac glycosides, reducing sugars and phlobatannins in M. myristica. The pharmacological effects seen with the extract may be attributed to some of these metabolites. Saponins, phenols, flavonoids, tannins and some glycosides have been reported to possess antinociceptive and/or anti-inflammatory activities.[30]

The role of antioxidant system in the observed effects was also investigated through DPPH-induced free radical generation. M. myristica yielded a significant comparably similar EC_{50} when compared with the standard (L-Ascorbic Acid) suggesting antioxidant property. DPPH radical is widely used as the model system to investigate the scavenging activities of several natural compounds.[31] DPPH is scavenged by antioxidants through the donation of proton forming the reduced DPPH which can be quantified by its decrease of absorbance. [53] Radical scavenging activity increases with increasing percentage of the free radical inhibition.[31] DPPH radical quenching of the extract as shown in the result indicated potent radical scavenging activity; an essential antioxidant property which offers protective effect against oxidants.

CONCLUSION

Findings from this study suggests that the hydroethanolic seed extract of M. myristica possesses antinociceptive activity through interaction with opioidergic, dopamine D_2 and $5HT_{2A/2c}$ receptors and anti-inflammatory activities possibly mediated through inhibition of release of chemical mediators of inflammation as well as enhancement of antioxidant defense system. The results obtained justify the use of the hydroethanolic seed extract of Monodora myristica for the treatment of painful and inflammatory conditions in traditional medicine.

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