

Full Length Research Paper

Aqueous and ethanol leaf-extracts of *Piliostigma thonningii* (Schum) increase locomotor activity in Sprague-Dawley rats

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Among other uses, aqueous and alcohol extracts of *Piliostigma thonningii* (Schum) have been claimed by traditional herbal medical practitioners in Nigeria to be effective tranquilizers. In our efforts to establish some of the tradomedical uses of the plant, we designed the present study in order to test the effects of the extracts on the locomotor activity (LA) of rats. Male rats were administered 25 or 50 mg/kg of the aqueous (AE) or ethanol (EE) extracts with or without 2 mg/kg dexamphetamine (DEX). Results show that the lower doses of both extracts did not significantly increase LA but the higher doses significantly ($P < 0.05$) increased the 90 min cumulative locomotor score although far less than 2 mg/kg DEX alone. Doses of 50 mg/kg of EE but not AE also significantly ($P < 0.05$) increased the 90 min cumulative DEX-induced locomotor score. The increase in LA by 50 mg/kg of the extracts has led us to conclude that they may not have any tranquilizing potentials.

Key words: *Piliostigma thonningii*, locomotor activity, tranquilization.

INTRODUCTION

Plant materials are central to tradomedical practices and have remained useful sources of new drugs. Although orthodox medical practice is generally acceptable, alternative healthcare is still relied on all over the world (O'Brien, 2004; Leckridge, 2004). In the developing countries of the world, traditional herbal medicine is often used side by side western medicine with herbal medicine taking the upper hand when the cost of western medicine is beyond reach (Busia, 2005). Treatment of mental illness is one area in which a lot of people in developing countries depend on herbal medicine (Fang and Schinke, 2007; Ushiroyama et al., 2005).

In Africa, *Piliostigma thonningii* Schum (Monkey bread or Camel's foot) of family Caesalpiniaceae is one of the plants with diverse ethnomedical and economic applications (Igoli et al., 2005; Togola et al., 2005). It is a perennial tree that grows luxuriantly in many parts of Africa and Asia (Burkill, 1995). In Nigeria it bears such lo-

cal names as Abefe (Yoruba), Kalgo (Hausa) and Okpoatu (Ibo). The leaves are 7.5 to 15 cm long, leathery, and very strongly reticulate (Burkill, 1995).

Various preparations of parts of the plant have been used to arrest bleeding, as laxative, to treat fevers and bacterial infections, as anthelmintic and as anti-inflammatory agents (Igoli et al., 2005; Togola et al., 2005; Ogundaini, 1999; Fakae et al., 2000; Ibewuiké et al., 1997). Some of the acclaimed pharmacological properties such as its antibacterial (Ogundaini, 1999; Ibewuiké et al., 1997), anthelmintic (Fakae et al., 2000), and anti-inflammatory (Ibewuiké et al., 1997) have been investigated scientifically. Our unpublished data confirm the tradomedical application of poultices of the aqueous extract for the arrest of bleeding through vasoconstriction. Although some herbalists have through personal communications claimed that the aqueous extracts of the leaves are used as tranquilizer, we are unable to find any scientific report in support of this.

Drugs which cause tranquilization may impair a vital component of neuronal activity leading to reduction in locomotor activity (Bardin et al., 2007; Duncan et al., 2006; Ozolua et al., 1996). For example, the typical antipsycho-

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tic drugs all impair dopaminergic transmission resulting among other effects in reduced locomotor activity in the animal (Duncan et al., 1996; Ozolua et al., 1996). Due to the absence of data on the central nervous system effects of leaf-extracts of *P. thonningii*, we sought to investigate how the aqueous and ethanol extracts would affect locomotor activity in rats and to relate this to the claim by some herbalists around us.

MATERIALS AND METHOD

Animals

The experiments were performed using male Sprague-Dawley rats weighing between 160 – 220 g. The animals were bred locally in the animal house, Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. They were housed in standard cages and allowed free access to growers' mash (Bendel Feeds and Flour Mill Nigeria Ltd) and tap water. Animals were exposed to natural lighting conditions with 24 h temperature ranging from 24 – 28°C. They were handled according to standard protocols for the use of laboratory animals (National Institute of Health USA: Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002).

Plant material

The leaves of *P. thonningii* were obtained from the premises of the University of Benin in the month of November and were identified and authenticated by pharmacy faculty from the Department of Pharmacognosy, University of Benin where a herbarium specimen exists. Identification was further confirmed by internet photographs of the plant from a recognized website for plant identification (www.zimbabweflora.co.zw). The leaves were sun-dried for 20 days and were thereafter ground to powder. The powder was divided into two portions and one portion was soxhlet-extracted over 8 h with absolute ethanol while the other portion was macerated with boiling water. Both the aqueous extract (AE) and ethanol extract (EE) were thereafter separately concentrated in a rotar vapour at 70°C, dried in an oven at 50°C and stored in labeled airtight bottles. At time of use, the injection solutions were prepared by suspending weighed quantities in appropriate volumes of normal saline or 3% polysorbate 80 for AE and EE, respectively.

Effect of extracts on locomotor activity

The animals were randomly allocated to 10 groups as follows:

Group 1: 0.2 ml of normal saline; Group 2: 2 mg/kg dexamphetamine (DEX); Group 3: 25 mg/kg AE; Group 4: 25 mg/kg EE; Group 5: 25 mg/kg AE + 2 mg/kg DEX; Group 6: 25 mg/kg EE + 2 mg/kg DEX; Group 7: 50 mg/kg AE; Group 8: 50 mg/kg EE; Group 9: 50 mg/kg AE + 2 mg/kg DEX; Group 10: 50 mg/kg EE + 2 mg/kg DEX.

All injections were given intraperitoneally except DEX which was given by the subcutaneous route but prior to drug administration, the animals were acclimatized to the activity cage for 90 min each as previously described by Ozolua et al. (1996). The activity cage is designed to measure animal's head and forepaw movements. Briefly, it is made of plywood and provided with a fan. Within the box is a sensitive electronic metre, 40fc (Motron products, Stockholm, Sweden) and consists of 40 photocells lined 8 by 5. On the upper inner part of the box directly above the photocells is a small 60-volt infrared light bulb. During experiments, all lights are turned

off except the infrared light which provides the beam for the photocells such that when the beam is interrupted by the animal's locomotion, a locomotor reader is triggered off and recorded. With the light, it is possible to make direct observations of the animal. The experiments were all carried out in a dark room (except for the infrared light in the box) equipped with an air conditioner to cool the room and provide background noise thereby preventing the animals from being disturbed from outside. Each animal was confined within the photocells by placing it on a small glass box which bottom dimensions are same as those of the photocells. By this arrangement, the animal moved only within the dimensions of the photocells. Readings were taken at time points 10, 30, 60, 90 min of putting the animal into the box and turning off the laboratory lights.

Drugs and chemicals

Dexamphetamine sulphate (dexamphetamine) was obtained from SKF (USA) and the injection solutions were prepared freshly before use by dissolving in distilled water. Polysorbate 80 (Tween 80[®]) was obtained from Halewood Chemicals, England. Sodium chloride (Sigma, UK) solution was prepared fresh each day. Absolute ethanol was obtained from Sigma (UK).

Statistics

The data are presented as mean \pm S.E.M (standard error of the mean) and *n* represents the number of rats used for each experiment. The 90th min cumulative locomotor activity (LA) score from the groups were compared by use of one-way analysis of variance (ANOVA) followed by Tukey post hoc tests. *P* < 0.05 was regarded as indicating statistically significant differences in all cases.

RESULTS

The effects of the extracts on locomotor activity in rats are shown in Figure 1. Panels A and B show the effects of 25 and 50 mg/kg doses of the extracts respectively. The score by groups treated with either of normal saline or dexamphetamine have been replicated in both panels.

The lower doses (panel A) of both AE and EE did not result in statistically significant differences in the 90 min cumulative locomotor activity score when compared to the score by the saline-treated group (AE: 2018 \pm 459; EE: 1814 \pm 415; Saline: 1512 \pm 223). The cumulative locomotor activity score following the administration of 2 mg/kg dexamphetamine was not significantly increased by concurrent administration of the lower doses of the extracts (AE + DEX: 9011 \pm 902; EE + DEX: 9420 \pm 901; DEX: 8379 \pm 859).

In panel B the cumulative locomotor activity score is significantly (*P* < 0.05) increased by 50 mg/kg of both extracts when compared to the score by the saline-treated group (AE: 2492 \pm 202; EE: 3789 \pm 686; saline: 1512 \pm 223). While the locomotor activity score by 2 mg/kg of dexamphetamine has been significantly increased by the concurrent administration of 50 mg/kg of EE, it is not significantly increased by the concurrent administration of 50 mg/kg AE (AE + DEX: 9729 \pm 927; EE + DEX: 10728 \pm 937; DEX: 8379 \pm 859). Although the

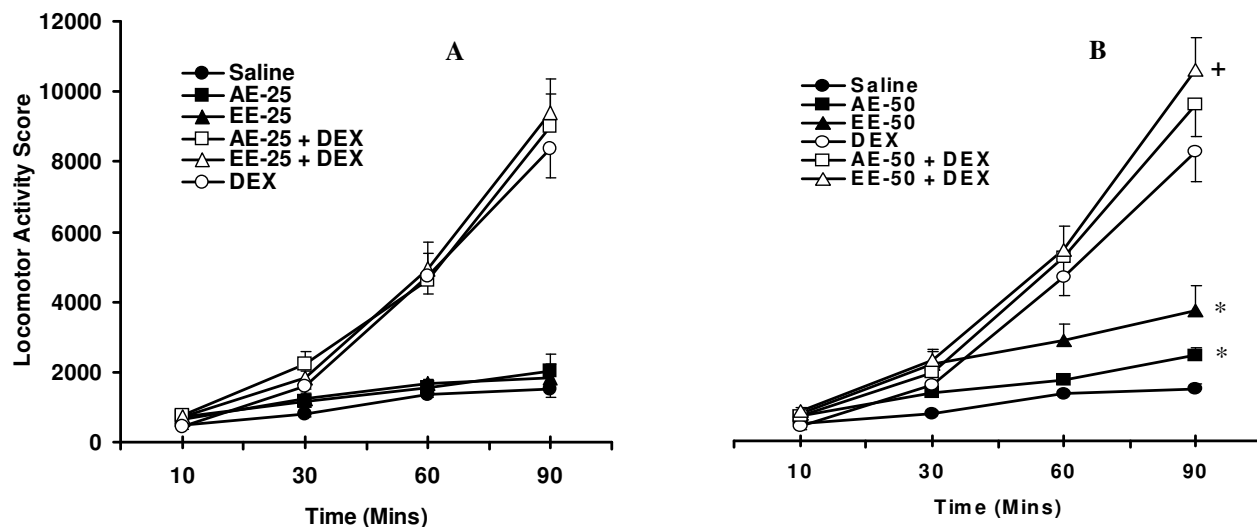


Figure 1. Effects of aqueous (AE), ethanol (EE) extracts of *P. thonningii* on the locomotor activity (LA) in rats. Doses of 25 mg/kg of AE or EE did not significantly increase LA compared to saline and did not increase the effect of DEX (A). Higher doses (50 mg/kg) of the extracts significantly ($*p < 0.05$) increased LA compared to saline and EE significantly ($*p < 0.05$) increased the LA by DEX (B). $n = 5$ per group.

ethanol extract appears to be superior to the aqueous extract in increasing locomotor activity in rats, the difference is not statistically significant.

DISCUSSION

This study has showed that the aqueous and ethanol extracts of *P. thonningii* at doses of 50 mg/kg increase locomotor activity in rats. The results suggest that the extracts are not likely to possess tranquilizing effects as claimed (through personal communications) by local herbalists. The acclaimed uses of herbal preparations have quite often been at variance with scientific evaluations of the same preparations (Oyekan and Lanionu, 1984; Wong, 1979). One possible reason for this is that the herbalists may not have sufficient pathological knowledge of specific disease states as to characterize them correctly. Such misapplication of terms may result in the use of the wrong medicine for the right ailment or vice versa. However, very high doses of dexamphetamine or apomorphine induce stereotype behaviour in animals which manifests as continuous biting, licking and gnawing with decrease in locomotor activity (Ozolua et al., 1996; Costall and Naylor, 1977). It is not immediately known if intoxicating doses of the extracts would produce a state of immobility which herbalists could mistaken for tranquilization.

A wide variety of experimental methods are used in the evaluation of drugs which modify central nervous system function. In the present study, we have used locomotor activity to evaluate the possible central nervous system effects of *P. thonningii*. Whereas drugs such as the typical antipsychotics cause tranquilization leading to

decrease in locomotor activity, drugs such as amphetamine, apomorphine which are CNS stimulants cause an increase in locomotor activity (Ozolua et al., 1996; Costall and Naylor, 1977; Obianwu et al., 1997). It is believed that these drugs act by facilitating the actions of monoamines such as 5-HT and dopamine (Giros et al., 1996; Gainetdinov et al., 1999). Although our phytochemical tests (not presented) indicate the presence of flavonoids, saponins, glycosides, tannins and alkaloids in the leaves of *P. thonningii*, we are unable to ascribe the increase in locomotor activity to any of these constituents. Flavonoids (Coleta et al., 2006), saponins (Kim et al., 2006), and tannins (Takahashi et al., 1986) from different plant species have been reported to cause central nervous system depression and act as anxiolytics. While some alkaloids stimulate, others depress the central nervous system. The present data (Figure 1B) have shown that the ethanol extract is superior to the aqueous extract in stimulating locomotor activity and suggests that the active compounds may be more abundant in the ethanol phase.

Conclusion

In conclusion, we have shown that aqueous and ethanol extracts of *P. thonningii* increase locomotor activity in rats and therefore not likely to be effective as tranquilizers.

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