Anti-inflammatory and anti-nociceptive effects of crude extract and fractions of *Morinda morindoides* root bark

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This study was carried out to assess the anti-inflammatory and anti-nociceptive effects of the extract of *Morinda morindoides* root bark to confirm folkoric claims. Anti-inflammatory and analgesic effects of the methanol extract, solvent fractions and Chromatographic sub-Fractions (CsF) A-F of the bioactive fractions of *Morinda morindoides* root bark were determined using carrageenan induced rat paw oedema model, thermal and acetic-acid induced writhing method in mice. Extracts of *Morinda morindoides* root bark at 400, 200 and 100mg/kg of methanol, solvent fractions (hydro methanol, hexane, chloroform and ethyl acetate) and bioactive CsF of the root bark were assessed in rats, which were compared with controls; a negative control given 10ml/kg Tween 80, positive control groups administered with Indomethacin, aspirin, paracetamol and morphine. The mean percentage inhibition of paw volume was highest in rats to which indomethacin was administered (85.65%), followed by rats administered aspirin (68.40%) which was comparable with that of rats dosed with 400mg/kg of *Morinda morindoides* root extract (65.52%).While the chloroform fraction and chloroform CsF A of *M. morindoides* at 200, and 100mg/kg respectively showed bioactivity with the highest percentage inhibition of increase in paw volume (93%). Also, extract increased significantly (P<0.05) the reaction time in the hot plate test (26.0+0.7)s and the mean number of writhing was significantly lower (P<0.05) in groups dosed with 400mg/kg methanol extract and 100mg/kg of ethyl acetate CsF D of *Morinda morindoides* root bark extract. The study establishes the anti-inflammatory and anti-nociceptive potential of *M. morindoides* root bark extract in methanol, chloroform and ethyl-acetate solvents.

Keywords: *Morinda morindoides,* root-bark, anti-inflammatory, anti-nociceptive.

INTRODUCTION

Inflammation has been known to be a regular occurrence in many disease conditions denoting the reaction of the body immune systems to physical, chemical or biological assault. It is a way by which the body removes the injurious stimuli as well as initiates the healing process (Denko, 1992). During inflammatory conditions, there is the activation of the body defense cells, the release of complements, cytokines, other inflammatory mediators and prostaglandins (Cotran et al., 2001). Erythema, pain (nociception), swelling and loss of function are the pathognomonic features of inflammatory conditions.

Before synthetic drugs became available for man's usage, medicinal plants have been used for the management of diseases. The synthesis of aspirin in the 19th Century opened a new vista in the history of anti-inflammatory and anti-nociceptive agents (Vane, 1971) but most conventional anti-inflammatory and analgesic agents are well known for their adverse effects including gastrointestinal irritations (Rainsford and Whitehouse, 1980). Pain is a common distressing feature of many disease conditions and anti-nociceptive drugs relieve pain by acting on the Central Nervous System or on peripheral pain mechanisms, without significantly altering consciousness.

Many plants have long been recognized as important sources of therapeutically effective medicines (Cragg et al., 1997). Herbs such as *Tithonia diversifolia* and *Tridax procumbens, Jatropha curcas* (Diwan et al., 1989; Owoyele et al., 2003; Olukunle et al., 2011) have been shown to possess anti-inflammatory activities while *Boswellia serrata* and *Pluchea indica* plants have analgesic effects (Vohora and Dandiya, 1992). Medicinal plants of this type can be useful as sole therapeutic agent in managing inflammatory conditions or as complementary therapy to conventional antiinflammatory drugs.

Plants of the *Morinda spp* (Family- Rubiaceae) are important plants in traditional medicine. In Nigeria, *M. lucida* is one of the four most used traditional medicines against fever (Burkill, 1997). Other species of Morinda group of plants are *M. morindoides*, *M. citrifolia* (noni), *M. longiflora* and *M. germinate*(Burkill, 1997).

M. morindoides popularly called brimstone tree in English, Oju ologbo in Yoruba (Southwest, Nigeria), is a climbing shrub that grows up to 10m high and is widely distributed in different parts of Africa such as Senegal, Cameroun and Nigeria (Burkill, 1997). Members of the *Morinda spp* has been used as decoctions among the natives against malaria (Tona et al., 2001; Awe et al., 1998; Asuzu, 1990)*,* diarrhoea, haemorrhoids, gonorrhoea, amoebiasis, rheumatism and other painful inflammatory diseases (Cimanga et al., 2006; Kambu, 1990; Meite et al., 2009). Biological studies have supported some of these traditional uses (Tona et al., 2004). A previous study by Cimanga et al., (1995) led to the isolation of nine flavonoids and flavonoids o-glycosides from the leaves of *M. morindoides.*

Although the use of these plants in traditional medicine have a rational background, it is essential to investigate the rationality of their use in modern scientific terms, this study is aimed at investigating the anti-inflammatory and anti-nociceptive properties of the crude methanol extract, solvents fractions and chromatographic sub fractions of *M. morindoides* root bark as claimed by folklore.

MATERIALS AND METHODS

Animals

Six matured Wistar rats of either sex weighing 150-200g were used per each group in all protocols involving rats and six swiss mice of either sex weighing 25-30g were used per each group in all protocols involving mice in this study.

All experimental protocols carried out on the animals were in accordance with the internationally accepted principles for the laboratory animal usage and were approved by Ethics Committee on the Laboratory Animal Use of the College of Veterinary Medicine of University of Agriculture, Abeokuta.

The animals were kept at the Experimental Animal House, College of Veterinary Medicine, University of Agriculture, Abeokuta.

They were provided feed (Vital feeds Limited, Ibadan, Nigeria) and water *ad- libitum.* The animals were kept for two weeks prior to the commencement of the study for acclimatization to laboratory conditions.

The housing provided had the following conditions; controlled lighting of 12:12 hours of light: dark, temperature of 25^0C and relative humidity of approximately 50%.

Plant material and preparation of extract

Fresh root bark of *Morinda morindoides* were obtained from a farm area in Odeda, Abeokuta and authenticated at both the Forestry Research Institute of Nigeria (F.R.I.N), Ibadan, and the Botany Department, College of Natural Sciences, University of Agriculture, Abeokuta, Nigeria where voucher specimen has been deposited.

The roots were air-dried, pulverized, finely sieved and 2kg of the powder was measured and soaked in 5 litres of methanol for 48hours, after which it was filtered and the filtrate was concentrated and evaporated to dryness in vacuo at 40°C, using rotary evaporator. The yield was calculated and the dry extract was stored in a refrigerator at -4°C until needed for experiments.

Two grams of the solid residue from concentrated methanol filtrate was also dissolved in 20ml of undiluted slightly warmed Tween-80 to make a working concentration of 100mg/ml. These working solutions were administered to the different animal test groups.

Bio-activity Guided Fractionation

The methanol extract of *M. morindoides* root bark was subjected to solvent –solvent fractionation in separating funnel. 100g of crude methanol extract of the root bark was suspended in 1:3methanol-water (Hydro methanol or MEOH), n-Hexane, chloroform, and ethyl acetate. This was done in a separating funnel at each stage of partitioning, the organic fraction (Phase) were pooled, and the solvent removed by concentrating the fraction on a water bath at 60ºC. This procedure was carried out for n-Hexane, chloroform, ethyl acetate and MEOH respectively. The bioactive fractions of the solvents were therefore subjected to further fractionation using standard procedure of accelerated gradient chromatography (Davies and Johnson, 2007) and the chromatographic sub-fractions were pooled into six groups (A-F) according to their TLC. All fractions and reference drugs were suspended in Tween-80 and administered orally to the animals for the acetic acid induced writhing and

carrageenan induced paw oedema test.

Drugs

Aspirin (100mg tablet of Acetylsalicylic acid, Jubel Nigeria Limited),
Indomethacin, (Greenfield Pharmaceuticals Nigeria Limited) and 500mg of Paracetamol
(May and Baker Nigeria Plc.) were administered orally to the animals while 15mg/ml
Morphine Sulphate (Antigen Limited, Ireland) and Carrageenan (Sigma-Aldrich, Belgium) were administered intraperitoneally to the animals.
Pure polyoxyethylene sorbitan monooleate (Tween 80 Sigma-Aldrich, Belgium) was administered orally as delivery vehicle for the plant extract.

Anti-inflammatory test

Carrageenan-induced rat paw oedema

Acute inflammation was induced using the carrageenan induced oedema model (Winter et al., 1962). The rats were starved for 12 hours after which they were divided into four groups (Groups A-D) of six animals each.

Group A rats, the negative control (untreated) were given (Tween 80) at 10ml/kg one hour before 0.1ml of (1% suspension of carrageenan in normal saline) was injected into the plantar surface of the right hind paw of each rat. Group B rats were given extract of *M. morindoides* root dissolved in Tween 80 orally at the dose of 400mg/kg one hour before carrageenan injection. Rats in Group C were given aspirin, a standard anti-inflammatory drug at the dose of 150mg/kg orally one hour before carrageenan injection while Group D rats were administered indomethacin, another standard anti-inflammatory drug at a dose of 10mg/kg orally one hour before carrageenan injection.

In two other separate experiments, (200) mg/kg of the solvents fraction(MeOH, hexane, ethyl acetate & Chloroform) and 100mg/kg Chromatographic sub Fractions A-F of *Morinda morindoides* root bark were respectively administered to the test groups while the control groups in the 2 experiments remain as described above.

The linear circumference of the paw was measured at zero hour on injecting the carrageenan suspension and three hours after carrageenan injection using a loop of thread tied round the paw such that it was neither too loose nor too tight. The length of the thread round the paw was then measured on a ruler and rounded off to the nearest centimetre, the readings were repeated thrice and the average taken to avoid errors. The percentage

inhibition was calculated according to the formula:)(Olukunle et al., 2011)

Eq. (A.1), Percentage Inhibition =

$$\frac{[C1-C0] \text{ control} - [C1-C0] \text{ test}}{[C1-C0] \text{ control}} \times 100$$

Where Co= Mean paw size at the 0 hour of carrageenan administration.

C1= Mean paw size at 3 hours after the carrageenan administration.

Anti-nociception test

Hot plate test

This was done as described by Hikino et al., (1985). The mice were divided into three groups of six mice each. The mice in group 1 were administered with methanol root extract of *M. morindoides* (400mg/kg), mice in group 2 were dosed 2mg/kg morphine while those in the group 3 which was the control were given 10ml/kg of Tween 80. The treatment was done one hour before the mice were placed on a hot plate (55 ± 1^0C) and the reaction time (licking the paw and jumping) to thermal stimulus was observed within 1 minute.

Acetic acid-induced abdominal writhing in mice

This was done as described by Koster et al., (1959). The mice were divided into three groups (Groups I- III) of six mice per group in each of the experiments carried out. Tween 80 at 10ml/kg was administered orally to the mice in the control group (Group I), 50mg/kg paracetamol was given orally to Group II animals and 400mg/kg of extract of *M. morindoides* root dissolved in Tween 80 was given orally to mice in Group III one hour before intraperitoneal injection of acetic acid (0.6%, v/v in distilled water,given at a dose of 10ml/kg).

In the fractionation phase, two separate tests were carried out in which 200mg/kg of the solvents fraction and 100mg/kg of the ethyl acetate Chromatographic sub Fractions A-F of *Morinda morindoides* root bark were administered to the test groups while the control groups remained as previously described.

The number of writhing exhibited by each animal was counted for 10 minutes beginning
10 minutes after acetic acid injection and computed according to the following formula: (Olukunle et al., 2011)

Eq. (A.2),   Percentage Inhibition =

$$\frac{\text{Mean of writhing (control)- Mean of writhing (test)}}{\text{Mean number of writhing (control)}} \times 100$$

Statistical analysis

Results were expressed as mean ± SEM. Analysis of the

Table 1. Effect of methanol extract of *M. morindoides* root bark on carrageenan induced paw oedema in Wistar rats.

Groups (n=6)	Dose mg/kg p/o	CBF(cm) 0hr	CAF(cm) 3hr	% increase in rat paw volume	% inhibition in rat paw volume
Group A (Control)	10	2.80 ± 0.47	3.38 ± 0.04	18.99± 1.32	13.09 ± 3.71
Group B (*M.morindoides*)	400	2.82 ± 0.65	3.02 ± 0.70	7.01 ± 1.40	65.52±13.10*
Group C (Indomethacin)	150	2.88 ± 0.03	2.96 ± 0.03	2.88 ± 0.58	85.65±13.40*
Group D (Aspirin)	10	3.03 ± 0.02	3.23 ± 0.42	6.59 ± 1.21	68.40±10.23*

* $P< 0.05$,   Values are Mean ± SEM
CBF – Circumference of the rat paw immediately after carrageenan injection (cm).
CAF - Circumference of the rat paw 3hours after carrageenan injection (cm).

Table 2. Effect of methanol extract of *M. morindoides* root bark on hot plate-induced pain in mice

Groups (n=6)	Dose mg/kg p/o	Reaction time (sec)
Group I (Tween 80)	10	18.22 ± 0.27
Group II (Morphine)	50	31.81 ± 1.50
Group III (*M. morindoides*)	400	25.73 ± 0.95

*$P<0.05$, Values are Mean ± SEM

data was done using the one-way Analysis of Variance (ANOVA) followed by the Duncan multiple range test. P value < 0.05 was considered significant in all cases.

RESULTS

Anti-inflammatory test

Crude methanol extract of *M. morindoides* root bark at a dose of 400mg/kg exhibited significant anti-inflammatory activity in carrageenan induced rat paw oedema model.

The mean percentage inhibition of paw volume was highest in rats to which indomethacin was administered (85.65%), followed by the rats administered with aspirin causing (68%) inhibition of increase in paw volume which was comparable with the result obtained from rats given *M. morindoides* (65.52%)(Table 1).

Anti-nociception test

The crude methanol extract of *M. morindoides* root bark, administered at a dose of 400mg/kg exhibited significant anti-nociceptive activity in mice (Table 2).

The extract caused significant increase in the mice reaction time of (25.73 ± 0.95)/s in the hot plate test when compared to the untreated control group reaction time of (18.22 ± 0.27)/s thereby indicating increase in pain threshold level.

Acetic acid- induced abdominal writhing in mice

The mean number of writhing movements was significantly lower (P<0.05) in mice dosed with 400mg/kg crude methanol extract of *M. morindoides* root (26.0±0.7) when compared with the control group (46.7±1.4) but was higher than value obtained from mice that was administered with the standard analgesic agent, paracetamol (23.5±1.3) (Table 3).

Pharmacological testing of the Solvent fractions and Chromatographic sub-fractions for anti-inflammatory effect

Each of the four solvents fraction (Meoh, hexane, chloroform and ethyl acetate) was tested for anti - inflammatory potential and the group of rats

Table 3. Effect of methanol extract of *M. morindoides* root bark on acetic acid-induced writhing in mice

Groups (n=6)	Dose mg/kg p/o	Number of writhing (per 10 min.)	% Inhibition of writhing
Group I (Control)	5ml/kg (Tween 80)	46.67 ± 1.36	0.00
Group II (Paracetamol)	50	23.50 ± 1.28	49.64*
Group III (*M.morindoides*)	400	26.00 ± 0.73	44.29*

Values are Mean ± SEM
*Statistically significant at P< 0.05
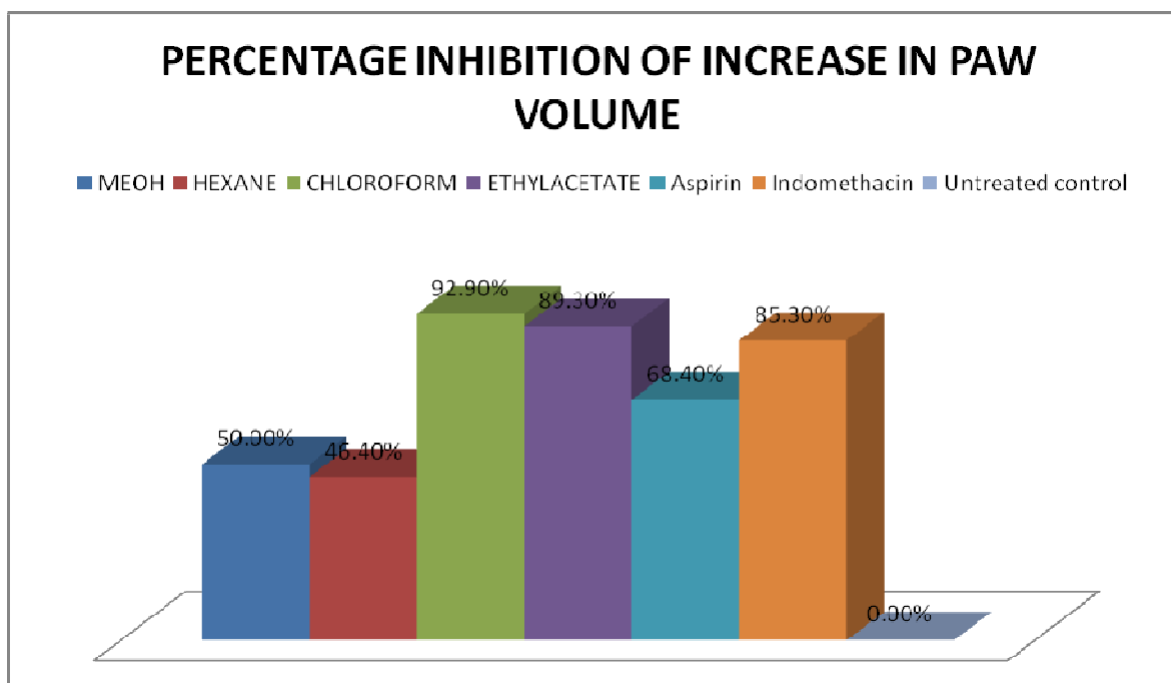
N.B: MEOH = Hydro methanol fraction.
Figure 1. Effects of 200mg/kg dose of Meoh, hexane, chloroform &ethyl acetate extracts of *M.morindoides* on carragenaan induced rat paw oedema

administered with 200mg/kg chloroform fraction of *M. morindoides* showed bioactivity with the highest percentage inhibition of increase in paw volume (93%), a value that is higher than what was obtained from the control groups that were administered with of standard anti-inflammatory agents' indomethacin (85%) and aspirin (68%)(Figure 1)
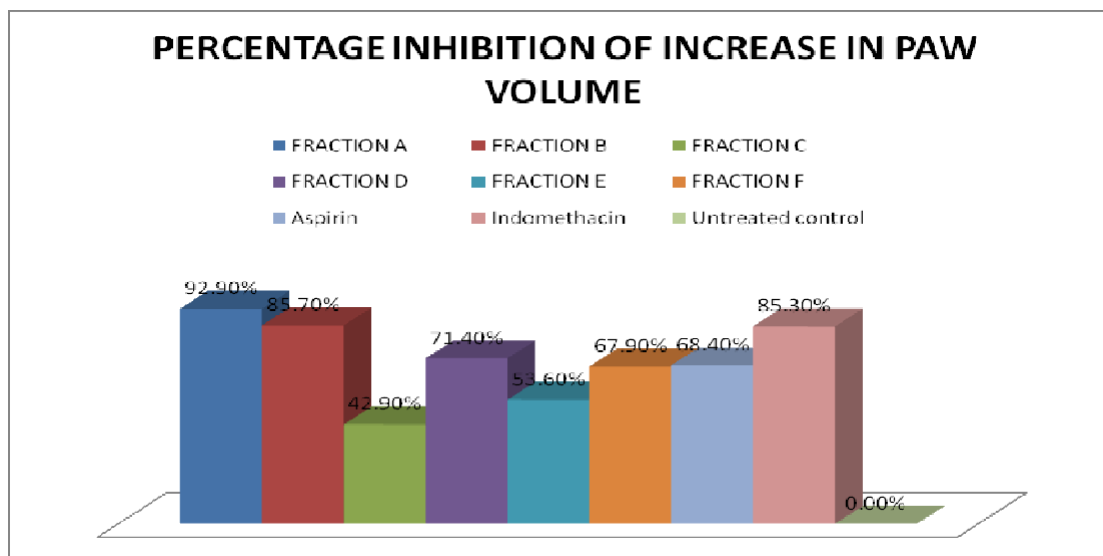
Each of the 6 sub-fractions (A-F) of chloroform fraction of *M. morindoides* was tested for anti-inflammatory potential at 100mg/kg dose. The chloroform chromatographic fraction A of *M. morindoides* showed the highest bioactivity with percentage inhibition of increase in paw volume (93%), a value higher than the performance of standard anti-inflammatory agent indomethacin (85%) and aspirin (68%) (Figure 2).

Pharmacological testing of the solvents and chromatographic fractions for analgesic Effect
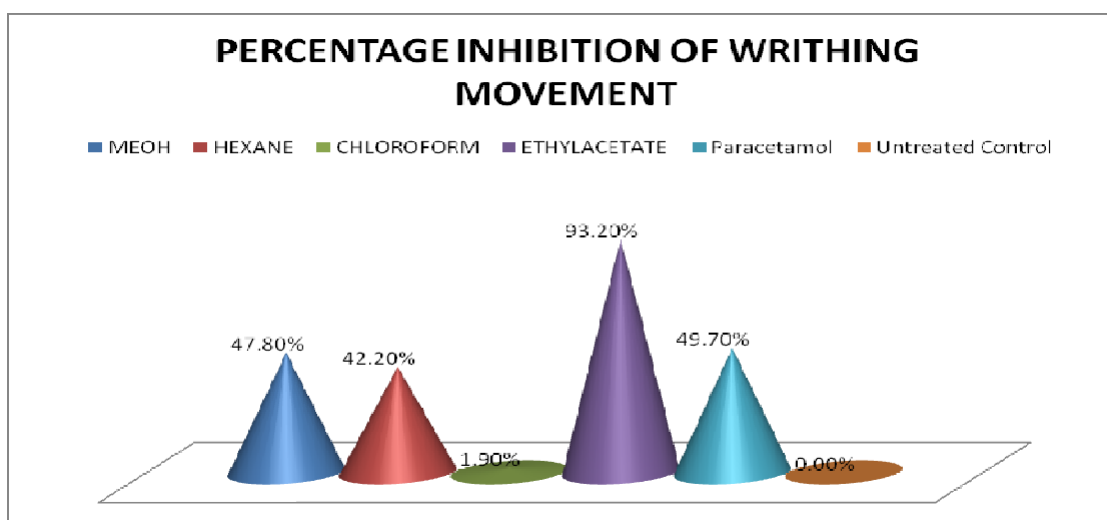
Each of the four solvents fraction (Meoh, hexane, chloroform and ethyl acetate) was assessed in the writhing movement experimental protocol for analgesic potential and the

200mg/kg dose group of ethyl acetate fraction of *M. morindoides* showed bioactivity with the highest percentage inhibition of writhing (93%) a value higher than the performance of reference analgesic agent paracetamol (50%) (Figure 3)

The 6 chromatographic sub fractions (A-F) obtained from the bioactive ethyl acetate fraction of *M. morindoides* were also assessed for analgesic potential

PERCENTAGE INHIBITION OF INCREASE IN PAW VOLUME

Legend:
- ■ FRACTION A
- ■ FRACTION B
- ■ FRACTION C
- ■ FRACTION D
- ■ FRACTION E
- ■ FRACTION F
- ■ Aspirin
- ■ Indomethacin
- ■ Untreated control

Values: 92.90%, 85.70%, 42.90%, 71.40%, 53.60%, 67.90%, 68.40%, 85.30%, 0.00%

Figure 2. Effects of 100mg/kg dose of chromatographic fractions of chloroform extract of *M.morindoides* on carragenaan induced rat paw oedema. NB: (n=6; p>0.05)

PERCENTAGE INHIBITION OF WRITHING MOVEMENT

Legend:
- ■ MEOH
- ■ HEXANE
- ■ CHLOROFORM
- ■ ETHYLACETATE
- ■ Paracetamol
- ■ Untreated Control

Values: 47.80%, 42.20%, 1.90%, 93.20%, 49.70%, 0.00%

Figure 3. Effects of 200mg/kg dose of meoh, hexane, chloroform and ethyl acetate extracts of *M. morindoides* plant on acetic acid induced writhing test in mice.
NB: MEOH = Hydro methanol fraction

in writhing experimental protocol and the 100mg/kg dose of ethyl acetate chromatographic sub fraction D of *M. morindoides* showed the highest bioactivity with percentage inhibition of writhing (71%) a value higher than the performance of reference analgesic agent paracetamol (50%) (Figure 4)

DISCUSSION

This study has evaluated for the activity of *M. morindoides* root bark. Our result suggests that methanol extract of *M. morindoides* root bark has anti-inflammatory effect comparable with those of the standard drugs, aspirin and indomethacin. Sub-fraction of the plant extract also shows stronger anti-inflammatory activity (Table 1; Figure 1 & 2).

Carrageenan-induced inflammatory process is believed to be biphasic (Vinegar et al., 1969). The initial phase seen at the 1st hour is attributed to the release of histamine and serotonin (Cruckhon and Meacock, 1971). The second accelerating phase of swelling is due to the release of prostaglandin, bradykinin and lysozyme. Katzung, (1998) reported that the second phase of
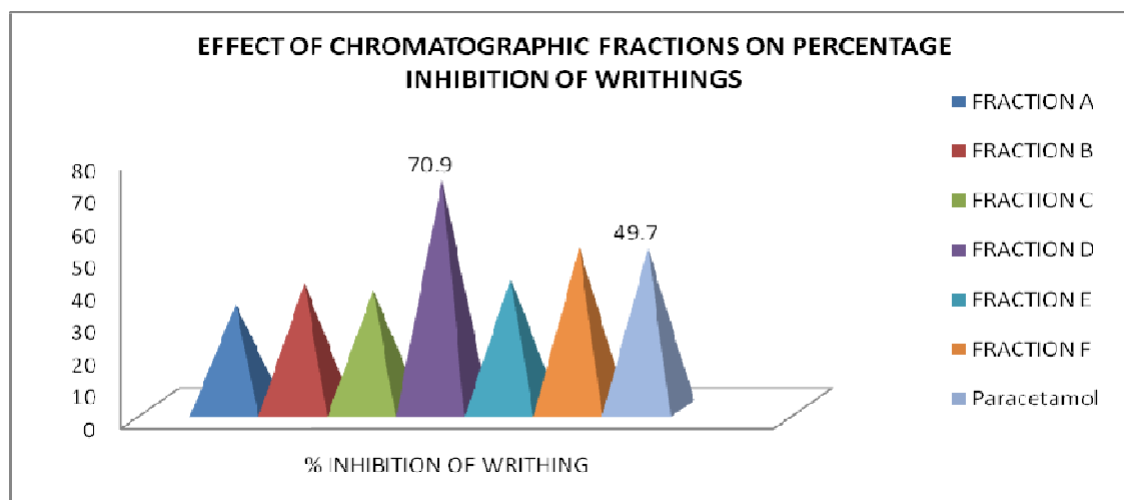
Figure 4. Effects of 100mg/kg dose of chromatographic fractions of ethyl acetate extracts of *M. morindoides* on acetic acid induced writhing test in mice.

oedema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents.

The anti-inflammatory activity exerted by the extracts and fractions of *Morinda morindoides* root bark suggests that it could have acted by affecting the release of these inflammatory mediators.

The presence of flavonoids in plants which has been shown to be part of the constituents of *Morinda morindoides* (Cimanga et al., 1995) is believed to be the factor responsible for the anti-inflammatory effects because flavonoids are known to exert strong antiiflammatory activity (Alejandra et al., 2003).

An important effect of flavonoids is the scavenging of oxygen-derived free radicals (Van Acker et al., 1995) and thus decrease the oxidative stress caused by NF-κB activation, a factor that has been found responsible for the coding of oxidative cytokines in most inflammatory conditions.

The anti-nociceptive effect of *M. morindoides* plant was also evident in this study, ethyl acetate fraction and chromatographic sub- fraction D exhibited significant anti-nociceptive bioactive compound responsible for the observed higher bioactivity.

The reduction in acetic acid induced writhing and the increase in the reaction time to the thermal stimulus, suggest that the anti-nociceptive effect of *M. morindoides* are mediated both peripherally and centrally (Adeyemi et al., 2002) since thermally induced painful stimuli are known to be selective to centrally but not peripherally acting analgesic drugs (Chau, 1989).

In conclusion, this work provides scientific evidence to support the utilization of this herb in traditional medicine for the treatment of rheumatism and other conditions that can cause inflammation.

Further tests are needed to isolate the active compounds, determine their structures to be able to hypothesize the exact mechanism of action of the bioactive constituents of the plant at the molecular level.

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