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Antinociceptive, Anti-inflammatory and Antipyretic Effects of Aqueous Extracts of *Morinda Lucida* Leaves in Experimental Animals

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ABSTRACT

Morinda lucida Linn, (Rubiaceae) is a tropical plant indigenous to West Africa, has crown dense branchelets, slender leaves, with yellow wood when fresh and changes colour when exposed, and is used in folk medicine for various purposes. The antinociceptive, anti-inflammatory and antipyretic activities of aqueous extract of the leaves of *Morinda lucida* was studied using animal models. Results showed that the aqueous extract of *M. lucida* was devoid of severe toxicity (LD_{50} 986.6 ± 2.65 mg/kg body weight), raised the painthreshold in rats using the hotplate or thermal method, reduced drug-induced abdominal constrictions or algesic effect, reduced carrageenan-induced rat-paw oedema and demonstrated substantial antipyretic properties in vaccine-induced hyperthermia in rabbits. These results are comparable to standard non-steroidal anti-inflammary agent (acetylsalicylic acid). It is concluded that the aqueous extract of the leaves of *M. lucida* can demonstrate strong analgesic, anti-inflammatory and antipyretic potency comparable in a time and dose-dependent manner to a nonsteroidal anti-inflammatory drug.

Key words: *Morinda lucida* analgesic, antipyretic anti inflammantory.

INTRODUCTION

Morinda lucida Linn family Rubiaceae is a tropical plant indigenous to some West African countries such as Nigeria, Ghana, Cameroons etc (Iwu and Anyanwu, 1982). The plant which grows to a height of about 15m, has crown dense branchlets, possesses slender leaves which are broadly elliptic to ovate acuminate entire and often dark purplish or black when dry. It produces white flowers about 1.5cm long twice annually, that is, between January and July, and September and October. The wood is yellow when fresh but darkens on exposure, becoming dark brown with lighter yellowish brown sapwood, and because of its chameleon-like changes, the Igbos of Southeast Nigeria call it “Ezeogwu”, (Igwe et al, 2009).

Morinda lucida has long history of use in folk medicine. In Ghana and the Southern Cameroons, a decoction of the leaves is used in the treatment of yellow fever, as bitter tonic, as an astringent for dysentery with fever and treatment of colic associated with intestinal worms. In Ivory Coast, the stem bark is used as decoction while the leaves are used as enema, steam bath, or as liniment in the treatment of yellow fever, haemoglobinurea and haematuria. On the other hand, Nigerians use it as an emetic, diuretic and purgative, useful as an antidote in the treatment of poison, astringent for dysentery, leprosy, and bitter tonics (Iwu and Anyanwu, 1982) and as antimalarial decoction (Igwe et al, 2009). However, there are no scientific studies in support of these traditional claims hence in the present study, an attempt has been made to investigate the antinociceptive, anti-

inflammatory and antipyretic effects of leaf extracts of *Morinda lucida* in experimental animals.

MATERIAL AND METHODS

Plant Material

Morinda lucida was obtained from Azia in Ihiala LGA of Anambra State Nigeria, during the month of April, and identified by Prof. C.U. Okeke of the Department of Botany and Biotechnology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria and a voucher specimen is deposited in the University Herbarium.

Preparation of aqueous extract

Dried leaves of *Morinda lucida* (2kg) was pulverized in an electric blender and macerated in a percholator using double-distrilled water, and allowed to stand for 24 hours. Thereafter, it was filtered using a whatman No. 1 filter paper and cotton wool. The filtrate was concentrated under reduced pressure using the rotary evaporator at 40^{o}C and the yield was about 18.5%. This was further evaporated to dryness in a oven and stored in a desicator until required for the animal experiments.

Animals

The following animals: mice weighing between 20 and 25g, Wistar rats weighing between 150 and 200g and rabbits, whose weights ranged between 1.5 and 2.0kg, of either sex were procured and maintained in the Departmental Animal House at room temperature 28-32^{o}C and fed with standard Animal Feed supplied by Livestock Feeds Nigeria Ltd. The animals were kept in separate cages in a 12hr dark-light cycle, with free access to food and water *ad libitum.* No animal was used more than once.

Phytochemical Test

The test for the detection of phytoconstitutents of *M. lucida* was carried out using standard techniques, reagents and thin larger chromatography as described by previous workers (Wagner and Bladt, 2001).

Preliminary screening and acute toxicity studies

Mice selected for this study were divided into eight groups of ten animals per group. The extract of *Morinda lucida* was administered orally in varying doses ranging from 0.5 to 64.0mg/kg body weight. The animals were continuously observed for 4hours to detect changes in the autonomic or behavioral responses such as alertness, spontaneous activity, irritability, pinnal reflex, corneal reflex, urination, salivation, piloercetion, etc.

Any mortality during experimentation and the following seven days was also recorded. A group of animals treated with the vehicle (distilled water) served as the control. Based on the outcome of the preliminary toxicity screening, the following doses 50, 100, 200, 400, 800, 1600, 3200 and 5000 mg/kg of *Morinda lucida* were chosen for the determination of LD_{50}

Antinociceptive activity using phenylbenzoquinone

This was investigated in mice based on methods described by Otterness and Bliven (1985) for measuring analgesic potency of non-steroidal anti-inflammatory drugs derived from writhing model. Mice of either sex, about 4 weeks of age and weighing between 20 and 25g were fasted for 24hours before drug administration with free access to food and water and *libitum were used for this study*. The fasting period maximizes the pain response to the irritant agent (phenlybenzon-quinone). The mice were randomly divided into 5 groups of A, B, C, D and E of six mice each and were given the drug (extract) orally by gavage. The control animals were treated with 100mg/kg aspirin (positive control) and the negative control received 25ml/kg per body weight of normal saline. 30min after the oral administration, 2mg/kg of pheynlbenzoquinone was administered. After 5 minutes, the number of abdominal stretches was counted for the next 30min at 5 minutes interval. Analgesic efficacy was expressed as the percentage decrease in abdominal stretches of treated vs untreated animals.

Ethacrynic acid-induced writhing in rats

Male Wistar rats weighing 130-150g were first fasted for 24hours with free access to water, then divided into 5 groups of 6 rats per group and were given various doses of the extract 175, 350, 700mg/kg per body weight respectively. Untreated animals received 100mg/kg aspirin (+ve control) while the negative control received 25ml/kg of normal saline per body weight. Thirty minute after

the administration of *Morinda lucida,* 15mg/kg of ethacrynic acid containing Evans Blue was injected into each rat intraperitonealy. From the injection of ethacrynic acid to 30min the number of abdominal contrictions was counted every 5 minutes. Antinociceptive effect was evaluated as the percentage decrease in abdominal contrictions (writhing) in the treated animals vs the untreated animals.

Hot plate method

Adult Wistar rats of either sex weighing (200 and 250g) were treated orally with different doses of *Morinda lucida* 175, 350, 700 mg/kg body weight respectively and were placed on a hot plate maintained at 45 ± 0.5°C. The time taken to lick the hind paw was taken as the reaction time. The rats were randomly divided into 5 groups of 6 rats per group. Groups 1-3 received 175, 350 and 700 mg/kg body weight of the aqueous extract respectively while groups 4 and 5 were given 100mg/kg and 25ml/kg body weight of acetylsalicylic acid (positive control) and normal saline (negative control) respectively (Igwe and Akunyili, 2005).

Anti-inflammatory activity

Male rats weighing 1.5 – 2.0kg fasted overnight to increase absorption were randomized in groups of five: A,B,C,D, and E, the aqueous extract of *Morinda lucida* was administered orally to groups A, B and C at doses of 175, 350, and 700mg/kg body weight respectively while the control animals, groups D and E received 100mg/kg of aspirin and 25ml/kg body weight of normal saline respectively. All animals had free access to food and water *ad libitum.*

One hour after the oral administration of the aqueous extract of *Morinda lucida* inflammation was induced by subplantar injection of 0.1ml carrageenan solution (0.1% in normal saline) into the right hand footpad. The degree of inflammation was assessed by measuring the volume of the animals right foot by means of water plethysmograph (Ugo Basile) at 1-, 3-, and 6-h intervals after inducing the inflammation. The volume of untreated left hind limb of each animal was also measured as a reference. Throughout the study, the animals were kept at room temperature to promote oedema formation.

Anti-inflammatory effect was expressed as the mean percentage inhibition of inflammation compared with the control 3hours after induction of inflammation for each treatment group.

Antipyretic activity

Rabbits weighing 250-400 kg, fasted overnight to increase absorption were randomized into groups of five: A,B,C,D, and E. were used for the study. The aqueous extract of *Morinda lucida* was administered orally to groups A, B and C at doses of 175, 350, and 700mg/kg body weight respectively while the control animals, groups D and E received 100mg/kg of aspirin (positive control) and 25ml/kg of normal saline (negative control) body weight respectively. All animals had access to food and water *ad libitum*.

Antipyretic activity was studied according to the methods described by Taesotiku et al, (2003) TAB vaccine 0.1ml was injected into the marginal ear vein of the rabbits and the rectal temperature was measured every 15min using electrolab 18 channel telethermometer, upto 3hours.

Antipyretic effect was expressed as the mean percentage decrease in hyperthermina compared with the control 3hr after induction of pyrexia for each treatment group.

Drugs used

The following drugs used in this study were purchased from Sigma Chemical Company (St. Louis, Mo, USA): phenylbenzoquinone, acetylsalicylic acid, (aspirin), carrageenan, all drug solutions were prepared fresh prior to use. The TAB vaccine was obtained from the National Veterinary Research Institute Vom, Jos, Nigeria.

Statistical Analysis

Data are expressed as means ± SEM. Differences between groups (extract-treated and controls) were considered to be significant at $p<0.05$ using ANOVA.

RESULTS

Preliminary phytochemical analysis by thin layer chromatography using specific reagents (Marini-Beltolo, et al, 1981) showed that the extract

Table 1: Analgesic activity (hot plate method) of the extract of *Morinda lucida*. The upper values represent the mean analgesic time in seconds ± SEM while the lower ones (in brackets) represent the percentage increase of ATRT against PTRT. (n=6)

Animal group	Drug and dose mg/kg		Pre-treatment reaction time (PTRT) (Sec)	After Treatment Reaction Time (ATRT) taken at 30 mininterval (upper values) and % increase of ATRT against PTRT (lower values)				
				30	60	90	120	150
A	Extract	175.0	16.8 ± 1.2	25.1 ± 1.6 (46.2)	36.6± 2.0 (93.7)	36.2 ±1.8 (65.8)	30.7 ±1.8 (31.0)	26.0 ±1.3 (1.3)
B	Extract	350.0	18.2 ± 2.0	30.2 ± 1.2 (55.6)	38.4± 1.6 (112.3)	38.2 ±1.9 (74.1)	33.3 ±1.0 (44.8)	27.1 ±1.8 (5.6)
C	Extract	700.0	19.6 ± 1.8	38.1 ± 1.6 (87.3)	46.6 ±1.8 (144.6)	42.1 ±1.8 (93.4)	35.3 ±1.9 (52.4)	28.9 ±1.4 (1.8)
D	Aspirin,	100.0	15.8 ± 1.6	29.2 ± 1.8 (84.8)	39.1 ±1.8 (147.5)	48.2 ±1.1 (205.1)	40.2 ±1.7 (153.2)	34.9 ±1.8 (120.9)
E	Normal saline 25ml/kg		15.9 ± 1.0	16.3 ± 1.2 (2.5)	16.4 ±1.0 (3.1)	17.9 ±1.9 (12.6)	16.9 ±2.0 (6.3)	16.1 ±1.8 (1.3)

was rich in alkaloids, flavonoids, glycosides, lipids, saponins tanins, steroids, and so on.

Acute toxicity studies in mice

The LD_{50} value after intraperitoneal administration of aqueous extract of the leaves of *Morinda lucida* in mice was 986.6 ± 3.4 mg/kg body weight. It was also observed, during the study that the extract did not produce any external or behavioral symptoms, such as wet fur, alertness, irritability and the animals moved freely.

Hot plate (thermal pain)

Table 1 shows the results of the analgesic testing (thermal pain) leaf extract of *Morinda lucida* using the hot plate method. The analgesic effect of the extract had a maximum percentage increase of 144.6% at a dose of 700mg/kg, while at 175 and 350 mg/kg, the extract gave percentage increases of 93.7 and 112.3% respectively. For each dose of the extract, the analgesic activity increased with time upto 80 minutes after which the effect declined. However, acetylsalicylic acid, a potent analgesic and the positive control, continued to demonstrate analgesic activity up to 90 minutes before declining. Using the student's t-test and a 95% confidence limit, there was no significant differences between the pretreatment reaction time (PTRT) and after treatment reaction time (ATRT) when normal saline was given.

Writhing pain induced in mice

Five minutes after i.p injection of pheynlbenzoquinone in mice, the number of writhing was counted for 5 min. The mean value was 16 ± 1.5 constrictions with a range 6 – 38. After oral administration of *Morinda lucida* extract, there was a significant reduction (inhibition) of writhing compared with the control. Potent analgesic activity was demonstrated by the extract with about 76% inhibition at the highest dose (700mg/kg) and 40% inhibition at the least dose of 175 mg/kg. there was a clear dose-dependent relationship (Table 2)

Ethacrynic acid-induced writhing

Furthermore, i.p injection of ethacrynic acid in rats caused algesic writhing response of about 24 constrictions during the 30 min after the injection. The analgesic potency of *Morinda lucida* extract increased in a dose-dependent manner and

at 700 mg/kg body weight was equivalent to aspirin (100 mg/kg) in reducing the abdominal constrictions (58 and 67%) respectively. The result is significant at $p<0.05$.

Rat paw oedema

Parallel analgesic activity was demonstrated by the aqueous extract of *Morinda lucida* with a dose-dependent anti-inflammatory activity similar to acetylsalicylic acid but lower in

Table 2: Analgesic effects of aqueous extract of *Morinda lucida* and acetylsalicylic acid against writhing test in mice and carragenan-induced rat paw oedema

	Treatment group	Percentage inhibition		
		Dose mg/kg (p.o)	Writhing	Rat paw oedema(mm)
A	Aqueous extract	175.0	40	16
B	Aqueous extract	350.0	65	16
C	Aqueous extract	700.0	76	16
D	Acetylsalicylic acid	100.0	80	54
E	Normal saline	25ml/kg	0	0

Table 3: Inhibition of phenylbenzoquinone-induced abdominal constrictions by the aqueous extract of *Morinda lucida* in mice. Each group received in addition 2 mg/kg of phenylbenzoquinone intraperitoneally. (n=6)

Group	Drug and dose (mg/kg)	No. of constrictions (±SEM)	% inhibition
1	Extract 175.0	18.4 ± 2.4	55.0
2	Extract 350.0	12.4 ± 3.2	70.0
3	Extract 700.0	8.2 ± 1.6	80.0
4	Aspirin, 100.0	2.4 ± 0.4	95.0
5	Phyenylbenzoquinone only 2.0	40.0 ± 0.0	0
6	Normal saline 25ml/kg	39.3 ± 4.2	5.0

Table 4: Inhibition of ethacrynic acid-induced abdominal writhing in rats by the aqueous extracts of *Morinda lucida*. Each group received in addition 15mg/kg ethacrynic acid intraperitoneally. (n=6)

Group	Drug and dose (mg/kg)	No. of constrictions (±SEM)	% inhibition
1	Extract 175.0	19.5 ± 2.2	17.0
2	Extract 350.0	15.4 ± 3.2	38.0
3	Extract 700.0	10.2 ± 1.3	58.3
4	Aspirin, 100.0	8.4 ± 12	66.7
5	Ethacrynic acid only 15.0	22.4 ± 3.2	8.3
6	Normal saline, 25ml/kg	24.0 ± 0.0	0

intensity and efficacy. Acetylsalicylic acid inhibited inflammation by 54% at 100mg/kg body weight, whereas the aqueous extract achieved about 20% inhibition at 700mg/kg body weight (Table 2). The aqueous extract of *Morinda lucida* did not prevent the formation of odema but facilitated the use of the leg, thus demonstrating analgesic activity whereas in the negative control (saline-treated group) there was total or complete demobilization.

Antipyretic activity

Administration of TAB vaccine to rabbits produced a significant increase in rectal temperature at 60 min and was gradually decreasing after 120 min. Treatment with acetylsalicylic acid (100 mg/kg) significantly reduced the rectal temperature. However, the groups treated with the aqueous extract of *Morinda lucida* also demonstrated dose-dependent reduction in hyperthermia and the difference between the extract-treated groups and the control (positive control) was significant at $p<0.05$ (Fig. 1).

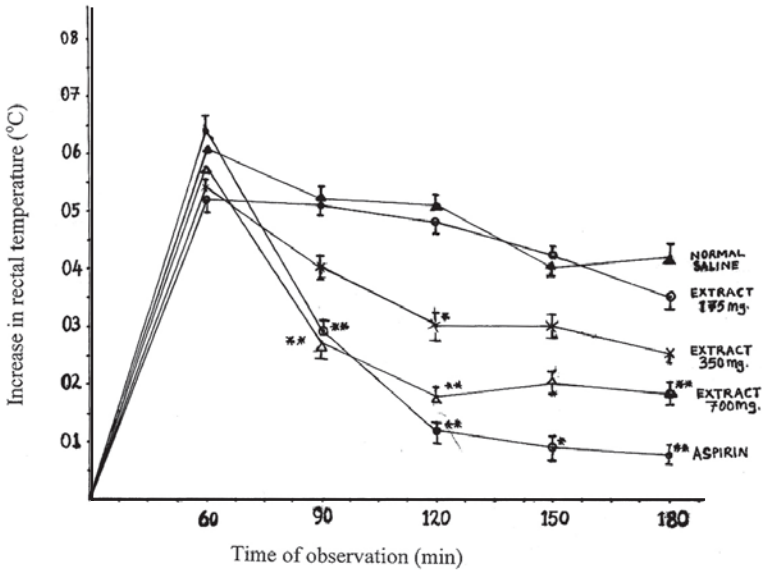

Fig. 1: Effect of *M. lucida* on vaccine-induced pyrexia in rabbits; **p<0.05; *p< 0.01 compared to normal saline treated group. Each point represents the mean ±SEM increase in rectal temperature recorded in six rabbits

DISCUSSION

Scientifically, a measure of assessing analgesic potency or efficacy of materials in experimental animals is failure to respond to pain within a given period under standard conditions. In the current investigation, the indices used for the assessment of analgesic activity were the prolongation of response of rats exposed to heat stimuli (hotplate or thermal pain) maintained at 45°C, decrease in writhing or abdominal stretches (constrictions) in mice induced by drugs (phenylbenzoquinone and ethacrynic acid) while drug induced rat-paw oedema was used to assess the anti-inflammatory effects of the plant extracts. On the other hand, vaccine-induced hyperthermia was used to determine the antipyretic activity of *M. lucida*.

Among the several traditional claims on *M. lucida* only the effects on pains, inflammation and fever have been demonstrated in the present study. Data obtained in this study show that the extract has a high antinociceptive action in mice and the analgesic potency is comparable or equivalent to that described elsewhere (Igwe and Akunyili,2005, Igwe et al, 2010), which seems to be related to the presence of several active compounds in the extract that enhance analgesic process in several ways (Table 1). Moreover, the data obtained from the

acute toxicity studies in mice indicated that the LD_{50} was 986.6 ± 3.4 mg/kg body weight, and at the doses investigated the extract of *M. Lucida* was devoid of toxic effect such as spontaneous activity, irritability, wet fur, and so on.

Furthermore, at the current dose level, the extract decreased drug induced writhing or abdominal stretches in mice and the analgesic potency of the extract is equivalent to that obtained with aspirin (Table 2). On the other hand, a potent anti-inflammatory activity of *M. lucida* was evidenced by the significant reduction in drug-induced rat paw oedema and the standard anti-inflammatory drug aspirin was only 1.3 times more potent than the extract on weight for weight basis (Table 2).

The greater anti-inflammatory activity demonstrated by aspirin might be due to its capacity to inhibit peripheral prostaglandin biosynthesis which also may contribute to its analgesic action. The antipyretic activity of *M. lucida* was made manifest when the aqueous extract caused a reduction in pyrexia induced by vaccine in rabbits. The response in higher doses was comparable to that of aspirin. Aspirin (fig 1) causes vasodilation which might contribute to reduction in inflammation, pain and fever due to antagonistic effect to bradykinin, a potent algesic agent. These mechanism may be lacking in the aqueous extract of *M. lucida* hence its inferior or weak anti-inflammatory effect in rat paw oedema, low analgesic effect and equivalent antipyretic actions.

Taken together, the combination of antinociceptive, anti-inflammatory and antipyretic effect of *M. lucida* indicated a likelihood of interaction with prostaglandin synthesis because prostaglandins have been established as a common mediator in all these responses or for the three end points. (Ali et al 1995).

In conclusion, the data reported here show that the extract *M. lucida* is a potent *invivo* analgesic, anti-inflammatory and antipyretic agent. Although, the potency of the extract in significantly lower than that of acetylsalicylic acid in some instances, work is currently going on to elucidate the active component of *M. lucida* that is responsible for its activities as well as its exact mechanism of action.

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