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Short communication

Evaluation of anxiolytic-like effect of 7-epiclusianone isolated from *Garcinia brasiliensis* in mice

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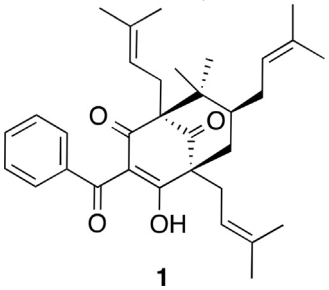
ABSTRACT

Garcinia brasiliensis Mart., Clusiaceae, species became the target of studies for some years because it has several compounds including polyprenylated benzophenones, as 7-epiclusianone. This benzophenone has several properties, such as leishmanicidal, anti-inflammatory and antinociceptive effects, however still did not be studied anxiolytic activity. For this, the open field and elevated plus maze tests were used in order to evaluate the effect of administration of 7-epiclusianone (isolated from *G. brasiliensis*) on behavioral performance. Swiss male mice ($n = 10$ per group) were pre-treated with vegetable oil (10 ml/kg; *i.p.*) or 7-epiclusianone (1, 3 or 10 mg/kg, *i.p.*) or diazepam (0.2 mg/kg, *i.p.*). After 1 h, the animals were submitted to the open field and elevated plus maze tests. The administration of 7-epiclusianone exerted a possible anxiolytic effect in the open field, increased the number of central crossings and anti-tigmotactic effect. In pre-treated group with 7-epiclusianone (10 mg/kg) was also possible to determine a possible anxiolytic effect in the elevated plus maze due to increased permanence of animals in the open arms. The results suggest a possible anxiolytic-like effect presented by the 7-epiclusianone and suggest its potential for the treatment of anxiety.

Introduction

The use of medicinal plants in health recovery has evolved over the times from the simplest forms of local treatment, up technologically sophisticated forms of industrial manufacturing currently used (de Abreu Matos, 2002). Many plants used in folk medicine are able to act in behavior, mood and feelings, and the understanding of their action, safety and efficacy is a challenge for researchers. Among these plants we can cite *Passiflora incarnata*, *Valeriana officinalis* and *Piper methysticum* (Carlini, 2003).

The *Garcinia brasiliensis* Mart., Clusiaceae, known as "bacupari", belongs to *Garcinia* genus, also known as *Rheedia*. It is cultivated throughout the Brazilian territory and is presenting as a medium-sized tree, containing yellow and oval fruits, leaves in format of spear and abundant flowers (Santa-Cecília et al., 2013). *Garcinia* genus presents a remarkable diversity of metabolites, such as oxygenated and prenylated phenolic derivatives, among which may be mentioned xanthones, flavonoids, phenolic acids and benzophenones, such as 7-epiclusianone (**1**) (Fiigueiredo, 2013). The *G. brasiliensis* and its isolated compounds showed antimicrobial, anti-inflammatory, antiproliferative, antioxidant and leishmanicidal activities but did not have reports in the literature regarding its anxiolytic effects (Naldoni et al., 2009; Murata et al., 2010; Pereira et al., 2010; Santa-Cecília et al., 2011).



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Anxiety is a normal emotion experienced in threatening situations, just like fear (Gelder et al., 2002). Those are common emotions already been experienced at some point in life. Conceptually, fear is an emotion caused by the presence of a real or imaginary

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danger, whereas anxiety is an emotion caused by anticipation of these same natures of danger described (Cavalli et al., 2009). Neurobiological studies of anxiety seek to emphasize the aspects related to brain mechanisms associated with phenomena anxious (Ramadam and Assumpção, 2005). Many neurotransmitters are involved in anxiety process, including the noradrenaline, serotonin, dopamine and gamma-aminobutyric acid (GABA) (Margis et al., 2003; Cavalli et al., 2009).

Therefore, still did not be reports in the literature regarding to potential anxiolytic activity of 7-epiclusianone, isolated from the medicinal plant *G. brasiliensis*. This study aimed to evaluate these effects through of the open field and elevated plus maze tests that are predictive models of anxious behavior.

Materials and methods

The 7-epiclusianone (**1**) was isolated and characterized, as previously reported by Santa-Cecília et al. (2011). In brief, to isolate the bioactive compound, *G. brasiliensis* hexane extract (35 g) was chromatographed on a silica gel (230–400 mesh) column (8 cm × 100 cm) and eluted with crescent polarity mixtures (hexane, hexane/ethyl acetate [95:5, vol/vol], hexane/ethyl acetate [80:20, vol/vol], hexane/ethyl acetate [50:50, vol/vol], hexane/ethyl acetate [20:80, vol/vol], and ethanol) to give 25 fractions. These fractions were pooled into four groups according to their similarities after analysis using thin-layer chromatography and compared with the standard 7-epiclusianone previously isolated from hexane extracts of the fruit of *G. brasiliensis*. Fractions 4–10 were chromatographed on a silica gel (230–400 mesh) column (8 cm × 100 cm) eluted with crescent polarity mixtures of hexane/ethyl-acetate (90:10, vol/vol) and ethyl acetate/ethanol (50:50, vol/vol) to purify the prenylated benzophenone 7-epiclusianone with a yield of 5%. Its structure was determined using spectroscopic techniques (infrared, ultraviolet, mass spectrometry, and ^{1}H and ^{13}C nuclear magnetic resonance).

The 7-epiclusianone (**1**) was dissolved in vehicle (vegetable oil) and was administered per gavage (*p.o.*), in doses of 1, 3 and 10 mg/kg. The standard drug was diazepam (DZP; Cristália, Itapira, SP, Brazil) in a dose of 0.2 mg/kg.

Adult male Swiss mice (40 ± 5 g) were obtained from the Central Animal Facility of the Federal University of Alfenas. The animals were fed on commercial diet and water "*ad libitum*" and were kept in controlled environmental conditions through the whole experiment, which ensured its adaptation for 7 days, under laboratory condition with alternating light and dark cycle of 12 h each, and 23 ± 2 °C. Immediately after the experiment, animals were sacrificed with isoflurano (Cristália, Itapira, SP, Brazil). All experiment was conducted in accordant with the principles of Declaration of Helsinki, guaranteeing the welfare of experimental animals, and with the approval of the Ethics Committee of the Federal University of Alfenas (protocol 532/2013).

In the experimental evaluation the animals were divided in groups and submitted to open field and elevated plus maze tests. To evaluate the involvement of 7-epiclusianone in the anxiolytic activity, the mice ($n = 10$) received the following pre-treatments: vehicle 10 ml kg (*i.p.*), 7-epiclusianone at doses of 1, 3 or 10 mg/kg (*i.p.*) and diazepam at a dose of 0.2 mg/kg (*i.p.*). One hour later, the animals were placed in the apparatus elevated plus maze or open field.

The elevated plus maze consisted of an apparatus of two open arms (30 cm × 5 cm × 0.5 cm), two closed arms (30 cm × 5 cm × 15 cm) and a central platform (5 cm × 5 cm) where the animals were placed, facing one of the closed arms and recorded for 5 min. The registered behavioral measures were: frequency of entries and time spent in the open arms were equivalent than in closed arms. An increase in the parameter corresponding to open arms (inputs and time) reveals an anxiolytic effect (Carobrez and Bertoglio, 2005).

The open field consisted of an apparatus with a floor (30 cm × 30 cm) with a circular base 40 cm in diameter and 40 cm high, divided in twelve squares. The eight square along the walls are considered periphery and the four others are central. The mice were placed in the center of the apparatus and filmed with a digital video camera for 5 min. The anxiolytic activity of the animals was evaluated considering the number of intersections with all four paws in the center. An increase of this parameter indicates a possible anxiolytic activity (Gomes et al., 2008). Typically animals tend to stay at the periphery for a longer time compared to the center area, this preference is known as tigmotaxia and reverse leads to an anti-tigmotactic effect can be observed in the open field and can be referred to as an anxiolytic action (Valle, 1970).

The results obtained were analyzed using GraphPad Prism version 6.0 and expressed as the mean ± S.E.M. Statistically significant differences among groups were calculated by the application of an analysis of variance (ANOVA) followed by the Newman–Keuls test. *P*-values less than 0.05 ($p < 0.05$) were considered statistically significant.

Results and discussion

As demonstrated in previous study performed by Santa-Cecília et al. (2011), 7-epiclusianone (**1**), a polyprenylated benzophenone extracted of the *Garcinia brasiliensis*, presented pharmacological effects in the central nervous system. In the present study, animal models that mimicking anxiety were used in order to evaluate the anxiolytic effect of 7-epiclusianone.

According to literature (Santa-Cecília et al., 2011), three doses were used: 1, 3 and 10 mg/kg. For the evaluation of anxiolytic effect, the open field test was chosen by suggesting anxiolytic activity when accomplished with experimentation animals (Gomes et al., 2008). In this test, four parameters were observed: number of crossings with the four paws in the center, number of crossings in the periphery, total number of entrances and the anti-tigmotactic effect.

In the open field test, pre-treatment with 7-epiclusianone increased the number of central entries in the doses of 1, 3 and 10 mg/kg ($p < 0.01$) compared with the control group (Fig. 1A). In addition, pre-treatment with 7-epiclusianone increased anti-tigmotactic effect at doses of 1, 3 and 10 mg/kg ($p < 0.01$) compared with the control group (Fig. 1D). The number of peripheral and total entries did not be different between the experimental groups ($p > 0.05$; Fig. 1B and C, respectively). The positive control used, diazepam, increased the number of entries in the center ($p < 0.01$) (Fig. 1A) and increased anti-tigmotactic effect ($p < 0.05$) (Fig. 1D).

Therefore, a significant increase of the number of entrances in the center was observed in the three doses of 7-epiclusianone (1, 3 and 10 mg/kg), when compared to the control group. These results demonstrate a significant increase of the effect anti-tigmotactic in all the groups pre-treated with the doses of 7-epiclusianone, when compared to the control group.

When analyzed the total of entrances and the total of crossings in the periphery, did not be difference in the experimental groups showing that the movement and exploratory activity of the mice did not be modified. It is known that the open field is a model anxiety predictive and that naturally, animals prefer areas protected as walls and they avoid the central area (Vilela et al., 2009). This preference is known as tigmotactic response and the inverse takes to an effect anti-tigmotactic, that can be observed in the open field and it can be referred as an anxiolytic action (Valle, 1970).

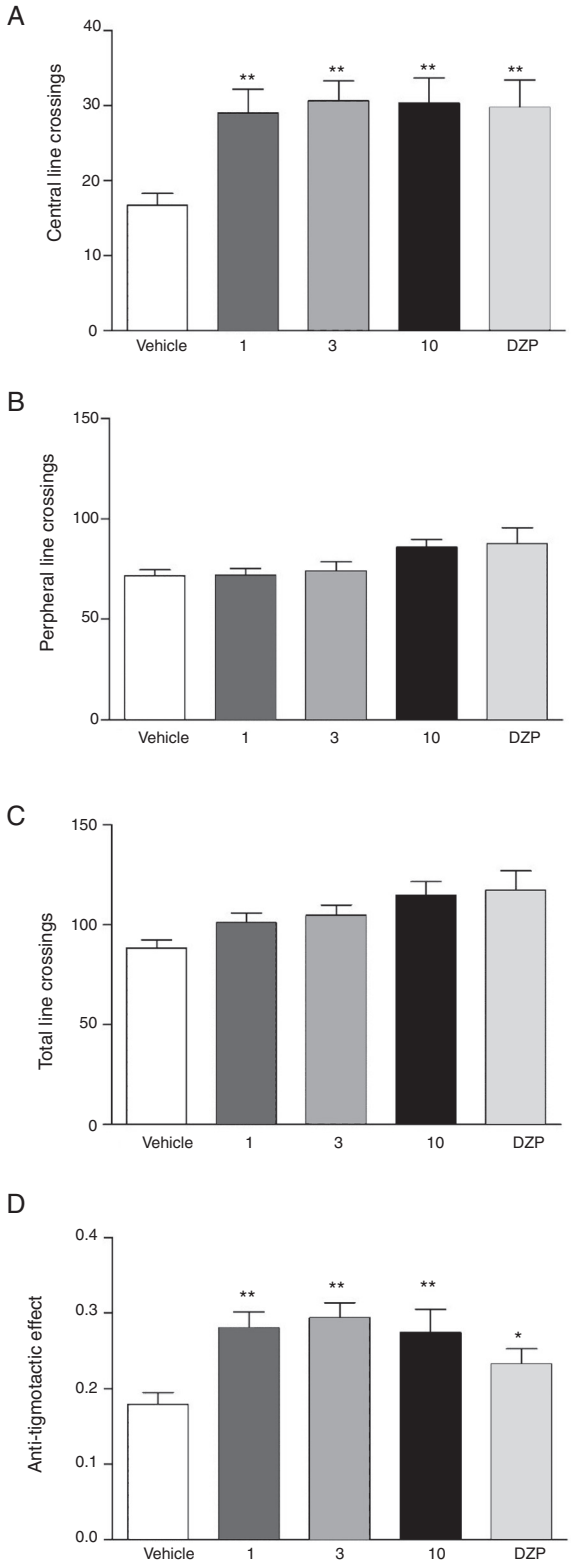

Fig. 1. Effect of treatment with 7-epiclusianone (1, 3 or 10 mg/kg, *i.p.*) or vehicle (10 ml/kg, *i.p.*) or diazepam (0.2 mg/kg, *i.p.*) in animals subjected to the open field test. (A) Total entries in the center with four paws. (B) Total entries in the periphery. (C) Total number of entries and (D) Anti-tigmotactic effect. The results obtained were expressed with mean ± S.E.M. $^{*}p < 0.05$ and $^{**}p < 0.01$ when compared with the group control (vehicle).

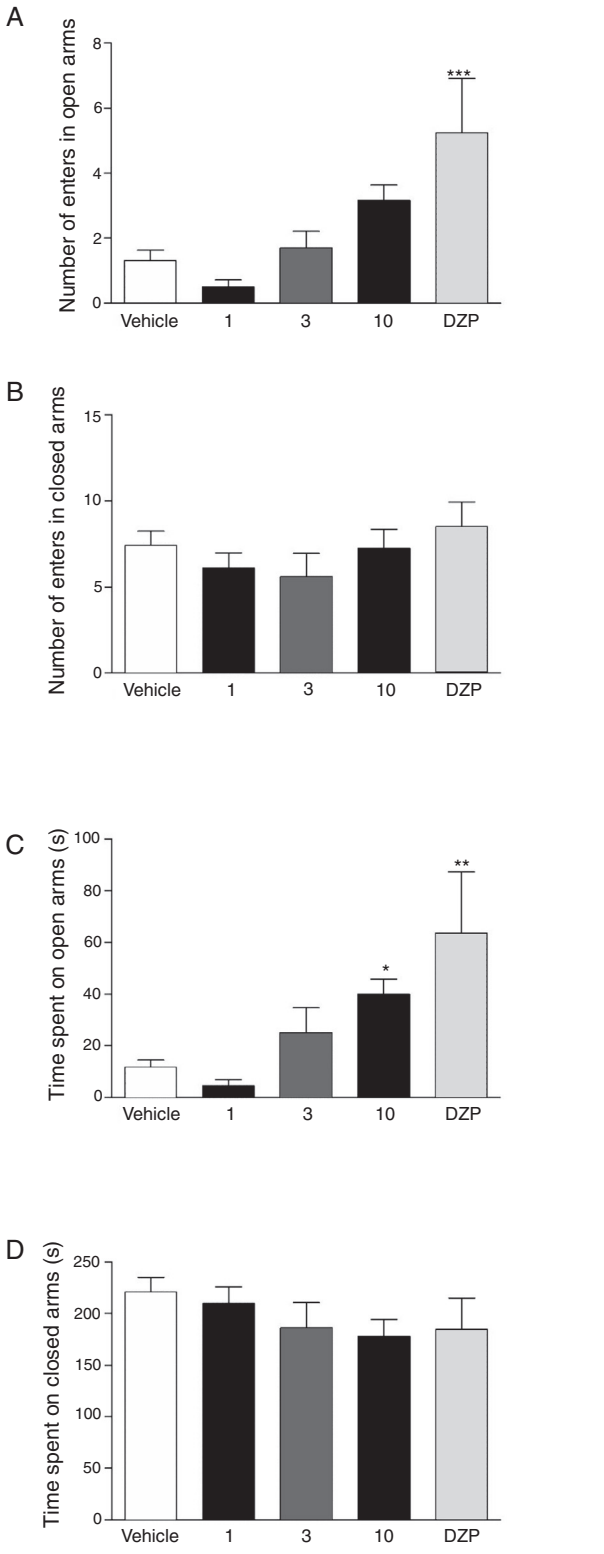

Fig. 2. Effect of treatment with 7-epiclusianone (1, 3 or 10 mg/kg, *i.p.*) or vehicle (10 ml/kg, *i.p.*) or diazepam (0.2 mg/kg, *i.p.*) in animals submitted to the elevated plus maze tests. (A) Total entries in the open arms. (B) Total entries in the closed arms. (C) Time spent in the open arms. (D) Time spent in the closed arms. The results obtained were expressed as mean ± S.E.M. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ when compared to the control group (vehicle).

Another test used to determine the anxiolytic activity, it was the elevated plus maze, described initially by Pellow et al. (1985). The results from the elevated plus maze test with administration of 7-epiclusianone (**1**) at a dose of 10 mg/kg increased the time spent on the open arms of the apparatus ($p < 0.05$), suggesting a possible anxiolytic effect when compared to the control group (Fig. 2C). According to the results, the other parameters did not be changed between experimental groups. The positive control group pre-treated with diazepam increased the number of entries in the open arms ($p < 0.001$; Fig. 2A) and also the time spent in the open arms ($p < 0.01$; Fig. 2C). The number of entries in the closed arms and the time spent on the closed arms of the apparatus did not be different between the experimental groups ($p > 0.05$; Fig. 1B and C, respectively).

Therefore, the results suggest a possible anxiolytic effect demonstrated by the preference of the animals to the open arms, when 7-epiclusianone was administered in the dose 10 mg/kg compared to the control group. The elevated plus maze if bases on the permanence time and in the total of entrances in the open arms. The increase of these parameters suggests a possible anxiolytic effect. Naturally, the animals prefer the closed arms of the elevated plus maze, due to the aversion to open places (Carobrez and Bertoglio, 2005). The others analyzed parameters did not be changed, however the importance of the permanence in the open arms is enough to propose a possible effect anxiolytic.

In conclusion, the results suggest a possible anxiolytic-like effect presented by the 7-epiclusianone and suggest its potential for the treatment of anxiety.

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.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Carlini, E.A., 2003. Plants and the central nervous system. Pharmacol. Biochem. Behav. 75, 501–512.
Carobrez, A.P., Bertoglio, L.J., 2005. Ethological and temporal analyses of anxiety-like behavior: the elevated pluz-maze model 20 years on. Neurosci. Biobehav. Rev. 29, 1193–1205.
Cavalli, J., Bertoglio, J.L., Carobrez, A.P., 2009. Pentylenetetrazole as an unconditioned stimulus for olfactory and contextual fear conditioning in rats. Neurobiol. Learn. Mem. 92, 512–518.
de Abreu Matos, F.J., 2002. Farmácias Vivas: sistema de utilização de plantas medicinais projetado para pequenas comunidades, 4. ed. rev. ampliada./F.J. de Abreu Matos. Editora UFC, Fortaleza.
Figueiredo, S.A., (Dissertação Mestrado em Ciências Farmacêuticas) 2013. Avaliação *in vitro* e *in vivo* do potencial fotoprotetor e/ou fotoquimioprotetor do extrato etanólico do epicarpo de *Garcinia brasiliensis*. Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo.
Gelder, M., Mayou, R., Geddes, J., 2002. Psiquiatria, 2nd ed, Rio de Janeiro.
Gomes, P.B., Noronha, E.C., De Melo, C.T., Bezerra, J.N., Neto, M.A., Lino, C.S., Vasconcelos, S.M., Viana, G.S., De Sousa, F.C., 2008. Central effects of isolated fractions from the root of *Petiveria alliacea* L. (tipi) in mice. J. Ethnopharmacol. 120, 209–214.
Margis, R., Picon, P., Cosner, A.F., Silveira, R.O., 2003. Relação entre estressores, estresse e ansiedade. Rev. Psiquiatr. RS. 25 (Suppl. 1), 65–74.
Murata, R.M., Yatsuda, R., Dos Santos, M.H., Kohn, L.K., Martins, F.T., Nagem, T.J., Alencar, S.M., De Carvalho, J.E., Rosalen, P.L., 2010. Antiproliferative effect of benzophenones and their influence on cathepsin activity. Phytother. Res. 24, 379–383.
Naldoni, F.J., Claudina, A.L., Cruz, J.W.J.R., Chavasco, J.K., Faria, E., Silva, P.M., Veloso, M.P., Dos Santos, M.H., 2009. Antimicrobial activity of benzophenones and extracts from the fruits of *Garcinia brasiliensis*. J. Med. Food 12, 403–407.
Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open, closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.
Pereira, I.O., Marques, M.J., Pavan, A.L., Codonho, B.S., Barbiéri, C.L., Beijo, L.A., Doriguetto, A.C., D'Martin, E.C., Dos Santos, M.H., 2010. Leishmanicidal activity of benzophenones and extract a from *Garcinia brasilienses* Mart. fruits. Phytomedicine 17, 339–345.
Ramadam, Z.B.A., AssumpçãoF F.B. JR., 2005. Psiquiatria da magia à evidência? Manole, São Paulo.
Santa-Cecília, F.V., Freitas, L.A., Vilela, F.C., Veloso, C.C., Da Rocha, C.Q., Moreira, M.E., Dias, D.F., Giusti-Paiva, A., Dos Santos, M.H., 2011. Antinociceptive and anti-inflammatory properties of 7-epiclusianone, a prenylated benzophenone from *Garcinia brasiliensis*. Eur. J. Pharmacol. 670, 280–285.
Santa-Cecília, F.V., Abreu, F.A., Da Silva, M.A., De Castro, E.M., Dos Santos, M.H., 2013. Estudo farmacobotânico das folhas de *Garcinia brasiliensis* Mart.(Clusiaceae). Rev. Bras. Pl. Med. 15, 397–404.
Valle, F.P., 1970. Effects of stain, sex and illumination on open-field behavior of rats. Am. J. Psychol. 83, 103–111.
Vilela, F.C., Soncini, R., Giusti-Paiva, A., 2009. Anxiolytic-like effect of *Sonchus oleraceus* L. in mice. J. Ethnopharmacol. 124, 325–327.