**ORIGINAL PAPER**

https://dx.doi.org/10.4314/njpr.v17i1.7

**Nig. J. Pharm. Res. 2021, 17 (1) pp 63-70**
**ISSN 0189-8434** **e-ISSN 2635-3555** **Available online at http://www.nigjpharmres.com**

# Toxicity Study and Anticonvulsant Effect of Ethanol Leaf Extract of *Piliostigma thonningii* Milne-Redhead (Fabaceae)

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

## Abstract

**Background:** *Piliostigma thonningii* (Schumach) Milne-Redhead [Fabaceae] is a plant widely used locally for the treatment and management of several ailments which include epilepsy in Northeastern Nigeria.
**Objectives:** This study aimed at evaluation of the toxicity and anticonvulsant effect of ethanol leaf extract of *Piliostigma thonningii* in rats and mice with a view to determining the efficacy of the plant as an anticonvulsant drug.
**Methods:** Fresh leaves of *Piliostigma thonningii* were air-dried, pulverized and extracted using soxhlet extraction apparatus. Acute toxicity study was carried out by Lorke's method and the anticonvulsant activity of the ethanol leaf extract was carried using pentylenetetrazole and strychnine-induced convulsion model on Wistar strain albino rats and mice respectively.
**Result:** The soxhlet extraction yielded 21.04% $^{w}/_{w}$ of extract after being concentrated. The oral and intraperitoneal $LD_{50}$ were ≥ 5000 mg/kg implying that the extract is relatively safe according to literatures. Anticonvulsant effect of the ethanol leaf extract using pentylenetetrazole (PTZ), revealed the ability of the extract to confer protection on rats treated with doses of 200, 400 and 600 mg/Kg bd. wt. by exerting 60%, 80% and 80% protection on rat against PTZ induced convulsion respectively in a dose dependent manner as well as protected 20%, 60% and 80% of mice against death induced by strychnine when treated with 100, 200 and 400 mg/kg of ethanol extract.
**Conclusion:** The ethanol leaf extract of *Piliostigma thonningii* was able to provide anticonvulsant effect and is relatively safe for consumption as medicine.
**Keywords:** Phytochemicals; Acute Toxicity; Strychinine; Pentylenetetrazole; Anticonvulsant.

## INTRODUCTION

Plant products have been part of phytomedicines since time immemorial. The use of medicinal plants in West Africa is probably as old as the duration of human settlement in the region (Abdulrahman *et al*., 2010; Sodipo *et al*., 2011). These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. (Newman and Cragg, 2001). The pharmacological properties of African medicinal plants are immense: remedies made from plants play an important role in the health of millions of people especially in the rural areas (Grabley and Thiricke, 1999). The availability, low cost and accessibility of these plants in Tropical and Sub-tropical Africa coupled with the global crisis of drug resistance incidences, adverse effects amongst other appalling negative scientific reports of conventional drugs make it convenient for in-depth survey of medicinal plants from this part of the world (Usman *et al*., 2009).
Medicinal plants are known to owe their curative potentials to certain biological active substances, which exist in parts of the plants. The chemicals which are referred to as active principles or phytochemical substances include terpenes, flavonoid, bioflavonoid, benzophonones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthrax quinones (Iwu, 1993; Asaolu, 2003).

*Piliostigma thonningii* (Schumach) Milne-Redhead [Fabaceae] is a plant used for medicinal purposes in many African countries. Different parts of the plant have been used traditionally for the treatment of various diseases in humans and animals (Djuma, 2003). For example, the roots and twigs have been used locally in the treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin infections (Iwalewa *et al*., 1990; Jimoh and Oladeji, 2005). The leaf is useful in the treatment of malaria fever (Akinniyi and Sultanbawa, 1983; Rabo and Sanusi, 2001). The leaves of *Piliostigma thonningii* are used to treat wounds, chronic ulcers, diarrhoea, toothache and gingivitis, cough, and bronchitis (Watt and Breyer-Brandwijk, 1962). The dry leaf powder has been reported to contain alkaloids, saponins, flavonoids and tannins (Stahl, 2005).

Toxicology is the science that deals with the study of the adverse effects caused by chemicals or physical agents in living organisms under specific conditions of exposure (Doull *et al*., 2008). Toxicity studies are conducted to provide greater understanding of the potential intrinsic hazard of the test item and to estimate safety margins (Robbinson *et al*., 2009). Plants used in traditional medicine are relatively safe, but some may have undesirable adverse effects which may be due to over dosage or certain factors and these may lead to toxicity and death (Okigbo *et al*., 2009).

Epilepsy is a major neurological disorder and up to 5% of the world population develops epilepsy in their lifetime (Sander and Shorvon, 1996). The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy (Mattson, 1995).

Despite of the use of *Piliostigma thonningii* in traditional medicine by the local people in the North-Eastern part of Nigeria in the management of epileptic conditions, only a few studies have been carried out to evaluate the convulsant activity and the safety of the leaves of *Piliostigma thonningii*, given its rich phytochemical composition. It is, therefore, pertinent to examine the phytochemicals responsible for the plant's medicinal uses in Tropical Africa particularly those found in the Arid and Semi-Arid region of Nigeria.

This study was carried out to evaluate the acute toxicity and anticonvulsant efficacy of ethanol extract of the leaf *Piliostigma thonningii*.

## METHODOLOGY

### Sample Collection, Identification and Preparation

Fresh leaves of the plant, *Piliostigma thonningii* were collect from Jumu'a, a village in Potiskum, Yobe State, Nigeria. The leaf was identified and authenticated to be *Piliostigma thonningii* by a Plant Taxonomist in the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria. It was given a voucher specimen number 548A and deposited at the Postgraduate Research Laboratory of the Department of Chemistry, Faculty of Science, University of Maiduguri.

The leaves were cleaned by hand picking foreign materials and air-dried under shade at room temperature for seven days and pulverized using mortar and pestle and then subjected to the following analysis.

### Plant Extraction

Eight hundred grammes (800 g) of the powdered leaves was exhaustively soxhlet extracted using ethanol as solvent. The extract fraction was concentrated to dryness by slow evaporation process at room temperature. It was weighed, labelled and kept in a desiccator until required.

### Experimental Animals

All the experiments performed on laboratory animals in this study followed the standard procedure for the treatment of animals. Ethical approval was obtained on 30$^{th}$ 03, 2015 from the University of Maiduguri Teaching Hospital by the Research and Ethical Clearance Committee. The animals were handled according to the International Guiding Principle for Biomedical Research involving animals, CIOMS and ICLAS, (2012).

A total of forty-eight albino rats (100-180 g) and twenty-five (25) mice of both sexes were purchased from the animal house of the Faculty of Pharmacy, University of Maiduguri, Borno State. They were housed in clean plastic, well-ventilated cages with saw dust as beddings under 12 hours light/12 hours dark cycle conditions of normal room temperature and humidity in the Pharmacology, Physiology and Biochemistry Laboratory, Faculty of Veterinary Medicine, University of Maiduguri for the analysis. They were fed with standard fed (ECWA, Jos) and allowed water *ad libitum.*

### Extract Preparation

Ethanol leaf extract of *Piliostigma thonningii* (2 g) was dissolved in 10 ml distilled water, to give a stock solution of 200 mg/ml.

$$\text{Stock solution} = \frac{2000\ \text{mg}}{10\ \text{ml}} = 200\ \text{mg/ml}$$

$$\text{Volume to be administered} = \frac{\text{Dose x Body Weight in kg}}{\text{Concentration of the Extract in mg}}$$

**Acute Toxicity Evaluation ($LD_{50}$)**

The acute toxicity ($LD_{50}$) of the crude leaf ethanol extract of *Piliostigma thonningii* was determined using standard conventional procedure as described by Lorke (1983). In this study, two different routes of administration were considered; the oral and intraperitoneal. In phase I, rats were divided into 3 groups of three rats each for each route (a total of nine rats) and then treated with the crude ethanol extract at doses of 10, 100 and 1000 mg/kg bd. wt. intraperitoneally and orally and observed for 24 hours for mortality. In the phase II, the animals of each group (for each route) were divided into three groups of one animal each and the ethanol extract was administered at doses that were determined after the phase I. The rats were observed for signs of toxicity and mortality for the first critical four hours and thereafter daily for 7 days. The $LD_{50}$ was then calculated using the formula:

$$LD_{50} = \sqrt{a \times b}$$

Where a = least dose that killed a rat
b = highest doses that did not kill a rat

**Pentylenetetrazole Induced-convulsant in Rats**

The methods of Nwafor (1998) was adopted for this experiment with some modifications. Adult rats of both sexes were used for this experiment, food was withdrawn 12 hours before the experiment, but water was made available *ad libitum* until the start of the experiment. The rats were randomly divided into five groups of five rats each and treated as follows: group A rats were given a convulsive dose of pentylenetetrazole (PTZ) (60 mg/kg bd. wt. *s.c*.) Groups B-D received doses of 200, 400 and 600 mg/kg b. wt.) of the ethanol extract (*i.p*.), pentylenetetrazole (100 mg/kg) was injected subcutaneously (*s.c*.) on the back of the neck of the rats after 30 minutes while group E were treated with 200 mg/kg of Sodium Valproate. Seizures manifested as tonic convulsions (tonic hind limb extension). The ability to prevent this feature or prolong the latency or onset of the tonic hind-limb extension over a 24-hour period was taken as indication of anticonvulsant activity. The onset of tonic convulsions and number of mice presenting convulsions per minute and the duration of convulsions was recorded. Rats that did not show tonic hind limb extension during the period of observation were considered as not having convulsed. The rats were also monitored for instances of death up to 24 hours after the experiment.

$$\%\ \text{Survival} = \frac{\text{Number of survived animals}}{\text{Total Number of Animals}} \times 100$$

**Strychnine-induced Convulsion in Mice**

The method used is as described by Lehmann *et al.* (1988). In brief, strychnine convulsions followed by death was induced in mice by the subcutaneous injection of 1mg/kg of strychnine nitrate. Thirty minutes prior to administration of strychnine three groups of 5 mice each were intraperitoneally pretreated with ethanol extract of *Piliostigma thonningii*. The fourth group was treated with phenobarbitone sodium (20mg/kg *i.p*) which served as the positive control while the fifth group received normal saline 10ml/kg as the negative control. Mice were observed for tonic extensor jerks of the hind limbs followed by death in 30 minutes. Abolition of tonic extensor jerks of the hind limb was considered an indicator that *Piliostigma thonningii* could prevent strychnine-induced seizures (Raza *et al*., 2001).

**Statistical Analysis**

Results of pharmacological study was analysed using GraphPad Prism version 8.0 2016 Model for windows One-way Analyses of Variance (ANOVA) test followed by Tukey-Kramer's Multiple Comparison test was used to analyse and compare the results at 95 % confidence level. Values of $p<0.05$ was considered significant. Results were expressed as mean ± standard error of mean.

## RESULTS

**Extraction Profile of *Piliostigma thonningii* Leaf**

The extraction of the leaf of *Piliostigma thonningii* using ethanol produced extract with 21.04% weight which was brownish in colour and gummy in texture. The result of the extraction profile is shown on Table 1.

**Table 1. The extraction profile of air-dried powdered leaf of *Piliostigma thonningii***

| S/N | Fraction | Mass | %Yield ($^{w}/_{w}$) | Colour | Texture |
|---|---|---|---|---|---|
| | Ethanol | 148.29 | 21.04 | Brown | gummy mass |

**Acute Toxicity ($LD_{50}$)**

Table 2 represents the result of acute toxicity of *P. thonningii* on rats. No death was recorded on administration of up to 5000 mg/kg dose of the extract via both the oral and intraperitoneal routes. Though behavioural signs of toxicity were observed in rat when 5000 mg/kg of the extract was administered via intraperitoneal route which included; paw licking, stretching and reduced activity but it revived 5 hrs after the exhibition of clinical signs. Thus, $LD_{50}$ of the crude ethanol leaf extract of *Piliostigma thonningii* in rats administered via both oral and intraperitoneal route were calculated as ≥ 5000 mg/kg bd. wt. which made it impossible to estimate the $LD_{50}$ via Lorke's method.

**Table 2.** Acute toxicity effect of ethanol leaf extract of *Piliostigma thonningii* on rats

| **Phase** | **Dose (mg/kg)** | **No. of rat** | **Mortality rate** | |
|---|---|---|---|---|
| **Oral route** | **IP route** | | | |
| I | 10 | 3 | 0/3 | 0/3 |
| | 100 | 3 | 0/3 | 0/3 |
| | 1000 | 3 | 0/3 | 0/3 |
| II | 1600 | 1 | 0/1 | 0/1 |
| | 2900 | 1 | 0/1 | 0/1 |
| | 5000 | 1 | 0/1 | 0/1 |

**Effect of Ethanol Extract of *Piliostigma thonningii* on Strychnine-induced Convulsion in Mice**

The ethanol extract of *P. thonningii* at doses of 200, 400 and 600 mg/kg bd. wt. exerted 60 %, 80 % and 80 % protection to rats against PTZ induced convulsion respectively. It was observed that the mean number of spasms of group B and D were significantly ($p<0.05$) different from the control group. Meanwhile, the mean time of onset of convulsion increased with increasing doses of extract from 18.00±0.71 min to 29.60±0.51 min for 200 mg/kg bd. wt. to 600 mg/kg bd. wt. of the extract when compared to the negative control that had 4.00±0.32 min. There was no significance difference of the effect extract administered to animals of groups B and C. However, the mean onset of death was also dose dependent for the rats treated with 200, 400 and 600 mg/Kg of the ethanol extract. The mean onset of deaths were significantly different from the control group at $p<0.05$. Valproic acid (200mg/kg) protected all the rats (100%) against clonic spasm induced by pentylenetetrazole as presented in Table 3.

**Effect of Ethanol Extract of *Piliostigma thonningii* on Strychnine-induced Convulsion in Mice**

The crude extract of *Piliostigma thonningii* at doses of 100, 200 and 400mg/kg body weight protected 20%, 60% and 80% of mice respectively against death induced by strychnine. However, crude extract of *Piliostigma thonningii* significantly ($P<0.05$) prolonged the onset of convulsion in a dose-dependent manner from 10.2±0.37min. In normal saline treated group by 60%, 111% and 127% at doses of 100, 200 and 400 mg/kg body weight respectively. Similarly, in the time of death, the crude extract of *Piliostigma thonningii* significantly ($P<0.05$) prolonged the time of death of convulsed mice from 11.8±0.37min. in normal saline treated group by 52%, 103% and 129% at doses of 100, 200 and 400 mg/kg body weight respectively. Result of the study is shown in Table 4.

**Table 3:** Effect of ethanol leaf extract of *Piliostigma thonningii* on pentylenetetrazole induced convulsion

| Extract pretreatment (mg/kg | Mean±SEM onset of spasm (min) | Mean±SEM onset of convulsion (min) | Mean±SEM onset of death (min) | Quantal death | Survival (%) |
|---|---|---|---|---|---|
| *control + 60 mg/kg of PTZ | 9.60±1.14 | 4.00±0.71 | 13.00±2.92 | 1/5 | 20 |
| 200 mg/kg + 60 mg/kg of PTZ | 18.20±5.89[a] | 18.00±1.14[a] | 44.40±1.40[a] | 3/5 | 60 |
| 400 mg/kg + 60 mg/kg of PTZ | 28.40±2.07[b] | 24.80±2.35[b] | 50.00±0.00[b] | 4/5 | 80 |
| 600 mg/kg + 60 mg/kg of PTZ | 28.40±2.07[bc] | 29.60±1.60[c] | 60.00±0.00[c] | 4/5 | 80 |
| Sodium Valproate (200mg/kg) | 00 | 0.0 0.00 | 0.00.00 | 5/5 | 100 |

*Control= Rats administered distilled water,
Values across column with different alphabets as superscript are statistically (p>0.05) significant
*Data presented as Mean ± SEM, n =5; *represent p<0.05 by student t-test.*

**Table 4: Effects of Crude Extract of *Piliostigma thonningii* on Strychnine-induced Convulsion in Mice**

| Treatment (mg/kg) | Mean Onset of Convulsion (min.±SEM) | Mean Time of Death (min.±SEM) | Quantal Protection | %Protection |
|---|---|---|---|---|
| N/Saline (10ml/kg) | 10.2±0.37 | 11.8±0.37 | 0/5 | 0 |
| 100 | 16.3±1.20* | 18.0±1.53* | 1/5 | 20 |
| 200 | 21.5±0.50* | 24.0±1.00* | 3/5 | 60 |
| 400 | 23.2±0.86* | 27.0±0.00* | 4/5 | 80 |
| Phenobarbitone (20mg/kg) | 24.0±0.00* | 24.00±0.00* | 4/5 | 80 |

*Data presented as Mean ± SEM, n =5; *represent p<0.05 by student t-test.*

## DISCUSSION

The observation of present study indicates that ethanol extract of *Piliostigma thonningii* possesses anticonvulsant activity in rats. There was no mortality at all dose levels of *Piliostigma thonningii* administered to the laboratory rats. The intraperitoneal and oral ≥ 5000 mg/kg b. wt. obtained in this study is about 25 times greater than the minimum effective dose of 200 mg/Kg. Earlier reports have shown that if the $LD_{50}$ of a test substance is three times more than the minimum effective dose; the substance is considered a good candidate for further studies (Madara *et al*., 2010) reported that oral administration is about 100 times less toxic than the intraperitoneal. Clarke and Clarke (1977) were of the opinion that compounds with $LD_{50}$ of 1500 mg/kg and above have low toxicity. The extract is therefore safe and this could explain the safe use of the plant by the local people who have been using it in traditional management of depressive illnesses in North-Eastern Nigeria (Idris *et al*., 2014).

The leaf extract was also observed to have an effect on pentylenetetrazole (PTZ) induced convulsion on rats. The extract appeared to reduce the convulsant effect in a dose dependent manner. It depressed the central nervous system stimulation from convulsing which agrees with the reports of Abdulrahman *et al*., (2005). This finding also agrees with the reports in rats using different plant extracts (Sandabe *et al*., 2003; Abdulrahman *et al*., 2005; 2007; Usman *et al*., 2008, Abdulrahman *et al*., 2012). The PTZ is a known convulsant and anticonvulsant activity in (PTZ) (s.c) test identifies compounds that can raise the seizure threshold in the brain (White *et al*., 1998; Raza *et al*., 2001). It acts by stimulation of the medulla (Franz, 1975). The ability of the plant's ethanol extract to protect rats stimulated with PTZ may be an indication of its depressant effects on both the spinal cord and brain stem. Triterpenoidal saponins are reported to possess anticonvulsant activity in some experimental seizure models such as Maximal electroshock (MES) and PTZ (Chauhan *et al*., 1988; Kasture *et al*., 2002).

Some flavonoids are also reported to have protective effects against PTZ, picrotoxin and N-methyl-D, L-aspartic acid (NMDLA)-induced convulsions (Johnston and Beart, 2004).

In the strychnine-induced seizure study, it is known that strychnine directly antagonizes the inhibitory spinal reflexes of glycine (Sayin *et al.,* 1993). The ethanol extract at the dose of 200mg/kg and 400mg/kg protected 20%, 60% and 80% of the mice against strychnine-induced death. The convulsing action of strychnine is due to the interference with postsynaptic inhibition mediated by glycine, an important inhibitory transmitter of the motor neurons and interneurons in the spinal cord. Strychnine sensitive postsynaptic inhibition in higher centers of the CNS is also mediated by glycine. Strychnine acts as a selective, competitive antagonist at all glycine receptors (Rajendra *et al.,* 1997). The ability of the ethanol extract to prevent the strychnine-induced seizures demonstrate additional anticonvulsant effects mediated via glycine receptors (Ogbonnia *et al.,* 2003).

The depressant activity of the ethanol leaf extract of *Piliostigma thonningii* may be attributed to the presence of phytochemicals reported by Yakubu *et al*. (2016) which may have singly or acted synergistically with one another and might be responsible for the anticonvulsant effect.

## CONCLUSION

The ethanol leaf extract of *Piliostigma thonningii* exhibited anticonvulsant activity in experimental animal models. The leaf extract had no observable toxic effect on rats. The results of this study provide support for the traditional use of *P. thonningii* as an anticonvulsant drug.

## ACKNOWLEDGEMENTS

The authors thankfully acknowledge the technical assistance of Mr. Fine Akawo, Department of Chemistry, University of Maiduguri, Maiduguri and Mr. Bitrus Wampana, Department of Pharmacology, Faculty of Veterinary Medicine, University of Maiduguri, Borno State.

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Conflict of Interest: None declared
Received: December 6,2020
Accepted: May 10, 2021