

Anxiolytic Activity of *Costus afer* (Costaceae) in Albino Wistar Rats

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ABSTRACT

This study was designed to assess the anxiolytic effects of Costus afer in rats. The aqueous extract of the plant is used in Cameroon traditionally in hepatocellular protection, as an anti-hyperglycemic, a central nervous system depressant, an analgesic and an antioxidant. Ninety rats were partitioned into 6 groups and treated as follow: Group 1 received distilled water; groups 2, 3, 4 and 5 were treated with C. afer (100, 250, 500 and 1000 mg/kg) respectively; and group 6 received either diazepam or phenobarbital. One hour post-treatment, Stress-induced hyperthermia, elevated plus maze and open field were the rodent behavioural models used in this study. Some biochemical parameters were analyzed and brain histological sections examined. Results showed that, C. afer (500; 1000 mg/kg) decreased stress-induced hyperthermia at 0.3°C compared to 0.6°C in the negative control. In the elevated plus maze, C. afer (500 mg/kg) significantly increased the percentage of open arms entries to 72.8 % compared to 24.15 % recorded in the group 1 animals. In the open field, C. afer (1000 mg/kg) raised the number of crossings from 8 ± 00 in the group 1 animals to 39.6 ± 5.8. C. afer (1000 mg/kg) significantly ($P < 0.001$) increased GABA level from 267.67 µg/g of tissue in the group 1 to 392.6 µg/g of tissue. The plant protects brain against stress-induced pyknosis of neurons. Its antipyretic and anxiolytic activities in rats could be used in traditional medicine to treat fever and anxiety.

Keywords

Anxiety, Antipyretics, Anxiolytic, *Costus afer*, Psychopharmacological tests.

Introduction

Anxiety can be defined as a reaction of threatening and/or stressful situations, which appears in the form of illness, pre-occupation and tension. Anxiety is usually a temporary phenomenon, but it should be treated as a serious illness if it lasts longer than usual or if it occurs in the absence of threatening or stressful situations [1]. Anxiety interferes with daily activities and can impair a person's family, social and school or working life. An estimated 4 % of the global population currently experiences an anxiety disorder [2,3]. In 2019, 301 million people in the world had an anxiety disorder, making anxiety disorders the most common of all mental disorders [3].

Although highly effective treatments for anxiety disorders exist,

only about 1 in 4 people in need (27.6 %) receive any treatment [4]. Access to adequate care is a complex subject which is still a source of much dissatisfaction for people suffering from mental illness and for public authorities. Unmet care needs, low use of services, inadequacy of treatments and therapeutic interruptions are all problems that deserve to be improved [5]. Anxiolytics available on the market help alleviating anxiety symptoms in around 70 % of people who take them, as many have side effects [6]. A third of the inhabitants of underdeveloped countries have very little access to these drugs due to their unaffordable cost and sometimes their unavailability in reception facilities [7]. In Africa, up to 80 % of the population uses traditional medicinal plants for their treatment. In China, traditional medicine represents 40 % of health care administered [8]. The use of traditional medicine is very widespread and is of growing health and economic importance. According to WHO health statistics, 25 % of medicines produced and marketed worldwide come from plants. Access to safe and effective traditional therapies could be decisive for the development

of health care for all [7].

Costus. afer as a medicinal plant is commonly used for traditional therapeutic and other socio-cultural purposes such as wrapping of indigenous food items, mat making, feed to small ruminants, treatment of cough, measles, malaria, diabetes mellitus, stomach ailments, arthritis, inflammation, eye defects, hunch back and evil repellants [9]. Experimental studies on various parts of the plant have shown biological activities such as anti-hyperglycemic [10], hepatocellular protection, cardioprotection, nephroprotection, central nervous system depressant, analgesic, antiarthritis, antibacterial and antioxidant [9]. This wide range of biological properties of *C. afer* would be largely attributed to the essential oil compounds such as Sabinene, β -pinene and β -caryophyllene which are among the major compounds of this plant. According to the pharmacological profile of *C. afer*, it is reasonable to assume that *C. afer* might have some other neuroactive activities. Therefore, the present study was designed to investigate the anxiolytic effects of *C. afer* in rats.

Materials and Methods

Plant Material and Extract preparation

The leaves of the plant were harvested from Sangmelima in the South Region on January 3rd, 2021. Voucher specimens (HNC11708) were deposited at the National Herbarium Yaounde. The leaves were dried under shade for three weeks and then ground in a hand machine to produce powder. 260 g of powder of the leaves of *C. afer* were introduced into the beaker containing 600 mL of distilled water. The unit was carried to boiling on a regulated hotplate at 100°C for 20 min. The mixture was filtered after cooling using Wattman paper n°1 and was evaporated in the drying oven at 40°C. This operation yielded about 18.3 g of dry extract of *C. afer*, giving a 7.04 % yield of extraction. 10 mL/kg was administered to the rats to serve as the doses of 100, 250, 500 and 1000 mg/kg following the traditional uses.

Chemicals

Chemicals used in this study were Diazepam (valium ®) 5 mg/mL in the form of bulbs (La Roche Laboratory), Phenobarbital (Gardenal ®) 40 mg/mL in the form of bulbs (Novartis Laboratory) obtained from local pharmacy.

Animals and ethical consideration

Adult *Wistar* albino male rats were bred in the Animal House in the University of Buea for three months. They had access to food pellets and their cages cleaned twice a week, and were maintained on a 12 h light/dark cycle. Animals which had weights between 150 to 180 g were used for the experiment. The study was conducted in accordance with the Cameroon National Ethics Committee (Reg. No. FWA-IRB00001954) and in accordance with the international guidelines [11]. More efforts were made to minimize animal pain and to reduce the number of rats used in the tests. An ethical clearance was gotten from UB-IACUC.

Behavioral Assessments: Stress-Induced Hyperthermia (SIH) test

The method described by [12] and reviewed by [13] was followed to assess the anti-stress-induced hyperthermic property of *C. Afer* extract in *Wistar* rats. Indeed, 60 rats were randomly shared into 6 groups of 10 rats each and marked. Rats within a given group were removed from the cage one after another in a precise order and were consecutively orally administered distilled water (negative control), 0.25 mL of phenobarbital (40 mg/mL) (diluted in 9.5 mL of distilled water, obtaining 2 mg/mL) which was administered intraperitoneally (positive control) and doses 100, 250, 500 and 1000 mg/kg of *C. afer* for the tested groups. After 1 h of post-treatment, rats were once again consecutively removed from the cage and their rectal temperature was recorded using a thermometer (Hugo Sachs model H11). In fact, “rats removed earlier had a lower anal temperature compared to those removed later in the same cage”. The difference between the body temperature of the first 3 rats and the body temperature of the last 3 rats constituted the stress induced hyperthermia [12].

Elevated plus maze (EPM)

The method used was that described by [14]. The experiment was carried out in a quiet room lit by daylight, in the Zoology Laboratory of the Department of Animal Biology and Conservation of the University of Buea. The rats were distributed in a homogeneous way in 6 groups of 5 animals per group. One group of the rats was administered distilled water that served as the negative control; another group was treated with diazepam (3 mg/kg) for the positive control; while the other groups received the doses of *C. afer* (100; 250; 500 and 1000 mg/kg). At the end of the treatment, the rats were returned in their respective cages to reduce the neophobic reactions to environment [15]. One hour after administration, each rat was placed in the center of the EPM facing an open arm. The number of entries and the time spent into open and closed arms were registered for 5 min by visual tracking. A rat considered taken to entered an arm when its four legs were on the arm. In addition to the total number of arm entries, the number of entries into the open arms expressed as a percentage of total arm entries, the time spent on the open arms expressed as a percentage of the time spent on the all arms and rearing, head-dipping were assessed. These parameters were used as indices of anxiety.

Open Field (OF)

The method used during our experiment is that described by [16]. The rats were distributed in a homogeneous way in 6 groups of 5 animals per group. One group of the rats was treated with distilled water that served as the negative control; another group received diazepam (2 mg/kg) for positive control; while the last groups received the doses 100; 250; 500 and 1000 mg/kg of *C. afer*. At the end of the treatment, the rats were returned in their respective cages to reduce the neophobic reactions to environment [15]. 1 h after administration, each animal was centrally placed in the open field apparatus for 5 min, during which the parameters quantified were: line crossing, rearing, self-grooming and duration at centre of open field.

Biochemical Analyses

At the end of the tests, the animals were immediately sacrificed by decapitation to reduce pains and suffering. Their brains were dissected and washed with ice-cold saline solution (0.9 %, w/v) to take off the hippocampus. Then, the hippocampi were weighed. To achieve biochemical analyzes, 10 % (w/v), homogenates prepared with ice-cold 0.1 M phosphate buffer (pH 7.4) were centrifuged (10,000 x g, 15 min). The aliquots of the supernatants were taken in and manipulated to quantify, according to different protocols, the level GABA, GABA transaminase (GABA-t) and Glutamate decarboxylase (GAD) activity.

Measurement of GABA Level

Ninhydrin reacts with succinic semialdehyde acid to form a complex. The concentration of GABA in the hippocampus was assessed by the colorimetric technique with absorbance at 512 nm wavelength as described by [17]. A reagent consisting of a mixture of 0.2 mL of 0.14 M ninhydrin solution was prepared in a bicarbonate buffer solution (0.5 M; pH 9.9) and 0.1 ML of 10 % glacial trichloroacetic acid (TCA). A homogenate sample (100 µL) was introduced into the working reagent. The mixture was incubated at 60°C for 30 min. After cooling at room temperature, the solution was introduced into 5 mL of copper tartrate solution prepared from 0.16 % disodium carbonate, 0.03 % copper sulphate, and 0.033 % tartaric acid. At 25°C for 10 min, the whole set-up was incubated, and the coloration resulting from the reaction between ninhydrin and GABA was measured using a spectrophotometer at 450 nm. The wavelength of GABA in the homogenate was determined using GABA standards (50, 100, 150, 200, 250, 300, 350, and 400 µg), each mixed with 1.5 mg of glutamate dissolved in 2 mL of TCA (10 %). The GABA level was expressed in µg/g of tissue.

Quantification of GABA-t activity

The coloration of the complex generated by succinic semialdehyde acid and 3-methyl-2-benzothiazolone-2-hydrazone in the presence of 12 % FeCl₃, is proportional to the activity of GABA-t in the homogenate. The activity of GABA-t was assessed using the colorimetric method described by [18]. Into test tubes, 5 µmol of α-oxoglutarate, 10 µg of pyridoxal phosphate, 15 µmol of GABA, and 0.1 mL of homogenate were introduced, while in the blank tube, 0.1 mL of methanol (5 %) was introduced. The final volume of the mixture was made up to 3 mL with Tris-HCl buffer (50 mM HCl; 150 mM KCl; pH 7.4). The tubes were then incubated at 37 °C for 30 min. The reaction was completed by adding 0.5 mL of TCA 20 %. The amount of semialdehyde succinic acid generated during the incubation was quantified against the blank at 610 nm by a spectrophotometer at 30 and 90 sec. The GABA-t activity was expressed in µg/min/mg of tissue.

Determination of Glutamate decarboxylase (GAD) activity

In the presence of glutamic acid, increase in GABA is proportional to glutamate decarboxylase activity [19]. The activity of GAD was determined using the amount of GABA during incubation with glutamic acid [17]. To 1.0 mL of 0.05 M glutamic acid (neutralized at pH: 6.3-6.7 and 1.0 mL of homogenate, 0.1 mL of pyridoxal-

5-phosphate (25 pg) was mixed. The mixture was incubated at 37°C for 30 min. After the incubation period, the reaction was stopped by heating at 100°C for 10 min using a hot plate. The increase in GABA was monitored at 30 and 90 sec at 455 nm using a spectrophotometer. The GAD activity was expressed in µg/min/mg of tissue.

Histological Analyses

After sacrifice, the brains were isolated and kept in 10 % formalin to preserve the tissues. 50 mm coronal sections were made from the brain in the hippocampus region using the rat brain atlas with the following coordinate (Anterior/Posterior D = 2.0 mm, Medial/lateral D = 1.5 mm, and dorsal/ventral AP D = 2.0 mm). The procedure for histopathological evaluation was carried out as described by [20]. Brain samples were washed and dehydrated using a series of ethanol solutions of increasing concentrations (50, 70, 95 and 100 %), to gradually remove water from the tissues. After dehydration, brain tissues were then 'cleared' using a 'clearing agent' of relatively high refractive index (xylene) for 2 h 30 min. The brain tissue specimens were then infiltrated by plunging it in paraffin wax (58 - 60°C) three times for a total of 4 h 30 min. This was to provide support to prepare them for cutting with a microtome. The tissue specimens were then embedded in paraffin wax for 24 h to prepare a wax template, surrounded by paraffin wax. Each specimen was then shaped using a sharp blade so it can easily be applied to the edge of the knife microtome. The paraffin blocks were then cut into thin sections (~6µ) using a microtome. The tissues were then spread on hot plates and mounted on glass slides. Afterwards, they were carefully rehydrated using a series of alcohol solutions of descending concentrations (100, 95, 70 and 50 %). Later, they were stained with Eosin stain and Haematoxylin to differentiate their different components. Finally, the tissues were observed under a light microscope for changes in their structure and then pictures were taken with the aid of a digital camera attached to the eyepiece of the light microscope.

Statistical analysis

For data collected for each group after parameters assessment, mean ± S.E.M was calculated for the observed values in each experimental group. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Dunnett test (bilateral) carried out to compare the values of the various parameters: the statistical package used was Graph pad Prism 8.4.3 (686). The threshold of significance was 5 %.

Results

Anxiolytic Properties of *Costus. afer*

Activity of aqueous extract of *Costus. afer* in SIH test

In comparison with the negative control group, *C. afer* aqueous extract (figure 1a) significantly reduced the body temperature of the rats at dose 1000 mg/kg from 35.54°C to 34.64°C. The phenobarbital (20 mg/kg) reduced it to 33.96°C. There was a high SIH observed in rats treated with distilled water, *C. afer* extract (figure 1b) brought a great reduction on it at doses 500 and 1000 mg/kg to 0.3°C respectively.

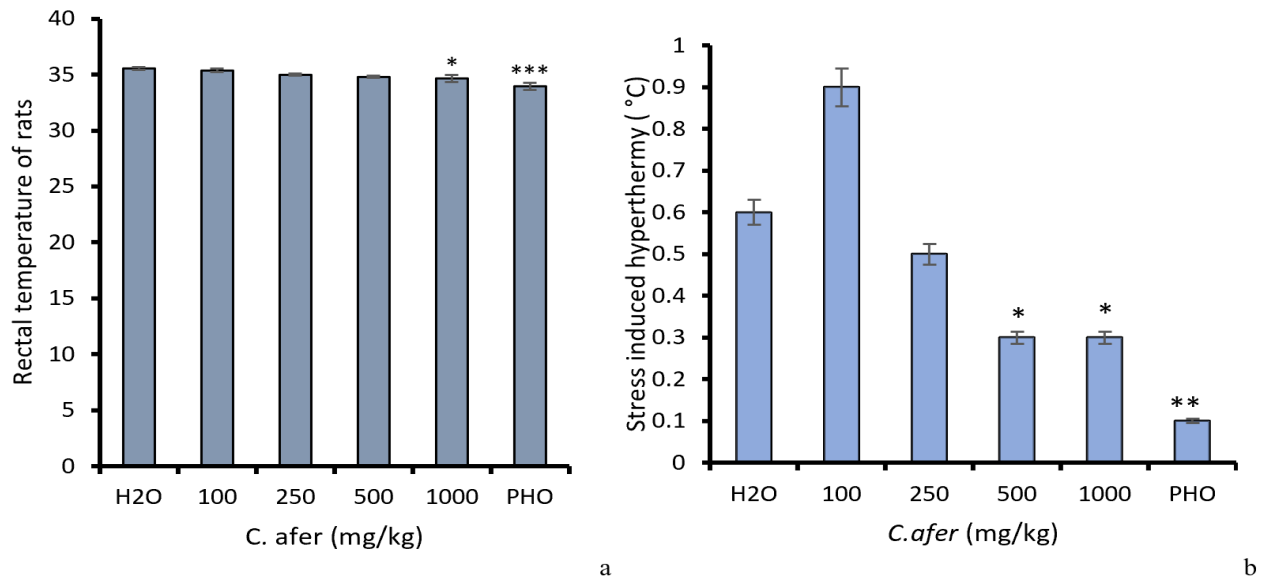


Figure 1a, b: Activity of *C. afer* extract on the SIH. Histograms are expressed as mean $\pm$ S.E.M., $n=10$ per dose where * $P < 0.05$, ** $P < 0.01$, Data were analysed by Dunnett's multiple comparison Test, significantly different to the vehicle. H₂O (distilled water), PHO = Phenobarbital (20 mg/kg).

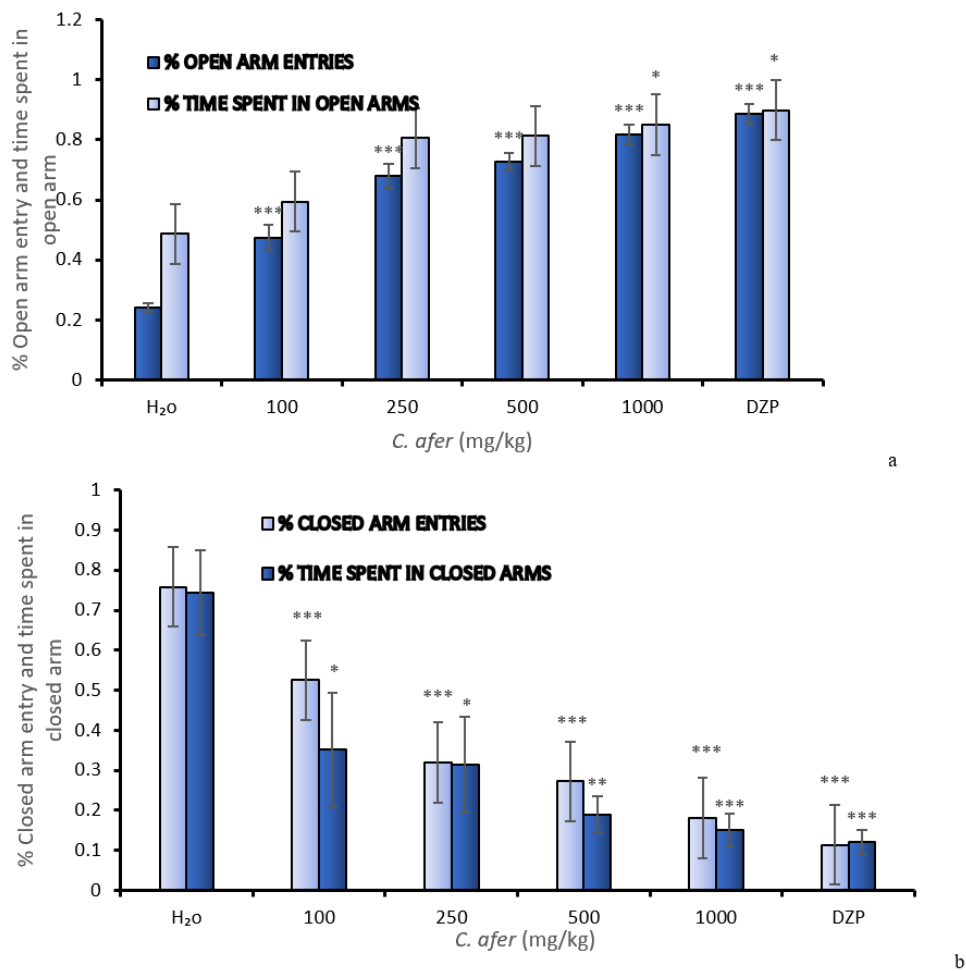


Figure 2 a, b: Effects of *Costus afer* as Percentage of entries and time spent in open and closed arms in the EPM. Illustrated are the Percentage of entries and time spent in open and closed arms. $N=5$ rats per dose. Data were analysed by Dunnett's multiple comparison Test, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ significantly different to the vehicle. H₂O (distilled water), DZP (Diazepam, 3 mg/kg).

Activity of *Costus. afer* on anxious behavior in rats on Elevated plus maze (EPM)

Percentage of entries and time passed in open and close arms in the EPM

The figure 2a shows an increase percentage of entries and time passed in open arms observed in groups rats treated with the extract which were significantly higher at 100, 250, 500 and 1000 mg/kg, and the same thing was observed for the positive control that received diazepam. On the other hand. For the closed arms of the EPM as seen on figure 2b, there was a significant decrease in percentage of entries and time passed in closed arms noted in extract plant.

Effects of *Costus afer* on the number of head-dipping and rearing in the EPM. In figure 3a, head-dipping illustrated no significant change observed with the extract treated at 100 mg/kg while there was a significant decrease observed with the extract treated groups at 250, 500 and 1000 mg/kg and a significant decrease was also observed at the positive control. While for rearing, it could be observed from figure 3b that there was significantly decrease

observed in the extract groups at 100, 250, 500 and 1000 mg/kg.

Effect of *Costus. afer* on Open Field (OF)

For the duration at the center, crossing and grooming, it could be observed that there was an increase observed in rats treated with doses at 250, 500 and 1000 mg/kg of *C. afer* and the positive control. The number of rearing decreased significantly in groups rats receiving extract plant at 500 and 1000 mg/kg and the diazepam compare to the negative control (distilled water).

Biochemical assessments

In figure 4a, *C. afer* has induced a significant ($P < 0.01$) increase in GABA concentration from 267.67 $\mu\text{g/g}$ of tissue (negative control) to 375.50 and 392.67 $\mu\text{g/g}$ of tissue in rats treated with doses 500 and 1000 mg/kg of plant. In figure 4b, GABA-t activity is 100.67 pg/ min/mg of tissue in the negative control; doses 500 and 1000 mg/kg of *C. afer* reversed it at 55.83 and 39.33 pg/min/mg of tissue. In figure 4c, *C. afer* reversed the decreased GAD activity from 1.50 $\mu\text{g/min/mg}$ of tissue (negative control) to 1.56 and 1.72 $\mu\text{g/min/mg}$ of tissue ($P < 0.01$) at the respective doses of 500 and

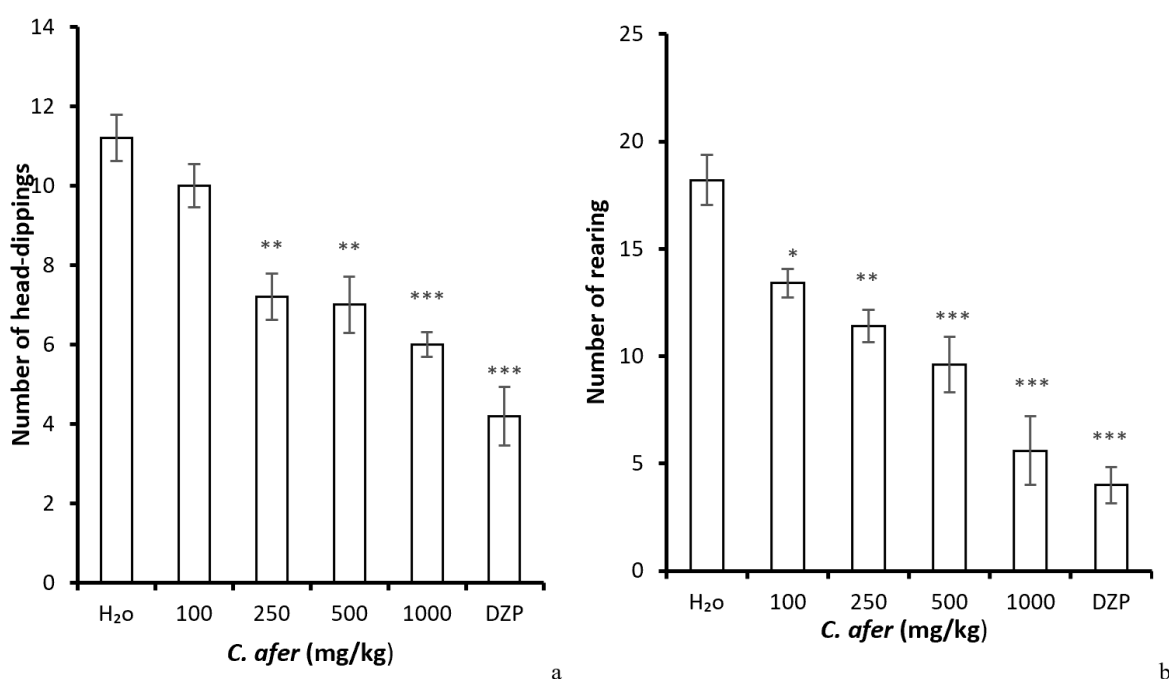


Figure 3a, b: Activity of *C. afer* on the number of head dipping a) and rearing b) in the EPM. Exposed are the number of head-dipping and rearing in the EPM. N=5 rats per dose. Data were analysed by Dunnett's multiple comparison Test, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ significantly different to the vehicle. H₂O (distilled water), DZP (Diazepam, 3 mg/kg).

Table 1: Effects of *C. afer* on the duration at center, crossing, rearing and grooming in the OF.

Parameters	H ₂ O	Dose de <i>C. afer</i> (mg/kg)				Diazepam (2 mg/kg)
		100	250	500	1000	
Duration center	1.2±0.4	2.8±0.3	4.8±0.3	7.2±0.5*	9.6±0.6**	16.4±3.5***
Crossing	8±0.7	14.6±1.9	21±2.9*	26.2±2.1**	39.6±3.6***	62.6±5***
Rearing	21.6±3.8	15.8±2.2	14±2.7	8±1.5**	5.6±1.6***	3.6±1.1***
Grooming	1.8±0.4	2.6±0.8	5.6±0.7**	8±0.4***	9.4±0.5***	11.2±0.5***

Data are mean ± S.E.M., n = 5, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ANOVA followed by Dunnett (bilateral) comparison test. H₂O (distilled water).

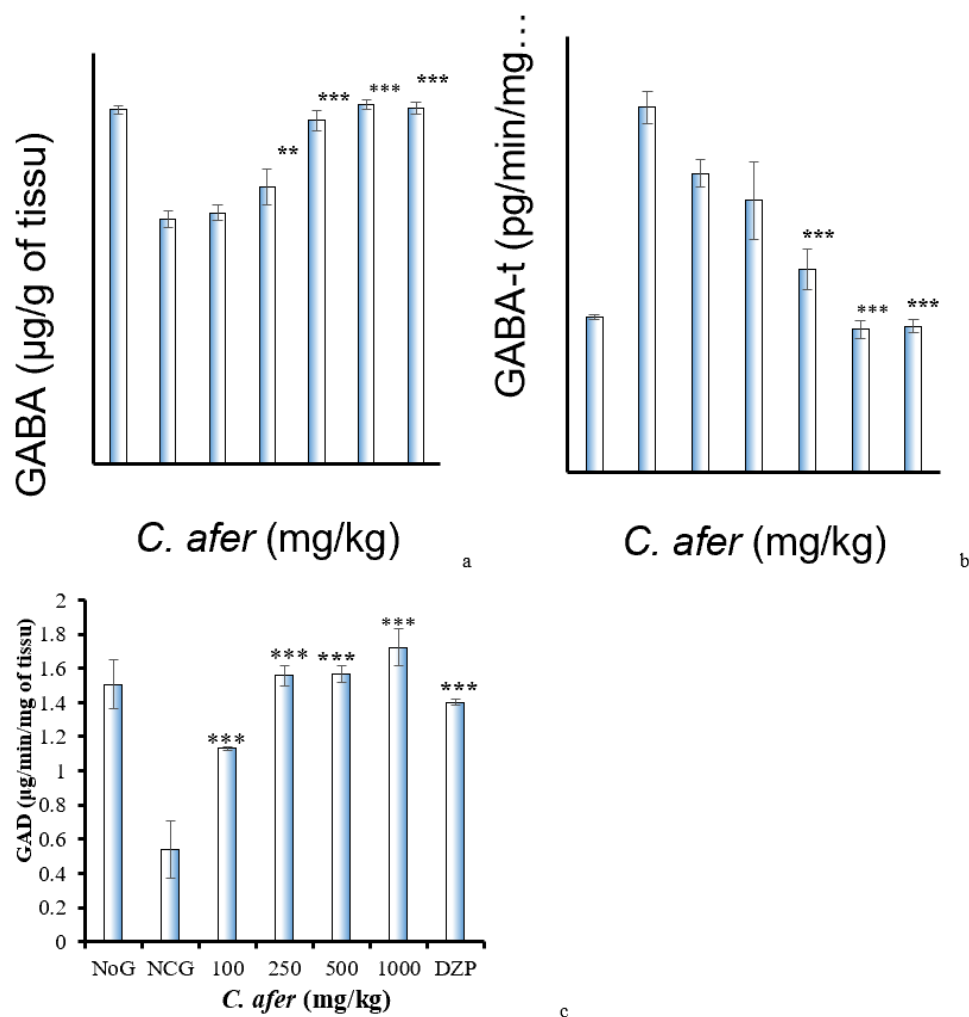


Figure 4a, b, c: Effects of *C. afer* on a GABA, b GABA-T and c GAD activities in the brain. Each bar indicates the mean $\pm$ S.E.M; n = 5. ***p < 0.001, vs. negative control. NoG= normal control group; NCG= negative control group; DZP= diazepam.

1000 mg/kg of plant.

Histological Analysis

The histological analysis showed that in the negative control, compared to the extract treated groups at doses 100, 250, 500 and the positive control group, there was a normal structure and architecture of neurons aspects and hippocampus. But at 1000 mg/kg of extract of *C. afer* leaves, there was signs as vacuolation of neuronal cells, which represents the hydropic degeneration in the dorsal ganglia due to neuronal death and may result in the formation of nodules to replace the lost neurons. And also, Pyknosis which can be defined as the irreversible condensation of chromatin in the nucleus of a cell enduring apoptosis or necrosis for a new growth followed by a fragmentation of the nucleus into 2 smaller nuclei to form new cells. However, Pyknosis is maturation of erythrocytes and the neutrophils.

Discussion

Results obtained from the SIH test revealed a significant ($P < 0.05$) dose-dependent reduction in the rats' rectal temperature treated with *C. afer* extract. Indeed, antipyretic drugs decrease the body temperature [21]. Then aqueous extract of *C. afer* may possess antipyretic properties. That would justify its use in folk medicine to lower the fever in the case of malaria [2]. Moreover, SIH is antagonized through the anxiolytic drugs, confirming *C. afer* extract having anxiolytic properties. These properties could be mediated through the intermediary of the serotonin receptors, because it is known that SIH is sensitive to the anxiolytic activity of drugs acting on the serotonergic system [13].

Developed by [22], The EPM is a widely used behavioural assay in the study of anxiety and anxiety-related disorders in Neuroscience [23]. When placed in the plus maze for the first time, a rat's behaviour is largely built on its levels of anxiety and fear. Normal rats that have not received any anxiolytic will become moderately

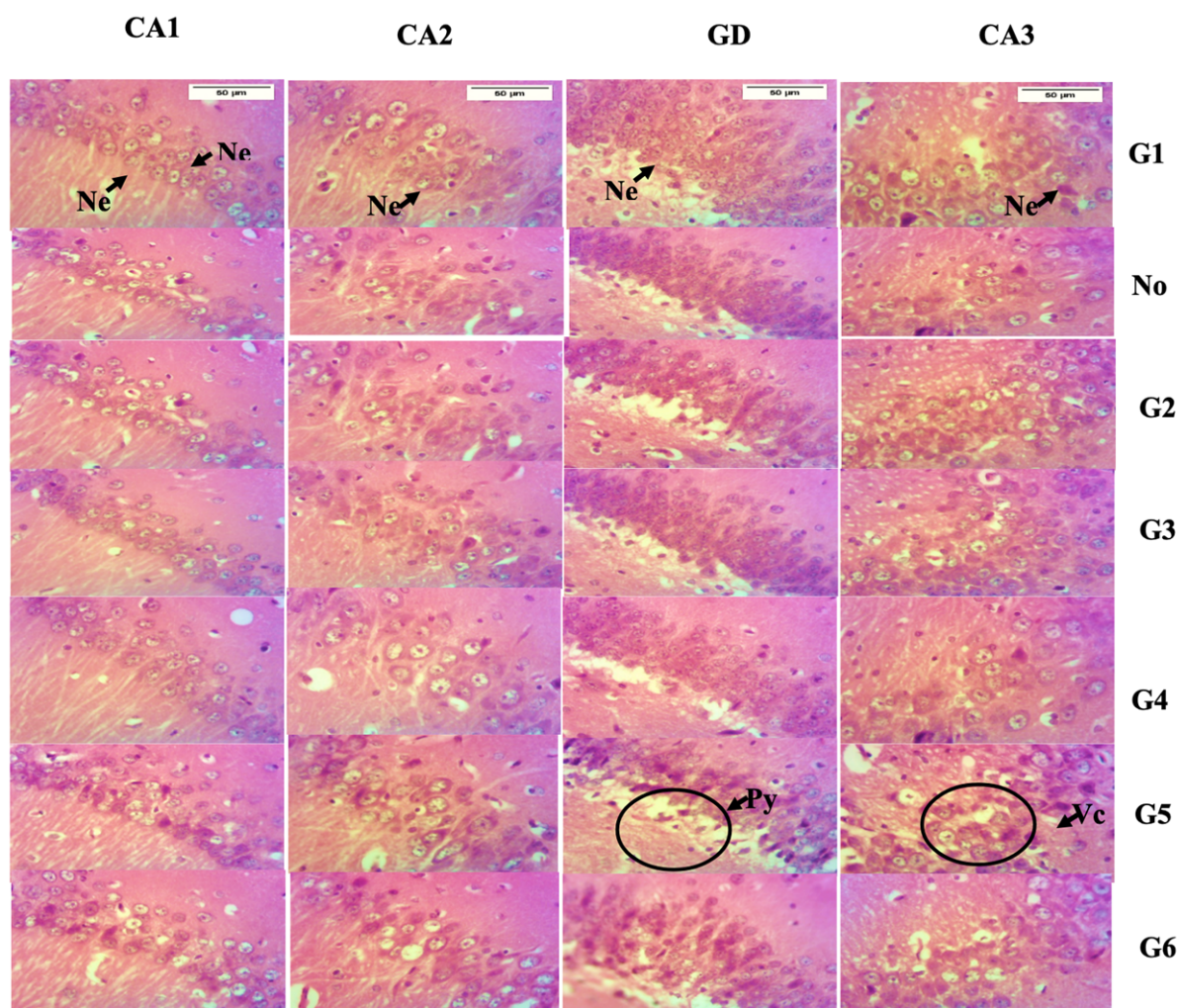


Figure 5: Microphotography of hippocampus and CA1, CA2 and CA3 zones (X250); Coloration with hematoxyline-eosine. Ne = Neurone; Vc = Vacuolation of neurons; Py = Pyknosis, NoG = normal control group.

anxious in this new environment. Thus, they tend to prefer the closed arms over the less secured open arms. This is seen in figure 2a where at dose 500 mg/kg of *C. afer*, the number of entries in open arm is significant than that observed in rats treated with distilled water. Whereas, the rats treated with anti-anxiety drugs tend to be less fearful, so they spend much time in the open arms compared to the negative control group. The percentage of the time spent and arm entries in closed and open arms are sensitive factors known to act via GABA receptors complex justifying the use of diazepam as a reference in this study. In agreement with previously published reports, diazepam increased the percentage of entries and the time passed in the open arm confirming its anxiolytic effects [24]. The aqueous extract of *C. afer* had similar effects on these parameters. An increase in the activity of rats in the open arms signifies to a decrease in anxiety and a decrease in these behavioural parameters in the closed arms reflects a reduction in stress [14], this insinuates that the extract of *C. afer* leaves has anxiolytic properties.

Other behavioural assessments of the present study showed a reduction of rearing in rats treated with the plant when compared

to the negative control (distilled water). The reduction in rearing activity in the open arms indicates a decrease in anxiety and demonstrates to the presence of the anxiolytic effects of *C. afer*. That could be facilitated by GABAergic neurotransmission which increases inhibitory neural responses and causes a reduction in brain cell activities, thereby reducing body activities as rearing which is an indicator of an anxious state of the animal.

The results obtained through the open field test showed that the aqueous extract of *C. afer* increased the number of crossings, grooming, the time spent at the centre of the OF and decrease in the number of rearing. The increase of these parameters is a manifestation of the locomotion and exploration activities in rats [23]. Thus, the significant difference is accounted by the fact that the rat turns to explore the OF more due to low level of anxiety that could enhance seek of refuge of the rat and enable an increased number of crossings and any substance that reduces stress under stress conditions as imposed by the OF is anxiolytic [25].

In an attempt to find possible mechanisms of actions of the

aqueous extract of *C. afer*, the involvement of the GABA pathway was explored, since this neurotransmitter plays a major function in anxiety [17].

Anxiety behaviour decreased GABA and GAD levels, while it increased GABA-t activity, as previously mentioned [18]. According to study, the decrease is an obvious manifestation of a loss of GABAergic interneurons and a decrease in GABA_A receptor expression in the different parts of brain. The effect of *C. afer* increased GABA and GAD levels, but decreased GABA-t, suggesting an interference with the GABA system. These findings might also suggest a reduction in the level of anxiety in rats through an activation of GABAergic transmission.

On the microphotography of the brain of each animal from each group, the histological analysis showed a normal architecture of the brain in rats (negative control), rat groups treated at doses 100, 250, 500 mg/kg of *C. afer* and the positive control group. While in group rats treated at dose 1000 mg/Kg of *C. afer*, there was signs as vacuolation of neuronal cells characterized by degeneration of old and death neurons in the dorsal ganglia, which resulted to development of nodules of Nageotte; and a hydropic degeneration in the dorsal ganglia due to neuronal death and may result in the formation of nodules to replace the lost neurons. And also, Pyknosis which can be defined as the condensation is not reversible of chromatin in the nucleus of a cell enduring apoptosis or necrosis for a new growth followed by a division of the nucleus into 2 smaller nuclei to form new cells. However, Pyknosis in brain cells can also be considered as the maturation of erythrocytes and neutrophils. This is justifiable for by the fact that there were no physiological changes in groups that received distilled water, *C. afer* (100, 250, 500 mg/kg) and diazepam. That is, there was no formation of nodules for replace of old or death neurons and no observed condensation of nucleus which is accountable by the fact that at the high dose of *C. afer* (1000 mg/kg), which is more efficient.

Conclusion

The research findings led us to the following conclusions; The effects of *C. afer* reduced the level of anxiety and stress in naive treated rats by increasing the number of entries, the percentage of entries and time passed in the open arms of the EPM test, increased the number of crossing, grooming, the time spent at the centre and decreased the number of rearing in the OF. The extract also showed positive anxiolytic activities by increasing GABA, GAD and decreasing GABA-t. *C. afer* also showed positive activities on brain architecture on histological studies. From the results, the aqueous of *C. afer* possessed antipyretic and anxiolytic effects in mice. The use of the plant in traditional medicine in Africa, particularly in northern part of Cameroon in 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.

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References

1. Craske MG, Rauch SL, Ursano R, et al. What Is an anxiety disorder. *Depress Anxiety*. 2009; 26: 1066-1085.
2. Kimbi H, Fagbenro-Beyioku A. Efficacy of *Cymbopogon giganteus* and *Enantia chrantha* against chloroquine resistant *Plasmodium yoelii nigeriensis*. *East Afr Med J*. 1996; 73: 636-637.
3. Javaid SF, Hashim IJ, Hashim MJ, et al. Epidemiology of anxiety disorders: global burden and sociodemographic associations. *Middle East Current Psychiatry*. 2023; 30: 44.
4. Alonso J, Liu Z, Evans-Lacko S, et al. Treatment gap for anxiety disorders is global: Results of the World Mental Health Surveys in 21 countries. *Depress Anxiety*. 2018; 35: 195-208.
5. Lamboy B. La santé mentale: état des lieux et problématique. *Santé publique*. 2005; 583-596.
6. Rector NA, Bourdeau D, Kitchen K, et al. Les troubles anxieux: guide d'information CAMH, Centre for Addiction and Mental Health= Centre de toxicomanie. 2009.
7. WHO. Allocution du Dr Gro Harlem Brundtland, Directeur général, à la Cinquante-sixième Assemblée mondiale de la Santé, Genève. 2003.
8. WHO. World Health Organization Traditional medicine strategy 2002-2005. Geneva. 2022.
9. Omokhua G. Medicinal and socio-cultural importance of *Costus afer* (Ker Grawl) in Nigeria. *African research review*. 2011; 5: 282-287.
10. Soladoye M, Oyesika O. A textbook of medicinal plants from Nigeria. University of Lagos Press. 2008; 628.
11. Guide for the care and use of laboratory animals. US Department of Health and Human Services. Public Health Service. 1986.
12. Borsini F, Lecci A, Volterra G, et al. A model to measure anticipatory anxiety in mice. *Psychopharmacology*. 1989; 98: 207-211.
13. Lecci A, Borsini F, Volterra G, et al. Pharmacological validation of a novel animal model of anticipatory anxiety in mice. *Psychopharmacology*. 1990; 101: 255-261.
14. Rodgers R, Cao BJ, Dalvi A, et al. Animal models of anxiety: an ethological perspective. *Brazilian journal of medical and biological research*. 2009; 30: 289-304.
15. Bourin M, Dhonnchadha B, Colombel MC, et al. Cyamemazine as an anxiolytic drug on the elevated plus maze and light/dark paradigm in mice. *Behav Brain Res*. 2001; 124: 87-95.

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16. Belzung C. The genetic basis of the pharmacological effects of anxiolytics: a review based on rodent models. *Behav Pharmacol.* 2001; 12: 451-460.
 17. Lowe IP, Robins E, Eyerman GS. The fluorimetric measurement of glutamic decarboxylase and its distribution in brain. *J Neurochem.* 1958; 3: 8-18.
 18. Nayak P, Chatterjee A. Effects of aluminium exposure on brain glutamate and GABA systems: an experimental study in rats. *Food Chem Toxicol.* 20021; 39: 1285-1289.
 19. de Souza EAP, Salgado PCB. A psychosocial view of anxiety and depression in epilepsy. *Epilepsy Behav.* 2006; 8: 232-238.
 20. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys.* 1959; 82: 70-77.
 21. Sundgren-Andersson AK, Östlund P, Bartfai T. Simultaneous measurement of brain and core temperature in the rat during fever hyperthermia hypothermia and sleep. *Neuroimmunomodulation.* 1998; 5: 241-247.
 22. Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze exploration model of 'fear'-motivated behaviour. *Naunyn Schmiedebergs Arch Pharmacol.* 1984; 327: 1-5.
 23. Taiwe G, Moto F, Ayissi E, et al. Effects of a lyophilized aqueous extract of *Feretia apodanthera* Del. Rubiaceae on pentylenetetrazole-induced kindling, oxidative stress and cognitive impairment in mice. *Epilepsy Behav.* 2015; 43: 100-108.
 24. Moser P. An evaluation of the elevated plus-maze test using the novel anxiolytic buspirone. *Psychopharmacology.* 1989; 99: 48-53.
 25. Moto F, Talla E, Taiwe G, et al. Anxiolytic-like Effects of the Decoction of *Psorospermum febrifugum* in Mice. *Asian Journal of Pharmaceutical and Health Sciences.* 2003; 3.