

Research Article

Anticonvulsant activity of methanol leaf extract of *Celtis integrifolia* Lam (Ulmaceae) in chicks and mice

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Background: *Celtis integrifolia* commonly known as (African Hackberry) and locally called *Zuwo* in Hausa, is a medicinal plant whose root, leaves and bark are used in northern Nigeria in the treatment of epilepsy.

Objective: The study evaluated the anticonvulsant activity of the methanol leaf extract of *Celtis integrifolia* in chicks and mice.

Methodology: Preliminary phytochemical screening and acute toxicity studies were carried out. The anticonvulsant activity of methanol leaf extract of *Celtis integrifolia* (200, 400 and 800 mg/kg, intraperitoneally) was assessed using maximal electroshock seizure test in chicks, pentylenetetrazole, 4-aminopyridine and strychnine tests in mice.

Results: Alkaloids, saponins, flavonoids, tannins and glycosides were detected in *Celtis integrifolia*, while the interperitoneal median lethal dose was estimated to be 2,154 mg/kg in mice. The methanol leaf extract of *Celtis integrifolia* significantly ($p < 0.01$) delayed onset of seizure induced by MES and provided 20.0% protection to chicks at 200 mg/kg dose. The onset of seizure behavior and latency to death was significantly ($p < 0.01$) increased by the extract, while 16.7 and 33.3% protection (200 and 400 mg/kg, respectively) was conferred to mice against pentylenetetrazole induced seizure. The extract at the dose of 200 mg/kg protected 50.0% of mice against 4-Aminopyridine induced seizure and significantly ($p < 0.01$) delayed onset of seizure behavior. The extract did not show any activity in the strychnine induced seizure model.

Conclusion: The study shows that *Celtis integrifolia* methanol leaf extract possess anticonvulsant activity, thus lending credence to the ethnomedicinal claim for the use of the plant in the management of epilepsy.

Keywords: *Celtis integrifolia*, anticonvulsant, maximal electroshock seizure, pentylenetetrazole, 4-aminopyridine

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1. Introduction

Epilepsy is the second most common disorder of the central nervous system after stroke and up to 5% of world population develops epilepsy in their lifetime (Rao and Subbalakshmi, 2010).

An epileptic seizure is a transient paroxysm of uncontrolled discharge of neurons causing an event that is discernible by the person experiencing the seizure and/or by an observer (Roger and Cate, 2012). About 50 million people worldwide have epilepsy of which

nearly 90% of the people with epilepsy are found in developing countries (WHO, 2016).

In Africa, up to 80% of the population uses traditional medicine for primary health care. The most effective method of identifying medicinal plants today is Ethnopharmacological studies (WHO, 2012).

An important medicinal plant species which has been useful in the maintenance of man's health is the *Celtis* species, commonly called (Hackberry). The genus is found all over the world but most predominant in Africa, is *Celtis integrifolia* (African Hackberry). *Celtis*

Integrifolia has been used for various soup preparations in some parts of northern Nigeria. It is locally called *Zuwo* in Hausa, *Aspe* in Yoruba, *Ngezo* in Kanuri, *Gimachi* in Nupe, *Gamki* in Fulfulde and *Abun gatu* by Shuwa Arabs.

Celtis Integrifolia is a medicinal plant whose root, leaves and bark are well known in northern Nigeria in the treatment of diseases like, leprosy (Hussaini and Karatela, 1989), microbial infections (Bum et al, 2011) and measles (Tolu, 2008). A report by Muazu and Kaita,(2008) indicated that *Celtis integrifolia* is one of the components of a polyherbal formulation for the treatment of epilepsy in northern Nigeria. However, only limited data are available concerning the anticonvulsant activity of this plant. The present work was undertaken to evaluate the anticonvulsant activity of an extract of *Celtis integrifolia* leaves.

2. Materials and Methods

2.1 Animals

Swiss albino mice of either sex weighing (18 to 25 g) were obtained from Animal House Facility of Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria and one day old ranger chicks were obtained from (NAPRI) National Animal Production and Research Institute Shika, Zaria. Mice were housed and allowed to acclimatize with free access to food and water in the animal house of the Department of Pharmacology and Therapeutics, Bayero University Kano and maintained under standard laboratory conditions in accordance with national academy of science, guides for the care and use of laboratory animals.

2.2 Drugs, Chemicals and Equipments

Pentylentetrazole (Sigma Chemical Co. St Louis USA), 4-aminopyridine (Merck-Schuchardt, Germany) and strychnine (Sigma Chemical Co. St Louis USA) were used to induce seizure in the experimental animals. The standard drugs used include Phenytoin Sodium (Hospira, UK Limited), Phenobarbitone (Lab Renavudin, France) and Sodium Valproate (Sanofi Aventis). Solvents used include Methanol (Sigma Aldrich, USA) and distilled water. An Electroshock Convulsive Machine (Ugobasile, Model 7801) was used to induce electrical seizure in chicks

2.3 Plant material

The leaves of *Celtis integrifolia* were collected from Igabi in Kaduna State, Nigeria in June, 2016. It was authenticated by a botanist at the Department of Biological Sciences Herbarium Ahmadu Bello University, Zaria Kaduna State and a voucher specimen number (11035) was deposited in the herbarium for future reference.

2.4 Preparation of plant extract

Fresh leaves of *Celtis integrifolia* were dried under shade for three weeks after which they were blended using mortar and pestle, and sieved until a fine powder that weighed 384 g was produced. The powdered plant material was cold macerated with 2.5 L 70%^{v/v}

methanol with constant shaking for 3 days and then filtered using Whatman filter paper No 1. The filtrate was then concentrated to dryness in an oven at 55°C, which was then kept in desiccators for use in the study. The percentage yield was then calculated.

2.5 Phytochemical screening

Freshly prepared methanol extract of *Celtis integrifolia* was subjected to phytochemical tests for the detection of various chemical constituents (Prashant et al, 2011).

2.6 Acute toxicity studies

The median lethal dose (LD₅₀) of the extract was determined using the method described by Lorke, 1983. The method is biphasic in nature and a total of 13 mice of either sex were used. Three groups of three mice each were administered with the extract intraperitoneally at doses of 10, 100 and 1000 mg/kg body weight and were observed for sign and symptoms of toxicity and death for 24 hrs. In the second phase which was determined by the first phase, three groups of one mouse each were treated with the extract at more specific doses 1600, 2900 and 5000 mg/kg. They were observed for sign and symptoms of toxicity and death for 24 hrs and number of deaths recorded. The LD₅₀ was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived.

2.7 Anticonvulsant studies

Maximal electroshock induced convulsion test in chicks

The method described by Swinyard and Kupferberg, (1985) and Browning, 1992 was employed. Fifty, day-old cockerels were randomly divided into five groups of ten chicks each. The first group were pre-treated with normal saline 10 ml/kg *i.p.*, while the second, third and fourth groups were pre-treated with 200, 400 and 800 mg/kg of the methanol leaf extract of *Celtis integrifolia* *i.p.*, and the fifth group pre-treated with 20 mg/kg of phenytoin *i.p.* Thirty minutes later, maximum electroshock was administered to induce seizure in the chicks using Ugobasile electroconvulsive machine (model 7801) connected to a stabilizer with corneal electrodes placed on the upper eyelids of the chicks after dipping them in normal saline. A current (80 mA) which induces tonic seizures in 90% of the control groups of chicks was used. The shock duration, frequency and pulse width was set and maintained at 0.8 sec, 100 pulse/sec and 0.6 ms respectively which were used throughout the study. Seizures were manifested as hind limb tonic extension. The ability to prevent this feature or prolong the latency and or onset of the hind limb tonic extension was considered as an indication of anticonvulsant activity.

Pentylentetrazole-induced convulsion test in mice

The method of Swinyard et al, (1989) was employed. Thirty mice were divided into five groups of six each. The first group was pre-treated with normal saline 10 ml/kg *i.p.*, while the second, third and fourth groups were pre-treated with 200, 400 and 800 mg/kg of the methanol leaf extract of *C. integrifolia* intraperitoneally.

The fifth group was pre-treated with 200 mg/kg body weight of Sodium valproate intraperitoneally. Thirty minutes later, mice in all the groups were injected with a convulsive dose CD₉₀ of PTZ 85 mg/kg body weight subcutaneously and observed for a period of thirty minutes. The absence of clonic spasm indicates the compound's ability to abolish the effect of PTZ on seizure threshold. PTZ induces seizures by loss of righting reflex with tonic forelimb extension

4-aminopyridine-induced convulsion test in mice

The method described by Yamaguchi and Rogawski, (1992) was used. Thirty mice were divided into five groups of six each. The first group was pre-treated with normal saline 10 ml/kg *i.p.*, while the second, third and fourth groups were pre-treated with 200, 400 and 800 mg/kg of the methanol leaf extract of *Celtis integrifolia* (*i.p.*). The fifth group was pre-treated with 20 mg/kg body weight of phenobarbitone (*i.p.*). Thirty minutes post treatment; 4-aminopyridine was administered at a dose of 14 mg/kg body weight subcutaneously to each mouse and observed after a period of thirty minutes for characteristic behavioral signs, such as hyperactivity, trembling, intermittent forelimb extension, tonic seizures and death. Ability of the extract to protect the mice from lethality within 30 minutes observation period was considered as an indication of anticonvulsant activity.

Strychnine-induced convulsion test in mice

The method was carried out as described by Porter et al, (1984). Thirty mice were divided into five groups of six each. The first group was pre-treated with normal saline 10 ml/kg *i.p.*, while the second, third and fourth groups were pre-treated with 200, 400 and 800 mg/kg of the extract (*i.p.*). The fifth group was pre-treated with 20 mg/kg body weight of phenobarbitone (*i.p.*). Thirty minutes post treatment, convulsive dose CD₉₀ of strychnine 0.25 mg/kg body weight subcutaneously was administered to each mouse and observed after a period of thirty minutes. The proportion of mice presenting convulsion as well as the onset of tonic convulsion was recorded. Abolition of tonic extension jerks of the hind limbs within 30 minutes after

strychnine administration was considered as an indication of anticonvulsant activity.

2.8 Statistical analysis

Results were expressed as Mean \pm Standard Error of the Mean (SEM) in form of tables. Statistical analysis for difference between means were carried out using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Values of $p < 0.05$ were considered significant.

3. Results

Percentage yield of methanol leaf extract of *Celtis integrifolia* was 13.53%^{w/w}. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, tannins, glycosides and saponins.

The intraperitoneal median lethal dose of methanol leaf extract of *Celtis integrifolia* in mice was estimated to be 2,154 mg/kg body weight.

Effect of methanol leaf extract of *Celtis integrifolia* on maximal electroshock test (MEST) in chicks

The methanol leaf extract of *Celtis integrifolia* produced significant ($p < 0.01$) increase in the mean onset of seizures in chicks at 200 and 400 mg/kg doses. However there was no significant effect on the mean recovery time from seizure. Protection of 20% against seizures was recorded at 200 mg/kg dose of extract while 80% was recorded for the standard drug, phenobarbitone 20 mg/kg (Table 1).

Effect of methanol leaf extract of *Celtis integrifolia* on pentylenetetrazole-induced convulsion in mice

The extract of *Celtis integrifolia* showed a significant ($p < 0.01$) increase in the mean onset of seizure and latency to death at 200 and 400 mg/kg doses. The same doses conferred 16.7 and 33.3% protection against PTZ-induced convulsion in mice respectively while standard anticonvulsant (Sodium valproate) gave 66.7% protection (Table 2).

Table 1: Effect of methanol leaf extract of *Celtis integrifolia* on maximal electroshock test (MEST) in chicks

Treatment (mg/kg)	Mean Onset of Seizure (sec)	Mean Time of Recovery (min)	Quantal Protection
NS 10 ml/kg	2.3 \pm 0.2	6.1 \pm 0.8	0/10
MLECI (200)	4.5 \pm 0.4**	7.1 \pm 1.2	2/10
MLECI (400)	4.0 \pm 0.2**	7.3 \pm 1.1	0/10
MLECI (800)	3.4 \pm 0.2*	7.5 \pm 0.7	0/10
PHN (20)	5.0 \pm 0.0**	6.0 \pm 0.0	8/10

Values are presented as Mean \pm SEM. Mean onset of seizure and Mean recovery time of seizure compared to normal saline group. * $P < 0.05$, ** $P < 0.01$ One way ANOVA followed by Dunnett's post hoc test, n=10, NS- Normal saline, MLECI- Methanol leaf extract of *Celtis integrifolia*, PHN- Phenytoin.

Table 2: Effect of methanol leaf extract of *Celtis integrifolia* on pentylenetetrazole – induced convulsion in mice

Treatment (mg/kg)	Mean Onset of Seizure (min)	Mean Latency to Death (min)	Quantal Protection
NS 10 ml/kg	7.3 ± 1.3	7.6 ± 1.2	0/6
MLECI (200)	15.0 ± 1.3**	27.5 ± 0.5**	1/6
MLECI (400)	20.0 ± 2.6**	26.0 ± 1.6**	2/6
MLECI (800)	14.2 ± 1.2*	14.8 ± 1.0*	1/6
SV (200)	21.0 ± 3.1**	-	4/6

Onset of seizures presented as Mean ± SEM. * $p < 0.05$, ** $p < 0.01$ compared to normal saline group using One way ANOVA followed by Dunnett's post hoc, n=6, NS- Normal saline, MLECI- Methanol leaf extract of *Celtis integrifolia*, SV- Sodium Valproate.

Table 3: Effect of methanol leaf extract of *Celtis integrifolia* on 4-aminopyridine-induced convulsion in mice

Treatment (mg/kg)	Mean Onset of Seizure (min)	Quantal Protection	Mortality
NS 10 ml/kg	12.8 ± 0.7	0/6	6/6
MLECI (200)	20.0 ± 1.5**	3/6	3/6
MLECI (400)	14 ± 0.9	0/6	6/6
MLECI (800)	12.3 ± 0.4	0/6	6/6
PHEB (20)	22.6 ± 1.6**	3/6	2/6

Onset of seizures presented as Mean ± SEM. * $p < 0.05$, ** $p < 0.01$ compared to normal saline group using One way ANOVA followed by Dunnett's post hoc, n=6, NS- Normal saline, MLECI- Methanol leaf extract of *Celtis integrifolia*, PHEB- Phenobarbitone.

Table 4: Effect of methanol leaf extract of *Celtis integrifolia* on strychnine – induced convulsion in mice

Treatment (mg/kg)	Mean Onset of Seizure (min)	Quantal Protection	Mortality
NS 10 ml/kg	4.5 ± 0.4	0/6	6/6
MLECI (200)	5.7 ± 0.4	0/6	6/6
MLECI (400)	5.2 ± 0.5	0/6	6/6
MLECI (800)	4.2 ± 0.4	0/6	6/6
PHEB (20)	9.0 ± 0.5*	4/6	2/6

Onset of seizures presented as Mean ± SEM. * $p < 0.01$, compared to normal saline group using One way ANOVA followed by Dunnett's post hoc, n=6, NS- Normal Saline, MLECI- Methanol leaf extract of *Celtis integrifolia*, PHEB- Phenobarbitone.

Effect of methanol leaf extract of *Celtis integrifolia* on 4-aminopyridine-induced convulsion in mice

The methanol leaf extract of *Celtis integrifolia* at a dose of 200 mg/kg produced significant ($p < 0.01$) increase in mean onset of seizure and afforded 50.0% protection against 4-aminopyridine induced seizure to the mice. Phenobarbitone (20 mg/kg) also gave 50% protection against seizure. The mortality recorded for the leaf extract (200 mg/kg) and phenobarbitone was 50.0 and 33.3% respectively, while control and remaining doses of the extract recorded 100.0% (Table 3).

Effect of methanol leaf extract of *Celtis integrifolia* on strychnine - induced convulsion in mice

Methanol leaf extract of *Celtis integrifolia* did not protect the mice against strychnine induced convulsion.

However the standard drug phenobarbitone gave 66.7% protection (Table 4).

4.0 Discussion

Natural products have been used in folklore for the treatment of many illnesses and diseases. They have been lead sources for development of many effective drugs now available in orthodox medicine. The observations in this study suggest that the methanol leaf extract of *C. integrifolia* (MLECI) possesses anticonvulsant activity in the tested animals.

The medicinal value of plants lies in some phytochemicals that have definite physiological actions on the human body (Amin et al, 2013). Preliminary phytochemical investigation of MLECI reveals the presence of flavonoids, saponins, tannins, alkaloids and

glycosides. Flavonoids, saponins and alkaloids have been reported to demonstrate CNS modulating activities such as affinity for GABA_A receptors and anticonvulsant effects (Miliauskas et al, 2004; Kavvadias et al, 2004; Paramdeep et al, 2014). The intraperitoneal median lethal dose (LD₅₀) of MLECI was estimated to be 2,154 mg/kg body weight, thus relatively less toxic according to Lorke (1983). This is in conformity with the study conducted on the aqueous ethanol leaf extract by Matawalli and Ochebo, (2014) who reported an LD₅₀ greater than 1,500 mg/kg.

The use of animals in chemical and electrical methods of seizure inductions has played a fundamental role in advancing our understanding of basic mechanisms underlying ictogenesis and epileptogenesis. These methods contributed to the discovery and preclinical development of novel antiepileptic drugs (Löscher, 2011).

In the present study, the effect of MLECI on seizure induced by MES, PTZ, 4-Aminopyridine (4-AP) and Strychnine in chicks and mice was evaluated and the results demonstrated that the extract was able to produce anticonvulsant activity in MES, PTZ and 4-AP induced seizures.

In MES model MLECI partly suppressed induced seizures by significantly increasing mean onset of seizure and offering 20.0% protection to the chicks against seizure specifically at 200 mg/kg dose. The mean recovery time was however not affected by administration of the extract. Plants like *Acacia albida* (Danjuma et al, 2010) and *Asparagus adscendens* (Priyanka and Rajesh, 2016) that have been reported to be used traditionally for the treatment of convulsion had similar trend of activity.

MES-induced convulsion model is a widely used tool to screen drugs for generalized tonic-clonic seizures. MES causes several changes at the cellular level, disrupting the signal transduction in the neurons by facilitating the entry of Ca²⁺ into the cells in large amounts, prolonging the duration of convulsions (DeSarro et al, 1999). Apart from Ca²⁺, MES may also facilitate the entry of other positive ions like Na⁺, blockade of which, can prevent the MES-induced tonic extension (Gale, 1992). Currently available anticonvulsant drugs like sodium valproate and phenytoin act by modulation of these ion channels (Rang et al, 2003).

In the PTZ model MLECI significantly increased onset of seizure behavior and latency to death compared to control mice. The extract at doses of 200 and 400 mg/kg afforded 16.7 and 33.3% protection to mice against PTZ-induced convulsion respectively. Pentylentetrazole (PTZ) exerts its convulsant effect by inhibiting the activity of gamma amino butyric acid (GABA) at GABA-A receptors (Bum et al, 2010). Drugs that enhance GABA-A receptor mediated inhibitory neurotransmission such as benzodiazepines, phenobarbital, valproate and felbamate can prevent this type of seizure (Sayyah et al, 2002).

4-aminopyridine is a K⁺ antagonist and is a powerful chemoconvulsant in animals and humans. It readily penetrates the blood brain barrier and interferes with all aspects of neuronal excitability, including resting

membrane potential, responsiveness to synaptic inputs, frequency adaptation and neurotransmitters release (Wickenden, 2002). 4-aminopyridine induces tonic clonic convulsion and lethality (Yamaguchi and Rogawski, 1992). The MLECI at the dose of 200 mg/kg significantly prolonged the onset of seizure behavior and gave 50.0% protection against 4-AP induced seizure to the mice which were similar to the protection provided by phenobarbitone. The same dose offered protection to 50.0% of the animals against mortality as against remaining doses that recorded 100%. The ability of the extract to display these properties suggests that it may interact with K⁺ channel to produce anticonvulsant activity. The extract however did not produce any activity against seizures induced by strychnine. It can be deduced from the present study that MLECI may produce its anticonvulsant effects via non-specific mechanisms since it delayed the mean onset of seizure and protected some of the animals against seizures produced by MES, PTZ and 4-AP.

5.0 Conclusion

It can be concluded that the methanol leaf extract of *C. integrifolia* contains bioactive constituents that possess anticonvulsant properties and lend pharmacological credence to the folkloric claim for the use of the plant in the management of epilepsy.

Conflict of Interest declaration

The authors declare no conflict of interest.

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