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Mechanisms of Anticonvulsant and Sedative Actions of the Ethanolic Stem-bark Extract of *Ficus sur* Forssk (Moraceae) in Rodents

[1]Ismail O. Ishola, [1]Sunday O. Olayemi, [2]Omoniyi K. Yemitan and [1]Ngozi K. Ekpemandudiri
[1]Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, PMB 12003, Idi-Araba, Lagos, Nigeria
[2]Department of Pharmacology, Faculty of Basic Medical Sciences, Lagos State University, College of Medicine, P.M.B. 21266, Ikeja, Lagos, Nigeria

Abstract: *Ficus sur* Forssk (Moraceae) is used in traditional African medicine in the treatment of epilepsy, pain and inflammations. Anticonvulsant activity was investigated using picrotoxin (PTX), strychnine (SCN), isoniazid (INZ), pentylenetetrazole (PTZ) and N-methyl-D-aspartic acid NMDA models of convulsion. The phytochemical analysis of the extract revealed the presence of flavonoids, saponins, tannins, alkaloids and anthraquinone. Oral administration of *Ficus sur*, 1 h before intraperitoneal injection of chemical convulsants significantly ($p<0.05$) delayed the onset and prolonged the duration of convulsions in PTX, SCN, INZ, PTZ and NMDA-induced seizures. However, the anticonvulsant activity of the ethanolic extract of *Ficus sur* was significantly reversed following intraperitoneal pre-treatment with flumazenil (GABA receptor antagonist), cyproheptadine (5-HT_2 receptor antagonist) and L-NNA (nitric oxide synthase inhibitor) in picrotoxin-induced convulsion. The data obtained suggest that ethanol extract of *Ficus sur* possessed significant anticonvulsant effect, thereby confirming the traditional uses of *Ficus sur* in the treatment of epilepsies; mechanisms of which could involve interaction with GABAergic, glycinergic, serotonergic and glutaminergic system barks.

Key words: Epilepsy, GABA, L-nitro-arginine, picrotoxin

INTRODUCTION

According to World Health Organization fact sheet (WHO, 2012), Epilepsy is seen as a chronic non-communicable disorder of the brain that affects people of all ages. Around 50 million people worldwide have epilepsy. Nearly 80% of the people with epilepsy are found in developing regions. Epilepsy responds to treatment about 70% of the time, yet about three fourths of affected people in developing countries do not get the treatment they need. Epilepsy accounts for 0.5% of the global burden of disease, a time-based measure that combines years of life lost due to premature mortality and time lived in states of less than full health. Based on a report from the WHO, approximately 30% of patients suffering from epilepsy do not respond to currently available antiepileptic drugs and the remaining 70% do not achieve complete remission (Dhir, 2010).

Ficus sur Forssk (Moraceae) is widely distributed throughout tropical Africa (Ojokuku *et al*., 2010). It also occurs in Yemen. It is usually found on riverbanks or in riverine forest but can also be found in drier woodlands. In Nigeria, *F. sur* is used in Traditional African Medicine for the effective management of epilepsy. Other reported uses include treatment of dysentery and wound dressing (Igoli *et al*., 2005), circumcision, leprosy, rickets, infertility, gonorrhoea, oedema, respiratory disorders and as an emollient (Olowokudejo *et al*., 2008). Our phytochemical tests on *F. sur* had revealed the presence of alkaloids, tannins, anthraquinones, phlobatannins, cardiac glycosides and sugars, corroborating previous studies done by Oyeleke *et al*. (2008) and Omonkhelin *et al*. (2009) as well as Ojokuku *et al*. (2010). Based on literature search, little or no attempts had been made to study the anticonvulsive effects as well as the mechanism(s) of action of *F. sur* to scientifically validate the folkloric claim of effectiveness for the treatment of convulsive disorders; hence, the objective of this study.

MATERIALS AND METHODS

Plant material: Fresh stem-bark of *Ficus sur* was purchased from local herb sellers at the Mushin market in Lagos, Nigeria and was identified and authenticated by Mr. T.K. Odewo, a forestry expert of the Department of Botany Herbarium, in the Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria, where the herbarium voucher specimen (LUH 5080) was deposited for reference.

Corresponding Author: Sunday O. Olayemi, Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, P.M.B. 12003 Lagos, Nigeria Tel: +234-8023150200

Preparation of extract: Dried stem-bark of *Ficus sur* was chopped into small pieces and pulverized, of which 395 g was soaked in ethanol for 48 h. Thereafter, it was decanted and filtered with filter paper. The filtrate was dried in the oven at 40°C. A yield of 3.40% of the evaporated extract was obtained in the process. The extract was freshly dissolved and given in 0.5% v/v of dimethylsulfoxide in normal saline on each day of experiment for the assays.

Phytochemical analysis: The phytochemical analysis of the ethanolic extract of *Ficus sur* was carried out to determine the presence of various phytoconstituents using the methods of Trease and Evans (1989).

Drugs: Pentylenetetrazol (PTZ), picrotoxin, L-arginine, *L*-Nitroarginine, strychnine, *N*-methyl-D-aspartic acid (NMDA), hexobarbitone and cyproheptadine (Sigma Aldrich, St. Louis, MO,USA), phenobarbitone (May and Baker, Lagos, Nigeria), flumazenil (Hikma Farmaceutical,Portugal, S.A.), diazepam (Swipha pharmaceuticals, Nigeria), isoniazid (Mancleods Pharmaceuticals Ltd, India) and normal saline (Unique Pharmaceuticals limited, Lagos, Nigeria).

Animals: Albino mice of both sexes weighing between 20-25 g were purchased and kept at the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. The animals were maintained under standard environmental conditions and had free access to standard diet (Pfizer Feeds, PLC, Lagos, Nigeria) and water *ad libitum*. The experimental procedures were carried out in accordance with the United States National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985) and the study protocol was approved by the Research and Ethics Committee of College of Medicine, University of Lagos, Nigereia. Experimental sessions were conducted between 09:00 and 14:00 h.

Acute toxicity testing: Mice were assigned to eight groups of 5 mice each and doses of the extract (500, 1000 and 2000 mg kg^{-1}, p.o. and i.p., separately) were administered to the test groups, while the control group received distilled water (10 mL kg^{-1} p.o.). Following extract administration, the animals were observed for toxic manifestations for the next 5 h and subsequently, intermittently, for signs of morbidity and mortality for additional period of 14 days. Body weight measurements were taken before extract administration.

Anticonvulsant tests

Picrotoxin-or isoniazid-induced convulsions: For each chemoconvulsant, 60 mice were used (n = 10). Different groups of mice received treatment thus: Vehicle (dimethyl sulfoxide, 10 mL kg^{-1}, p.o., given to control group), phenobarbitone (40 mg kg^{-1}, i.p., given as a reference standard) or *F. sur* (200, 400 or 800 mg kg^{-1}, p.o.). Thirty minutes (i.p.) or 1 h (p.o.) after treatment, the mice were administered with picrotoxin (7 mg kg^{-1}, i.p.) or isoniazid (250 mg kg^{-1}, i.p.). Convulsions consisted of clonic-tonic seizures and wild-running around, followed by death). The presence or absence of clonic seizures was noted, with cut-off at 60 min following administration of each convulsant and protection from seizures, if any, were noted (Perazzo *et al*., 2003; Bernasconi *et al*., 1988).

Strychnine, Pentylenetetrazole-induced seizures: For each chemoconvulsant 35 mice were used (n = 7). Dosing pattern was similar to those stated in 2.7.1. Thirty minutes (i.p.) or 1 h (p.o.) after treatment, the mice were given strychnine (5 mg kg^{-1}, i.p.) or pentylenetetrazole (90 mg kg^{-1}, i.p.). The latency to convulsion and protection from seizures, if any, was recorded (Adeyemi *et al*., 2007; Gupta *et al*., 2012).

NMDA-induced seizures in mice: Thirty five mice of either sex were randomly allotted to 5 groups (n = 7). Treatment was carried out as follows: group 1: Vehicle (10 mL kg^{-1}, p.o.), group 2: Phenobarbitone (40 mg kg^{-1} i.p.) and group 3-5: *F. sur* (200, 400 or 800 mg kg^{-1}, p.o.) were administered to different groups of mice 1 h before NMDA (100 mg kg^{-1}, i.p.). Mice were observed for turning behaviour within 30 min. Turning behaviour was characterized by two consecutive 360°C cycles fulfilled by the same animal (Velisek, 2006). Animals that did not exhibit turning behaviour within the 30 min observation period were declared protected. The times of onset of this behaviour in non-protected animals were recorded.

GABAergic pathway involvement: Forty albino mice of either sex were randomly allotted to 3 groups (n = 10). They were pre-treated with flumazenil (3 mg kg^{-1}, i.p.) and 15 min later, group I mice were given dimethyl sulfoxide (10 mL kg^{-1}, p.o.), group II: phenobarbitone (40 mg kg^{-1}, i.p.)., group III: *F. sur* (400 mg kg^{-1}, p.o.). Thirty minutes (i.p.) or 1 h (p.o.) after treatment, the mice were given picrotoxin (7 mg kg^{-1}, i.p.). The latency to convulsion and percentage protection were recorded (Nogueira and Vassilieff, 2000).

Serotonergic pathway involvement: Thirty mice of either sex were randomly allotted to 3 groups (n = 10). They were pretreated with cyproheptadine (4 mg kg^{-1}, p.o) 15 min later, group I mice were given 0.5% v/v dimethyl sulfoxide in normal saline (10 mL kg^{-1}, p.o.), group II: Phenobarbitone (40 mg kg^{-1}, i.p.) and group III: *F. sur* (400 mg kg^{-1}, p.o). 1 h (p.o.) after treatment, the mice were

given picrotoxin (7 mg kg^{-1}, i.p.). The latency to convulsion and percentage protection were recorded (Michael, 2006).

Nitric oxide pathway involvement: Forty albino mice of either sex were randomly allotted to 3 groups (n = 10). They were pre-treated with nitric oxide synthase inhibitor-L-NNA (10 mg kg^{-1}, p.o). Fifteen minutes later, the animals were given 0.5% v/v dimethyl sulfoxide in normal saline (10 mL kg^{-1}, p.o.), or *F. sur* (400 mg kg^{-1}, p.o.). 1 h (p.o.) after treatments, the mice were given picrotoxin (7 mg kg^{-1}, i.p.). The latency to convulsion and percentage protection were recorded (Paul and Subramanian, 2002).

Hexobarbitone sleeping time: Five groups of 7 mice each were given normal saline (10 mL kg^{-1}, p.o.), *F. sur* (200, 400, or 800 mg kg^{-1}, p.o.) and diazepam (3 mg kg^{-1}, p.o.), respectively. One hour later, hexobarbitone (100 mg kg^{-1}, i.p.) was administered to each mouse, in turn. The mice were placed on their backs in separate chambers and the duration of loss of righting reflex, starting at the time of hexobarbitone administration, until they regained their righting reflexes were recorded. When there was any doubt, the animal was placed gently on its back again and if it righted itself within 1 min, this was regarded as the end-point (Vogel and Vogel, 1997; Mujumdar *et al*., 2000).

Statistical analysis: Results are expressed as Mean±SEM, Statistical analysis of data was done by a means of One-way Analysis of Variance (ANOVA), followed by Tukey's multiple comparison tests. Levels of significance ($p<0.05$, 0.01 or 0.001) were considered for each test.

RESULTS

Organoleptic test: Organoleptic test revealed that the extract possesses the following characteristics: brownish in color, aromatic odor, bitter taste, weak acid (pH 5.5) and slightly soluble in water.

Picrotoxin-induced seizure: Intraperitoneal injection of picrotoxin (6 mg kg^{-1}) elicited seizures in vehicle control treated with an onset of 252.00±28.57 and 210.00±25.29 sec duration of clonic seizure with 100% mortality. However, oral administration of *F. sur* extract (400 mg kg^{-1}) 1 h before the injection of the convulsant significantly ($p<0.05$) prolonged the onset of seizure (444.00±24.98 sec) and attenuated the duration of seizure (132.00±8.79 sec; $p<0.001$) which was similar to the protective effect of phenobarbitone (a standard anticonvulsant drug) but significantly ($p<0.01$) lower duration of seizure (88.00±20.44 sec) when compared to *F. sur* 400 mg kg^{-1} treated (Table 1).

Strychnine-induced seizure: Intraperitoneal injection of strychnine produced seizure duration of 26.67±8.74 sec in vehicle control treated. *F. sur* (200 mg kg^{-1}) significantly ($p<0.05$) increased onset (171.70±12.03 s) and decreased ($p<0.05$) the duration of seizure (10.83±3.17 sec) which was similar to the effect produced by phenobarbitone and both causing 100% mortality. However, *F. sur* (400 and 800 mg kg^{-1}, p.o), though prolonged the onset, but did not have any significant ($p<0.05$) effect on the duration, as well as mortality in this seizure model (Table 2).

Isoniazid-induced seizure: Isoniazid (250 mg kg^{-1}, i.p.) elicited seizures in 1924.00±180.0 sec and duration

Table 1: Effect of ethanol stem bark extract of *Ficus Sur* on picrotoxin-induced seizure in mice

Treatment	Dose (mg kg^{-1})	Onset of seizure (sec)	Duration of seizure (sec)	Protection (%)	Mortality (%)
Vehicle	10 (mL kg^{-1})	252.00±28.57	210.00±25.69	0	100
F. sur	200	276.00±57.13	284.00±50.44^{c}	40	60
F. sur	400	444.00±24.98*	132.00±8.79^{c}	0	100
F. sur	800	420.00±15.49*	300.00±24.85	0	100
Phenobarbitone	40	468.00±36.00**	88.00±20.44$^{c,\#}$	40	60

Values are expressed as Mean±SEM. *Statistical significant prolongation of onset of seizure *$p<0.05$, versus vehicle control treated; ^{c}Statistical significant decrease in the duration of seizure $^{c}p<0.05$ vs. vehicle control treated; $^{\#}p<0.001$ vs. *F. sur* 400 mg kg^{-1} treated group. Statistical analysis by one way ANOVA followed by Tukey's *post hoc* multiple comparison test. *F.sur* = ethanolic stem bark extract of *Ficus sur*

Table 2: Effect of ethanol stem bark extract of *Ficus sur* against strychnine-induced seizure in mice

Treatment	Dose (mg kg^{-1})	Onset of seizure (sec)	Duration of seizure (sec)	Protection (%)	Mortality (%)
Vehicle	10 mL kg^{-1}	67.67±2.950	26.67±8.74	0	100
F. sur	200	171.70±12.03*	10.83±3.17$^{\#}$	0	100
F. sur	400	140.00±14.56	21.83±7.45	0	100
F. sur	800	147.70±28.96	29.83±5.35	0	100
Phenobarbitone	40	170.00±30.80*	8.33±9.18$^{\#}$	0	100

Values are expressed as Mean±SEM: *Statistical significant prolongation of onset of seizure *$p<0.05$ versus vehicle treated control; $^{\#}$Significant decrease in the duration of seizure *$p<0.05$ versus vehicle treated control using one way ANOVA followed by Tukey's *post hoc* multiple comparison test. *F.sur* = ethanolic stem bark extract of *Ficus sur*; Vehicle = dimethyl sulfoxide

of seizure 1020±215.20 sec. Whereas, the extract (800 mg kg^{-1}, p.o,), significantly ($p<0.01$) prolonged the onset of seizure (2668.00±104.10 s) and decreased ($p<0.01$) the duration of isoniazid-elicited tonic convulsion in mice (262.00±34.30 sec). this effect was comparatively similar to that of phenobarbitone (40 mg kg^{-1}, p.o.) (Table 3).

Pentylenetetrazole-induced seizures: Pentylenetetrazole (90 mg kg^{-1}, i.p.) elicited clonic convulsion in 100% of the animals used in vehicle treated control with 52.83±10.70 sec onset of seizure and 102.00±10.58 sec duration of seizure. Prior administration of *Ficus sur* (200-800 mg kg^{-1}, p.o.) prolonged the onset of seizure with peak effect at 200 mg kg^{-1} extract treated which significantly ($p<0.001$) delayed the onset (555.70±146.50 sec) and reduced the durations of seizures induced by PTZ (90 mg kg^{-1}, i.p.) (58.80±5.90 sec; $p<0.01$). in addition, *Ficus sur* (200 mg kg^{-1}) pretreated produced 100% protection against PTZ-induced convulsion. Phenobarbitone (40 mg kg^{-1}, p.o), however, completely protected the animals against the tonic convulsion elicited by PTZ (90 mg kg^{-1}, i.p.) (Table 4).

N-methyl-D-aspartate (NMDA)-induced seizures: NMDA (100 mg kg^{-1}, i.p.) elicited turning behaviour in 100% of vehicle control treated mice with an onset of 120.80±0.31 sec to tonic seizures. *F. sur* (200-800 mg kg^{-1}, p.o.; $p<0.05$, $p<0.01$, $p<0.001$, respectively) dose dependently increased the onset of turning behaviour with peak effect at 800 mg kg^{-1} (1300.00±129.00 sec). However, it failed to attenuate the duration of convulsion but rather increased the duration of seizure from 324.30±2.09 sec in control treated to 2670.00±164.10 sec in extract 800 mg kg^{-1} treated. However, oral administration of phenobarbitone failed to increase the onset of turning behaviour (Fig. 1a, b).

Hexobarbitone sleeping time in mice: *Ficus sur* produced dose-dependent and significant ($p<0.001$) prolongation of sleep duration induced by hexobarbitone and the effect of *F. sur* at 800 mg kg^{-1} is comparable to that produced by phenobarbitone (Fig. 2a, b).

Possible Mechanism of anticonvulsant effect: The anticonvulsant effect produced by *F.sur* and phenobarbitone was reversed by flumazenil, cyproheptadine and *L*-nitro arginine pre-treatment (Table 5).

Table 3: Effect of the stem bark extract of *Ficus sur* on isoniazid-induced seizure in mice

Treatment	Dose (mg kg^{-1})	Onset of seizure (sec)	Duration of seizure (sec)	Protection (%)	Mortality (%)
Vehicle	10 mL kg^{-1}	1924.00±180.0	1020.00±215.2	0	100
F. sur	200	2076.00±196.9	228.00±29.10[b]	0	100
F. sur	400	2520.00±110.6*	296.00±33.23[b]	0	100
F. sur	800	2668.00±104.1*	262.00±34.30[b]	0	100
Phenobarbitone	40	2088.00±49.96*	272.00±56.71[b]	0	100

Values are expressed as Mean±SEM. *Statistical significant prolongation of onset of seizure *p<0.05 versus vehicle-treated control group; [b]Statistical significant reduction in the duration of seizure [b]p<0.01 versus vehicle-treated control using one way ANOVA followed by Tukey's *post hoc* multiple comparison test. *F.sur* = ethanolic stem bark extract of *Ficus sur*; Vehicle = dimethyl sulfoxide

Table 4: Effect of ethanol stem bark extract of *Ficus sur* against Pentylenetetrazol-induced seizure in mice

Treatment	Dose (mg kg^{-1})	Onset of seizure (sec)	Duration of seizure	Protected (%)	Mortality (%)
Vehicle	10 mL kg^{-1}	52.83±10.70	102.00±10.58	0	100
F. sur	200	555.70±146.50***	58.80±5.90[b]	100	0
F. sur	400	122.70±14.81*	67.20±5.90[a]	0	100
F. sur	800	82.00±0.93**	59.00±2.24[a]	0	100
Phenobarbitone	40	NC	NC	100	0

Values are expressed as Mean±SEM, *Statistical significant prolongation of onset of seizure *P<0.05, **P<0.01, ***P<0.001 versus vehicle-treated control group; [b]Statistical significant reduction in the duration of seizure [a]p<0.05; [b]p<0.01 versus vehicle-treated control using one way ANOVA followed by Tukey's *post hoc* multiple comparison test, NC: No convulsion, *F.sur* = ethanolic stem bark extract of *Ficus sur*; Vehicle = dimethyl sulfoxide

Table 5: Elucidation of mechanism of Anticonvulsant effect of stem bark extract of *F. sur*

Treatment	Dose (mg kg^{-1})	Onset of seizure (sec)	Duration of seizure (sec)	Protection (%)	Mortality (%)
Vehicle	10 mL kg^{-1}	252.00±28.57	210.00±25.69	0	100
F. sur	400	444.00±24.98*	132.00±8.79***	0	100
Phenobarbitone	40	468.00±36.00**	88.00±20.44***[c]	40	60
Flu+Vehicle	3 + 10	276.00±12.00[a]	280.00±20.00[c]	40	60
Flumazenil+*F. sur*	400	612.00±42.14*[a]	328.00±43.39	40	60
Flu+Phenobarbitone	40	360.00±25.82	572.00±83.56	100	0
Cyp+*F. sur*	400	360.80±35.91	429.00±47.71	0	100
Cyp+Phenobarbitone	40	384.30±32.23	451.20±17.10	0	100
L-NNA+*F. sur*	400	382.80±48.82	219.70±15.73	0	100

Values are expressed as Mean±SEM. Statistical significant level *p<0.05, **p<0.01, ***p<0.001 compared to control, [c]p<0.001 compared to *F. sur* 400 using one way ANOVA followed by Turkey's multiple comparison test. Flu: Flumazenil, Cyp: Cyproheptadine, LNNA: L-nitroarginine, Vehicle: 0.5% v/v dimethylsulfoxide in normal saline

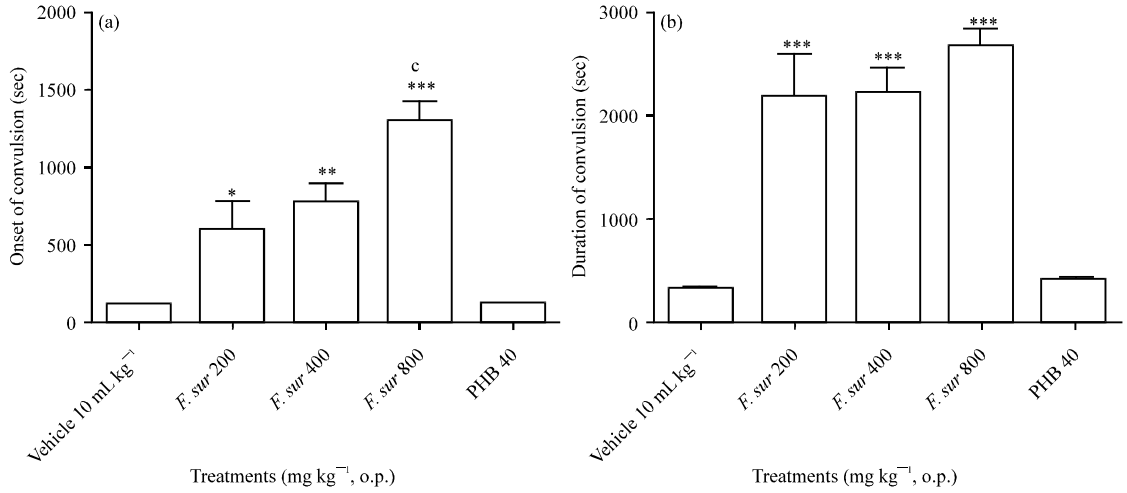

Fig. 1(a-b): Onset of seizures produced by *Ficus sur* on NMDA-induced seizures. Values are expressed in terms of Mean±S.E.M. Statistical significant Level *p<0.05, **p<0.01, ***p<0.001 compared to control., °p<0.01 versus phenobarbitone 40 mg kg^{-1}. Analyzed by one way ANOVA followed by Turkey's *post hoc* multiple comparison test. *F. sur* = ethanolic stem bark extract of *Ficus sur*; Vehicle = dimethyl sulfoxide

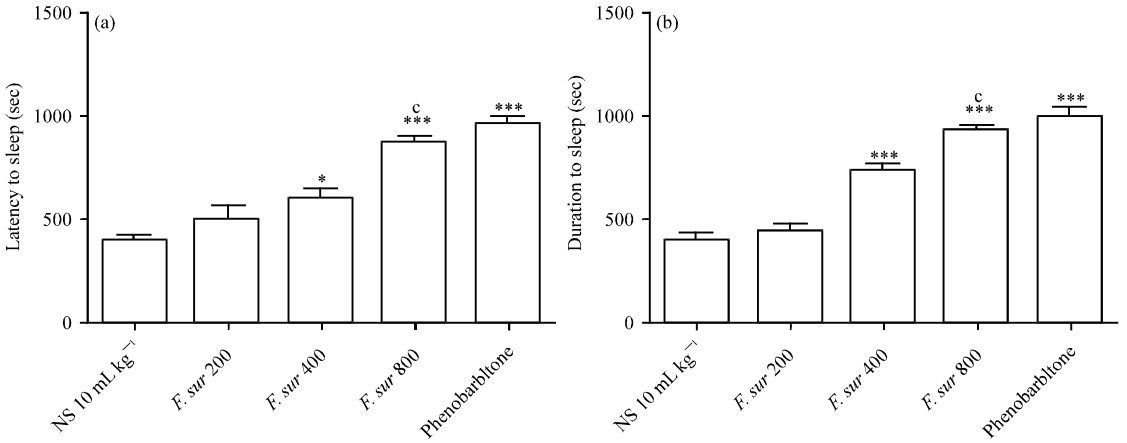

Fig. 2(a-b): Effect of *Ficus sur* on (a) Latency to sleep and (b) Duration of sleep of hexobarbitone-induced hypnosis test. Values are expressed as Mean±SEM. Statistical significant level *p<0.05, ***p<0.001 compared to control, °p<0.001 compared to *F. sur* 400. Analyzed by one way ANOVA followed by Tukey's *post hoc* multiple comparison test. *F. sur* = ethanolic stem bark extract of *Ficus sur;* Vehicle = dimethyl sulfoxide

DISCUSSION

Findings from this study showed that the ethanolic stem-bark extract of *Ficus sur* possesses anticonvulsant and hypnotic effects which were comparable to the effect of standard drug used for the management of epileptic seizures. The significant action that was shown by *F. sur* in prolonging the onset of seizure and attenuating the duration of seizure, as well as protecting against mortality, up to 100%, by different doses of the extract against the picrotoxin-induced seizure suggests that the extract might act to oppose the selective non-competitive antagonism of picrotoxin on gamma amino butyric acid (GABA), specifically at $GABA_A$ receptor, which has been widely implicated in epilepsy (Rang *et al*., 2000). GABA is the major inhibitory neurotransmitter in the brain and its inhibition is thought to be an underlying factor in epilepsy (Gale, 1992; Adeyemi *et al*., 2007).

The extract, at 200 mg kg^{-1}, produced a significant ($p<0.05$) increase in onset of convulsion that is comparable with phenobarbitone, but with 100% mortality in the strychnine-induced seizure model. Strychnine, a

potent convulsant, selectively blocks inhibitory inputs by glycine receptors, predominantly at the spinal cord, to induce excitatory responses in the CNS (Nicoll, 2001) a mechanism that might also define the action of *F. sur*.

To confirm the involvement of GABA enhancement in the anticonvulsant properties of *Ficus sur*, isoniazid-induced seizure model experiment was carried out. Isoniazid exerts its convulsive effect by inhibiting GABA synthesis (Costa *et al*., 1975). In addition, it is a potent monoamine oxidase (MAO) inhibitor and a glutamic acid decarboxylase (GAD) inhibitor (enzyme involved in GABA synthesis). Therefore, isoniazid systematically increases the brain monoamine content and inhibit GABA synthesis, respectively, r Coulsto esultantly leading to CNS excitation and convulsions (Wood and Peesker, 1973; Marcus and Coulsto, 1985). *Ficus sur* demonstrated dose-dependent and significant anticonvulsant activity of in isoniazid model of convulsion with effects comparable with phenobarbitone. This result indicate a possible enhancement of GABA synthesis as a result of a non-receptor mediated action against blockers of the GABA synthetic pathway.

Furthermore, NMDA is an agonist that produces effects similar to glutamic acid at the NMDA receptors and exerts its convulsant effect by activating the receptors to enhance excitatory glutaminergic neurotransmission (Watkins and Evans, 1981; Chapman and Meldrum, 1993). NMDA-induced seizures in rodents have previously been proposed as a model of refractory seizures and are significantly suppressed by NMDA receptor antagonists (Velisek, 2006). The effects produced by *Ficus sur* in this NMDA seizure model suggest that the extract might be inhibitory against glutaminergic excitatory responses, specifically at the NMDA receptors, thereby suggestive of its usefulness in refractory seizures, such as occurring in some forms of epilepsies.

Pentylenetetrazole is a GABA antagonist and is specifically used in seizure assays as a method of assessing the excitability of the central nervous system and GABA activity. The extract produced significant delays in onset of seizure at 200 and 800 mg kg^{-1}, effect which at 200 mg kg^{-1}, like phenobarbitone, producing 100% protection. This is a reverse effect in dose-dependency, where the lower dose is that prolonging onset as well as causing maximum protection and is an indication that *Ficus sur* is most potent and possibly efficacious against Pentylenetetrazole-induced seizure; the effectiveness of a drug against pentylenetetrazole seizures indicates its probable effectiveness against *Absence* epilepsy (McNamara, 2006). This suggests that the active ingredient(s) in the extract might promote possible future anti-Absence seizure activity.

To further investigate possible involvement of GABAergic pathway in the mechamisms of action of *Ficus sur*, flumazenil (GABA receptor antagonist) was used. The influence of flumazenil in this study is that it acts by competitively inhibiting the activity at the benzodiazepine recognition site on the GABA/benzodiazepine receptor complex. From the results, flumazenil antagonized the effect of *Ficus sur* significantly when compared to both vehicle control and phenobarbitone. Similarly, flumazenil antagonized the effect of phenobarbitone by significantly reducing the onset of convulsion, producing 100% protection. Moreover, cyproheptadine (5-HT_2 receptor antagonist) antagonized the effect of *Ficus sur* and phenobarbitone by significantly reducing their onset and duration of convulsion and consequently causing 0% protection from seizure. This is an indication of possible serotonergic, $5HT_2$ receptor antagonism by the extract.

Finally, *L*-nitroarginine antagonized the effect of *Ficus sur* by significantly reducing the onset and duration of convulsion, with resultant 0% seizure protection as in the serotonergic case; an action suggesting a NMDA-glutamate receptor antagonism by the extract, thus confirming its inhibitory effect in NMDA-induced seizure.

CONCLUSION

Due to the challenges and difficulties in the management of convulsion which is as a result of side effects associated with the use of synthetic anticonvulsant drugs, it is important to encourage the practice of traditional medicine which employs the use of natural plants. The extract of *Ficus sur* shows prospects as a future anticonvulsant activity in the experimental animal models used. The findings in this study have shown that the stem-bark extract of *Ficus sur* possessed anticonvulsant activity which confirmed its folkloric uses in traditional African medicine for even refractory-type epilepsies; and with possible mechanisms well suggested to involve GABAergic, glycinergic, anti-Glutaminergic as well serotonergic receptors and/or pathways.

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