Anti hyperalgesic potentials of Laggera aurita in Swiss Albino mice

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Abstract: The ethnomedical uses of *Laggera aurita* (LA), including its anti-nociceptive properties have been documented in literature. This study evaluated the anti-hyperalgesic effects of the methanolic extract of LA (MELA) using chemically and mechanically induced hyperalgesia models. Acute toxicity and preliminary phytochemical screening were also conducted. The intraperitoneal median lethal dose was found to be 3807.88 mg/kg, while saponins, tannins and carbohydrates were found to be present in the plant material. MELA exhibited significant analgesic activity in the acetic acid induced writhing and Randall-Siletto tests. The 400 mg/kg dose of MELA exhibited a significant ($p<0.001$) analgesic activity which offered 19.75% inhibition above piroxicam in the acetic acid test. At 200 and 400 mg/kg MELA demonstrated comparative analgesia with pentazocine in the Randall-Siletto test. The study shows that MELA possesses anti-nociceptive principles and the presence of saponins, tannins and carbohydrates which have been previously associated with anti-hyperalgesia may be responsible for the pharmacological actions, thus authenticating the ethnomedical rationale for its anti-nociceptive uses.

Keywords: anti-nociception, *Laggera aurita*, pain.

INTRODUCTION

Mankind has experienced pain right from historical times (Hasnain *et al.,* 2012), and a vast number of people suffer from pain the world over on a daily basis (Khatum *et al.,* 2012). The phenomenon of pain is essential to animal survival as it is a signal that prevents injuries from potential external damaging stimuli or internal tissue damage (Chen *et al.,* 2010), and may be nociceptive or neuropathic in nature (Dinh *et al.,* 2011). Despite its physiological function and importance, in many parts of Africa and the developing world, pain is a major reason for patronage of both orthodox and traditional health services. Today, millions of people use herbal remedies either being prescribed or otherwise. The use of natural products as alternatives to orthodox drugs has spread from developing to western countries (Talhouk *et al.,* 2008). In Nigeria, rheumatism and inflammatory conditions are among the commonest ailments and problems on which traditional medical practitioners thrive (Akah and Nwambie, 1994). As a result of the unpleasant nature and accompanying pain suffered by patients, herbal remedies continue to get patronage partly due to perceived safety of herbs. This is further predicated on the fact that despite current technological advancement, over 70% of people living in developing countries still depend on traditional medicine as their means of maintaining health (Okoli *et al.,* 2007). However, a large number of these herbal remedies have not been scientifically verified to be efficacious and safe. *Laggera aurita* (Asteraceae) also known as *Blumea aurita* (Family Compositae) is a shrub commonly found along pathways, in farmlands and around houses. Different ethnomedical formulations of the plant are utilized for several medical conditions in parts of Africa, and the plant has been reportedly used in the treatment of constipation, inflammation, dyspepsia and aiding of wound healing. It is also used for the treatment of constipation, dysentery and rheumatic pain (Burkill, 1985). In this study the possible justification for its purported anti-nociceptive effects was investigated using chemical and mechanical hyperalgesia models. Acute toxicity and preliminary phytochemical screening were also conducted.

MATERIALS AND METHODS

Plant collection and preparation

The whole plant was collected in the month of June, 2008 in Zaria, Nigeria. Identification and authentication was done in the Department of Biological Sciences, Ahmadu Bello University Zaria by Mr. Umar Gala. A specimen with voucher number 1160 is currently deposited at the herbarium for reference purposes. After collection, the plant was air dried for about two weeks away from sunlight, and subsequently followed by the extraction of 520 g of the pulverized stem bark material using 48 h cold maceration in methanol. The resulting macerate was filtered and concentrated at about 60°C.

Chemicals and drugs

Piroxicam (Pfizer), Pentazocine (Ranbaxy) and Acetic Acid (BDH) were used in this study.

Animals

Adult Swiss albino mice of weight range between 22-36 g of either sex were used for the studies. The animals were inbred in the animal facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, and were placed on standard feed and water *ad libitum* and maintained under a natural light and dark cycle. The experiments were conducted according to

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institutional guidelines and the NIH animal guide. The animals were grouped into four groups of five mice with group 1 served as negative control. Groups 2 and 3 received the extract at 200 mg/kg and 400 mg/kg respectively while Group 4 received the standard analgesic agent (piroxicam 5 mg/kg or pentazocine 20 mg/kg).

Acute toxicity and phytochemical testing

Since the intraperitoneal route was used in the study, intraperitoneal acute toxicity testing was conducted as previously described (Lorke, 1983). In summary this consisted of using three animals for each of three doses in the first phase and a single animal each for additional dose levels in the second phase. The geometric mean of the two adjacent doses that produce death and no death were calculated. Preliminary phytochemical screening was carried out (Evans, 1989) on the plant material to determine presence of some chemical constituents.

Chemically induced hyperalgesia

Acetic acid induced writhing is a chemically induced pain model for assessing peripherally mediated analgesia. The contractions resulting from intrapertoneal injection of 10 ml/kg acetic acid (0.6%), consisting of constriction of abdominal muscles together with stretching of the hind limbs, were counted over a cumulative twenty min duration according to the procedure of Collier and coworkers (1968). Animals were divided into four groups (n=5) with two dose levels (200 and 400 mg/kg) of the extract and a saline control as well as a piroxicam at a dose of 5 mg/kg as standard.

Mechanically induced hyperalgesia

Mechanically induced pain is a model used for the investigation of centrally mediated analgesia. This model usually consists of the application of a noxious mechanical stimulus applied in graded or incremental intensity with the result of characteristic reflex responses that may include vocalizations and complex motor behaviors, (Le Bars 2001). The tail pressure-Randall-Selitto test was adopted in the mechanical hyperalgesia protocol (Nassar *et al.,* 2005; Drel *et al.,* 2007) using the Ugo Basile analgesiometer. The machine was read off at the maximum tolerable pressure for each animal. Animals were divided into four groups as previously stated but pentazocine was administered as standard at a dose of 20 mg/kg. Readings were taken at times 0, 30, 60, 90 and 120 min.

STATISTICAL ANALYSIS

Data obtained from the study was entered into GraphPad Prism software and analyzed using One Way ANOVA followed by Student's Newman-Keuls multiple comparison tests, and p values less than 0.05 was considered statistically significant. Data are presented as mean ± standard error of the mean (SEM).

RESULTS

Following the methanolic extraction, the yield of the crude extract obtained was 3.85%. The intraperitoenal LD_{50} calculated using the method of Lorke (calculating the geometric mean) was found to be 3807.88 mg/kg. Table 1 shows the dosing for the acute toxicity test and the deaths observed. Preliminary phytochemical screening established the presence of tannins, saponins and carbohydrates (table 2). In the acetic acid induced writhing model, the extract at doses of 200 and 400 mg/kg both reduced the number of abdominal constrictions, with the 400 mg/kg dose producing a statistically significant reduction ($p<0.001$). The percentage inhibition produced by this dose was 84.50% while that by 5 mg/kg piroxicam was 64.75% (table 3). In the mechanical hyperalgesia model, the extract also produced significant reduction in the maximum tolerable pressure particularly between the 30 and 90 min time point (table 4).

Table 1: Lethality data in acute toxicity test

Phase 1		Phase 2	
Dose (mg/kg)	Number of deaths	Dose (mg/kg)	Number of deaths
10	0/3	1600	0/1
100	0/3	2900	0/1
1000	0/3	5000	1/1

Table 2: Results of phytochemical screening

Test	Phytochemical Group	Inference
Molisch; Fehling's	Carbohydrates	Present
Borntrager	Free Anthraquinones	Absent
Kella-Killiani, Kadde, Salkowsk	Cardiac Glycosides	Absent
Frothing, Hemolysis	Saponnins	Present
Liberman-Burchards	Steroids and riterpenses	Absent
Shinoda; Sodium hydroxide	Flavonoids	Absent
Lead sub acetate; Ferric Chloride	Tannins	Present
Meyer; Dragendoff, Wagner; Picric Acid	Alkaloids	Absent

Table 3: Effect of *Laggera aurita* on Acetic acid induced writhing in Mice

Group	Number of writhes	Percentage inhibition
Control	40.25±6.70	-
MELA 200 $mgkg^{-1}$	38.6±3.58	4.9%
MELA 400 $mgkg^{-1}$	6.2±1.20*	84.5%
Pir 20 $mgkg^{-1}$	14.2±2.26*	64.75%

Data was subjected to ANOVA followed by Newman-Keuls Multiple comparison test; *=$p<0.001$ compared with control group, n=5; Data is Mean ± SEM

Table 4: Effect of *Laggera aurita* in the Randall-Selitto experiment in mice

	Mean Pressure Tolerated in Grams				
Time	0 min	30 min	60 min	90 min	120 min
Control	85.4±9.1	86.0±10.7	94.40±6.735	102.0±16.0	65.0±5.0
MELA 200 $mgkg^{-1}$	102.0±7.6	122.8±9.6*	162.0±19.60*	172.0±14.1**	74.0±7.4
MELA 400 $mgkg^{-1}$	86.0±4.8	137.0±5.3*	176.0±19.39*	185.0±10.9**	90.0±5.9
Pent 20 $mgkg^{-1}$	75.0±7.5	176.0±6.6***	154.0±16.91*	140.8±7.3*	63.0±3.3

Data were subjected to ANOVA followed by Neuman-Keuls Multiple comparison test; *=p<0.05; **=p<0.01; ***=p<0.001, n=5

DISCUSSION

With an intraperitoneal LD_{50} of 3807.88 mg/kg, the extract is considered slightly toxic based on the toxic classification of chemicals (Matsumura, 1975). Surviving animals in both phases of the toxicity test were sedated suggesting possible central effects. In the acetic acid induced writhing which screens peripherally mediated analgesia, the significant inhibition of writhes strongly suggests the involvement of cyclooxygenase enzyme (COX) inhibition (*Vongtau et al.,* 2004). Drugs that act via this mechanism usually inhibit the synthesis of prostaglandins involved in pain mediation (Immer *et al.,* 2003). Results from the Randall-Siletto test showed significant anti-hyperalgesia which surpassed that of pentazocine a centrally acting agent. Opioid analgesics act on μ, δ and κ receptors, while partial agonists like pentazocine antagonize some receptors (Laurence *et al.*, 1999). However, peripheral presence of opioid receptors enables some opioids exert peripheral activity (Furst *et al*., 2005). The actions of herbal extracts are usually a function of their phytochemical constituents, and this knowledge may be useful for synthesis of complex chemical substances (Savithramma *et al*., 2011). Saponins, tannins and carbohydrates which are present in the plant have been documented to have anti nocicpetive properties (Pal *et al.,* 2009; Arrau *et al*., 2010). The presence of carbohydrates and tannins, have also been associated with reduction in both chemical and thermal hyperalgesia (Smiderle *et al.,* 2008; Zulfiker *et al.,* 2010). With the inhibitory effects of the extract in both the acetic acid and Randall-Selitto tests, its actions may be likened to that of acetaminophen which is believed to act centrally and peripherally on the eicosanoid system (Smith, 2009), and has been demonstrated to interact with the serotonergic system (Pickering *et al.,* 2006) and the descending pain control system (Vanegas *et al.,* 2010).

CONCLUSION

The data obtained from the current investigation points to the fact that the methanolic extract of *Laggera aurita* possesses analgesic effects that may have both peripheral and central components. The presence of constituents that have been consistently associated with anti-nociception also further validates the use of the plant in the treatment of rheumatism in folk medicine and may therefore offer alternative leads for the development of novel analgesic agents.

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