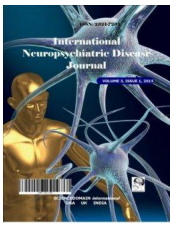

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Antipyretic and Anxiolytic Properties of Aqueous Extract of *Cymbopogon giganteus* in Rats Model

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ENB and SP designed the study. Author GST performed the statistical analysis. Authors ENB and SP wrote the protocol, and wrote the first draft of the manuscript. Authors NK, AKK, FCMO and JSKN managed the analyses of the study. Author SP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This present study aimed to assess the antipyretic and anxiolytic activities of *Cymbopogon giganteus* decoction in rats.
Place and Duration of Study: Experiments were conducted in the laboratory of Biological Sciences for bioassays and laboratory of Chemistry for phytochemical screening, Faculty of Science, University of Ngaoundere, Cameroon from August to October 2013.
Methodology: For its medicinal vertues, the decoction of this plant was evaluated for its anxiolytic properties using Elevated plus maze, stress-induced hyperthermia and open field tests in rats. The decoction of *C. giganteus* was administrated orally to rats at doses of 34, 85, 170 and 340 mg/kg.

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Results: The decoction of *C. giganteus* exhibited a significant anxiolytic activities in the three tests used. The plant decoction significantly reduced the body temperature in rats ranging from 36.83°C in the control to 35.43°C at the dose of 340 mg/kg. *C. giganteus* decoction antagonised the increase of temperature in which ΔT°C decreased from 1.06°C in the control to 0.30°C at 340 mg/kg dose in stress-induced hyperthermia test. The decoction of *C. giganteus* also significantly increased the number of entries ant time spent into the open arms and reduced the percentage of entries and time into the closed arms in the elevated plus maze test. Moreover in this test, the number of rearing and head dipping were also decreased. In the open field test, the decoction of the plant significantly reduced the number rearing and defecation and increased the number of crossing, centre time and grooming.
Conclusion: *C. giganteus* decoction possesses antipyretic and anxiolytic activities in rats and could be uses in traditional medicine to treat fever and anxiety.

Keywords: Antipyretic; anxiolytic; decoction; Cymbopogon giganteus.

1. INTRODUCTION

Generalised anxiety disorder is defined as a syndrome of ongoing anxiety and constancy general worry difficult to control about many events or thoughts that the patient generally recognises as excessive and inappropriate [1]. It is characterized by an unpleasant state of inner turmoil, often accompanied by nervous behavior, somatic complaints and rumination [2]. Anxiety disorder implied also a subjective wellbeing, physical health and longevity leading to a critical decrease the quality of life inducing several psychosomatic diseases [3]. With very high co-morbidity, anxiety disorders are the most prevalent mental disorder and its prevalence on the whole life in general population would be around 5% [2,3]. The comorbidity is high in the generalized anxiety disorder, particularly with the others anxious disturb and mood disorders [4].

Currently available drugs including benzodiazepines, β-blocking or the factors acting on the serotoninergic neurotransmission drugs, although effective were not specifically developed for anxiety disorder treatment and possess undesirable effects such as sedation and dependence [5]. Besides, overall clinical outcomes and the standard of care for most patients under treatment are still far from optimal. Moreover, current treatments have modest efficacy and many patients do not respond or are unable to tolerate pharmacological approaches. Therefore, the research for newer, better-tolerated and more efficacious for anxiety treatments is highly needed and encouraged and plant pharmacologically active compounds could provide an alternative for treatment of different mood and neurological disorders.

Nowadays, traditional medicine explores a wide variety of plant species to find new medicine to treat anxiety with less undesirable effects. Previously, several plant products with potent antidepressant, anxiolytic and memory enhancement activity have been reported in the literature [6-11]. In Cameroon, some plants including *Psorospermum febrifugum, Nauclea latifolia* and *Terminalia macroptera* have proven their anxiolytic properties in rodents [12-14].

The plant species *Cymbopogon giganteus* Chiov. (Poaceae) is a perennial grass found in tropical and savannah regions of Western Africa. The plant is used in folk medicine against skin disorders and exhibited strong antimalarial activity against chloroquine resistant *Plasmodium* [15]. *C. giganteus* is also used against mental illness, broncho-pulmonary affections, bilharzias, jaundice, cold, conjunctivitis, migraine, dermatoses, rheumatic pains, childhood coughs and hepatitises [16]. In Cameroon, the plant is used to treat Cough, arterial hypertension and showed high activity against gram positive and gram negative bacteria [17]. Cytotoxicity, anti-trypanosomal and antiplasmodial of the plant was reported by Kpoviessi et al. [18]. Among the plant belonging to the genus of *Cymbopogon*, no previous work was done on *C. giganteus* on brain disorder diseases. For its large medicinal properties listed above, this present investigation aimed to evaluate anxiolytic and antipyretic properties of this medicinal plant in rats.

2. MATERIALS AND METHODS

2.1 Plant Material

2.1.1 Harvesting and processing

The leaves of *C. giganteus* were harvested in september 2011 at Home, a village located at about 90 km from Touboro sub-division, North

region of Cameroun and identified by the herbalist of the Faculty of Science, University of Ngaoundere, Cameroon. The identity of the plant was confirmed under the registration number of 8256 HNC at the National Herbal of Yaoundé, Cameroon where voucher was deposit. The leaves of the plant were cleaned with tap water, dried under the shade in the room temperature and pulverized into powder until the particles passed through a 0.4 mm mesh size sieve. The powder was stored in the glass bottles until needed for uses.

2.1.2 Preparation of the decoction of *Cymbopogon giganteus*

For plant extraction, 10 g of the leaf plant powder was introduced into a beaker containing 100 ml of distilled water and boiled on a regulated hotplate set at 100°C during 20 min. After cooling, the mixture was filtered using paper Wattman N°1. The yellow clearly filtrate obtained, constituted the initial solution of 100 mg/ml concentration. From that initial solution, three other concentrations were made by dilution down of 1/2, 1/4 and with the 1/10 representing doses of 50, 25 and 10 mg/kg, respectively, using distilled water. To obtain the dry matter contained in the decoction, 50 ml of the initial filtrate solution obtained was evaporated at 60°C in the Oven apparatus. However, 1.70 g of dry plant extract was obtained representing 17% extraction yield.

2.1.3 Phytochemical screening

The phytochemical screening of *C. giganteus* decoction was carried out using the methods performed by Harbone [19] and Trease and Evans [20] for the determination of the principal chemical groups including alkaloids, flavonoids, tannins, saponins, phenols, anthroquinons, sterols and triterpenes.

2.2 Animal Material

For "in vivo" tests, wistar rats used were reared in the animal house of the Department of Zoology and Animal Physiology of the University of Buea, Cameroon. In each plexiglass cage used, rats were maintained in batch of 4 in each plexiglass cages (40 cm × 28 cm × 18 cm in size) in where food and of water are continuously supplied to the animals. The rats were maintained under the ambient temperature approximately about 25°C under 12 h light and 12 h darkness cycle. Experiments were conducted following the nationally (N°.FWA-IRB00001954) and US guideline principles for laboratory animal use and care internationally accepted.

2.3 Bioassays

2.3.1 Stress-Induced Hyperthermia (SIH) test

Method described by Borsini et al. [21], and reviewed by Lecci et al. [22] was followed to assess effect of *C. giganteus* decoction on SIH in Wistar rat. Indeed, groups of 10 rats were formed and marked. Rats within a given group were removed from the cage one after another in a precise order and were consecutively treated (1 min interval) with distilled water (negative control group), phenobarbital administrated at 20 mg/kg, intraperitoneally (positive control group) and 4 doses of 34, 85, 170 and 340 mg/kg of *C. giganteus* decoction for the tested groups. After 1 h post treatment, rats were once again consecutively removed from the cage and their body temperature (rectal temperature) was recorded using HUGO SACHS H11 thermometer. In fact, “Rats removed earlier had a lower compared to those removed later body temperature among rats in the same cage”. The difference between the body temperature of the first three rats and the body temperature of the last three rats constitutes the stress- induced hyperthermia. Then, mean temperature of the first three mice was compared to the mean temperature of the last three mice in each group.

2.3.2 Elevated Plus Maze (EPM) test

The method performed by Bourin et al. [23] was used to evaluate the anxiolytic effect of *C. giganteus* decoction in Wistar rat. Elevated to 50 cm high from the floor level, EPM apparatus was designed with two closed arms (16 cm x 5cm x 10 cm), two open arms (16 cm x 5 cm) and extended from a common central platform (5 cm x 5 cm). Groups of 6 rats each were formed and rats in each group was treated with distilled water (negative control group), diazepam at 3 mg/kg (positive control group) and with different doses of 34, 85, 170 and 340 mg/kg of *C. giganteus* decoction for the tested groups, ranging respectively from group 1 to group 6. After 1h post-treatment, rats were individually and consecutively placed on the centre of EPM facing an open arm. For 5 min observation of each animal, conventional parameters including the number of entries into the open or closed arms and the time spent on either open or closed arms as well as in the centre of the platform were

recorded. Some ethological parameters such as head dipping and rearing were also recorded.

2.3.3 Open field (OF) test

To evaluate the effects of *C. giganteus* decoction on anxiolytic and exploratory activities, Prut & Belzung [24] method was used. The open field apparatus used was a 40 cm x 40 cm x 45 cm wooden square box, with floor divided into 16 smaller squares of equal dimensions (10 cm x 10 cm). As a preliminary, six group of six rats each were made of and rats in each group was treated with distilled water (negative control group), diazepam at 3 mg/kg (positive control group) and with different doses of 34, 85, 170 and 340 mg/kg of *C. giganteus* decoction, ranging from group 1 to group 6, respectively. One hour after treatments, each rat was placed in the centre of the box and observed for 5 min. The number of rearing (number of times the animal stood on its hind legs), crossing (number of square floor units entered), grooming, defecation and centre time were recorded.

2.4 Statistical Analysis

Pharmacological parameter data were expressed in mean ± standard error of means (SEM). Data were submitted to variance analysis (ANOVA) using XL-Stat V. 2007 software. Dunnett (HSD) test was done to compare the negative control with the tested groups and positive control. A value of $P<0.05$ was considered significant.

3. RESULTS

3.1 Phytochemical Characterization of *Cymbopogon giganteus* Decoction

In the decoction of *C. giganteus* the phytochemical screening test revealed the presence of alkaloids, flavonoids, tannins, steroids, terpenoids, saponins, phenolic compounds and anthraquinons (Table 1).

3.2 Anxiolytic Properties of *Cymbopogon giganteus* Decoction

3.2.1 Effect of *C. giganteus* in SIH test

The effect of the *C. giganteus* decoction in the body temperature reduction in rats is presented in Fig. 1. The reduction of body temperature in rats was dose-dependent and reduced with the increasing doses. In comparison with the negative, the decoction of the plant significantly reduced the body temperature of the rats at doses of 170 mg/kg ($P<0.05$) from 36.83°C in the control to 35.55°C and 340 mg/kg ($P<0.01$) from 36.83°C in the control to 35.43°C. The phenobarbital used at dose of 20 mg/kg significantly ($P<0.001$) reduced the body temperature of rats ranging from 36.83°C in the control to 34.53°C with the phenobarbital.

The Fig. 2 shows a significant reduction ($P<0.01$) and dose dependent on the stress induced hyperthermia which varies from 1.06°C in the negative control rat group to 0.3°C for those treated at 340 mg/kg with *C. giganteus* decoction. The SIH is 0.10°C in the group of rats treated with phenobarbital (20 mg/kg). Plant extract tested at 340 mg/kg and phenobarbital induced a reduction in SIH of 71.7% and 90.6%, respectively.

Table 1. Phytochemical composition of *Cymbopogon giganteus* decoction

Phytochemical components	Status
Alkaloids	+
Flavonoids	+
Tanins	-
Steroids	+
Terpenoids	+
Saponins	+
Phenolic compounds	+
Anthroquinones	+

- = absent, + = present

3.2.2 Effect of *Cymbopogon giganteus* on elevated plus maze (EPM)

The decoction of *C. giganteus* administered at 340 mg/kg significantly increased the number of open arms entries Table 2. At that dose, the number of open arms entries increased significantly ($F_{(5,30)}=12$; $p<0.0001$) from 5.8 entries in control rat group to 38 entries with the increasing entries of 555.2%. Diazepam induced also a significant increase in open arms entries with 49.8 entries recorded. From the table, a significant decrease in the number of closed arms entries was recorded with the *C. giganteus* extract and was dose-dependent way. The number of closed arms entries of the labyrinth passed significantly ($F_{(5,30)}=24$; $p<0.0001$) from 7 entries in the control rat groups to 2.3 and 2 entries in the rat groups treated by the decoction of *C. giganteus* with 170 et 340 mg/kg, respectively. The diazepam (3 mg/kg) also induced a significant reduction in the number of

entries in the closed arms of the labyrinth of 1.6 entries compared to 7 entries recorded in the negative control. Compared to the negative control, the decoction of *C. giganteus* tested at 340 mg/kg and diazepam significantly reduced the closed arms entries of 67.8 and 77% respectively Fig. 3.

The number of "rearing" varied significantly ($F_{(5,30)}$=15 ; p<0.0001) from 12.8 in the negative control rat groups to 4.6 and 4 in the rat groups treated with doses of 170 and 340 mg/kg of thedecoction of *C. giganteus,* respectively. The number of "head-dipping" of 5.6 recorded in the negative control decreased significantly

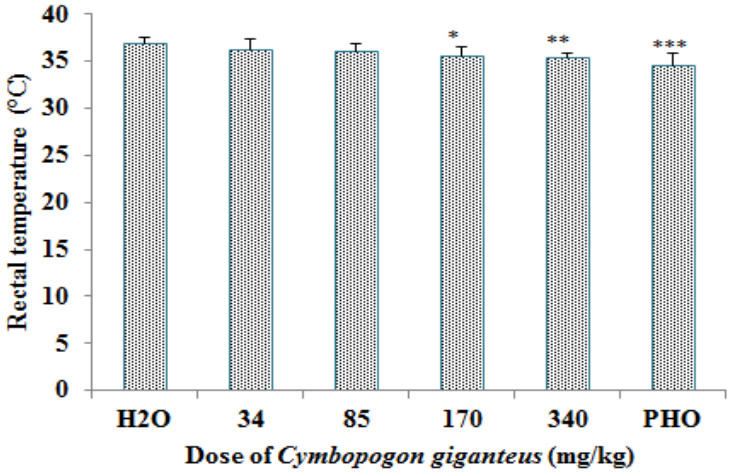

Fig. 1. Effect of *Cymbopogon giganteus* decoction on the body temperature in rats. Histograms are expressed as mean ± S.E.M., n =10 per dose, *P<0.05; **p < 0.01 and ***p < 0.001, ANOVA followed by Tukey test (P=.05). H2O = distilled water. PHO = Phenobarbital 20 mg/kg

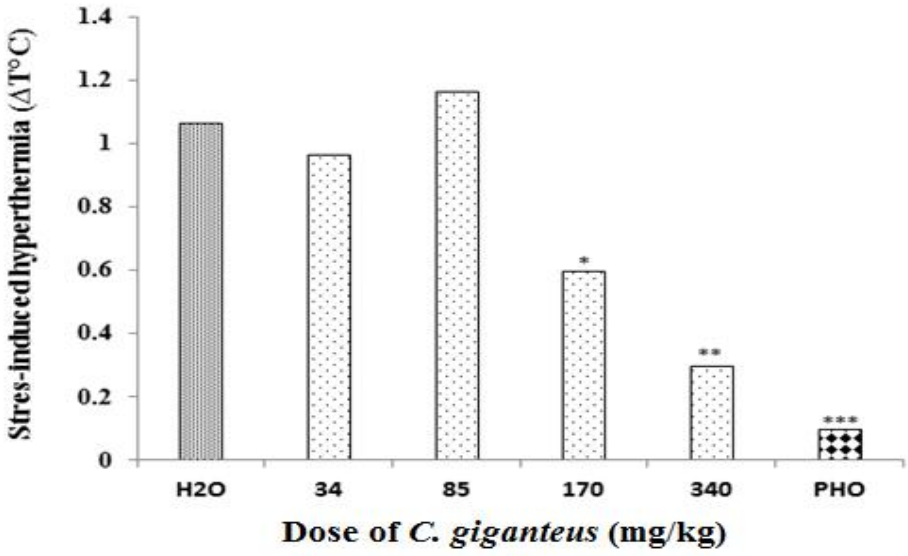

Fig. 2. Effect of *Cymbopogon giganteus* on Stress-Induced Hyperthermia in Wistar rat. The figure represents the temperature difference (T°C) between the first three rats and the last three rats. n = 10 per dose, *p < 0.01, **p < 0.01, ***p < 0.001, ANOVA followed by Dunnett (HSD). H2O = distilled water. PHO= Phenobarbital (20 mg/kg)

($F_{(5,30)}$=15; p<0.0001) of 2 in the rat group treated with 340 mg/kg of the decoction of *C. giganteus* decoction. The number of "stretched awaits posture" passed significantly from 2.3 in the negative control rat group to 0.3 and 0.0 in the rat group treated 340 mg/kg of the plant decoction and the diazepam.

3.2.3 Effect of *Cymbopogon giganteus* in open field test

The results showed a significant ($F_{(5,30)}$=59 ; p<0.0001) increase in the number of "grooming" ranging from 2.5 in the negative control to 5.1 and 6.0 in the rat groups treated with doses of 170 and 340 mg/kg of the plant decoction, respectively. The Diazepam (3 mg/kg) significantly increased the number of "grooming" up to 7.1 compared to the negative control. The number of crossing varied also significantly ($F_{(5,30)}$=21.2 ; p<0.0001) from 28.6 in the negative control to 57 in the rat groups treated with 170 mg/kg of plant decoction. The number of "crossing" also increased significantly to 65.5 in the rat groups' treated with the Diazepam (3 mg/kg). The number of "rearing" decreased considerably ($F_{(5,30)}$=12.9; p<0.0001) from 27.5 in the negative rat groups to 13.6 and 11.6 in the group of rats treated with plant decoction at doses of 170 and 340 mg/kg, respectively. This number was reduced to 8.5 in the group of rats treated by the diazepam (3 mg/kg).

A significant ($F_{(5,30)}$=14; p<0.0001) variation in the number of freezing reduction ranging of 2.5 in thein the negative control rat group to 0.6 s in both the tests groups treated at 340 mg/kg of the plant decoction and the Diazepam (3 mg/kg). The time spent in the center increased significantly ($F_{(5,30)}$=25.7; p<0.0001) from 4.1 s in the group of rats receiving distilled water (negative control) to 9.3s and 13.5s in the groups receiving 340 mg/kg of plant decoction and the Diazepam (3 mg/kg), respectively.

Table 2. The number of open arms entries, closed arms entries, rearing and head dipping on EPM

Parameters	Doses of *Cymbopogon giganteus* (mg/kg)						F-value
	Distilled water	34	85	170	340	DZP (3 mg/kg)	
Open arms entries	1,1±0,1	1,3±0,2	1,6±0,3	2,3±0,4	3,6±0,3^{c}	3,6±0,4^{c}	12.0***
Closed arms entries	7±0,6	4,6±0,5^{b}	2±0,4^{c}	2,3±0,4^{c}	2±0,2^{c}	1,6±0,2^{c}	24.0***
Total arms entries	8,1±0,7	5,9±0,7	3,6±0,7^{c}	4,6±0,8^{c}	5,6±0,5^{a}	5,2±0,6^{b}	7.30**
Head dipping	5,6±1,1	3,0±0,6^{c}	2,5±0,8^{c}	2,3±0,4^{c}	2±0,6^{c}	1,5±0,5^{c}	15.0***
Rearing	12,8±1,6	11,3±1,6	6,5±0,5^{b}	4,6±0,8^{b}	4±0,7^{b}	2,5±0,2^{b}	15.0***

*Data are mean ± standard error of the mean, n = 6, *** p < 0.001, ANOVA followed by Dunnett (HSD)*

Table 3. The number of rearing, crossing, grooming, freezing centre time and quantity of fecal boli on open file

Parameters	Distilled water	Doses of *Cymbopogon giganteus* (mg/kg)				DZP (3mg/kg)	$F_{(5,30)}$
		34	85	170	340		
Grooming	2,5 ± 0,2	3,8 ± 0,4	4,6 ± 0,3^{b}	5,1 ± 0,4^{b}	6 ± 0,5^{c}	7,1 ± 0,6^{c}	59.0***
Crossing	28,6 ± 2	39,8 ± 2^{a}	50,6 ± 1,8^{c}	57± 3,1^{c}	55,1 ± 4,5c	65,5 ± 2,7^{c}	21.2***
Rearing	27,5 ± 2,8	22,1 ± 2	20,5 ± 2,8	13,6 ± 0,8^{c}	11,6 ± 0,9^{c}	8,5 ± 0,5^{c}	12.9***
Freezing	2,5±0,2	2.00±0,2	1,5±0,2^{a}	1,5±0,2^{a}	0,6±0,2^{c}	0,6±0,2^{c}	14.0***
Freezing time	28,3±2,4	12,8±0,9^{c}	10,8±0,7^{c}	8,1±0,6^{c}	5,1±0,4^{c}	3,8±0,6^{c}	10.3***
Centre time (s)	4,1 ± 0,4	4,6 ± 0,4	5,1 ± 0,6	5,6 ± 0,8	9,3 ± 0,7^{c}	13,5 ± 1^{c}	25.7***
Fecal boli (g)	0,2±0	0,1±0,1	0,1±0,1	0±0,1^{a}	0±0^{b}	0±0^{b}	4.0***

*Data are mean ± standard error of the mean, n = 6, *** P<0.001, ANOVA followed by Dunnett (HSD). DZP= Diazepam*

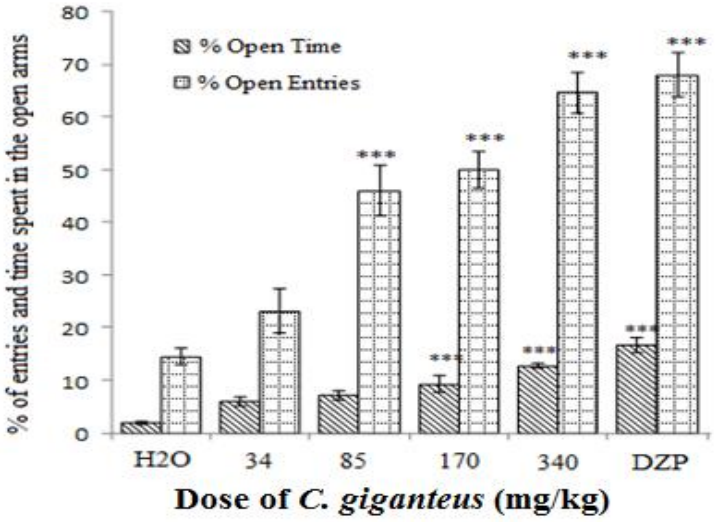

Fig. 3. Effect of *C. giganteus* in open arms entries and time (Elevated Plus Maze), n = 6 rats per dose, ***p < 0.001, ANOVA followed by Tukey (HSD). H2O = distilled water. DZP = diazepam 3 mg/kg

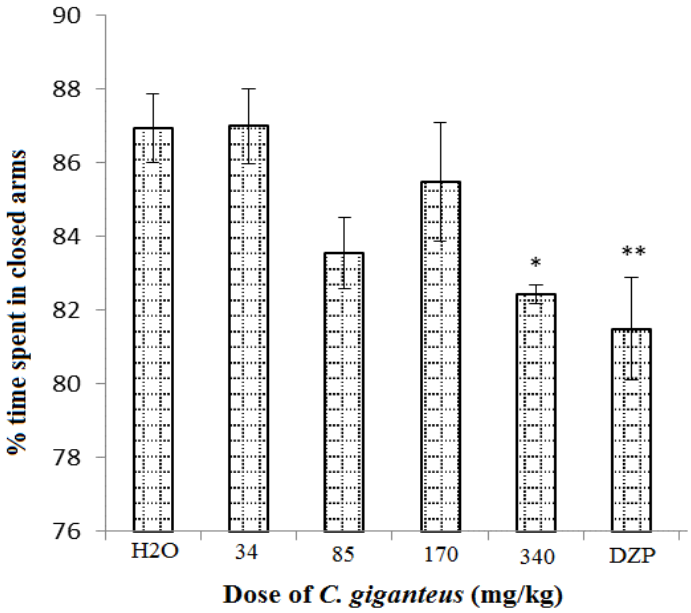

Fig. 4. Effect of *C. giganteus* in closed arms entries and time (Elevated Plus Maze). The figure represents the % of closed arms entries and time/total arms entries and time. N = 6 per dose, *p<0.05, **p<0.01, ANOVA followed by Tukey (HSD). H2O= distilled water. DZP = Diazepam 3 mg/kg

4. DISCUSSION

Results obtained from the Stress-induced hyperthermia test revealed a significant (P<0.05) dose-dependent reduction the rectal temperature on the rats treated with *C. giganteus* decoction. Indeed, antipyretic drugs decrease the body temperature [25] and the decoction of *C. giganteus* may possess antipyretic properties. These antipyretic properties would justify its use

in traditional medicine to lower the fever in the case of malaria [15]. Moreover, stress-induced hyperthermia is antagonized through the anxiolytic drugs [22], confirming *C. giganteus* extract having anxiolytic properties. These anxiolytic properties could be mediated through the intermediary of the serotonin receptors, because it is known that stress-induced hyperthermia is sensitive to the anxiolytic activity of drugs acting on the serotoninergic system [22].

The elevated Plus Maze test showed a significant increase in the percentage of entries and time spent in the open arms of the apparatus in the rats treated with the decoction of *C. giganteus*. At the same time, a significant reduction of the number of entries and percentage of time spent in the closed arms of the labyrinth were observed. Similarly in the Elevated Plus-Maze, ethanolic extract of the aerial parts of *Canscora decussate* and *Convulvulus pluricaulis* as well as *Evolvulus alsinoides* ethyl-acetate fractions showed an anxiolytic effect as evidenced by increase in the time spent in open arms and the number of open arm entries, compared to control group [6,11]. Based on the fact that a reduction in the activity in the closed arms represented a reduction of the stress and that an increase in the activity in the open arms determines a reduction in the anxiety on the labyrinth [26,27], *C. giganteus* extract may possess anxiolytic properties. Moreover, a significant reduction of the number of "rearing" and "head-dipping" was observed on the labyrinth. These results confirm the observation of Rodgers et al. [28] which showed a reduction of the number of "rearing" and of "head-dipping" in the labyrinth indicating an anxiety reduction in the rodents. The reduction of the anxiety could be attributed to the action of the plant extract on the sites of benzodiazepines of the complex GABA-receptors and/or by the antagonism of 5-HT receptors [29,30].

The Open Field test revealed a significant increase in the number of "crossing", the time spent in the center and the number of grooming in the rats treated with the extracts of *C. giganteus*. Similarly, the open field exploratory behavior was also increased on administration of the ethanolic extract of *Canscora decussate*, and *Convulvulus pluricaulis* and *Evolvulus alsinoides* ethyl-acetate fractions [6,11]. An increase in the number of "crossing", "grooming" and time spent in the center of the open arena indicate the increase in the locomotor activity and the level of exploration which concerns an intrinsic manifestation of the anxiety reduction [31]. This anxiolytic effect could be done through the action mechanism of the plant extract on the benzodiazepine sites contained in GABA receptors [32].

5. CONCLUSION

From the results, the decoction of *C. giganteus* possessed antipyretic and anxiolytic effects in Wistar rat. The use of the plant in traditional medicine in Africa, particularly in northern part of Cameroon fever and anxiety treatment is confirmed in this study and could be a new potential source of anxiolytic drugs. However further investigations on solvent extraction process and other pharmacological activity (epileptic, sedative, cell viability and oral mammalian toxicity, etc of the plant species will be done.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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