PROPERTIES OF Bauhinia *forficata* LINK IN RATS: BEHAVIORAL EVALUATIONS.

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Summary

The objective of the present study was to examine the possible central nervous system (CNS) effects of *Bauhinia forficata* Link (*Bf*) aqueous extract in rats using anxiety behavioral models. To observe the effect of *Bf* in rats, the extract was orally administered at dose of 5.0 g/kg body weight. Thus results suggest that *Bf* aqueous extract induced general changes in behavioral indicating anxiolytic effect.

Key words: *Bauhinia forficata* Link; Anxiolytic effect; Behavioral assays

Introduction

Bauhinia forficata Link (Fabaceae-Caesalpinioideae) commonly known as "paw-of-cow", is widely used in Brazilian folk medicine for the treatment of diabetes mellitus (DM) either together with or in place of conventional treatment[1,2]. Pepato et al.[3] (2003) showed that long-term oral treatment with leaf decoction of *Bf* significantly reduces the levels of blood glucose, urinary glucose and urinary urea. The antidiabetic activity of aqueous, ethanolic and hexanic extracts of *Bf* was investigated in a model of alloxan-induced diabetes in rats. The results showed that the plant extracts when administered by gavage may reduce glucose, triglycerides, total cholesterol and HDL-cholesterol levels. These results suggest the validity of the clinical use of *Bf* in the treatment of diabetes mellitus type II[4]. Silva et al.[5] (2002) evidenced that by means of use of a *n*-butanolic fraction *Bf* promotes a glycemic level reduction in either healthful and diabetic rats induced by alloxan. Medicinal plants, especially those that contain flavonoids, exhibit multiple pharmacological activities. In *Bauhinia forficata* leaves four different flavonoids were identified, with kaempferitrin predominating[6]. Pinheiro et al.[7] (2006) deal with the quantitative analysis of kaempferitrin from *Bf* medicinal extract (aqueous and hydro alcoholic) using the liquid chromatographic method to compare kaempferitrin content in leaves collected from two different

regions in the south Brazil. A marked difference in total flavonoid concentration was observed between kaempferitrin content for the fluid extract and for the aqueous extract. Khalil et al.[8] (2008) shows that aqueous extracts of leaves of *Bf* are a potential source of natural antioxidants and may be helpful in the prevention of diabetic complications associated with oxidative stress. Volpato et al.[9] (2008) showed that *Bf* treatment has no hypoglycemic effect, does not improve maternal outcomes in diabetic rats, but it contributes to maintain GSH concentration similar to non-diabetic groups, suggesting a relation with the decreased incidence of visceral anomalies. Our earlier results of the acute toxicity assay showed that the general activity of mice was not reduced with a 5.0 g/kg dose of aqueous extract of *Bauhinia forficate* Link. No death was observed with any of the tested doses[10]. The objective of the present study was to investigate the possible central nervous system (CNS) of *Bauhinia forficata* Link aqueous extract using anxiety behavioral models.

Materials and methods

Preparation of the aqueous fraction extract

The *Bauhinia forficata* Link specimen were collected in Sorocaba city (State of São Paulo, Brazil). A voucher specimen has been deposited on the herbarium at University of Sorocaba (Uniso) after identification was carried out by the Botanic Institute of São Paulo (Brazil) as authenticated by Dr. Sérgio Romaniuc Neto (PQC IV, Especialidade Florística de Mata Atlântica/Taxonomia de Moraceae). The fresh leaves (450 g) without petiole were dried, powdered and a hydro alcoholic (70%) extract was obtained by percolation. It was stored at room temperature free of light and humidity until the toxicological assays were performed. The extract was concentrated under reduced pressure and lyophilized providing 102.3 g of powder (22.7% efficiency). The aqueous extract was freshly prepared in distillated/deionizated water and previously administrated by oral route (p.o.).

Experimental animals

Adult swiss mice (male and female) weighing 25 g to 30 g and male wistar rats weighing 160 g to 200 g, of both gender, were obtained and kept in the UNISO/Pharmacy School facilities according to *"The Guide for the Care and Use of Laboratory Animal"* (National Research Council 1996) and "*European Community guidelines"* (EEC Directive of 1986; 86/609/EEC). All animals were maintained in groups (10 mice or 5 rats per cage) with food and water *ad libitum*, except during the experimental days. A twelve hour light/dark cycle and constant temperature (23 ± 1^{o}C) were maintained. All animals were previously adapted to laboratory conditions during one week before the experiments. The study design was previously approved by Uniso Ethical Committee for Experiments.

Acute toxicity Assay (LD_{50})

This assay was carried out according to previous studies[11]. Fifty mice (50% of each gender) were distributed into five groups (one control and four experimental) of five animals of each gender. Experimental groups received 0.5, 1.0, 2.5 and 5.0 g/kg/p.o. of *Bf* aqueous extract (w/w). Control group received the vehicle (deionized water).

Behavioral assays

Twenty rats were distributed into two groups (one control and one experimental). Experimental group received 5.0 g/kg/p.o. of *Bauhinia forficata* Link aqueous extract (w/w). Control group received the vehicle (deionized water). The *Bauhinia forficata* Link aqueous extract administration pretreatment time was 30 minutes for each assay.

Open field assay

The general physical activity was used to evaluate the spontaneous locomotive and exploratory activity[12]. The open-field was a cylindrical container made of transparent acrylic (97.0 cm diameter and 32.5 cm height walls). The open-field's floor had three concentric circles divided into 19 segments of equal area by lines radiating from the center. During experiments, light was provided by a 40 W white bulb located at 72 cm above the floor providing continuous illumination of the area. The room was also sound-attenuated. Hand-operated counters and an electronic timer were used to score the following parameters: ambulation, number of floor units entered using four paws, rearing frequency, number of times an animal stood on its hind legs, cleaning activity, number of times an animal licked its paws or fur, time of immobility, and total time (in seconds) without spontaneous movements. Before the introduction of the next rat, the open-field container was cleaned (5% ethanol) in order to avoid possible odors. Both control and experimental animals were intermixed during this assay. Each rat was individually placed for observation in the middle of the open field, these parameters were recorded for 5 min. The apparatus was washed with a 5% ethanol solution before each behavioral test, control and experimental rats were intermixed and the observations were made between 14 and 16h.

Pluz maze assay

The elevated pluz-maze was made of wood and had two open arms and two enclosed arms of the same size (50 x 10 cm) with 40 cm-high walls. It was elevated 50 cm above the ground [13]. Each rat was placed in the central square (10 x 10 cm) and observed for the number of entries into each type of arm (all four paws defining at entry) and the time in the open and closed arms. These parameters were recorded for 5 min. The apparatus was washed with a 5% ethanol solution before each behavioral test, control and experimental rats were intermixed and the observations were made between 14 and 16 h.

Pentobarbital sleep time

This assay was conducted as previously described by Amos et al.[14]. Briefly, each animal was injected with 30 mg/kg/i.p. sodium pentobarbital, after 30 minutes *Bauhinia forficata* Link aqueous extract administration. The time (in minutes) elapsed from the injection to the loss of the uprightness reflex (induction time), and the time from the loss of uprightness reflex to awakening (duration of sleeping), were both registered during four hours.

Statistical analysis

Normally distributed data were submitted to comparison between both groups by using Student's *t*-test or Chi-square test. The significance level was set at 5%.

Results

Acute toxicity Assay (LD_{50})

The results of the acute toxicity assay showed that the general activity of mice was slightly reduced with a 5.0 g/kg dose of aqueous extract of *Bf.* A cyanosis of small proportion was observed in 10% of mice (10% of male and 10% of female) with the same dose. It has not been observed any convulsions, contortions, straub, trembling and ataxy among other acute toxicity parameters under the dosages being studied, as well as no death was observed with any of the tested doses. Based on that, the 5.0 g/Kg dose was selected for the behavior studies.

Open field assay

The result in Table 1 indicate that the administration of *Bauhinia forficata* Link aqueous extract (5.0 g/kg) in the open field test showed decrease general activity.

Table 1: Effects of *Bauhinia forficata* Link aqueous extract administration in general activity.

Parameters	**Control**	***Bauhinia forficata* Link 5.0g/kg**
Open-field	mean ± S.E.M	mean ± S.E.M
Locomotion frequency	69.90 ± 5.94	42.70 ± 4.37*
Rearing frequency	30.60 ± 6.93	14.00 ± 1.37*
Grooming duration	32.70 ± 5.11	52.30 ± 5.14*
Immobility duration	19.14 ± 8.18	60.06 ± 7.06*

N: 10 rats per group. Data are reported in mean ± S.E.M. *p<0,05 (Student's *t*-test)

Pluz maze assay

In the present study, *Bauhinia forficata* Link extract administration, in spite of the slight increase of entries into open arms, considerably decreased time into open arms and crossings, so indicating the absence of any anxiolytic effect (Table 2).

Table 2: Effects of *Bauhinia forficata* Link aqueous extract in anxiolytic assay.

Parameters	**Control**	***Bauhinia forficata* Link 5.0g/kg**
Pluz-maze	%	%
% Entries into open arms	45.63	49.39
% Time into open arms	30.18	16.31 *
% Central time	8.90	4.80 *

N: 10 rats per group. Data are reported in percentage *p<0,05 (Chi-square tests)

Pentobarbital sleep time

The result in Table 3 indicates that the administration of *Bauhinia forficata* Link aqueous extract significantly augmented pentobarbital-induced sleep.

Table 3: Pentobarbital sleep inducing time and sleeping time of rats exposed to *Bauhinia forficata* Link aqueous extract.

Parameters	**Control**	***Bauhinia forficata* Link 5.0g/kg**
Open-field	mean ± S.E.M	mean ± S.E.M
Sleep inducing time	3.20 ± 2.48	4.20 ± 1.87
Sleeping time	46.50 ± 2.68	84.20 ± 4.88*

N: 10 rats per group. Data are reported in mean ± S.E.M. *p<0,05 (Student's *t*-test)

Discussion and Concluion

The use of medicinal plants as alternative therapy is wide spread in the populations of underdevelopment countries, which have a limited access to medical assitence[9].

Until the present time, the biological essays realized with the extract of Bauhinia forficate leaves, mainly refer to diabetes. Studies over its effects on the CNS are still rare. This way, our studies, although preliminary, are new and demonstrate that Bauhinia forficate presents effects over CNS.

The result indicate that the administration of *Bauhinia forficata* Link aqueous extract (5.0 g/kg) in the open field test showed decrease in locomotion frequency and increase in time of immobility, suggesting reduced general activity. Reduction in the locomotion as well as immobility increase seem not to be associated with the stomach fullness, once the observation in the open field occurred 30 minutes after administration of vegetal extract in animals under complete fasting.In addition, as far as the observed decrease in rearing behavior is concerned, it can be explained thinking that *Bf* would reduce the rat´s habituation process. Open field grooming duration was increased by *Bauhinia forficate* Link extract administration. Barros et al[15] observed that GABAergic agonists reduce grooming of rats in the open field, suggesting the usefulness of this parameter to detect the effects of GABAergic drugs and anxiolytic effects. The increase of grooming duration would be caused by a possible anxiogenic effect of *Bauhinia forficata* Link extract.

The elevated pluz maze is one most widely used model in pre-clinical research on anxiety and it is known that anxiolytic drugs increase entries and time into open arms of this apparatus[16]. The result reinforce that the administration of *Bauhinia forficata* Link aqueous extract in rats (5.0 g/kg) possible anxiogenic effect. This can be confirmed by the reduction in both, the number of entries in open-arms and the time in the crossing[17].

Table 3 showed that *Bauhinia forficata* Link extract significantly augmented pentobarbital-induced sleep, reflected by increased sleeping time assessed with the loss-of-righting reflex, suggesting these effects were potentiated by serotonin[18]. In summary, the above data suggest that *Bauhinia forficata* Link aqueous extract has promoted general alterations in behavior in anxilolytic models. Thus results suggest that *Bf* aqueous extract induced general changes in behavioral indicating toxicologic effect. In this regard, studies involving dosages for central neurotransmissors must be realized, in order Bauhinia forficata can be safely used by population.

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