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Anxiolytic and antipyretic activities in the decoction of leaves of *Piliostigma reticulatum*

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ABSTRACT

Piliostigma reticulatum is a plant used in traditional medicine in Cameroon to treat epilepsy, anxiety, agitation. Elevated plus maze, stress-induced hyperthermia and open field tests were used to determine anxiolytic properties. The doses of the decoction used were 35, 87.5, 175 and 350 mg/kg. *P. reticulatum* showed antipyretic properties by reducing in a dose dependent manner the body temperature that changed from 30.73°C to 28.28°C at the dose of 350 mg/kg. *P. reticulatum* showed also anxiolytic activities in the three tests. In stress-induced hyperthermia test, *P. reticulatum* antagonised dose dependently the increase of temperature. ΔT° decreased from 0.5°C in the control group to 0.10°C at the dose of 350 mg/kg. In the elevated plus maze test, *P. reticulatum* strongly and significantly increased the number of entries into the open arms, the % of entries and time into the open arms, and reduced the % of entries and time into the closed arms. Rearing and head dipping were as well decreased. In the open field test, *P. reticulatum* increased the number of crossing, centre time and grooming and reduced the number rearing and defecation. The results suggest that the decoction of *P. reticulatum* possesses anxiolytic and antipyretic properties in mice and could really be helpful in the treatment of anxiety in Traditional medicine in Cameroon.

INTRODUCTION

Anxiety disorders are the most prevalent mental disorder with very high co-morbidity [1] and [2]. With a complex etiology that is not fully known, anxiety disorders have severe impact on quality of life [3] and [4]. Besides, currently available drugs, although effective, were not specifically developed for treating anxiety disorders and possess unwanted side effects including sedation and dependence [5]. Traditional medicine that relies on the use of a wide variety of plant species could be explored to find new medicine to treat anxiety with less unwanted side effects. *Piliostigma reticulatum* (D.C.) Hoscht (Ceasalpiniacea) (*P. reticulatum*) also named *Bauhinia reticuta* D.C.; *Bauhinia benzoin* KOTSCH; *Bauhinia glabra* A. CHEW; *Bauhinia glauca* A. CHEW; *Elaguna biloba* RAF is one of the medicinal plants used in Cameroon to treat many diseases. A tree up to 9 m high, *P. reticulatum* is found from west Senegal to Central Africa. In Africa and Cameroon, leaves are used against cold, eyes problems, mumps, cough, head aches, migraines, and epilepsy. Leaves and barks are used as antiseptic, wound and injury healing. Barks are used against diarrhea, dysenteries, tooth aches, rheumatisms, and ulcer [6]. According to Cameroonian traditional healers, roots of *P. reticulatum* are also used in the treatment of anxiety, agitation and epilepsy. Previous experiments have shown that *P. Reticulatum* possesses antimicrobial, anti-inflammatory, anti-diarrheal, antioxidant, insecticidal and analgesic activities [7-12]. Chemical study revealed the presence tannins, glycosides, saponins, sterols, flavonols and oxychromonol compounds in *P. reticulatum* [12-13]. *Since P. reticulatum* is used to treat anxiety and agitated patients, our study was undertaken to look for anxiolytic properties of this medicinal plant. Part of these results has been published in abstract form [14].

MATERIALS AND METHODS

Animals

Adult male mice (*Mus musculus* Swiss; 22 2g) were used for

this study. The animals were housed in standard cages, at 25°C, on a 12/12 h light-dark cycle. They were supplied with food and water *ad libitum*. Mice were divided in 6 groups: one negative control group received distilled water as vehicle, one positive control group received an appropriate well known anxiolytic substance as a reference and four test groups received different doses of decoction. Treatments were administered intraperitoneally in a volume of 10 ml/kg of mice body weight. The study was conducted in accordance with the nationally (N°.FWA-IRB00001954) and internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH Publication No. 8023, revised 1996).

Plant material

Leaves of *P. reticulatum* used were collected in Cameroon in the immediate vicinity of Ngaoundéré, during the dry season (July 2008). A voucher specimen of the plant (4498/Geerling) was authenticated by the Botanist of our Faculty Professor PM *Mapongmetsem and deposited at the National Herbarium of Cameroon in Yaoundé. Dried leaves of P. reticulatum were* ground. 100 g of this powder were boiled in 500 ml of distilled water for 20 min. After cooling, the supernatant was collected, filtered with a Watman N°1 filter paper and was evaporated to dryness using a Rota vapor at a temperature of 60°C. The decoction obtained was 14.7 g (yield: 14.7%). The decoction was diluted in distilled water and was administered intraperitoneally (i.p.) 1 h before the test. The following doses were used: 35, 87.5, 175 and 350 mg/kg.

Chemicals

Phenobarbital is from Sigma Chemical, USA and diazepam from Roche.

Pharmacological tests

Tests were performed every day in the light cycle between 8 a.m and 2 p.m. with experimentally naïve mice.

Elevated Plus Maze (EPM) test

The apparatus was made up of two open arms (16 cm x 5 cm) and two closed arms (16 cm x 5 cm x 10 cm) that extended from a common central platform (5 cm x 5 cm). The entire maze was elevated to a height of 50 cm above the floor level. Naïve mice were treated with distilled water for the negative control group, with diazepam (3 mg/kg) for the positive control group and with different doses of *P. reticulatum* for the tested groups. 1h after treatment, mice were individually placed on the EPM centre platform facing an open arm and were observed for 5 min [15], [16] and [17]. The number of entries into the open or closed arms and the time spent on either open or closed arms (conventional parameters) were recorded for each animal with stopwatches. The centre platform time and some ethological parameters like rearing and head dipping were also recorded.

Stress-Induced Hyperthermia (SIH) test

Animals were marked and housed 10 per cage. Mice were removed from the cage one after another in a precise order and were treated with distilled water for the negative control group, phenobarbital (20 mg/kg, ip) for the positive control group and 4 doses of the decoction of *P. reticulatum* for the tested groups. All animals within a given cage were consecutively treated at 1min interval. After 60 min, mice were again consecutively removed from the cage (1min interval) and their body (rectal) temperature was recorded. This experiment is based on the fact that "among animals in the same cage, mice removed later had a higher body temperature compared to those removed earlier" [18] and [19]. The stress- induced hyperthermia was defined as the difference between the temperature of the first three mice and the temperature of the last three mice. The mean temperature of the first three mice was compared to the mean temperature of the last three mice in each group.

Open field (OF) test

One hour after appropriate treatment administration, naïve mice were placed in the centre of the open field. The open field used was a wooden square box: 40 cm x 40 cm x 45 cm, the floor was divided into 16 smaller squares of equal dimensions (10 cm x 10 cm). Animals placed one by one in the centre of the box could explore the box for 5 min. Mice were observed for 5 min in order to evaluate the effects of the plant both on exploratory and anxiolytic activities [20] and [21]. Hand operated counters and stopwatches were used to score the number of crossing (number of square floor units entered), rearing (number of times the animal stood on its hind legs), grooming, defecation and centre time. The positive control group received diazepam at a dose of 0.3 mg/kg.

Statistical analysis

The values of the negative control were compared to the values of the tested groups and positive control. The analyses of variance (ANOVA) followed by Tukey (HSD) were done. A value of $P<0.05$ was considered significant.

RESULTS

Effect of *P. reticulatum* on EPM

The administration of the decoction resulted in a significant increase in the number of open arms entries from 1.0 in the control group to 8.16 at the dose of 350 mg/kg [$F(6,29) = 54$; $p < 0.001$] (Figures 1). The % of entries into the open arms was dose dependently increased from 8.82% in the control group to 90.74% at the dose of 350 mg/kg [$F(6,29) = 277$; $p < 0.001$], as well as the % of time spent in the open arms from 6.13% in the control group to 95.27% at the dose of 350 mg/kg [$F(6,29) = 224$; $p < 0.001$]. As expected for a positive control group, diazepam 3 mg/Kg *i.p.* also induced an increase in the % of entries and time spent in the open arms (Figures 2). Like diazepam, the decoction induced a significant reduction in the % of closed arm entries from 91.17% in the control group to 9.26% at the dose of 350 mg/kg [$F(6,29) = 124$; $p < 0.001$] and in the % of closed arms time from 93.86% in the control group to 2.6% at the dose of 350 mg/kg [$F(6,29) = 254$; $p < 0.001$] (Figure 3). The number of rearing and head dipping were also reduced both by diazepam and the decoction [$F(6,29) = 113$; $p < 0.001$] and [$F(6,29) = 14$; $p < 0.001$], respectively (Table 1).

Effect of *P. reticulatum* on SIH

The difference of temperature between the first three and the last three mice was reduced as expected by phenobarbital. The decoction of *P. reticulatum* produced the same effect in a dose dependent manner from 0.5°C in the control group to 0.10°C at the dose of 350 mg/kg [$F(6,29) = 4$; $p < 0.001$] (Figure 4). In addition, the decoction decreased their body temperature from 35.5°C in the control group to 31.55°C at the dose of 350 mg/kg [$F(6,53) = 54$; $p < 0.001$] (Figure 5).

Effect of *P. reticulatum* on OF

Like in the EPM test, the number of rearing was strongly decreased both by diazepam and by the decoction from 6.66 in the

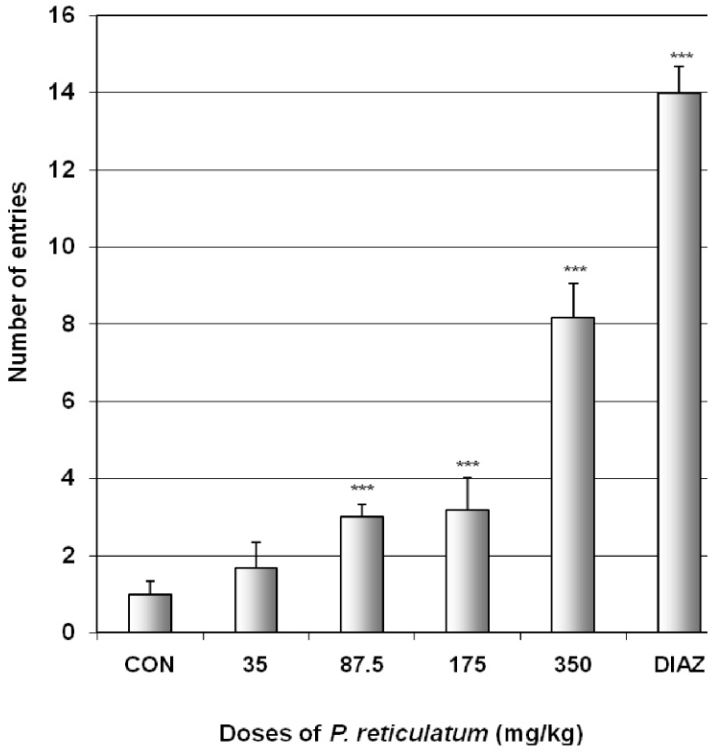

Figure 1: Effect of *P. reticulatum* on mice placed on EPM: Open arms entries.

The figure represents the number of open arms entries. N = 6 per dose, ***p < 0.001, ANOVA followed by Tukey (HSD). CON = distilled water. Diaz = diazepam 3 mg/kg

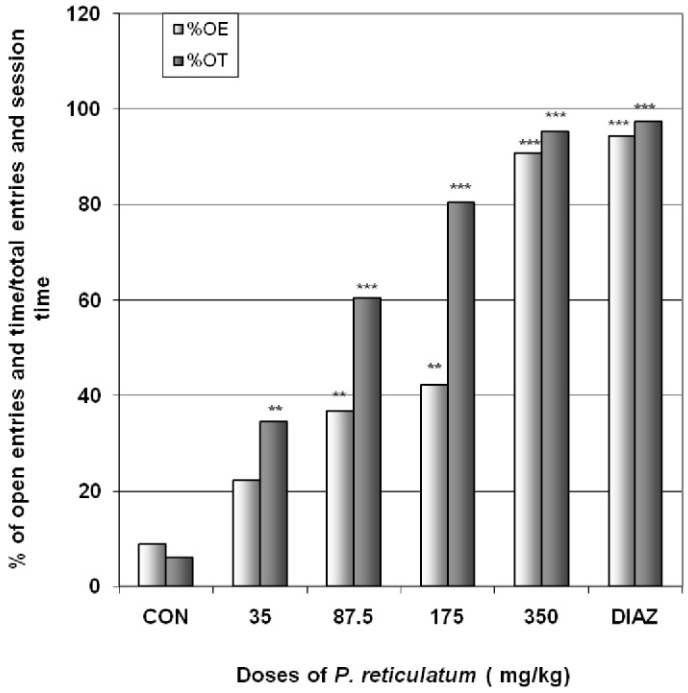

Figure 2: Effect of *P. reticulatum* on mice placed on EPM: Open arms entries and time.

The figure represents the % of open arms entries and time/total arms entries and time. N = 6 per dose, **p < 0.01, ***p < 0.001, ANOVA followed by Tukey (HSD). CON = distilled water. Diaz = diazepam 3 mg/kg

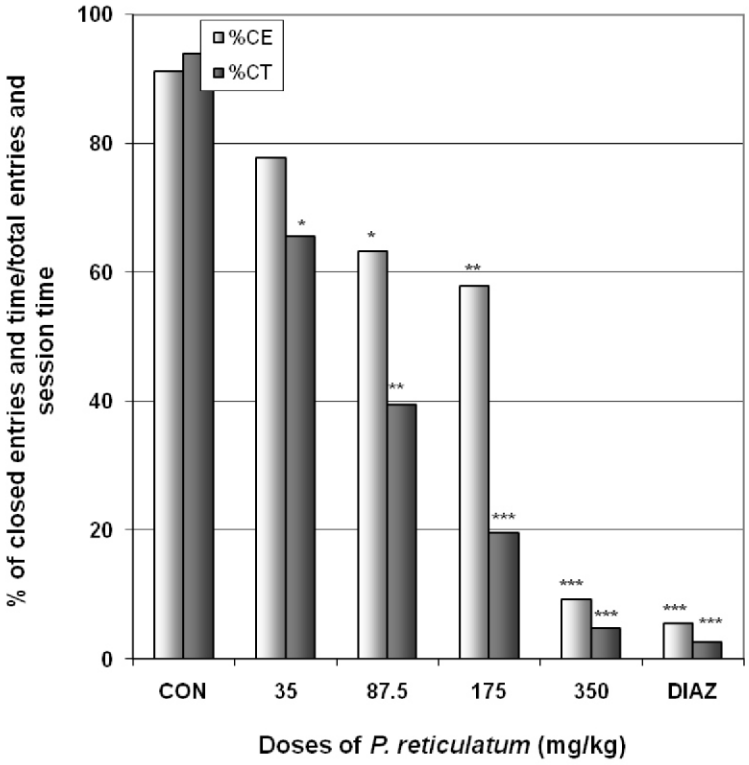

Figure 3: Effect of *P. reticulatum* on mice placed on EPM: Closed arms entries and time.

The figure represents the % of closed arms entries and time/total arms entries and time. N = 6 per dose, *p < 0.05, **p < 0.01, ***p < 0.001, ANOVA followed by Tukey (HSD). CON = distilled water. Diaz = diazepam 3 mg/kg

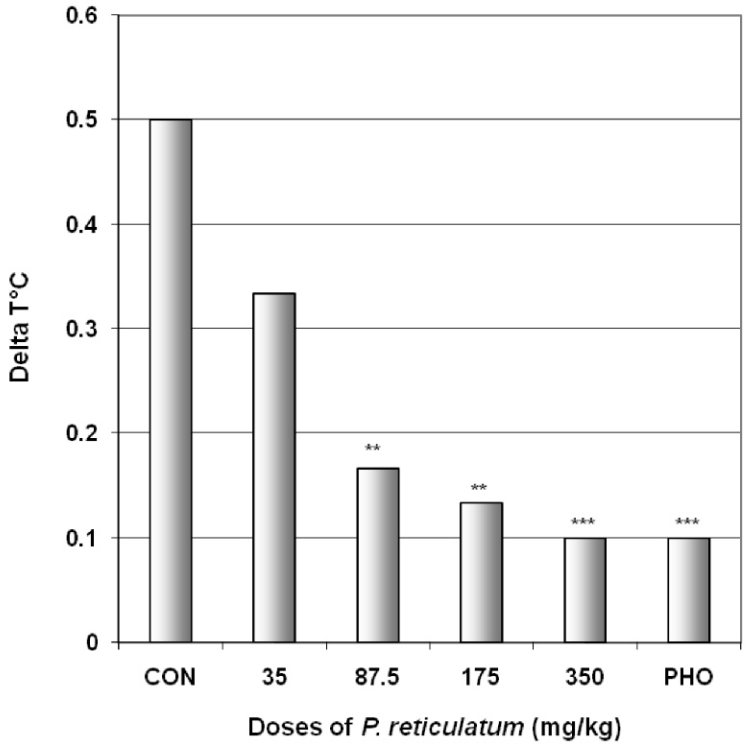

Figure 4: Effect of *P. reticulatum* on SIH in mice.

The figure represents the temperature difference (ΔT°C) between the first three mice and the last three mice. N = 10 per dose, **p < 0.01, ***p < 0.001, ANOVA followed by Tukey (HSD). CON = distilled water. PHO = Phenobarbital 20 mg/kg

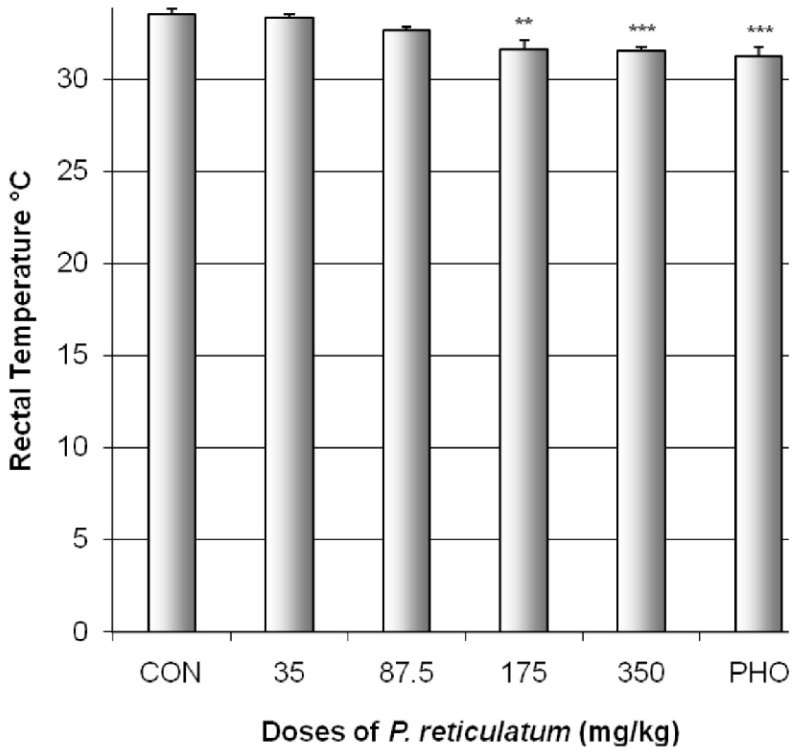

Figure 5: Effect of *P. reticulatum* on the body temperature in mice.

The figure represents the body temperature in the presence of the extract. Histograms are expressed as mean + S.E.M., n = 10 per dose, **p < 0.01, ***p < 0.001, ANOVA followed by Tukey (HSD). CON = distilled water. Pheno = Phenobarbital 20 mg/kg.

Table 1: The number of open arms entries, closed arms entries, rearing and head dipping on EPM

Doses of *P. reticulatum* (mg/kg)	Distilled water	35	87.5	175	350	Diazepam (3 mg/kg)
Closed arms entries	10.33±1.66	5.83±0.55	5.16±0.55*	4.33±1.00***	0.83±0.55**	0.83±0.27***
Total arms entries	11.33±1.16	7.50±0.66	8.16±0.88*	7.50±1.83***	9.00±1.00**	14.83±0.83***
Total arms time	274.50±8.66	291.83±5.16*	296.50±2.83**	249.83±3.44*	252.50±8.16	269.00±8.33
Rearing	15.50±0.71	7.66±1.14	5.33±0.66**	4.66±0.66**	1.00±0.66***	1.00±0.33***
Head dipping	11.83±3.94	8.16±0.47	4.83±0.55*	3.00±0.66**	1.00±0.33***	0.66±0.44***

Data are mean S.E.M, n = 6, * p < 0.05, ** p < 0.01, *** p < 0.001, ANOVA followed by Tukey (HSD).

control group to 2.83 at the dose of 350 mg/kg [F(6,29) = 72; p < 0.0001]. The decoction also decreased the mass of fecal boli [F(6,29) = 4; p < 0.003]. Controversially, the decoction increased the number of crossing from 17.66 in the control group to 39.16 at the dose of 350 mg/kg [F(6,29) = 27; p < 0.001]. The increase was also observed in the time spent by mice in the centre from 13.16 s in the control group to 52.83 s at the dose of 350 mg/kg [F(6,29) = 147; p < 0.001] (Table 2).

DISCUSSION

The decoction of P. reticulatum antagonized in a dose-dependent manner, the hyperthermia induced by stress. This antagonism, close to the effect of Phenobarbital, suggests the presence of anxiolytic-like activity of the plant, since anxiolytic drugs induce inhibition of SIH [22] [18] [23] and [24]. The presence of anxiolytic properties was confirmed in the EPM test where *P. reticulatum* increased the number of entries, the % of entries and time into the open arms, and reduced the % of closed arms time [17], [25] and [26]. The reduction of rearing and defecation by the decoction in EPM and OF tests also suggested the presence of anxiolytic properties [25] and [27]. In addition, since the closed and total arms entries and the head dipping in

Table 2: PThe number of rearing, crossing, grooming, centre time and quantity of fecal boli on OF.

Doses of *P. reticulatum* (mg/kg)	CON	35	87.5	175	350	Diazepam (0.3 mg/kg)
Rearing	6.66±1.33	4.83±1.22	4.16±1.16**	3.83±0.88**	2.83±0.88***	1.66±0.77***
Crossing	17.66±1.44	26.66±1.66	29.16±2.11*	35.00±2.66***	39.16±3.22***	52.66±6.55***
Grooming	0.83±0.55	1.16±0.66	2.00±1.00*	2.33±0.66**	3.83±0.55**	4.83±1.16***
Fecal boli (g)	0.54±0.07	0.23±0.17	0.15±0.13**	0.18±0.18***	0.10±0.13**	0.01±0.02***
Center time (s)	13.16±2.55	22.33±3.00	36.50±2.50	40.50±2.33*	52.83±3.16**	76.33±5.11***

Data are mean S.E.M, n = 6, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ANOVA followed by Tukey t (HSD).

EPM were reduced, the increase of crossing in OF suggested the increase of the exploration activity, but not the increase in the locomotion. The increase of the exploration activity suggested anxiolytic activities as anti-anxiety drugs decrease the stress-induced the inhibition of exploratory behaviour [21] [25], [27] and [28]. These anxiolytic properties could be mediated by some components in the decoction interacting with the benzodiazepine/$GABA_A$ receptors as agonists, with the 5-HT_{1A} receptors as agonists, with the NMDA receptors as antagonists, or with any other mechanisms [22], [24] and [29]. The reduction by the decoction of the closed and total arms entries in EPM test indicated a reduction of locomotion [30] that could suggest sedative properties in the plant. In the same experiment, *P. reticulatum* seemed to possess antipyretic properties which allowed the body temperature to fall in SIH test [20], [22] and [31].

CONCLUSIONS

The decoction of *P. reticulatum* possesses antipyretic and anxiolytic properties in mice. These properties could explain the use of this plant in traditional medicine in Africa, especially in Cameroon in the treatment of fever and anxiety. *P. reticulatum* could also be a new potential source of anxiolytic drugs.

List of abbreviations:

Closed Entries (CE), Negative Control (CON), Close Time (CT), Diazepam (Diaz), Elevated Plus Maze (EPM), Open Entries (OE), *Piliostigma reticulatum* (*P. reticulatum*), Open Field (OF), Open Time (OT), Phenobarbital (Pheno), Stress-Induced Hyperthermia (SIH), .

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