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Original Article

Antipyretic and analgesic activities of aqueous extract of *Acacia nilotica* root

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ABSTRACT: This study was designed to investigate the scientific basis for the use of *Acacia nilotica* root extract for treatment of fever and pain in traditional medical practice. Anti-Pyretic study was carried out using Brewer's yeast suspension to induce pyrexia. The hot plate, tail immersion and acetic acid-induced writhing tests were the nociceptive models used for analgesic study. Anti-pyretic and analgesic activity of the extract was compared with acetaminophen that was used as control drug. Five groups comprising five animals per group were used for each study. Group 1 was administered 10 ml/kg body weight of distilled water, Group 2 was administered 150 mg/kg body weight of acetaminophen while groups 3, 4 and 5 were administered 100, 200 and 400 mg/kg body weight of extract respectively as single oral dose. The extract produced significant dose-dependent reduction in rectal temperature of rats at 200 and 400 mg/kg body weight. Significant analgesic activities were also observed in the hot plate, tail immersion and acetic acid induced writhing, after administration of 200 and 400 mg/kg b.w of extract which is comparable to the control drug, acetaminophen. The results from this study showed that aqueous extract of *Acacia nilotica* root at 200 and 400 mg/kg body weight possess significant antipyretic and analgesic activities. This provides scientific support for its traditional medical use in the treatment of fever and pain.

KEYWORDS: Antipyrexia, Analgesia, *Acacia nilotica,* Brewer's yeast.

INTRODUCTION

Acacia nilotica (Linn.) Willd. Ex Del. (also called Gum Arabic tree; *'Gabaruwa'* in *Hausa* and 'Igi Kasia/Booni' in *yoruba*) is an important plant used in traditional medical practice in Northern Nigeria and many African countries (Bargal and Bargali, 2009), for the treatment of different ailments such as diabetes, diarrhea (van Wyk, 2000), tuberculosis (Oladosu *et al*., 2007) and malaria (Alli *et al*., 2011). It is rich in phytochemicals such as alkaloids, flavonoid, tannins, terpenes and phenolics [Banso, 2009; Alli *et al*., 2011) that are responsible for some of its pharmacologic activities. Although studies have been carried out to validate some of the local uses of the stem and bark extract of this plant, there is need to investigate the anti-pyretic and analgesic activity of the extract of *A. nilotica* root in order to validate the use of the root extract in traditional treatment of fever and pain. As pain and fever are the main symptoms of malaria, this study is therefore investigating the anti-pyretic and analgesic activity of aqueous extract of *Acacia nilotica* root, after its anti-plasmodial activity has been established from previous studies (Alli *et al*., 2011).

Pain and Pyrexia are common physiologic response to infection, inflammation and tissue damage (Spacer and Breder, 1994). Pain may occur as a result of nociceptive,

inflammatory or neuropathic conditions that could involve biochemical mediators like prostaglandins, substance and glutamate (Raffa, 2006). Pyrexia could occur as body's natural defense mechanism to prevent survival of infectious agents (Cooper, 1995; Woolf, 2010). It is initiated when infectious agents or damaged tissues triggers increase production of pro-inflammatory mediators (pyrogens such as, Tumour Necrosis Factor-α {TNF-α}, interleukin 1b and interleukin-6) which increase the synthesis of prostaglandin E-2 (PGE-2) near the pre-optic area of anterior hypothalamus. The hypothalamus is consequently triggered to elevate the body temperature (Akpan *et al*., 2010). The mechanism of action of commonly used anti-pyrexia involves inhibition of cyclooxygenase enzyme (COX), specifically COX-2, thereby interrupting the synthesis of prostaglandin E-2, and thus reducing body temperature (Cheng *et al*., 2005). Some of the commonly available synthetic antipyretic agents, with analgesic properties, (such as acetaminophen, acetyl salicyclic acid and other non-steroidal anti-inflammatory drugs) could be toxic to hepatic cells and glomeruli (Cheng *et al*., 2005). Therefore, search for safe and effective plant-based antipyretic and analgesic agents is highly essential and desirable.

MATERIALS AND METHODS

Experimental animals

Healthy Wistar albino rats (190 ± 10 g) and albino mice (25 ± 5 g) used for this study, were obtained from the animal facility center of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. They were housed in well ventilated cages, fed with rat pellets (Pfizer feeds Plc, Lagos, Nigeria) and water *ad libitum.* Standard laboratory conditions were maintained in accordance with the guideline for the care and use of Laboratory animals by National Academy of Sciences. The ethical committee on animal use and care of NIPRD approved the use of laboratory animals for this study.

Plant materials

Root samples of *Acacia nilotica* were collected by Mallam Muazzam, from Bamburu-Chaza village, Suleja, Niger State, Nigeria. The samples were identified and authenticated at the herbarium of NIPRD, by a taxonomist, Mrs Grace Ugbabe. Voucher specimen (NIPRD/H/6401) was prepared and deposited at the herbarium for referencing.

Preparation of Aqueous Extract of *Acacia nilotica* root

The root sample of *A. nilotica* was air dried under shade for two weeks and pulverized using grinding machine. The powder was stored in an airtight container and kept in a cool dry place. Aqueous extraction was carried out following the cold maceration method described by Adzu *et al.,* (2003). Two hundred grams of the powdered root of *A. nilotica* was soaked in 1 litre of distilled water and kept for 24 hours. The suspension was then filtered with muslin cloth followed by filtration with Whatman filter paper (No.1). The filterate was freeze-dried using AMSCO/FINN-AQUA GT2 Freeze dryer (Germany).

Reagents

Acetaminophen (Emzor Pharmaceutical brand, NAFDAC: 04-0411) was obtained from Novac Pharmacy, Kubwa, Abuja, Nigeria. Brewer's yeast was obtained from Nigerian Breweries Plc, Lagos, Nigeria. Solutions of acetic acid (1% v/v) and 150 mg/kg body weight acetaminophen were prepared with distilled water. All other reagents were of analytical grade and prepared in glass bottles using distilled water.

Acute toxicity study

Acute toxicity study of the aqueous extract of *A. nilotica* root was carried out following the Organization for Economic Cooperation and Development (OECD) Guideline 423 (OECD, 2001). Nine healthy, nulliparous and non-pregnant female albino mice weighing 20 ± 2 g were used for this study. They were weighed and randomized into three groups of three mice per group. The animals were fasted for 4 hours prior to dosing. After 4 hours of fasting, the control (Group 1) was administered 10 ml/kg body weight of distilled water, while Groups 2 and 3 were administered oral doses of the extract at 300 and 2000 mg/kg body weight respectively. The mice were observed individually for signs of toxicity during the first 30 minutes, periodically during the first 24 hours and daily for 14 days. Weight changes were recorded twice weekly and mortality recorded daily. LD_{50} of the root extract was determined by comparing number of mortality with fixed LD_{50} cut-off values in the guideline.

Phytochemical screening

Phytochemical screening of the crude extract was carried out following the methods described by Sofowora (1993) to determine the presence of secondary metabolites such as alkaloids, phenolics, flavonoids, saponins, terpenes, tannins, anthraquinones

Induction of Pyrexia and administration of extract

The method described by Singh *et al.,* (2010), using Brewer's yeast suspension, was followed to induce pyrexia in the rats. Subcutaneous injection of 15% brewer's yeast suspension into rats will produce pyrexia in the animals. The basal rectal temperatures of these rats were measured by inserting clinical thermometer (OMRON digital thermometer, Eco Temp Basic) up to 1 cm into the anus and recording the temperature after 1 minute. The baseline temperature was recorded at 0 hour, after which the rats were administered subcutaneous injection of 10 ml/kg body weight of 15% Brewer's yeast suspension in the abdominal region. Nineteen hours after administration of yeast, the rectal temperature of

each rat was observed and rats that showed an increase in rectal temperature of at least 0.5 ^{o}C were selected and randomized into five groups containing five rats per group.

Group 1 served as the control, and received 10 ml/kg body weight of distilled water, Group 2 received 150 mg/kg body weight of acetaminophen, while Groups 3, 4 and 5 received 100, 200 and 400 mg/kg body weight of extract respectively. All the animals received a single oral dose of treatment for each group. The rectal temperatures were taken again, at the 20th, 21st, 22nd and 23rd hour after brewer's yeast injection.

Table 1: Phytochemical constituents of aqueous extract of *A. nilotica* root.

Phytochemicals	Quantity (mg/kg)
Phenolics	34.50 ± 1.55
Tannins	27.00 ± 1.75
Alkaloids	23.30 ± 1.86
Saponins	9.80 ± 0.89
Anthraquinones	4.70 ± 0.97
Flavonoids	0.50 ± 0.06
Terpenes	0.10 ± 0.05
Sterols	0.10 ± 0.05

Values are expressed as mean ± S.E.M of 3 observations.

Analgesic Activity (Hot Plate method)

The hot-plate latency method described by Hosseinzadeh *et al*. (2000) was used. Healthy Wistar Albino rats (190 ± 10 g) were screened for thermal sensitivity by placing them one-by-one on a hot plate maintained at 55.0 ± 0.5 ^{0}C. Rats that stayed on the hot plate for ≥ 2 sec without jumping or licking paws (response to thermal stimulus) were selected for the study. The selected rats were fasted for 12 hours and then randomized into five groups of five rats each. Group 1 served as the control, and received 10 ml/kg body weight of distilled water, Group 2 received 150 mg/kg body weight of acetaminophen, while Groups 3, 4 and 5 received 100, 200 and 400 mg/kg body weight of extract orally, respectively.

The rats were each placed on a hot plate (maintained at 55.0 ± 0.5 ^{0}C) 30 minutes after administration of distilled water, acetaminophen and extract. The time taken for the rats to respond to the thermal stimulus was noted as the latency of response (in seconds) and the mean latency for each group was calculated. The effects of the administration of extracts, acetaminophen and distilled water on response of the rats to thermal stimulus (by licking paws or jumping) were also determined after 30, 60, 90 and 120 minutes. A latency time of 20 seconds was regarded as complete analgesia and measurement was terminated if 20 sec is exceeded to avoid tissue injury to the animals.

Tail Immersion Method

The tail immersion method described by Jansen and Jagenav (1959) was followed. Healthy Wistar Albino rats (190 ± 10 g) were screened for thermal sensitivity by putting the tip of their tail (lower 5 cm portion) inside a beaker containing hot water maintained at 55.0 ± 0.5 ^{0}C. Rats that maintained their tail in the hot water for ≥ 2 sec were selected for the study. Twenty five albino rats selected for this study were randomized into 5 groups of 5 rats each, and placed into restraining cages leaving the tail to hang out freely. Group 1 served as the control, and received 10 ml/kg body weight of distilled water, Group 2 received 150 mg/kg body weight of acetaminophen, while Groups 3, 4 and 5 received 100, 200 and 400 mg/kg body weight of extract orally, respectively.

The lower 5 cm portion of the tail was immersed in a beaker filled with hot water maintained at 55.0 ± 0.5 ^{o}C. The time (seconds) taken for the withdrawal of the tail from hot water induced pain recorded with a stop watch as the reaction time, was recorded for each rat at intervals of 30, 60, 90 and 120 minutes after the extract and drug administration. The maximum cut-off time for immersion in hot water was 15 seconds to avoid injury to tissues of the tail.

Acetic acid induced writhing test

Analgesic activity of the aqueous extract of *A. nilotica* root to peripheral pain was assessed using the method described by Salawu *et al.,* (2008). Twenty-five mice were randomly divided into five groups of 5 mice each. Group 1 served as the control, and received 10 ml/kg body weight of distilled water, Group 2 received 150 mg/kg body weight of acetaminophen, while Groups 3, 4 and 5 received 100, 200 and 400 mg/kg body weight of extract orally, respectively. Thirty minutes after treatment, each mouse received intraperitoneal injection of 10 ml/kg body weight of 1% (v/v) acetic acid solution and subsequently placed in a glass beaker for observation. The number of writhes (contraction of abdomen, twisting of trunk and extension of the hind limbs) per animal was counted within 30 minutes observation period, beginning from 5 min after acetic acid injection. Inhibition (%) was calculated using the following expression.

$$\text{Percentage Inhibition (\%)} = \frac{\text{Average writhe (Control)} - \text{Average writhe (Test)}}{\text{Average writhe (Control)}}$$

Table 2: Antipyretic activity of aqueous extract of *A. nilotica* (*A.N)* root

Treatment/Dose (mg/kg body weight)	Rectal Temperature (°C) of rats					
	0 hour pre-injection of yeast	19 hour post-injection of yeast	Temperature after administration of extract			
			20 hour	21 hour	22 hour	23 hour
Control	37.60 ± 0.25	38.50 ± 0.20	38.60 ± 0.25	38.70 ± 0.22	38.90 ± 0.24	39.00 ± 0.25
Acetaminophen 150	37.60 ± 0.26	38.60 ± 0.25	38.40 ± 0.23	38.00 ± 0.22*	37.80 ± 0.18*	37.60 ± 0.15*
Extract 100	37.50 ± 0.31	38.50 ± 0.20	38.40 ± 0.22	38.40 ± 0.25	38.30 ± 0.25	38.20 ± 0.20
200	37.50 ± 0.29	38.60 ± 0.28	38.40 ± 0.25	38.30 ± 0.22	38.20 ± 0.24	37.90 ± 0.23*
400	37.60 ± 0.26	38.60 ± 0.17	38.40 ± 0.25	38.20 ± 0.21	37.90 ± 0.19*	37.70 ± 0.15*

Values are expressed as mean ± S.E.M of five observations; * = significantly different at $P < 0.05$ when compared to control.

Table 3: Analgesic activity of aqueous extract of *A. nilotica* root using hot plate test

Treatment/Dose (mg/kg b.w)	Mean Basal time (sec)	Mean reaction time (sec) at interval of 30 min			
		30	60	90	120
Control	2.54 ± 0.15	2.55 ± 0.20	2.54 ± 0.18	2.56 ± 0.22	2.55 ± 0.20
Acetaminophen (150)	2.55 ± 0.17	5.50 ± 0.15*	6.10 ± 0.20*	6.85 ± 0.22*	7.65 ± 0.25*
Extract 100	2.55 ± 0.20	2.55 ± 0.25	2.65 ± 0.22	2.65 ± 0.27	2.75 ± 0.28
200	2.50 ± 0.17	3.45 ± 0.24	4.80 ± 0.23	5.30 ± 0.20*	5.75 ± 0.25*
400	2.55 ± 0.20	4.85 ± 0.22	5.45 ± 0.15*	5.95 ± 0.25*	6.30 ± 0.30*

Values are expressed as mean ± S.E.M of five observations; * = significantly different at $P < 0.05$ when compared to control

Statistical analysis

Data were expressed as mean ± standard error of mean of five replicates. Statistical analysis was done using Graph pad prism version 5.0. The differences between the mean were compared using analysis of variance (ANOVA). Values with $P < 0.05$ were considered statistically significant.

RESULTS

There were no remarkable behavioural (such as reaction to food supply, and contact) changes in the treated mice, during the acute toxicity study period. No mortality was recorded during the observation period following administration of the extract at the doses of 300 and 2000 mg/kg body weight. The oral median lethal dose (LD_{50}) of the extract was estimated to be 5000 mg/kg in mice following the OECD Guideline 423 (2001) for oral acute toxicity study. Absence of mortality in the experimental animals after administration of 2000 mg/kg body weight of extract corresponds to a LD_{50} value of 5000 mg/kg body weight on the guideline chart.

Initial tests were carried out to detect the presence of common phytochemicals in the aqueous extracts of *A. nilotica* root. The aqueous extract were found to produce positive reactions to phenolics, tannins, alkaloids, anthraquinones, flavonoids, terpenes and sterols (Table 1).

Table 4: Analgesic Activity of *A. nilotica* root extract using tail immersion test

Treatment/Dose (mg/kg b.w.)	Mean basal time (sec)	Mean reaction time (sec) at interval of 30 min			
		30	60	90	120
Control	2.20 ± 0.15	2.50 ± 0.20	2.55 ± 0.15	2.45 ± 0.18	2.50 ± 0.15
Acetaminophen (150)	2.35 ± 0.25	4.70 ± 0.22*	5.40 ± 0.25*	6.50 ± 0.22*	7.60 ± 0.20*
Extract 100	2.20 ± 0.20	2.25 ± 0.23	2.25 ± 0.20	2.40 ± 0.25	2.45 ± 0.22
200	2.25 ± 0.23	3.50 ± 0.25	4.20 ± 0.22	5.25 ± 0.21*	5.85 ± 0.23*
400	2.45 ± 0.20	3.85 ± 0.20	4.95 ± 0.26*	5.90 ± 0.23*	6.70 ± 0.25*

Values are expressed as mean ± S.E.M of five observations; * = significantly different at $P < 0.05$ when compared to control

Table 5: Analgesic activity of *A. nilotica* on acetic acid-induced writhes in rats

Treatment	Dose (mg/kg b.w)	Number of writhes	Inhibition (%)
Distilled water	10	32.50 ± 2.50	-
Acetaminophen	150	8.50 ± 0.65*	74.6*
A.nilotica	100	22.50 ± 1.50	30.7
A.nilotica	200	14.00 ± 1.00*	56.9*
A.nilotica	400	9.50 ± 0.57*	70.8*

Values are expressed as mean ± S.E.M of five observations; * = significantly different at $P < 0.05$ when compared to control

There was a significant dose-dependent reduction in the rectal temperature in rats administered with 200 and 400mg extract /kg body weight at 22 and 23 hr after extract administration respectively (Table 2).

Significant dose-dependent increase in reaction time, in the hot plate and tail immersion test, was observed after oral administration of 200 and 400 mg/kg b.w dose of aqueous extract of *A. nilotica* (Tables 3 and 4). The extract at 200 and 400 mg/kg body weight significantly inhibited the acetic acid induced writhing in the mice when compared with the control (Table 5).

DISCUSSION

The antipyretic and analgesic activity of aqueous extract of *A. nilotica* was studied in rats using standard models. Acetaminophen (paracetamol) is used in this study as control drug because it is one of the most widely used analgesic and antipyretic in both children and adults (Chou *et al.*, 2007; Zhang *et al.*, 2007; Loke, 2009). It has also been shown to have significant concentration in the central nervous system at analgesic dose (Courade *et al.*, 2001). The antipyretic activity of the extract at doses of 200 and 400 mg/kg b.w, is comparable to the antipyretic activity of acetaminophen that

was used as the control drug. This result is similar to the antipyretic activity of aqueous stem extract of *Enantia chlorantha* at 100 and 200 mg/kg dose (Adesokan *et al*., 2008). Several other plant extracts, such as methanol extract of *Bauhinia racemosa* stem bark (Gupta *et al*., 2005), aqueous leave extract of *Parquetina nigrescens* (Owoyele *et al*., 2009), methanolic extract of *Capparis zeylanica* plant (Padhan *et al*., 2010) and ethanol extract of *Strophanthus sarmentosus* (Agbaje and Ajidahun, 2011) have been reported to possess antipyretic activity in Wistar rats. The dose dependent antipyretic activity of *A. nilotica* may be due to the presence of phenolics and alkaloids which have been reported to possess antipyretic and analgesic properties (Kanagusuku *et al*., 2007; Owoyele *et al*., 2008). Flavonoids such as baicalin and alkaloids such as boldine have been reported to exert antipyretic effect by suppressing TNF-α (Chang *et al*., 2007) or inhibiting the synthesis of prostaglandin E_2 (Backhouse *et al*., 1994). Yeast-induced pyrexia is based on increase synthesis of prostaglandin (PGE_2) (Akpan *et al*., 2012), therefore anti-pyretic activity of the extract may be mediated via inhibition of PGE_2 synthesis. It may also be due to reduction of prostaglandin concentration in the hypothalamus through inhibition of cyclooxygenase pathway or through vasodilatation of superficial blood vessels leading to increase heat loss (Rang *et al*., 2007).

The analgesic activity of aqueous extract of *A. nilotica* root (using the hot plate and tail immersion test) at 200 and 400 mg/kg body weight dose is significant. This is similar to the result obtained for analgesic activity of aqueous extract of *Curcum longa* rhizome (Neha *et al*., 2009), aqueous extract of *Parquetina nigrescens* leaves (Owoyele *et al*., 2009) and aqueous extract of *Mimosa albida* root (Rejón-Orantes *et al*., 2013). Substances that can produce inhibitory effects in hot plate and tail immersion test can inhibit centrally-induced pain and therefore act as analgesics (Prado *et al*., 1990). The reason for this is that analgesic action in hot plate and tail immersion tests involves supra-spinal and spinal components of the central nervous system (Mustaffa *et al*., 2010). The writhes induced by acetic acid were used in this study to assess peripherally acting analgesics (Chou *et al*., 2007). Intraperitoneal injection of acetic acid can liberate prostaglandins and histamine which will stimulate local peritoneal receptors involved in the writhing response (Kanagusuku *et al*., 2007). Secondary metabolites from medicinal plants such as alkaloids (*myristica fragrance* seed) (Hayfaa *et al*., 2013), tannins and flavonoids (such as ethylquercetin (Picq *et al*., 1991), rutin and bioflavonoids (Calixto *et al*., 2000;Bittar *et al*., 2000) have been reported to be responsible for analgesic and antipyretic activities. The significant reduction in acetic acid-induced writhes by aqueous extract of *A. nilotica* suggests that the extract also possesses peripheral analgesic activity.

Conclusion: The observed activities of *A. nilotica* extract in the above tests suggest that the aqueous root extract possesses significant antipyretic and analgesic activities which may be due to the alkaloids and flavonoids present in the extract. It may therefore be used as a potent antipyretic and analgesic herbal medication alongside its common use as an antimalarial.

Author's Contributions

This work was carried out in collaboration between all authors. LAA contributed to conception and design, data acquisition, analysis and interpretation; and writing of the manuscript and its post-review revisions. All other authors contributed to data analysis and interpretation. They were involved in drafting and revising the manuscript for important intellectual content.

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