Revista Brasileira de Farmacognosia 28 (2018) 589–593

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

Original Article

Hydroethanolic extract of *Tropaeolum majus* promotes anxiolytic effects on rats

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ARTICLE INFO

Article history:
Received 6 February 2018
Accepted 25 June 2018
Available online 18 July 2018

Keywords:
Alternative treatment
Anxiety
Anxiolytic
Elevated plus maze
Hole-board
Medicinal plants

ABSTRACT

Tropaeolum majus L., *Tropaeolaceae*, popularly known in Brazil as 'capuchinha' is widely used due its anti-inflammatory, antiseptic, anti-hypertensive and anti-depressive properties. However, scientific investigations about its effects on the central nervous system are still scarce. This study investigated the central pharmacological actions of the prolonged treatment with a hydroethanolic extract of *T. majus* in male Wistar rats in the elevated plus maze and hole-board behavioral models. For this, rats were daily treated with distillated water (negative control); diazepam (1 mg/kg) or hydroethanolic extract of *T. majus* (75, 150 and 300 mg/kg), for 29 days (by gavage) and were submitted to elevated plus maze and hole-board. Animals treated with all hydroethanolic extract of *T. majus* or diazepam doses increased the percentage of entries in open arms when compared to control group. However, only treatment with diazepam increased the length of time spent in the open arms of the elevated plus maze. No differences between all groups were observed regardless rearing, grooming, stretched-attend postures and defecation rates. In the HB test, in opposite to diazepam, treatment with hydroethanolic extract of *T. majus* did not interfere in the exploratory activity of rats. The hydroethanolic extract of *T. majus* promotes anxiolytic-like effects when orally administered in rats.

Introduction

Anxiety and depression are two pathologies that lead the list of mental disorders, reaching one in ten people of the world population regardless of region, class or culture, leading in extreme cases to suicide. These disorders generate disability in patients, especially resistance to treatment, and are one of the major causes for non-fatal health problems among young individuals (Noda et al., 2015; Stonckings et al., 2016; WHO, 2016). Recent studies have shown that the treatments cost to the global economy is about one trillion dollars annually (Chisholm et al., 2016).

Frequently, conventional drugs used to treat anxiety (specially benzodiazepine drugs) produce several adverse reactions (which include drowsiness, dizziness, muscle weakness, constipation, nausea, dry mouth and blurred vision). These effects are dependent on the class of anxiolytic drug used and may compromise their safety and treatment adhesion. In the United States, approximately 8% of hospitalizations occur due to reactions caused by the consumption of synthetic drugs. It is estimated that about 100,000 deaths occur annually due to toxicity from such drugs, three times higher compared to traffic deaths caused by driving under the influence of alcohol (Haq, 2004). These effects, in addition to the popularity of this pathology, triggered studies on alternative treatments, such as the use of medicinal plants (Kolouri, 2016). Scientific studies on medicinal plants facilitate their use and increase the therapeutic options at reduced cost in relation to existing treatments in order to improve the quality of life of patients (Yunes et al., 2001; Leitão et al., 2009; Figueredo et al., 2014).

Originally from Brazil, Mexico and Peru, *Tropaeolum majus* L., Tropaeolaceae, popularly known in Brazil as "capuchinha", "chaguinha" and "nastúrcio", is an herbaceous of showy flowers, simple or folded, with flowering that reaches about 2–3 meters in length and 30–40 cm in height. It is considered an important medicinal, ornamental and edible plant (Silva et al., 2011).

This species has a variety of bioactive compounds including flavonoids, carotenoids and other polyphenols known for their anti-inflammatory activity (Butnariu and Bostan, 2011). Preclinical studies with *T. majus* have shown antibacterial action on the

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https://doi.org/10.1016/j.bjp.2018.06.006

urinary tract (Goss et al., 2006), diuretic action without renal calcium loss (Barboza et al., 2014), hypotensive and cardiorenal protective effects (Gasparotto Junior et al., 2017), antifungal and antiviral properties for the treatment of bronchitis and acute sinusitis (Conrad et al., 2006), and as a natural expectorant against influenza due to its large amounts of vitamin (Ribeiro et al., 2012).

In its empirical knowledge, *T. majus* is believed to act in the ascension of emotional energy expressed in different forms as manifestations of anguish, frustration, anxiety and depression (Campos, 1994); and, in recent years, this plant has been popularly used as antidepressant agent (Ferreira et al., 2004). However, there are no scientific investigations that demonstrate the efficacy of *T. majus* in the treatment of anxiety. So, the present study aimed to investigate the action of the prolonged use of the hydroethanolic extract of *T. majus* (HETM) and its pharmacological potential in the treatment of this pathology.

Material and methods

Botanical material and HETM preparation

Tropaeolum majus L., Tropaeolaceae, leaves were collected from the Garden of Medicinal Plants of Unipar, Umuarama-PR, 430 m of altitude above sea level (23°47′55S and 53°18′48W), in the morning. A specimen of this species is cataloged under the number 2230 in the official herbarium of the Paranaense University. The material collected was dried in oven with forced air circulation at 37 °C for a period of 5 days. After drying, the material was ground and stored in paper bags. Then, HETM was prepared by soaking at room temperature for seven days using 90% ethanol as solvent. The hydroethanolic extract obtained was filtered and concentrated at reduced pressure through rotary evaporator with temperature not exceeding 55 °C. Subsequently, the extract was lyophilized to obtain the yield. The 15.3% extract yield was diluted with distilled water for use in experiments.

The main classes of HETM compounds were investigated by high performance liquid chromatograph (HPLC-UV) and electrospray ionization-mass spectrometry (ESI-MS), evidencing flavonoid isoquercitrin as the major compound, from the subclass of flavanols. A previous study by our group analyzed the HETM constituents in more detail (Gasparotto Junior et al., 2011).

Animals

Fifty-five male Wistar rats with approximately three months of age and body weight ranging from 280 to 320 g were maintained in the animal facility of the Laboratory of Preclinical Research of Natural Products of Paranaense University. Animals were housed in groups of five animals per polypropylene cages (60 × 25 × 25 cm) and kept under controlled humidity and temperature conditions (22 ± 1 °C), with 12-h light–dark cycle (lights on from 6 h am to 6 pm), with *ad libitum* access to water and food. Before the beginning of behavioral experiments, animals went through an adaptation period (one week) and were de-wormed. Animals were randomized into control and experimental groups, with eleven rats by each experimental group ($n = 11$), for treatment with vehicle (distilled water, negative control group), diazepam (1 mg/kg; positive control group) or HETM (75, 150 or 300 mg/kg). Treatments were performed daily, for 29 days, by gavage. Doses were selected according to Gasparotto Junior et al. (2009), who reported pharmacological activity of HETM in these doses.

The ethical committee on animal use of the Paranaense University approved all procedures (No. 30272), and experiments were performed in accordance with international standards and ethical guidelines on animal welfare.

Behavioral evaluation

To evaluate the central activity of *T. majus*, two models of animal behavior were used: elevated plus maze (EPM), which evaluates anxiolytic and anxiogenic activity (Pellow et al., 1985; Neto et al., 2008; Campos et al., 2013) and hole-board (HB), which verifies ambulation and anxiolytic and anxiogenic action (Treit et al., 1981; Neto et al., 2008; Campos et al., 2013). The apparatuses were installed in an air-conditioned room (21 ± 2 °C) and illuminated by red light to keep the cycle dark. Two cameras were installed to capture the tests that took place during the last two days of treatment. Each animal received the daily dose of treatment 1 h before the start of the test. After this period, animals were submitted, one at a time, to EPM and then immediately to HB. During the exchange of animals, the apparatuses were cleaned with 10% alcohol. After experiment, animals returned to the animal facility. Subsequently, the behavioral videos of animals were analyzed by three blind evaluators. The experimental design was based on other published articles that evaluate the anxiolytic activity of other compounds in the same models (Vargas et al., 2006; Neto et al., 2008; Campos et al., 2013; Pandey et al., 2016).

Elevated plus maze (EPM)

The apparatus, made of wood, consists of two open arms (50 × 10 cm), and two closed arms on the opposite side (50 × 10 × 40 cm), elevated 50 cm from the ground. The junction area of the four arms (central platform) measures 10 × 10 cm. Each animal was placed in the center of the apparatus facing a closed arm. Animals were observed for a period of 5 min, and the following parameters were considered: number of entries in the open and closed arms, time spent in the open and closed arms (it was considered an entry when the four paws of the animal were inside the arm). These data were used to calculate the percentage of entries in open arms [% EOA: entries in open arms/(entry in open arms + entries in closed arms) × 100]; percentage of time spent in the open arms [% TOA: time in open arms/(time in open arms + time in closed arms) × 100]. The number of entries in closed arms was used as an index of locomotor activity (Rodgers et al., 1997). The grooming, rearing and stretched-attend postures were used to evaluate the possible anxiolytic or anxiogenic action of the extract and the defecation rates in the period was also analyzed.

Hole-board (HB)

Soon after the EPM test, animals were individually evaluated in the HB test. This equipment consists of a platform (50 × 50 × 30 cm), with black floor, marked with white lines with 10 cm^2 of area and with sixteen holes of 2 cm in diameter, equally distributed on the surface to the center of quadrants. Animals were individually placed on the center of the device and recorded for 5 min and the locomotor activity of animals included the number of crosses between different demarcated areas, in addition to rearing, grooming and time of immobility. The stretched-attend postures, head dipping and the defecation rates in this period were also recorded.

Statistical analyses

Data were analyzed for homogeneity of variance and normal distribution. Differences between means were determined using one-way analysis of variance (ANOVA) followed by Duncan's *post hoc* test. Non-parametric data were expressed as medians (interquartile ranges) and analyzed by Kruskal–Wallis followed by Dunn's *post hoc*. The significance level was set at 95% ($p < 0.05$) and results are expressed as mean ± standard error of the mean (S.E.M.).

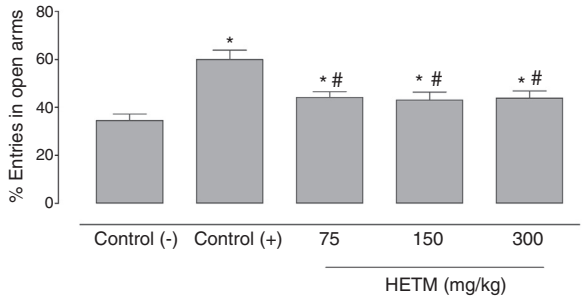

Fig. 1. Percentage of entries in open arms of rats daily treated with vehicle, diazepam or *Tropaeolum majus* hydroethanolic extract. Rats were daily treated with vehicle (negative control), diazepam (1 mg/kg; positive control) or *T. majus* hydroethanolic extract (75, 150 or 300 mg/kg), by gavage, for 29 days. Values are expressed as mean ± S.E.M. ($n = 11$). Statistical comparison was performed using one-way ANOVA followed by Duncan's test. *$p < 0.05$ when compared with negative control group; #$p < 0.05$ when compared with positive control group.

Results

The effects of 29 days of treatment with vehicle, diazepam or HETM (75, 150 and 300 mg/kg) on the percentage frequency of entry in the open arms of EPM are demonstrated in Fig. 1. One-way ANOVA indicated a difference between groups in the percentage of entries in the open arms of EPM ($F(4,50) = 9.06$, $p < 0.001$). Prolonged treatment with all HETM doses increased the percentage of entries in the open arms of the apparatus. The same occurs with diazepam when compared with negative control group. Regarding the number on entry in the enclosed arms of EPM, no differences were observed between groups ($H(4,55) = 6.46$, $p = 0.16$) (data not shown).

The group treated with diazepam obtained a significant increase in percentage of the time spent in the open arms of EPM compared with the negatives control group ($H(4,54) = 9.84$), which was an expected result due to its anxiolytic action. No difference was found in this parameter in groups treated with different *T. majus* doses (Table 1). Regarding the index of locomotion, no significant differences were observed between the groups (data not shown).

The effects of vehicle, diazepam or HETM on rats regardless of rearing, grooming, stretched-attend postures, defecation rates in the EPM test are shown in Table 1. Rearing, which indicates exploratory behavior, did not differ significantly between groups. Regarding the stretched-attend postures, which represent the risk assessment in which the animal anticipates a potential danger, no significant differences were observed between groups. Additionally, no significant differences were found for the defecation rates.

The results of the Hole-board test after 29 days of vehicle, diazepam or HETM on locomotor activity, rearing, grooming, head dipping, fecal rate after administration are present in Table 2. An increase in the locomotor activity (number of crosses) of the diazepam group compared to the vehicle group was observed ($H(4,55) = 9.74$). All HETM doses tested did not alter the locomotion of rats. No significant differences were observed between the groups for the other parameters analyzed.

Discussion

Anxiety disorders are quite common in the population: one in four adults has some anxiety at some point in their life and one in ten people probably had an anxiety in the past year (National Collaborating Centre for Mental Health, 2013). These anxiety disorders result in deep personal suffering and financial strain, since these disorders make hard for people to manage daily tasks, to work or study, and to relate to other people (Andreatini et al., 2001; Rector et al., 2005). The treatment to anxiety disorders involves psychological treatments (meditation, psychoeducation, cognitive conceptualization and cognitive restructuring), cognitive-behavioral therapy and medications (selective serotonin reuptake inhibitors, norepinephrine and serotonin reuptake inhibitors, benzodiazepines and beta blockers) (Oliveira, 2011; Rector et al., 2005). However, people who take these drugs are likely to experience side effect and new options of treatment with lower side effects are urgently necessary. In this way, this research reports the prolonged preclinical anxiolytic effects of *T. majus*, an important medicinal plant with several pharmacological properties.

In this investigation, rats treated with diazepam (positive control group) increased the number of entries in the open arms of elevate plus maze, indicating the effectiveness of the positive control group and validating the experimental procedure (Fernandez Espejo, 1997). Studies using EPM show that anxiolytic drugs, such as diazepam, increase the number of entries in the open arms and total entries, while anxiogenic agents, such as picrotoxin, decrease this proportion (Handley and Mithani, 1984). The same pattern of action of diazepam is expected in the Hole-Board test. Previous studies indicate that diazepam facilitates exploratory behavior by the anxiolytic effect that is promoted by a non-sedative dose (Crawley, 1985; Ohl et al., 2001; Silva and Elisabetsky, 2001). Our results showed that *T. majus* extracts at doses of 75, 150 and 300 mg/kg did not alter the animals' locomotion in the HB test. These results suggest that the *T. majus* extract does not interfere in the exploratory activity of the animal. Besides this, there was an increase in the locomotor activity (number of crosses) of the diazepam group compared to the vehicle group. Our data corroborate previous studies evaluating anxiolytic activity using diazepam as a positive control in the HB test that also found no anxiolytic effect at this dose tested (1 mg/kg) (Takeda et al., 1998; Akindele et al., 2012). These results indicate that, despite diazepam did not increase the head-dipping behavior, it maintains its exploratory activity (non-sedative effect). The anxiolytic-like effect of *T. majus* cannot be ruled out since in an increase in exploratory activity was observed in open arms in the elevated plus maze test. According to Blanchard et al. (2001), it is possible to identify a probable anxiolytic or anxiogenic action of a compound comparing results obtained in EPM with the group treated with diazepam. The prolonged treatment with all HETM doses (selected according to previous preclinical

Table 1
Effects of treatment with vehicle, diazepam or *Tropaeolum majus* hydroethanolic extract (HETM) on the elevate plus maze.

Treatment	Time spent in the open arms (s)	Rearing	Grooming	Stretched-attend postures	Fecal rate
Control (−)	51 [33–63]	16 [14–19]	4 [1–5]	8 [5–10]	3 [1–4]
Control (+)	86 [73–120][a]	15 [8–24]	3 [2–5]	11 [7–18]	2 [1–3]
HETM 75	92 [49–107]	13 [12–17]	2 [1–4]	12 [7–15]	3 [2–5]
HETM 150	52 [39–110]	15 [12–21]	4 [3–5]	9 [6–10]	1 [1–4]
HETM 300	68 [28–82]	18 [12–21]	2 [1–4]	9 [5–10]	4 [2–5]

Rats were orally treated with vehicle (negative control), diazepam (1 mg/kg, positive control) or HETM (75, 150 or 300 mg/kg), for 29 days. Values are expressed as median and interquartile intervals [Q1–Q3], ($n = 11$). Statistical comparison was performed using Kruskal–Wallis followed by Dunn's test. HETM: hydroethanolic extract of *Tropaeolum majus*.

[a] $p < 0.05$ when compared with negative control group.

Table 2
Effects of treatment with vehicle, diazepam or *Tropaeolum majus* hydroethanolic extract (HETM) on the hole-board.

Treatment	Locomotor activity	Rearing	Grooming	Head dipping	Fecal rate
Control (−)	62 [46–66]	23 [19–26]	5 [3–7]	13 [8–15]	1 [1–3]
Control (+)	81 [66–111][a]	29 [16–44]	4 [2–6]	13 [6–20]	1 [1–2]
HETM 75	75 [51–97]	29 [21–41]	3 [2–8]	18 [9–21]	2 [1–3]
HETM 150	76 [48–84]	26 [12–31]	5 [2–7]	14 [6–24]	1 [0–2]
HETM 300	72 [59–90]	33 [23–35]	3 [2–5]	14 [10–16]	1 [1–3]

Rats were orally treated with vehicle (negative control), diazepam (1 mg/kg, positive control) or HETM (75, 150 or 300 mg/kg), for 29 days. Values are expressed as median and interquartile intervals [Q1–Q3], ($n = 11$). Statistical comparison was performed using Kruskal–Wallis followed by Dunn's test. HETM: hydroethanolic extract of *Tropaeolum majus*.

[a] $p < 0.05$ when compared with negative control group.

studies) also promotes anxiolytic effects, since an increased in the percentage of entries of rats in the open arms of the apparatus was observed. Nevertheless, the action observed is less effective when compared to the anxiolytic effect of diazepam, considered the gold standard.

In the EPM, rearing (which indicates exploratory behavior) did not differ significantly between groups. The performance of this behavior is important to evaluate the integrity of the motor system, related to exploratory activity (Fernandez Espejo, 1997; Rodgers et al., 1997). Regarding grooming, there are indications that benzodiazepines, such as diazepam, cause a reduction in this behavioral because they reduce the response to the anxiogenic stimuli (Spruijt et al., 1992; Kalueff and Tuohimaa, 2005; Spazojevic et al., 2007); however, according to literature, the evaluation of this parameter is quite variable, and some studies report no correlation between grooming behavior and anxiety. In this experiment, no significant effect between groups was found for this parameter and for the stretched-attend postures, which represent the risk assessment in which the animal anticipates a potential danger. The same effect was observed in rearing, grooming and stretched-attend postures and defecations rates of rats in the hole-board test, suggesting that HETM does not interfere in the exploratory activity of the animal.

This anxiolytic-like effects of *T. majus* may be consequence of its main compounds: isoquercitrin and quercetin, important second metabolites of the plants that belongs to flavonoids class. In fact, the anxiolytic (Emamghoreishi et al., 2005; Zhang et al., 2012; Aguirre-Hernandéz et al., 2016) and central (Dos Santos et al., 2005; Can and Özkay, 2012) effects of this compounds were previously described in the literature. Confirming this information, the phytochemical profile of the extract was previously investigated and showed characteristic distributions of the flavonoids, including the isoquercitrin (Gasparotto Junior et al., 2017).

The potential pharmacological effect of *T. majus* and its popular indication are evident. However, until now, no studies have evaluated the activity of this plant in the central nervous system to confirm the possible central effects. This study is a start point and provides scientific evidence that the hydroethanolic extract of *T. majus* has anxiolytic-like effects when orally administered in rats, for a prolonged period (29 days).

In conclusion, hydroethanolic extract of *T. majus* at doses of 75, 150, 300 mg/kg has anxiolytic-like effects when orally administered, for 29 days, in rats. Further studies are necessary to verify the mechanisms of action of this extract and to elucidate which active principles are involved in this central activity.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Author contribution

ACM, SCAC, AFC, ANVS, SWS and ECWL conduced the experiments. FARL, ELBL, IPB and ECWL were responsible for data analysis and preparation of the manuscript.

Funding

Our immense gratitude to CNPq, Brazil (Process no. 800074/2016-5) and Paranaense University for the financial support to students.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

Our immense gratitude to Mikhael dos Santos Theodoro, Cleiton José de Carvalho Magalhães, Victor Felipe Pereira and Eloara Caroline Rodrigues Rebonato for the inestimable help in the experiments.

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