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Anticonvulsant Activity of Methanol Extract of *Harungana madagascariensis* Leaf on Mice Model of Isoniazid-induced Siezure

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Authors' contributions

This work was carried out in collaboration among all authors. Authors UOC and NSN designed the study, performed the statistical analysis and wrote the protocol. Author AIJ wrote the first draft of the manuscript. Authors FKA and ORM managed the analyses of the study. Author UOC managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Epilepsy is a neurological illness that affects people of all ages and is characterized by excessive electric activity in the brain. This causes social embarrassment coupled with side effect of orthodox medication; hence, the needs to search for plant with antiepileptic agent.
Aims: This study aimed at investigating the anticonvulsive effects of Methanol extract of *Harungana madagascriensis* leaves on the isoniazid-induced (300 mg/kg, i.p) seizure in adult mice.
Study Design: This is an original research carried out in the Department of Pharmacology, Faculty of pharmaceutical Sciences, Enugu State University of Science and Technology (ESUT), Agbani, Enugu State, Nigeria, between Jan and June, 2021.
Methodology: The pulverized leaf of *Harungana. madagascariensis* was extracted using cold maceration and the phytochemical screening was carried out by the method of Treas and Evans The acute toxicity study was evaluated by the method of Lorke's and Anticonvulsant study was

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carried by the method of Webster and Velluci. Data generated was statistically analyzed using one way ANOVA.
Results: Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, phenols, glycosides, saponins, terpenoids and steroids. Flavonoids, Phenols, and terpenoids appeared in abundant concentration (4264.00 ±360.2,14065.00 ±538.4, 5484.00±30.4). Acute toxicity tests showed no toxicity and mortality at doses up to 5000 $mgkg^{-1}$. Anticonvulsant study revealed that the extract significantly (p =.05) delayed the onset of clonic seizure in a dose dependent manner and abridge the duration of siezure on the group treated with 100, 500 and 1000 mg/kg b.w of extract compared with normal control when induced with isoniazid (300 mg/kg, i.p.).
Conclusion: The results suggest that methanol extract of *Harungana. madagascariensis* leaves may have anticonvulsant activity, coupled with the presence of active secondary metabolites such as saponins, steriods and flavoniods which has a potentials for the management of epilepsy.

Keywords: Harungana madagascariensis; anticonvulsant; seizure; methanolic extract.

1. INTRODUCTION

According to the World Health Organization (WHO), epilepsy is a neurological illness that affects people of all ages and is characterized by excessive or abnormal electric activity in either all or part of the brain. Seizures that might occur repeatedly and without warning are referred to as the external symptoms of epilepsy. Numerous illnesses, including a stroke, brain tumor, a head injury, or an infection of the central nervous system, may be to blame for the seizures [1]. According to estimates, fifty million people worldwide today suffer with epilepsy, which accounts for 1% of the world's illness burden [2], especially in low- and middle-income countries [3]. Pharmacovigilance is one of the most important clinical concerns in the management of the condition, despite the wide range of pharmacological medicines that have been approved for use in patients with epilepsy. Many people are still nonresponsive or refractory to antiepileptic medication therapy. Additionally, according to Clossen and Reddy [4], these medications merely treat the symptoms of epilepsy and do not effectively halt seizures or prevent them from happening in the first place. It is necessary to create antiepileptic medicines (AEDs) that are more effective, safer, and have improved clinical characteristics. Status epilepticus (SE), also known as an isoniazid-induced seizure, is a serious disorder marked by frequent convulsive episodes that does not respond well to the currently available anticonvulsant drugs [5]. Status Epilepticus (SE) is one of the major side effects of isoniazid, a first line medication used for the treatment of tuberculosis. Isoniazid causes persistent seizures by inhibiting glutamate decarboxylase, an enzyme that regulates the production of GABA, a substance that slows down the rate at which brain nerve cells fire. Repeated convulsions, which frequently result in the creation of poisonous chemicals that harm the brain cells, are the primary symptom of SE in individuals who have consumed too much isoniazid. Although intravenous diazepam is still used to control seizure episodes in the absence of pyridoxine, it is known that isoniazid-induced seizures do not respond well to the anticonvulsant medications currently on the market [6-7]. On this note, diazepam serving as reference drugs, was compared with the current study test substance. The use of animal models has made vital contributions to our understanding of seizures [8]. *Harungana madagascariensis* Lam. ex Poir, of the Guttiferae family, is commonly known as haronga, orange-milk or dragon‟s blood tree. It is an evergreen shrub or tree with a much branched, heavy, spreading canopy, approximately 12 meters tall, with occasional specimens up to 27 meters. The bole is straight and cylindrical [9]. It is a multipurpose tree and particularly valued for its multiple medicinal uses and as a dye.

Harungana madagascariensis, a traditional herbal remedy is acclaimed to possess antioxidant and anticonvulsant properties. The preliminary phytochemical analysis has reported active constituent. This might be a better prognosis in the treatment of seizure in alternative /complementary medicine and provide raw source of new effective pharmacotherapy for convulsion. Biological claims include antimicrobial activity (leaf extract) [10], antibacterial [11], antioxidant [12] as well as antiplasmodial and antitrichomonal [13] activities. Therefore, in light of its biological activities on animal models, this study aims at evaluating its anticonvulsant activities in Isoniazid-induced seizure.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Leaves of *Harugana madagasccariensis* were used for this study. The leaves were collected from Oba in Nsukka Local Government Area in Enugu state, and were identified by Mr. Alfred Ozioko of Bioresource Development and Conservation Programme (BDCP) research centre, Nsukka, Enugu State.

2.1.1 Preparation of plant material

The leaves of *Harungana madagascariensis* were air dried and pulverized to coarse powder. Pulverized leaves (1000 g) were macerated in 5 liters of methanol for 72 hours at room temperature with constant agitation. The suspension was filtered with muslin cloth, followed by Whatman No. 1 filter paper. The filtrate was concentrated at 40^{oC} using a water bath. Then, the extract was evaporated to be in slurry form. The extract was then transferred into an air tight container and stored at $4^{o}+2^{oc}$ in a refrigerator until needed.

2.1.2 Study animals

Twenty four (24) adult male Albino rats of weight (19-30 g) were used for the anti-convulsant studies and eighteen adult male Albino mice of weight (16-26 g) were used for the median lethal dose (LD_{50}) study. All the animals were obtained from the animal house of the Department of Pharmacology, Enugu state University of science and Technology Agbani. The rats were fed with water and standard Grower's mash rat pellets (Grand Cereals Nig. Ltd, Enugu).

2.1.3 Instruments/ Equipment

Beakers (Pyrex), Conical flask (Pyrex), Filter paper, (Whatman), Refrigerator(Haier thermocool, England), Spatula (Pyrex), Syringe (Life Scan), Mechanical grinder(Vickas Ltd, England), Water bath (Gallenkamp, London), Weighing balance (Metler HAS), Rotary evaporator (Vickas Ltd, England).

2.2 Quantitative and Qualitative Phytochemical Screening

The phytochemical constituents were screened according to standard method described by Trease and Evans [14] and Harbone [15].

2.2.1 Acute toxicity test

The acute toxicity test of methanol extract of *Harungana madagascariensis* was conducted in accordance with the method of Lorke [16]. The study was conducted in two phases using a total of 18 mice. In the first phase, nine mice were divided into 3 groups of 3 mice each. Groups 1, 2, 3 animals were treated with 10, 100 and 1000 mg/kg body weight (b.w) of the extract respectively. Clinical signs of toxic effect and mortality were observed within 24 hrs. In the second phase, 9 mice were divided into 3 groups of 3 mice each. Three groups of three (3) mice each were treated with 1600, 2900, and 5000 mg/kg b.w of the extract respectively. The extract was dissolved in tween 80 and the route of administrate was oral (p.o).

2.2.2 Study design: A preventive study

Twenty five (25) adult male mice, which had previously been fed standard Pfizer diet and allowed free access to water, were used. Rats were fasted for 18 hours with free access to water were randomly divided into five (5) groups of five (5) rats each were treated as follow:

Group I: Served as normal control and mice in this group received 0.25 ml of 10% Tween 80.
Group II: Served as positive control and mice in this group received 0.5 $mgkg^{-1}$ (diazepam).
Group III: Received 100 $mgkg^{-1}$b.w of methanolic extract of *H. madagascariensis* leaves.
Group IV: Received 500 $mgkg^{-1}$ b.w of methanolic extract of *H. madagascariensis* leaves.
Group V: Received 1000 $mgkg^{-1}$ b.w of methanolic extract of *H. madagascariensis* leaves.

2.2.3 Anticonvulsant study

The chemical induced method described by Velluci and Webster [17] were used for this study. The 25 adult male mice were randomized into five(5) groups of five animals each. The normal control group (I) received equivalent amount of vehicle (10% Tween 80, i.p.) and the standard control group (II) received diazepam (0.5 $mgkg^{-1}$, i.p.). Isoniazid (300 $mgkg^{-1}$) was used to induce seizures in all groups 30 mins

after pretreatment. Groups III, IV and V were pretreated with doses of the extract: 100 mgkg-^{1}, 500, mgkg^{-1} 1000 mgkg^{-1}b.w intraperitoneally (i.p.). Latency to convulsion, animals that convulse and duration of seizure across the groups were observed over a period of 2 hours and recorded. Animals that did not convulse within the stipulated 2 hours were recorded as "no convulsion".

2.2.4 Statistical analysis

All results obtained were expressed as mean ± SEM. Data obtained were analyzed by One-way analysis of variance (ANOVA) and was subjected to Turkey Post-hoc test using Graph Pad Prism Version 8.3 (GraphPad Software, San Diego, CA, USA) for multiple comparisons. Differences between means were considered significant at P =.05.

3. RESULTS

3.1 Percentage Yield of Crude Extract

The percentage yield of the crude extract was obtained to be appreciably 28.70%.

$$\%Yield = \frac{weight\ of\ crude\ extract}{wieght\ of\ pulverized\ coarse\ powder\ soaked} \times \frac{100}{1}$$

3.2 Phytochemical Screening

The results of the phytochemical screening from Table 1 indicated the presence of flavonoids, alkaloids, phenols, glycosides, saponins, terpenoids and steroids. Flavonoids, Phenols, and terpenoids appeared in abundant concentration (4264.00 ±360.2, 14065.00 ±538.4, 5484.00±30.4), the alkaloids, tannins and steroids appeared in moderate concentration (642.00±215.7, 867.70±8.6, 96.77±3.7 whereas saponins, glycosides appeared in low concentration (39.67±0.6, 12.20±0.4) respectively.

3.3 Acute Toxicity Studies

The results of acute toxicity test showed that the Methanol extract of *H. madagascariensis* leaves exhibited no toxic effect and there was no sign of behavioral changes up to a dose of 5000 mgkg^{-1} body weight.

The result showed from Table 3 reveled that group treated with the methanol leaf extract of *Harungana madagascariensis* cause a significant (P =.05) delay in latency of clonic convulsion and abridge duration of seizure on the group treated with 100,500 and 1000mg/kg b.w of extract compared with normal control. Group treated with standard drug (diazepam) offered a better protection of 60% than group treated with extract with 0%.

Table 1. Quantitative and Qualitative phytochemical screening

Phytoconstituents	Concentrations (mg/100g)	Qualitative remarks
Tannins	867.76±86	+
Total phenolics	4065.00±538.8	+
Alkaloids	264.20±215.7	+
Flavonoids	4264.00±360.2	+
Glycosides	12.20±0.4	+
Saponnins	39.67±0.6	+
Terpenoids	5484.00±30.4	+
Steroids	96.77±3.7	+

Key: += present

Table 2. LD_{50} of methanolic extract of *H. madagascariensis* leaves

Groups	Dose of extract (mg/kg. b.w.)	Mortality	Behavioral changes
Phase 1			
Group 1	10	0/3	Nil
Group 2	100	0/3	Nil
Group 3	1000	0/3	Nil
Phase II			
Group 1	1600	0/3	Nil
Group 2	2900	0/3	Nil
Group 3	5000	0/3	Nil

Table 3. Effects of MEHML on latency to siezure on isoniazid-induced convulsion in mice

Dose (mg kg^{-1})			No. of animals convulsed/ No. used	Animals protected against siezure(%)	Latency of clonic convulsion (min) Mean ± S.D.	Duration of siezure (sec) Mean ± S.D.
Tween 80	MEHML	DZP				
0.25 ml	-	-	5/5	0.00	8.10 ± 1.24**	45.64 ± 0.99**
-	100.00	-	5/5	0.00	20.00 ± 0.71**	32.09 ± 0.8481***
-	500.00	-	5/5	0.00	23.60 ± 0.89***	27.50 ± 0.9721***
-	1000.00	-	5/5	0.00	26.20 ± 1.10***	22.46 ± 0.5921***
-	-	0.50	2/5	60	44.20 ± 6.64***	16.40 ± 0.9282***

*Values represent the mean ± S.D. *P < 0.05, **P < 0.01, ***P < .001 compared to control (10% Tween 80).*
** Indicates multiple comparisons with control group.*
***Key:** MEHL = Methanolic extract of Harungana madagascriensis leaves, DZP = Diazepam*

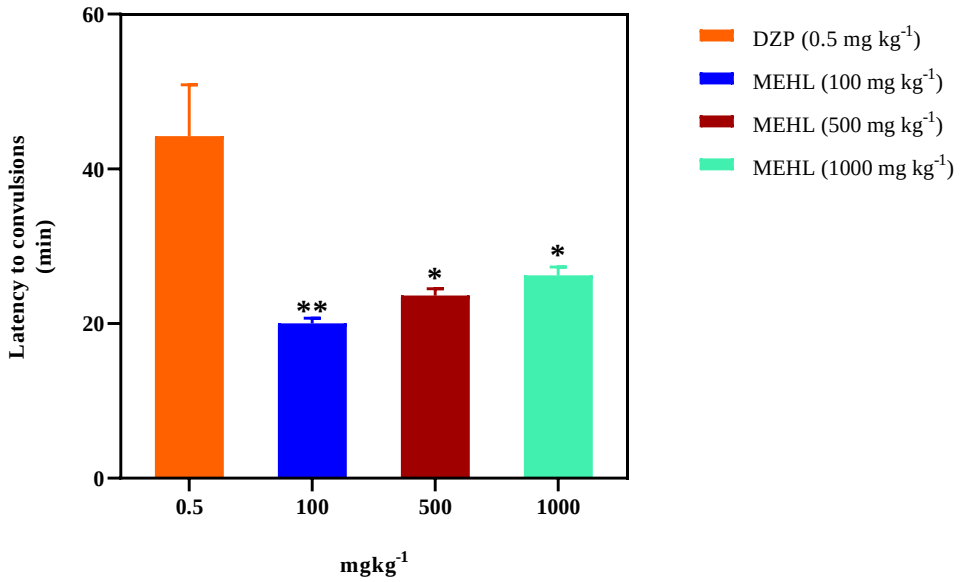

Fig. 1. Effect of MEHL and DZP on Latency to convulsion

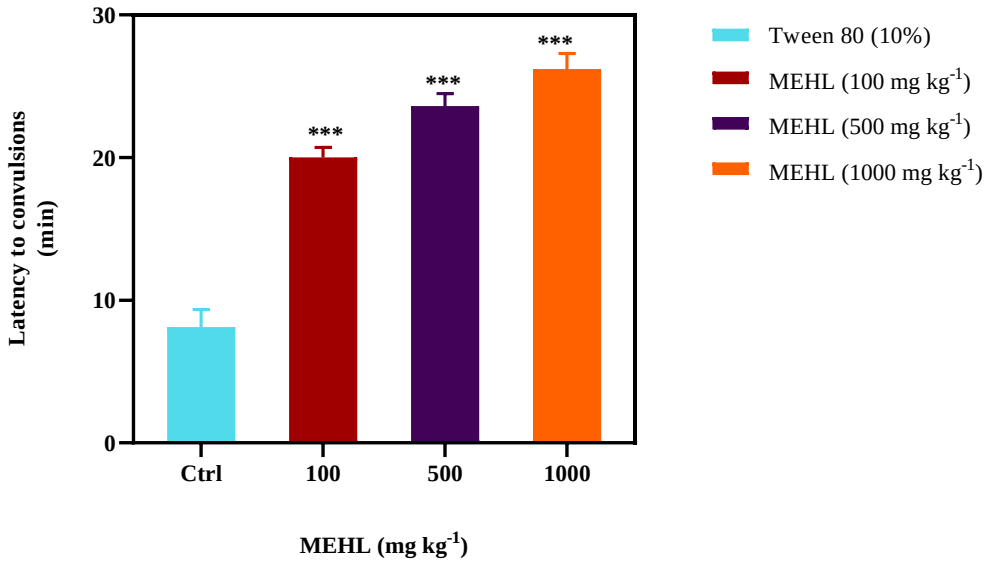

Fig. 2. Effect of MEHL on Latency to convulsion

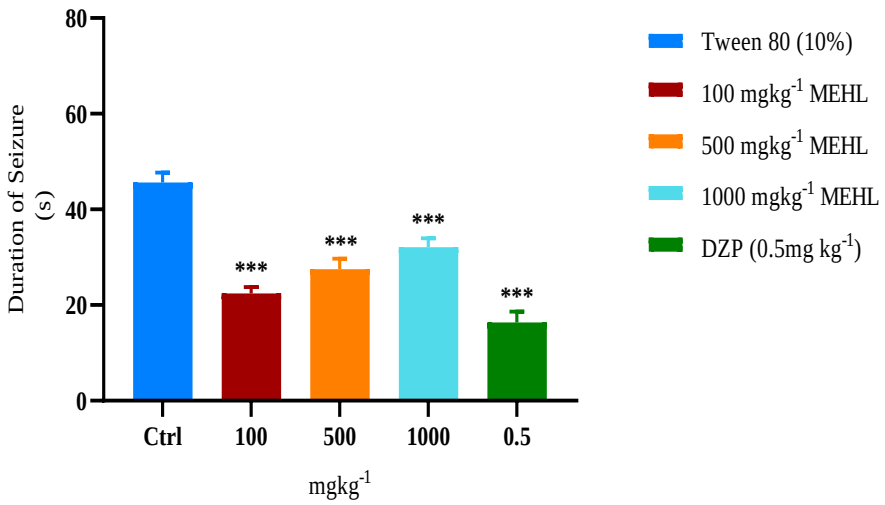

Fig. 3. Effect of extract and standard drug (Diazepam) on duration of seizure

4. DISCUSSION

Medicinal value of plants lie in the presence of phytochemicals that have a definite physiological action on the human body. Our Findings from the qualitative and quantitative phytochemical screening of the extract revealed presence of flavonoid, phenols, saponins, steroids, tannins, terpenoids and alkaloid as shown in Table 1. However, flavonoid, phenols and terpenoids are richly presence in varying proportions as shown in Table 1. This findings were in accordance with previous studies on the plant [18-19]. Moreover, previous report stated that saponins, alkaloids, and steroids, in the plant, may be responsible for the anticonvulsant effect of the plant [20]. The results of the acute toxicity study indicated that the extract is safe even at high dose of 5000mg/kg body weight as shown in Table 2. The anticonvulsant study indicated that methanol extract of *Harungana madagascariensis* leaves significantly (p = 0.5) delay the onset of clonic convulsion and abridge the duration of siezure in a dose dependent manner. The standard drug at dose of 0.5mg/kg body weight offered a better protection against seizure when compared to the extract at doses of 100, 500 and 1000mg/kg body weight. The treated groups offered better delay seizure when compared to normal control as shown in Table 3. The plant extract demonstrated inhibition of neurotransmitter associated with seizure. GABA is the major inhibitory neurotransmitter in the brain while glutamate is an excitatory neurotransmitter in the brain. The inhibition of GABA neurotransmitter and the enhancement of the action of glutamic acid have been shown to be the underlying factors in epilepsy [21]. Status epilepticus by isoniazid is related to the inhibition of glutamate decarboxylase (GAD), an enzyme required for GABA synthesis [22-23]. Again, decreased levels of GABA in the brain are associated with ongoing seizures seen in animals exposed to high amounts of isoniazid [24]. Therefore, GABA shortage might manifest as seizure, particularly in the setting of acute poisoning. Only at its maximum dose did diazepam reduce the delay to death. The primary limitations of DZP's clinical efficacy in the treatment of isoniazid toxicity include the variability and quick availability of the amount of isoniazid (INH) consumed [25-26]. Once more, earlier research showed that the effectiveness of anticonvulsant medications depends more on their capacity to prolong the latency of seizures than on their ability to prevent convulsions [27].

5. CONCLUSION

The methanol extract of *H. madagascariensis* indicated phytochemical constituent which may be responsible for the observed anticonvulsant activity via non-specific mechanisms. The plant extract is safe validating the use of the plant in traditional medicine. However, extensive studies are needed to isolate and characterize bio-active anticonvulsant agent, evaluate the mechanism(s) of action and the safety profile of the plant in the management and treatment of convulsive disorders.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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