Revista Brasileira de Farmacognosia 26 (2016) 595–600

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original article

Effects of bark flour of *Passiflora edulis* on food intake, body weight and behavioral response of rats

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ARTICLE INFO

Article history:
Received 30 September 2015
Accepted 11 February 2016
Available online 20 June 2016

Keywords:
Behavioral
Passiflora edulis
Sedative
Open field

ABSTRACT

Effects of treatment with the bark flour of *Passiflora edulis* Sims, Passifloraceae, were evaluated. Adult male *Wistar* rats were treated for 30 days (130 mg/kg, *p.o.*) with the albedo flour, flavedo and full bark of *P. edulis*, corresponding to albedo associated with flavedo. Behavioral response observed after treatment with bark flour *P. edulis* showed sedative effects by the reduction of exploratory activity and increased duration of immobility in the open field test for the group of animals that received the albedo flour associated with the flavedo. Sedative effects were observed in the absence of motor incoordination or muscle relaxation. Food intake of experimental animals was not changed, but the weight gain was decreased both in animals that received only albedo flour, and in those who received the full bark flour. The full bark flour of *Passiflora* showed sedative effects, without anxiolytic effect detectable and muscle relaxation or motor incoordination, and reduces body weight gain.

Introduction

Species of *Passiflora* genus are popularly indicated for treatment and prevention of central nervous system disorders such as depression, insomnia and anxiety. Thus, several studies have been conducted to evaluate these therapeutic actions. The anxiolytic activity of *Passiflora edulis* Sims, Passifloraceae, leaf extract (Petry et al., 2001) as well as the sedative properties of the aerial parts and the pericarp extracts (Sena et al., 2009; Deng et al., 2010; Klein et al., 2014), and antidepressant effect of leaves and stems ethanolic extract have been reported (Wang et al., 2013).

Besides, the evaluation of anti-inflammatory activity of the leaves extract and isolated chemical components of *P. edulis* (Montanher et al., 2007; Zucolotto et al., 2009) and antioxidant action of *P. edulis* fruit have also been described (Zeraik et al., 2011; Martínez et al., 2012). Active phytochemical compounds with antioxidant properties can inhibit oxidative damage and prevent the emergence of inflammatory diseases and disorders, including, the neurodegenerative ones (Praticò, 2010; Del Rio et al., 2013).

The *P. edulis* fruits are widely used by the food industry, and the bark is considered a byproduct of the industrial process (Ishimoto et al., 2007; Gomes et al., 2010). This consists of the exocarp or flavedo, part with color, and mesocarp or albedo, middle white part (Canteri et al., 2010).

The bark of passion fruit (*P. edulis*) is traditionally used as a functional food due to its high concentration of soluble and insoluble fiber. The use of mesocarp of passion fruit is an important dietary supplement for the treatment of Diabetes mellitus due to its potential hypoglycemic effect, and its ability to reduce triacylglycerides and cholesterol levels (Corrêa et al., 2014; Grosseli et al., 2014). The investigation of biological activities is critical to recovery and valorization of byproduct that may induce considerable environmental, economic and therapeutic impacts.

Although there has been described the activity of *P. edulis* as an anxiolytic or sedative agent, most studies have investigated the potential of its aerial part and the biological activity of different fruit parts, such as peels, seeds and pulp, its nutritional and therapeutic aspects have been little explored (Gosmann et al., 2011).

This study investigated the central effects from albedo flour, flavedo and full bark of passion fruit (*P. edulis*) using behavioral model in rat (plus maze) and others tests evaluated adverse effects (open field and rota rod), as locomotor and exploratory activity. Physiological effects were also evaluated, food intake and body weight, thereby investigating their potential nutraceutical properties.

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http://dx.doi.org/10.1016/j.bjp.2016.02.010

Materials and methods

Animals

Forty male albino Wistar (*Rattus norvegicus*) rats, with the same mean weight (200 g), 60 days old, were used in the study. Divided into four groups ($n = 10$): control: CG, albedo: AG, flavedo: FG, Albedo + Flavedo: AFG, housed in boxes ($n = 5$ per box) of the same treatment group or control. All animals were provided from the *vivarium* Prof. Dr. Thomas George from the Universidade Federal da Paraíba and kept throughout the experiments in with water and standard food ad libitum under illumination standards (light/dark cycle, 12/12 h), in a temperature range of $23 \pm 2\,^{\circ}C$, and humidity ($55 \pm 10\%$). Every experiment was approved by the Ethics Committee on Animal Use (CEUA) of UFPB with certificate No. 1405/12. All animals were under care according to the ethical principles of the National Council Control of Animal Experimentation – CONCEA/2013.

Flour preparation

The fruits of *Passiflora edulis* Sims, Passifloraceae, were collected and identified by the State Company of Agricultural Research of Paraíba S.A., Brazil – EMEPA. The bark flour of *P. edulis* was developed in the Laboratory of Food Microbiology and Biochemistry of UFPB, following protocol of the respective laboratory and Ishimoto et al. (2007). The passion fruits were washed in running water, the mesocarp or albedo and the exocarp or flavedo were manually separated. The barks were immersed in water and then 70% alcohol, both dips for 12 h. Shortly after, the barks were taken into a drying oven with air circulation at 64 °C for 24 h. Once dry, these were ground in an industrial blender and then sieved into a fine-mesh sieve to obtain thinner flour. Being thus prepared the albedo flour, flavedo flour and full bark flour, Albedo associated to Flavedo.

Treatment

The animals received 130 mg/kg of flour, safe amount for animals without toxicity problem (Medeiros et al., 2009), corresponding to the experimental group to which they belonged. Control group, Albedo group, Flavedo group, and Albedo + Flavedo Group. The flour was diluted with filtered water according to the animal weight and administered by gavage technique, daily, always at the same times (7–9 a.m.), for 30 days. The CG received only filtered water.

Behavioral tests to investigate the central action

Open field test

On the first day after the end of treatment, from 10 to 12 a.m., the animals were subjected to the Open Field apparatus. The open field apparatus consist in a box divided by quadrants drawn at the bottom, protected by an acrylic cylinder, where they can move freely during the test time. The locomotor and exploratory activity (ambulation) were evaluated by the number of quadrants traversed, and the behavioral parameters of Grooming, Rearing and immobility time were analyzed and registered in a 5 min period of observation for each animal (Bailey et al., 2008).

Motor coordination

The effect on motor coordination was evaluated using the Rota Rod apparatus. The Rota Rod Test measures the muscle relaxation effect or motor incoordination produced, for example, by anxiolytics and/or sedatives (Torres and Escarabajal, 2002; Martínez-Vázquez et al., 2012). This test was conducted through two sessions (habituation in apparatus and test). The first session (habituation in apparatus) was performed before the start of the 30 days of treatment. The animals were placed in the device for a total time of 3 min, including falls and repositioning, for habituation to the apparatus (speed = 6 rpm). The second session (test) was conducted on the first day after the end of treatment, from 2 to 4 p.m., and the time that the animal remained on the rotating rod was recorded for period till 3 min.

Elevated plus maze test

The methodology of the elevated plus maze (EPM) test was created by Lister in 1987 being a validated method to explore the neurobiological basis of anxiety (Torres and Escarabajal, 2002). The parameters evaluated in this test were: number of entries into the open and closed arms and time spent in the open and closed arms. Each animal was individually subjected to the apparatus once for 5 min, on the last day of treatment from 10 to 12 a.m.

Food intake and body weight

The body weight of the animals was measured once a week, always at the same time, from 7 to 9 a.m., using the digital electronic scale (Bioprecisa, BS3000A model), 3 kg capacity and sensitivity of 0.1 g, during the 30 days of treatment of the animals. Food intake controlling was performed by weighing up the leftover food and subtracting the amount that was stipulated and placed as standard. Three times a week were offered 500 g of feed for box, from 7 to 9 a.m. However, every day at the same time it was checked the amount of feed per box and added more, if necessary, because the purpose of the experiment was not control consumption, but only quantify it. The leftovers were quantified, clean reject (CR) and dirty reject (DR), between 7 and 9 a.m., three times a week, throughout the treatment period. It was obtained the total consumption per cage, and averaged the consumption per animal. The feed consumption (FC) was determined by the following equation:

$$FC* = \frac{((1500 + \text{additional}) - (CR + DR))/5 **}{7}$$

where *g/day/animal, and **number of animals per box.

Statistical analysis

Results were statistically evaluated using GraphPad Prism 4.00 (GraphPad Software, San Diego, CA, USA). Data were expressed as mean ± standard error of the mean for parametric data and median (percentile 25–75) for the non-parametric results. Variables were examined using variance analysis (One way ANOVA), followed by Tukey's Post Hoc or Kruskal–Wallis test followed by Dunn's Post Hoc. Open Field test and EPM variables were examined using Kruskal–Wallis test followed by Dunn's post hoc and/or ANOVA followed by Tukey's Post Hoc. Rota Rod test, food intake and body weight the differences were analyzed by ANOVA followed by Tukey's Post Hoc. Differences were considered significant when $p < 0.05$.

Results

Behavioral tests to investigate the central action

Open field test

The median of spaces traversed by the AFG animals was 10 (percentile 7–20) space while CG animals was observed a median of 31 (percentile 31–37) space, $p < 0.001$ and the other experimental groups, AG and FG, showed a median of 32 (percentile 31–37) space, $p < 0.001$ and 29 (percentile 24–39) space, $p < 0.01$ respectively (Fig. 1A). The albedo flour associated with the flavedo altered

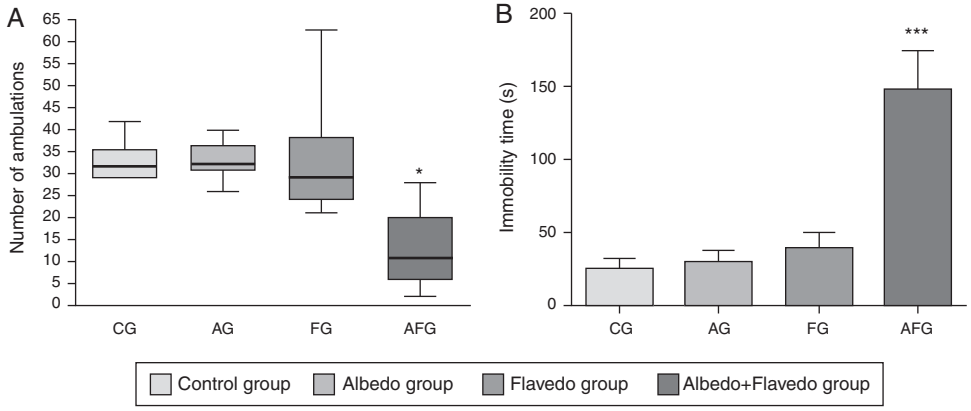

Fig. 1. Effect of bark flour of *Passiflora edulis* in the open field test. The animals were treated for 30 days (*p.o.*). Each bar represents the mean ± S.E.M. or Median (interquartile range) ($n = 10$). $^{*}p < 0.05$; $^{***}p < 0.001$ when compared with the control group. (A) Kruskal–Wallis test followed by Dunn's post hoc and (B) ANOVA followed by Tukey's Post Hoc.

the immobility time of the tested animals using the open field apparatus (Fig. 1B). Statistical analysis confirmed that AFG (148.1 ± 26.0) showed higher immobility time compared to both CG (24.0 ± 7.0; $p < 0.001$) and the experimental groups treated with albedo flour only (29.3 ± 7.6; $p < 0.001$) and flavedo flour (39.0 ± 10.4; $p < 0.001$). In relation to other behavioral parameters settings evaluated on the Open Field apparatus (Grooming and Rearing) no significant difference was found.

Motor coordination

The treatment with the passion fruit bark did not change the animals performance on the Rota Rod apparatus in the three-minute period of observation, CG (148.6 ± 12.7), AG (136.5 ± 15.5), FG (166.4 ± 3.0), AFG (125.1 ± 14.7), indicating that the bark flour of *P. edulis* does not cause muscle relaxation in animals (Fig. 2).

Elevated plus maze test

The entries number of animals in open and closed arms and the time spent in closed arms showed no statistical difference between groups. However, animals treated with albedo flour associated to flavedo (62.89 ± 22.06) had a higher time (s) spent in the open arms of the apparatus compared to CG (12.67 ± 5.09; $p < 0.05$). Fig. 3 shows the data obtained in the EPM.

Food intake and body weight control

Weight gain (g) of animals treated with albedo flour associated to flavedo (79.6 ± 5.60) and with the albedo flour (86.3 ± 3.22) were lower when compared to CG (102.8 ± 1.52). However, there was no significant variation between the average food intake (Table 1). Fig. 4 shows the body weight (g) of AFG animals (292.3 ± 7.72) at the end of the experiment, it was significantly lower than the CG (319.0 ± 4.76; $p < 0.05$) and the FG (321.9 ± 5.29; $p < 0.01$). In the third week of experiment the AFG (265.1 ± 8.91) already showed reduction in body weight compared to CG (289.7 ± 3.39; $p < 0.05$) and FG (300.3 ± 4.30; $p < 0.01$).

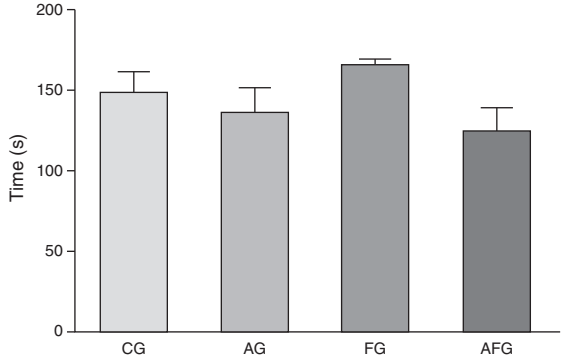

Fig. 2. Effect of *Passiflora edulis* bark flour in the Rota Rod test. The animals were treated for 30 days with the albedo flour, flavedo and full bark of *P. edulis* (130 mg/kg, *p.o.*) or control. Each bar represents the mean ± S.E.M. ($n = 10$). ANOVA followed by Tukey's Post Hoc.

Discussion

Species of *Passiflora* are popularly used and evaluated in experimental studies as agents anxiolytic, sedatives and tranquilizers (Dhawan et al., 2004; Gosmann et al., 2011; Sampath et al., 2011). Unlike studies described in the literature, used only extract of plant, in this study was evaluated the activity of bark of passion fruit on the CNS over a month, and *in natura.*

Regarding anxiolytic and sedative properties of the genus *Passiflora* the treatment with albedo flour associated to flavedo caused a decrease in ambulation of animals in the open field device and an increase in immobility time, when compared to CG, demonstrating the sedative property of the species.

Our data are in line with previous studies in which the administration of extracts made from the pericarp of *P. edulis* presented sedative effect in the induced sleep ethyl ether test (Sena et al., 2009), and in the telemetry test by the reduction of exploratory activity (Klein et al., 2014).

The elevated plus maze test is a preclinical test commonly used to investigate new anxiolytic agents. Nutraceuticals with anxiolytic properties reduce the animal's natural aversion to promote the exploration of the environment in the open arm of the apparatus. Studies with nutraceuticals or plant extracts with anxiolytic properties prove that the time spent in the open arms and the number of these entries is increased compared with control group

Table 1
Mean values of food intake and weight gain of the animals at the end of 30 days of administration of flour from the bark of *Passiflora edulis.*

Groups	Feed consumption (g/day/animal)	Weight gain (g)
CG	26.82 ± 2.05	102.8 ± 1.52
AG	26.15 ± 2.15	86.3 ± 3.22[a]
FG	27.26 ± 2.37	102.2 ± 2.55
AFG	26.48 ± 2.46	79.6 ± 5.60[a]

CG, control group; AG, albedo group; FG, flavedo group; AFG, albedo + flavedo group.
Data are expressed as mean ± standard error ($n = 10$/group).
The differences were analyzed by ANOVA followed by Tukey's Post Hoc ($p < 0.05$).
[a] $p < 0.05$ compared to CG.

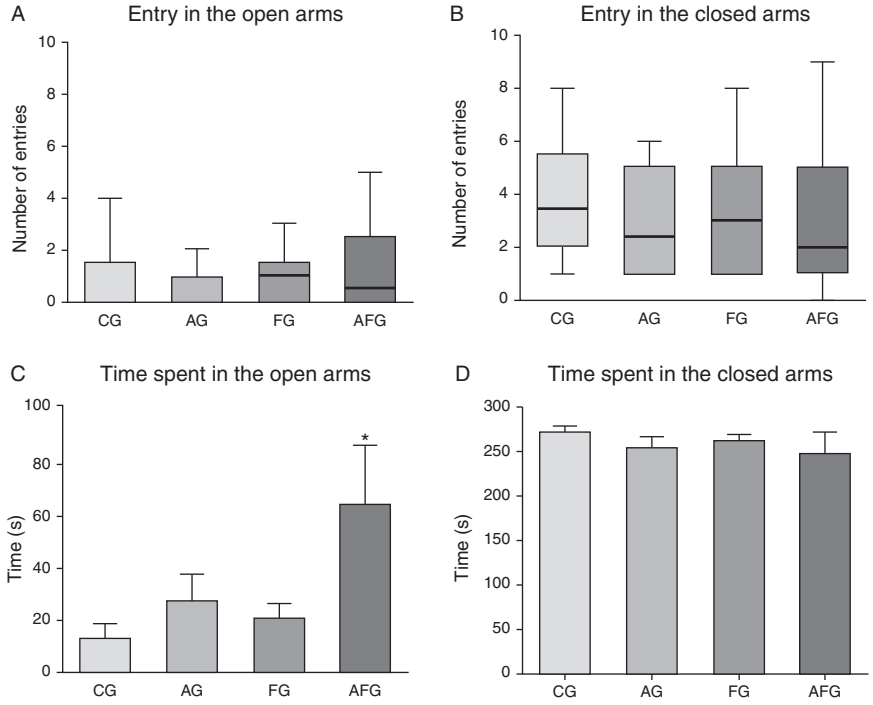

Fig. 3. Effect of *Passiflora edulis* in the elevated plus maze test. The animals were treated for 30 days with the albedo flour, flavedo and full bark of *P. edulis* (130 mg/kg, *p.o.*) or control. Each bar represents the mean ± S.E.M. or Median (interquartile range) ($n = 10$). $^{*}p < 0.05$ when compared with the control group. ANOVA followed by Tukey's Post Hoc or Kruskal–Wallis test followed by Dunn's post hoc.

animals (Kuribara et al., 2001; Carvalho-Freitas and Costa, 2002; Landaverde et al., 2009).

AFG showed a greater time spent in the open arms when compared to other groups, however, the number of entries into the open arms was not significant compared with control group. A locomotion reduction is predictive of a pronounced sedative effect, then, in order to prove that the animals spent more time immobile in the open arms was due to its lower motor activity observed in the open field, suggesting that the albedo flour associated with flavedo of *P. edulis* presents possible sedative activity.

According to previous studies, the sedative effect often appears at doses higher than the dose that presents the anxiolytic effect (Deng et al., 2010; Li et al., 2011), which may explain the failure to detect a possible anxiolytic effect from the bark flour of *P. edulis*, since this was administered for one month and not in a single dose and we tested only one dose for each group.

Using extracts from the aerial parts of *P. edulis* f. *flavicarpa* Deng et al. (2010) demonstrated anxiolytic effects at low doses and sedation at higher doses of extracts. The aqueous extract of *P. alata* and *P. edulis* leaves showed anxiolytic effects in elevated plus maze test, without the memory process was changed. And both species showed no change in spontaneous locomotor activity in the open field test (Barbosa et al., 2008). While no anxiolytic effect was seen for the leaf extract of *P. edulis* by Li et al. (2011), and sedative activity suggested. Different from our data, administration of aqueous extract of the *P. edulis* pericarp induced anxiolytic behavior in mice in the light/dark box test.

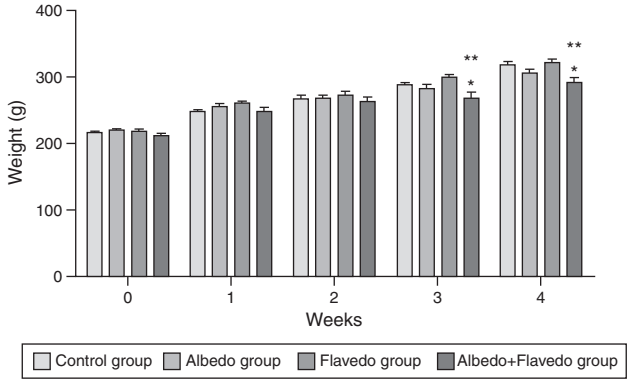

Fig. 4. Evolution of body weight of the animals during the administration of bark flour from *Passiflora edulis*. Each bar represents the mean ± S.E.M. ($n = 10$). $^{*}p < 0.05$ compared CG; $^{**}p < 0.01$ compared to FG. ANOVA followed by Tukey's Post Hoc.

The differences observed in the various studies how much anxiolytic and sedative properties of extracts from *Passiflora* may be attributed to differences in experimental procedures, preparation of extracts, doses, regimens and used administration ways, and different parts of the used plants (Petry et al., 2001; De-Paris et al., 2002; Reginatto et al., 2006; Deng et al., 2010; Li et al., 2011).

Studies suggest that the flavonoids present in passion fruit may be partly responsible for the anxiolytic and/or sedative activity of the many *Passiflora* genus species, but there are few data on action mechanisms of plant components and their activity (Coleta et al., 2006; Sena et al., 2009; Zucolotto et al., 2012). Flavonoids most frequently cites for species of *Passiflora* are glycosylated (Zucolotto et al., 2009; Zhou et al., 2009).

Treatment with Albedo Flour, Flavedo Flour and full bark flour, and Albedo associated Flavedo resulted in no change in motor coordination of animals within the 3-min period of observation in the Rota Rod apparatus. According to Farkas et al. (2005) a possible muscle relaxant effect is often observed by classical anxiolytic-sedative drugs which can compromise the functionality of the behavioral property. The full bark flour of *P. edulis* has a sedative effect in the absence of motor incoordination or muscle relaxation.

Besides the behavioral response observed, in this study, the full bark flour, and albedo associated with flavedo decreased weight gain of the animals during the trial period, without changing the food intake, the AFG having smaller body mass at the end of the experiment. The bark of *Passiflora* is rich in soluble fiber, especially pectin (Yapo and Koffi, 2008; Kulkarni and Vijayanand, 2010). A

high intake of fiber has been associated with weight loss or less weight gain (Koh-Banerjee et al., 2003; Bes-Rastrollo et al., 2006; Liu et al., 2003; Schroder, 2010). Therefore our findings are supported by the literature, since the increased intake of fibers is accompanied by a loss weight in AFG.

Several mechanisms have been suggested to explain how dietary fiber helps weight controlling (Slavin, 2005). The fibers decrease gastric emptying, absorption of nutrients and alter the secretion of gut hormones, in addition to influencing the oxidation and storage of fat (Pereira and Ludwig, 2001). According to Schroder (2010) fiber intake is inversely associated with weight and body fat loss.

Conclusion

It was found that full bark flour – albedo associated with flavedo – from *P. edulis* when administered for one month promote sedative effect without muscle relaxant effects. Additionally, it assists in reducing body weight gain without causing change in food consumption. Thus, before their beneficial health effects, it is important to note that the albedo flour associated with flavedo may be interesting for the production of nutraceuticals, since it showed both pharmacological, like sedative drugs, and nutritional properties.

Authors' contribution

DAFF, LCMP, MJCC and MCRG are responsible for the conception and design of the study, analysis and interpretation of data, and preparation of article. LLP, RMB, DVF and BSS participated in the behavioral tests and provided support during the execution of work. KHDO performed critical revision of the article. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The Universidade Federal da Paraíba, Brazil, for allowing us to conduct this research and EMEPA by the fruits of *Passiflora edulis* provided.

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