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The aqueous extract of *Terminalia macroptera* possess anxiolytic and antipyretic activities in mice

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ABSTRACT

Terminalia macroptera is a plant used in traditional medicine in many countries in Africa to treat headaches, epilepsy, anxiety, agitation, depressions, etc. *Terminalia macroptera* is found in Cameroon. Elevated plus maze, stress-induced hyperthermia and open field tests were used to determine anxiolytic properties of the plant. The doses of the plant used were 20, 50, 100 and 200 mg/kg. *T. macroptera* showed antipyretic properties by reducing in a dose dependent manner the body temperature that changed from 30.73 ± 0.70°C to 28.28 ± 0.41°C at the dose of 200 mg/kg. *T. macroptera* showed also anxiolytic activities in the three tests. In stress-induced hyperthermia test, *T. macroptera* antagonised dose dependently increase of temperature. ΔT° decrease from 1.20°C in the control group to 0.26°C at the dose of 200 mg/kg. In the elevated plus maze test, *T. macroptera* 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, *T. macroptera* increased the number of crossing, centre time and grooming and reduced the number rearing and defecation. The results lead to conclude that the decoction of *T. macroptera* possesses anxiolytic and antipyretic properties in mice and could really be helpful in the treatment of fever and anxiety in Traditional medicine in Cameroon.

INTRODUCTION

Anxiety disorders are the most prevalent mental disorder with very high co-morbidity [1-2]. With a complex etiology that is not fully known, anxiety disorders have severe impact on quality of life [3-4]. 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. *Terminalia macroptera* Guill And Perr (Combretaceae) (*T. macroptera*) is a medicinal plant used in Cameroon to treat many diseases. *T. macroptera* grow in many countries in Africa. *T. macroptera* is a 13 meters high tree found Senegal, Cameroon, Democratic Congo, Ghana, Mali, Niger, Nigeria, Sudan and Uganda [6]. Barks, leaves and roots of the plant are used against head aches, migraines, epilepsy, depressions, asthenia, cold, fever, hypertension, hepatitis, gastritis, colic, leprosies, tuberculosis, syphilis and as tranquilizer [6-7]. According to Cameroonian traditional healers, roots of *T. macroptera* are used in the treatment of anxiety, agitation, epilepsy and fever. Previous experiments have shown that *T. macroptera* possesses antibacterial, antiplasmodial and anti-Neisseria gonorrhoeae activities [8-11]. Chemical study revealed the presence of tannins, triterpene and phenolic compounds in *T. macroptera* [12-14]. *Since T. macroptera* is used to treat fever, anxiety and to tranquilize agitated patients, our study was undertaken to look for anxiolytic and antipyretic properties of this medicinal plant.

MATERIAL AND METHODOLOGY

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 extract. 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.

Plant material

Barks of roots of *T. macroptera* used were collected in Cameroon in the immediate vicinity of Ngaoundéré, during the dry season. A voucher specimen of *T. macroptera* (3053/SRFK) were *authenticated and deposited at the National Herbarium of Cameroon in Yaoundé*. The dried barks of roots of *T. macroptera* were ground. 200 g of the plant powder were boiled in 1000 ml of distilled water for 20 min. After cooling, the supernatant was collected, filtered with a Whatman n°1 filter paper and was evaporated to dryness using a Rota vapor at a temperature of 50°C. The aqueous extract obtained was 12 g (yield: 6%). The extracts were diluted in distilled water and were administered intraperitoneally (i.p.) 1 h before the test. The following doses were used: 20, 50, 100 and 200 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 *the extracts* 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 [15-17]. 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 extracts 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" [18-19]. 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 [20-21]. 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 Dunnett (HSD) were done. A value of $P<0.05$ was considered significant.

RESULTS

Effect of *T. macroptera* in EPM test

The administration of the extract resulted in a significant increase in the number of entries into open arms from 0.66 in the control group to 2.83 at the dose of 200 mg/kg [$F(6,29) = 14$; $p < 0.0001$] (Table 1). The percentage of entries into open arms was dose dependently increased from 10.5% in the control group to 70.8% at the dose of 200 mg/kg [$F(6,29) = 247$; $p < 0.0001$], as well as the % of time in open arms from 0.2% in the control group to 88.1% at the dose of 200 mg/kg [$F(6,29) = 247$; $p < 0.0001$]. 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 (Figure 1). Like diazepam, the extract induced a significant reduction in the percentage of entries into closed arm from 89% in the control group to 29% at the dose of 200 mg/kg [$F(6,29) = 24$; $p < 0.0001$] and in the percentage of time in closed arms from 99% in the control group to 11% at the dose of 200 mg/kg [$F(6,29) = 24$; $p < 0.0001$] (Figure 2). The number of rearing and head dipping were also reduced both by diazepam and the extract [$F(6,29) = 113$; $p < 0.0001$] and [$F(6,29) = 38$; $p < 0.0001$], respectively (Table 1).

Effect of *T. macroptera* in SIH test

$\Delta T°$, the difference of temperature between the first three and the last three mice was reduced as expected by phenobarbital. The extract of *T. macroptera* produced the same effect in a dose dependent manner from 1.2°C in the control group to 0.26°C at the dose of 200 mg/kg [$F(6,29) = 28$; $p < 0.0001$] (Figure 3). In addition, the extract decreased their body temperature from 30.73°C in the control group to 28.28°C at the dose of 200 mg/kg [$F(6,53) = 27$; $p < 0.0001$] (Figure 4).

Effect of *T. macroptera* in OF test

In OF, like in the EPM test, the number of rearing was strongly decreased both by diazepam and by the extract from 15.83 in the control group to 3 at the dose of 200 mg/kg [$F(6,29) = 23$; $p < 0.0001$]. The extract also decreased the mass of fecal boli from the dose 50 mg/kg [$F(6,29) = 4$; $p < 0.003$]. Controversially, the

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

Doses of *T. macroptera* (mg/kg)	Distilled water	20	50	100	200	Diazepam (3 mg/kg)
Open arms entries	0.67±0.44	1.00±0.33	1.50±0.50*	1.67±0.77*	2.83±0.55***	3.00±0.33***
Closed arms entries	5.66±0.88	4.83±0.55	3.00±1.33*	1.83±0.55***	1.16±0.55**	0.66±0.44***
Total arms entries	6.33±0.77	5.83±0.55	4.50±1.16*	3.50±0.83***	4.00±1.00**	3.66±0.44***
Rearing	14.00±0.85	10.16±0.76	9.16±0.83**	7.5±0.83**	2.33±1.00***	0.66±0.44***
Head dipping	5.66±0.77	4.66±0.57	4.00±0.66*	1.83±0.88**	1.16±0.27***	0.0±0.0***

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

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

Doses of *T. macroptera* (mg/kg)	CON	20	50	100	200	Diazepam (0.3 mg/kg)
Rearing	15.83±2.16	10.00±3.00	8.00±1.00**	5.83±1.50**	3.00±1.00***	1.0±1.0***
Crossing	17.66±1.33	21.83±3.55	27.33±4.00*	37.16±3.44***	52.00±5.33***	63.66±3.33***
Grooming	1.33±0.44	1.50±0.50	2.33±0.66*	2.66±0.66**	2.66±0.66**	3.5±1.16***
Fecal boli (g)	0.49±0.10	0.34±0.13	0.17±0.14**	0.15±0.18***	0.14±0.14**	0.00±0.00**
Center time (s)	6.83±0.88	5.5±0.83	9.33±0.77	12.00±1.66*	47.16±8.11**	89.50±3.66***

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

extract increased the number of crossing from 17.66 in the control group to 52 at the dose of 200 mg/kg [$F(6,29) = 76$; $p < 0.0001$]. The increase was also observed in the time spent by mice in the centre from 6.83 s in the control group to 47.16 s at the dose of 200 mg/kg [$F(6,29) = 277$; $p < 0.0001$] (Table 2).

DISCUSSION

The extract of T. macroptera 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] [23-24]. The presence of anxiolytic properties was confirmed in the EPM test where *T. macroptera* increased the number of entries, the % of entries and time into the open arms, and reduced the % of closed arms time [17], [25-26]. The reduction of rearing and defecation induced by the extract in EPM and OF tests also suggested the presence of anxiolytic properties [25], [27]. In addition, since the closed and total arms entries and the head dipping in 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], [27-28]. These anxiolytic properties could be mediated by some components in the extract 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], [29]. The reduction by the extract 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, *T. macroptera* seemed to possess antipyretic properties which allowed the body temperature to fall in SIH test [20], [22], [31].

CONCLUSION

The extract of *T. macroptera* 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. *T. macroptera* could also be a new potential source of anxiolytic drugs.

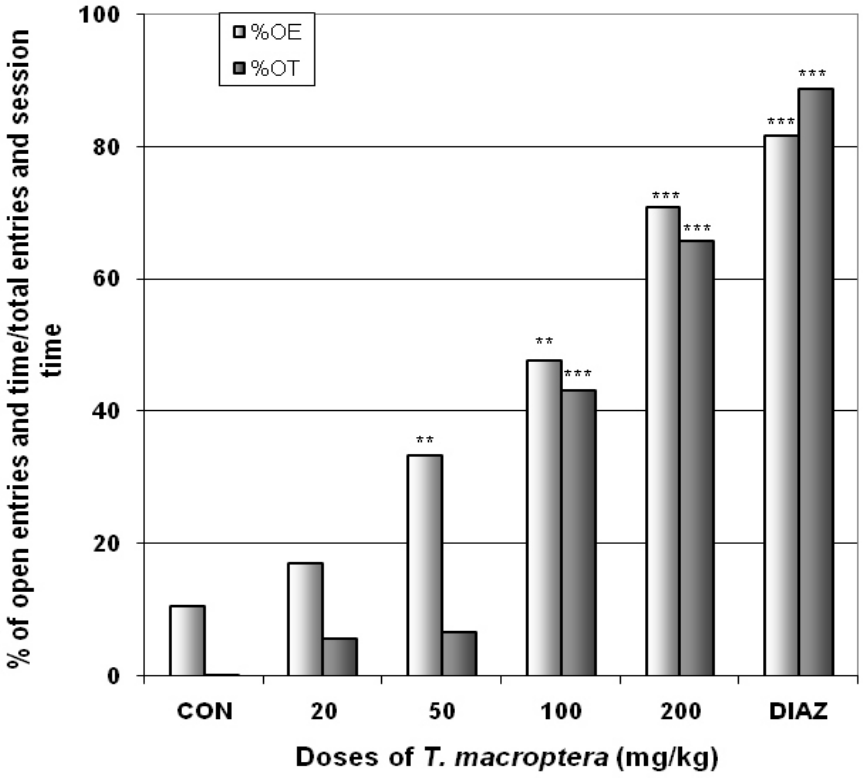

Figure 1: Effect of T. macroptera in open arms entries and time (EPM)
The figure represents the % of open arms entries and time/total arms entries and time. N = 6 per dose, ***p < 0.001, ANOVA followed by Tukey (HSD). CON = distilled water. Diaz = diazepam 3 mg/kg

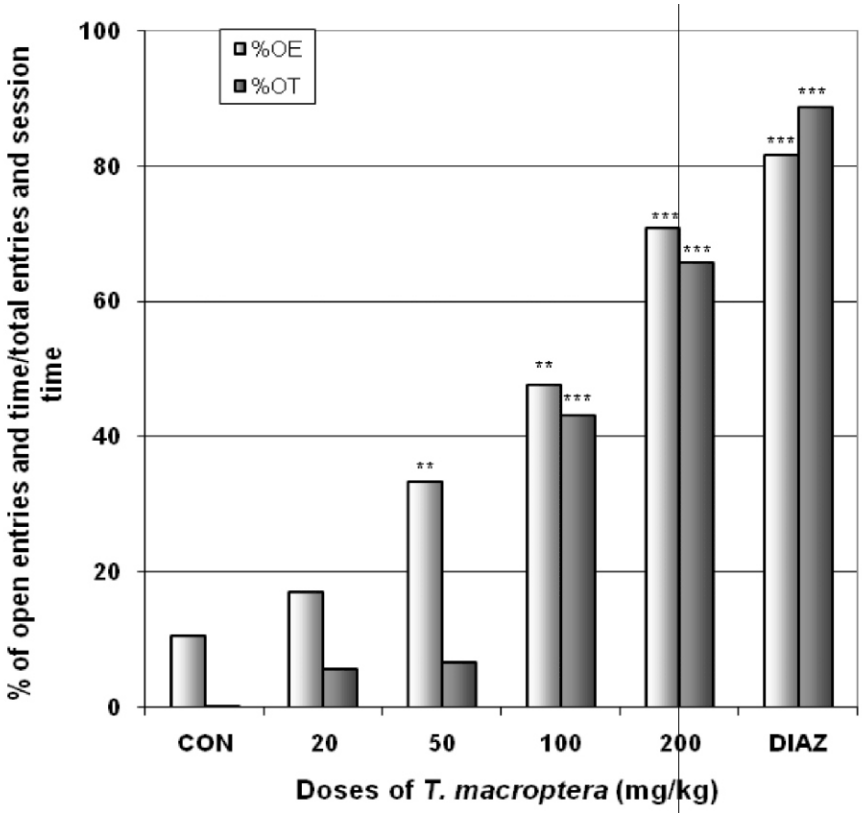

Figure 2 : Effect of T. macroptera in closed arms entries and time (EPM)
The figure represents the % of closed arms entries and time/total arms entries and time. N = 6 per dose, *p < 0.05, ***p < 0.001, ANOVA followed by Tukey (HSD). CON = distilled water. Diaz = diazepam 3 mg/kg

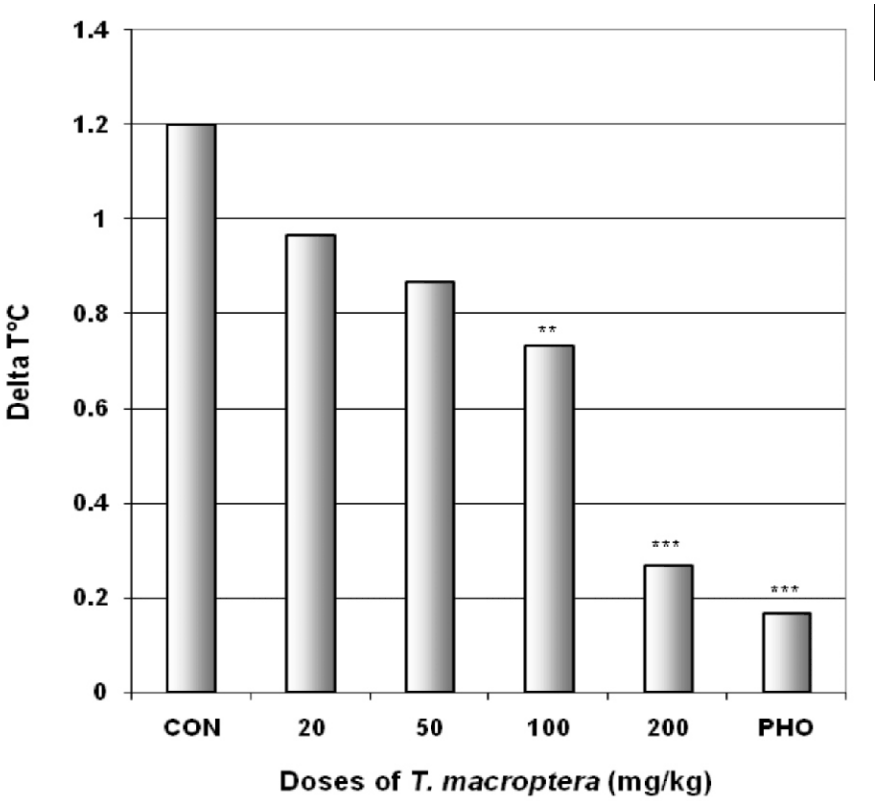

Figure 3: Effect of T. macroptera on SIH in mice.
The figure represents the temperature difference (T°C) between the first three mice and the last three mice. N = 10 per dose, **p < 0.01, ***p < 0.001, ANOVA followed by Tukey (HSD). CON = distilled water. Pheno = Phenobarbital 20 mg/kg

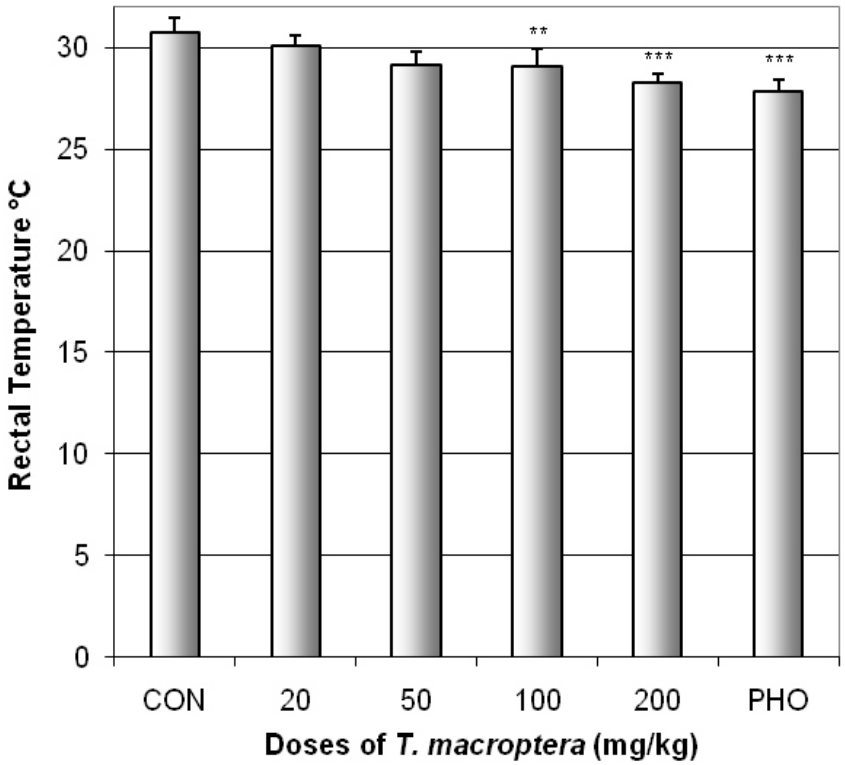

Figure 4: Effect of T. macroptera on the body temperature in mice.
The figure represents the body temperature in the presence of the extract. Histograms are expressed as mean + S.E.M., n = 10 per dose, **p < 0.01, ***p < 0.001, ANOVA followed by Tukey (HSD). CON = distilled water. Pheno = Phenobarbital 20 mg/kg.

List of abbreviations: Closed Entries (CE), Negative Control (CON), Close Time (CT), Diazepam (Diaz), Elevated Plus Maze (EPM), Open Entries (OE), *Terminalia macroptera* (*T. macroptera*), Open Field (OF), Open Time (OT), Phenobarbital (Pheno), Stress-Induced Hyperthermia (SIH).

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