Arch. Bas. App. Med.11 (2023):14-20
www.archivesbamui.com
www.ojshostng.com/index.php/abam

Research Article

Evaluation of the analgesic and anticonvulsant properties of the ethanol extract of *Lannea acida* leaves in mice

Adebayo S.A.[2], Akadri B.A.[1], Alegbeleye B.T.[1], *Bakre A.G.[1,2] and Gbotosho G. O.[1,2]
[1]*Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Nigeria.*
[2]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Ibadan, Nigeria.*
Accepted: 20 July, 2023

Abstract
Lannea acida A. Rich. (family: Anacardiaceae) is a multipurpose medicinal tree that is indigenous to several West African countries. It is used traditionally for the treatment of several ailments including pain and epilepsy. In this study, *Lannea acida* leaf ethanol extract's analgesic and anticonvulsant effects are assessed using convulsion and pain models in animals. The phytochemical constituents of the ethanol extract of *Lannea acida* leaves (EELAL) as well as its acute oral toxicity were determined. Swiss mice weighing between 18-25 g were allotted to treatment groups (n = 5) and the analgesic property of EELAL at doses of 100, 200 and 400 mg/kg administered orally was evaluated using acetic acid-induced writhing test, formalin-induced paw licking test and hot plate test. The doses of 500, 750 and 1000 mg/kg were selected for the evaluation of the anticonvulsant activity of EELAL using pentylene tetrazole, strychnine and isoniazid-induced convulsion models. The results of the phytochemical studies showed that EELAL had an abundance of tannins and flavonoids, while the oral median lethal dose (LD50) was determined to be >2000 mg/kg. Acetic acid-induced abdominal constrictions were significantly inhibited by 100, 200 and 400 mg/kg, EEAL (58.20, 34.73 and 95.62%) compared with negative control animals. The inflammatory phase of the formalin test was significantly inhibited by EELAL. This was observed as the reduction in paw-licking time compared to normal saline-treated animals. The extract did not show any significant effect on the neurogenic phase of the formalin test, hot plate test and chemical-induced (pentylene-tetrazole, strychnine, and isoniazid) epilepsy tests. The leaves of *Lannea acida* possess significant peripheral analgesic activity but lack an anticonvulsant effect.

Key Words: Pain, Epilepsy, *Lannea acida*

INTRODUCTION

From time immemorial, plants have been employed as a source of medicinal agents for the treatment of diseases and ailments plaguing humanity. Several effective medicines in current use were originally obtained from plants (Atanasov *et al*., 2015). In the recent past, there has been a renewed interest in the exploration of plants for new, therapeutic principles that will be an improvement on current drugs with regard to efficiency and safety (Ahn, 2017; Atanasov *et al*., 2021).

Pain has been defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Raja *et al*., 2020). When pain is nagging or interferes with daily activities, relief is sought from therapeutic measures and medicines. Pain relievers in current use include non-opioid analgesics (paracetamol, and NSAIDS such as aspirin, ibuprofen and diclofenac) and the opioid analgesics (codeine, pentazocin, fentanyl and morphine) (Yang *et al*., 2020). However, the use of these conventional analgesics have been plagued with unacceptable adverse effects ranging from organ toxicities to physical dependence and abuse (Cazacu *et al*., 2015).

Epilepsy is a neurological condition affecting up to 50 million people around the world (WHO, 2019). It is typically characterized by seizures due to abnormal and excessive electrical discharges in the brain which could be localized or widespread (Beghi, 2020; Sarmast *et al*., 2020). Even though great success has been reported in its management, about 30% of patients experience therapeutic failure with current antiepileptic drugs which include viagabatrin, lamotrigine, valproic acid, ethosuximide and carbamazepine (Kwan *et al*., 2010; Löscher and Klein, 2021).

The challenges being encountered in the management of pain and epilepsy have led to the search for newer, safer and more effective medicines. The ethno-medicinal use of several plants have served as a valuable lead in drug discovery. Numerous plants have been reported to have analgesic and anticonvulsant effects. One of such plants is *Lannea acida* .

Lannea acida A. Rich (family: Anacardiaceae) is a tree indigenous to West Africa, usually growing wild in the tropical savannah regions (von Maydell, 1990). It is locally called 'bembé, bembey, bembéńań' in Senegal; 'boukinebelege, chukon-kyodi' in Gambia; 'kuntunkori, samtuluga, ekualokpoe' in Ghana; and 'faruhi, faànin mútaàneé, ekan aja' in Nigeria (Burkil, 1985). It can attain a height of up to 10 meters or more and has narrow, oval-shaped, glabrous leaves which are densely-packed, and clusters of berry-like fruits during the fruiting season (Burkil, 1985; Arbonnier, 2004). It is regarded as a useful tree, traditionally

**Author for Correspondence:* Tel: +234 8081062365 E-mail: *bakreeyitemi@live.com*

serving multiple purposes ranging from nutritional to material and medicinal.

The fruits of *Lannea acida* are consumed fresh, dried or processed into drinks; the leaves are boiled to make vegetable soups, while dyes and gum are obtained from the tree bark (Burkil, 1985; Mayori, 2018). More importantly, *Lannea acida* has been regarded as a source of valuable herbal medicines in several West African countries. Its bark, leaves and roots have been employed, alone or in combination with other herbal plants, in several preparations for the management of a wide range of medical conditions including inflammation, pain, epilepsy, helminth infestation, gastro-intestinal disorders, fever, malaria, gynaecological conditions, infertility, venereal diseases, hemorrhoids, skin infections, snake bite and diabetes, as well as infections among cattle such as New castle disease and foot and mouth disease (Burkil, 1985; Mayori, 2018).

Ethno-medicinally, the whole plant has been used in the treatment of central nervous conditions such as pain and epilepsy (Mayori, 2018). While extensive research has been done on the phytochemical constituents and pharmacological effects of *Lannea acida* stem bark and some research on its roots, there are inadequate studies on its leaves (Kone *et al*., 2004; Ahmed *et al*., 2010; Ouattara *et al*., 2011; Muhaisen, 2013; Oumarou *et al*., 2017; Owusu and Ofori-Amoah, 2017; Onoshe *et al*., 2018; Tetsatsi *et al*., 2019). The leaves of a tree can be harvested easily without inflicting much damage to the plant, unlike the stem bark or root. Therefore, this study aims to determine if the leaves of *Lannea acida* are able to produce analgesic and anticonvulsant effects, in order to authenticate the traditional use of *Lannea acida* for these effects. The findings of this study may go a long way in increasing the therapeutic options available for pain and epilepsy, especially in developing countries where accessibility and affordability of synthetic drugs continue to pose a challenge.

MATERIALS AND METHODS

Chemicals and Drugs: Aspirin, acetic acid, formalin (2.5%), tramadol injection (25 mg/mL), diazepam injection (5 mg/mL), pyridoxine, strychnine, pentylene tetrazole, isoniazid, normal saline (0.9 %w/v), distilled water. The drugs were dissolved in or diluted with distilled water to obtain stock solutions which were appropriately diluted to make the concentration required for each experiment. Drug solutions were freshly prepared daily.

Experimental Animals: Male Swiss mice (18-25 g) were obtained from the Central Animal House, College of Medicine, University of Ibadan, Nigeria. They were kept in plastic cages in a well-ventilated environment, and provided with water and commercial rodent pellets ad libitum. The National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals were observed in the conduct of experimental procedures.

Collection and preparation of plant material: Fresh leaves of *Lannea acida* were collected from the forest along Iseyin road, Oyo State. They were identified and authenticated by Mr. Esimekhuai, a plant taxonomist at the herbarium section of Department of Botany, University of Ibadan, and a voucher specimen (UIH-23303) was deposited at the herbarium. The leaves were air-dried in the shade for four weeks. The air-dried leaves (375 g) were pulverized and macerated with 70 % ethanol for 72 hours. The extract was filtered, and then concentrated in a rotary evaporator yielding an extract of 262 g. The extract was then stored in a desiccator and, when required for experimental procedures, it was weighed and reconstituted with distilled water to obtain appropriate concentrations.

Phytochemical screening: Standard techniques were used in preliminary analyses of the ethanol extract of *Lannea acida* leaves (EELAL) for the qualitative identification of its phytochemical contents. (Trease and Evans, 1989).

Acute toxicity studies: The Organization for Economic Co-operation and Development (OECD) guidelines No. 423 were followed to determine the acute toxicity of EELAL. The extract was administered orally at a dose of 2000 mg/kg to three mice and they were observed continuously for two hours, then at four-hour intervals and after 24 hours for signs of toxicity or mortality. No toxicity or mortality was observed for 24 hours after the oral administration of 2000 mg/kg. Hence, lower doses of 100 mg/kg, 200 mg/kg and 400 mg/kg (p.o.) were selected for the evaluation of the analgesic effect of EELAL, while doses of 500 mg/kg, 750 mg/kg and 1000 mg/kg (p.o.) were selected for anticonvulsant studies.

Evaluation of analgesic effect of EELAL

1. Acetic acid-induced writhing test: Using the previously described method, the analgesic efficacy of EELAL on acetic acid-induced abdominal writhing was evaluated. (Woode *et al*., 2009; Owusu and Ofori-Amoah, 2017). Five groups of 5 mice each were treated as follows: Group I, normal saline (10 mL/kg, p.o.), groups II through IV, EELAL (100, 200, and 400 mg/kg, p.o.), Group V, aspirin (100 mg/kg, p.o.). Thirty minutes after the treatments, 0.6%v/v acetic acid (10 mL/kg) was administered intra-peritoneally to each mouse. The number of writhing episodes that occurred within a 30-minute period was measured, along with the latency time to the initial abdominal muscle contraction. The percentage of inhibition was then calculated.

2. Formalin-induced paw-licking test: The analgesic effect of EELAL on formalin-induced paw licking was assessed as described by Sofidiya *et al*. (2014) and Bhuiyan *et al*. (2020). Groups I through IV were treated as described above, while animals in Group V were pre-treated with tramadol (50 mg/kg, p.o.). After thirty minutes, nociception was induced by the administration of 20 µL of 2.5% formalin in the sub-planter space of the right hind paw of each mouse. The duration of paw licking as an index of pain was determined at 0-5 minutes for the early (neurogenic) phase, and then at 20-30 minutes for the late (inflammatory) phase after the formalin injection.

3. Hot plate test: The hot plate test was performed as described previously using a hot plate apparatus maintained at 55±0.5ºC (Shankar *et al*., 2013; Bhuiyan *et al*., 2020). The animals were grouped and treated as described for formalin-induced paw licking. Thirty, 60, 90 and 120 minutes after drug treatment, each mouse was individually placed on the hot plate maintained at 55±0.5ºC and the reaction time due to thermal stimulation was recorded. The latency of nociceptive responses (i.e. the time it took the mouse to begin to withdraw or lick its paws, or attempt to jump off the plate) was then determined. A post-treatment cut-off of 20 seconds was taken to prevent injury to the mice (Bhuiyan *et al*., 2020).

Evaluation of anticonvulsant effect of EELAL

1. Pentylene tetrazole-induced convulsions: The anticonvulsant effect of EELAL on pentylene tetrazole-induced convulsions was assessed as described by Amole *et al*. (2009) and Agbafor *et al*. (2015). Forty-five mice (n = 9) were divided up into five groups and were pretreated as follows: group I (normal saline, 10 mL/kg, p.o.), groups II through IV (EELAL, 500, 750, and 1000 mg/kg, p.o. respectively) and group V (diazepam, 10 mg/kg, p.o.). Pentylene tetrazole 85 mg/kg was given intraperitoneally 30 minutes after pretreatment, and animals were observed for latency to convulsion, duration of convulsion and mortality. Animals that were protected for the first 2 hours were observed for protection the next day (24 hours).

2. Strychnine-induced convulsions: The anticonvulsant effect of EELAL on strychnine-induced convulsions was assessed as described by Perazzo *et al*. (2003) and Murtala *et al*. (2020). Forty-five mice (n = 9) were divided up into five groups and were pretreated as follows: group I (normal saline, 10 mL/kg, p.o.), groups II through IV (EELAL, 500, 750, and 1000 mg/kg, p.o. respectively) and group V (diazepam, 10 mg/kg, p.o.). Strychnine 2 mg/kg was given intraperitoneally 30 minutes after pretreatment, and animals were observed for latency to convulsion, duration of convulsion and mortality. Animals that were protected for the first 2 hours were observed for protection the next day (24 hours).

3. Isoniazid-induced convulsions: The anticonvulsant effect of EELAL on strychnine-induced convulsions was assessed as described by Corda *et al*. (1982) and Asehinde *et al*. (2018). Forty-five mice (n = 9) were divided up into five groups and were pretreated as follows: group I (normal saline, 10 mL/kg, p.o.), groups II through IV (EELAL, 500, 750, and 1000 mg/kg, p.o. respectively) and group V (pyridoxine, 300 mg/kg, p.o.). Isoniazid 300 mg/kg was given intraperitoneally 30 minutes after pretreatment, and animals were observed for latency to convulsion, duration of convulsion and mortality. Animals that were protected for the first 2 hours were observed for protection the next day (24 hours).

Statistical Analysis: The results were expressed as the mean and standard error of the mean (S.E.M.) One-way Analysis of Variance (ANOVA) was used to analyze all data, followed by the Newman-Keuls post hoc test was performed. $p < 0.05$ was taken as the level of significant difference from the control.

RESULTS

Acute Toxicity Test: The median lethal dose (LD50) of EELAL was estimated to be >2000 mg/kg as no mortality or toxic effects were observed in the mice for 24 hours after the oral dose of 2000 mg/kg was administered.

Phytochemical screening: The phytochemical analysis shows that EELAL is rich in tannins and flavonoids. The extract does not possess alkaloids, cardiac glycosides or anthocyanins. However, minute quantities of glycosides are present in the extract (Table 1).

Table 1:
Phytochemical Constituents of Ethanolic Extract of *Lannea acida* Leaves (EELAL)

Constituents	Tests	Observation	Inference
Tannins	Ferric chloride Test	Dark greenish color	+++
Flavonoids	Ferric chloride Test	Dark greenish color	+++
Anthocyanin	Ammonium hydroxide Test	No change was observed	-
Alkaloids	i. Mayer's test	No change was observed	-
	ii. Wagner's test	No change was observed	-
	iii. Dragendoff's test	No change was observed	-
Glycosides	Fehling's test	Yellow color	+
Cardiac glycoside	Kedde test	No change was observed	-

Key: +++ highly present; ++ moderately present; + minutely present; - not present

Effect of EELAL on acetic acid-induced writhing in mice: The administration of 100 and 400 mg/kg produced a significant ($p < 0.05$) decrease in the number of writhes caused by the intraperitoneal injection of 0.6% glacial acetic acid in mice when compared with control (normal saline 10 mL/kg). The percentage inhibition of writhes produced by 400 mg/kg of the extract (95.62%) was higher than 100 mg/kg acetylsalicylic acid (77.31%). The other doses, 100 and 200 mg/kg, produced 58.20% and 34.73% inhibition of writhes respectively (Table 2).

Effect of EELAL on formalin-induced paw licking in mice: Neither the standard drug tramadol (50 mg/kg, i.p.) nor any of the doses of the extract altered response to pain in the early (neurogenic) phase I the mice compared with control (10 mL/kg normal saline). However, in the late (inflammatory) phase, the extract significantly ($p < 0.05$) inhibited formalin-induced pain as there was significant reduction in the duration of paw licking at all the doses used (19.60±6.49, 30.40±8.80 and 14.20±9.55 minutes for 100, 200 and 400 mg/kg respectively) compared to the control animals (99.60±17.96 minutes). The duration of paw licking produced by tramadol was 3.60±1.94 minutes (Table 3).

Table 2:
Effect of EELAL on acetic acid-induced writhing in mice

Treatment/Dose (mg/kg)	Number of Writhes	Inhibition of Writhes (%)
NS 10 ml/kg	50.25±13.03	0
EELAL 100	21.00±8.82*	58.20
EELAL 200	32.80±9.42	34.73
EELAL 400	2.20±2.20*	95.62
Aspirin 100	11.40±1.69*	77.31

Values are represented as mean ± S.E.M (n=5), NS - normal saline, EELAL - ethanolic extract of *Lannea acida* leaves * p <0.05 compared to normal saline/control (One-way analysis of variance followed by Newman – Keuls test),

Table 3:
Effect of EELAL on formalin-induced paw licking in mice

Treatment/Dose (mg/kg)	Duration of paw licking	
	Early Phase (0-5 mins)	Late Phase (20-30 mins)
NS 10 ml/kg	36.00 ±3.81	99.60±17.96
EELAL 100	51.00±9.41	19.60±6.49*
EELAL 200	45.40±15.08	30.40±8.80*
EELAL 400	23.00±10.56	14.20±9.55*
Tramadol 50	12.00±6.11	3.6±1.94*

Values are represented as mean ± S.E.M (n=5), NS - normal saline, EELAL - ethanolic extract of *Lannea acida* leaves, mins – minutes,* $p < 0.05$ compared to normal saline/control (One-way analysis of variance followed by Newman – Keuls test).

Effect of EELAL on thermally-induced pain in mice: Administration of the extract at all the doses used did not significantly delay the paw licking or flinching response of the mice to noxious heat from the hot plate when compared to control at 30, 60, 90 and 120 minutes. The standard drug tramadol (50 mg/kg, i.p.) produced significant analgesic effect at 30, 90 and 120 minutes, producing reaction times of 10.36±1.11, 12.52±2.92 and 10.56±1.43 seconds respectively, compared to control (Table 4).

Table 4:
Effect of EELAL on thermally-induced pain in mice

Treatment/ Dose (mg/kg)	Reaction Time (seconds)			
	30 mins	60 mins	90 mins	120 mins
NS 10 ml/kg	6.26±0.60	6.26±0.60	6.26±0.60	6.26±0.60
EELAL 100	4.90±0.39	5.74±0.41	5.58±1.04	6.72±0.91
EELAL 200	5.88±0.95	7.04±1.23	5.38±0.35	6.74±0.80
EELAL 400	7.28±1.53	8.92±1.76	6.84±1.14	5.88±0.59
Tramadol 50	10.36±1.11*	9.28±1.25	12.52±2.92*	10.56±1.43*

Effect of EELAL on pentylene tetrazole-induced convulsions in mice: The oral administration of EELAL at doses of 500, 700 and 1000mg/kg did not significantly increase the mean onset of pentylene tetrazole-induced convulsions or the latency of death as compared with control. The mean onset of convulsion and latency of death with normal saline was 39.25 ± 4.23 and 181.30 ± 50.77 seconds respectively. EELAL at doses of 500 mg/kg, 750 mg/kg and 1000 mg/kg produced a mean onset of convulsion at 52.80 ± 24.72, 77.80 ± 28.05 and 33.25 ± 3.30 seconds respectively, while the latency of death produced were 168.60 ± 56.04, 212.00 ± 57.86, and 156.00 ± 29.27 seconds respectively. However, diazepam (10 mg/kg, i.p.) produced 100 % protection against pentylene tetrazole-induced convulsions (Table 5).

Table 5:
Effect of EELAL on pentylene tetrazole-induced convulsions in mice

Treatment/ Dose (mg/kg)	Convulsion Onset (seconds)	Death Latency (seconds)	% Mortality
NS 10 ml/kg	39.25 ± 4.23	181.30 ± 50.77	100
EELAL 500	52.80 ± 24.72	168.60 ± 56.04	100
EELAL 750	77.80 ± 28.05	212.00 ± 57.86	100
EELAL 1000	33.25 ± 3.30	156.00 ± 29.27	100
Diazepam 10	3600 ± 0.00*	3600.00 ± 0.00*	0

Values are represented as mean ± S.E.M (n=4-5), * $p < 0.05$ compared to normal saline/control (One-way analysis of variance followed by Newman – Keuls test), NS - normal saline, EELAL - ethanolic extract of *Lannea acida* leaves.

Effect of EELAL on strychnine-induced convulsions in mice: The administration of EELAL at doses of 500, 750 and 1000 mg/kg (p.o.) did not significantly increase the onset or latency of death in strychnine-induced convulsions compared to control. The mean onset of seizure and latency of death with normal saline was 219.70 ± 24.84 and 311.70 ± 84.23 seconds respectively. EELAL at doses of 500, 750 and 1000 mg/kg (p.o.) produced a mean onset of seizure at 350.40 ± 73.03, 241.00 ± 39.21, 205.40 ± 19.99 seconds respectively. The latency of death produced by EELAL was 372.60 ± 79.53, 265.00 ± 38.09, and 227.00 ± 18.31 seconds at 500 mg/kg, 750 mg/kg and 1000 mg/kg respectively. Mortality of 100 % was witnessed after administration of all test doses of EELAL, while diazepam (10 mg/kg, i.p.) produced 100 % protection against strychnine-induced convulsions (Table 6).

Table 6:
Effect of EELAL on strychnine-induced convulsions in mice

Treatment/ Dose (mg/kg)	Convulsion Onset (seconds)	Death Latency (seconds)	% Mortality
Normal saline	219.70 ± 24.84	311.70 ± 84.23	100
EELAL 500	350.40 ± 73.03	372.60 ± 79.53	100
EELAL 750	241.00 ± 39.21	265.00 ± 38.09	100
EELAL 1000	205.40 ± 19.99	227.00 ± 18.31	100
Diazepam 10	3600.00 ± 0.00*	3600.00 ± 0.00*	0

Values are represented as mean ± S.E.M (n=4-5), * $p < 0.05$ compared to normal saline/control (One-way analysis of variance followed by Newman – Keuls test), NS - normal saline, EELAL - ethanolic extract of *Lannea acida* leaves.

Effect of EELAL on isoniazid-induced convulsions in mice: The administration of EELAL at doses of 500, 750 and 1000 mg/kg (p.o.) did not significantly increase the onset of convulsion compared to control, neither did it significantly reduce the latency to death against isoniazid-induced seizures. The administration of pyridoxine (300 mg/kg) also did not significantly alter the onset of convulsion (34.04 ± 4.53 seconds) or latency of death (34.40 ± 4.53 seconds) compared to positive control. The mean onset of seizure and latency of death with normal saline was 46.50 ± 5.17 and 311.70 ± 84.23 seconds respectively. EELAL at doses of 500, 750 and 1000 mg/kg (p.o.) produced a mean onset of seizure of 62.20 ± 1.80, 52.00 ± 5.65, and 57.00 ± 10.70 seconds respectively, while death latency produced by EELAL was 372.60 ± 79.53, 265.00 ± 38.09, and 227.00 ± 18.31seconds at doses of 500, 750 and 1000 mg/kg (p.o.) respectively. Mortality of 100 % was obtained after administration of all test doses of EELAL, while diazepam (10 mg/kg, i.p.) produced 100 % protection against isoniazid-induced convulsions (Table 7).

Table 7:
Effect of EELAL on isoniazid-induced convulsions in mice

Treatment/ Dose (mg/kg)	Convulsion Onset (seconds)	Death Latency (seconds)	% Mortality
Normal saline	46.50 ± 5.17	311.70 ± 84.23	100
EELAL 500	62.20 ± 1.80	372.60 ± 79.53	100
EELAL 750	52.00 ± 5.65	265.00 ± 38.09	100
EELAL 1000	57.00 ± 10.70	227.00 ± 18.31	100
Pyridoxine 300	34.04 ± 4.53	34.40 ± 4.53	0

Values are represented as mean ± S.E.M (n=4-5), * p <0.05 compared to normal saline/control (One-way analysis of variance followed by Newman – Keuls test), NS - normal saline, EELAL - ethanolic extract of *Lannea acida* leaves.

DISCUSSION

The present study evaluated the analgesic and anticonvulsant effects of ethanol extract of *Lannea acida* leaves (EELAL) which have been documented to be used in Traditional African Medicine (TAM) for treatment of pain and epilepsy. This was done with a view to confirming these pharmacological effects scientifically, and providing newer and potentially more effective, less toxic drugs as alternatives to conventional drugs for the management of these conditions.

Acetic acid-induced writhing test is commonly used to screen potential analgesics, even though positive results can be produced by non-analgesic substances such as adrenergic antagonists and muscle relaxants (Le Bars *et al*., 2001; Alexandre-Moreira *et al*., 1999; Liu *et al*., 2012). The intraperitoneal administration of dilute acetic acid (0.6%) causes local inflammation in the peritoneal cavity which triggers the release of pain mediators (prostaglandins, kinins, serotonin and histamine), resulting in the characteristic abdominal contortions, and hind-leg extensions observed (Bhuiyan *et al*., 2020). EELAL at 400 mg/kg produced a significant percentage reduction in abdominal writhes which was better than that of aspirin. This result suggests that the phytochemical constituents of EELAL may possess peripheral analgesic activity. In order to confirm this activity, the formalin test was undertaken.

The formalin test, which involves the administration of formalin into the sub-plantar space of the mouse hind paw, evokes a biphasic behavioral response. The mouse exhibits an early phase of intense paw licking within 5 minutes of formalin administration and then a late phase after about 15 to 30 minutes. While the early phase is said to be neurogenic due to the direct stimulation of nociceptors, the late phase is said to be peripheral and inflammatory in nature (Le Bars, 2001; Sofidiya *et al*., 2015). EELAL significantly decreased paw licking time only in the late phase of the formalin test compared with control. The inability of the extract to inhibit the early phase suggests its lack of central antinociceptive effect. The late phase involves inflammation triggered by the release of pain mediators like kinins and prostaglandins, and is peripheral in origin. The effect of EELAL against the late phase of the formalin test as well as its significant effect against acetic acid-induced writhing are indicative of peripheral analgesic activity. It is possible that EELAL is able to prevent the synthesis of pain mediators that cause inflammation or inhibit their release due to its phytochemical constituents. Sultana and Ilyas (1986) reported the identification of a flavonone, 6,7-(2”,2”-dimethyl chromeno)-8-γ,γ-dimethyl allyl flavanone-1 from the acetone extract of *Lannea acida* leaves. The phytochemical screening of EELAL in this study show that the extract is rich in tannins and flavonoids. These phytoconstituents may be responsible for the anti-inflammatory effect observed. Previous studies reported the anti-inflammatory and analgesic effects of an aqueous extract of *Lannea acida* stem bark (Owusu and Ofori-Amoah, 2017). The result of this study indicates that the ethanol extract of *Lannea acida* leaves also possesses anti-inflammatory and analgesic effects.

The hot plate test is used to evaluate the central analgesic activity and the opioid-like effect of substances. It induces thermal pain which elicits reactions that are supraspinal and centrally mediated (Wildor and Wicox, 1987; Le Bars *et al*., 2001; Bhuiyan *et al*., 2020). Opioids or morphine-like drugs abolish all forms of pain modalities, an action attributed to their ability to raise pain threshold and to alter the patient’s reaction to pain (Gibson and Helme, 2000; Chen *et al*., 2021; Paul *et al*., 2021). Opioids produce their analgesic activity by interacting mainly with mu-opioid receptors at supra-spinal, spinal and peripheral sites (Imam *et al*., 2018; Paul *et al*., 2021). This interaction leads to presynaptic inhibition of neurotransmitter release from C-fibre terminals, postsynaptic inhibition of evoked activity in nociceptive pathways, or disinhibition of other circuits regulating nociceptive transmission. In this study, none of the extract doses administered produced a significant analgesic effect on thermally-induced pain, as the standard drug, tramadol did. This result buttresses the inability of the extract to inhibit the early phase of the formalin test and indicates that EELAL does not possess central analgesic activity, as the hot plate test evaluates the analgesic effect of central origin.

The study suggests that the extract does not possess anticonvulsant effect. In all three models of chemically-induced convulsions in mice used in this study, the extract did not produce any significant anticonvulsive effect. It has been documented that the whole plant is used in local communities in Burkina Faso and Ghana for the treatment of epilepsy (Ziblim *et al*., 2013; Kinda *et al*., 2017). It is possible that the phytochemicals present in the leaf extract of *Lannea acida* do not confer it with an anticonvulsant effect, or that their quantity in the leaf extract is not adequate to produce an anticonvulsant effect. In particular, *Lannea acida* bark extracts have been reported to possess significant antioxidant properties which have been attributed to flavonoids and phenolics (Ouattara *et al*., 2011; Muhaisen, 2013; Oumarou *et al*., 2017 and Onoshe *et al*., 2018). It is thought that antioxidant property enhances anticonvulsant effects of plant extracts as it mitigates the oxidative stress that is associated with neurological conditions like epilepsy (Asehinde *et al*., 2018). Again, it is possible that the quantity of flavonoids in the leaf extract of *Lannea acida* is not adequate to produce a significant antioxidant effect.

CONCLUSION

The findings of this study lend credence to the ethno-pharmacological use of *Lannea acida* leaves for the treatment of pain and inflammation, and predict that the leaves would be effective when used alone. Further studies are required to identify and isolate the active components of ethanolic extract of *Lannea acida* leaves. The extract does not seem to possess anticonvulsant activity, based on the result of this study. Since the whole plant is traditionally used in the treatment of epilepsy, further research into the anticonvulsant effect of *Lannea acida* bark and root is recommended.

REFERENCES

Agbafor, K.N., C. Ezeali, C. and Akubugwo, E.I. 2015. Anticonvulsant and analgesic properties of leaf and root extracts of Newbouldia laevis. *Asian J. Biochem*. 10(6):299-305.

Ahmed, M.K., Mabrouk, M.A., Anuka, J.A., Attahir, A., Tanko, Y., Wawata, A.U., and Yussuf, M.S. 2010. Studies of the effect of methanolic stem bark extract of *Lannea acida* on fertility and testosterone in male wistar rats. *J. Med. Sci.* 7:6-7.

Ahn, K. 2017.The worldwide trend of using botanical drugs and strategies for developing global drugs. *BMB Reports*. 50:111–116.

Alexandre-Moreira, M.S., Piurezam, M.R., Araujo, C.C. and Thomas, G. 1999. Studies on the anti-inflammatory and analgesic activity of Curatella americana L. *J. Ethnopharm*. 67: 171-177.

Amole, O.O., Kayode, Y.O. and Adeola, O.K. 2009. Anticonvulsant activity of Rauvolfia vomitoria (Afzel). Afr. *J. Pharm. Pharmacol.* 3:319-322.

Arbonnier, M. 2004. Trees, shrubs and lianas of West African dry zones. Paris: CIRAD. 141 pp. ISBN 2-87614-579-0. OCLC 56937881.

Asehinde, S., Ajayi, A., Bakre, A., Omorogbe, O., Adebesin, A., and Umukoro, S. 2018. Effects of Jobelyn® on isoniazid-induced seizures, biomarkers of oxidative stress and glutamate decarboxylase activity in mice. *Basic Clin. Neurosci*. 9(6):389-396.

Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E.M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H. and Rollinger, J.M. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotech. *Advances.* 33(8):1582-1614.

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M. and Supuran, C.T. 2021. Natural products in drug discovery: advances and opportunities. Nature Rev. Drug Discov. 20:200–216.

Beghi, E. 2020. The Epidemiology of Epilepsy. *Neuroepidem*. 54:185–191.

Bhuiyan, M.R., Bhuiya, N.M.M.A., Hasan, M.N. and Nahar, U.J. 2020. In vivo and in silico evaluation of antinociceptive activities of seed extract from the Holarrhena antidysenterica plant. Heliyon 6. e03962.

Burkil, H.M. 1985. The useful plants of West Tropical Africa. London: Royal Botanic Gardens, Kew. 960 pp.

Cazacu, I., Mogosan, C. and Loghin, F. 2015. Safety issues of current analgesics: an update. Clujul Medical. 88(2):128-136.

Chen, R., Coppes, O.J., and Urman, R.D. 2021. Receptor and molecular targets for the development of novel opioid and non-opioid analgesic therapies. *Pain Physician* 24:153-163.

Corda, M.G., Costa, E. and Guidotti, A. 1982. Specific proconvulsant action of an imidazobenzodiazepine (Ro 15-1788) on isoniazid convulsions. *Neuropharmacol*. 21(1):91-94.

Fattorusso, A., Matricardi, S., Mencaroni, E., Dell'Isola, G.B., Di Cara, G., Striano, P. and Verrotti A. 2021. The Pharmacoresistant Epilepsy: An Overview on Existant and New Emerging Therapies. Front. Neurol. 12:674483. doi: 10.3389/fneur.2021.674483.

Gibson, S. J. and Helme, R. D. 2000. Cognitive factors and the experience of pain and suffering in older persons. *Pain*. 85:375-383.

Imam, M. Z., Kuo, A., Ghassabian, S., and Smith, M. T. 2018. Progress in understanding mechanisms of opioid-induced gastrointestinal adverse effects and respiratory depression. *Neuropharmacol*. 131:238-255.

Khan, H., Saeed, M., Gilani, A. U. H., Khan, M. A., Khan, I. and Ashraf, N. 2011. Antinociceptive activity of aerial parts of Polygonatum verticillatum: Attenuation of both peripheral and central pain mediators. *Phyto. Res*. 25.7:1024-1030.

Kinda, P.T., Zerbo, P., Guenné, S., Compaoré, M., Ciobica, A., Kiendrebeogo, M., and Kiendrebeogo, M. 2017. Medicinal plants used for neuropsychiatric disorders treatment in the Hauts Bassins region of Burkina Faso. Medicines (Basel) 4:32.

Koné W.M., Atindehou, K.K., Terreaux, C., Hostettmann, K., Traoré D. and Dosso M. 2004. Traditional medicine in North Côte d'Ivoire: Screening of 50 medicinal plants for antibacterial activity. *J. Ethnopharmacol*. 93:43-49.

Kwan, P., Arzimanoglou, A., Berg, A.T., Brodie, M.J., Allen Hauser, W., Mathern, G., Moshe, S.L., Perucca, E., Wiebe, S. and French, J. 2010. Definition of drug resistant epilepsy: consensus proposal by the ad hoc task force of the ILAE commission on therapeutic strategies. *Epilepsia* 51:1069–77.

Le Bars, D., Gozariu, M. and Cadden, S.W. 2001. Animal models of nociception. *Pharmacol. Rev*. 53:597-652.

Liu, Z., Gao, W., Zhang, J. and Hu, J. 2012. Antinociceptive and Smooth Muscle Relaxant Activity of Croton tiglium L Seed: An In-vitro and in-vivo Study. *Iranian J Pharm. Res*. 11(2):611-620.

Löscher, W. and Klein, P. 2021. The Pharmacology and Clinical Efficacy of Antiseizure Medications: From Bromide Salts to Cenobamate and Beyond. CNS Drugs 35:935–963.

Mayori, A. 2018. *Lannea acida* : A review of its medicinal uses and phytochemistry and pharmacological properties. *Asian J. Pharm.* Clin. Res. 11(11):69-74.

Muhaisen, H.M. 2013. Chemical constituents from the bark of *Lannea acida* rich (Anacardiaceae). *Scholars Res. Lib*. 5:88-96.

Murtala, A.A., Akindele, A.J. and Oreagba, I.A. 2020. Anticonvulsant, muscle relaxant and in-vitro antioxidant activities of hydroethanol leaf extract of Costus afer Ker Gawl (Costaceae) in Mice. Trop. *J. Nat. Product Res*. 4(5):195-202.

Onoshe, S., Azubuike, M.M., Hitler, L., Oghenekevwe, I.E., Mary, M.A. and Amos, P.I. 2018. Free radical scavenging activity and preliminary phytochemical screening of ethylacetate fraction of stem bark of *Lannea acida* (A. Rich). *J. Nat. Product Plant Res.* 4:182-184.

Ouattara, L., Koudou, J., Zongo, C., Barro, N., Sawadogo, A., Bassole, I.H., Ouattara, A.S. and Traore, A.S. 2011. Antioxidant and antibacterial activities of three species of Lannea from Burkina Faso. *J. Applied Sci*. 11:157-62.

Oumarou, M.R., Zingue, S., Bakam, B.Y., Ateba, S.B., Foyet, S.H., Mbakop, F.T., and Njamen, D. 2017. *Lannea acida* A. Rich. (Anacardiaceae) ethanol extract exhibits estrogenic effects and prevents bone loss in an ovariectomized rat model of osteoporosis. Evidence-Based Compl. *Altern. Med. Article* ID: 7829059.

Owusu, G. and Ofori-Amoah. J. 2017. Anti-inflammatory and analgesic effects of an aqueous extract of *Lannea acida* stem bark. *British J. Pharm. Med. Res*. 16:1-8.

Paul, A.K., Smith, C.M., Rahmatullah, M., Nissapatorn, V., Wilairatana, P., Spetea, M., Gueven, N. and Dietis, N. 2021. Opioid analgesia and opioid-induced adverse effects: A review. *Pharmaceuticals*. 14(11):1091.

Perazzo, F.F., Carvalho, J.C., Carvalho, J.E. and Rehder, V.L. 2003. Central properties of the essential oil and the crude ethanol extract from aerial parts of Artemisia annua L. *Pharmacol. Res*. 48:497-502.

Raja, S.N., Carr, D.B., Cohen, M., Finnerup, N.B., Flor, H., Gibson, S., Keefe, F., Mogil, J.S., Ringkamp, M., Kathleen, A. Sluka, K.A., Song, X., Stevens B., Sullivan, M., Tutelman, P., Ushida, T., and Vader, K. 2020. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *Pain* 161(9):1976-1982.

Sarmast, S.T., Abdullahi, A. and Jahan, N. 2020. Current classification of seizures and epilepsies: scope, Limitations and recommendations for future action. *Cureus J. Med. Sci.* 12(9): e10549.

Shankar, P., Banga, H. and Dixit, R.K. 2013. Practical Manual of Experimental and Clinical Pharmacology. Asian Man (The) - An International Journal 7:232–233.

Sofidiya, M.O., Imeha, E., Ezeania, C., Flora, R. Aigbe, F.R. and Akindele, A.J. 2014. Antinociceptive and anti-inflammatory activities of ethanolic extract of Alafia barteri. Revista Brasileira de Farmacognosia 24: 348-354.

Sultana, S. and Ilyas, M. 1986. A flavanone from *Lannea acida* . *Phytochem*. 25:963-964.

Tetsatsi, A.C.M., Nkeng-Effouet, P.A., Alumeti, D.M., Bonsou, G.R.F., Kamanyi, A. and Watcho, P. 2019. Colibri® insecticide induces male reproductive toxicity: Alleviating effects of *Lannea acida* (Anacardiaceae) in rats. *Basic Clin. Androl*. 29:16.

Trease, G.E. and Evans, W.C. 1989. A textbook of Pharmacognosy. 13th Ed. London: Bacilliere Tinal Ltd.

von Maydell, H. 1990. Trees and shrubs of the Sahel: Their characteristics and uses. Germany: Deutsche Gesellschft fur Zusammenarbeit. ISBN 3-8236-1198-1204.

Wigdor, S. and Wilcox, G.L. 1987. Central and systemic morphine-induced antinociception in mice: contribution of descending serotonergic and noradrenergic pathways. *J. Pharmacol. Exp. Therap.* 242:90–95.

Woode, E., Amoateng, P., Ansah, C. and Duwiejua, M. 2009. Anti-nociceptive effects of an ethanolic extract of the whole plant of Synedrella nodiflora (L.) Gaertn in mice: Involvement of adenosinergic mechanisms. *J. Pharmacol. Toxicol.* 4:17–29.

World Health Organization. 2019. Epilepsy: a public health imperative: Summary. World Health Organization. https://apps.who.int/iris/handle/10665/325440. License: CC BY-NC-SA 3.0 IGO.

Yang, J., Bauer, B.A., Wahner-Roedler, D.L., Chon, T.Y. and Xiao, L. 2020. The Modified WHO Analgesic Ladder: Is It Appropriate for Chronic Non-Cancer Pain? *J. Pain Res*. 13:411-417.

Ziblim, I.A., Timothy, K.A. and Deo-Anyi, E.J. 2013. Exploitation and use of medicinal plants, Northern region, Ghana. *J. Med. Plants Res*. 7:1984-1993.