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Chronic Administration of *Bauhinia forficata* Link Reduced Exploratory Activity Without Affecting Blood Glucose in Healthy Rats

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Abstract: This study provides information about the effect of chronic ingestion of a decoction of *Bauhinia forficata* on the behavior of rats. The aim of this study was to investigate the exploratory activity, anxiety level, and blood glucose of healthy rats submitted to prolonged treatment with a decoction of *B.forficata*. Twenty male rats, aged two months and weighing approximately 145g were randomly distributed into two groups. The Bauhinia group freely ingested a decoction of *B.forficata* for 4 weeks, while the control group received water. Body weight, food and fluid intake, and glycemic index were quantified. The open field (OF) and elevated plus maze (EPM) tests were applied at the end of the experiment. Data were analyzed by Student's t test for unpaired samples, with a significance level of $P<0.05$. The results of the EPM test were analyzed by the Wilcoxon Signed Rank Test and blood glucose by the Bonferroni test. There were no significant differences in body weight, food intake or glycemic index between the Bauhinia and control groups. Net liquid intake was higher in the Bauhinia group. EPM results did not differ between groups, but in the OF test, the frequencies of rearing and locomotion were lower and immobility time was increased for rats in the Bauhinia group. In conclusion, *B.forficata* reduced exploratory activity and did not alter blood glucose in healthy rats.

Keywords: Behavior, Exploratory Activity, Ethnopharmacology

1. Introduction

Due to their limited access to medical assistance, the populations of underdeveloped countries use medicinal plants extensively. Among the plants with medicinal interest are those of the *Bauhinia* genus (Fabaceae) popularly known as "pata de vaca" or" mororó do sertão" [1-2]. A commonly used species is *Bauhinia forficata*, which is well known in folk-medicine for its antidiabetic activity [3] and less so for its calmative effect [1, 4].

Studies examining the calmative effect of plants from the Bauhinia genus on the central nervous system (CNS) have investigated alterations in rodent behavior using various combinations of behavioral assessment methods such as the open field (OF), elevated plus maze (EPM), and rotarod (RR) tests. These studies include reports on the depressant and anxiolytic activities of two species in the *Bauhinia* genus [5, 6] and on the anxiolytic activity of *B. forficata* [7]. The one study of *B.forficata* examined the effect of acute administration of the plant decoction [7]. However, continuous consumption of the decoction for its antidiabetic properties is popularly indicated and reported [8], and the effect of continuous use on behavior remains unknown. We

subjected healthy male Wistar rats to continuous ingestion of a decoction of *B.forficata* and assessed its effect on blood glucose and behavior.

2. Material and Methods

2.1. Experimental Groups

Twenty male Wistar rats two months of age and with a mean body weight of 145±9g were used. Animals were distributed equally into two groups and housed in plastic boxes. The *Bauhinia* group ingested the plant decoction freely for 30 days, while the control group received water. Both groups were fed Presence® rodent chow (Purina, SãoPaulo, Brazil). The animals were kept in the animal house of the Department of Morphology and Physiology of FCAV-UNESP, Univ Estadual Paulista (Brazil). The study followed the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation and was approved by the institutional animal care and use committee of the FCAV (Protocol number 008115/13).

2.2. Preparation of the B.forficata Decoction

Dried *B.forficata* leaves (lots 07SDM and 08SDM) were provided by the herbal pharmacy "Oficina das Ervas" in Ribeirão Preto (SãoPaulo, Brazil). The species was identified by Ademar Menezes Jr., the agronomist of the pharmacy (CREA 5060000803). The decoction was prepared by boiling 50g of dried leaves in 1L of water for 5min ,a method commonly used in folk-medicine [3]. Mean daily dose of *B.forficata* decoction was 24.04±4.99ml/100g body weight.

2.3. Analyzed Variables

Rats were weighed daily in the morning. Each animal received 100g feed plus 150mL of either water or decoction per day. Food and fluid intake were quantified.

2.4. Glycemic Index

Rats were fasted for 12h. Subsequently, a 50-µL blood sample was collected from the tail end at times 0 (before) and 5, 10, 15, 30, 60, 120, and 180 min after oral administration of dextrose solution (only dose of 2g/kg), prepared from 60g dextrose diluted with 100 ml distilled water. Blood glucose was determined by the electro-enzymatic method in a YSI2300 Stat Plus bioanalyzer.

2.5. Behavioral Tests

The OF test was performed in a wooden circular arena, 96cm diameter, bordered by a 32.5cm wall. The arena bottom was divided into 25 divisions (areas/zones), delimited by three circles which are intercepted by radial segments. This test is used to measure the overall activity of the animals in an unfamiliar environment, and the following behavioral variables were determined: rearing, locomotion, grooming, frequencies of defecation and urination, and time of immobility (7). The test was performed three times (tests1, 2, and 3) on each rat, with 8-min intervals between repetitions lasting 5 min each. The animals returned to their home cages during the intervals.

Anxiety was evaluated using an EPM, a wooden device that has two open arms crossed perpendicularly by two closed arms. Each arm is 50cm long and 10cm wide while the closed arm has a 40cm wall. During a period of 5min, the number of entries into and time spent in the open and closed arms were quantified, and the percentage of entries into and time spent in the open arms was determined for each animal, which were evaluated only once.

In both behavioral tests, the assessment was conducted via video, outside the laboratory, always by the same observer.

2.6. Statistical Analysis

The Shapiro-Wilk test was used to determine the normality of the data. Data were expressed as mean±standard error and analyzed by Student's *t* test using Sigma Plot 12.0. EPM test results were analyzed by the Wilcoxon signed rank test. Blood glucose versus time data were analyzed by the Bonferroni test. The significance level used was $P<0.05$.

3. Results

Daily ingestion of the *B.forficata* decoction by rats was significantly higher than their water intake (56.9±0.89 mL and 45.5±1.17mL, respectively; $P<0.001$). There was no difference between the *Bauhinia* and control groups for body weight (Figure 1).

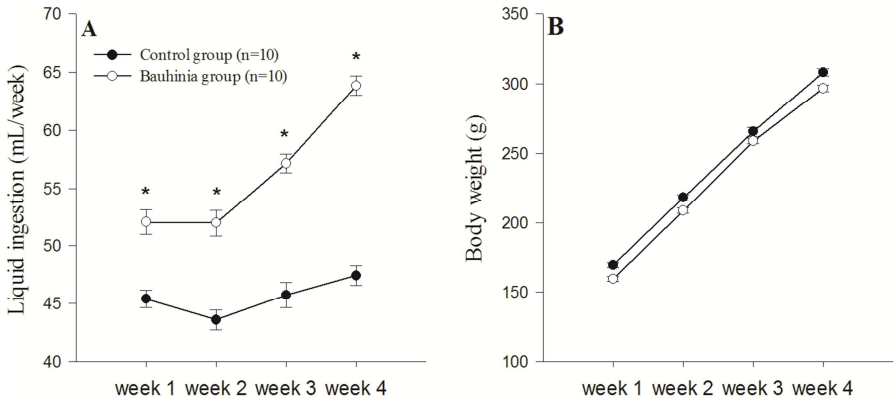


Figure 1. *Effect of Bauhinia forficata on the liquid ingestion and body weight of Wistar rats. Liquid ingestion of water and Bauhinia decoction (A) and body weight (B) for 4 weeks of treatment. Bar whiskers represent the standard error of the mean. *Indicates increased related to the control group (P<0.05).*

3.1. Glycemic Index

Blood glucose concentrations increased in both groups (P<0.01). However, no differences in the glycemic index were found between them (Figure 2).

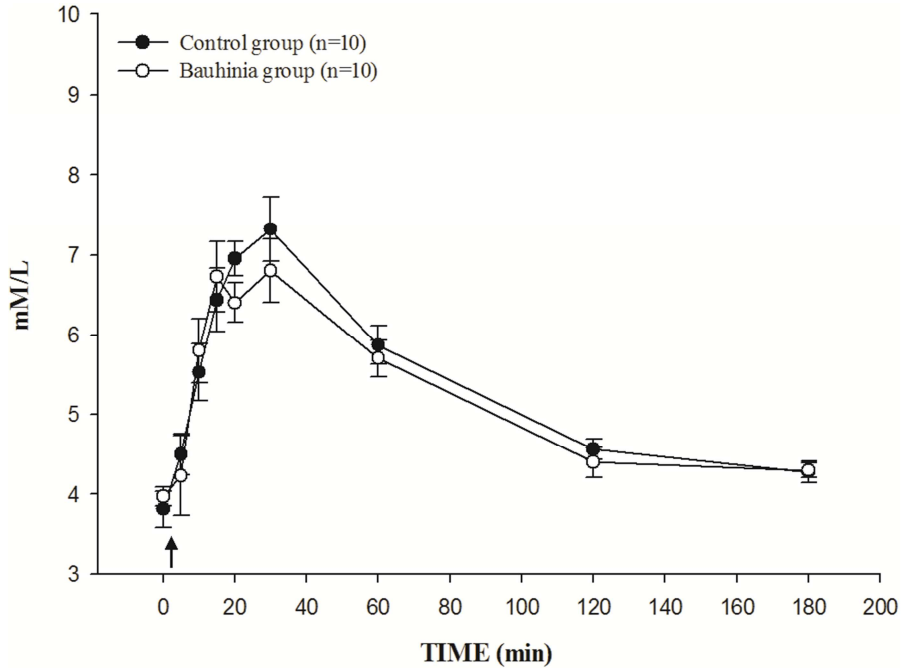
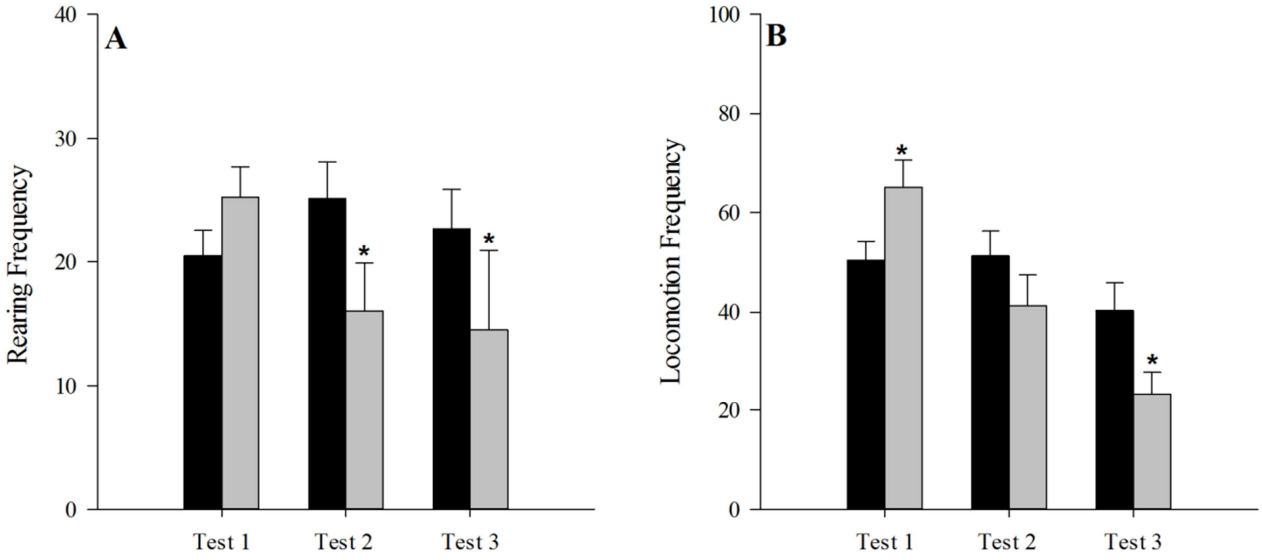


Figure 2. *Effect of Bauhinia forficata on the glycemic index of Wistar rats. Blood glucose concentrations were measured after administration of dextrose to animals receiving the decoction (Bauhinia group ,n=10) or not (Control group, n=10). The arrow indicates the time of administration of dextrose solution (P<0.01). Barwhiskers represent the standard error of the mean.*

3.2. Open Field Test

Frequencies of grooming, defecation, and urination were equal between the *Bauhinia* and control groups, but rearing and locomotion frequencies as well as immobility time differed significantly (Figure 3). In test 1, locomotion frequency was higher (P=0.047) in the *Bauhinia* group than in the control group (13.0±1.12 and 10.3±0.79 locomotions/min, respectively). In test 2, the *Bauhinia* group showed lower rearing frequency (P=0.041; control, 5.00±0.59 rearings/min; *Bauhinia*, 3.22±0.77 rearings/min). Finally, in test 3, compared to the control group, the *Bauhinia* group exhibited lower frequencies of rearing (P=0.037; control, 4.54±0.62 rearings/min; *Bauhinia*, 2.90±1.28 rearings/min), lower locomotion (P=0.027; control, 8.00±1.1 locomotions/min; *Bauhinia*, 4.66±0.88 locomotions/min) and increased immobility time (P=0.017; control, 111±20 sec; *Bauhinia*, 188±20 sec).



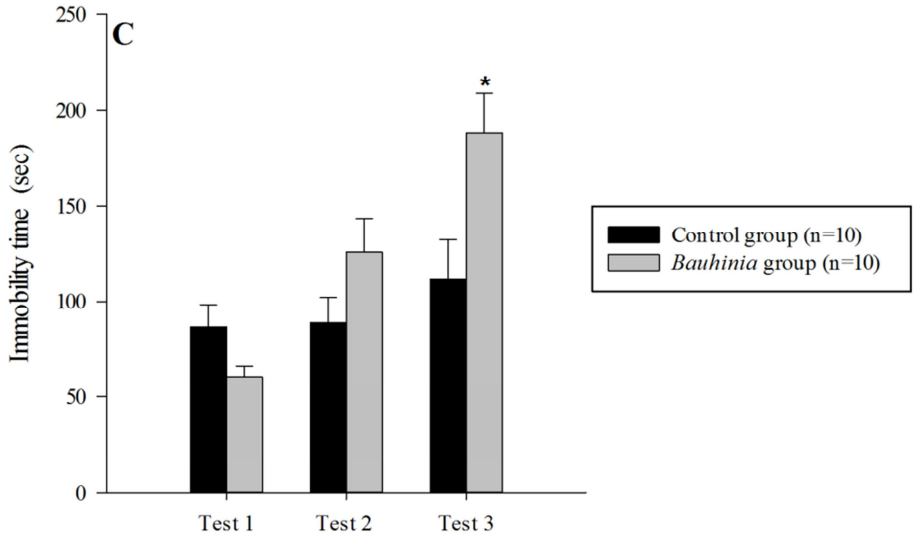


Figure 3. *Effect of chronic ingestion of Bauhinia forficata decoction on the behavior of Wistar rats assessed by the Open Field test using three successive 5-min trials per rat: rearing frequency (A); locomotion frequency (B); immobility time (C). Data expressed as mean±standard error of the Control (n=10) and B.forficata (BG, n=10) groups.*Indicates statistical difference (P<0.05).*

3.3. Elevated Plus Maze Test

Behavioral variables evaluated in the EPM test did not differ between groups (Figure 4).

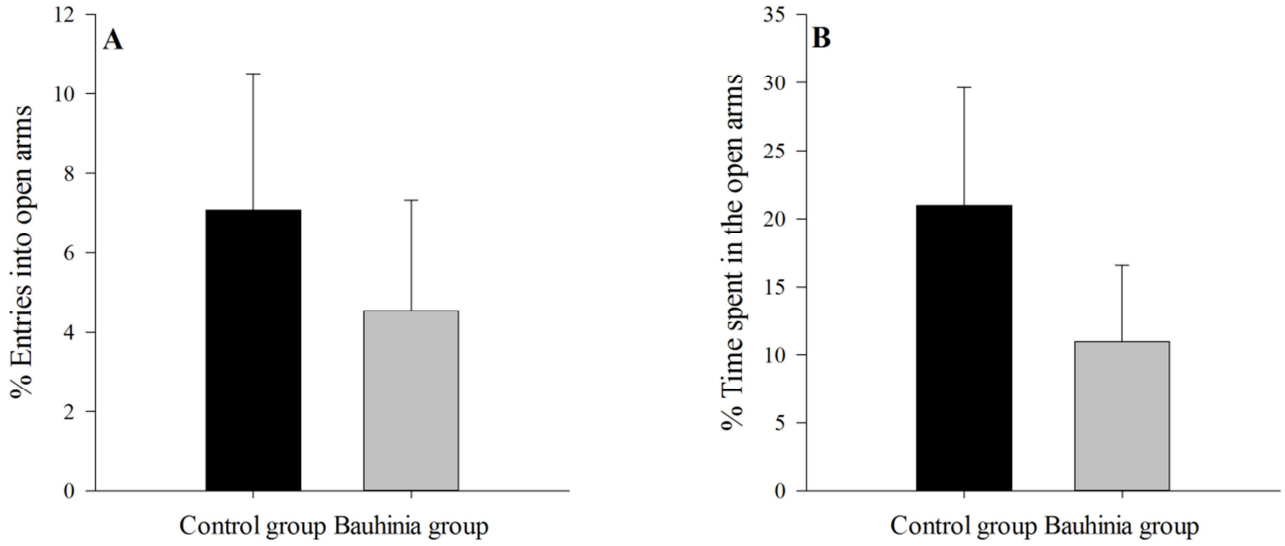


Figure 4. *Effect of chronic ingestion of Bauhinia forficata decoction on the behavior of Wistar rats assessed by the Elevated Plus Maze test using percentage of entries into open arms (A) and percentage of time spent in the open arms (B). Data expressed as mean±standard error of the control (n=10)and Bauhinia (n=10) groups.*

4. Discussion

The main finding of this study was the possible decreased of exploratory activity induced by continuous intake of *B.forficata* decoction.

The OF test measured exploratory, psychomotor, depressive, and sedative behavior in an experimental animal model. We found that the locomotion frequency in Bauhinia-treated group was significantly higher in the first test and lower in the third test (compared to the control group). Despite a possible conflicting results on locomotor activity when the other variables (rearing and immobility time) are interpreted together, we observed a decrease of locomotor activity. In a previous study using this test, [7] reported increased immobility in rats receiving a single dose of *B.forficata* by gavage. Another behavioral study with mice receiving an ethereal fraction of *Bauhinia platypetala* reported a decrease in locomotor activity and suggested this plant has sedative and depressant activities [6]. Similar CNS effects have also been described using behavioral assessment methods such as the OF, EPM and RR tests in rodents receiving *Erythrina* genus [9] and *Dioclea virgara* [10], which are plants belonging to the same family as the *Bauhinia* genus.

The reduced exploratory activity observed in this study may be explained by the depressant activity of *B.forficata* on the CNS, probably due to the presence of flavonoids, which have GABAergic activity [5]. Indeed, flavonoids have been shown to cause anticonvulsant and anxiolytic effects in the CNS [11]. However, further studies will be needed to test this hypothesis.

The EPM test is a classical method for evaluating anxiety. The behavioral variables assessed by the EPM test did not differ between the two groups studied, indicating that *B.forficata* has no anxiolytic effect when it is continuously administrated. In [7], investigating acute effects of *B.forficata* using the same test, reported that treated rats spent less time in the open arms, again indicating no anxiolytic effect. However, in mouse studies that also used EPM assessment, anxiolytic activity was reported for them ethanol extract of *Bauhinia racemosa* [12] and the ethereal fraction of *Bauhinia platypetala* [6]. These discrepancies may be due to differences in extraction methodology and administration method as well as to the particularities of the animal and plant species used in these studies. It is important to mention that the use of different doses of any compound or extract may conduct to a general view of its effects. Compounds acting at the CNS level and at different doses may cause a particular level of anxiety or a reduction of exploratory activity.

Blood glucose concentrations did not differ between groups, indicating that *B.forficata* had no hypoglycemic effect on healthy animals, a result that raises doubts about its use for diabetes prevention. Indeed, previous studies have also shown that *B.forficata* did not affect blood glucose levels in nondiabetic rats [3, 13]. Moreover, the pharmacodynamics of *B.forficata* has been associated with a possible reduction of insulinase activity [14].

Weight gain and food intake did not differ between the *Bauhinia* and control groups, indicating that this herbal extract did not affect the physiology of the gastrointestinal system. These results were similar to those obtained by [13], who reported that body weight was not altered by *B.forficata* in either normal or diabetic rats. Because fluid intake was higher in the *Bauhinia* group, we suggest that the decoction was palatable to these animals. However, in [14] investigated *B.forficata* and found that it did not affect fluid intake by nondiabetic rats. This discrepancy is probably due to the different concentrations of the active compound in the decoction preparations.

Because the reduction in rat exploratory behavior observed in this study occurred as a result of continuous exposure to high amounts *B.forficata*, we speculate that equivalent exposure would also lead to anxiolytic and depressant effects in humans. Thus, the calmative effects reported for *B.forficata* decoctions used empirically by communities located in the Caatinga ecoregion of Brazil [1,4] may result from high, and perhaps even toxic, exposure to the plant's chemical constituents. Further research is needed to address this issue and to elucidate the mechanism underlying *B.forficate* induced alterations in the CNS and in behavior.

5. Conclusions

Ingestion of a *Bauhinia forficata* decoction reduced exploratory activity in healthy rats without altering blood glucose levels. These findings highlight the need for further studies on the triad *B.forficata*-behavior-CNS depressant activity.

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