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PHYTOCHEMICALS AND ANALGESIC PROPERTIES OF THE METHANOL STEM BARK EXTRACT OF *ADANSONIA DIGITATA* L. IN MICE

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ABSTRACT
There is an urgent need to find cheap, safe and effective alternative analgesic agents to replace or supplement the currently available non-steroidal anti-inflammatory drugs (NSAIDs) and opioids which are associated with serious gastrointestinal and central nervous system side effects, respectively. In this work, analgesic activities of the methanol stem bark extract of *Adansonia digitata* were carried out in mice using acetic acid induced abdominal writhing and hot plate models for peripheral and central analgesic activities, respectively. Piroxicam (20 mg/kg, *i.p*) and pentazocine (20 mg/kg, *i.p*.) were used as standard drugs to validate the two models, respectively. In the acetic acid induced abdominal writhing test, the extract has significantly ($p<0.05$) and dose-dependently caused a decrease in the number of abdominal writhes in mice at all the doses tested when compared to the control group. In the hot plate test, the extract at 1000 mg/kg dose has significantly ($p<0.05$) increased the reaction time at 30 & 60 minutes with no significant ($p<0.05$) increase in reaction time at 90 and 120 minutes when compared to the control group. However, at doses of 250 & 500mg/kg dose, there was no significant ($P<0.05$) increase in reaction time by the extract when compared to the control group during the entire two hours duration. The result obtained concluded that the methanol stem bark extract of *Adansonia digitata* possesses analgesic activities which constitute the scientific basis of the traditional uses of the plant.

KEYWORDS: *Adansonia digitata*, analgesics, hot plate test, acetic acid, abdominal writhes.

INTRODUCTION
Although there have been great improvement in pain management, the medical community still requires additional analgesics that are safe, effective, and potent for the treatment of various painful conditions such as cancer pain and pain associated with surgery.[1] Pain is an obnoxious sensory and emotional occurrence associated with the actual or potential tissue damage which can be described in terms of such damage[2] and are caused as a result of the stimulation of pain receptors which are free nerve endings that are located outside the spinal column in the dorsal root ganglion.[3] In medical diagnosis, pain is regarded as a major symptom.[4] Pains are treated and sometimes managed using classical analgesic drugs (opiates and non-steroidal anti-inflammatory drugs) with origin in natural products and are associated with serious adverse effects such as gastrointestinal ulceration, gastrointestinal bleeding, respiratory distress, additive potential and drowsiness which require urgent intervention.[4] This necessitates the need to search for a more cheaper, safer and effective alternative through careful pharmacological screening of medicinal plants with a long history of traditional use such as *Adansonia digitata*.

Adansonia digitata L. (malvaceae), native to the African continent, is a massive deciduous tree with a round or spreading crowns, it can grow to a height of 20 m (approximately 65 ft) and can be up to 12 m (39 ft) in diameter.[5] The leaves, bark, and fruits are used for food and medicinal purposes in Africa.[5] It is used ethnomedicinally as an analgesic, astringent, anti-inflammatory, antiperspirant, anti-diarrheal, anti-asthmatic, anti-viral and anti-pyretic agents.[6]

MATERIALS AND METHODS
Animals
Swiss Albino mice of both sexes (18-25g) were obtained from the animal facility, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Bayero University Kano-Nigeria. They were housed in well ventilated cages, fed with standard rodent pellet diet & allowed free access to water. They were maintained under standard laboratory conditions in accordance with

the protocols approved by the University ethical committee on use and care of experimental animals. All procedures involving the use of animals in this experiment have been checked and approved by the ethical committee, Faculty of Basic Medical Sciences, Bauchi State University Gadau, Bauchi State Nigeria.

Drugs & chemicals
Methanol (Sigma chemical co. St Louis, USA), normal saline (Fidson Pharma., 268 Ikorodu Rd, Obanikoro, Lagos, Nigeria), pentazocin & piroxicam were used. All drugs and chemicals were locally procured.

Equipment
Hot plate, pestle and mortar, dry oven, weighing balance, syringe & needles, Whatman's filter paper no.1, spatula, beaker, stainless tray & medical adhesive tape.

Plant material
Fresh stem bark of *Adansonia digitata* were collected from Darazo, Darazo L.G.A of Bauchi state-Nigeria. The plant material was identified and authenticated by Dr Haladu Ali Gagman of the herbarium section, Department of Biological science, Faculty of Sciences, Bauchi State University Gadau with a voucher number (021036) for easy reference.

Preparation of the extract
The fresh stem bark of *Adansonia digitata* was cleaned, air-dried with adequate ventilation and crushed in to fine powder with the aid of pestle and motor. About 200grams of the fine powdered material was soaked in 2L of 70% v/v methanol for 3 days (72hrs) with regular shaking (morning & evening). The mixture was filtered using whatman's filter paper No 1, concentrated and evaporated to dryness using electric oven at temperature of 45^0C and finally air dried. The extract was weighed and kept in an air tight container marked MEAD until used.

Preliminary phytochemical screening
Phytochemical screening of the methanol stem bark extract of *Adansonia Digitata* was carried out as described by Sofowora.[7]

Acute toxicity studies
Oral median lethal dose (LD_{50}) was determined in mice using method described by Lorke.[8] In phase 1, three (3) groups of three (3) mice each were administered with the crude extract at doses of 10, 100 and 1000mg/kg body weight orally for group 1, 2 and 3 respectively and observed for signs of toxicity and mortality for 24 hours. The phase 2, commenced after 24 hours, four (4) groups of one (1) mouse each were administered with four (4) specific doses (1200, 1600, 2900 and 5000mg/kg respectively) of the extract. The Animals were observed for signs of toxicity and mortality for another 24 hours.

Analgesic screening
Acetic acid induced abdominal writhes in mice
Method described by koster[9] was followed. Thirty (30) mice were randomly divided into 5 groups of 6 mice each. Group I received normal saline (10ml/kg), Group II, III and IV were pre-treated with the extract orally at doses of 1000, 500 and 250 mg/kg body weight respectively, while group V received piroxicam 20mg/kg, ip. One hour post-treatment, each mouse was then injected intraperitoneally with 10ml/kg of aqueous solution of acetic acid (0.6%) and placed in a transparent cage. After five minutes lag period, the number of abdominal constriction accompanied with backward stretching of hind limbs were counted for each mouse, using tally counters for a period of 10 minutes.

$$\% \text{ Inhibition} = \frac{\text{Mean no. of writhing (control)} - \text{Mean no. of writhing (test)}}{\text{Mean no. of writhing (control)}} \text{ X } 100$$

Hot plate model
This was carried out according to the method described by Eddy and Leimbach.[10] Mice were individually placed on a hot plate for pre-test at 45^OC. Those having latency period of more than 15sec on hot plate during pre-testing were excluded; only those having latency period of less than 6seconds were selected. Thirty (30) mice were randomly divided into 5 groups of 6 mice each (n=6). Group-I received normal saline 10ml/kg, Group-II, III & IV received the extract orally at the doses of 1000, 500 and 250 mg/kg respectively while group-V received pentazocin (10mg/kg, ip). 1hour post treatment, the mice were individually placed on hot plate at 45^oc and the time at which the animal licked the paw or jumped was taken as reaction time and recorded. A cut-off time of 20sec was used to avoid paw tissue damage. The latency was observed and recorded after 30, 60, 90, and 120min. The prolongation of the latency time was taken as an analgesic response.

$$\% \text{ Analgesia} = \frac{\text{Test latency} - \text{Control latency}}{\text{Cut-off time} - \text{Control latency}} \text{ X } 100$$

Statistical analysis
Data were analyzed (using SPSS statistical software version 20) and expressed as Mean ± SEM. Analysis for difference between means were carried out using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Values of $P<0.05$ were considered statistically significant.

RESULTS

Percentage yield
The percentage yield of the extract (dark brown in colour) obtained was calculated to be 10.06%.

Preliminary phytochemical screening
The preliminary phytochemical screening of the extract showed the presence of flavonoids, glycosides, saponins, tannins, and cardiac glycosides (Table I).

Table I: Phytochemical constituents of the methanol stem bark extract of *Adansonia degitata.*

Constituents	Inference
Alkaloids	-
Flavonoids	+
Glycosides	+
Saponins	+
Tannins	+
Cardiac glycosides	+

Key: - = absent, + = present

Acute toxicity study

The oral LD_{50} of the methanol stem bark extract of *Adansonia digitata* in mice was found to be 2900 mg/kg.

Acetic acid-induced abdominal writhes in mice

MEAD has caused a significant ($p<0.05$) decrease in the number of abdominal writhes in mice at all doses tested when compared to the control group in a dose dependent manner (Table II).

Table II: Effect of MEAD on acetic acid induced abdominal writhes in mice.

Grp	Treatment (mg/kg)	Mean No. of writhing (± SEM)	Inhibition (%)
1	N/S (Control) 10ml/kg	18.4±2.6	
2	MEAD 1000	0.0±0.0 *	74.61
3	MEAD 500	1.8±1.4 *	70.78
4	MEAD 250	2.4±1.1*	69.25
5	Piroxicam 20	3.4±1.9*	50.78

Values presented as Mean ± SEM, n=6, *= significantly different from control at p< 0.05 using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Grp = group, N/S = normal saline, MEAD = methanol stem bark extract of *Adansonia digitata*.

Hot plate model

The MEAD has significantly ($p<0.05$) increased the reaction time at the dose of 1000 mg/kg at 30 & 60 minutes and there was no significant ($p<0.05$) increase in reaction time at 90 and 120 minutes when compared to control group. However, at doses of 250 & 500 mg/kg body weight there were no significant ($P<0.05$) increase in reaction time by the extract when compared to normal saline treated group during the entire 2-hour duration (Table III).

Table III: Effect of MEAD on hot plate induced nociception in mice.

			Mean reaction time (sec)±SEM				
Grp	Treatment	Dose (mg/kg)	0 min	30min	60min	90min	120min
1	N/S	10ml/kg	1.63±0.11	1.62±0.26	1.68±0.15	1.78±0.11	1.72±0.10
2	MEAD	1000	1.93±0.74	2.68±0.36*	2.92±0.15*	1.73±0.05	1.73±0.20
3	MEAD	500	1.73±0.14	2.31±0.34	1.70±0.40	1.68±0.17	1.69±0.21
4	MEAD	250	1.50±0.20	2.01±0.39	1.67±0.12	1.66±0.20	1.63±0.11
5	PENTA	20	1.98±0.07	2.97±0.40*	3.33±0.23*	3.31±0.41*	2.00±0.24*

Values presented as Mean±SEM, n=6, *= significantly different from control at p< 0.05 using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Grp = group, N/S = normal saline, PENTA = Pentazocine, MEAD = methanol stem bark extract of *Adansonia digitata*.

DISCUSSION

Several animal models have been employed in the screening of analgesic activities of plant extracts. In the present study, hot plate and acetic acid induced writhing tests was used to screen for the central (narcotic) and peripheral (non-narcotic) analgesic activities of methanol stem bark extract of *Adansonia digitata* in mice, respectively. Pentazocine (20 mg/kg, *i.p*.) and piroxicam (20 mg/kg, *i.p*) were used as standard drugs to validate the two models, respectively.

The hot plate method which is specific for centrally mediated nociception is one of the most common tests of nociception that is based on a phasic stimulus of high intensity[11] which produces norciceptive pain via opioid receptors.[12] The acetic acid induced abdominal constriction response is a sensitive procedure used in the evaluation of peripherally acting analgesic agents.[13] Acetic acid causes increased capillary permeability within peritoneal cavity leading to general increase in concentration of prostanoids, PGE_2 and $PGF_{2\alpha}$ in the peritoneal exudates[14] which then enhances inflammatory pain[15] through interaction with local peritoneal receptors which have been shown to be involved in the abdominal constrictions response.[16] Generally, there is also liberation of other endogenous substances such as substance P, serotonin, bradykinins and histamine, as well as lipoxygenase products.[17] The ability of the extract at the highest test dose (1000 mg/kg) to significantly prolong the latency of reaction time to thermally-induced pain (hot plate test) compared to the control group suggests that it possesses mild central analgesic activity. In the acetic acid model, the extract at all the doses tested, has significantly and dose-dependently reduced the number of abdominal writhes

when compared to the control group which indicates marked peripheral analgesic activities. Non-steroidal anti-inflammatory drugs possess the intrinsic ability to inhibit cyclooxygenase enzymes in the peripheral tissues and hence, interfere with the mechanism of transduction of primary afferent nociceptors.[12] It is important to note that, the activity observed with all the graded doses, were comparable to that of piroxicam at 20mg/kg body weight. This shows that MEAD could block the effect or release of endogenous substances that excite pain nerve endings in a similar fashion to that of the standard drug, piroxicam (NSAID).

Phytochemical constituents are responsible for the various pharmacological activities of plants. The analgesic activities observed in this study could be linked to the presence of flavonoids, saponins and tannins in the extract which have been reported in literature to possess analgesic activity.[18]

CONCLUSION

The result of this study indicates that, MEAD possesses mild central and marked peripheral analgesic activities which constitute the scientific basis of the traditional uses of the plant in pain management. It could be of great therapeutic benefits in the management of pains associated with various disorders.

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