

*Full Length Research Paper*

# Evaluation of the anxiolytic effects of acute administration of *Passiflora alata* extract in wistar rats submitted to swimming

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Received 16 December 2021; Accepted 1 February 2022

Anxiety is present in several psychiatric disorders, and pharmacological treatment has limitations. Therefore, there is a search for new therapeutic approaches. This study aimed to evaluate the anxiolytic effect of acute administration of the dry extract of *Passiflora alata* (*P. alata*) leaves in male Wistar rats submitted to swimming. The animals were randomly divided into four groups (n = 8) (G1 to G4). G2 to G4 were submitted to swimming for 15 min, and 23 h later, all groups were treated via gavage: G1 (control); G2, treated with 0.9% saline solution; and G3 and G4, treated with 22 mg/kg and 66mg/kg of *P. alata* extract, respectively. After 1 h of administration, G2-G4 were again submitted to swimming for 5 min. All groups were submitted to the Elevated Plus Maze (EPM). The results showed that all animals submitted to swimming (G2-G4) remained less time in the open arms when compared to the control group (G1). The results demonstrate that the swimming model was adequate to induce anxiety in the animals. However, no significant differences were observed between the groups in the times of movement and immobility (in the swimming model) and the length of time in the open arms in the EPM model, indicating that in the model and doses used, *P. alata* extract had no anxiolytic effect.

**Key words:** *Passiflora alata* extract, anxiety, swimming, wistar rats, elevated plus maze.

## INTRODUCTION

Disorders such as anxiety and post-traumatic stress (PTSD) are very prevalent in the population, and is associated with physical comorbidities, increased use of health services, and decreased productivity at work,

leading to a significant social impact (Bandelow, 2020; Pereira-Lima et al., 2016; Remes et al., 2016). They present similar aspects, sharing common clinical characteristics such as excessive fear and anxiety

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(American Psychiatric Association, 2013; Liu et al., 2015).

Treatment can be carried out using benzodiazepines, antipsychotics and antidepressants (Bandelow, 2020; Liu et al., 2015). However, the use of benzodiazepines is related to the potential for abuse, dependence and abstinence. Antidepressants and antipsychotics can cause undesirable effects such as postural hypotension, the potential for arrhythmias, constipation, and urinary retention (Akinnusi and El Solh, 2019; Fonseca et al., 2020). Thus, the search for new therapeutic alternatives is very relevant, and, in this context, therapy research based on the use of plants has increased worldwide (Hou et al., 2020; Kim and Song, 2012; Kim et al., 2017; Rahbardar and Hosseinzadeh, 2020). In this sense, there is consistent evidence of the effectiveness of several plants in the treatment of psychiatric disorders (Sarris, 2018), highlighting the genus *Passiflora* (Aman et al., 2016; da Cunha et al., 2021; Dantas et al., 2017; Giovannini and Howes, 2017; Janda et al., 2020; Kim et al., 2019), especially *Passiflora incarnata*, used to treat insomnia and anxiety (Kim et al., 2019; Miroddi et al., 2013; Patel et al., 2009; Schäfer et al., 2021). The anxiolytic activity seems to be due to flavonoids, which would act on the GABAergic system (Fonseca et al., 2020). Although *Passiflora incarnata* is the most described worldwide; in Brazil, the species *Passiflora edulis* and *Passiflora alata* (*P. alata*), known as sweet passion fruit, are common and have been popularly used as anxiolytics (Barbosa et al., 2008; Boeira et al., 2010). However, few studies have evaluated the effects of *P. alata* on anxiety models. Thus, the present study aimed to evaluate the anxiolytic effect of the dry extract of the leaf of *P. alata* in male Wistar rats submitted to swimming.

## METHODS

### Ethics

This study started after approval by the Animal Use Ethics Committee (CEUA) of the University of Marília (UNIMAR), Marília, Sao Paulo, Brazil; protocol number 018/2019.

### Vegetal extract

*Passiflora alata* leaf dry extract (using water as a solvent), was purchased from the local commercial establishment in the city of Marília, Sao Paulo, Brazil. As informed by the manufacturer, the extract had a total flavonoid content (expressed as apigenin) of 0.19%. For administration, the dry extract was prepared in concentrations of 22 and 66 mg/mL, diluted in saline solution (0.9%). The effect of acute administration of *P. alata* at doses of 22 and 66 mg/Kg was evaluated. These doses were determined based on a previous study by our group (Rizzi et al., 2017).

### Modified model of forced swimming

To the stress induction, the animals performed a modification of the Porsolt Model (Porsolt et al., 1978), in which the animals are

submitted to swimming for 15 min and 24 h later, to a new swimming period of 5 min, both in a column of 15 cm of water. In the present work, the water column was increased from 15 to 30 cm.

### Elevated plus maze

An apparatus characterize the elevated plus maze (EPM) is built of wood (100 cm from the ground), includes two open and opposed arms (50 × 10 cm) and two enclosed arms (50 × 10 × 40 cm) (Boerngen-Lacerda et al., 2000). Platforms with the same extension of the open arms cross them perpendicularly, resulting in a delimited central area (10 cm<sup>2</sup>). Wistar rats were placed in the apparatus for 5 min to evaluate the frequency and time spent in the center and the open and closed arms.

The tests at EPM were carried out in an isolated room with attenuated sound, temperature and light control, and air exhaustion, in which no other activity was performed at the time of the experimental protocol. The experimental sessions were carried out all during the day to avoid variations due to the circadian cycle. The animals were filmed for 5 min to assess the frequency of entrances and the time spent in open and closed arms and passages through the middle of the EPM. Additionally, based on these parameters, the anxiety index was calculated, as described by Huynh (Huynh et al., 2011):  $1 - [(time\ in\ the\ open\ arm / 300) + (entries\ in\ the\ open\ arm / total\ entries / 2)]$ .

### Experimental protocol

Thirty-two male albino Wistar rats weighing between 180 and 220 g were used. During the experimental protocol, the animals received water and rat food *ad libitum*.

Before experimentation, the animals were randomly divided into four groups (G1-G4) (n = 8), placed in plastic boxes (40 × 30 × 17 cm), four animals per box, and then acclimated for ten days to the laboratory conditions in a room with temperatures 20 to 22°C and controlled light / dark cycle (12/12 h). Groups G2-G4 underwent swimming according to a modification of Porsolt Model. Twenty-three hours later, all groups received treatment via gavage as follows:

G1: Control group that was treated with saline solution at a dose of 1 mL/kg - not submitted to swimming;  
 G2: Treated with saline solution at a dose of 1 mL/kg;  
 G3: Treated with a dose of 22 mg/kg of *P. alata* extract;  
 G4: Treated with a dose of 66 mg/kg of *P. alata* extract.

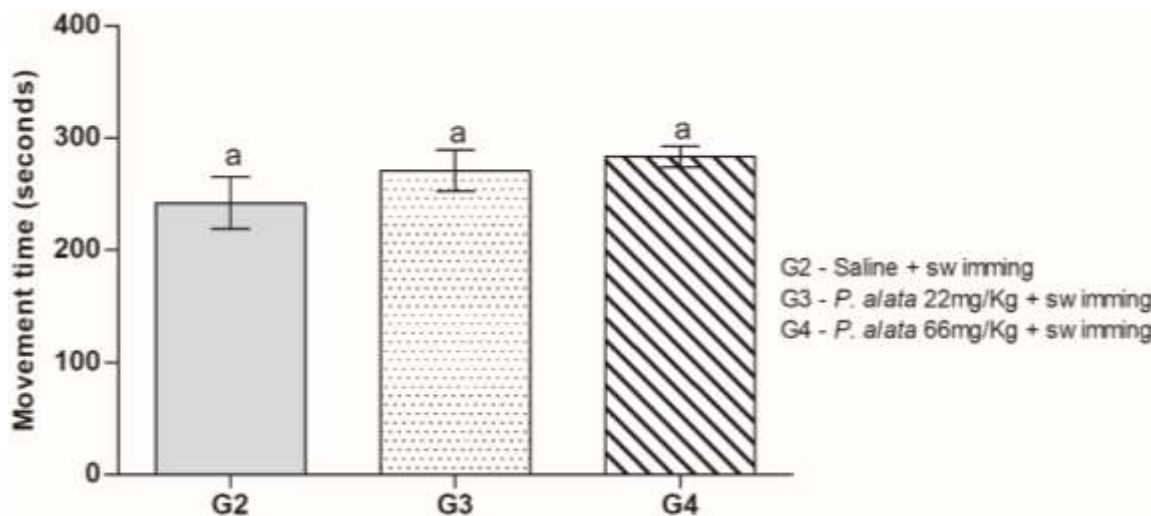
Sixty minutes after the administration, G1 was evaluated in the EPM, and the groups G2 to G4 were submitted to a new swimming period for 5 min. After swimming, the animals in G2 to G4 were dried and evaluated in the EPM.

### Statistical analysis

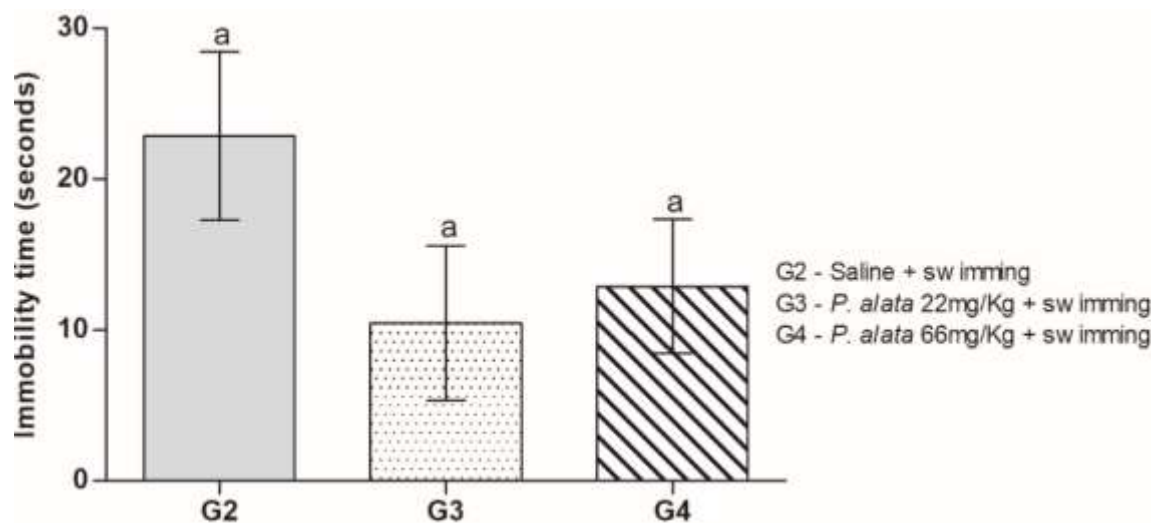
For statistical evaluation, the GraphPad Prism 5.0 software was used. The results were submitted to the normality test and, subsequently, the analysis of variance complemented by the Tukey test. The variables evaluated in swimming and the Elevated Plus Maze were expressed as mean ± standard deviation. The level of significance adopted was 5% (p < 0.05).

## RESULTS AND DISCUSSION

Figures 1 and 2 show the effect of acute administration of



**Figure 1.** Effect of acute administration of *P. alata* extracts on movement time in the forced swimming model. Results expressed as mean  $\pm$  mean standard error. According to the Tukey test, there was no statistically significant difference ( $p > 0.05$ ).



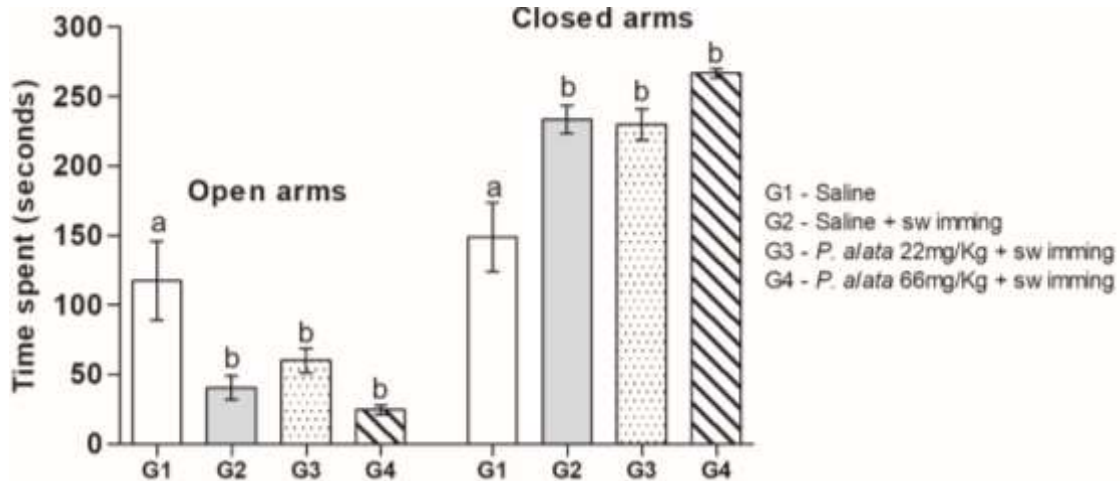
**Figure 2.** Effect of acute administration of *P. alata* extract on immobility time in the forced swimming model. Results expressed as mean  $\pm$  mean standard error. According to the Tukey test, there was no statistically significant difference ( $p > 0.05$ ).

*P. alata* extract, respectively, on movement and immobility times. In the doses used, there was no significant difference between the groups.

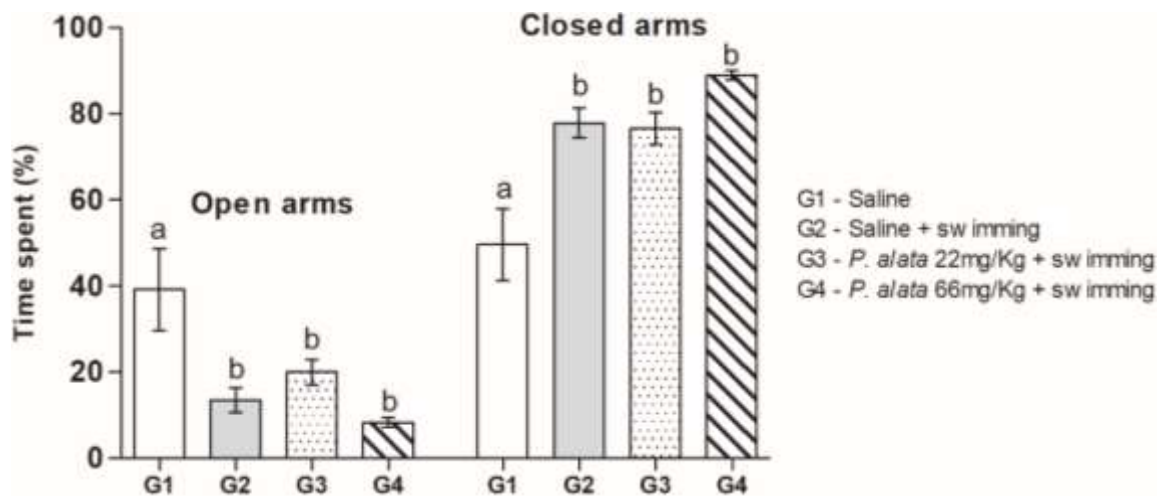
Figures 3 and 4 show the effects of swimming and acute administration of *P. alata* extract on time spent in open and closed arms, respectively, in absolute numbers and percentages. It is observed that the group that was not submitted to swimming (G1), compared to the others (G2, G3, and G4), remained longer in the open arms. On the other hand, the groups submitted to swimming remained longer in closed arms. At the doses used, the

*P. alata* extract did not significantly alter the length of stay of the animals in the EPM arms.

Figure 5 shows the effects of swimming and the acute administration of *P. alata* extract on locomotion, assessed through the number of passes through the center and the total number of entries into the arms of the EPM. It is observed that animals submitted to swimming had less locomotion than animals that were not swimming. Additionally, it can be observed that the administration of *P. alata* extract did not significantly alter animal movement.



**Figure 3.** Effects of swimming and acute administration of *P. alata* extract on time spent in open and closed arms in the elevated plus-maze model. Results expressed as mean  $\pm$  mean standard error. Different letters indicate statistically different groups according to Tukey's test ( $p < 0.05$ ).



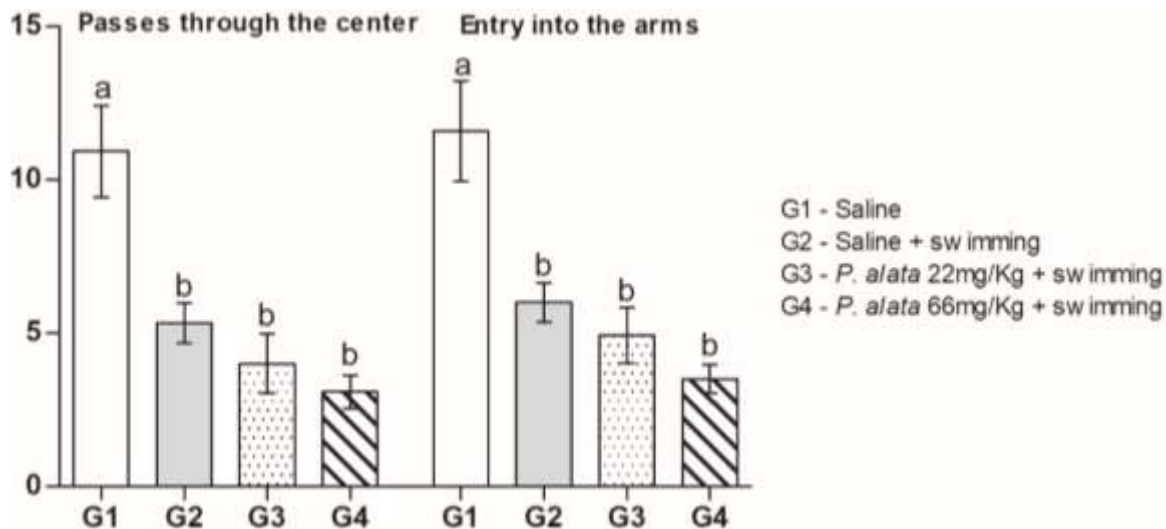
**Figure 4.** Effects of swimming and acute administration of *P. alata* extract on percentages of time in open and closed arms in the elevated plus-maze model. Results expressed as mean  $\pm$  mean standard error. Different letters indicate statistically different groups according to Tukey's test ( $p < 0.05$ ).

The effects of swimming and acute administration of *P. alata* extract on the anxiety index are shown in Figure 6. It is observed that the anxiety index was higher in animals submitted to swimming. There were no significant differences in the anxiety index between the animals that received a saline solution and *P. alata* extract.

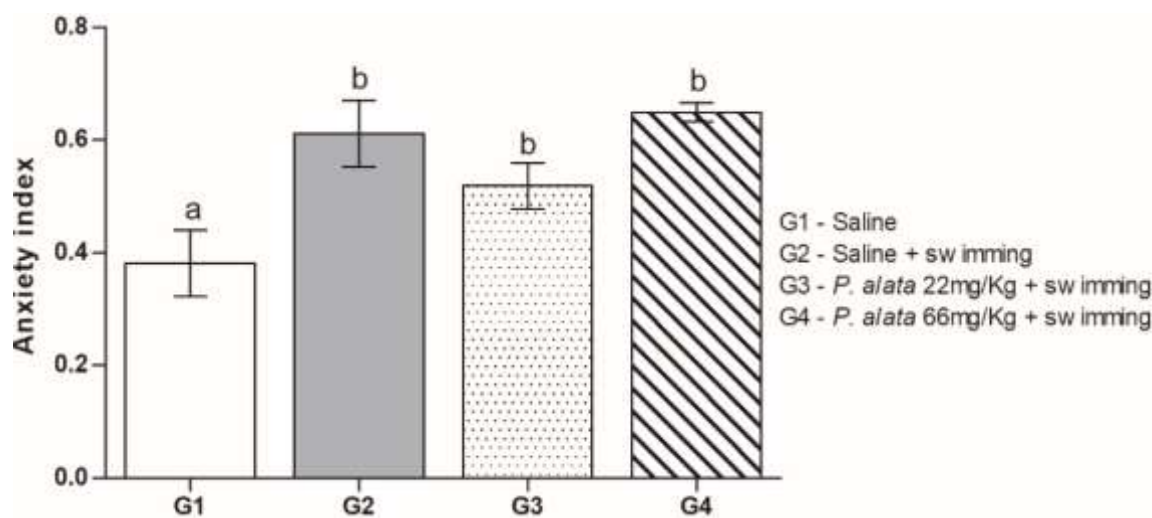
Plants of the *Passiflora* genus, especially *P. incarnata*, have several compounds emphasizing flavonoids such as chrysin, isovitexine, orientin and vitexin. Other compounds such as alkaloids (harmine), amino acids, and essential oils are also present (Kim et al., 2019b; Lans, 2019; Schäfer et al., 2021; Savage et al., 2018). The

anxiolytic effect appears to be due to an action on the gabaergic system (Fonseca et al., 2020; Jawna-Zboinska et al., 2016; Kim et al., 2019b), showing that flavonoids play a similar role to GABA (gamma-Aminobutyric acid) (Otify et al., 2015). Thus, the genus *Passiflora* has been considered to treat anxiety, a component present in depression and anxiety and post-traumatic stress disorders (Aman et al., 2016; Kim et al., 2019a).

For this purpose, models that induce anxiety in animals have been used. According to Arantes et al. (2013) and Campos et al. (2013), fear and anxiety behavior can be induced by exposing the animal to stressful or traumatic situations through physical stressors (underwater trauma,



**Figure 5.** Effects of swimming and acute administration of *P. alata* extract on locomotion in the elevated plus-maze test, assessed through the number of passes through the center and total number of entries into the open and closed arms. Results expressed as mean  $\pm$  mean standard error. Different letters indicate statistically different groups according to Tukey's test ( $p < 0.05$ ).



**Figure 6.** Effects of swimming and acute administration of *P. alata* extract on the anxiety index assessed in the elevated plus-maze model. Results expressed as mean  $\pm$  mean standard error. Different letters indicate statistically different groups according to Tukey's test ( $p < 0.05$ ).

electric shock, and physical restraint), social stressors such as housing instability or psychological stressors such as exposure to the predator or its odor or early maternal withdrawal (Whitaker et al., 2014). Such approaches to inducing anxiety states do not seek to generate anxiety disorders but rather a state of anxiety present in various diseases (Campos et al., 2013; Kraeuter et al., 2019).

Our protocol showed anxiety induction through a modification in the swimming model described by Porsolt

et al. (1978). In this model, the animal was exposed to swimming in a 15 cm water column and, according to Calil et al. (2002) the immobility of the animal is usually interpreted as helplessness. It can be used to assess the effect of antidepressant drugs. Indeed, the Porsolt model was initially developed to evaluate antidepressant drugs. However, the Porsolt model was modified in this study as the animals were exposed to a 30 cm water column, not allowing the escape and forcing the animal to swim. This new situation generated by swimming involves an

emotional component. For this reason, this model can generate a stressful situation, and, in this case, there is a relationship between the immobility time and the adaptive response of the animal to the stress situation. Thus, less movement indicates a lower level of anxiety (Calil et al., 2002).

Our results also showed that the administration of *P. alata* leaf extract promoted increased movement time and decreased immobility time (Figures 1 and 2) of animals submitted to swimming. However, the results were not statistically significant, suggesting no anxiolytic effect at the doses used.

The animal behavior was also evaluated in the EPM model. EPM, often used for the study of anxiety, is based on the premise that the presence of a new environment or situation can cause conflicting reactions between anxiety and curiosity / exploratory behavior. Thus, when exposed to a new situation, a rodent would explore new environments, conflicting with the fear of heights and the aversion to closed arms. Thus, the avoidance of the open arm would be indicative of anxiety (Arantes et al., 2013; Campos et al., 2013; Kraeuter et al., 2019; Padurariu et al., 2017; Rico et al., 2019; Verbitsky et al., 2020).

In this work, when comparing groups G1 and G2, it is possible to observe that swimming significantly increased the time the animals remained in the closed arms and decreased the time in the open arms of the maze (Figures 3 and 4), demonstrating that the Porsolt modified model was able to increase the levels of anxiety in the animals.

According to Sturman et al. (2018) and Kraeuter et al., 2019, less anxious animals tend to explore the environment more. The present study evaluated locomotion in the EPM through the total number of entries in the arms and passages through the center (Figure 5). The results demonstrate that the animals submitted to swimming had less locomotion, which was associated with fewer entries in the open arms, indicating less exploratory behavior, suggesting that the animals submitted to swimming had a higher level of anxiety. Indeed, the anxiety index (Figure 6) was higher in group G2 than G1.

In comparing groups G2, G3, and G4, there was no significant difference in the length of stay (absolute and in percentage) in the open and closed arms (Figures 3 and 4). In addition, the exploratory locomotor activity of the animals (Figure 5) showed no difference between the groups, indicating that the administration of *P. alata* extract (at the doses used) was not effective in reducing the anxiety state generated by swimming. This fact is corroborated by the anxiety index, which showed no significant difference between groups undergoing swimming, regardless of treatment (Figure 6). Dhawan et al. (2001) observed that the methanolic fraction of the extract of *P. incarnata* produced an anxiolytic effect at an oral dose of 10 mg/kg in mice performing the EPM model. Similarly, Petry et al. (2001) used the EPM to evaluate

the anxiolytic effect of the hydroalcoholic extract of leaf of two species of the genus *Passiflora* (*Passiflora edulis* and *Passiflora alata*). The plants were administered intraperitoneally in female Wistar rats at 25, 50, 100 and 150 mg/Kg doses. The authors observed that the animals that received the extract of *P. alata* at doses of 100 and 150 mg/Kg and *P. edulis* at doses of 50, 100 and 150 mg/Kg remained longer in the open arms and less time in the closed arms, showing thus an anxiolytic effect. The phytochemical characterization of the extracts demonstrated the presence of flavonoids in both species and triterpenes only in the extract of *P. alata*.

Otify et al. (2015) demonstrated that acute oral administrations of the hydroalcoholic extract of *P. edulis* in mice and some of its fractions (200 mg/kg) increased the length of animals' stay in the open arm, suggesting an anxiolytic effect.

Jawna-Zboinska et al. (2016) evaluated the effects of a standardized extract of *P. incarnata* in male Wistar rats and demonstrated that daily doses of the extract of 30, 100 and 300 mg/kg, administered orally over four weeks promoted a dose-dependent decrease in anxiety. Additionally, they observed depletion in the levels of glutamic acid in the hippocampus, parallel to an increase in its metabolites, suggesting an effect related to GABA.

Barbosa et al. (2008), using the EPM model, demonstrated that intraperitoneal administration of *P. alata* extract (at doses of 100 and 150 mg/kg in male Wistar rats) showed anxiolytic effect. The authors determined that the flavonoid content of the extract was 2.1%.

In addition to animal studies, the effects of *P. incarnata* have been investigated in clinical trials. Dantas et al. (2017), in a randomized, double-blind clinical trial, evaluated the anxiolytic effect of oral administration of *P. incarnata* at a dose of 260 mg in patients undergoing tooth extraction. The anxiolytic effect of *Passiflora* was similar to that of midazolam.

Previous studies conducted by Rizzi et al. (2017) demonstrated the administration of 22 mg/kg of dry extract of *Passiflora* spp. by intraperitoneal route decreased the levels of anxiety evaluated in the open field model.

The safety of the aqueous extract of *P. alata* was evaluated by Boeira et al. (2010), which did not show mortality with doses of 300 mg/kg administered for 14 days. Regardless of the safety, there is a possibility that the use of higher doses may interfere with the locomotor activity of the animals and consequently would interfere with the results in swimming and in EPM.

Indeed, based on Rizzi et al. (2017) study, the study evaluated the effect of oral administration of doses of 22 and 66 mg/Kg of dry extract of *P. alata* leaf. In the swimming and EPM model, the acute administration of this extract could not lower anxiety levels. It is important to highlight that the doses used were lower than those that effectively demonstrated an anxiolytic effect in the

mentioned studies.

Moreover, most of the above-commented studies evaluated the *P. incarnata* extract, which is described in some studies as the species with the highest anxiolytic activity. Some of them used different routes than the oral route, and none used swimming as a stressor.

The present study has some limitations, especially for not using a reference anxiolytic drug. In addition, the dry extract used showed a low flavonoid content (0.19%, expressed as apigenin). In the model and the doses used, no anxiolytic effect was observed. However, this effect should not be discarded since several studies have demonstrated the potential of species of the genus *Passiflora* to treat disorders in which anxiety is present. Thus, further studies are needed to better assess *P. alata* in models that use swimming to induce stress and anxiety.

## Conclusion

The swimming model used in this study was adequate to induce anxiety behavior in the animals. However, at the doses used, the extract of *P. alata* could not reduce the anxiety behavior. For these reasons, we suggest further studies with higher doses to assess the anxiolytic effects of *P. alata* in Wistar rats.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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