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EVALUATION OF ANTICONVULSANT ACTIVITY OF ETHANOL LEAF EXTRACT OF *FICUS SYCOMORUS* LINN IN PENTYLENETETRAZOLE AND STRYCHNINE INDUCED SEIZURES IN MICE

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ABSTRACT

Epilepsy is a chronic and often progressive disorder characterized by the occurrence of epileptic seizures, affecting about 50 million people worldwide. The prescribed synthetic drugs for the treatment of epilepsy are associated with severe side effects and addiction liabilities upon long term uses. Thus, researchers around the globe are searching for newer, effective and safer drugs from natural resources. *Ficus sycomorus* Linn. (Moraceae) is used traditionally in Northern Nigeria for the management of epilepsy without any scientific validation. The aim of this study is to evaluate the phytochemical constituents and the anticonvulsant activity of the ethanol leaf extract of *F. sycomorus* L. in mice. The basic phytochemical screening was carried out based on some standard procedures, while Lorke's method was used to determine the lethal dose that kills 50% of laboratory animals. Pentylenetetrazole and strychnine were used to induce seizures in mice for the anticonvulsant screening.The plant extract revealed the presence of tannins, saponins, carbohydrates, alkaloids, flavonoids, steroids, terpenoids and cardiac glycosides. The intraperitoneal LD_{50} in mice was estimated to be >2900 mg/kg body weight. The plant extract at the highest tested dose protected the animals from death against the chemically induced seizures by pentylenetetrazole and strychnine while the time for onset of convulsion was dose dependent. An extremely significant difference ($p<0.05$) in the mean onset of convulsion was observed at highest dose of the extract against PTZ and strychnine when compared to the control and the mice were protected from death by the extract. However, the extract showed better activity against strychnine induced seizures. The data suggest that the ethanol leaf extract of *F. sycomorus*, at the tested doses and under the experimental conditions reported, contains many phytochemical constituents that may be responsible for the anticonvulsant activity. Thus, justifying the ethnomedical use of this plant extract in the management of epilepsy as claimed by the traditional medicine practitioners.

Keywords: Anticonvulsant, Ethanol, Leaves, *Ficus sycomorus*, Pentylenetetrazole, Strychnine

INTRODUCTION

Epilepsy is a chronic disorder characterized by the periodic and unpredictable occurrence of epileptic seizures, which are caused by an abnormal discharge of cerebral neurons. Many different types of seizures can be identified because of their clinical phenomena (Löscher, 1998). It is one of the most common diseases of the brain, affecting at least 50 million persons globally (Scheuer and Pedley, 1990). Seizures are fundamentally divided into two major groups: partial and generalized. Partial (focal, local) seizures are those in which clinical or electrographic evidence exists to suggest that the attacks have a localized onset in the brain, usually in a portion of one hemisphere, while generalized seizures are those in which evidence for a localized onset is lacking (CCTILE, 2003).

The practice of using plants to treat and prevent diseases started since prehistoric times and flourishes today as the primary form of medicine especially on the African continent. Humankind for millennia has used medicinal plants, and their use is as old as humanity itself. The range of species used and their scope for healing is vast (Butler, 2004). It was reported by the World Health Organization (WHO) that over 80% of the world's population depend mainly on plants and plant extracts for health care (WHO, 2002). It has been estimated that over 250,000 higher plant species occur on earth, more than 80,000 species are reported to have at least some medicinal values, and around 5,000 species have specific therapeutic value (Joy *et al*., 1998). One of such plants that holds great therapeutic potential is *Ficus sycomorus* (*F. sycomorus*). The plant belongs to the family Moraceae. It is commonly known among the Hausa people of Northern Nigeria as Farin Baure. The roots, bark and leaves of this plant are traditionally used for the treatment of epilepsy, diarrhoea, dysentery, painful urination and vaginal infections (Abubakar *et al*., 2015).

The *in vitro* antimicrobial screening of the methanol root bark extract of *F. sycomorus* revealed that the extract exhibited varying activity against *Enterococcus faecalis, Escherichia coli, Salmonella typhi, Shigella dysenteriae and Candida albicans* (Abubakar *et al*., 2015), while its leaves possess antidiabetic, anti-oxidant (70% methanol extract), antitumor and antibacterial activities (Mousa, 1994). Also, aqueous extract of stem bark exhibits sedative and muscular activities (Sandabe *et al*., 2003; 2006).

The current therapy of epilepsy with modern anti-epileptic drugs (AEDs) is associated with dose-related side effects, chronic toxicity and approximately 30% of the patients continue to have seizures with current antiepileptic drug therapy. Therefore, there is a need for new AEDs (especially from plants) with greater efficacy and novel mechanisms of action to serve as alternative therapy for the treatment of resistant epilepsy.

MATERIALS AND METHODS

Plant material

The plant was collected in Maiduguri City, Borno State, Nigeria. It was identified and authenticated by a Taxonomist, Prof. S.S Sanusi of the Biological Sciences Department, University of Maiduguri

Preparation of Extract

The leaves of *F. sycomorus* were carefully plugged from the tree. It was then air-dried and ground into powder using mortar and pestle. The powdered plant material was weighed accurately (500 g) and was macerated with 500 mL of 95% ethanol in a glass container with intermittent shaking for 48 hours. The filtrate was concentrated using rotary evaporator and was dried over a water bath at 45°C.

Drugs

Valproic acid, pentylenetetrazole, strychnine, DMSO, and 95% ethanol which were all obtained from the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri.

Animals

Swiss albino mice of both sexes, weighing 18-25 g were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Gombe State University, Gombe state, Nigeria. They were housed under standard conditions of temperature (25 ± 2^{o}C), light/dark cycles, fed with standard diet (Growers Mesh), and given water *ad libitum*. All experiments performed in this work were carried out in accordance with the National Institute of Health guide for the care and use of laboratory animals.

Preliminary phytochemical screening

The leaf extract of *Ficus sycomorus* were prepared in suitable forms for the screening of alkaloids, anthraquinones, tannins, saponins, cardiac and cyanogenetic glycosides, flavonoids, and carbohydrates using the standard laboratory procedures described by Harbone (1991), Trease & Evans (1989, 2002) and Sofowora (1993, 2008).

Acute toxicity tests of ethanol leave extract of *F. sycomorus*

Acute toxicity study for ethanol extract of *F. sycomorus* leaves was carried out by using the modified Lorke's method (1983). The study was conducted in two phases. In the first phase three groups of rats were administered the extract at respective oral doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg. The animals were observed for signs of toxicity and possible mortality for 24 hours. In the second phase, another two groups of three rats each were administered doses of 1600 mg/kg and 2900 mg/kg respectively. They were also observed for signs of toxicity and mortality, the LD_{50} will then be determined by the square root of the dose that killed and the dose that did not kill.

Experimental groups

Acute convulsive groups (pilot study)

The first of two groups of (n=3) mice each were treated with a single convulsive dose of Pentylenetetrazole (PTZ) 25, 50 and 75 mg/kg, (i.p). Only the dose of 50 mg/kg and 75 mg/kg initiated an onset of convulsion with sudden death respectively. Similarly the second group (n=3) were given with strychnine 2.5, 5, and 7.5 mg/kg without valproic acid. The onset of convulsions and mortality was only seen with 5 and 7.5 mg/kg strychnine respectively. Hence, only doses of 50 mg/kg PTZ and 5 mg/kg strychnine were used to induce convulsion in this experiment.

Kindling groups

Pentylenetetrazole induced-seizure test in mice

The method of Swinyard *et al* (1952) was employed to induce convulsion using PTZ in mice. Twenty-five mice were randomly divided into five groups of five mice (n=5) each weighing between 18-25 g. The mice in the first group received 10 ml/kg normal saline intraperitoneally, while the mice in the second group received 200 mg/kg Valproic acid intraperitoneally as a standard positive control. The third, fourth, and fifth groups were administered doses of 250, 500 and 1000 mg/kg of the ethanol extracts of *F. sycomorus* respectively. Thirty minutes later, mice in all the groups were administered with freshly prepared solution of PTZ at a dose of 50 mg/kg body weight (i.p). The mice were observed for 30 min for the onset and incidence of seizures. An episode of tonic extension of the hind limbs or clonic spasm, which persisted for a minimum of 30 s, was considered as a threshold convulsion. Lack of threshold convulsion during 30 min of observation was considered as protection.

Strychnine induced-seizure test in mice

The same protocol was carried out as in the PTZ-induced seizures, twenty-five mice were also randomly divided into five groups of five mice (n=5) each weighing between 18-25 g. The mice in the first group received 10 ml/kg normal saline i.p. while the mice in the second group received 5 mg/kg strychnine. The third, fourth, and fifth groups were given i.p. doses of 250, 500 and 1000 mg/kg of the ethanol extract of *F. sycomorus* respectively. Thirty minutes later, mice in all the groups were administered with freshly prepared solution of PTZ at a dose of 50 mg/kg body weight i.p. The mice were observed for 30 minutes for the onset and incidence of seizures.

Statistical analysis

Results were expressed as mean ± standard error of the mean (S.E.M) and analyzed using Computer software GraphPadInStat® @ USA; 2003. The significant difference between mean was determined using student T-test. Values of $p<0.05$ were considered significant.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical constituents identified in the ethanol leaf extract of *F.*

sycomorus include: carbohydrates, saponins, cardiac glycosides, cyanogenetic glycosides, flavonoids, tannins and alkaloids. However, the Borntrager test showed a negative result which depicts the absence of anthraquinones (Table 1).

Table 1: Phytochemistry of ethanol leaf extract of *F. sycomorus* L

Phytoconstituents	**Results**
Alkaloids	
Dragendroff's reagent	+
Mayer's reagent	+
Carbohydrates	
Molish test	+
Fehling's test	+
Barfoed test for monosaccharide	+
Flavonoids	
Shinodas test	+
Ferric chloride test	+
Tannins	
Ferric chloride test	+
Cardiac glycosides	
Buchard test for glycosides	+
Salkwoski test for steroidal nucleus	+
Keller kilani test	+
Saponin glycosides	
Frothing test	+
Steroids and triterpenoids test	+
Cyanogenetic glycosides	
Guignard test	+
Anthraquinones	
Borntragers test	-

+ = present, - = absent

Acute toxicity

The acute toxicity of the ethanol leaf extract of *F. sycomorus* in mice was found to be greater than 2900 mg/kg intraperitoneally. However, neither neurobehavioral effects nor death were observed (Table 2).

Table 2: Acute toxicity study of ethanol leaf extract of *F. sycomorus* L

Phase	**Dose(mg/kg)**	**Observation**
Phase1	10	-
	100	-
	1000	-
Phase 2	1600	-
	2900	-
	5000	X
LD_{50} = >2900 mg/kg		

-= no death, x = not administered

Effect of ethanol leaf extract of *F. sycomorus* in pentylenetetrazole induced convulsion in mice

The ethanol leaf extract of *F. sycomorus* was able to increase onset of convulsion, and increase percentage protection from death. The activity of *F. sycomorus* ethanol leaf extract in pentylenetetrazole induced convulsion was found to be dose dependent. The anticonvulsant activity of the extract at 1000 mg/kg was found to be statistically significantly higher than the control ($p<0.001$), and protected 100% of the animals from death. Furthermore, Valproic acid used as positive control also protected 100% of the animals (mice) from death. In contrast, the extract at 250 mg/kg and 500 mg/kg were not found to be statistically significantly higher than the control and could protect only 20% and 60% of the mice respectively (Table 3).

Table 3: Effects of different doses of ethanol leaf extract of *F. sycomorus* L on pentylenetetrazole induced convulsion in mice

Treatment	Mean onset of clonic convulsion (mins)	Percentage protection from death (%)
NS (10 ml/kg)	4.00±0.45	0
VA (200 mg/kg)	-	100
FSE (250 mg/kg)	4.20±0.58	20
FSE (500 mg/kg)	6.20±1.24	60
FSE (1000 mg/kg)	11.40±1.03***	100

NS = Normal saline, VA = Valproic acid, FSE = *Ficus sycomorus* extract, * = $p<0.05$ (significant), ** = $p<0.01$ (highly significant), *** = $p<0.001$ (extremely significant), $p>0.05$ = not significant

Effect of ethanol leaf extract of *F. sycomorus* L in strychnine induced convulsion in mice

The ethanol leaf extract of *F. sycomorus* was also able to increase onset of convulsion and also increase protection from death. In strychnine induced convulsion, the activity of the ethanol leaf extract of *F. sycomorus* was also dose dependent. The tested doses of the extract at 500 mg/kg and 1000 mg/kg were found to be statistically significantly higher than the control ($p<0.05$ and $p<0.001$ respectively). Valproic acid used as positive control also protected 100% of the mice. However, the tested dose of the extract at 250 mg/kg was found to be not statistically significantly higher than the control and protected 60% of the mice (Table 4).

Table 4: Effects of different doses of ethanol leaf extract of *F. sycomorus* L. on strychnine induced convulsion in mice

Treatment	Mean onset of clonic convulsion(mins)	Percentage protection from death (%)
NS (2mg/kg)	6.40±0.51	20

VA (200mg/kg)	-	100
FSE (250mg/kg)	7.20±0.73	60
FSE (500mg/kg)	8.40±0.51*	80
FSE (1000mg/kg)	19.40±1.63***	100

NS = Normal saline, VA = Valproic acid, FSE = *Ficus sycomorus* extract, * = $p<0.05$(significant), ** = $p<0.01$(highly significant), *** = $p<0.001$(extremely significant), $p>0.05$ = not significant

DISCUSSION

The different phytochemical constituents identified in the ethanol leaf extract of *F. sycomorus* are considered as important biological active compounds of plant origin. That may be responsible for the anticonvulsant activity of the plant extract. This study is in agreement with the study on anticonvulsant activity reported by Abubakar and his colleagues (2015) where similar phytoconstituents were obtained.

Rang et al (2001) reported that acute toxicity test gives a clue on the range of doses that could be toxic to the animals, and can also be used to estimate the therapeutic doses of drugs and xenobiotics The acute toxicity study of the ethanol leaf extract of *F. sycomorus* showed that the plant is relatively less toxic at the doses <2900 mg/kg. However, this study disagrees with the report of Abubakar *et al* (2015) where the methanol root bark extract caused toxicity such as loss of appetite, restlessness and generalized weakness. The reason for the disagreement may be due to the differences in the solvent systems or in the parts of the plant used. In the present study leaves were used instead of the root bark used by Abubakar and his colleagues (2015).

PTZ is a well-known convulsant, and the chemically induced seizure using PTZ test usually identifies compounds that raise seizure threshold in the brain. PTZ has been shown to interact with GABA neurotransmitter and $GABA_A$ receptor complex (De Deyn *et al*., 1992). The ethanol leaf extract of *F. sycomorus* did not protect the animals against the chemically induced convulsion of PTZ, but increases the latency time to onset of seizure. This finding indicates that the extract may not contain compounds that can raise seizure threshold in the brain as previously reported by White *et al* (1998). Furthermore, the highest tested dose of the leaf extract of *F. sycomorus* conferred 100% protection from death to the animals treated with a convulsive dose of PTZ. The ability of the extract to increase the latency time to onset of a seizure in the PTZ test suggested a possible interaction of the extract with GABAnergic neurotransmission. This finding is in contrast to a similar test conducted on *F. abutilifolia*, which failed to show anticonvulsant activity against PTZ (Danmalam *et al*., 2012), and this may be due to differences in chemical constituents between the two *Ficus* species.

The effects of ethanol leaf extract of *F. sycomorus* on strychnine induced seizures in

mice was found to increase the latency time of onset of seizures and reduced number of convulsions and also protected the mice from death in a dose-dependent mannaer. This suggested possible interaction with glycine receptors. However, this study is in contrast with the study of anticonvulsant activity of aqueous stem bark of *F. sycomorus* by Sandabe *et al* (2003) where the rats used were not protected from death. This may likely be due to the difference in plant part (in which the constituents may differ) or the difference in solvent system used. When compared with the PTZ experimental study, the extract was found to have more activity on the glycine receptors than the GABA receptors. This may suggest that *F. sycomorus* may be acting on glycine receptors. However, more experimental studies are encouraged in order to determine the exact mechanism of action of the plant extract.

CONCLUSION

The result of this study shows that leaf extract of *F. sycomorus* is relatively safe and contains significant phytochemical constituents which may be responsible for the anticonvulsant activity and thereby supports its traditional usage by rural dwellers and Traditional Medicine Practitioners in Northern Nigeria. Further investigation should be done to elucidate the main mechanism of action of ethanol extract of *F. sycomorus*. More research should be done so as to isolate and characterized the specific compound(s) responsible for the observed activity. The Government should encourage cultivation of the plant and its subsequent integration in to orthodox medicine that may be affordable for people in local communities.

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