

RESEARCH ARTICLE

Superoxide dismutase and catalase activities in rat hippocampus pretreated with garcinielliptone FC from *Platonia insignis*

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Abstract

Context: *Platonia insignis* Mart. (Clusiaceae), commonly known as “bacuri,” is a timber and fruit native species of the Brazilian Amazon. Some plants of the Clusiaceae family have their pharmacological properties associated with the presence of xanthone and polycyclic polyprenylated acylphloroglucinols derivatives, which have antioxidant and anticarcinogenic activities.

Objective: The aim of this study was to assess the *in vivo* potential of extracts, fractions, and garcinielliptone FC isolated from *Platonia insignis* seeds as a natural antioxidant.

Materials and methods: Male Wistar rats (250–280 g; 2 months old) were treated with Tween 80 0.05% dissolved in 0.9% saline (i.p., vehicle – control group), ethanol extract (EE), hexane extract (HE), dichloromethane fraction (DMF), ethyl acetate fraction (EAF), and garcinielliptone FC (GFC) isolated from *P. insignis* at doses 2 mg/kg (i.p.). All groups were observed for 24 h after the treatment. The antioxidant enzymatic activities [superoxide dismutase (SOD) and catalase (CAT)] were measured using spectrophotometric methods.

Results: There were no marked alterations in SOD and CAT activities in rat hippocampus after pretreatment with EE, HE, DMF, EAF, and GFC. However, the pretreatment with GFC induced a significantly increase of 13, 17, 19, and 13% in SOD activities when compared to EE, HE, DMF, or EAF groups, respectively.

Discussion and conclusion: Our findings strongly support the hypothesis that GFC isolated from *P. insignis* has a significant potential to be used as a natural antioxidant agent probably due to the modulation of enzymatic activity of hippocampal SOD.

Keywords: Catalase, superoxide dismutase, hippocampus, mice

Introduction

Endogenous antioxidant mechanisms inhibit or delay specific substrate oxidation by reactive oxygen species (ROS) and are important in preventing many diseases (Halliwell et al., 1992; Ferreira et al., 2008; Freitas et al., 2010). Their effectiveness can be improved by exogenous antioxidants

of natural and synthetic origin that are present in the diet. Phenolics, also known as high and low molecular weight polyphenols, represent the main class of natural antioxidants. These bioactive compounds participate in defense against oxidative damage associated with their scavenging of harmful reactive species (Mujic et al., 2011).

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The Clusiaceae family includes 40 genera, divided into 1200 species, distributed in tropical regions of the world. Several species are well known for their hulls manufacture due to the quality of their wood and for the healing properties of their latex used traditionally against dermatoses (Marti et al., 2009). This family is known to be a rich source of polycyclic polyprenylated acylphloroglucinols (PPAPs) with a large spectrum of biological activities (Cuesta-Rubio et al., 2005; Marti et al., 2009). Besides, some plants of Clusiaceae family have their pharmacological properties associated with the presence of xanthone derivatives, which has antioxidant and anticarcinogenic activities (Sundaram et al., 1983; Chen et al., 1996; Iinuma et al., 1996; Suksamrarn et al., 2003; Ee et al., 2008; Chomnawang et al., 2009; Hung et al., 2009).

Among the Clusiaceae family, *Platonia insignis* Mart., commonly known as “bacuri,” is a timber and fruit native species of the Brazilian Amazon (Sundaram et al., 1983; Chen et al., 1996; Iinuma et al., 1996; Suksamrarn et al., 2003; Ee et al., 2008; Chomnawang et al., 2009; Hung et al., 2009). The bacuri fruit has a thick-skin, and is 7–15 cm long and 5–15 cm in diameter, weighing 200–1000 g, containing a large quantity of resins. The pulp wrapping the seeds is white and bitter, with a pleasant smell and taste. The *P. insignis* fruit can be consumed raw or as juice, ice-cream, or jam (Alves & Jennings, 1979). While, the *P. insignis* seed oil has been used to treat various skin diseases in both men and animals, the seed decoction is used in human diarrhea treatment (Agra et al., 2007). In the search for biologically active constituents in Clusiaceae plants, we investigated bioactive constituents of the seeds of *P. insignis* and reported a novel tautomeric pair PPAPs, named garcinielliptone FC (Figure 1), isolated from the seeds of *P. insignis*, were identified by extensive analysis of spectroscopic (Costa Júnior et al., 2011). It was reported that phloroglucinols, especially PPAPs, are common constituents of members of the family Clusiaceae (Ciochina & Grossman, 2006).

Since previous investigations in our laboratory (Costa Júnior et al., 2010) demonstrated an antioxidant and

anticonvulsant effects of *P. insignis*, we investigated the effect of acute administration of *P. insignis* on oxidative free radical scavenging enzymes (superoxide dismutase, SOD; catalase, CAT) in order to assess the role of a possible antioxidant effect in the cognition-facilitating action of this medicinal plant.

The antioxidant effects ethanol extract (EE), hexane extract (HE), dichloromethane fraction (DMF), acetate ethyl fraction (EAF), and garcinielliptone FC (GFC) isolated from the seeds of *P. insignis* were assessed in *in vivo* tests.

Methods

The protocols for the animals experiments described in this study were performed in accordance with international (EEC Directive of 1986, 86/609/EEC) and national rules and institutional guidelines as prescribed by the ethical committee for animal experiments of the Federal University of Piauí (UFPI).

Plant material and chemistry study

The *P. insignis* fruits were collected at Barras, Piauí State, Brazil, in March 2009. A voucher specimen has been identified by botanist Roseli Farias de Melo Barros and deposited at the “Graziela Barroso,” Herbarium of Biology Department of Federal University of Piauí, Brazil (Voucher No.: ICN TEPB27164).

The seeds were dried at 55°C and powdered. Crushed seeds (848.2 g) were extracted with hexane (63%, w/w), followed by ethanol (EtOH) (5.8%, w/w) in a Soxhlet apparatus (8 h for each solvent). The ethanol extract (EtOH) was added to 100 mL of water and then partitioned with solvents of increasing polarity. The EtOH extract was fractionated with dichloromethane (8 × 100 mL) to obtain DMF (3.4%, w/w), acetate ethyl (7 × 100 mL) to obtain EAF (0.4%, w/w).

The new PPAP compounds, the known compound GFC, were isolated from the HE of *P. insignis*. GFC: yellow oil; EIMS m/z (%): 602 [M]⁺ (1), 465 (6), 341 (8), 231 (10), 177 (3), 137 (20), 109 (11), 69 (100). Their structure and molecular formula (C₃₈H₅₀O₆; m/z 603.3) was confirmed by ESI(+)/MSⁿ and ¹H NMR and ¹³C NMR data (Costa Júnior et al., 2011). In the present work, the GFC was suspended in 0.05% Tween 80 distilled in 0.9% saline, and sonicated before use.

Animals and treatment protocols

Adult male Wistar rats (250–280 g; 2 months old) were maintained in a temperature controlled room (26 ± 1°C) with a 12 h light/dark cycle and food and water *ad libitum* (Nutrilabor, Campinas, Brazil). Animal care followed the official governmental guidelines in compliance with the Society Policy and was submitted by the Ethics Committee of the Federal University of Piauí, Brazil. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All doses are expressed in milligrams

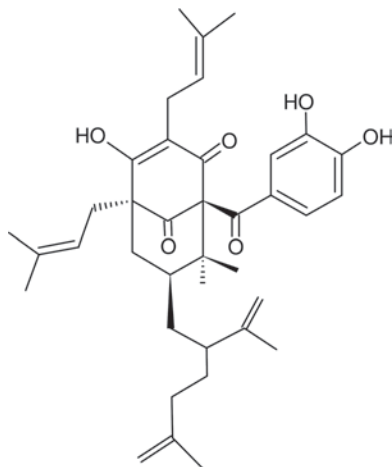


Figure 1. Structure chemistry of GFC isolated from the seeds of *P. insignis*.

per kilogram and were administered in a volume of 10 mL/kg injected intraperitoneally (i.p.).

A total of 57 rats were treated with either 2 mg/kg of EE, HE, DMF, EAF, or GFC from *P. insignis* (i.p.) or vehicle (saline/Tween 80 0.05% dissolved in 0.9% saline, i.p.): group 1, vehicle treatment serves as control ($n=12$); group 2, DMF 2 mg/kg ($n=9$); group 3, EAF 2 mg/kg ($n=9$); group 4, EE 2 mg/kg ($n=9$); group 5, HE 2 mg/kg ($n=9$); and group 6, GFC 2 mg/kg ($n=9$). After the treatment, the animals were recorded in 30 cm $\times$ 30 cm chambers to behavioral studies for 24 h. At the end of observations, the survivors were sacrificed by decapitation and their brains were dissected on ice to remove hippocampus for SOD and CAT determinations.

The drug dosages of EE (Costa Júnior et al., 2010) and fractions (Costa Júnior et al., 2011) were determined by previous studies in our laboratory. The drug doses used in this present study are not equivalent to those used by humans because rats have different metabolic rates.

Behavioral effects on adult rats pretreated with GFC and extracts from *P. insignis*

Behavioral screening of the rats was performed following parameters described by Almeida and collaborators (1999) and animals were observed at 24 h after i.p.

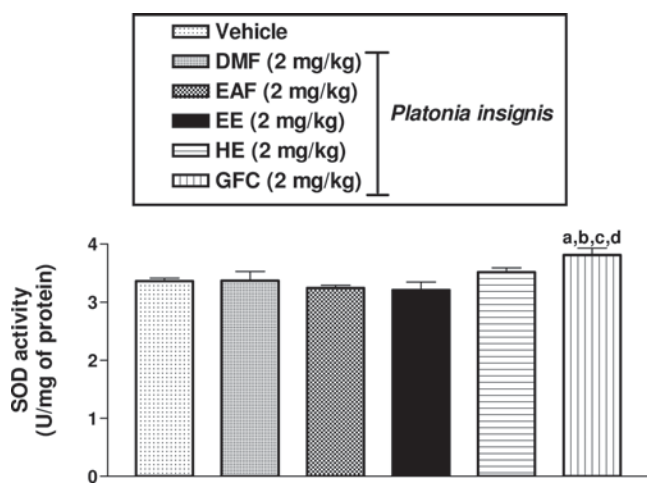


Figure 2. Effects of DMF, EAF, EE, HE, and GFC on SOD activities in rat hippocampus. Male rats (250–280 g; 2 months old) were treated with a single dose of DMF (2 mg/kg, intraperitoneal, i.p., $n=9$), EAF (2 mg/kg, intraperitoneal, i.p., $n=9$), EE (2 mg/kg, intraperitoneal, i.p., $n=9$), HE (2 mg/kg, intraperitoneal, i.p., $n=9$), GFC (2 mg/kg, intraperitoneal, i.p., $n=9$), and the vehicle (Tween 80 0.05% dissolved in 0.9% saline) (i.p., $n=12$, Control). Results are expressed as means $\pm$ S.E.M. for the number of animals shown above in parenthesis. Results were expressed as U/mg of protein. The Student-Newman-Keuls t -test was used for multiple comparisons of means of two groups of data. Differences in experimental groups were determined by two-tailed analysis of variance; $^a p < 0.05$, when compared to vehicle group (ANOVA and Student-Newman-Keuls as *post hoc t*-test); $^b p < 0.05$, when compared to DMF group (ANOVA and Student-Newman-Keuls as *post hoc t*-test); $^c p < 0.05$, when compared to EAF group (ANOVA and Student-Newman-Keuls as *post hoc t*-test); $^d p < 0.05$, when compared to EE group (ANOVA and Student-Newman-Keuls as *post hoc t*-test).

administration of EE, HE, DMF, EAF, or GFC from *P. insignis* (2 mg/kg, i.p.). During the 24 h, they were observed for the occurrence of general signs of toxicity: piloerection, prostration, writhing, increased evacuation, grooming, dyspnea, sedation, analgesia and palpebral ptosis.

SOD and CAT activity determinations in the hippocampus of adult rats pretreated with GFC, fractions and extracts from *P. insignis*

The SOD and CAT activity determinations were estimated as previously described by Santos and collaborators (2008). The hippocampus was ultrasonically homogenized in 1 mL 0.05 M sodium phosphate buffer, pH = 7.0. Protein concentration was measured by the method of Lowry and collaborators (1951). The 10% homogenates were centrifuged (800 $\times g$, 20 min) and supernatants were used to assay SOD and CAT. The SOD activities in the EE (2 mg/kg; $n=9$), HE (2 mg/kg; $n=9$), DMF (2 mg/kg; $n=9$), EAF (2 mg/kg; $n=9$), GFC (2 mg/kg; $n=9$), and control ($n=12$) groups were assayed by using xanthine and xanthine oxidase to generate superoxide radicals (Flohe & Otting, 1984), and the results expressed as U/mg of protein.

The CAT activities were measured in the EE (2 mg/kg; $n=9$), HE (2 mg/kg; $n=9$), DMF (2 mg/kg; $n=9$), EAF (2 mg/kg; $n=9$), GFC (2 mg/kg; $n=9$), and control ($n=12$) groups by the method that uses H_2O_2 to generate H_2O and O_2 (Chance & Maehly, 1955). Results are expressed as mmol/min/mg of protein.

Statistical analysis

Results of SOD and CAT activity determinations were compared by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls t -test ($p < 0.05$) (Graphpad program Intuitive, Software for Science, San Diego, CA, USA).

Results

SOD activities in rat hippocampus pretreated with GFC, fractions, and extracts from *P. insignis*

The SOD activities in rat hippocampus were not markedly altered in of EE, HE, DMF, EAF groups in comparison with the vehicle group ($p > 0.05$). On the other hand, there was a significant increase in SOD (13, 17, 19, and 13%) ($p < 0.001$) activity of rats pretreated with GFC (2 mg/kg) in comparison with vehicle, DMF, EAF, and EE groups, respectively (Figure 2).

CAT activities in rat hippocampus pretreated with GFC, fractions, and extracts from *P. insignis*

The CAT ($p > 0.05$) activities in rat hippocampus were not markedly altered in of EE, HE, DMF, EAF, and GFC groups in comparison with the vehicle group ($p > 0.05$). Correspondingly, there was no significant change in CAT activities of animals pretreated with extracts and fractions isolated from *P. insignis* ($p > 0.05$) (Figure 3).

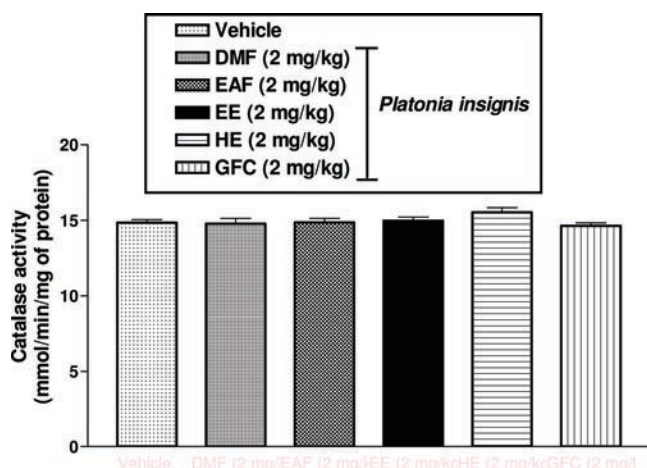


Figure 3. Effects of DMF, EAF, EE, HE, and GFC on CAT activities in rat hippocampus. Male rats (250–280g; 2 months old) were treated with a single dose of DMF (2 mg/kg, intraperitoneal, i.p., $n=9$), EAF (2 mg/kg, intraperitoneal, i.p., $n=9$), EE (2 mg/kg, intraperitoneal, i.p., $n=9$), HE (2 mg/kg, intraperitoneal, i.p., $n=9$), GFC (2 mg/kg, intraperitoneal, i.p., $n=9$), and the vehicle (Tween 80 0.05% dissolved in 0.9% saline) (i.p., $n=12$, Control). Results are expressed as means $\pm$ S.E.M. for the number of animals shown inside in parenthesis. Results were expressed as mmol/min/mg of protein. The Student–Newman–Keuls t -test was used for multiple comparisons of means of two groups of data. Differences in experimental groups were determined by two-tailed analysis of variance.

Discussion

In present study, we investigated the effects of GFC, fractions, and extracts from *P. insignis* in the antioxidant enzymatic activities within hippocampus rats. The rats treated with the substances isolated from *P. insignis* presented behavioral alterations, such as increased ambulation, palpebral ptosis, and stimulation. These behavioral changes suggest a possible effect on CNS.

Recently, several research groups reported that PPAPs-type diterpenoids have shown antioxidant activity and reduction of ROS, and protection of subsequent cell death (Ciocchina & Grossman, 2006; Lin et al., 2009). Our present study demonstrated that GFC isolated from *P. insignis* displays *in vitro* antioxidant effects (Costa Júnior et al., 2011). In addition, in the present study, we investigated the influence of DMF, EAF, EE, or HE on antioxidant enzymatic activities of SOD and CAT in rat hippocampus. Our data shows a possible antioxidant effect of GFC through the scavenging of radical O_2^- . This consequent scavenging of O_2^- produces a decrease in the H_2O_2 levels generated by superoxide dismutation in hippocampus, causing increase of the SOD activity, suggesting this can be the antioxidant action mechanism of the GFC. Free radical formation elevations are frequently accompanied by an immediate compensatory increase in the activity of free radical scavenging enzymes (Tomé et al., 2010a).

It is well known that free radicals produce several diseases, such as Parkinson disease, Alzheimer type

dementia, seizures, and others (Reuter et al., 2010; Tomé et al., 2010b). The production of free radicals and the activity of the scavenger enzymes against those radicals such as SOD are correlated with the life expectancies. We have demonstrated that GFC isolated from *P. insignis* seeds were capable of inhibiting and quenching free radicals, acting as reducing agents. Furthermore, phenolic compounds present in the plant kingdom are mainly responsible for the antioxidant potential of plants (Larson, 1988).

The GFC demonstrated a significant scavenging effect on hydroxyl radicals and NO generation *in vitro* (Costa Júnior et al., 2011). Our findings showed that GFC, DMF, EAF, EE, or HE did not produce alterations in the hippocampus CAT activity, since they did not protect the hippocampus from neuronal damage induced by lipid peroxidation products. It is unlikely that the unaltered CAT activity is related to the mechanisms involved in maintenance of oxidative stress (Freitas et al., 2005). These data suggest that hippocampus of rats pretreated with GFC, fractions, and extracts from *P. insignis* do not use CAT as the major free radical scavenging system.

Conclusions

Accordingly in this study, a significant relationship was found between the antioxidant activity and phenolic compounds, indicating that phenolic compounds could be major contributors to the antioxidant activity. Although the antioxidant activities found *in vivo* analyses were only indicative of the potential health benefit, these results remain important as the first step in screening antioxidant activity of *P. insignis* seeds. Thus, it can be concluded that GFC isolated from *P. insignis* seeds can be used as an accessible source of natural antioxidants.

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Declaration of interest

The authors declare that there are no conflicts of interest.

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