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Anti-cataleptic, skeletal muscle relaxant and cognitive properties of the ethanol extract of *Lophira alata* Banks ex C.F. Gaertn. (Ochnaceae) stem bark in mice

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Abstract
Lophira alata is a perennial tree which grows in many parts of sub-Saharan and East Africa and is used in Ethnomedicine for a wide range of disorders including insomnia, algesia, psychosis and memory enhancement. Phytochemical screening was carried out and acute toxicity of ethanol stem bark extract of *L. alata* was determined. The central nervous system modulating activities of *Lophira alata* stem bark extract (200, 400 and 800 mg/kg, per oral) were evaluated via haloperidol and morphine induced catalepsy, rotarod performance test, diazepam induced sleep, the novel object recognition and Y-maze tests. Phytochemical screening revealed the presence of phytochemicals such as alkaloids, flavonoids, saponins and tannins; the LD_{50} of *L. alata* was estimated to be greater than 5000 mg/kg. *L. alata* significantly ($p<0.05$) attenuated catalepsy in a non-dose dependent manner, decreased latency time on the rotarod (all dose levels), did not reduce onset of sleeping time nor increase duration of sleep. *L. alata* also significantly ($p<0.05$) increased time spent with the novel object (200, 400 and 800 mg/kg) and increased percentage spontaneous alternation in the Y-maze test (800 mg/kg). *L. alata* possess CNS activity which may account for its use in ethnomedicine for management of psychosis and cognitive enhancement.

Keywords: *Lophira alata;* Diazepam; Catalepsy; Haloperidol; Novel object

INTRODUCTION

Lophira alata is a monoecious plant found in the evergreen forests of tropical and sub-tropical regions of Africa. This plant which grows mainly in Nigeria, the Democratic Republic of Congo, Cameroun, Ivory Coast, Equatorial Guinea, Sierra Leone, Libya, Uganda and Sudan has wide ethnomedicinal and industrial uses [1-4]. It is an endangered species due to deforestation and a high demand for its timber which is one most durable forms of timber available. Its common names are azobe or iron redwood while its local names in Nigeria include *aba* (Igbo), *akufo, ekki* (Yoruba), and *namijin kadai* (Hausa); *Iku luo* or *bongosi* in Cameroun and *lugbara* in Uganda [1-6]. Roots, stembark, stemheart wood, leaves, seeds and flowers of *Lophira alata* are used in ethnomedicine for the management of headaches, toothaches, infertility, cough, diarrhoea and hepatic disorders [3,7-10]. Biological activities

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documented from studies on extracts of various parts of the plant include antimicrobial, antiprotozoan, central nervous system activities, antioxidant, insecticidal and anti tumourinogenic properties [10-17].

Neurodegenerative diseases which are common in many countries of the world with higher prevalence amongst the elderly include Parkinson's disease, Alzheimer's disease, Huntington's disease, amyolateral sclerosis and multiple sclerosis. These disorders currently have no cure as underlying cytotoxic changes are irreversible and pharmacotherapy help to alleviate symptoms and improve quality of life [18,19]. Characteristic symptoms of Parkinson's disease include resting tremors, muscular rigidity, hypokinesia, akinesia and postural imbalance while in Alzheimer's there is memory loss and cognitive dysfunction [18-20].

Plants are valuable sources of drugs and could be potential targets for drug discovery and development [21]. *L. alata* has been documented to modulate central nervous system functioning [12,13 22], thus in this study, we seek to evaluate the effects of *L. alata* in murine models of Pakinson's disease using morphine and haloperidol induced catalepsy; the skeletal muscle effects on the rotarod performance test; the sleep potentiating property using benzodiazepine induced sleep and the cognitive enhancing potential using the Y-maze and novel objects recognition tests.

EXPERIMENTAL METHODS

Plant materials. Leaves and stem bark of *Lophira alata* collected from Okeigbo in Ondo State in November 2019, were identified and authenticated by Mr. Adeniji of the Forestry Research Institute of Nigeria, Ibadan (Herbarium Unit) where a voucher specimen (FHI 112824) was deposited for future reference. The stem barks were thoroughly cleaned, air dried and ground to the powdered form in a mill. Three litres of 98% v/v ethanol was added to 1 kg of *Lophira alata* powder, the mixture was left at room temperature for 72 hours with gentle shaking. Thereafter filtration was carried out using Whatman's filter paper, the filtrate was subsequently concentrated on a rotary evaporator and dried in an oven at 40 °C until a constant weight was obtained. The final product, dark brown crystals referred to as *L. alata* extract was stored in a refrigerator at 4°C**.** The percentage yield was calculated using the formula

% Yield = (Weight of *L. alata* crystals obtained/Weight of powdered stem bark used for extraction) * 100

Fresh solutions of *L. alata* dissolved in distilled water were prepared daily for use.

Drugs/ chemicals. Haloperidol Injection, (CHI Pharmaceuticals), benzhexol tablet (Pharma base, Nigeria), distilled water, Morphine (Sterop, Belgium), Ethanol (Sigma Aldrich, UK), Diazepam (Swipha, Nigeria), Scopolamine (Sigma Aldrich, USA), Donepezil (Pfizer).

Phytochemical screening. Phytochemical screening was carried out in accordance with the standard procedures described by Trease and Evans [23]. The extract was screened for the presence of alkaloids, tannins, saponins, flavonoids, glycosides, phytosterols and triterpenoids.

Animals. Male Swiss albino mice weighing 18-25 g procured from the Animal House Facility of the Department of Pharmacology and Toxicology, University of Benin, Benin-City, Edo State were used for the study. They were kept in polypropylene cages and maintained under natural light/dark cycle, room temperature (26-28 °C), atmospheric humidity (68-83%), and were fed with standard laboratory animal feed and clean water *ad libitum.* Adequate hygiene was maintained through daily cleaning of the cages. Handling of the animals was done according to standard protocols for the use of laboratory animals of the National Institute of Health [24]. Ethical approval from the Institutional Ethics Committee of the Faculty of Pharmacy,

University of Benin, Nigeria was obtained prior to the commencement of the study (EC/FP/019/09).

Acute toxicity study. The method described by Lorke [25] with some modifications was employed in the determination of the median lethal dose (LD_{50}) of *L. alata* extract in mice. It was carried out in two phases; in the first phase, nine mice were weighed and randomly divided into three groups (n=3). Graded doses 10, 100 and 1000 mg/kg *L. alata* extract were administered orally to mice in each group. The mice were observed for 24 hours for signs of toxicity and death. Based on the outcome of the first phase, 1600, 2900 and 5000 mg/kg *L. alata* extract was administered to a fresh batch of animals (n=3 per group) and observed for number of deaths for 24 hours.

Behavioural studies

Morphine-induced catalepsy. For this experiment, thirty mice randomly distributed into five groups of six animals each were used. Mice in group I received 10 ml/kg distilled water orally; mice in groups II, III and IV were treated with 200, 400 and 800 mg/kg *Lophira alata* orally while those in group V were administered 0.5 mg/kg benzhexol intraperitoneally. Thirty minutes later, each mouse received 20 mg/kg morphine subcutaneously and cataleptic score was determined for 2 hours at intervals of 30 minutes post morphine administration by observers unaware of drug treatment Catalepsy was measured as the time an animal maintained an imposed position with both front limbs raised and resting on a 3 cm high wooden bar. End point for catalepsy was removal of the front limbs from the bar with a maximum cut off point of 3 minutes [26].

Haloperidol-induced catalepsy. The effect of *Lophira alata* on haloperidol-induced catalepsy was evaluated using the method described by Ahtee and Buncombe [27]. Briefly, thirty mice were divided into five groups of six animals each. The animals were treated with 10 ml/kg distilled water orally (group I); 200, 400 and 800 mg/kg *Lophira alata* orally (groups II, III and IV respectively) with the aid of an orogastric tube for mice, and 0.5 mg/kg benzhexol intraperitoneally (group V). Thirty minutes post drug administration, each mouse received 1 mg/kg haloperidol intraperitoneally and cataleptic score was determined by observers unaware of drug treatment. Cataleptic score was determined for four hours at intervals of one hour post haloperidol administration as previously described.

Rotarod performance test. The effect of the ethanol extract of the stem bark of *Lophira alata* on motor coordination was assessed using the rotarod apparatus, which consists of a base platform and an iron rod of 3 cm diameter and 30 cm length, with a non-slip surface. Mice were trained on the rotarod set at a constant speed of 20 rpm, animals that fell off the horizontal bar in less than 30 seconds were excluded from the study. Thirty mice which met the inclusion criteria were randomly distributed into five groups of six each. Mice in group one received 10 ml/kg distilled water while those in groups II, III and IV were treated with 200, 400 and 800 mg/kg of *Lophira alata* stem bark extract orally, mice in group V were administered 1 mg/kg diazepam intraperitoneally. Thirty minutes post drug administration, each mouse was placed on the rotarod apparatus and the length of time the animals stayed on the horizontal bar before falling off was recorded by unbiased observers. Animals were placed on the rotarod 60 and 90 minutes after drug administration and latency time to fall off the rotarod determined as previously was described [28,29].

Diazepam induced sleep test. The sleep potentiating effect of the ethanol extract of the stem bark of *Lophira alata* was evaluated using the diazepam induced sleeping test as described by Beretz *et al.,* [30] and modified by Rakotonirina *et al.,* [31]. Thirty Swiss albino mice were randomly divided into five

groups, each group containing six mice. The first group served as control and was treated with 10 ml/kg distilled water orally. The second, third and fourth groups were treated with 200, 400, and 800 mg/kg of the extract respectively by oral route (oral administration was done with the aid of an orogastric tube). The fifth group was treated with 1 mg/kg haloperidol intraperitoneally. Thirty minutes post-treatment, each mouse was administered diazepam at a dose of 30 mg/kg intraperitoneally. The mice were then placed individually in cages. The time interval between the administration of diazepam and loss of righting reflex was considered onset of sleep while the duration between loss and regain of righting reflex was taken as duration of sleep.

Novel object recognition test. A modified method of Ennaceur and Delacour [32] was used. Thirty mice were randomly divided into five groups of six animals each. Animals in all five groups were administered 1.4 mg/kg scopolamine thirty minutes prior to drug administration. The first group served as control and was given distilled water 10 ml/kg orally. The second, third and fourth groups received graded doses (200, 400, 800 mg/kg) of ethanol extract of *Lophira alata* orally respectively, while the fifth group received donepezil at a dose of 1 mg/kg intra-peritoneally. Thirty minutes post drug administration, each mouse was placed in the open field arena for a training period of two minutes and then returned to their cages. Five minutes after the training period the mice were again placed individually in the open field arena with two similar objects placed equidistant from each other for five minutes and then returned to their cages. Five minutes later, the mice were again placed in the open field arena this time with a familiar object and a novel object for five minutes. The time spent licking, sniffing or observing the familiar and novel object was scored by unbiased observers. After each test, the apparatus was cleaned and wiped with 70% alcohol [32].

The Y-maze test. The method of Sarter et al [33] was used. Thirty mice were randomly divided into five groups of six animals each. Animals received 1.4 mg/kg scopolamine and thirty minutes later 10 ml/kg distilled water orally (group 1); 200, 400 and 800 mg/kg of *Lophira alata* orally (groups II to IV respectively) and donepezil at a dose of 1 mg/kg intra-peritoneally (group V). Thirty minutes post drug administration, each mouse was placed at the centre of the Y-maze for five minutes. The total number of entry sequence into each arm of the Y-maze were scored by unbiased observers blinded to treatment. An arm entry was scored when all four paws were placed in that arm. Spontaneous alternation (SA), defined as consecutive entries into all three arms, was calculated using the ratio of the actual number of alternations to the possible/maximum number of alterations using the following formula:

SA= (Actual Alternations / Maximum Alternations) *100

After each animal was removed, the apparatus was cleaned and wiped with 70% alcohol. Each animal was used only once [33,34].

Statistical analysis. The results were statistically analyzed for significance using analysis of variance and Tukey post-test with Sigmastat^{R} software version 13, p values less than or equal to 0.05 were considered significant.

RESULTS

The percentage yield obtained from the extraction of *L. alata* was 12.4%.

Phytochemical screening. The results of phytochemical screening revealed the presence of alkaloids, tannins, saponins, steroids, anthraquinone glycosides, carbohydrates, flavonoids and cardiac glycosides. Results of phytochemical screening are presented in Table 1.

Acute toxicity study. The oral LD_{50} of *L. alata* was determined to be greater than 5,000 mg/kg (Table 2).

Effect of ethanol extract of *L. alata* stem bark on morphine induced catalepsy. *L. alata* at 200 and 800 mg/kg dose levels significantly decreased ($p<0.05$) the cataleptic score at 30, 60, 90 and 120 minutes post treatment compared to vehicle treated animals while the middle dose - 400 mg/kg - produced a significant reduction of this index at 60 and 90 minutes post treatment. There was no significant difference between benzhexol and the three dose levels of *L. alata* at the four time frames evaluated. Results are presented in Fig. 1.

Effect of *L. alata* stem bark on haloperidol induced catalepsy. In the haloperidol induced catalepsy, significant reduction ($p<0.05$) in the cataleptic score was observed with 200 mg/kg dose at the four time points evaluated. The middle dose reduced this index at the last timepoint - 240 minutes post treatment. Details are shown in Fig. 2.

Effect of *L. alata* on rotarod performance test. In the rotarod test, time spent on the rotarod was significantly decreased ($p<0.05$) compared to the vehicle at 30, 60 and 90 minutes post treatment for 400 and 800 mg/kg while the 200 mg/kg produced this effect at 60 and 90 minutes post treatment. There was no significant difference between test groups and diazepam at the various time points. Data is presented in Fig 3.

Effect of *L. alata on* diazepam induced sleep. *L. alata* significantly ($p<0.05$) prolonged the onset of sleep and decreased sleep duration at all three dose levels used in this study, these effects were more noticeable with the highest dose. Results are presented in Fig 4.

Effect of *L. alata* in the novel object recognition test. The lowest and highest doses of *L. alata* produced a significant increase ($p<0.05$) in time spent on the novel object, these were significantly different from the control animals but not from the donepezil group. The middle dose did not improve this index. Results are represented in Fig 5.

Effect of *L. alata* in the Y-maze test. The highest dose -800 mg/kg- used for this study significantly increased ($p<0.05$) spontaneous alternation in the Y-maze test. Details are shown in Fig 6.

Table 1: Phytochemicals present in ethanol extract of *L. alata* stem bark

Phytochemicals	Remarks
Alkaloids	+
Tannins	+
Saponins	+
Anthraquinone glycosides	+
Steroids	+
Carbohydrate	+
Flavonoids	+
Cardiac glycosides	+
Reducing sugars	-

+ present, - absent

Table 2: Oral lethal dose (LD50) of ethanol extract *L. alata* stem bark in mice

Phases	Groups	n	Dose (mg/kg)	Mortality (%)
Phase 1	1	3	10	0
	2	3	100	0
	3	3	1000	0
Phase 2	1	3	1600	0
	2	3	2900	0
	3	3	5000	0

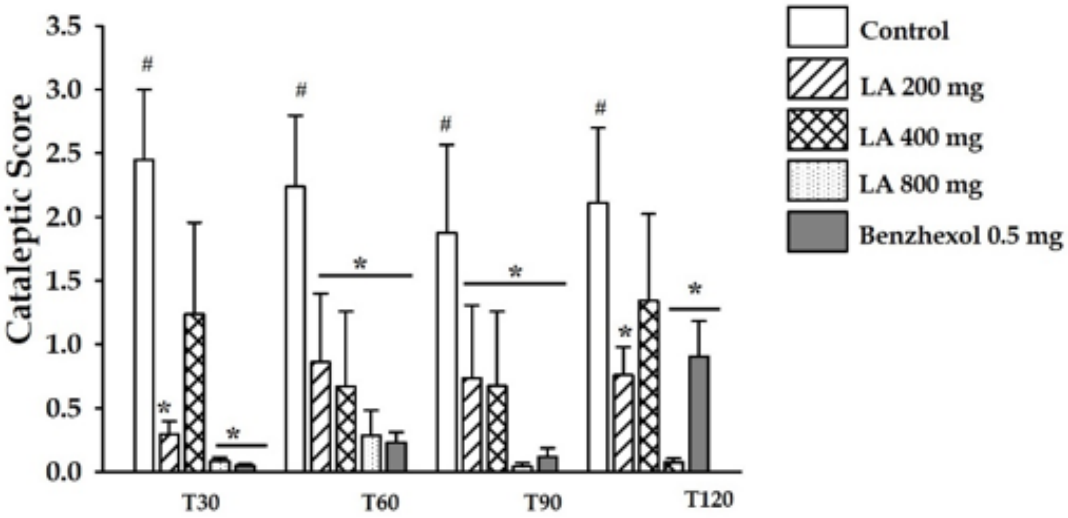

Fig 1: Effect of *L. alata* on cataleptic score in the morphine induced catalepsy test. Data is expressed as mean ± SEM. *p < 0.05 compared to vehicle, #p < 0.05 compared to benzhexol, n = 6 per group.

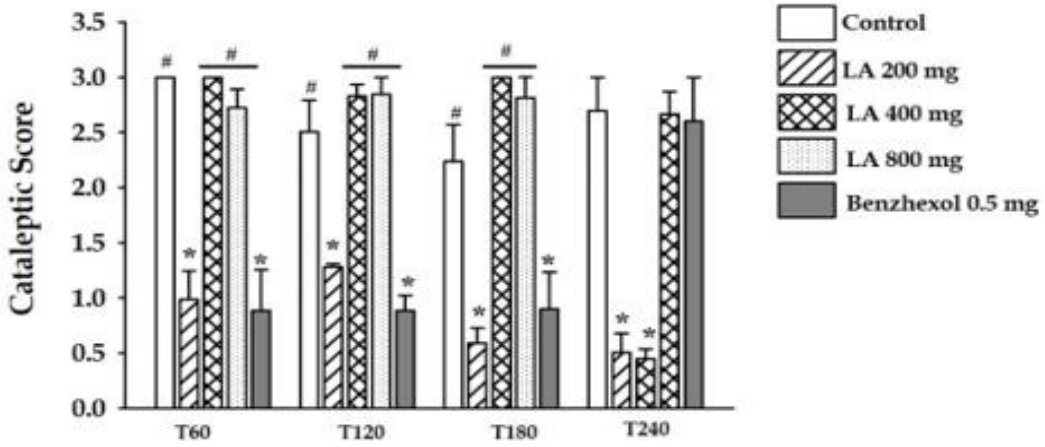

Fig 2: Effect of *L. alata* on cataleptic score in haloperidol induced catalepsy. Data is expressed as mean ± SEM. *p < 0.05 compared to vehicle, #p < 0.05 compared to benzhexol. n = 6 per group.

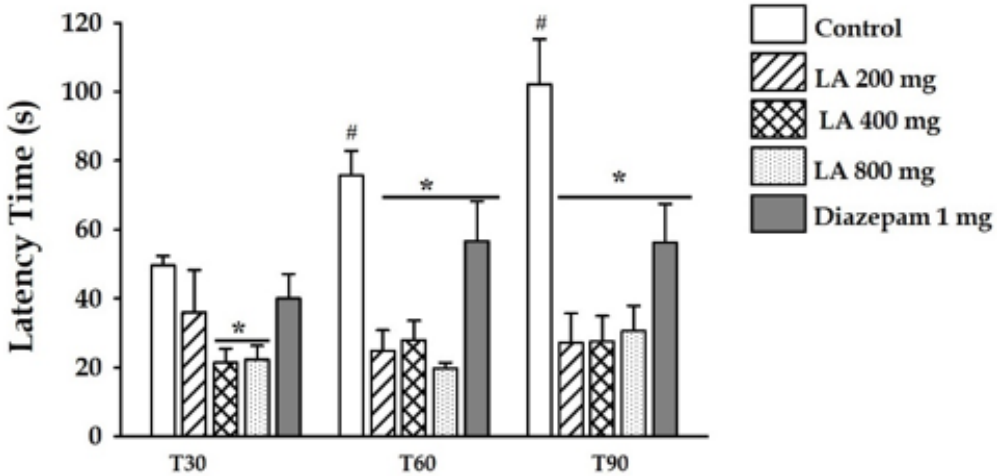

Fig 3: Effect of *L. alata* in the rotarod performance test. Data is expressed as mean ± SEM. *p < 0.05 compared to vehicle, #p < 0.05 compared to diazepam. n = 6 per group.

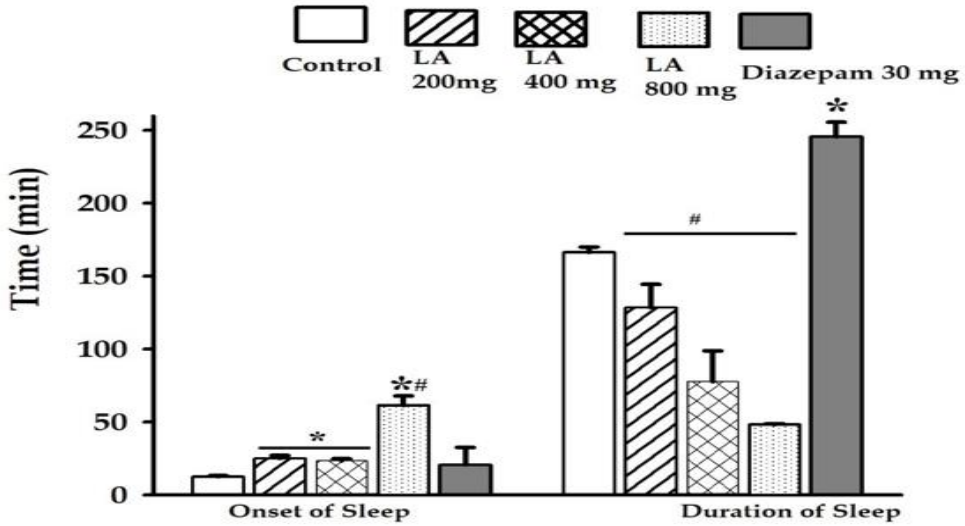

Fig 4: Effect of *L. alata* on diazepam induced sleep. Data is expressed as mean ± SEM. *p < 0.05 compared to vehicle, #p < 0.05 compared to diazepam. n = 6 per group.

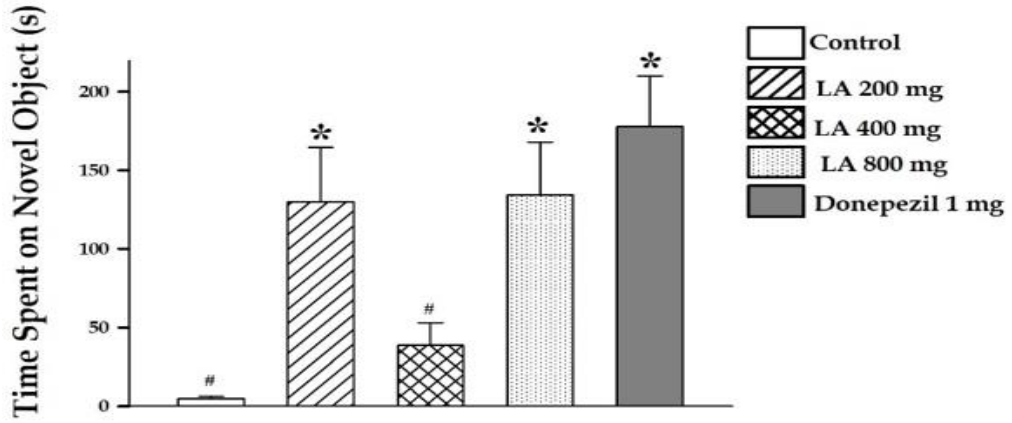

Fig 5: Effect of *L. alata* in the novel object recognition test. Data is expressed as mean ± SEM. *p < 0.05 compared to vehicle, #p < 0.05 compared to donepezil. n = 6 per group.

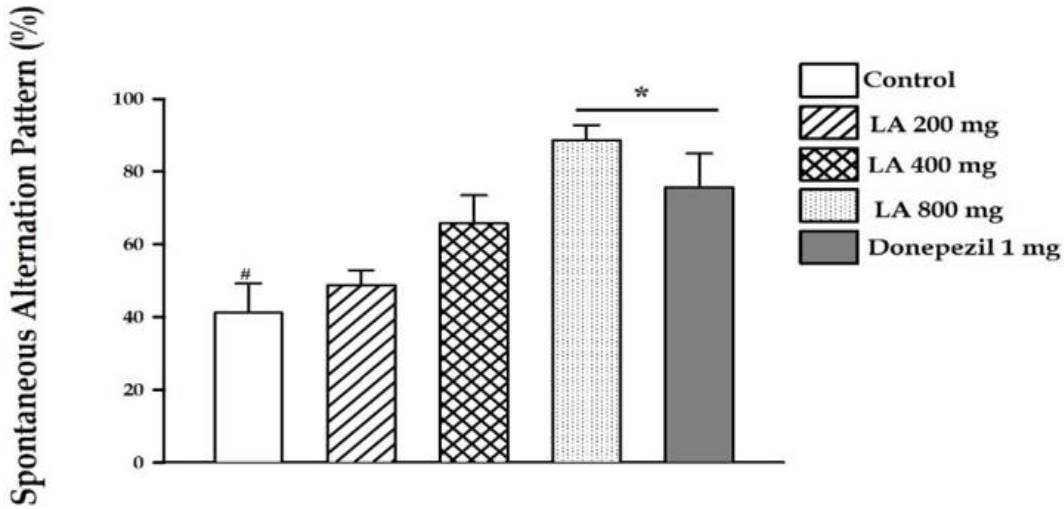

Fig 6: Effect of *L. alata* in spontaneous alternation performance test. Data is expressed as mean ± SEM. *p < 0.05 compared to vehicle, #p < 0.05 compared to donepezil. n = 6 per group.

DISCUSSION

In this study, ethanol extract of *L. alata* stem bark attenuated haloperidol and morphine induced catalepsy, reduced time spent on the rotarod, improved spontaneous alternation in the Y-maze test and increased time spent with the novel object in the novel object recognition test in a non -dose dependent manner. However, this extract did not reduce onset nor prolong duration of diazepam induced sleep.

Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, steroids and tannins which have been reported to possess biological activities including central nervous system modifying actions [35,36].

The median lethal dose of ethanol extract of *L. alata* was estimated to be greater 5000 mg/kg using the method described by Lorke. This high median lethal dose (LD_{50}) value is indicative of relative non-toxicity of oral administration of *L. alata* stem bark extract [25].

Catalepsy is an important animal model for studying compounds with potential antipsychotic or anti-Parkinsonism activity because of its similarities to clinical conditions of human neurological disorders such as Parkinsonism, catatonic schizophrenia and damage to parts of the basal ganglia [37,38]. Catalepsy in laboratory animals such as rodents is manifested as acceptance and retention of an abnormal posture i.e. failure to correct an externally imposed posture over a period of time [36]. Several compounds and neurotransmitters are implicated in the pathogenesis of catalepsy such as dopamine, acetylcholine, serotonin, noradrenaline, GABA and opiates. Dopamine antagonists such as typical antipsychotics like haloperidol and chlopromazine which reduce nigrostriatal dopamine levels induce catalepsy in rodents while neuroleptics with little or no inhibitory activity on nigrostriatal dopamine are unable to induce catalepsy in rodents, thus drugs which augment or ameliorate neuroleptic induced catalepsy have been found to aggravate or attenuate extrapyramidal side effects [39-41]. Dopamine has an inhibitory role on acetylcholine, thus hypodopaminergic neurotransmission leads to hyperactivity of acetylcholine. Catalepsy can also be induced by cholinergic agonists, cholinergic antagonists such as benzhexol, benztropine and scopolamine are used to reverse catalepsy in experimental subjects. [39,42-45]. Opiates also interact with dopaminergic pathways and high doses of opiates such as morphine can induce catalepsy Although the *nucleus accumbens* is not involved in neuroleptic induced catalepsy, it plays a role in opiate induced catalepsy [39,46,47]. In this study, *L. alata* was more effective in reducing morphine induced catalepsy than haloperidol induced catalepsy, indeed only the lowest dose of *L. alata* reversed haloperidol induced catalepsy. This may be due to differences in mechanism of action or other pharmacodynamic mechanisms. This interesting finding calls for mechanistic studies which we intend to undertake in the next phase of this study

The rotarod performance test which is one of the oldest tests used in behavioural assessments of test compounds produces a simple and rapid estimation of neuromuscular coordination effect. The length of time an animal spends on the apparatus is a function of balance, grip strength, coordination, physical condition and muscle tone. Animals with intact muscle tone and those that are not neurologically deficient are able to stay longer while those with neurological deficits fall off in no time. Drugs which cause muscle relaxation such as benzodiazepines reduce muscle tone and decrease time spent on the rotarod [28,29, 48]. In this study, *L. alata* decreased latency time on the rotarod, decrease produced was greater than that of the standard drug diazepam; this is indicative of muscle relaxant activity. The diazepam induced sleeping time evaluates sleep promoting property of test compounds. Compounds

which decrease onset of sleep and prolong duration of sleep are potential somnofacient agents [31]. *L. alata* decreased skeletal muscle tone without potentiating sedative activity at doses used in this study. Some compounds such as diazepam act as muscle relaxants at lower doses while at higher doses cause sedation and hypnosis [49,50]. Though the highest dose used in this study was 800 mg/kg, it is possible higher doses could produce sedative effects.

Acetylcholine plays a key role in cognition and memory, the role of acetylcholine in cognitive function in neurodegenerative diseases such as Alzheimer's disease and its role in learning and memory led to the development of the cholinergic hypothesis [51,52]. Drugs which potentiate central cholinergic function improve memory while cholinergic antagonists such as scopolamine result in cognitive dysfunction and are used in experimental studies [53,54]. The novel object recognition test relies primarily on rodents' natural exploratory tendency in the absence of externally applied rules or reinforcements. It can be used to study memory alternation and can be configured to measure working memory, attention, and preference for novelty in rodents [32,55,56]. The Y-maze relies on the rodent's natural to explore new environments, neither food restriction nor training is required. This exploratory capacity is expressed by a high frequency of spontaneous alternation between the arms; thus, the rodent will naturally enter individual arms successively without returning to the last visited arm. Memory impairment can therefore be estimated by the frequency of same arm return or alternate arm return [33,34,57]. In this study, *L. alata* non dose dependently increased time spent with the novel object while only the highest dose improved memory impairment caused by scopolamine in the Y-maze test, suggestive of improved cognitive function and working memory in rodents.

Taken together, results from this study confirm the central nervous system modulatory activity of *L. alata* and lends credence to its ethnomedicinal uses such as memory enhancing agents.

Conclusion. Ethanol extract of *Lophira alata* stembark possesses anti-cataleptic, skeletal muscle relaxant and memory enhancing properties and can be a potential candidate for development of lead compounds or adjunct therapy in the management of such neurodegenerative disorders as Parkinson's disease.

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