



The Decoction of *Cymbopogon citratus* (Poaceae) Exerts Anxiolytic and Antioxidant Effects in Mice on Stress Paradigm Tests

Jean Pierre Omam Omam^{1,*}, Andre Hamadou², Antoine Kavaye Kandeda³, Juliette Koube⁴, Djibrilla Mana Moussa⁴, Brice Kevin Tchoupe Djoumsie⁴, Mireille Delphine Minkoulou Ze³, Roger Sébastien Ndjock⁵, Elisabeth Ngo Bum^{5,6}

¹Department of Biological Sciences, High Teacher Training College, University of Yaounde I, Yaounde, Cameroon

²National Institute of Youth and Sport, Yaounde, Cameroon

³Department of Animal Biology and Physiology, Faculty of Sciences, University of Yaounde I, Yaounde, Cameroon

⁴Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon

⁵Department of Biological Sciences, Faculty of Sciences, University of Ngaoundere, Ngaoundere, Cameroon

⁶Department of Biological Sciences, University of Maroua, Maroua, Cameroon

Email address:

jpbolikol@yahoo.fr (Jean Pierre Omam Omam)

*Corresponding author

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Abstract: *Cymbopogon citratus* has many medicinal properties: it is used to treat headaches, cough, malaria and anxiety or fear in the national diet. The interest of this study was to evaluate the anxiolytic properties and acute toxicity of the decoction of *Cymbopogon citratus* leaves. *C. citratus* was harvested in the Wouri division, Littoral Region of Cameroon. The identification of the families of secondary metabolites was carried out on the leaves decoction through staining and precipitation tests. The animal material consisted of male and female *Mus musculus* Swiss mice of 18 g at 30 g over aged about 9 weeks and not previously tested. They were used for acute toxicity assessment by administering a single dose of 5000mg/kg. Then, the anxiolytic activity assessment of the decoction on basic and chronic anxiety was done using the following devices: the elevated plus maze, the open field, the hole board and the restrictor. Finally, some oxidative stress parameters such catalase, sulphoxide dismutase, reduced glutathione and malondialdehyde were measured. The extraction yield of the *C. citratus* decoction was 15.12%. No signs of toxicity were observed at the dose of 5000mg/kg, 14 days after treatment. The Screening of the decoction revealed the presence of saponins, phenolic compounds and sterols. The anxiolytic activity assessment of the decoction on basic and chronic anxiety through the different devices shows that the most effective dose of the *C. citratus* decoction was 151 mg/kg. In the case of the elevated plus maze test, there was an increase of the number of entries into the open arms, the time spent in the open arms and their respective percentages. However, there was a decrease of the number of entries into the closed arms, the time spent in the closed arms and their percentages. The number of rearing and the mass of stool produced were significantly reduced in the maze, indicating a decrease of the level of anxiety in these mice. The open field and hole board tests also showed that, the *C. citratus* decoction would have anxiolytic properties. This could be justified may be by the presence of saponins and phenolic compounds. The determination of oxidative stress markers showed that, mice given the decoction and diazepam had elevated catalase, sulphoxide dismutase and reduced glutathione, but their malondialdehyde was low. The results of the study showed that the decoction of *Cymbopogon citratus* has anxiolytic properties.

Keywords: *Cymbopogon citratus*, Decoction, Acute Toxicity, Anxiolytic

1. Introduction

The brain dysfunction is a public health problem where pharmacological treatments play a major role in reducing its impact on society [1]. Approximately 12% of the world's population is affected by an anxiety disorder each year and between 5% and 30% at some point in their lives [2]. Neuropsychiatric disorders are, according to the World Health Organization (WHO), a group of mental health problems characterised by abnormalities in thinking, feeling, behaving and relating to others. Genetic, social, environmental and psychotropic factors are the main causes of these mental disorders. They also account for 13% of the total burden of disease worldwide [3], and affect everyone regardless of race, gender and age [4]. Among them, anxiety disorders: they come in various forms: generalized anxiety disorder (GAD), panic disorder, phobia or obsessive compulsive disorder (OCD) [5]. Among these stated pathologies, generalized anxiety focuses our attention. The anxiety is defined as an emotion and is considered as "an unpleasant emotional condition or state that is characterised by subjective sensations of tension, apprehension and worry and by activation of the nervous system [6]. The somatic manifestation of this includes fatigue, heart palpitations, headaches, insomnia and excessive sweating. The anxiety disorders are one of the most common mental disorders [7, 8]. The treatment of anxiety disorders is based on seven approaches: psychoeducation, dietary management, psychotherapy, alternative therapies, treatment of comorbidities, medical and social care and drug treatments with marketing authorisation for anxiety disorders. The latter are generally benzodiazepines (BZDs), barbiturates and tricyclic antidepressants (TCAs). They have long been used to treat anxiety disorders. But they show side effects like insomnia, sedation, withdrawal and tolerance (BZD, barbiturates), sexual dysfunction, anticholinergic [9]. In addition, these modern treatments are expensive, complex and not easily accessible to African populations in rural areas [10, 11]. Many of these psychoactive molecules have plant origins [12, 13], which could justify the use of plants in traditional African medicine to treat neuropsychiatric diseases [14, 15]. For this and other reasons, many pharmaceutical companies are conducting studies to find an alternative medicine or drugs are derived from plants with more specific anxiolytic effects [4]. It is currently considered that nearly 75% of the African population uses only the plants around them to treat themselves and does not have access to so called "mother of medicine". Over the past thirty years, numerous studies have attempted to verify the action of traditional medicines and their toxicity. Many plants are as effective as the drugs imported from Africa and unknown to the majority of the population [16]. *Cymbopogon citratus*, more commonly known as lemongrass, is a species in the Poaceae family. *C. citratus* is widely used in traditional medicine. It is used for example, in the treatment of coughs, influenza, gingivitis, headaches, leprosy, malaria, ophthalmic problems,

pneumonia and vascular pathologies. It is also known for its detoxifying properties for the liver, pancreas, kidney, bladder and digestive tract. *C. citratus* is also used for skin problems such as acne [17]. Other populations use it as an analgesic, diuretic, febrifuge, spasmolytic and tranquilliser [18]. It is antirheumatic, digestive, diuretic and antipyretic drug [19]. The main objective of this study is to evaluate the anxiolytic properties of the decoction of the dried leaves of *Cymbopogon citratus* and its safety in the white mouse.

2. Material and Methods

2.1. Material

2.1.1. Plant Material

The plant material consisted of the leaves of *Cymbopogon citratus*. The choice of this plant was motivated on the one hand by its use in traditional medicine where the decoction of its leaves is used against anxiety. In addition, by the review of the literature this had shown that few studies had been conducted on *C. citratus* with regard to toxicity and anxiolytic activity, none of which on the decoction of the dried leaves powder. Thus, the leaves of *C. citratus* were collected very early in the morning in the Littoral Cameroon, more precisely in the Department of Wouri, Douala V Subdivision, Ndogbong district. Then, this sample was dried in the shade for 21 days, crushed and kept in a sealed jar. Finally, *Cymbopogon citratus* collected was identified in the National Herbarium of Cameroon in comparison with the sample of herbarium N° 18628/SRF cam. From Daniel Dang N° 202.



Figure 1. *Cymbopogon citratus* plant (Omam, 2022).

2.1.2. Animals

The animals were previously untested male and female *Mus musculus* Swiss mice, weighing between 18 g and 30 g, aged approximately 9 weeks. They had free access to tap water and their cages were cleaned daily. The mice were regularly fed a standard composition diet consisting of: peanut meal, soybean meal, palm kernel meal, fish meal, bone meal, vitamins and corn meal. The animals were

obtained from the animal house of the laboratory of Animal Physiology of the University of Yaounde I. They were housed in standard cages with the temperature maintained at $25 \pm 3^\circ\text{C}$ and 12 h alternating light and dark cycles. They were supplied with food and water ad libitum. All animal handling procedures were done in accordance with National Ethic Guidelines (FWA- IRB00001954), and the experiments were designed to minimize the number of animals used and to minimize their suffering.

2.2. Methods

2.2.1. Preparation of the Aqueous Extract of *C. citratus*

Forty (40) g of *C. citratus* leaves powder was introduced into an Erlen-meyer flask containing 400 mL of distilled water at a concentration of 100 mg/mL. Subsequently, the Erlen-meyer flask was closed and boiled for about 20 min on a hot plate. After cooling, the mixture was filtered through Watmann paper No. 3, the filtrate collected was considered as the stock solution. The solutions were administered to the animals at a rate of 10mL/kg body weight. Dilutions of this stock solution were then made to 1/2, 1/4 and 1/10 with distilled water. Finally, the calculation of the yield made it possible to determine the doses of the decoction administered. To do this, the same procedure was reproduced and the filtrate obtained was dried at 45°C in the oven and a dry extract mass was obtained.

2.2.2. Preparation of the Diazepam (DZP)

The DZP used for the preparation of the solutions was contained in 10 mg/2mL ampoules, i.e. a concentration of 5 mg/mL under the conditions where the volume of administration was fixed at 10 mL/kg. For the preparation of the different concentrations of DZP used in the chronic tests, knowing that the final concentration was 0.2 mg/mL, a volume of DZP of 1 mL was taken, introduced into the beaker, then completed with distilled water to 25 mL for a dose of 2 mg/kg. This solution was prepared for the chronic stress tests. For the preparation of the concentration of DZP used in the classical stress tests (EPM and OF) and knowing that the final concentration was 0.3 mg/mL, a volume of DZP of 1 mL was taken and introduced into the beaker, then completed with distilled water to 16.6 mL for a dose of 3 mg/kg. For the preparation of the concentration of DZP used in the classical stress tests (the hole board) and knowing that the final concentration was 0.5 mg/mL, a volume of DZP of 1 mL was taken and then introduced into the beaker, completed with distilled water to 10 mL for a dose of 5 mg/kg.

2.3. Pharmaceutical Tests

2.3.1. Elevated Plus Maze Test (EPM)

The Elevated Plus Maze (EPM) used is the one described by Handley and Mithiani (1984) [20]. It is at a height of 50 cm from the floor and consists of two opposite open arms of (15 × 5 cm) and two opposite closed arms of (15 × 5 × 10 cm) with a platform in the Centre. The test was carried out in a quiet room with daylight. The principle of the test is based

on the approach/avoidance conflict of the open arms. An animal which explores the open arms, will be described as “little anxious” and an animal, that remains confined to the closed arms of the device, will be described as “anxious” [21]. The mice, classified in 6 groups of 5 animals each, were treated with distilled water (10 ml/kg) for the negative control group, diazepam (3 mg/kg; i.p) for the positive control group and different doses of *C. citratus* decoction (151mg/kg; 75mg/kg; 37mg/kg; 15mg/kg; p.o) for the test groups. One hour after the administration of different treatments, the mice were placed one after the other in the Centre of the maze platform. The behaviour of each mouse was observed and recorded during a period of 5 minutes. Among the classical variables measured, there were the number of entries and the time spent in the different arms. There was also the percentage of the time spent in closed arms in the maze [21]. The experimental paradigm was cleaned with ethyl alcohol (70°) after each mouse's passage.

2.3.2. Open Field Test

The Open Field (OF) is a square enclosure with elevated edges, illuminated at the Centre, which does not allow the animal inside to escape or hide. The exploration surface is divided into 17 tiles: 16 tiles dividing the interior surface of the experimental paradigm and one Central tile. The dimensions of the OF were 40 cm square and 19 cm high [22]. The open field test is commonly used to assess the level of locomotor activity, exploration and emotional reactivity in rodents [23-25]. The mice were evenly divided into six groups of five animals each. These animals were treated with distilled water for the negative control, with different doses of *C. citratus* decoction (151mg/kg; 75mg/kg; 37mg/kg; 15mg/kg; p.o) for the test groups and with diazepam (3 mg/kg; i.p) for the positive control group. After administration of the different substances to the mice, they were returned to their original cages to reduce neophobic responses due to the experimental environment [26]. One hour after the administration of the different substances to the mice, they were placed one after the other in the Centre of the experimental paradigm. The behaviour of each mouse was observed and noted for a period of 5 minutes, among parameters recorded, there were the number of crossings and the time spent in Centre. After the observation, the mouse was returned to its original cage and the experimental paradigm was cleaned with ethyl alcohol (70°) [26].

2.3.3. The Hole Board Test

The experimental device used in this test is a board measuring 40 × 40 × 2.2 cm. It has 16 holes of 3 cm diameter. The Hole-Board (HB) is raised 25 cm above the ground. The principle is based on an unconditional conflict between a motivation to explore the new situation and a trend to show fear/anxiety behaviours towards this newness [27]. The HB is a paradigm designed to study the behaviour of a mouse in a new environment. One hour after administration of the different treatments, the mice were placed one after the other in the Centre of the HB and several behavioural parameters were observed and recorded for a period of 5

minutes. Among these parameters were the latency time of the first head dipping and the number of head dipping. The experimental paradigm was cleaned each time with ethyl alcohol before the start of each test. The mice were divided into 6 groups of 5 animals, and were treated with distilled water (10 ml/kg; p.o) for the negative control group, different doses of *C. citratus* decoction (151mg/kg; 75mg/kg; 37mg/kg; 15mg/kg; p.o) for test groups and diazepam (5 mg/kg; i.p) for the positive control group [27].



Figure 2. Mice in falcon tubes during the chronic immobilisation test (Omam, 2022).

2.3.4. Chronic Immobilisation Test

In order to induce chronic stress, 6 mice per group were placed in a rodent restrictor like described by Mei *et al.* 2011 and confirmed by Omam, 2018 [28]. The animals were subjected to repeated immobilisation stress, which consists of completely immobilising them in a falcon tubes called “restrictor”. The restrictor is a cylindrical Plexiglas tube of 3 cm in diameter and 8 cm high. This device does not give the animal any possibility of mobility, hence the term restriction stress or immobilisation [28]. During the test, the animals had no access to food or water. The animals were divided into 7 homogeneous groups of 6 mice each and were subjected to the restriction each day for duration of 2 successive hours during 10 consecutive days. With the aim of ruling out any possibility of the development of habituation behaviour in the mice, immobilisation was carried out at variable times. The different groups were treated successively with distilled water (p o) for the normal control and the stressed negative control groups, the different doses of the decoction (151mg/kg; 75mg/kg; 37mg/kg; 15mg/kg; p.o) for groups and diazepam for the positive control group. Thirty minutes later, induction procedures have done by chronic immobilisation test. Twenty-four hours after the last stress, the mice were treated and one hour after these treatments, the anxiolytic effect of *Cymbopogon citratus* decoction was assessed in these mice, placed on the EPM followed by the OF test. The classical behavioural parameters for measuring anxiety were observed during a

period of 5 minutes [28] followed by the animal sacrifice for *in vivo* antioxidant analysis.

2.4. Statistics

Values were expressed as mean \pm SEM (Standard Error of the Mean). All data were analysed by one way analysis of variance (ANOVA). Post hoc tests were then performed using Dunnet's or Turkey's test by graphpad Insert or Prism, with the level of significance set at $P < 0.05$.

3. Results

3.1. Anxiolytics Effects of *Cymbopogon citratus* Decoction on the Elevated Plus Maze

During the 5 min observation of each mouse, the figure 3A shows that *C. citratus* decoction induced a significant ($p < 0.001$) increase of the number of open arm entries from 7.8 ± 2.1 in negative control mice to 45.2 ± 6.4 in Cc151 mice group. The diazepam also induced a significant ($p < 0.001$) increase of this number to 46.6. Similarly, the figure 3B shows that *C. citratus* decoction induced a significant ($p < 0.001$) increase of the percentage of open arm entries from 14.35% in negative control mice to 78.50% in Cc151 group. The diazepam induced also a significant ($p < 0.001$) increase of this percentage to 84.59% compared to the negative control. Secondly, the figure 3C shows that *C. citratus* decoction induced a significant ($p < 0.001$) increase of the time spent of the open arms from 92.2 ± 21.13 in negative control mice to 262 ± 5.61 in Cc151 mice group. The diazepam also induced a significant increase ($p < 0.001$) of this time compared to the negative control. Finally, the figure 3D shows that *C. Citratus* decoction induced a significant decrease ($p < 0.001$) of the number of entries into the closed arms from 46 ± 2.91 in the negative control mice to 12.4 ± 2.60 of the Cc151 mice group. The diazepam also induced a significant ($p < 0.001$) decrease of the number of entries into the closed arms to 8.8 ± 2.17 .

3.2. Anxiolytic Effects of *Cymbopogon citratus* Decoction on the Open Field Test

The figure 4A shows that *C. citratus* decoction induced a significant ($p < 0.001$) increase of time spent in the centre from 1 ± 0.71 s in negative control mice group to 10.6 ± 1.52 s in Cc 151 mice group. Diazepam also induced a significant ($p < 0.001$) increase of time spent in the centre to 11.8 ± 2.28 s. Similarly, figure 4B shows that *C. citratus* decoction induced a significant ($p < 0.001$) increase in the number of crossing from 44.2 ± 5.12 in negative control mice to 177.8 ± 9.55 in Cc 151 group. Diazepam also induced a significant increase ($p < 0.001$) of the number of crossing to 169.8 ± 11.03 . The figure 4C shows that *C. citratus* decoction induced a significant ($p < 0.001$) decrease of the number of rearing from 109.8 ± 12.55 in negative control mice to 26.6 ± 2.97 of Cc 151 group. The diazepam also induced a significant ($p < 0.001$) decrease of the number of rearing to 9.2 ± 1.48 .

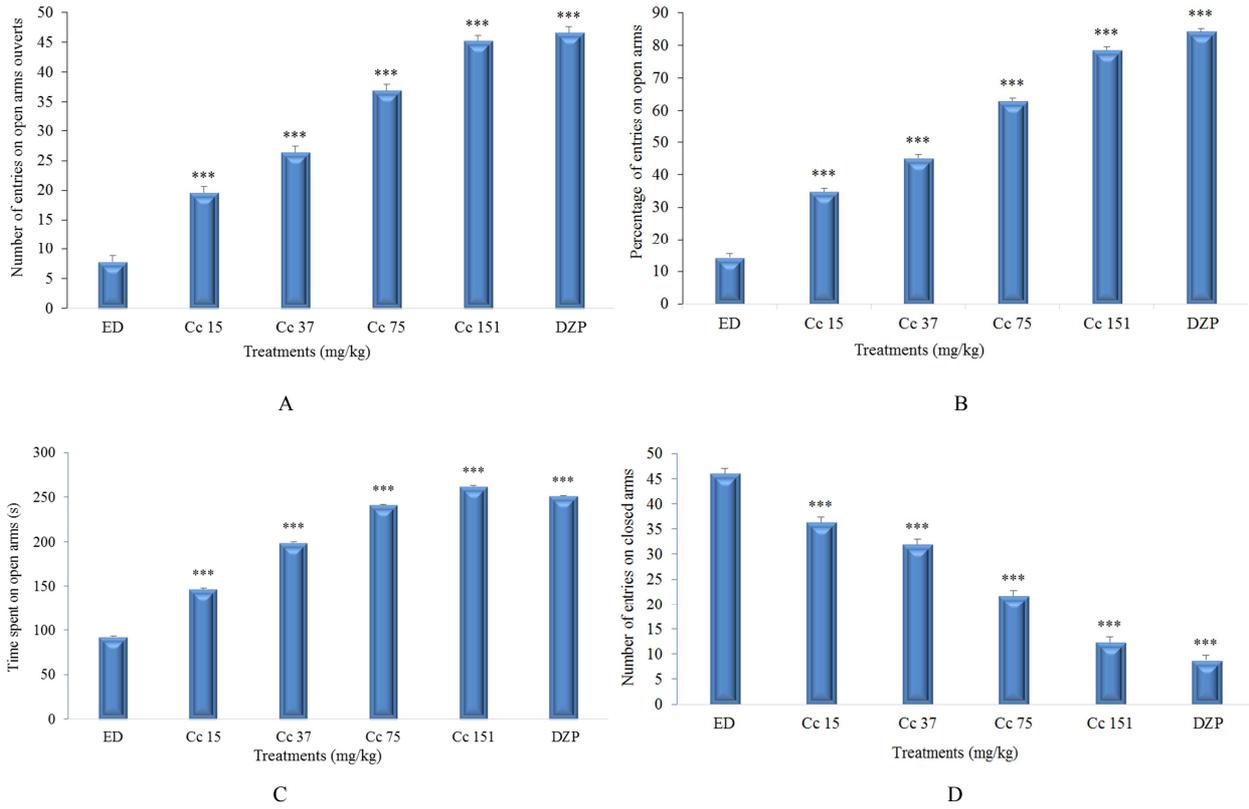


Figure 3. Anxiolytic effects of *Cymbopogon citratus* decoction on the EPM. A (on the number of entries in the open arms); B (on the percentage of entries on the open arms); C (on the time spent on the open arms); D (on the number of entries on the closed arms). Each bar represents the parameters of the EPM, n = 5. ***p<0.001; significant difference from negative control; ED: distilled water; Cc15, Cc37, Cc75, Cc151: different doses of *Cymbopogon citratus*; DZP: Diazepam at 3mg/kg.

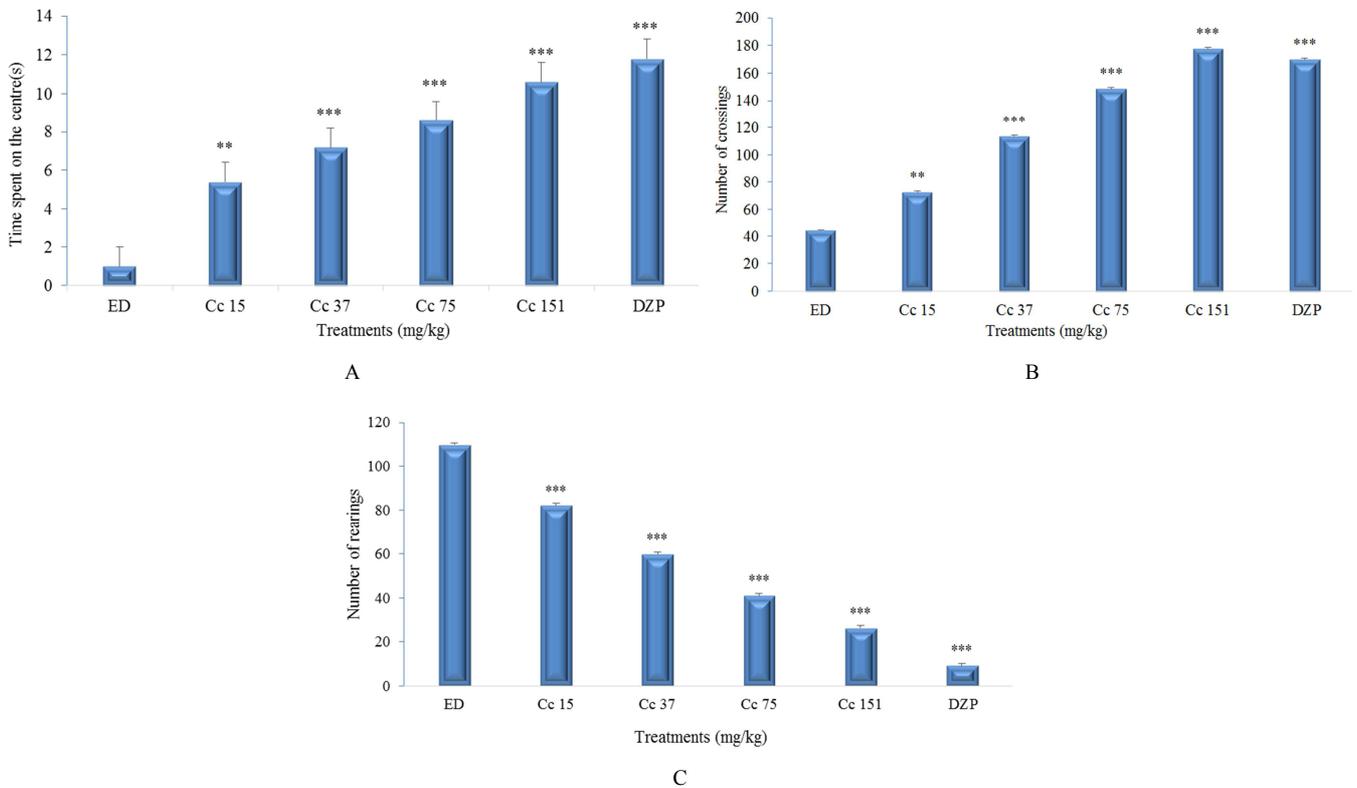


Figure 4. Anxiolytic effects of *Cymbopogon citratus* decoction on the Open field test. A (on the number of crossing); B (on the time spent in the Centre) C (on the number of rearing); Each bar represents the parameters of the OF, n = 5. *p<0.05; ***p<0.001; significant difference from negative control; ED: distilled water; Cc15, Cc37, Cc75, Cc151: different doses of *Cymbopogon citratus*; DZP: Diazepam at 3mg/kg.

3.3. Anxiolytic Effects of *Cymbopogon citratus* Decoction on the Hole Board Test

During the 5 min observation period for each mouse, figure 5A shows that *C. citratus* decoction induced a significant ($p<0.001$) increase of the number of grooming from 0.4 ± 0.55 in the negative control mice group to 3.6 ± 0.55 in the Cc 151 mice group. The diazepam also induced a

significant increase ($p<0.001$) of this number to 4.2 ± 0.45 . In contrast, the figure 5B shows that *C. citratus* decoction induced a significant ($p<0.001$) decrease of the mass of stools produced from 5 ± 0.71 mg in the negative control mice group to 0.4 ± 0.55 mg in the Cc151 mice group. The diazepam also induced a significant ($p<0.001$) decrease of the mass of stools produced.

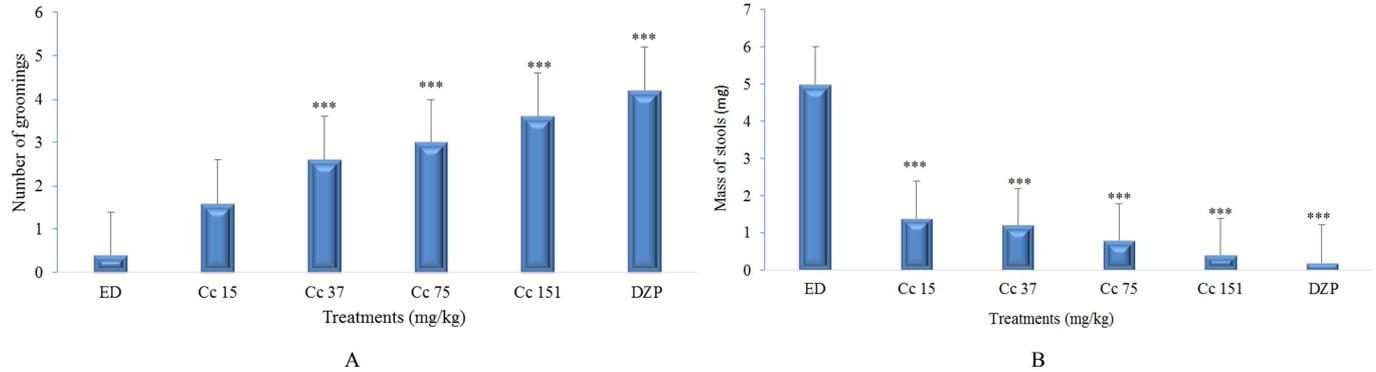
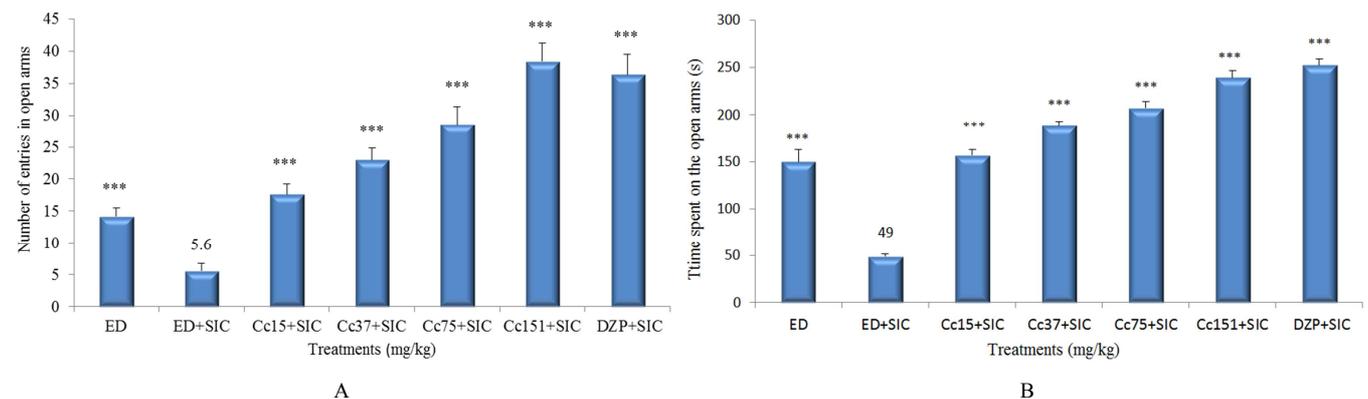


Figure 5. Anxiolytic effects of *Cymbopogon citratus* decoction on the Hole Board test. A (on the number of grooming); B (on the mass of stools); Each bar represents the parameters of the HB, $n = 5$. *** $p<0.001$; significant difference from negative control; ED: distilled water; Cc15, Cc37, Cc75, Cc151: different doses of *Cymbopogon citratus*; DZP: Diazepam at 5mg/kg.

3.4. Anxiolytic Effects of *Cymbopogon citratus* Decoction by Chronic Immobilisation Test on the EPM and OF

During the 5 minutes observation of mice in each group, the figure 6A shows that, the CIS induced a significant ($p<0.001$) decrease of the percentage of entries into the open arms from 45.59% in mice of the normal control group to 12.55% in those in the negative control group. *C. citratus* decoction induced a significant ($p<0.001$) increase of the percentage of entries into the open arms from 12.55% in the negative control mice group to 78.42% of Cc151 mice group. The diazepam at 2mg/kg also induced a significant ($p<0.001$) increase of this percentage to 83.01%. Also, figure 6B shows that, the CIS induced a significant ($p<0.001$) decrease of the time spent in the open arms from 149.6 ± 12.97 s in the normal control mice to 49 ± 2.91 s in the negative control. *C. citratus* decoction induced a significant ($p<0.001$) increase of the time spent in the open arms from 49 ± 2.91 s in the negative control mice to 239.4 ± 7.63 s of Cc151 mice group. The

diazepam at a dose of 2mg/kg also induced a significant increase ($p<0.001$) of this time to 252.4 ± 6.65 s. Secondly, the figure 6C shows that, the CIS induced a significant ($p<0.001$) decrease of the number of crossing from 64.4 ± 6.02 in the normal control mice to 28.2 ± 2.49 in the negative control mice. *C. citratus* decoction induced a significant increase ($p<0.001$) of the number of crossing from 28.2 ± 2.49 in the negative control mice to 128.2 ± 4.92 of the Cc151 mice group. The diazepam at a dose of 2mg/kg also induced a significant increase ($p<0.001$) of the number of crossings to 116.4 ± 11.01 . Finally, the figure 6D shows that, the CIS induced a significant ($p<0.001$) decrease of time spent in the centre from 13 ± 2 s in the normal control mice to 3.6 ± 0.55 s in the negative control mice. *C. Citratus* decoction induced a significant ($p<0.001$) increase of time spent in centre from 3.6 ± 0.55 s in negative control mice to 55 ± 3.08 s of Cc151 mice group. The diazepam at a dose of 2mg/kg also induced a significant ($p<0.001$) increase of time spent in centre to 52 ± 3.67 s.



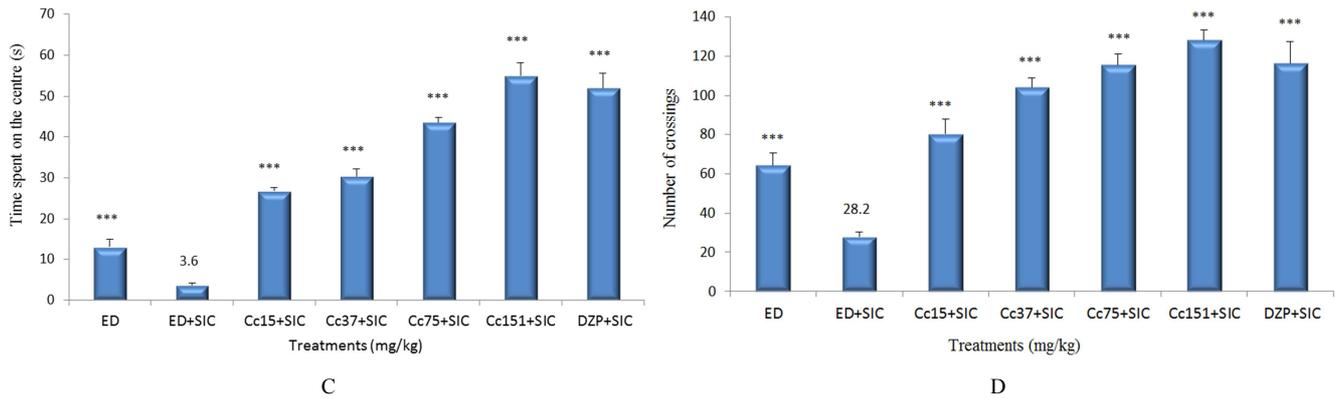


Figure 6. Anxiolytic effects of *Cymbopogon citratus* decoction on the EPM and OF. A (on the number of entries in open arms); B (on the time spent on the open arms); C (on the time spent in the centre) D (on the number of crossing); Each bar represents the parameters of the EPM or OF, n = 5. ***p<0.001; significant difference from negative control; ED: distilled water; Cc15, Cc37, Cc75, Cc151: different doses of *Cymbopogon citratus*; DZP: Diazepam at 2mg/kg.

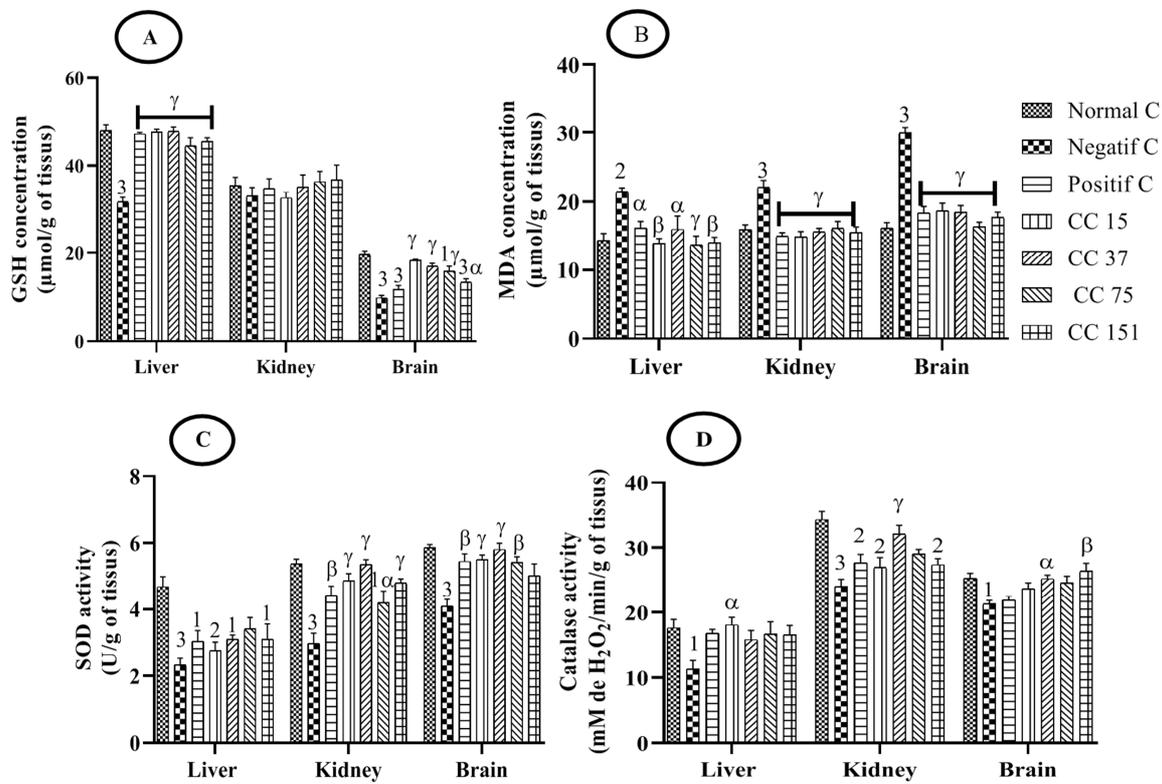


Figure 7. Anxiolytic and antioxidant Properties of *Cymbopogon citratus* decoction in vivo. A (on the concentration of reduced glutathione); B (on the concentration of Malonaldehyde), C (on the concentration of Superoxyde dismutase) D (on the concentration of catalase); Each bar represents the parameters of the EPM or OF, n = 5. 1p<0,05; 2p<0,01; 3p<0,0001; significant difference from negative control; ED: distilled water;; Cc15, Cc37, Cc75, Cc151: different doses of *Cymbopogon citratus*; DZP: Diazepam at 2mg/kg.; DZP: Diazepam at 5mg/kg.

3.5. Anxiolytic and Antioxidant Effects of *Cymbopogon citratus* Decoction in Vivo

The figure 7A shows the effect of *C. citratus* decoction on the activity of reduced glutathione (GSH) in the different organs of brain, liver and kidney. A significant difference (p<0.001) was found between the normal control and the stressed negative control groups. *C. citratus* decoction induced a significant (p<0.001) increase in GSH concentration compared to the stressed negative control group. The diazepam also induced a significant (p<0.001)

increase of GSH concentration. Then, the figure 7B shows the effect of *C. citratus* decoction on MDA concentration in the same organs. There was a significant difference (p<0.001) between the normal control and the stressed negative control groups. The decoction of *C. citratus* induced a significant (p<0.001) increase of GSH concentration compared to the stressed negative control group. Also, the figure 7C shows the effect of *C. citratus* decoction on the concentration of superoxide dismutase (SOD) in the different organs like brain, liver and kidney. There was a significant difference (p<0.001) between the normal control and the

stressed negative control groups. *C. citratus* decoction induced a significant ($p < 0.001$) increase of SOD concentration compared to the stressed negative control group. The diazepam also induced a significant ($p < 0.001$) increase of SOD concentration. Finally, the figure 7D shows the effect of *C. citratus* decoction on catalase (CAT) activity in these same organs. A significant difference ($p < 0.05$) between the normal control and the stressed negative control groups have been observed. The decoction of *C. citratus* induced a significant ($p < 0.05$) increase of CAT concentration compared to the stressed negative control group.

4. Discussion

In this study, the extraction yield of the decoction of *C. citratus* leaves in relation to the initial mass of powder was 15.12%. This result shows that the active principle of the plant distills fairly well in water [29, 30]. Also, the phytochemical screening performed on the decoction of *C. citratus* shows the presence of several chemical families such as sterols, phenolic compounds, saponins and coumarins. As well as the acute oral toxicity study of *C. citratus* leaves decoction was carried out at a lethal dose (DL50) of 5000mg/kg. The mice used in this experiment showed no signs of toxicity 24 hours after administration of the decoction. No mortality also was recorded after 14 days of experimentation. Then, through classical behavioural tests, this work showed that the decoction of *C. citratus* caused an increase of the number of entries and the time spent in the open arms, the percentages of entries in the open arms, and a decrease of the number of entries in the closed arms. These results led to the conclusion that *C. citratus* the decoction would have anxiolytic properties because it was demonstrated by Santos *et al.* in 2006 that substances that increase the number of entries and the time spent in open arms have anxiolytic effects. As the diazepam, the extract of our plant would act on the GABA-A receptor complex, precisely at his different receptor sites for reducing the opening time of the voltage-dependent chloride channel [31]. Analysis of the behavioural parameters of mice showed that the decoction of this plant decreased the number of rearings. This has suggested yet the presence of anxiolytic effects of *C. citratus* decoction [32, 33]. In addition, it was found that when animals were treated with distilled water and placed on the paradigm platform, they would hide in the closed arms. This behaviour corroborated the observations of Rodgers and Cole whose showed that when placed on the central platform of the EPM, mice not treated with anxiolytic substances avoided the open arms and the light because they were anxious. The aqueous extract of this plant would act through to the complex GABA-A receptor at the level of barbiturates receptor sites, benzodiazepine, alcohol, thiroidian or gaba sites too, by extending the opening of voltage-dependent chloride channel to produce the anxiolytic effect [34, 35]. The parameters evaluated through the open field test showed that the increase of the time spent in the central zone and the number of crossing as well as the decrease of the number of

rearing indicated a decrease of the avoidance of open spaces. This exploration of the open field is a factor that shows that the mouse is no longer anxious. Therefore, like DZP, *C. citratus* decoction has anxiolytic properties [36]. The feeling of insecurity or the desire to flee in the HB test, which results of an increase in exploration of the holes, may be due to an increased amount of brain serotonin. As the treated mice do not feel the urge to flee and the feeling of insecurity in the new environment, they do not attempt to explore the holes but rather become familiar with them. This leads to the hypothesis of inhibition of serotonin reuptake in presynaptic neurons after its release into the synaptic cleft [37, 38]. Because both *C. citratus* decoction and DZP caused a reduction in the mass of stool produced and an increase of the number of grooming by mice. These results indicate the presence of anxiolytic properties of *C. citratus* decoction. This anxiolysis would take place by acting on the benzodiazepine site contained in GABA receptors, [39-42]. The increase in the number of entries and time spent in the open arms, the time spent in the Centre and the number of crossing on the EPM and OF paradigms shows the animal's desire to explore its environment and the more time the animal spends in the open arms or in the Centre, the less anxious it is. This shows that the decoction of *C. citratus* would have anxiolytic properties which could be explained through certain hypotheses that reveal its mechanism of action. First of all, this chronic immobilisation stress test would stimulate the hypothalamic-pituitary-adrenal (HPA) axis through the production of biogenic amines (catecholamine and indolamine) which promote the release of glucocorticoids responsible for the anxiety state [43, 46]. In addition, *in vivo* biochemical tests have demonstrated antioxidant enzyme activity in the brain, liver, and kidney by *C. citratus* decoction. A significant increase of the activity of SOD, GSH CAT as well as a reduction of the lipid deposition of MDA, which are compounds responsible of antioxidant activity, was observed. This means that the decoction of the plant would stimulate these enzymes to reduce free radicals and thus treat anxiety [42]. Thus, the plant would possess efficiency with visible behavioural effects and stable enzymatic and neurochemical markers [44-46].

5. Conclusion

In this last part of the study, it should be recalled that the aim was to evaluate the anxiolytic activity of *Cymbopogon citratus* in mice. For this purpose, the EPM, OF and HB tests for classical and chronic anxiety results revealed that the increase of the number of entries and time spent in the open arms, the percentages of entries in the open arms and contrastly the decrease of the number of entries in the closed arms were noted. As well as the increase of the time spent in the central zone and the number of crossings as well as the decrease of the number of rearing indicated a reduction of avoidance of the open areas. This exploration of the open area is a factor that shows the mouse is no longer anxious. The phytochemical screening carried out on the *C. citratus*

decoction shows the presence of several chemical families such as sterols, phenolic compounds, saponins and comarins, which are probably involved in reducing anxiety. Just as the acute oral toxicity study of *C. citratus* decoction was carried out at a dose of 5000 mg/kg and caused no major consequences. The mice used in this experiment showed no signs of toxicity 24 hours after administration of the decoction. Then, no mortality was recorded after 14 days of experimentation. In addition, in vivo biochemical tests demonstrated antioxidant enzyme activity in the brain, liver, and kidney by the *C. citratus* decoction. For that and other reasons, this plant is frequently used in traditional medicine. The results of this study showed that the aqueous extract of *Cymbopogon citratus* exerts its anxiolytic effects by interfering with neurotransmission systems GABA and through its antioxidant properties. These results justify the empirical use of this plant for the management of nervous system disorders such as anxiety.

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