

# Anxiolytic and Antioxydant Effects of Aqueous Extract of *Hiptis spicigera* Lam in Mice Exposed to Classical Paradigms and Chronic Immobilisation Test

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**Abstract:** Anxiety is defined as an exaggerated feeling of apprehension, uncertainty and fear. The pharmacological treatments used for this disease show many side effects, which has limited their use by the patients. However, it is worth mentioning that in Africa medicinal plants are invaluable resources for the vast majority of rural populations, where more than 80% use them for their primary health care. This reality could justify the use of plants in African traditional medicine to treat neuropsychiatric diseases. The aim of this study is to assess the anxiolytic properties of *Hyptis spicigera* Lam leaves decoction on chronic immobilisation after treated by classical tests as EPM, Open Field and Hole Board. In order to assess the anxiolytic activity of the decoction on acute and chronic anxiety, the mice were divided by groups and treated with distilled water, Diazepam and different doses of the decoction (22.5mg/kg, 56.25mg/kg, 112.5mg/kg and 225mg/kg). Finally, some oxidative stress parameters such like catalase, sulfoxide dismutase, reduced glutathione, nitrite and malondialdehyde were measured. Concerning EPM test, the figure 1A and B showed a significant increase respectively of number of entries  $6.8 \pm 1$  and time spent in the open arms  $94 \pm 22.74$  s from the negative control to  $13.6 \pm 2.3$  and  $210 \pm 11$  s in 225mg/kg dose of plant. Also, the figure 1C showed a significant decrease of the percentage of time spent in the closed arms from 68.7 to 30%. These parameters showed the reduction of the level of anxiety in these mice. The Open Field and Hole Board tests, by their parameters also showed that the *Hyptis spicigera* decoction would have anxiolytic properties. This could be justified may be by the presence of secondary metabolites such as saponins and flavonoids. The assay of oxidative stress markers showed that mice group which received the decoction and diazepam, had elevated catalase, sulfoxide dismutase, reduced glutathione and nitrites, but their malondialdehyde was low. The results of our study have shown that the decoction of *Hyptis spicigera* Lam would have anxiolytic and antioxydant properties.

**Keywords:** Hyptis Spicigera, Decoction, Anxiolytics, Antioxidant

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## 1. Introduction

Brain disorders or dysfunctions are a major concern for human society. They represent an area in which pharmacological intervention plays a key role [1]. Roughly 12% of people are affected by an anxiety disorder during a year and between 5% and 30% are affected at some point in their lives [2]. According to the World Health Organisation (WHO), neuropsychiatric disorders are a set of “mental health problems”, which are characterised by abnormalities in thinking, feeling, behaving and relationships with others. These pathologies disable the person involved and affect people around them. The causes of these disorders are mainly genetic, social, environmental and psychotropic. Mental and neurological disorders account for 13% of the total disease burden worldwide [3]. These pathologies affect all categories of people, race, gender and age [4]. Among these, there is anxiety. This disease is defined as an exaggerated feeling of apprehension, uncertainty and fear. It is a state of unpleasant tension with an anticipation of imminent danger [5]. The somatic manifestations of anxiety include: tiredness, palpitations, headaches, insomnia and excessive sweating. It is associated with almost all mental disorders and frequently with physical illness. Anxiety disorders are one of the most common mental disorders [6, 7]. Pharmacological treatments that have long been commonly used to treat anxiety disorders are benzodiazepines (BZDs), barbiturates, monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs). These agents mainly used to relieve symptoms and to offer palliation of a temporary nature show side effects such like insomnia, sedation, muscle relaxation, weaning and tolerance (as BZDs, barbiturates or alcohol), sexual dysfunction, anticholinergic effects, antihistaminic effects (as TCAs) which has limited their use in patients [5]. However, these modern treatments are expensive, complex and unaffordable to African populations living in rural areas [8, 9]. Moreover, many of these psychoactive molecules are of plant origin [10, 11]. It should be noted that in Africa, medicinal plants are invaluable resources for the vast majority of rural populations, where more than 80% use them for their primary health care [5]. This could justify the use of plants in traditional African medicine to treat neuropsychiatric diseases [12, 13]. For this reason, many pharmaceutical companies are carrying out studies to find alternative medicine or plant-derived drugs with more specific anxiolytic and antioxidant effects [4]. These recent years, the use of alternative medicine in particular, derived from plants, has been screened and marketed as drugs [8]. Plants of the genus *Hyptis* are members of the Lamiaceae family. They have a wide range of properties and uses, especially for medicinal and culinary purposes. The Lamiaceae include many medicinal species, all parts of which can be used. Among the plants of this family, *Hyptis* specy (also called *Ourdi Djoulde* in Foulfoulde) is the subject of our present study. This specy has many medicinal virtues. In this study, we will assess the anxiolytic and antioxidant effects of *Hyptis spicigera* Lam decoction leaves.

## 2. Material and Methods

### 2.1. Material

#### 2.1.1. Plant Material

The leaves of *Hyptis spicigera* L. were harvested in the Far North region of Cameroon, precisely in the Maroua subdivision, Zokok Laddeo village. The identification of the plant was carried out at the National Herbarium of Cameroon in Yaounde. Sample identified in comparison with Letonzey's material R 10908 of specimen of the Herbarium collection n°28063 SRF/cam.

#### 2.1.2. Animals

Swiss albino naive mice of either sex weighing approximately 18-30 g, aged about 2 to 3 months were used for experimental purpose. 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

The decoction and doses of *Hyptis spicigera* were obtained based on the traditional medicine protocol. The leaves of *Hyptis spicigera* were dried for 24 days at room temperature. After grinding, we obtained the powder. Then, 40g of this powder were introduced in Erlen-meyer and completed with 400 ml of distilled water for a concentration of 100 mg/ml. The Erlen-meyer flask was then closed and boiled for about 20 min. After cooling down, the mixture was filtered through Watmann paper N°. 3, the filtrate collected was considered as the stock solution. The dilution have been done by  $\frac{1}{2}$ ,  $\frac{1}{4}$  and  $\frac{1}{10}$  factors in order to determinate the doses of administered decoction. In other experiment, the filtrate obtained was dried at  $45^\circ\text{C}$  in the oven to obtain a dry extract mass. This gave us a yield of 22.5 %.

#### 2.2.2. Preparation of Diazepam (DZP)

The DZP which served to the preparation of used solutions was contained in ampoules of 10 mg/2mL with a concentration of 5 mg/ml under conditions where the volume of administration was defined at 10ml/kg. For the preparation of different concentrations of DZP which used in the chronic test, knowing that the final concentration wished was 0.2 mg/ml, a volume of DZP of 1 ml was taken and introduced into the beaker, completed with distilled water to 25 ml for a dose of 2 mg/kg. Then, for the preparation of the concentration of DZP used in the classical tests (EPM and OF) and knowing that the final concentration wished was 0.3 mg/ml, a volume of DZP of 1 ml was taken and introduced into the beaker, completed

with distilled water to 16.6 ml for a dose of 3 mg/kg. Finally, for the preparation of the concentration of DZP used in the HB test, and knowing that the final concentration wished was 0.5 mg/ml, a volume of DZP of 1 ml was taken and introduced into the beaker, then completed with distilled water to 10 ml for a dose of 5 mg/kg.

### 2.3. Pharmacological Tests

#### 2.3.1. Elevated Plus Maze Test (EPM)

The Elevated Plus Maze (EPM) used is the one described by Handley and Mithiani (1984) [14]. 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” [15]. 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 *H. spicigera* decoction (225mg/kg; 112.5mg/kg; 56.25mg/kg; 22.5mg/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. 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 [16]. The open field test is commonly used to assess the level of locomotor activity, exploration and emotional reactivity in rodents [17-19]. 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 *H. spicigera* decoction (225mg/kg; 112.5mg/kg; 56.25mg/kg; 22.5mg/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 [20]. 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 5 minutes of observation, the mouse was returned to its original cage and the experimental paradigm was cleaned with ethyl alcohol (70°).

#### 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 [21]. 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 *H. spicigera* decoction (225mg/kg; 112.5mg/kg; 56.25mg/kg; 22.5mg/kg; *p.o*) for test groups and diazepam (5 mg/kg; *i.p*) for the positive control group.

#### 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 [22]. The animals were subjected to repeated immobilisation stress, which consists of completely immobilising them in a falcon tube 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. 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 (225mg/kg; 112.5mg/kg; 56.25mg/kg; 22.5mg/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 *H. spicigera* 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.

2.4. Statistics

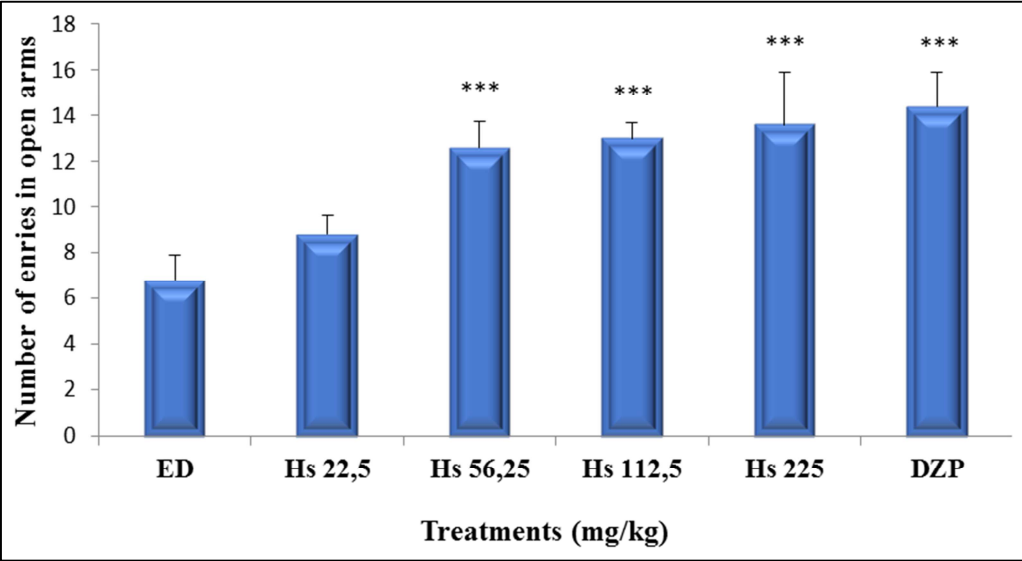
Values were expressed as mean ± SEM (Standard Error of the Mean). All data were analysed by one way analysis of variance (ANOVA). Post hoc tests were then performed using Dunnet's or Turkey's test by graphpad Insert or Prism, with the level of significance set at P<0.05.

3. Results

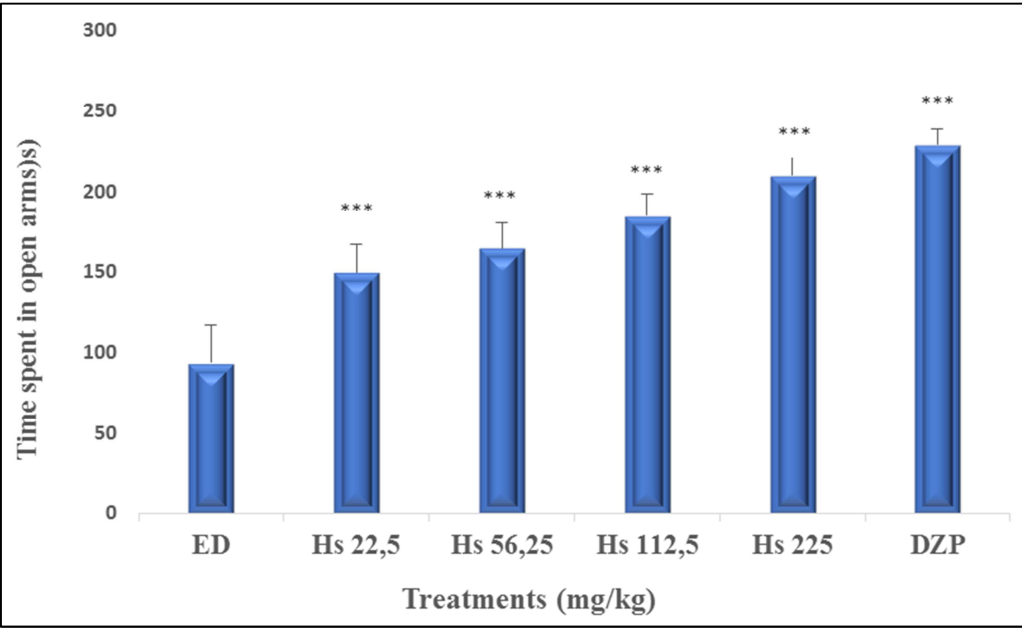
3.1. Anxiolytic Effects of *Hiptis Spicigera* Decoction on the Elevated Plus Maze

During the 5 minutes observation of each mouse, figure 1A shows that *Hyptis spicigera* decoction induced a significant increase (p<0.001) of the number of open arms

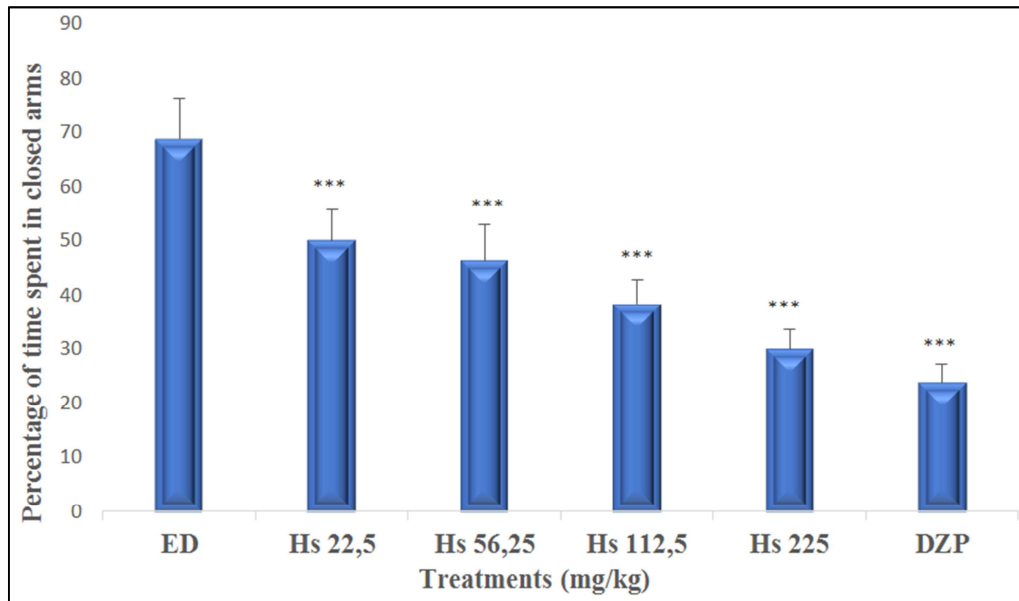
entries from 6.8±1.1 in the negative control mice to 13.6±2.3 in those of the group treated with the dose of 225mg/kg. Diazepam also induced a significant increase (p<0.001) of this number. Also, figure 1B shows that *H. spicigera* decoction induced a significant increase (p<0.001) of the time spent in open arms from 94±22.75 s in the negative control group of mice to 210 ± 11 s in those of the group treated with the dose of 225mg/kg. Diazepam equally induced a significant increase (p<0.001) of this time. The end, Figure 1C shows that *H. spicigera* decoction induced a significant decrease (p<0.001) of percentage of time spent in closed arms from 68.7±7.58% in the negative control group of mice to 30 ±3. 67% in the group treated with 225mg/kg. Diazepam equally induced a significant decrease (p<0.001) i of this percentage to 23.8± 3.39%.



A



B



C

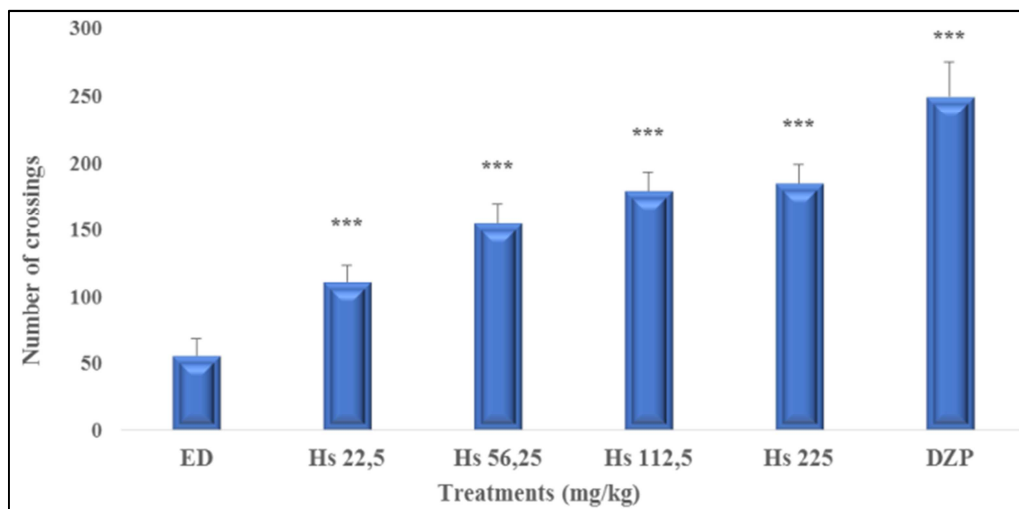
**Figure 1.** Anxiolytic effects of *Hiptis spicigera* decoction on the EPM. A (on the number of entries in the open arms); B (on the time spent in the open arms); C (on the percentage of time spent in the closed arms). Each bar represents the parameters of the EPM,  $n = 5$ . \*\*\* $p < 0.001$ ; significant difference from negative control; ED: distilled water; Hs22.5, Hs56.25, Hs112.5, Hs225.5: different doses of *Hiptis spicigera*; DZP: Diazepam at 3mg/kg.

### 3.2. Anxiolytic Effects of *Hiptis Spicigera* Decoction on the Open Field Test

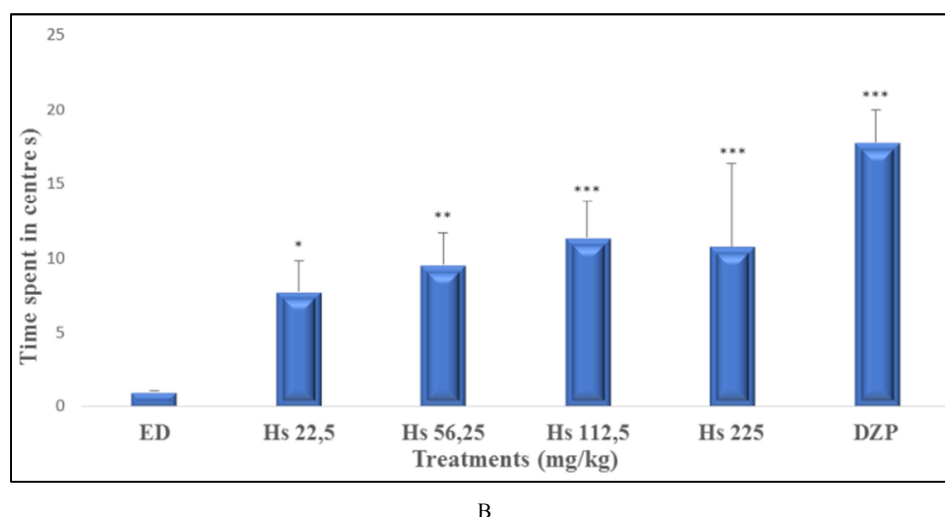
The figure 2A shows that *H. spicigera* decoction induced a significant increase ( $p < 0.001$ ) of the number of crossings from  $55.8 \pm 12.05$  in the negative control group of mice to  $185 \pm 13.69$  in the group treated at 225mg/kg. Diazepam also induced a significant increase ( $p < 0.001$ ) of the number of crossings to  $249.8 \pm 25.16$ . Also, the figure 2B shows that *H. spicigera* decoction induced a significant increase ( $p < 0.001$ ) of the time spent in open arms from 0 s in the negative control mice to  $10.8 \pm 5.54$  s in those of the group treated with the dose of 225mg/kg. Diazepam also induced a significant increase ( $p < 0.001$ ) of this time to  $17.8 \pm 2.17$  s.

### 3.3. Anxiolytic Effects of *Hiptis Spicigera* Decoction on the Hole Board Test

The figure 3A shows that *H. spicigera* decoction induced a significant decrease ( $p < 0.001$ ) of the time spent of first head dipping from  $3.4 \pm 0.55$  s in the negative control group of mice to 1 s in the group treated at 225mg/kg. Diazepam also induced a significant decrease ( $p < 0.001$ ) of the time spent of first head dipping to  $1.2 \pm 0.4$  s. Also, the figure 3B shows that *H. spicigera* decoction induced a significant decrease ( $p < 0.001$ ) of the number of head dipping from  $57.4 \pm 7.50$  in the negative control group of mice to  $26.8 \pm 2.86$  in the group treated with 225mg/kg. Diazepam also induced a significant decrease ( $p < 0.001$ ) of this number of head dipping to  $16.8 \pm 2.05$ .

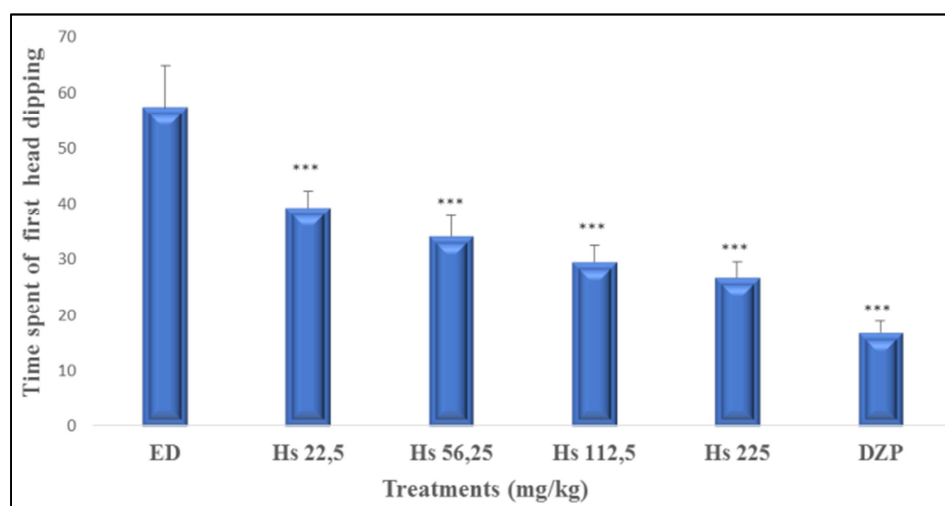


A

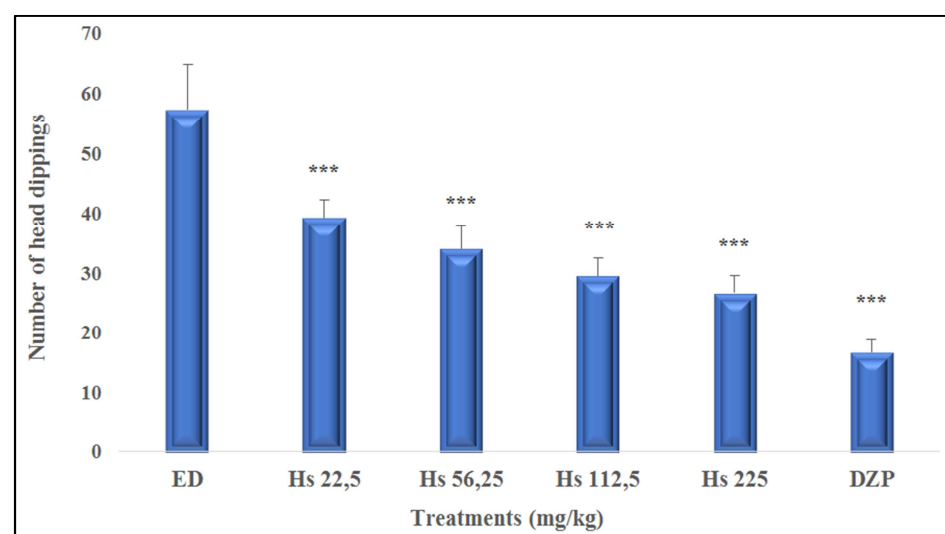


B

**Figure 2.** Anxiolytic effects of *Hiptis spicigera* decoction on the Open field test. A (on the number of crossings); B (on the time spent in the Centre); Each bar represents the parameters of the OF,  $n = 5$ . \* $p < 0.05$ ; \*\*\* $p < 0.001$ ; significant difference from negative control; ED: distilled water; Hs22.5, Hs56.25, Hs112.5, Hs225.5: different doses of *Hiptis spicigera*; DZP: Diazepam at 3mg/kg.



A



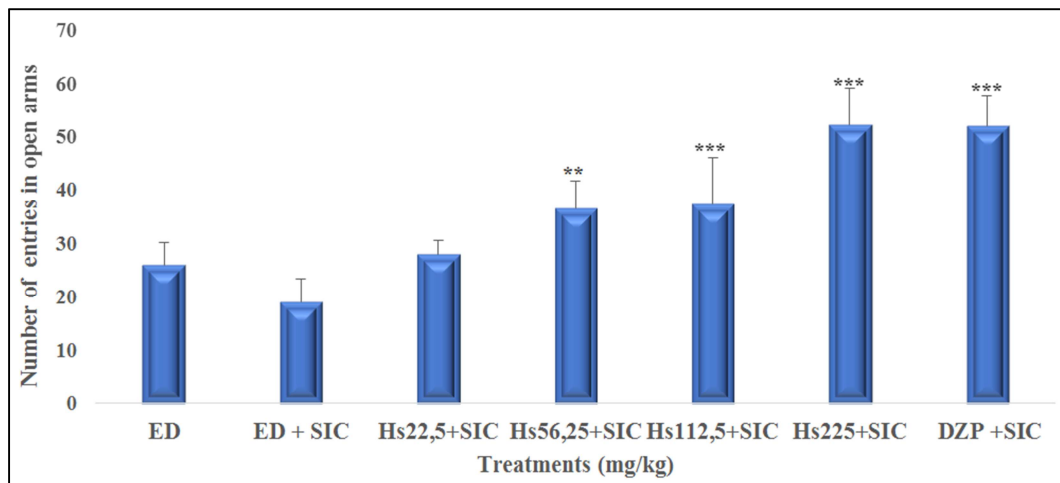
B

**Figure 3.** Anxiolytic effects of *Hiptis spicigera* decoction on the Hole Board Test. A (on the time spent of the first head dipping); B (on the number of head dipping); Each bar represents the parameters of the HB,  $n = 5$ . \*\*\* $p < 0.001$ ; significant difference from negative control; ED: distilled water; Hs22.5, Hs56.25, Hs112.5, Hs225.5: different doses of *Hiptis spicigera*; DZP: Diazepam at 5mg/kg.

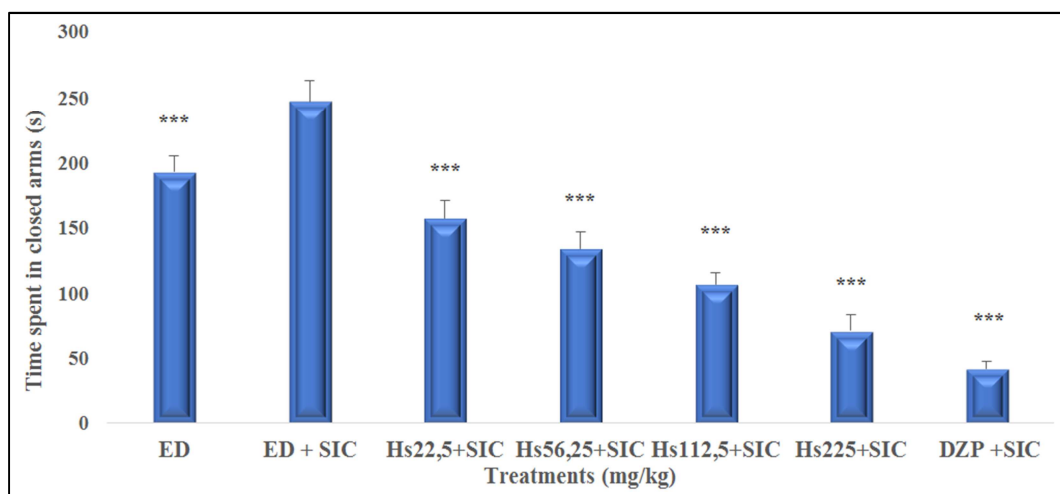
### 3.4. Anxiolytic Effects of *Hiptis spicigera* Decoction by Chronic Immobilisation Test on the EPM and OF

Figure 4A reveals that the Chronic Immobilisation stress (CIS) test caused a non-significant decrease of the number of entries into the open arms from  $26 \pm 4.18$  in the normal control group of mice to  $19 \pm 4.36$  in the negative control group. *H. spicigera* decoction induced a significant increase ( $p < 0.001$ ) of the number of entries into the open arms from  $19 \pm 4.36$  in the negative control group of mice to  $52.2 \pm 6.98$  in the group treated with 225mg/kg dose of plant. Diazepam at 2mg/kg induced equally a significant increase ( $p < 0.001$ ) of the number of entries into the open arms at  $52 \pm 5.79$ . Also, the figure 4B shows that, the SIC induced a significant increase of the time spent in the closed arms from  $193 \pm 12.04$  s in the normal control group of mice to  $247.4 \pm 16.02$  s of the negative control group. *H. spicigera* decoction induced a significant decrease ( $p < 0.001$ ) in the time spent in the closed arms from  $247.4 \pm 16.02$  s in the negative control group of mice to  $71 \pm 12.25$  s in the 225mg/kg treated group. The

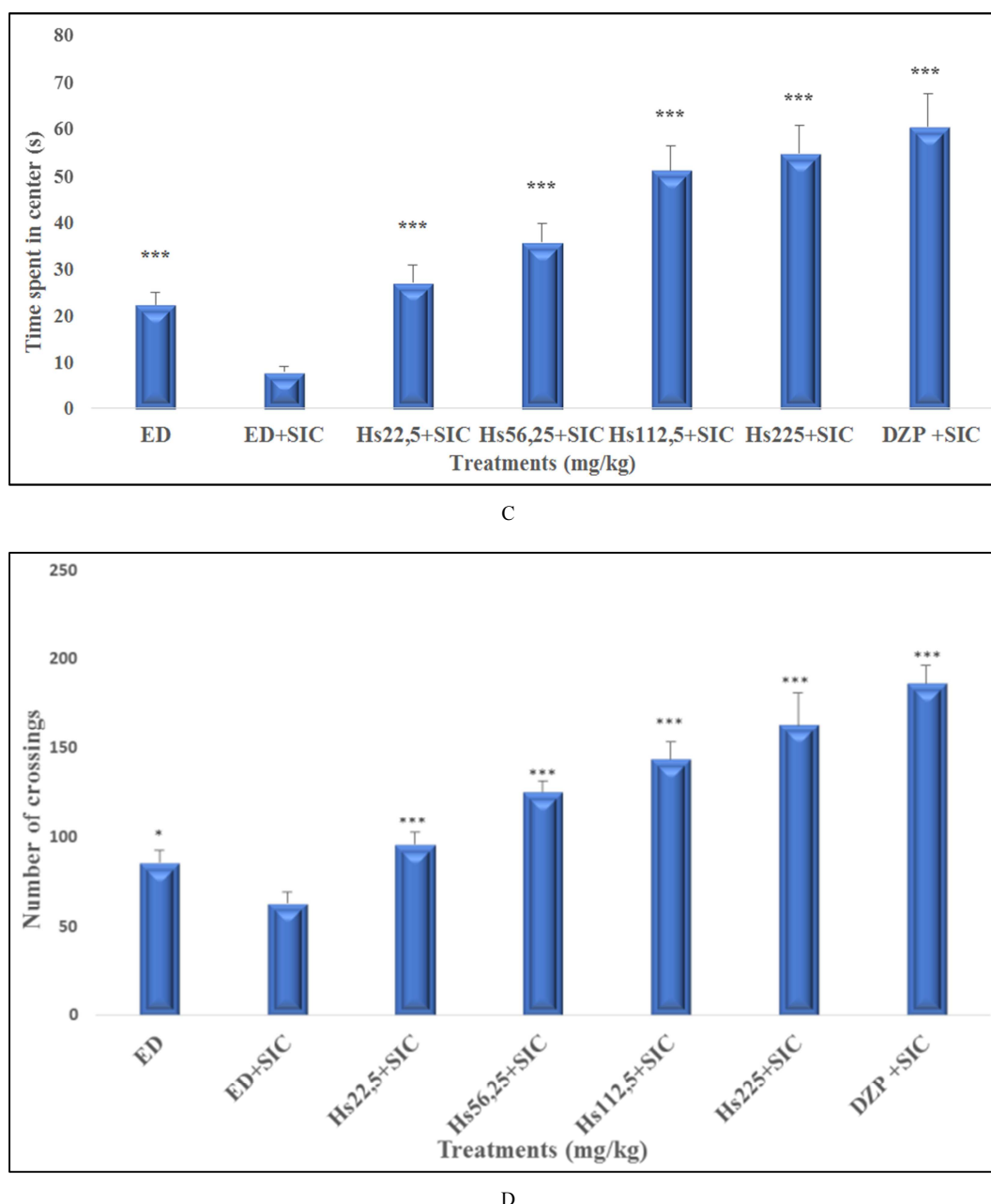
diazepam induced equally a significant decrease ( $p < 0.001$ ) on this time to  $41.8 \pm 5.81$  s. The figure 4C shows that CIS induced a significant decrease ( $p < 0.001$ ) of time spent in the Centre from  $22.4 \pm 2.61$  s in normal control group of mice to  $8 \pm 1.22$  s in negative control group. Meanwhile *H. spicigera* decoction induced a significant increase ( $p < 0.001$ ) of time spent in the Centre from  $8 \pm 1.22$  s in the negative control group of mice to  $54.8 \pm 5.93$  s in the 225mg/kg treated group. The diazepam induced also a significant increase ( $p < 0.001$ ) of time spent in the Centre to  $60.4 \pm 7.3$  s. The end, the figure 4D shows that, the CIS induced a significant decrease ( $p < 0.001$ ) of crossings from  $86.4 \pm 6.75$  in normal control group of mice to  $63.6 \pm 5.77$  in negative control group. Meanwhile *H. spicigera* decoction induced a significant increase ( $p < 0.001$ ) of crossings from  $163 \pm 18.15$  in the negative control mice to  $186.4 \pm 9.96$  in the 225mg/kg treated group. The diazepam also induced a significant increase ( $p < 0.001$ ) of crossings to  $60.4 \pm 7.3$ .



A



B



**Figure 4.** Anxiolytic effects of *Hiptis spicigera* decoction on the EPM and OF Tests. A (on the time spent of the first head dipping); B (on the number of head dipping); C (on the time spent in the Centre); D (on the number of crossings). Each bar represents the parameters of the EPM or OF,  $n = 5$ . \* $p < 0.05$ , \*\*\* $p < 0.001$ ; significant difference from negative control; ED: distilled water; Hs22.5, Hs56.25, Hs112.5, Hs225.5: different doses of *Hiptis spicigera*; DZP: Diazepam at 5mg/kg.

### 3.5. Anxiolytic and Antioxydant Effects in Vivo

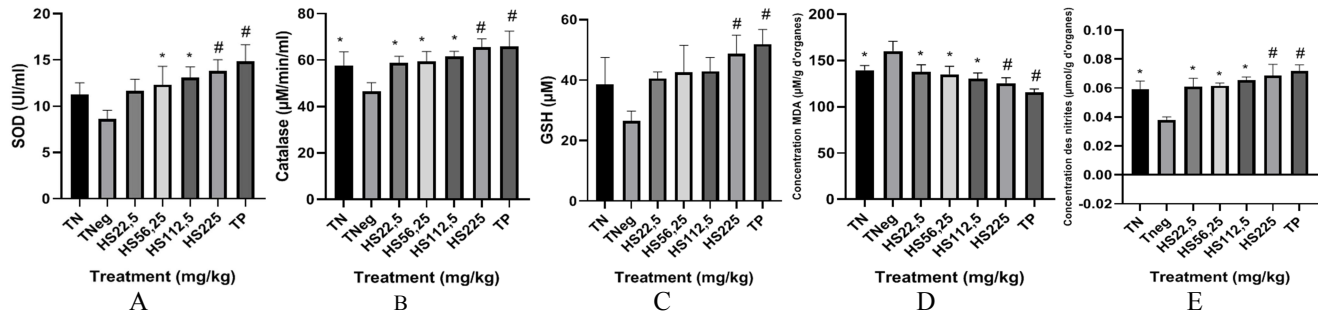
Figure 5A shows the effect of *H. spicigera* decoction on SOD concentration in the brain. There was a difference in SOD concentration between the normal control and the stressed negative control. The *H. spicigera* decoction at HS225 dose induced a highly significant increase ( $p < 0.001$ ) of SOD concentration compared to the stressed negative control group. Diazepam also induced a very significant increase ( $p < 0.001$ ) of SOD concentration. The figure 5B shows the effect of *H. spicigera* decoction on catalase activity in the brain. There was a significant difference

( $p < 0.01$ ) between the normal control group and the stressed negative control group. *H. spicigera* decoction at Hs225 dose induced a highly significant increase ( $p < 0.001$ ) of CAT concentration compared to the stressed negative control group. Diazepam also induced a highly significant increase ( $p < 0.001$ ) of CAT concentration in the brain. The Figure 5C shows the effect of *H. spicigera* decoction on reduced glutathione activity in the brain. There was a small difference between the normal control and the stressed negative control groups. *H. spicigera* decoction at HS225 induced a highly significant increase ( $p < 0.001$ ) of GSH concentration compared to the stressed negative control group. The



diazepam also induced a very significant increase ( $p < 0.001$ ) of GSH concentration. The Figure 5D shows the effect of *H. spicigera* decoction on MDA concentration in the brain. It is a significant difference ( $p < 0.001$ ) of MDA concentration between the normal control and the stressed negative control groups. *H. spicigera* dose induced a very significant decrease ( $p < 0.001$ ) of MDA concentration decoction at HS225 compared to the stressed negative control group. The Figure

5E illustrates the effects of *H. spicigera* decoction on the nitrite concentration in the brain. There was a significant difference in nitrite concentration between the normal control and the stressed negative control groups. *H. spicigera* decoction at HS225 dose induced a significant ( $p < 0.001$ ) increase of nitrite concentration compared to the stressed negative control group. The Diazepam also induced a significant increase ( $p < 0.001$ ) in nitrite concentration.



**Figure 5.** Anxiolytic and antioxidant Effects of *Hyptis spicigera* decoction in vivo. A (on the concentration of Superoxyde dismutase); B (on the concentration of catalase); C (on the concentration of reduced glutathione); D (on the concentration of Malonaldehyde); E (on the concentration of Nitrites). Each bar represents the parameters of the EPM or OF,  $n = 5$ . \* $p < 0.05$ , # $p < 0.001$ ; significant difference from negative control; ED: distilled water; HS22.5, HS56.25, HS112.5, HS225.5: different doses of *Hyptis spicigera*; DZP: Diazepam at 5mg/kg.

## 4. Discussion

The results of this work demonstrated that *H. spicigera* decoction led to an increase of the number of entries and time spent in the open arms, a decrease of percentage of time spent in the closed arms on EPM. These results led to the conclusion that *H. spicigera* decoction would have anxiolytic properties because substances that increase the number of entries and time spent in open arms have been shown to have anxiolytic effects [23]. Knowing that EPM test is based on the natural aversion of rodents to open spaces and large areas. Analysis of the behavioural parameters of the mice revealed that the decoction of this plant reduced also the percentage of time spent in closed arms. That suggested the presence of anxiolytic effects in *H. spicigera* decoction [24-26]. In addition, it was seen that when animals were treated with distilled water and placed on the paradigm platform, its moved in the closed arms. This behaviour confirmed the observations of Rodgers and Cole who showed that once 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 [27].

Moreover, the OF test was developed to measure emotional reactivity in rodents [28]. This test presents the approach/avoidance conflict in mice: approach or exploration is reflected by the mouse passing through the central area and avoidance is translated by its immobility or refusal in the area near the wall of the arena by the straightenings. The increase of the time spent in the centre and crossings by the mouse is an indication of a decrease of the avoidance of open spaces. This exploration of the OF is a factor that shows that the mouse is no longer anxious [29]. The parameters evaluated through the results obtained from the OF test showed that, as

DZP, *H. spicigera* would have anxiolytic properties through their binding action to GABAergic receptors of type A and B may be by flavonoid and saponin components which have been founded. In the HB test, the feeling of insecurity or the urge to escape those results in increased exploration of the holes may be due to an increased amount of brain serotonin. As the treated mice do not feel the urge to escape and the feeling of insecurity in the new environment, they do not attempt to explore the holes but rather become familiar with it. This observation leads to the hypothesis of inhibition of serotonin reuptake in presynaptic neurons after its release into the synaptic space [30]. This study revealed that *H. spicigera* decoction, as DZP, caused a reduction of the latency time of the first head dipping and the number of head dippings. These results equally reflected the presence of anxiolytic properties of *H. spicigera* decoction [31-33]. Indeed, the decoction decreased, dose-dependently, the latency time of the first head dipping and the number of head dippings. The reduction of these stress assessment parameters in this paradigm demonstrates that the mice have acquired a capacity to adapt to their new environment, and are therefore no longer anxious [33-35]. Additionally, the decoction significantly decreased the number of head dippings and the number of crossings in the HB test. Considering that head dipping behaviour was an indicator of the animal's emotional state [33] and that the decrease of head dipping was an expression of anxiety calming in the animal, it is typical of the presence of the anxiolytic effects in the decoction [36, 37]. Some authors have furthermore shown that flavonoids and alkaloids are responsible for the anxiolytic activity [38] several hypotheses could be put forward, either the anxiolytic activity of the decoction reinforces the inhibitory effect of GABA which gradually reduces fear [39] or it could also act by reducing the effect of excitatory agents such as serotonin

and catecholamines. The results obtained from the chronic immobilisation test on the EPM showed that the number of open arms entries is increased and the time spent in closed arms is decreased in mice of the group that was under the CIS and treated with different doses of the plant decoction; these parameters are also increased in the diazepam and CIS group. Conversely, these parameters are decreased in the normal control (DW) to negative control (DW+CIS) groups. These results show that, *H. spicigera* decoction would possess anxiolytic effects. [40, 41]. The number of head dipping and the number of crossings are increased in mice of the group of CIS which treated with different doses of the plant decoction (HS22.5+CIS-HS225+CIS) as well as in those of the group treated with the diazepam and stressed. The increase of these parameters may reflect that the animal feels comfortable and is not afraid to explore the EPM. Also, the time spent in Centre and number of crossings have increased by CIS in OF. These results indicate that the *H. spicigera* decoction would possess anxiolytic properties which could be explained through some assumptions giving details about the mechanism of action. This chronic immobilisation test would stimulate may be the hypothalamic-pituitary-adrenal (HPA) axis through the production of biogenic amines (catecholamine). These amines favor the release of glucocorticoids, responsible for the anxiety [42]. This anxiolytic effect could equally be explained by a possible action of the plant decoction on the GABAergic way through the GABA-A receptor [43, 44]. This plant decoction would have been active by influencing the benzodiazepine site or the barbiturate sites [45-48]. Concerning *in vivo* antioxidant tests, studies after the CIS test have revealed that the plant decoction showed an activity on antioxidant parameters in the brain. A significant increase of the activity of SOD, CAT and reduced GSH, which are enzymes responsible for antioxidant activity, was observed. These results suggest that the plant decoction proceeds through the antioxidant enzyme way to reduce free radicals and therefore treat anxiety [49]. Similarly, *H. spicigera* decoction revealed antioxidant effects through a reduction of lipid deposition of MDA. This is because a significant decrease in MDA activity was observed in all the mice of the groups treated with different doses of *H. spicigera* decoction as well as in those treated with diazepam. This reflects the action of *Hyptis spicigera* through the MDA way [49-51]. Thus, it could be concluded that the antioxidant enzyme way is used by the plant decoction to transfer its anxiolytic effects [52, 53].

## 5. Conclusion

The plant decoction would possess anxiolytic and antioxidant effects in mice on classicals and chronic immobilisation tests. This could be justified by the fact that the results obtained on the different paradigms, such as the EPM, the Open Field and the Hole Board, show that the decoction significantly increased some classical behavioural parameters of anxiety in mice that received the decoction of

*Hyptis spicigera*. The assay of oxidative stress markers showed that antioxidant analysis organs like brain had concentration of CAT, SOD, GPx, GSH and Nitrites increased. But their malondialdehyde was low. These parameters were improved by the plant extract at all doses, suggesting that this extract would have antioxidant properties. Also the LD<sub>50</sub> of the plant extract was estimated to be greater than 5000 mg/kg suggesting a relatively low toxicity. Reason is why this plant is used frequently in the traditional medicine in subsaherian Africa.

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