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Effects of *Monodora myristica* (Gaertn, Dunal.) (Annonaceae) root bark on acute and chronic inflammation

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Several researches have established the antioxidant, antibacterial and anti-inflammatory activities of the seeds and stem bark of *Monodora myristica* while scientific data on the pharmacological activities of the roots is sparse. Hence, this work evaluated the effects of methanol extract of the root bark of *M. myristica* on acute and chronic inflammation. The root bark was extracted by cold maceration in methanol to yield methanol crude extract (MME). MME was subjected to phytochemical screening and acute toxicity test. The effects of MME on acute inflammation were evaluated using carrageenan induced rat paw edema and xylene induced topical ear edema in mice. While formaldehyde induced arthritis and cotton pellet induced granuloma in rats were used to investigate its effects on chronic inflammation. The MME was found to be relatively safe (LD50 >5000 mg/kg). Phytochemical screening revealed the presence of glycosides, carbohydrates, reducing sugars, resins, terpenoids, steroid and proteins in MME. The MME elicited 58.1% inhibition of xylene induced topical ear edema and dose-dependent reduction of carrageenan induced rat paw edema with 800 mg/kg causing 60.9% inhibition after 1 to 2 h. However, there was no activity against chronic inflammation. Results demonstrate the ability of root bark of *M. myristica* to ameliorate acute inflammation thus justifies the ethnomedicinal use of the plant to manage inflammation. There is therefore the need for further studies on the root bark of *M. myristica* so as to maximize its medicinal potential.

Key words: *Monodora myristica*, carrageenan induced pedal edema, formaldehyde induced arthritis, cotton pellet induced granuloma, xylene induced topical ear edema, acute inflammation, chronic inflammation.

INTRODUDUCTION

Morphologically, *Monodora myristica* is a perennial plant of the Annonaceae or custard apple family of flowering plants (Burubai et al., 2009; Ojiako et al., 2010). The tree grows naturally in the evergreen forest of the sub-Saharan African regions (Burubai et al., 2009) in West Africa: from Libya to Nigeria, Cameroon and Ghana, as well as in Angola, Uganda and West Kenya (Weiss, 2002; Okafor, 1976). Globally, the plant is widely

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distributed from Africa to Asia, Central and South America and Australia (Omobuwajo et al., 2003; Ekeanyanwu et al., 2012). The tree can reach a height of 35 and 2 m in diameter (Weiss, 2002). Fruiting occurs from August to November (Omobuwajo et al., 2003). The fruit of *M. myristica* is a berry of 20 cm in diameter, smooth, green and spherical and becomes woody at maturity. It is attached to a long stalk, which can be up to 60 cm long. Inside the fruit are the numerous oblong, pale brown seeds which are usually 1.5 cm long and are embedded in a white sweet-smelling pulp (Weiss, 2002; Onyenibe et al., 2015). It has been observed that an average of 119 to 122 seeds can be found in one fruit (Burubai et al., 2009).

M. myristica is currently listed under Kew's difficult seeds due to inability to grow easily outside natural habitat (Burkill, 1985). The plant is largely harvested from the wild and greatly affected by wild fires, urbanization, reckless and uncontrolled felling of trees for timber and firewood without replanting (Uyoh et al., 2014). It is variously known as *Iwor* among the Itsekiri's, *Ikposa* in Benin; *Ehiri* or *Ehuru* in Ibo land, *Gujiya dan miya* in Hausa land, and *Ariwo, arigbo, Abo lakoshe* or *eyi naghose* in Yoruba land, *Ehinawosin* in Ikale, *Uyengben* in Edo, and *Fausse noix de muscade* in French (Keay, 1989; Feyisayo e al., 2013; Enabulele et al., 2014; Bouba et al., 2016). The timber is hard, easy to work with and is used for carpentry, house fittings and joinery while the seeds are also made into necklaces (Ngueface et al., 2004). The presence of bioactive compounds in the plant makes it possible for the seeds to be used in traditional medicines as well as spice in local foods (Okwu et al., 2005).

One of the major medicinal uses of this plant is in inflammation. Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Ferrero-Miliani et al., 2007). The five classical signs of inflammation are heat, pain, redness, swelling, and loss of function (Ferrero-Miliani et al., 2007).

Inflammation can be classified as either acute or chronic. Acute inflammation is a short-term process, usually appearing within a few minutes or hours and begins to cease upon the removal of the injurious stimulus (Cotran et al., 1998).

Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. It is characterized by five cardinal signs designated by the acronym 'PRISH' (Parakrama and Clive, 2005).

Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation, such as mononuclear cells, and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

Several researches have established the anti-oxidant (Akinwunmi et al., 2013), anti-fungal (Okwu et al., 2007), anti-bacterial (Enabulele et al., 2014), anti-spasmodic, anti-ulcer (Komolafe, 2012), anti-hypertensive (Koudou, 2007), anti-cancer (Bakarnga-Via, 2014), hepato-protective (Oyinloye et al., 2016), cholesterol lowering (Nwozo et al., 2015), and anti-inflammatory (Ishmail et al., 2016) activities of the seeds and stem bark of *M. myristica* while scientific data on the pharmacological activities of the roots is sparse. Hence, this work evaluated the effects of methanol extract of the root bark of *M. myristica* on acute and chronic inflammation.

MATERIALS AND METHODS

Chemicals

Extraction of analytical grade methanol

Phytochemical screening: Molisch's reagent, Fehling's solutions I and II, dilute and concentrated sulphuric acid, Dragendorff's reagent, potassium hydroxide solution, Millon's reagent, ethanol, distilled water, chloroform, Mayer's reagent, Wagner's reagent, Picric acid solution, aluminium chloride solution, acetone, hydrochloric acid solution, nitric acid solution, and copper sulphate solution.

Anti-inflammatory test: Carrageenan, xylene, tween 80, distilled water, piroxicam (Hovid®) 10 mg capsule, formaldehyde, water and Goya® extra virgin olive oil.

Apparatus

Syringes, animal weighing balance, electronic weighing balance, spatula, bijou bottles, beakers, conical flasks, cotton wool, test tubes, retort stands, animal cages, hand towels, gloves, litmus papers, Bunsen burner and rotor evaporator.

Animal

(1) Acute toxicity (LD_{50}): Swiss albino male and female (non-pregnant) mice (15-30 g)
(2) Xylene induced topical ear edema: Swiss albino male and female (non-pregnant) mice (15-30 g)
(3) Carrageenan induced pedal edema: Swiss albino male and female (non-pregnant) rats (60-100 g)
(4) Formaldehyde induced arthritis: Swiss albino male and female (non-pregnant) rats (60-100 g)
(5) Cotton pellet induced granuloma: Swiss albino male and female (non-pregnant) rats (90-150 g)

Collection and preparation of plant

The root of *M. myristica* was collected from Obinagu in Udi LGA, Enugu State Nigeria on July 2012 and was authenticated by Mr. Ozioko of Centre for Bioresources Development and Conservation Programme (BDCP), Nsukka, Enugu State. The root bark was separated from the root wood. The root back was then washed and shade dried for 14 days and subsequently milled to a coarse powder using a mechanical milling machine.

Extraction of the plant

A 5.0 kg of the milled coarse root bark powder was extracted by

cold maceration using methanol at 25°C for 48 h with intermittent shaking. It was subsequently concentrated using a rotor evaporator under reduced pressure and completely dried over a water bath at 60°C to yield 113.86 g of the methanol crude extract (MME).

Determination of the yield (%)

The yield (%) of methanol extract was calculated using the formula:

$$\frac{\text{Total yield of the methanol extract}}{\text{Weight dried milled root bark}} \times 100$$

Phytochemical screening of the MME

Phytochemical analysis entails performing simple chemical tests to detect the presence of secondary metabolites e.g. alkaloids, glycosides, tannins, etc. The phytochemical tests carried out on the methanol extract were based on the procedures outlined by Trease and Evans (1996).

Determination of acute toxicity (LD_{50})

Acute toxicity (LD_{50}) determination was carried out using the Lorke (1983) procedure. A preliminary test was carried out using three different doses of the MME. The mice were placed in 3 groups of 3 animal each and were administered the following: group 1 received 10 mg/kg of the extract, group 2 received 100 mg/kg of the extract, and group 3 received 1000 mg/kg of the extract. These were administered via the oral route. The animals were constantly observed for the first 2 h, intermittently for the next 4 h and then overnight and the observation was recorded at the end of 24 h. From the results obtained, the second phase of the acute toxicity test was performed using 3 groups of one animal each. One group received a dose of 1600 mg/kg, another group received a dose of 2900 mg/kg and the last group received a dose of 5000 mg/kg. The general behavior of the mice was continuously monitored similarly.

$$LD50 = \sqrt{x_1 x_2}$$

where x_1 = least lethal dose after a 24 h observation period and x_2 = the highest non-lethal dose after a 24 h observation period.

Test for acute anti-inflammatory activities

Carrageenan induced rat paw edema (systemic)

The rat paw edema of Winter et al. (1962) was used. Here, 5 groups of 5 animals each were used. Zero time volume of water displaced by the left hind paw of each rat measured. Groups 1, 2 and 3 were then treated with 200, 400 and 800 mg/kg of the MME, respectively while groups 4 and 5 received 5 ml/kg of olive oil and water (3:1)/vehicle/negative control and 50 mg/kg of piroxicam/ positive control, respectively via oral route.

In an hour later, inflammation was induced by injecting 0.1 ml of 1% w/v carrageenan in normal saline into the sub plantar region of the left hind paw of each rat (Winter et al., 1962). After 30 min, the volume of water displaced by the left hind paw of each rat was taken and this was repeated every 1 h for 6 h.

Edema formation was assessed in terms of the difference between the zero time volume of the water displaced by the left hind paw (V_0) and the volume of water displaced by the left hind paw at the different time intervals (V_t) after carrageenan injection (Okoli et al., 2006). The level of inhibition was calculated using the relation (Perez, 1996):

$$\% \text{ inhibition} = 100\left[1 - \left(\frac{a-x}{b-y}\right)\right]$$

where a = mean volume of water displaced by treated rats after carrageenan injection; x = mean volume of water displaced by treated rats before carrageenan injection; b = mean volume of water displaced by control rats after carrageenan injection; and y = mean volume of water displaced by control rats before carrageenan injection.

Xylene induced ear edema (topical)

Here, 3 groups of 5 animals each were used and the treatment was as follows: group 1 received 5 mg/ear of the methanol extract, group 2 received 5 mg/ear of piroxicam (positive control), group 3 received 5 ml/ear of olive oil and water (3:1), that is, vehicle. These treatments were applied on the anterior surface of the right ear. Topical inflammation was instantly induced on the posterior surface of the same ear by application of xylene (0.1 ml/ear).

Two hours after the induction of the inflammation, the mice were sacrificed by over dose of chloroform. Circular section (6 mm diameter) of both the right ear (treated) and left ear (untreated) were punched out using a cork borer and weighed. Edematous response was quantified as the weight difference between the two earplugs. The anti inflammatory activity was evaluated as percentage inhibition in the treated animals relative to control animals (Tubaro et al., 1985; Asuzu et al,. 1999) using the relation:

$$\text{Edema inhibition (\%)} = 100\left(1 - \left(\frac{Rt - Lt}{Rc - Lc}\right)\right)$$

here R_t = mean weight of the right earplug of treated animals, L_t = mean weight of the left earplug of treated animals, R_c = mean weight of the right earplug of control animals, and L_c = mean weight of the left earplug of control animals.

Test for chronic anti inflammatory activity

Formaldehyde induced arthritis

The method of Seyle (1949) was used, 4 groups of 5 animals each were used here and the duration was 10 days. Zero time volume of water displaced by the left hind paw of each rat was measured. Subsequently, groups 1 and 2 were given 200 and 400 mg/kg of the methanol, respectively while groups 3 and 4 received the vehicle (olive oil and water in the ratio of 3:1), that is, negative control and 10 mg/kg of piroxicam (positive control) all via the oral route. After 2 hour, inflammation was induced by injecting 0.1 ml of 2% w/v formaldehyde into the sub-plantar region of the left hind paw of each rat. After 4 h, the volume of water displaced by the left hind paw of each rat was then determined. On the 2nd day, the rats were treated and soon thereafter, the volume of water displaced by the left hind paw of each rat was determined. On the 3rd day, the volume of water displaced by the left hind paw of each rat was determined also. Inflammation was subsequently re-induced. On the 4th day, the rats were treated and the volume of water displaced by the left hind paw of each rat was determined. This was then repeated on days 5, 6, 7, 8, 9 and 10. The animals were then sacrificed afterwards. Edema formation was assessed in terms of the differences between the zero time volume of water displaced by the left hind paw (V_o) and the volume of water displaced by the left hind paw at the different time intervals (V_t) after 0.1 ml of 2% w/v formaldehyde injection. The % inhibition was calculated using the

Table 1. Phytochemical constituents of the methanol crude extract.

Phytochemical constituents	Methanol crude extract
Alkaloids	++
Glycosides	+++
Saponins	+
Flavonoids	+
Tannins	++
Acid compounds	+
Resins	+++
Steroids	+++
Terpenoids	++++
Proteins	+++
Reducing sugars	+++
Carbohydrates	++++
Fats and oil	+

+ Mildly present, ++ moderately present, +++ highly present, ++++ abundantly present.

relation:

$$\% \text{ inhibition } = 100[1 - (\frac{c-x}{d-y})]$$

where c = mean volume of water displaced by the left hind paw by the treated rats after formaldehyde injection; x = mean volume of water displaced by the left hind paw by treated rats before formaldehyde injection; d = mean volume of water displaced by the left hind paw by control rats after formaldehyde injection; y = mean volume of water displaced by the left hind paw by control rats before formaldehyde injection.

Cotton pellet induced granuloma

Four groups of 5 animals were used and the duration was for 8 days. Known weights (30 mg) of sterile cotton pellets (2) were surgically implanted subcutaneously into the axillae region of each rat with the aid of a sedative (xylazine) and anesthetic (ketamine). After 5 h when the effects of the anesthetic and sedative had worn off, groups 1 and 2 were treated with 200 and 400 mg/kg of the methanol via the oral route, respectively while groups 3 and 4 received 10 mg/kg of piroxicam (positive control) and the vehicle (negative control) via the oral route, respectively. This treatment was repeated on days 2, 3, 4, 5, 6, and 7 and on day 8, the animals were sacrificed and the cotton pellets coated with granuloma tissue were surgically removed. The cotton pellets were then dried at 60°C to a constant mass and weighted. The granuloma formed was determined as the difference between the final and initial weights of the cotton pellets.

Statistical analysis

The results obtained were analyzed by SPSS version 22.0 using one way ANOVA and expressed as mean ± standard error of mean (SEM). Differences between treated and control groups were evaluated further with LSD Post hoc test and considered significant at P<0.05.

RESULTS AND DISCUSSION

Extractive yield (%) of the MME

The yield of the MME calculated as percentage of the starting material was 2.28% w/w. After the cold maceration in methanol, the methanol extract obtained was 113.86 g. The weight of the starting material, that is, the weight of the dry milled root bark that was subjected to methanol maceration was 5 kg. After the extraction of the root bark, the MME was observed to have a dark brown colour, a coffee aroma and when warm, the MME was in a molten state but when cool, it was very viscous.

Phytochemical analysis

MME tested positive to alkaloids, glycosides, saponins, resins, flavonoids, tannins, proteins, steroids, carbohydrates, reducing sugars, terpenoids, acidic compounds and fats and oil Table 1.

Acute toxicity (LD_{50}) of the MME

The MME was found to be relatively safe (LD_{50} >5000 mg/kg) since no death was recorded at 5000 mg/kg Table 2.

Effect of methanol extract on carrageenan induced pedal edema

The MME elicited a dose dependent and significant (*P<0.*05) reduction of carrageenan induced rat paw edema. The early phase of the inflammation (90 to 120

Table 2. Acute toxicity (LD_{50}) of the methanol crude extract.

1st phase of LD50 test		2nd phase of LD50 test	
Dose (mg/kg)	**Mortality**	**Dose (mg/kg)**	**Mortality**
10	0/3	1600	0/1
100	0/3	2900	0/1
1000	0/3	5000	0/1

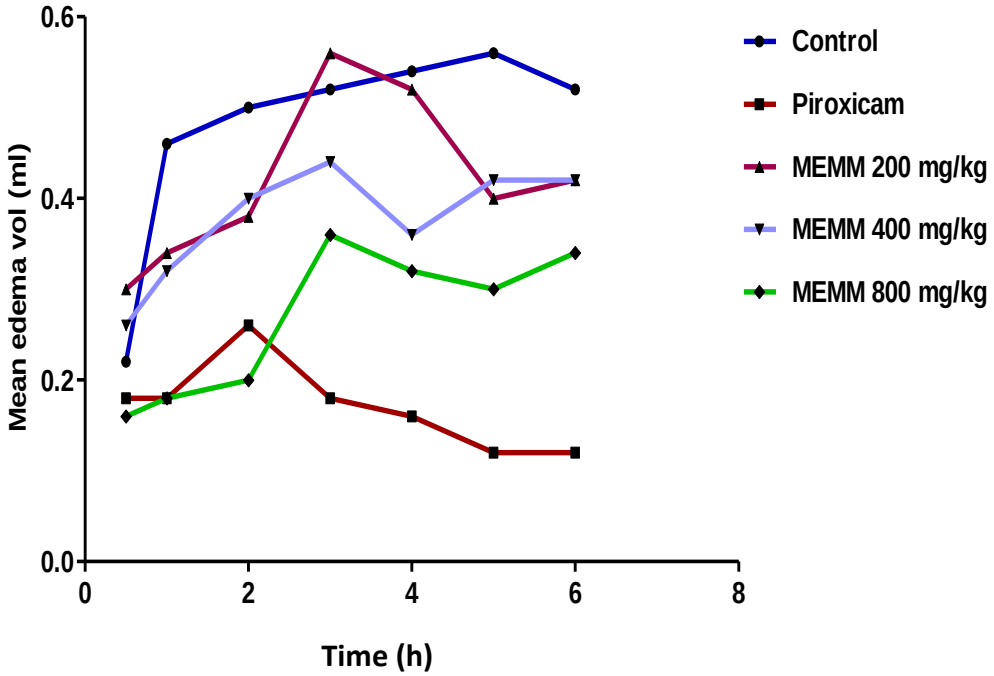

Figure 1. Effect of methanol extract on carrageenan induced rat paw edema.

min) is due to the release of histamine and serotonin while the later phase is due to activation of kinin and prostanoids. The ability of the MME to reduce both early and late phases of carrageenan induced rat paw edema suggests the inhibition of histamine, serotonin, kinin and prostanoids. At 1 to 2 h, 800 mg/kg of methanol extract elicited a 60.9% inhibition of carrageenan induced rat paw edema similar to 50 mg/kg of Piroxicam. However, after 3 to 6 hours, the percentage inhibition of the MME declined to less than 50% indicating that the activity of the methanol extract is short-lived lasting for about 2 h Table 3 and Figure 1.

Effect of MME on xylene induced topical ear edema

The MME also elicited a dose dependent and significant (*P<0.*05) reduction of xylene induced topical ear edema. Upon topical application of xylene, there was fluid accumulation and edema which is a characteristic of acute inflammation response. Suppression of this response is a likely indication of anti-phlogistic effect (Akah and Alkohafi, 1998) and suggests it has acute topical anti-inflammatory activity. MME elicited a 58.1% inhibition of xylene induced topical ear edema which was greater than piroxicam (46% inhibition) at a dose of 5 mg/ear. This indicates that MME may be a better choice in alleviating inflammation than piroxicam Table 4 and Figure 2.

Effect of MME on formaldehyde induced arthritis

There was no activity on chronic inflammation. The MME was unable to inhibit formaldehyde induced arthritis. In formaldehyde induced arthritis, articular changes induced by formaldehyde injection is similar to those that occur in rheumatoid arthritis.

Although, at a dose of 200 mg/kg, there was 52.7, 45.7 and 25.9% inhibition (which was non-significant P<0.05)

Table 3. Effect of methanol extract on carrageenan induced pedal edema.

Treatment	Mg/kg	Edema (ml)						
		0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
MME	200	0.3±0.05 (-36.4)	0.34±0.07 (26.1)	0.38±0.12 (24.0)	0.56±0.09 (-7.7)	0.52±0.12 (0.0)	0.4±0.09 (28.6)	0.42±0.1 (19.2)
	400	0.42±0.1 (19.2)	0.32±0.37 (30.7)	0.4±0.00 (20.0)	0.44±0.07 (15.4)	0.36±0.02 (30.8)	0.42±0.02 (25.0)	0.42±0.02 (19.2)
	800	0.42±0.1 (19.2)	0.18±0.04 (60.9)	0.2±0.04 (60.0)	0.36±0.07 (30.8)	0.32±0.06 (38.5)	0.3±0.05 (46.4)	0.34±0.08 (38.5)
Piroxicam	50	0.18±0.16 (18)	0.18±0.06 (60.9)	0.26±0.09 (48)	0.18±0.05 (65.4)	0.16±0.07 (69.2)	0.12±0.05 (78.6)	0.12±0.02 (76.9)
Control (Vehicle)	-	0.22±0.06	0.46±0.09	0.5±0.13	0.52±0.07	0.54±0.37	0.56±0.07	0.52±0.04

Table 4. Effect of Methanol extract on Xylene induced topical Ear Edema.

Treatment	Dose (mg/ear)	Average edema (ml)	Inhibition (%)
MME	5	4.4±2.11	58.1
Piroxicam	5	5.67±0.8	46.0
Control (Vehicle)	-	10.5±0.5	-

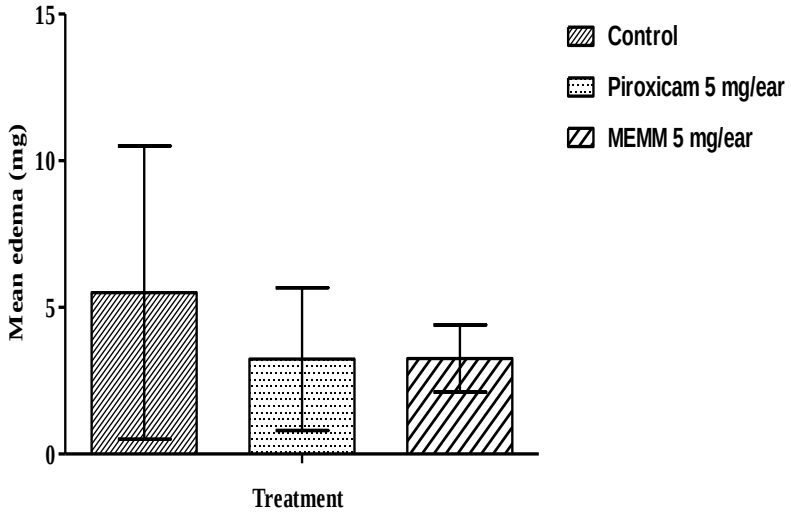

Figure 2. Effect of methanol extract on onxylene induced mouse ear edema.

Table 5. Effect of methanol crude extract on formaldehyde induced arthritis.

Treatment	mg/kg	Edema (ml)									
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
MME	200	0.3±0.03 (13.4)	0.2±0.02 (-50)	0.08+0.02 (0)	0.14±0.04 (-16.7)	0.34±0.04 (-70)	0.3±0.05 (-7.21)	0.3±0.05 (-11.1)	0.18±0.02 (52.7)	0.13±0.05 (45.7)	0.2±0.03 (25.9)
	400	0.3±0.03 (13.4)	0.12±0.07 (-50)	0.12±0.05 (-50)	0.24±0.06 (-100)	0.26±0.05 (-30)	0.24±0.06 (-14.3)	0.24±0.06 (11.1)	0.16±0.05 (56.8)	0.16±0.05 (30.4)	0.22±0.05 (18.5)
Piroxicam	10	0.38±0.09 (-46)	0.08±0.05 (233.3)	0.04±0.02 (50	0.008±0.05 (125)	0.15±0.02 (25)	0.15±0.02 (46.4)	0.2±0.03 (25.9)	0.25±0.02 (32.4)	0.05±0.03 (78.3)	0.12±0.02 (63)
Control (Vehicle)	-	0.22±0.11	0.08±0.06	0.06±0.05	0.12±0.07	0.2±0.09	0.28±0.06	0.27±0.05	0.37±0.05	0.233±0.07	0.26±0.6

Table 6. Effects of methanol crude extract cotton pellet granuloma.

Treatment	Dose (mg/kg)	Average edema (mg)	Inhibition (%)
MME	200	0.099±0.01	-32
	400	0.1±0.01	-33.3
Piroxicam	10	0.066±0.01	12
Control (Vehicle)	-	0.075±0.00	-

on days 8, 9 and 10, respectively, this cannot be attributed to the MME. Rather, this is due o the self-resolving nature of inflammation after 1 week which was observed from days 8 to 10. A similar incident was also observed at a dose of 400 mg/kg of MME on days 8, 9 and 10 with % inhibition of 56.8, 30.4 and 18.5, respectively (which was also non-significant *P<0.05*). As earlier stated, this activity was not significant and cannot be attributed to the MME due to self resolving nature of inflammation after about 1 week (Table 5).

Effects of MME cotton pellet granuloma

There was also no activity observed on cotton pellet induced granuloma. The MME showed a % inhibition of -32 which was not significant (*P<0.05*). Although, piroxicam (10 mg/kg) had a 12% inhibition which was greater than the MME, it was also not significant (*P<0.05*) (Table 6).

Conclusion

Though, the MME showed no sufficient and significant activity on chronic inflammation (formaldehyde induced arthritis and cotton pellet induced granuloma), the results demonstrate the ability of root bark of *M. myristica* to ameliorate acute inflammation thus justifies the ethnomedicinal use of the plant to manage inflammation. There is therefore the need for further studies on the root bark of *M. myristica* so as to maximize its medicinal potential.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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