African Journal of Pharmacology and Therapeutics Vol. 5 No. 2 Pages 65-73, 2016
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Research Article

Acute Toxicological, Analgesic and Anti-Inflammatory Effects of Methanol Extract of *Laggera aurita* Linn F (Compositae) in Mice and Rats

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Background: The plant *Laggera aurita* is an annual or biannual plant belonging to the family Compositae that has been used for management of pain related conditions locally. It reportedly has anti-oxidant as well as antimicrobial properties.

Objectives: To conduct LD_{50} and phytochemical studies, evaluate the analgesic and anti-inflammatory properties of the methanol extract of *L. aurita* and determine possible mechanism of action.

Methodology: Analgesic and anti-inflammatory properties of the extract were investigated using acetic acid induced writhing, thermally-induced pain, and formalin induced inflammation in rats and mice. Phytochemical and acute toxicological screenings were also conducted.

Results: The LD_{50} was found to be above 5000 mg/kg with slight changes in histological architecture observed in the kidney, liver, lungs and stomach. The extract at dose 200, 400 and 800 mg/kg significantly ($p<0.05$) inhibited acetic acid induced writhes in mice and increased mean reaction time in the thermal pain model, both dose dependently. The effect on thermally induced pain was blocked by naloxone, a non-specific opioid antagonist, suggesting opioid receptor involvement in analgesia. The extract also significantly ($p<0.05$) decreased formalin induced paw edema dose dependently.

Conclusion: These findings suggest that the methanol extract of *L. aurita* possesses analgesic and anti-inflammatory properties that justify its ethnomedicinal use in management of pain and inflammation.

Keywords: *Laggera aurita*, anti-inflammatory, analgesic, acute toxicity.

Received: January, 2016
Published: May, 2016

1. Introduction

The use of herbal drugs in the treatment of pain is a common practice in many African countries (Usman et al. 2008). Despite the immense technological advancement in modern medicine, many people in developing countries still rely on traditional medicine and healing practices for their daily health care needs (Gedif and Hahn, 2003). The Plant *L. aurita* (Linn F) belongs to the family Compositae. It is called *Abanaadene* (vulture's excrement) in Igbo (Awka) and *Eru-taba* (Slave of tobacco) in Yoruba (Ilorin) (Burkill, 1985). *L. aurita* is a widely used medicinal plant in African countries like Nigeria, Senegal and Ghana. The plant was also listed among anti tubercular plants of foreign origin (Vikrant, 2011). The anti-oxidant and antibacterial activities of the steam distilled volatile oil of *L. aurita* grown in Pakistan have been reported (Shahwar et al. 2012). Recent studies on the ethanol crude extract of *L. aurita* showed the presence of

 ISSN 2303-9841

triterpenes, saponins, flavonoids, coumarins, tannins and also anti-inflammatory effects (Abdulla et al. 2013). Olurishe and Mati, (2014) reported some preliminary antihyperalgesic potentials. The current study was conducted to investigate the acute toxicological and anti-nociceptive effect of the methanol extract of *L. aurita* using rodent models.

2. Materials and Methods

2.1 Plant Collection

The whole plant was collected from Zaria, Kaduna State in December, 2012. The plant specimen was identified at the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University Zaria- Nigeria by comparing with a specimen voucher number 510 previously deposited in the herbarium.

2.2 Extraction Procedure

The plant material was dried under shade with intermittent weighing until a constant weight was obtained. The stalk part of the plant was removed and whole plant was size-reduced using mortar and pestle. About 1500 grams of the powdered materials was extracted with methanol using soxhlet apparatus at a temperature of 65 °C. The extract was concentrated under reduced pressure and temperature 45 °C. The extract was then stored in a dessicator until required for each study.

2.3 Animals

Wistar strain rats (150-200 grams) and swiss albino mice (20-25 grams) of either sex were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. They were housed in standard propylene cages and kept under natural day and light cycle. The animals were fed on standard laboratory animal diet and water *ad libitum*. Food was withdrawn during the experimental hours to verify pharmacological outcomes. All experimental protocols were approved by the University Animal Ethics Committee.

2.4 Drugs

Acetic acid (Ranbaxy Laboratories Ltd, Punjab); Ketoprofen (Lek; Slovenia); Piroxicam (Pfizer laboratories, Pakistan); Naloxone (Pfizer laboratories, Pakistan); Morphine sulphate BP (Martindale Pharmaceuticals, Ramford, Essex). All drugs or reagents were freshly prepared to the desired concentration with distilled water or normal saline just before use.

2.5 Phytochemical Screening of *Laggera aurita*

Phytochemical screening was carried out on the methanol extract of the plant *L. aurita* using standard protocol as described by Trease and Evans (1983).

2.6 Acute Toxicity Studies in Mice and Rats (LD_{50})

LD_{50} determination was conducted using Organization for Economic Co-operation and Development (OECD 420) guidelines in rats and mice. In this method, two groups each of three animals were fasted prior to dosing (food but not water was withheld overnight for the rats and for 3 hours for mice). The fasted body weight was determined for each animal and the dose was then calculated according to the body weight. Food was further withheld for 3-4 hours in rats and 1-2 hours in mice after the extract was administered. The extract was administered in a single oral dose using an oral canula. A start dose of 2000 mg/kg was used for each animal in the first phase. Animals dosed in the first phase were observed for 48 hours after which there was no death and the test proceeded to the second phase. The same procedure was adopted but with a dose of 5000 mg/kg.

Animals were observed individually at least once during the first 30 minutes after dosing and periodically during the first 24 hours with careful observation during first 4 hours, and then daily for 14 days. Observations included changes in skin and fur, eyes and mucous membranes, somator activity and behaviour pattern, autonomic and central nervous systems. Animals were also observed for tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma, with onset of toxic symptoms and disappearance also noted. Individual weights of animals were determined at least weekly. At the end of the test, surviving animals were weighed and then euthanized using chloroform. The liver, kidney, spleen, lungs, heart and stomach of the animals were harvested fresh and fixed in 10% v/v formalin for histological observations.

2.7 Histological studies

Tissues were prepared and slides were examined microscopically for pathological lesions as described by Arthur and John (1978).

2.8 Antinociceptive Studies

Acetic acid induced writhing test

The acetic acid induced writhing test in mice as described by Koster et al. (1959) was employed. Thirty albino mice were divided into five groups of six mice. The first group received 10 ml/kg of normal saline orally and this served as control. Group 2 received Piroxicam 20 mg/kg as positive control. Group 3, 4 and 5 received 200, 400 and 800 mg/kg oral doses of the methanol extract of *Laggera aurita* (MELA) respectively. Thirty minutes after pretreatment, mice in all groups were treated with acetic acid (0.6% $^{v}/_{v,}$ 10 ml/kg body weight i.p.). Mice were then placed in individual cages and the number of abdominal writhes was counted for each mouse for a period of 10 minutes after 5 minutes latency period.

Hot plate test in mice

The method previously described by Eddy and Leimbach (1953) was employed for the study. Mice were grouped into five groups of six mice each. Group 1 received 10 ml/kg normal saline orally. Groups 2, 3 and 4 received 200, 400 and 800 mg/kg of MELA orally respectively, while group 5 received 5 mg/kg morphine solution orally. Thirty minutes after treatment, each mouse was placed on a hot plate (Gallenkamp thermostat) which was set and maintained at 55 ± 1^{o}C.

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The pain response latency was determined using a stop watch.

Effect of naloxone pretreatment on analgesic effect of methanol extract of *Laggera aurita* in the thermally induced pain model

Four groups of 10 mice each were used. Initially all the animals received naloxone at a dose of 2 mg/ kg i.p. After 15 minutes, group 1 and 2 received 400 and 800 mg/ kg of MELA orally. Group 3 received normal saline 10 ml/kg orally and group 4 received morphine 5 mg/ kg orally. The index of pain response latency was assessed at 60, 120 and 180 minutes using hot plate method (Younos et al. 1990).

2.9 Anti-inflammatory activity

Formalin induced inflammation

Thirty rats were divided into five groups each of six rats. Group 1 was treated with 10 ml/kg normal saline orally; group 2 was treated with 10 mg/kg ketoprofen orally; while group 3, 4 and 5 received 200, 400 and 800 mg/kg of MELA respectively orally. Inflammation was induced in rat hind paw by injecting formalin 0.1 ml (1% w/v in 0.9% normal saline) into the subplanter surface of the left hind paw 30 mins after treatment. Paw thickness (mm) was measured using a digital vernier caliper (MR 2002) at 0, 1, 2, 3, 4 and 5 hours after formalin injection (Winter et al. 1963).

2.10 Data Analysis

The results were expressed as Mean ± SEM and analyzed using one way ANOVA for acetic acid test and repeated measures ANOVA for hot plate test, interaction of extract with naloxone, and formalin induced inflammation, followed by Bonferoni post hoc test. Results were considered significant at $P \leq 0.05$.

3. Results and Discussion

3.1 Phytochemical screening

The phytochemical constituents found in the methanol extract of *L. aurita* include the flavonoids, alkaloids, glycosides, saponins, cardiac glycosides, phenols, tannins, steroids and carbohydrates.

3.2 Acute Toxicity Study

The oral acute toxicity (LD_{50}) was found to be greater than 5000 mg/kg body weight in both mice and rats.

Histological studies

Liver: The groups that received normal saline showed normal hepatocytes and intact portal tract in both mice and rats. At 2000 mg/kg of the methanol extract of *L.aurita* (MELA), there was slight vascular congestion in mice and there was intense vascular damage with kupffer cell hyperplasia and slight hepatic necrosis in rats. At 5000 mg/kg of MELA, there was intense vascular congestion with kupffer cell hyperplasia and lymphocyte hyperplasia in mice (**Plate I**) and there was vascular congestion with lymphocyte hyperplasia in rats (**Plate II**).

Kidneys: The normal saline group showed normal renal tissue, normal glomeruli, tubular epithelium, interstitial tissue and vessels. At 2000 mg/kg of MELA, there was slight glomerular necrosis in mice, slight glomerular necrosis and tubular distortion in rats. At 5000 mg/kg, there was slight glomerular necrosis with tubular damage in mice (**Plate III**) and rats (**Plate IV**).

Lungs: The saline groups showed normal alveoli in both mice and rats. At 2000 mg/kg, alveoli were normal in both mice and rats. At 5000 mg/kg, there was slight alveoli congestion in mice (**Plate V**), while vacuotation with lymphocyte hyperplasia and congestion were observed in rats (**Plate VI**).

Spleen: The saline group and at 2000 mg/kg extract, red and white pulp in both mice and rats were normal. At 5000 mg/kg there was lymphocyte hyperplasia (**Plate VII**).

Stomach: The normal saline group showed normal mucosa lining in both mice and rats. At 2000 mg/kg, both mice and rats showed normal mucosa lining. At 5000 mg/kg, moderate erosion of stomach epithelium in mice and slight mucosa damage in rats (**Plate VIII**) were observed.

Heart: Cardiac muscles in both mice and rats were normal in the normal saline group, at 2000 mg/kg and 5000 mg/kg of MELA.

3.2 Analgesic activity

Effect of methanol extract of *L. aurita* on acetic acid induced writhes test in mice

MELA significantly ($p<0.05$) decreased the number of acetic acid-induced writhes in mice in a dose dependent manner. The effect of MELA at all doses tested showed significant ($p<0.01$) decrease in number of acetic acid-induced writhes than piroxicam at a dose of 20 mg/kg (**Figure 1**).

Effect of the methanol extract of *L. aurita* on thermally induced pain

MELA significantly ($p<0.05$) increased the mean reaction time in a dose dependent manner. At 800 mg/kg, MELA showed significant ($p<0.01$) increase in mean reaction time than morphine (5 mg/kg) at three and four hours treatment time (**Table 1**).

Effect of methanol extract of *L. aurita* interacted with naloxone and assessed by hot plate test

MELA co-administered with naloxone significantly ($p<0.01$) decreased the reaction time in a dose dependent manner. At 800 mg/kg, MELA showed significant ($p<0.001$) decrease in reaction time than morphine 5 mg/kg at the three hours treatment time (**Table 2**).

 ISSN 2303-9841

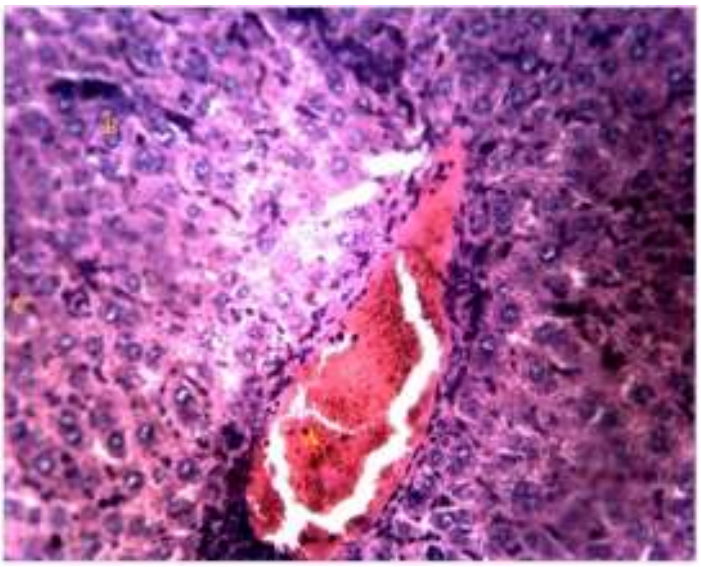

Plate I: Photomicrograph of a liver section of mouse treated with 5000 mg/kg of methanol extract of *L. aurita* and observed for fourteen days showing A = intense vascular congestion, B = Kupffer cell hyperplasia, C = lymphocyte hyperplasia. H & E stain (× 400)

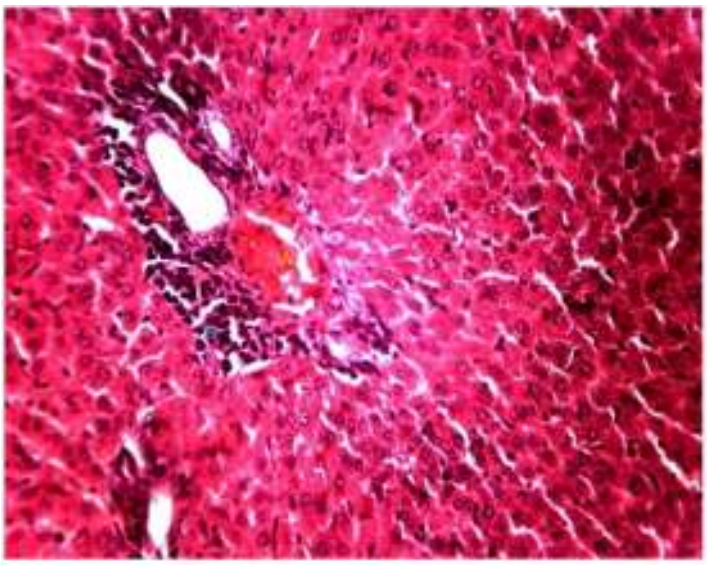

Plate II: Photomicrograph of a liver section of rat treated with 5000 mg/kg of methanol extract of *L. aurita* and observed for fourteen days showing A = vascular congestion, B = lymphocyte hyperplasia. H & E stain (× 400)

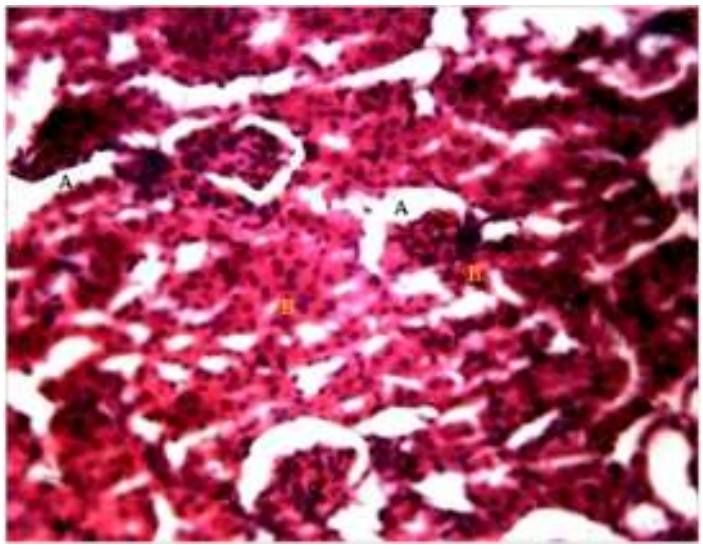

Plate III: Photomicrograph of a kidney section of mouse treated with 5000 mg/kg of methanol extract of *L. aurita* and observed for fourteen days showing A = slight glomerular necrosis, B = tubular damage. H & E stain (× 400)

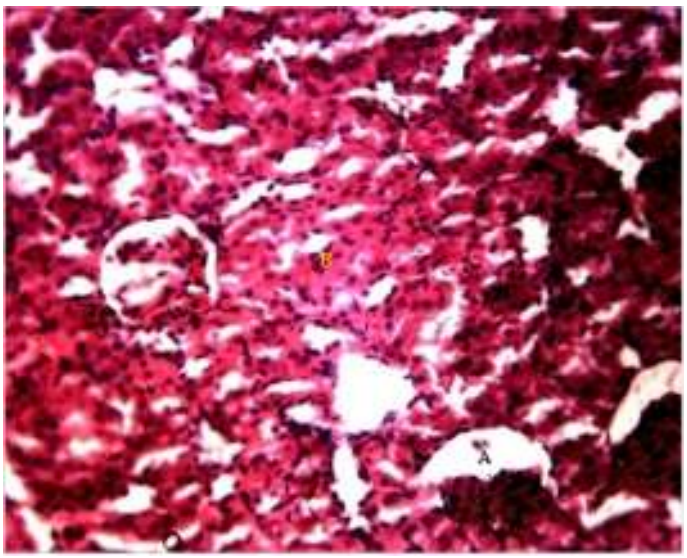

Plate IV: Photomicrograph of a kidney section of rat treated with 5000 mg/kg of methanol extract of *L. aurita* and observed for fourteen days showing A = slight glomerulus necrosis, B = tubular damage. H & E stain (× 400)

3.3 Anti-inflammatory activity

Effect of the methanol extract of *L. aurita* on formalin induced inflammation in rats

Formalin (0.6% v/v) produced local edema in rats paw which was significant as compared to time zero in all the groups ($p<0.001$). The red color denote peak of edema and blue color denotes significant statistical decrease in mean paw edema size. Peak of inflammation was reached in the third hour in normal saline and extract dose 200 mg/kg, and in the second hour for Ketoprofen 10 mg/kg, MELA 400 and 800 mg/kg. The extract significantly ($p<0.001$) decreased formalin-induced paw edema dose dependently at the fourth and fifth hours post treatment time (**Table 3**).

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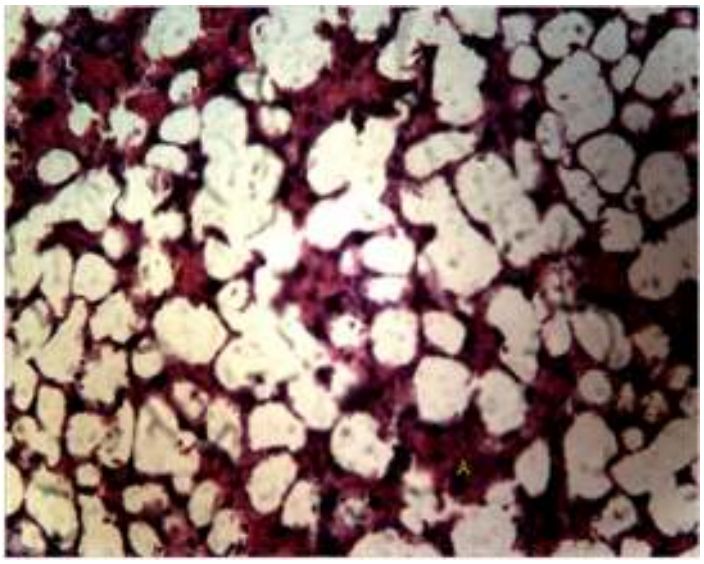


Plate V: Photomicrograph of a lung section of mouse treated with 5000 mg/kg of methanol extract of *L. aurita* and observed for fourteen days showing A = slight alveoli congestion. H & E stain (× 400)

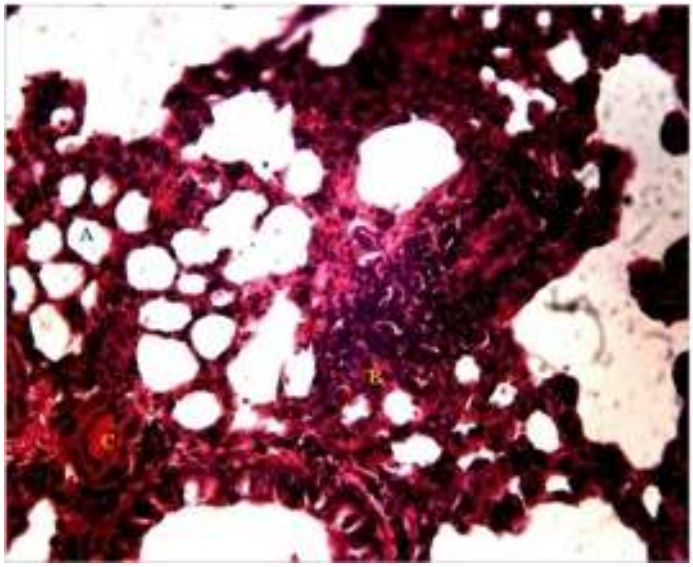


Plate VI: Photomicrograph of a lung section of rat treated with 5000 mg/kg of methanol extract of *L. aurita* and observed for 14 days showing A = vacuotation, B = lymphocyte hyperplasia, C = congestion. H & E stain (× 400)

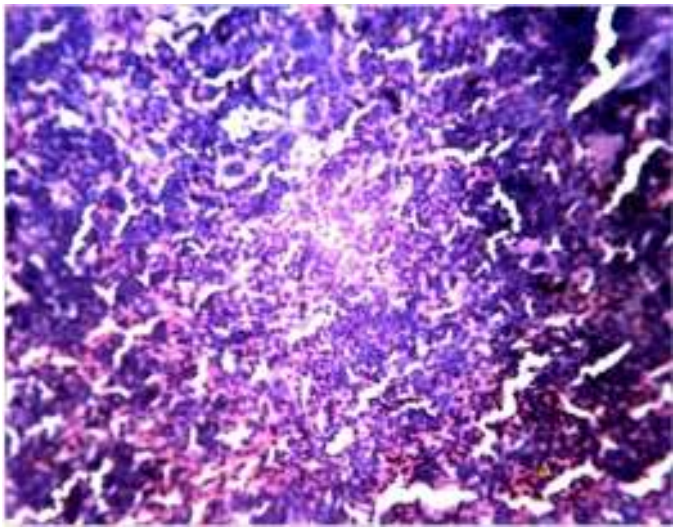


Plate VII: Photomicrograph of a spleen section of mouse treated with 5000 mg/kg of methanol extract of *L. aurita* and observed for fourteen days showing A = lymphocyte hyperplasia. H & E stain (× 400)

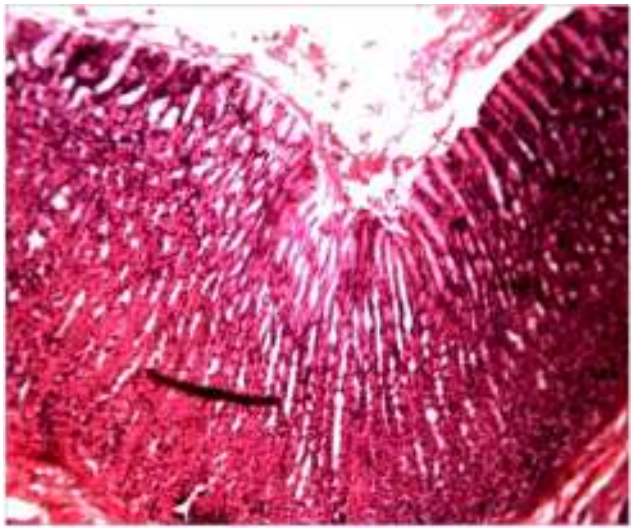


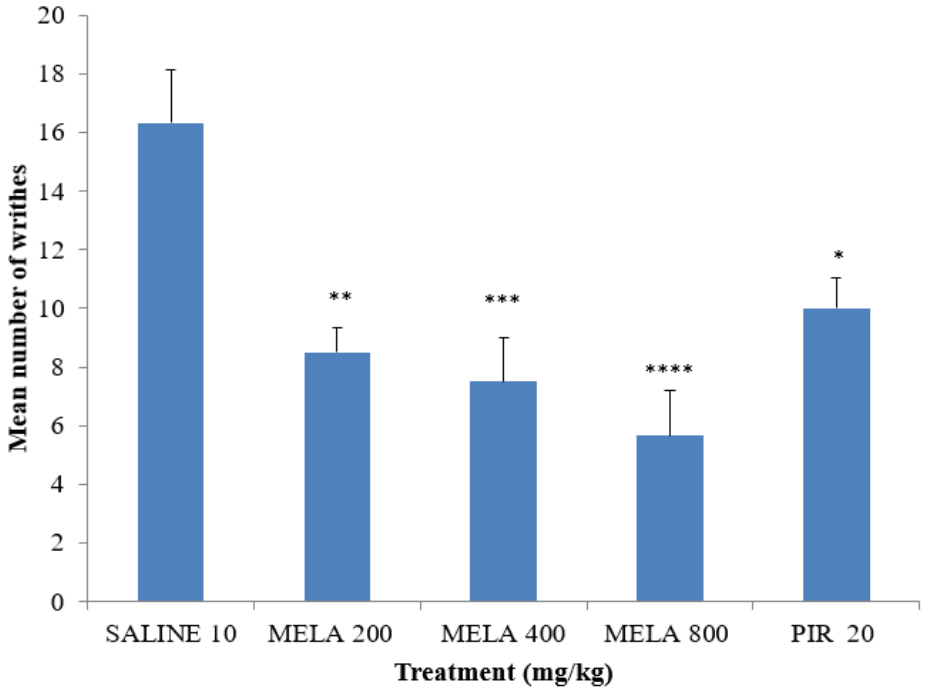
Plate VIII: Photomicrograph of a stomach section of rat treated with 5000 mg/kg of methanol extract of *L. aurita* and observed for fourteen days showing A = slight mucosa damage. H & E stain (× 400)

4. Discussion

The biological or pharmacological actions of plant extracts are known to be due to the presence of specific phytochemical constituents. Analgesic and anti-inflammatory effects have been observed and reported with flavonoids (Amin et al. 2012) and tannins (Ahmadiani et al. 2000). There are also reports on alkaloidal analgesic effects (Reanmongkol et al. 2005), and with saponins (Choi et al. 2005, Arrau et al. 2010). Certain flavonoids possess potent inhibitory activity against many enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A_2 and phosphodiesterases (Middleton, 1998). Flavonoids were also reported to be effective against inflammation (Narayana et al. 2001) by inhibiting enzymes involved in prostaglandin synthesis (Kamil, 1993; Kim et al. 2000).

Thus, the antinociceptive and anti-inflammatory effect exhibited by the plant *L. aurita* may be due to the presence of saponins, flavonoids, alkaloids, carbohydrates, tannins or combination of all found present in the plant.

ISSN 2303-9841



*Data was analyzed using one way ANOVA followed by Bonferoni Post Hoc test and presented as mean ± SEM, * = p≤0.05, ** =p≤0.01, *** =P≤0.001, **** =P≤ 0.0001 significant statistical difference as compared with Control group, n=6, MELA= methanol extract of* L. aurita*, PIR = piroxicam 20 mg/kg*

Figure 1: Effect of methanol extract of *L. aurita* on acetic acid induced writhing in mice

Table 1: Effect of the methanol extract of *L. aurita* on thermally induced pain in mice

Groups (mg/kg)	Mean reaction time ± SEM (secs)				
	0 min	30 mins	60 mins	90 mins	120 mins
Control	2.29±0.10	2.18±0.22	2.19±0.23	2.43±0.28	2.49±0.19
LA(200)	1.67±0.14	3.25±0.39	3.32±0.14**	3.01±0.24*	3.02±0.29*
LA(400)	1.81±0.25	2.82±0.14**	3.02±0.16	3.28±0.31	3.29±0.18
LA(800)	2.08±0.20	2.99±0.19	2.69±0.19	3.87±0.27**	3.81±0.24**
Mor (5)	2.15±0.19	3.37±0.20*	3.07±0.41	3.73±0.16*	3.72±0.27*

*Data was analyzed using repeated measures ANOVA followed by Bonferroni post hoc test, * = P< 0.05, ** = P< 0.01 significant statistical increase in mean reaction time compared with time zero; n=6. Values are Mean ± SEM. LA= methanol extract of* Laggera aurita*, Mor = morphine 5 mg/kg*

Table 2: Effect of methanol extract of *L. aurita* interacted with naloxone and assessed by hot plate test

Treatment (mg/kg)`	Mean reaction time ± SEM (secs)			
	0 mins	60 mins	120 mins	180 mins
Control	2.09±0.17	2.00±0.14	1.97±0.09	2.04±0.14
LA (400)	1.75±0.13	1.90±0.13	1.81±0.09	1.69±0.07
LA (800)	2.16±0.19	1.99±0.21*	2.03±0.19*	1.88±0.18**
Mor (5)	2.21±0.12	2.13±0.16	2.05±0.11	1.94±0.10*

*Data was analyzed using repeated measures ANOVA followed by Bonferroni post hoc test, * = p<0.01, ** = p<0.001 significant statistical difference compared with time zero; n=10. Values are Mean ± SEM. LA= methanol extract of* L. aurita*, Mor= morphine 5 mg/kg*

 ISSN 2303-9841

Table 3: Effect of methanol extract of *L. aurita* on formalin induced inflammation in rats

Treatment (mg/kg)	Mean Paw Diameter (millimetre)					
	0 hrs	1 hrs	2 hrs	3 hrs	4 hrs	5 hrs
Control	2.24±0.06	2.78±0.10##	3.18±0.05##	3.4±0.05####	3.03±0.06####	2.7±0.06#
LA (200)	2.08±0.05	2.76±0.06##	2.73±0.04##	2.88±0.04##	2.57±0.03**##	2.46±0.01*##
LA (400)	2.19±0.07	2.86±0.02##	2.87±0.02##	2.86±0.06###	2.65±0.04**	2.34±0.05**
LA (800)	2.21±0.09	2.86±0.03#	2.82±0.04#	2.78±0.03##	2.46±0.06**	2.33±0.04**
Ket (10)	2.21±0.05	2.55±0.06##	2.69±0.09##	2.32±0.04##	2.3±0.02**	2.19±0.01**

*Data was analyzed using repeated measures ANOVA followed by Bonferoni Post Hoc test, *= p<0.05, **= p<0.0001, significant statistical decrease in mean paw edema size (blue) as compared to the peak of edema (red), # = p<0.05, ## = p<0.01, #### = p<0.000 significant statistical increase in mean paw diameter as compared to time zero; n= 6. Values are Mean ± SEM. LA= methanol extract of* L. aurita*, Ket= ketoprofen 10 mg/kg, hrs= hours*

The oral LD_{50} was above 5000 mg/kg for MELA, showing relative safety. LD_{50} is a useful index in assessing the safety margin of a substance. The OECD (Walum, 1998) classification of acute systemic toxicity based oral LD_{50} of >500 ≤ 2000 mg/kg as non-toxic or harmful. Based on this, the oral LD_{50} greater than 5000 mg/kg established in both mice and rats indicated relative oral safety. Other toxicity scales (Hodge and Sterner, 1943) reported that compound with an oral LD_{50} of between 500 – 2000 mg/kg should be considered practically non-toxic.

Acetic acid induced writhing test is usually employed to screen for peripheral analgesic activity (Bentley et al. 1981, Gene et al. 1998). Intraperitoneal administration of acetic acid to mice leads to the release of prostaglandins like PGE_2 and $PGF_{2\alpha}$ which have been implicated as mediators of pains and inflammations (Santosh et al. 2008). Thus, activity shown by MELA suggests that analgesic effects may be due to their action on visceral receptors sensitive to acetic acid, or inhibition of the production of algogenic substances, or the inhibition at the central level of painful messages transmission or combination of all the three mentioned above.

The thermally induced pain model is utilized as a standard method for the evaluation of centrally mediated analgesia. Narcotic agents like morphine, pentazocine and codeine mediate their analgesic effect through this mechanism (Leonard et al. 2006). The analgesic activity observed in MELA may be due to its activity via central mechanism similar to the narcotic analgesic agents (Vogel and Vogel, 1997). This centrally mediated effect which is through interaction with opioid receptors (Leonard *et al.* 2006) was blocked by prior administration of naloxone. Naloxone is a specific antagonist of opioid receptors (Stein, 1995, Almeida et al. 2011), and thus the inhibition of the analgesic action in the presence of this agent suggests possible central and opioid receptor involvement in the analgesic effect of the plant extract.

Formalin induced inflammatory response is usually bi-phasic in nature. The first phase is mainly due to the release of histamine, serotonin and kinins (1-3 hours) while the second phase is mainly attributed to the release of prostaglandins (Vinegar et al. 1969; Chan et al. 1995). MELA inhibited both phases of inflammation which may be due to its ability to inhibit the release of the mediators of inflammation.

Some analgesic drugs act by inhibiting prostanoids biosynthesis. They act as analgesic and anti-inflammatory agents by inhibiting COX-2 dependent prostanoids in the cells at inflammatory sites (Mishra et al. 2011) and in the spinal cord (Ramer et al. 1998). They also inhibit the action of phospholipase A_2, which releases arachidonic acid from the cell membrane (Burke et al. 2006). These classes of drugs are usually associated with side effects like stomach problems such as bleeding, ulcer, stomach upset (Traversa et al. 1995), kidney problems, heart problems (Gislason *et al.* 2009), high blood pressure and fluid retention (Knight et al. 2010). Thus, the histological changes observed in the organs from animals exposed to 2000 and 5000 mg/kg may be resultant from inhibition of prostanoid biosynthesis.

5. Conclusion

The methanol extract of *L. aurita* has been shown to possess significant analgesic and anti-inflammatory activities which may be responsible for it use in painful and inflammatory conditions. Based on this, studies should be carried out to isolate, characterize and elucidate the structure of the bioactive constituents responsible for the observed pharmacological effects.

The results of histological studies showed effects on kidney and liver at dose of 2000 mg/kg and on kidney, liver, spleen, lungs and stomach at dose of 5000 mg/kg, thus detailed toxicological screening on long term repeated dosing should be conducted.

Conflict of Interest Declaration

The authors declare no conflict of interest.

ISSN 2303-9841

Acknowledgement

The authors acknowledge the effort of Dr Jamilu Yau for providing us with pure naloxone powder, Ibrahim Adamu for providing us with laboratory animals and all technical staff of Pharmacology Department, Ahmadu Bello University, Zaria, Nigeria.

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 ISSN 2303-9841