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Evaluation of anxiolytic activity of decoction of *Parkia biglobosa* in mice exposed to chronic immobilisation and physical activity test

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Abstract

Anxiety is a major current issue for both athletes and sedentary people. Several strategies for fighting this condition, aimed at limiting its sometimes adverse effects on the health and performance of athletes, are currently being developed. This study aims at assessing the combined effect of the decoction of dried *Parkia biglobosa* bark and aerobic physical activity in white mice. For this purpose, the elevated plus maze test (EPM) and the open field test were used. Thirty male and female mice of approximately eight weeks of age and 22 g body weight were used. They were divided into six groups of five mice each. This makes three test groups, one of which received the plant decoction by gavage and underwent anxiety induction; the other plant decoction and underwent anxiety induction and physical activity, and the third the anxiety induction and physical activity. A normal group received distilled water by gavage, a negative control group received distilled water by gavage and underwent anxiety induction and a positive control group received diazepam intraperitoneally and underwent anxiety induction. Thus, the results in the EPM give a significant difference ($p<0.001$) in the open arms of the number of entries from 5 ± 1 in the negative control mice to 27 ± 2.54 in those treated with 56 mg/kg of the batch (Pb56+SIC+AP), the percentage of entries from 18.08 ± 4.54 in the negative control mice to 84.46 ± 2.57 in those treated with 56 mg/kg of the group (Pb56+SIC+AP). Then, the open field test showed a significant increase ($p<0.001$) in the time spent in the centre from 6 ± 1second in the negative control mice to 42 ± 2 in those treated with 56 mg/kg of the group (Pb56+SIC+AP), in the number of crossing from 53 ± 4, 24 in the negative control mice to 101.8 ± 7.15 in those treated with 56 mg/kg of the group (Pb56+SIC+AP), the number of grooming from 2 ± 0.70 in the negative control mice to 11 ± 1.87 in those treated with 56 mg/kg of the batch (Pb56+SIC+AP). The decoction of dried barks of P. biglobosa combined with physical activity produces anxiolytic effects, that is, the ability to treat anxiety.

Keywords: *Parkia biglobosa*; Anxiolytic; Decoction; Physical activity

1. Introduction

Physical activity is one of the most fundamental aspects of life. The human body is actually a complex organism, capable of performing extremely diverse tasks [1]. Indeed, the body does not function at the same intensity all the time [2]. Physiological, psychological and emotional aspects, among others, may cause this variability.

According to Doron and Parot in 2007 [3], there are several basic emotions: joy, anger, fear, surprise and disgust. These are at the source of many other types of emotion, including anxiety. The latter refers to an emotion "provoked by the

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anticipation of a diffuse danger, difficult to foresee and control. It turns into fear when faced with a clearly identified danger".

In sport psychology, the traditional approach of emotions can be characterised with two axes, a negative axis focused on anxiety and another axis focusing on its influence on performance analysed through the concept of activation [2].

For Beaucage in 1997 [4], performance anxiety is related to the fear of failure and is mainly characterised by a strong apprehension when faced with assessments or any other situation where one may feel judged. It can manifest itself through high stress, panic or anxiety attacks, exaggerated perfectionism, or a range of somatic disorders in the run-up to deadlines (migraines, digestive disorders, etc.).

Four main factors influence performance. These are social, structural, physiological and psychological factors. These may not be in the athlete's immediate environment [5].

Managing anxiety to prevent it from undermining performance is an issue of great interest in sport. Most athletes, coaches and psychologists claim that the ability to manage anxiety before, during and after competitions is a critical element of sports performance. This management is achieved through psychological methods and the uses of natural substances, in this case those of the traditional pharmacopoeia, which have properties to tackle stress and anxiety. Thus, *Parkia biglobosa* is widely used as a source of ethnomedicine in the dry areas of Africa thanks to its numerous antineuralgic, diuretic, febrifuge, tonic, anti-bleeding, vermifuge and antiseptic properties [6]. Moreover, this plant has shown its effectiveness in various pathologies such as sterility, venereal diseases, bronchitis, hepatitis and leprosy [7]. In this respect, several studies have been carried out, including those by Omam in 2018 [8], on *Parkia biglobosa* effects on anxiety. Few studies investigated the combined effects of *Parkia biglobosa* and physical activities in order to establish a link with sports performance.

With regard to the above, our main objective is to evaluate the combined effects of the consumption of *Parkia biglobosa* dried bark decoction and aerobic performance on anxiety parameters in white mice. More specifically, the aim is to evaluate the combined effects of the consumption of the decoction of dried *Parkia biglobosa* barks and physical activity on anxiety parameters in white mice on the elevated plus maze test and the locomotor activity of *Parkia biglobosa* and physical activity on anxiety parameters in white mice in the open field test.

2. Material and methods

2.1. Plant Material

Parkia biglobosa barks were collected in Yaounde in the Centre region in December 2019. These barks were washed with tap water and dried in an unlit area to avoid reactions to light.

2.2. Decoction Preparation

Fresh bark of *Parkia biglobosa* was washed with tap water, cut and dried in the shade. A decoction of the latter was prepared by introducing 5 g of plant powder into a beaker containing 50 ml of distilled water. With the beaker closed, the mixture was boiled on a hot plate for about 20 minutes. After the mixture had cooled, it was filtered through a Wattman number 3 paper to obtain a liquid called stock solution. This is the solution to be administered to the animals. The administration was made according to the weight of the animal.

2.3. Animals

The animals used were 30 healthy male and female called *Mus musculus,* Swiss white mice that had not previously been subject of experiments. The average weight of the mice was 22 g and they were approximately 8 weeks old. These mice came from the animal house of the Faculty of Science of the University of Ngaoundere and were brought to the animal house of the animal physiology laboratory of the Higher Teachers' Training College in Yaounde for experiments where they had a one-week acclimatisation period. They were housed in Plexiglas cages lined with wood shavings with a maximum of five mice per cage under ambient temperature conditions. There was sufficient aeration with a natural light cycle (12 h light, 12 h dark). They had free access to tap water and food.

2.4. Chemical

Diazepam was used as a reference molecule. For its preparation, a volume of 0.4 ml of diazepam was taken and put into a beaker, then added with 9.6 ml of distilled water to obtain a diluted solution of 10 ml corresponding to a dose of 2 mg/kg of body weight.

2.5. Physical Training Programme

The swimming programme lasted 10 days. The training frequency was one session per day. The duration of the sessions varied from 10 to 20 minutes. The mice swam individually and continuously until the time limit was reached, then were taken out of the pool, dried with a suitable towel and returned to their cage. The water used for training was immediately replaced. The swimming was done between 3 p.m. and 6 p.m. The animals had free access to food and water after the day experiment.

2.6. Experiment Procedure

Chronic anxiety was induced in the animals by immobilising them in a narrow tube each day for 2 hours for 10 consecutive days. The mice were divided into 6 groups of 5 mice each. Group 1, also called the normal group, received distilled water; group 2 (negative control) received distilled water and stress induction; group 3 received the plant decoction and stress induction; group 4 received the plant, stress induction and physical activity; group 5 received stress induction and physical activity and group 6 received diazepam and stress induction. After these different treatments, the mice were placed one after the other on the elevated plus maze test and the open field test.

2.7. Elevated Plus Maze Test (EPM)

According to Elhage in 2012 [9], the EPM test is a cross-shaped device raised 65 cm above the ground. It consists of four arms (L = 50 cm × W = 10 cm) facing each other; two of these arms are closed by 49 cm high sides while the other two are open. The arms are connected with a central platform (10 cm × 10 cm). This device is topped by a video camera to record the animal's behaviour during the test. The mouse is placed in the centre of the device, facing an open arm, and left free to explore it for 5 min. The floor was cleaned with 10% ethanol between each mouse to avoid any odour that might affect the behaviour of the next animal. Several parameters were measured by analysis of the video recordings: the number of entries and the time spent in the open arms (OA) and closed arms (CA). An entry is counted when the animal's four legs cross the threshold of the arm. These data are used to calculate the time spent in the central platform, considered as an indicator of hesitation between approaching and avoiding the most anxiety-provoking arms, the total number of entries in all the arms, considered as a good indicator of the animal's locomotor activity, as well as the percentage of time spent in the open arms (OA) (time spent in OA*100/time OA + time CA) considered as reflecting the animal's anxiety state [10].

2.8. Open Field Test

The open field paradigm is a square enclosure with high edges, lighted in the centre, which does not enable the animal inside to escape or hide. The exploration area is divided into 17 tiles separating the inner surface of the experimental set-up and 1 central tile. The open field was 40 cm square and 19 cm high [11]. The open field test is commonly used to assess locomotor activity, exploration and emotional reactivity in rodents [12; 13; 14].

2.9. Statistical Analysis

Values were compared using the analysis of variance (ANOVA) test and where differences prevailed, Tukey's comparison tests (HSD) were used to separate them. From $p \leq 0.05$, the values were considered to be different, thus significant.

3. Results

3.1. Anxiolytic Effects of *Parkia biglobosa* Decoction and Physical Activity on the Elevated Cross Maze

Figure 1A shows that chronic stress caused a non-significant decrease in the number of entries into the open arms from 7±2 in the normal batch mice to 5±1 in the negative control. *P. biglobosa* decoction and physical activity induced a significant increase ($p<0.001$) from 5±1 in negative control mice to 27± 2.54 at dose 56 mg/kg in the (Pb56+SIC+AP) group. Figure 1B shows that chronic stress caused a significant decrease ($p<0.001$) in the percentage of open arm entries from 34.95±8.40 in the normal batch mice to 18.08±4.54 in the negative control. *P. biglobosa* decoction and physical activity also induced a significant increase ($p<0.001$) from 18.08 ± 4.54 in the negative control mice to 84.46 ± 2.57 at dose 56 mg/kg in the (Pb56+SIC+AP) batch.

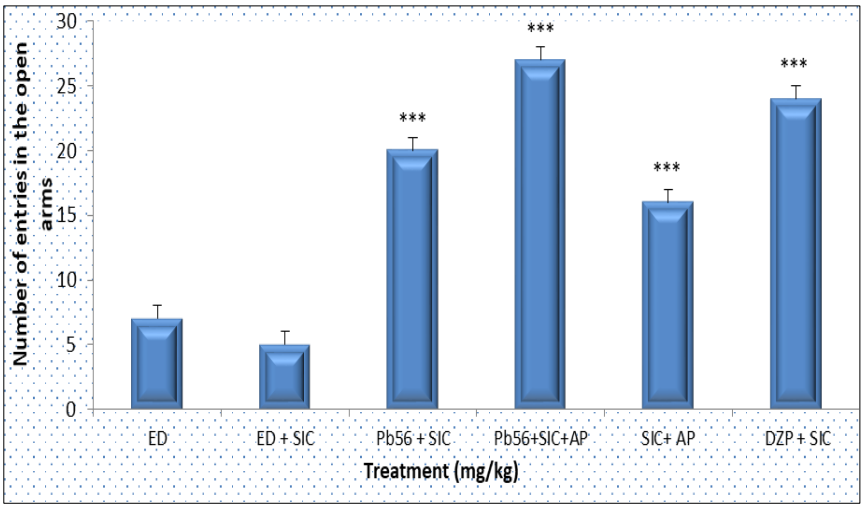

Figure 1 A

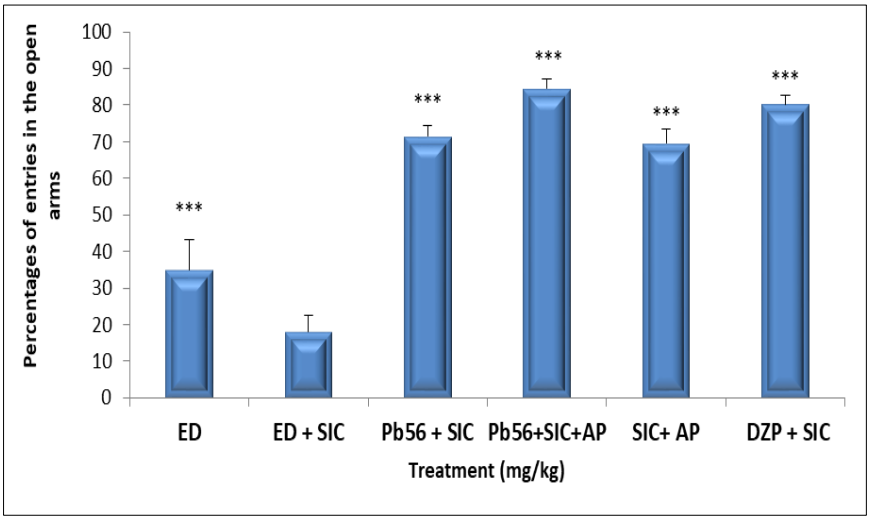

Figure 1 B

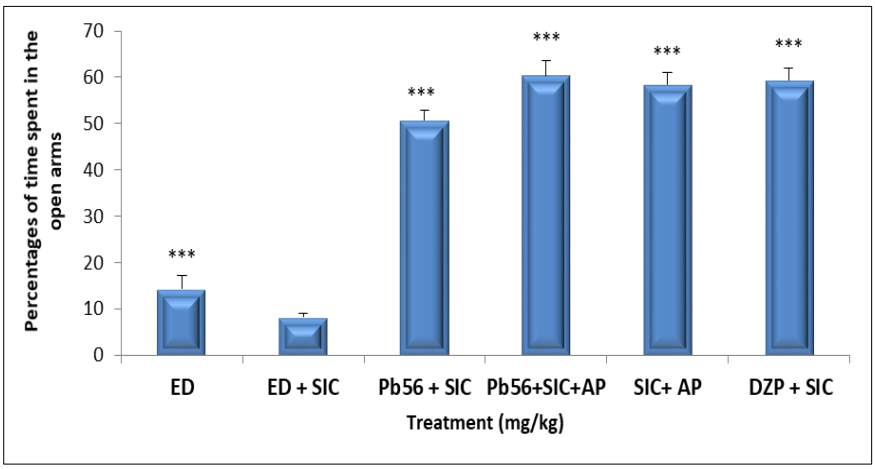

Figure 1 C

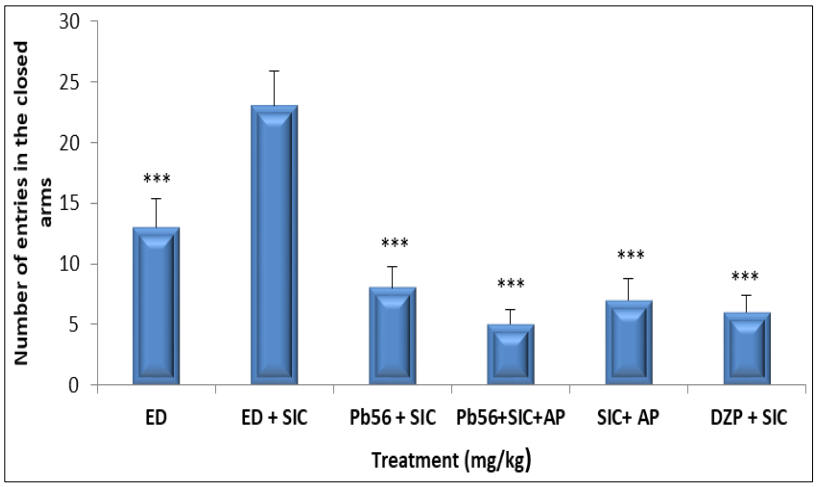

Figure 1 D

Figure 1 Anxiolytic effects of *Parkia biglobosa* decoction and physical activity. A (on the number of entries in the open arms); B (on the percentage of entries in the open arms); C (on the percentage of time spent in the open arms); D (on the number of entries 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; SIC: chronic immobilisation stress, PA: physical activity, Pb56: *Parkia biglobosa* at dose 56; DZP: diazepam

Figure 1C shows that chronic stress caused a significant decrease (p<0.001) in the percentage of time spent in the open arms from 34.95 ± 8.40 in the normal batch to 18.08 ± 4.54 in the negative control. *P. biglobosa* decoction and physical activity also induced a significant increase (p<0.001) from 8.31 ± 0.74 in the negative control mice to 60.37 ± 3.24 at the 56 mg/kg dose in the (Pb56+SIC+AP) group. Figure 1D shows that chronic stress caused a significant increase in the number of entries into the closed arms from 13.00 ± 2.34 in the normal batch to 23 ± 2.91 in the negative control. *P. biglobosa* decoction and physical activity induced a significant decrease (p<0.001) from 23 ± 2.91 in the negative control mice to 8 ± 1.73 at dose 56 mg/kg in the (Pb56+SIC+AP).

3.2. Anxiolytic Effects of *Parkia biglobosa* Decoction and Physical Activity on the Open Arena

Figure 2A shows that chronic stress caused a significant decrease (p<0.01) in time spent in the centre from 12 ± 2.34 s in the normal group of mice to 6 ± 1 in the negative control. *P. biglobosa* decoction and physical activity induced a significant increase (p<0.001) from 6 ± 1 s in negative control mice to 42 ± 2 at 56 mg/kg in the (Pb56+CIS+PA) batch. Figure 2B demonstrates that chronic stress caused a significant decrease (p<0.001) in the number of crossings from 80 ± 3.16 in the normal batch to 53 ± 4.24 in the negative control. *P. biglobosa* decoction and physical activity induced a significant increase (p<0.001) from 53 ± 4.24 in negative control mice to 101.8 ± 7.15 at 56 mg/kg in the (Pb56+CIS+PA) group.

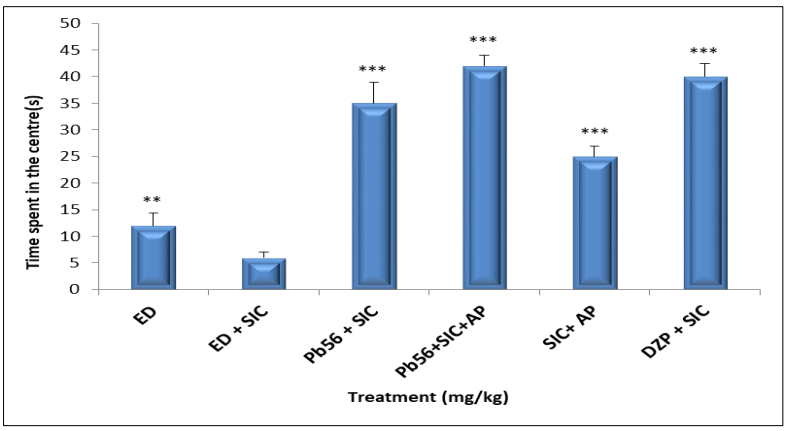

Figure 2 A

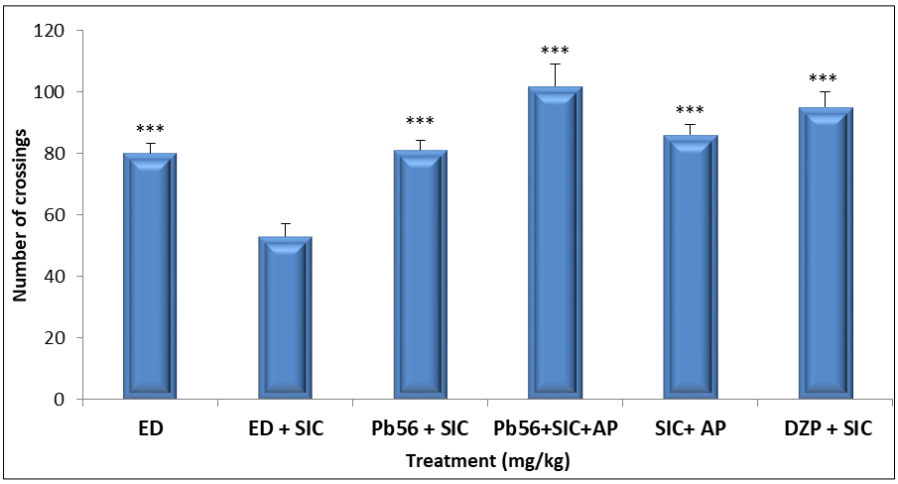

Figure 2 B

Figure 2 Anxiolytic effects of *Parkia biglobosa* decoction and physical activity. A (on time spent in the centre); B (on the number of crossings). Each bar represents the time spent in the centre and the number of crossings, n = 5. ***p<0.001; significant difference from negative control; ED: distilled water; SIC: chronic immobilisation stress, PA: physical activity, Pb56: *Parkia biglobosa* at dose 56; DZP: diazepam

4. Discussion

The results obtained through the chronic immobilisation stress test showed that the number of entries in the open arms, the percentage of time spent in the open arms and the percentage of entries in the open arms of the elevated plus maze were high in mice from the group that received the plant decoction, anxiety and physical activity (Pb56 + CIS + PA); in mice from the anxiety and diazepam group (DZP + CIS); in mice from the anxiety and physical activity group (CIS + PA); and in mice from the plant and anxiety group (Pb56 + CIS). On the other hand, these parameters were lower in mice from the normal group (ED) and the negative control (ED + CIS). All these results indicate that *Parkia biglobosa* decoction combined with physical activity alleviates anxiety. In the same vein, the number of entries in the closed arms was low in the mice of the plant decoction, anxiety and physical activity (Pb56 + CIS + PA); those of the anxiety and physical activity group (CIS + PA); the plant and anxiety group (Pb56 + CIS) and those of the anxiety and diazepam group (DZP + CIS). On the contrary, mice from the negative control group (ED + CIS) and the normal group (ED) had the highest parameters. This is because it has been found that substances that increase the number of entries and time spent in the open arms have anxiolytic effects [10]. This reduction of the parameters in the closed arms might be because the animal feels comfortable in the experimental device. All these results show that *Parkia biglobosa* decoction combined with physical activity would possess anxiolytic properties through some hypotheses explaining the action mechanism. Firstly, this chronic immobilisation stress test would stimulate the hypothalamic-pituitary-adrenal (HPA) axis through the production of biogenic amines (catecholamine and indolamine) which facilitate the release of glucocorticoids responsible for the anxiety state [8]. However, the results suggest that the combined effect of the plant and physical activity would have prevented or reduced the production of corticosterone, which is a marker of anxiety, through the serotonergic pathway. The GABAergic pathway could be involved through the GABA-A receptor complex where the decoction combined with physical activity could be mediated by the benzodiazepine site and of course a possible intervention of other sites of this complex. These results are in line with Grudman and collaborators in 2007 and Venault and Chapouthier in 2017 [15; 16] who thought that the fixing of the plant decoction compounds at this level would cause an increase in the opening time of the chloride channels leading to a membrane hyperpolarisation due to an increased flow of chloride ions into the cell. In the light of these findings, the combined effect of *Parkia biglobosa* and physical activity would further induce this increase. Under the same conditions and concerning the open field, the time spent in the centre and the number of crossings were higher in mice from these groups (Pb56 + CIS + PA), (CIS + PA), (Pb56 + CIS) and (DZP + CIS). This increase could reflect the fact that the animal does not feel stressed. *Parkia biglobosa* decoction combined with physical activity could also induce anxiolytic effects through the GABAergic system of benzodiazepines and even barbiturates or GABA. This could also be mediated by the HPA axis and to lesser extent NMDA receptors [17; 18; 19; 20].

5. Conclusion

In short, we wanted to show whether the combined effect of the plant and the aerobic physical activity were potentiating and that the plant stimulated the locomotor action of the animals. The decoction of *Parkia biglobosa* combined with physical activity revealed better anxiolytic properties in mice whose anxiety was induced. This is justified by the fact that in the elevated plus maze, the classical anxiety parameters such as the number of entries in the open arms, the percentage of entries in the open arms, the percentage of time spent in the open arms significantly increased in mice having received the plant decoction combined with physical activity in the elevated cross maze test. This was also the case in the open field with the time spent in the centre and the number of crossings. These results explain that the decoction of *P. biglobosa* combined with physical activity could treat anxiety. It is probably for these virtues that this Minosaceae is more used empirically in traditional medicine.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

These mice came from the animal house of the Faculty of Science of the University of Ngaoundere and were brought to the animal house of the animal physiology laboratory of the Higher Teachers' Training College in Yaounde for experiments.

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