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The Analgesic Effect of Ethanolic Extract of *Myristica Fragrans Houtt* (Nutmeg) on Mice

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ABSTRACT

The analgesic effect of the ethanolic extract of *Myristica fragrans* was studied in rats using the following models; Acetic acid induce writing, Thermal test (HOT PLATE), Formalin induced pain test. The results for all the models showed dose dependent and were significant (P<0.05). The control for acetic acid induced- writhing test showed 72±0.41, the extract at dose of 200mg/kg showed 34.25 ± 0.85 while dose of 500mg/kg showed 20.5±65 and Aspirin drug group showed 3.5± 0.65. There was an increase in pain threshold for thermal induced pain at 60mm pre-treatment. For the control group, the result showed 2.59± 0.08 which was significant (P<0.05). When *M. fragrans* was given at 200mg/kg, the result was 8.75± 0.72 and when given at dose 500mg/kg, the reaction time was 11.75 ± 0.60. For the Aspirin drug group, the reaction time was 33.30 ±1.52. The formalin induced pain was inhibited in both phases i.e 0-5mins and 15-30 mins, the result was still significant for both phases. The results for the control group were 97.25±0.48 and 90.5±0.65 for 0-5mins and 15-30mins respectively. For the extract (*M. fragrans*) at dose 200mg/kg the result for 0-5 min was 72.75 ±0.48 and for 15-30 min, it was 44.75± 1.32, at the dosage of 500mg/kg, the results were 57.5± 0.65 and 31.75± 0.72 at 0-5min and 15-30min respectively. The results for the aspirin drug group were 52.0 ± 0.41 and 22.25 ± 0.63 at 0-5min and 15-30min respectively. The results indicated that the analgesic effect of *Myristica fragrans houtt* (NUTMEG) ethanolic extract is both peripherally and centrally significant.

Keywords: *Myristica fragrans houtt*, Analgesic effects, Acetic acid writhing, Thermal test, formalin pain test, mice.

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INTRODUCTION

Myristical fragrans (Nutmeg) is the dried seed of nutmeg plants. The nutmeg plant requires a hot, humid climate and is widely cultivated in the tropics, particularly on the spice islands (the Moluccas) around the strait of molaccas in the Carribbean[1]. M. fragrans is widely used as a spice because of its characteristic pleasant fragrance and its warm taste it is used to flavor many kinds of baked foods, confections puddings, meat etc, it is also used as components of curry powder, teas and soft drinks or mixed in milk and alcohol.[2]

Medicinally, M. fragrans houtt has been reported to have Aphrodisiac[3], Stomach carminative[4], Tonic[5], Nervous stimulant[6], Anomatic, narcotic astringent, hypolipidemic, antithnombotic, antifiungal and anti-inflammatory properties[7].

It has also been shown to be useful in paralysis[8] and increased blood circulation[9], it also possesses antioxidant property[10].

The chloroform extract of nutmeg showed analgesic and anti-inflammatory activity in rodents[11] and also exhibited antidarrhoea activity by increasing tissue contents of Na^+ and Cl ions[12].

The petroleum either extract of M. fragrans fruits possesses antidiarrhoea property[13] and its hexane extract has been reported to have memory enhancing effect in mice[14]. The purpose of the present study was, therefore to evaluate the analgesic effect of the ethanolic extract using several acute and chronic models of pain in mice.

MATERIALS AND METHODS

Preparation of plant extract

Dried kernels of M. fragrans were purchased from the Choba market Prot- Harcourt, River state and authenticated by Mr. Jefta of the Department of Botanical Science; University of Port-Harcourt.These seeds were further dried under shade for 2 weeks. The dried seeds were milled with a manual engine into powder. 360g of the powdered seed sample was suspended in 500ml of ethanol (%, BDH) (vol) and left for 2days. The suspension was filtered with a filter membrane with a pore size of about 0.1-0.5µm. The ethanolic filtrate was concentrated using air pressure exploratory with high pressure and temperature (40%) the concentrate was freeze-dried and kept frozen until used, total amount of the extract gotten was 34.4g

Animals:

Adult albino mice weighing (18-25g) of either sex were kept at the animal house of Madonna University, River State. The animals maintained under standard environmental conditions had free access to standard diet (Pfizer feeds, PLC) and water ad libitum. The research study and

animal use was under the permission of Ethical Committee on The Use of Laboratory Animal for Reasearch, Madonna University, Nigeria.

Thermal Test

The method of *Hikino et al*[15] was used. The extract (200mg/kg and 500mg/kg) was administered orally to the test group, morphine (10ml/kg) was administered subcutaneously as reference group and the control group was given normal saline (5ml/kg) were administered 1hr before the mice were placed on a hot plate regulated at 56 $\pm^{o}$C and the reaction time (delay to lick the paw) to the thermal stimulus was observed with a cut off time of 60sec.

Chemical Test

The method of *Koster et al*[16] was used. The extract (200mg/kg and 500mg/kg) was administered orally to two different groups respectively. Aspirin (100mg/kg) was administered subcutaneously to the third group while the fourth group received distilled water (5mg/kg) and serve as the control group.

1hr after, acetic acid (0.6%,v/v in normal saline, 10ml/kg) was injected intraperitoneally. The writhings exhibited by each animal were counted for 10min, starting for 10min of the acetic acid injection).

Formalin Test

The methods of *Shibata et al.*[17] was used. Twenty microlitres of 1% formalin was injected subcutaneously into the right hand paw of mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Reponses were measured for 5min after formalin injection (first phase) and 15-30min after formalin injection (second phase). Extract (200-500mg/kg, oral) and morphine (10mg/kg subcutaneously) were administered 60 and 30min, respectively before formalin injection. Control animals received distilled water (10ml/kg)

Statistical Analysis

All data were expressed as mean t SEM and analyzed by one way ANOVA using S.P.S.S. (version 17).

RESULT AND DISCUSSION

The acetic acid induced writhing on the extract M. fragrans caused a significant ($P<0.05$) and dose dependent inhibition compared to the control writhes (Table 1). The effect or inhibition produced at the highest dose (500mg/kg) of the extract was significantly ($P<0.05$) lower than that by Aspirin (100mg/kg).

Table 1. Effect of ethaholic extract of M. fragrans on acetic acid- induced writhing test.

Group	Dose (mg/kg)	No of writhes (per 30min)	Inhibition (%)
Control	___	72.00 ± 0.41	___
Myristica	200	34.25 ± 0.85	52.43
Fragrans	500	20.50 ±0.65	71.53
Aspirin	100	3.50 ± 0.65	95.14

Values are mean ± SEM P<0.05 significantly differed from control: ANOVA (n=5).

The thermal induced pain test on the extract M. fragrans caused a significant (P<0.05) and dose dependent inhibition of control (Table 2). The inhibition produced at the highest dose (500mg/kg) of the extracts was significant (P<0.05) lower than that by morphine (10mg/kg). There was a significant dose dependent inhibition of both phases of the formalin induced pain response in mice (Table 3) with a more potent effect on the second than the first phase morphine also inhibited both phases of the pain.

Table2. Effect of ethanolic extract of M. fragrans on thermal- induced pain.

Group	Dose (mg/kg)	No of writhes (per 30min)	Inhibition (%)
Control	___	2.59 ± 0.08	___
Myristica	200	8.75 ±072	237.8
Fragrans	500	11.75 ± 0.60	353.7
Morphine	10	33.30 ± 1.52	1185.7

Table3. Effect of ethanolic extract of M. fragrans formalin- induced pain.

Group	Dose (mg/kg)	0-5 min	% inhibition	15-3 30min	Inhibition (%)
Control	___	97.25 ± 0.48	__	90.5 ±0.65	___
Myristica	200	72.75± 0.48	25.19	44.75 ± 1.32	50.55
Fragrans	500	5.7.5 ±0.65	40.87	31.75 ± 0. 72	64.92
Morphine	100	52.0. ±0.41	46.52	22.25 ± 0.63	75.41

Several acute tests were employed in evaluating the analgesic effect of the ethanolic effect of M. fragrans. It is necessary to apply tests which differ with respect to stimulus quality, intensity and duration, to obtain as complete a picture as possible of the analgesic properties of a substance using behavioral nociceptive tests[18]. The results obtained indicated that the extract possesses a moderate dose-dependent analgesic effect on the various pain models used. A potent inhibitory effect was exerted by the extract on the mouse writhing assay (a test useful for evaluating mild analgesic non-steroidal anti-inflammatory agents). This suggests that the analgesic effect of the extract many be peripherally mediated.

The extract also had a significant effect on the various acute (Phasic) pain models, namely, thermal-induced pain test and acetic acid-induced pain test. Centrally acting analgesic drugs elevate pain threshold of animal towards heat and pressure. The effect of the extract on the pain models indicates that it might be centrally acting.

The extract inhibited both phases of the formalin- induced pain with a more potent effect on the second than the first phase. The formalin pain test is very useful for evaluating the mechanism of pain and analgesia[18]. Drugs which act mainly centrally, such as narcotic analgesics, inhibit both phases of pain in this models while peripherally acting drugs such as aspirin or indomethacin, only inhibit the late phase[19].

The results obtained in this study indicate that the extract possesses analgesic properties, which are mediated via peripheral and central inhibitory mechanisms. This could provide a rationale for the use of this plant in pain and inflammatory disorders in folk medicine.

CONCLUSION

The results indicated that the analgesic effect of *Myristica fragrans houtt* (NUTMEG) ethanolic extract is both peripherally and centrally significant as presented variously by tests which differ with respect to stimulus quality, intensity and duration, however, the actual mechanism of action is for this action is yet to be fully elucidated.

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