

Pharmacological effects of *Piliostigma thonningii* leaf extract on anxiety-like behaviour and spatial memory in Wistar albino rats

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Received: 20 January 2012, **Revised:** 28 February 2013, **Accepted:** 1 March 2013

Abstract

Preparation of *Piliostigma thonningii* Schum. (Caesalpiniaceae) leaf is used traditionally in managing fever, toothache, amnesia and anxiety. The aim of the study was to evaluate the effect of the extract on anxiety-like and spontaneous alternation behaviour in rats. The oral median lethal (LD₅₀) dose of *Piliostigma thonningii* ethanol leaf extract (PTLE) was evaluated using modified Lorke's method in rats. The effect of PTLE (50-200 mg/kg p.o), diazepam (2.5 mg/kg, i.p) and 10 ml normal Saline/kg on anxiety-like behavior and escape latency of rats were assessed on EPM. The oral median lethal (LD₅₀) dose of PTLE was estimated to be 5000 mg/kg weight in rats respectively. The extract significantly ($F_{4, 25} = 1840, p < 0.0001$) increased the percentage of time spent and the number of entries ($F_{4, 25} = 28, p < 0.0001$) into the open arms. The administration of the extract produced significant ($F_{3, 16} = 3.5, p < 0.001$) decrease in escape latency of rats from the open arm of the Elevated Plus Maze. The results of the present study provided evidence for anxiolytic and nootropic effects of the PTLE thus providing scientific basis for its use in the management of brain disorders characterized by apprehension and amnesia.

Keywords: Rats, amnesia, anxiety, *Piliostigma thonningii*

Introduction

Anxiety has been described as a frequent and serious disorder affecting the world's population, independent of ethnicity, and is considered a cardinal symptom of many psychiatric disorders (Cassano *et al.*, 1999; Goddard *et al.*, 2001, Tijani *et al.*, 2012). Research findings in the area of behavioral pharmacology has contributed immensely to the identification, diagnosis and treatment of anxiety disorders, such as generalized anxiety disorder, panic disorder, phobias, obsessive compulsive disorder, acute stress, and posttraumatic stress disorder. Substantial numbers of patients suffering from anxiety disorders have to bear the con-

quences of the adverse effects of available treatment regimens and present comorbid difficulties in memory and cognitive tasks (Eysenck & Calvo, 1992).

Medicinal plants have found wide acceptability and utility in management of various ailments including neuropsychiatric disorders such as anxiety, cognition impairment and depression. One of such plants used in folk medicine include *Piliostigma thonningii*. *Piliostigma thonningii* (Caesalpiniaceae) is a shrubby tree with alternate compound leaves. The fruits are often pod-like with pods containing one to many seeds. The plant is widely distributed in Africa and Asia. In Nigeria it grows abundantly as uncultivated tree in Zaria, Bauchi, Ilorin Plateau, Lagos and Abeokuta (Madara et al., 2010). In Nigeria it is represented by two species that are much alike - *Piliostigma thonningii* and *Piliostigma reticulatum* (Keay et al., 1964). The powdered bark of the young stem and the pods are applied as dressing for wounds. The leaves are believed to have expectorant property, and are used in infusions or chewed for chest complaints, diarrhoea, amnesia and anxiety (Dalziel, 1937).

A warm infusion of the leaves is used traditionally as antipyretic and analgesic to relieve fever, toothache and for management of diarrhoea (Salawu et al., 2007). Previously the antimalarial activity of the *Piliostigma thonningii* leaf was reported by Madara et al., (2012).

The purpose of the present study was to determine the effects of PTLE on anxiety and memory. The elevated plus maze (EPM), a standard behavioral model in which the aversive behavior of rats in response to open and elevated areas is considered an index of anxiety. The retention of memory in rats exposed to open arm of EPM was also assessed using escape latency as index of memory retention.

Materials and Methods

Experimental Animals

Wistar rats aged 8-10 weeks weighing (150 – 200g) were obtained from the Animal Facility Centre, (NIPRD), Idu, Abuja. The rats were fed standard laboratory diet, given water ad libitum and maintained under laboratory conditions of temperature $23\pm 2^{\circ}\text{C}$, relative humidity 60% and 12-hour light; 12-hour dark cycle. Food was withheld for 24 hours prior to each experiment. All animal experiments complied with the “Principles of Laboratory Animal Care” (NIH Publication No. 85-23, revised in 1996).

Collection and Identification of plant material

Fresh leaves of *Piliostigma thonningii* were collected from Suleja, Niger State Nigeria. It was identified by Mal. Ibrahim Muazzam of the Medicinal Plant Research and Traditional Medicine (MPR & TM) Department, NIPRD Idu, Abuja. A sample with voucher number NIPRD/H/6268 has been deposited for future reference at the department's (MPR & TM) Herbarium.

Preparation of extract

The plant's leaves were cleaned, air dried under room temperature and pulverized usi-

ng a mortar and pestles into powders. The powder was cold macerated with 1L of aqueous ethanol for 24hr with constant shaking using a GEL shaker, the resultants mixture was filtered using whatman filter paper and the filtrate concentrated by rotary evaporator at discs. The filtrate was dried on steam bath. The dried sample was stored in specimen bottle and kept in a refrigerator until required for use.

Acute toxicity study

Acute toxicity study of *Piliostigma thoningii* leaf extract (PTLE) was carried out according to method described by Lorke (1983). The study was carried out in two phases. In the first phase, nine rats were randomized into three groups of three rats per group and given 10, 100 and 1000 mg /kg body weight orally (via cannula), respectively. Animals were observed for 24 hours after treatment for signs of toxicity and mortality. Absence of mortality in animals used for the first phase of the study at 24 h, informed the choice of doses for the second phase, in which 1600, 2900 and 5000 mg PTLE/kg were given orally to another fresh set of three rats per group. All the rats were subsequently observed for 14 days for possible signs of delayed toxicity. The final median lethal (LD₅₀) dose value was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e. the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second stage.

Anxiety Study

Elevated plus maze

The elevated plus maze is an anxiety paradigm based on the rodent's natural aversion to a novel and potentially dangerous environment represented by the open and elevated spaces (Lister, 1987). The elevated plus maze apparatus is a plus (+) shaped wooden structure, consisting of two open arms (40cm×5cm×10 cm) and two enclosed arms (40 cm×5 cm×10 cm) extended from a central platform (10 cm×10 cm). The maze was elevated 50 cm from the room floor. Rats were habituated to the testing room under dim light for at least 1 h before the test and then randomly divided into seven groups. The control group received 10 ml normal saline/kg body weight orally, PTLE (50, 100 and 200 mg/kg body weight orally) and diazepam (2.5 mg/kg body weight i.p)- treated groups. One hour after oral treatment with PTLE and thirty minutes after intraperitoneal administration of diazepam, each rat was placed at the center of the maze, facing one of the open arms and allowed to explore the maze freely for a 5 min testing period. The time spent and frequencies of entries in open and enclosed arms were recorded. The maze was thoroughly cleaned between tests with a tissue paper moistened with 70% ethanol.

Effect of extract on short term memory

This experiment was carried out to assess cognitive performance of rats after oral treatment with PRLE using elevated plus maze paradigm. The elevated plus maze consists of two opposite open arms (50 x 10 cm), crossed with two closed arms of same dimensions with 10 cm high walls. The arms are connected with Central Square (10 x10 cm). Acquisition of memory was assessed on day 1 before pre-treatment with PRLE. Rats were placed indivi-

dually at one end of an open arm facing away from the central square. The time taken to move from open arm and enter into the closed arms was recorded as initial transfer latency (ITL). If a rat did not enter an enclosed arm within 90 s, it was gently pushed into the enclosed arms and the transfer latency (TL) was assigned 90 s. Retention of memory was assessed by placing a rat at one end of the open arm facing away from the central square. The time taken by the rat to enter into the enclosed arm was noted as the retention latency after 24 h of ITL (14). Rats were grouped into 4 groups of 6 rats each and treated orally as follow: Group 1: 5 ml normal saline/kg body weight; group II: 50 mg PRLE/kg body weight; group III: 100.0 mg PRLE/kg body weight and group IV: 200 mg PRLE/kg body weight

Results

Acute toxicity study

Neither sign of clinical toxicity nor mortality to *Piliostigma thoningi* leaf extract administration was observed at all the doses used in the study. All the rats used for the study were alive, healthy and active during the observation period. The oral median lethal (LD₅₀) dose of *Piliostigma thoningi* leaf extract was therefore estimated to be greater than 5000 mg /kg body weight in rats.

Effect of extract on anxiety parameters in rats on elevated plus mazes (epm)

Effect of extract on time spent in closed arm of EPM

The extract significantly ($F_{4, 25}=3.9, p<0.05$) decreased the time spent in the closed arm of the Elevated Plus Maze when compared to the control (Fig 1).

Effect of extract on time spent in open arm of EPM

The extract significantly ($F_{4, 25}= 1840, p<0.0001$) increased the time spent in the opened arm of the Elevated Plus Maze when compared to the control (Fig 2).

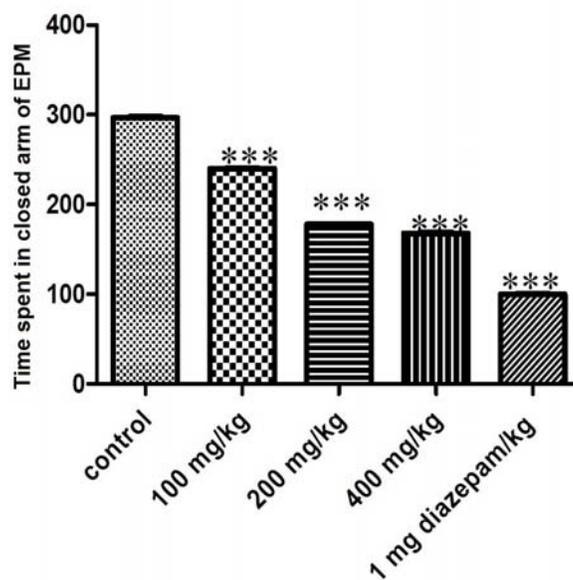


Figure 1. Shows the graph of mean time spent by mice in the closed arm of elevated plus maze(EPM). *** Significantly different from the control at $p<0.0001, n=6$

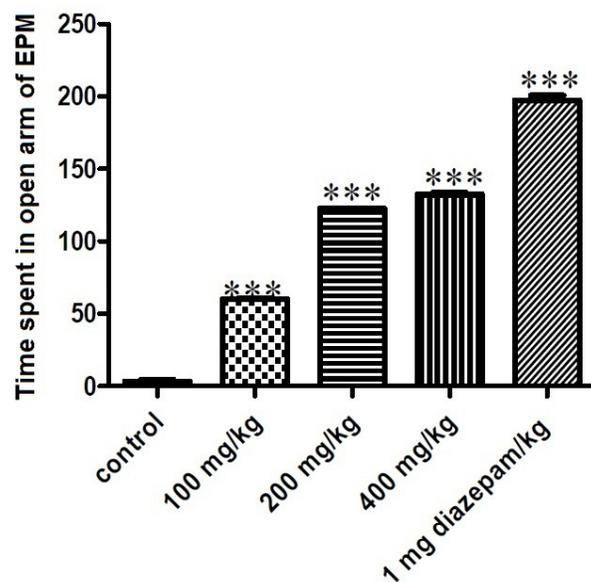


Fig 2: Shows the graph of mean time spent by mice in the open arm of elevated plus maze(EPM). *** significantly different from the control at $p < 0.0001$, $n = 6$.

Effect of extract on entry into closed arm of EPM

The extract significantly ($F_{4,25} = 72$, $p < 0.0001$) decreased frequency of entry into the closed arm of the Elevated Plus Maze when compared to the control (Fig 3)

Effect of extract on entry into open arm of EPM

The extract significantly ($F_{4,25} = 28$, $p < 0.0001$) increased frequency of entry into the open arm of the Elevated Plus Maze when compared to the control (Fig 4)

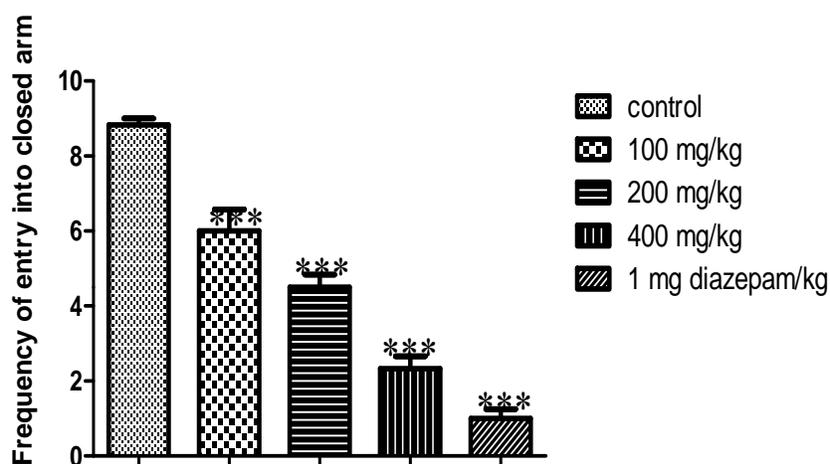


Fig 3: Shows the graph of mean frequency of entry into closed arm of Elevated Plus Maze (EPM). *** Significantly different from the control at $p < 0.0001$, $n = 6$.

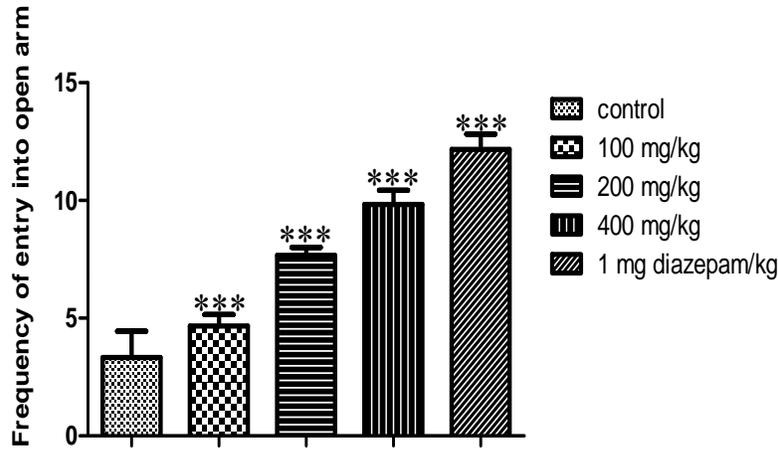


Fig 4. Shows the graph of mean frequency of entry into open arm of Elevated Plus Maze (EPM). *** Significantly different from the control at $p < 0.0001$, $n = 6$.

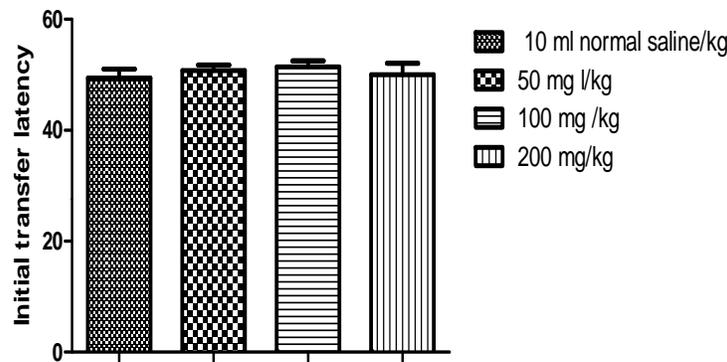


Fig 5: shows the graph of the initial transfer latency of rats on the Elevated Plus Maze before treatment with graded doses of *Piliostigma thoningii* leaf extract.

Effect of extract on short term memory

Effect of PTLE on escape latency on EPM

There was no significant ($F_{3, 16} = 0.34$, $p = 0.4637$) difference in the initial escape latency of rats in all the treatment groups (Fig. 5). However, the extract significantly ($F_{3, 16} = 3.5$, $p < 0.001$.) and dose- dependently shortened the retention latency on the elevated plus maze (Fig 6)

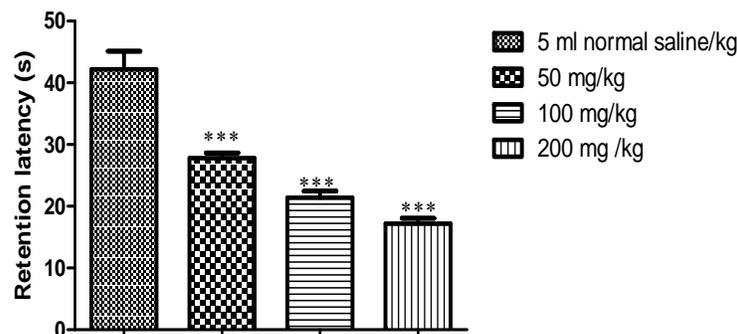


Fig 6: shows the graph of retention latency of rats treated with graded doses of *Piliostigma thoningii* leaf extract. *** Significantly different from the control at $p < 0.0001$, $n = 6$.

Discussion

The results of this study showed that the aqueous methanolic leaf extract of *Piliostigma thoningii* did not produce any sign of clinical toxicity and had oral median lethal dose greater than 5000 mg/kg body weight. The extract produced significant anxiolytic effect in rats on Elevated plus Maze (EPM) and had positive nootropic effect on rats as reflected in shortened escape latency on EPM.

The oral median lethal dose of greater than 5000 mg/kg body weight suggests that the extract is practically non-toxic acutely via oral administration in rats (Salawu *et al.*, 2010). This wide margin of safety may in part explain its wide spread use for managing various ailments in Africa folk medicine.

The elevated plus-maze (EPM) test represents one of the most widely used animal models for screening anxiolytics (Lister, 1987). This test is able to reproduce anxiolytic or anxiogenic effects in rodents such that anxiolytics produce increase in the number of entries into the open arms of the maze and the time spent there, while anxiogenic substances produce the opposite effect (Lister, 1987).

The indices of anxiety (percentage of open-arm entries, and percentage of time spent in the open arm) are sensitive to agents thought to act via the GABAA receptor complex, justifying the use of diazepam (DZP) as a positive control in this study. Diazepam, a benzodiazepine binds to GABAA receptors to increase the frequency of chloride channel openings resulting in hyperpolarization (Carobrez and Bertoglio, 2005). It increased the frequency of open-arm entries and the time spent in the open arms (Tijani *et al.*, 2012), confirming its anxiolytic effects. *Piliostigma thoningii* leaf extract produced similar effects on these parameters in treated rats.

Piliostigma thoningii improved the memory retention of rats as reflected by diminished transfer latency values on elevated plus maze. The transfer latency is an index used for assessment of memory retention potential (Rahmat *et al.*, 2012). The first trial was to expose the animals to a novel arena while the second assessment in the open arm of the Elevated Plus Maze queries ease of recognition and retrieval of acquired memory. The extract significantly shortened time require for retrieval of this information as indicated by the shortened escape latency from the open arm of the elevated plus maze.

Conclusion

It may therefore be concluded that the aqueous methanol leaf extract of *Piliostigma thoningii* possesses anxiolytic and nootropic effects thus providing evidence for its use in management of agitated patients in folkloric medicine.

Acknowledgement

The authors are grateful to the technical staff of the Department of Biological Sciences of the University of Abuja for providing technical support for the conduct of this study.

Conflict of Interest statement

The authors alone are responsible for the content of this manuscript and have declared no conflict of interest.

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