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Acute and subchronic toxicological evaluation of the semipurified extract of seeds of guaraná (*Paullinia cupana*) in rodents

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ABSTRACT

We evaluated the toxicity of a semipurified extract (EPA fraction, containing caffeine and several flavan-3-ols and proanthocyanidins) of seeds of the native Amazon plant *Paullinia cupana* (guaraná) in rodents. Acute toxicity was tested in male Swiss mice, which received different doses orally (OR) and intraperitoneally (ip); control groups received water. These tests produced acute mortality, with LD_{50} of 1.825 g/kg (OR) and 0.827 g/kg (ip), and a significant weight decrease in lungs of mice receiving a dose of 0.1 g/kg. In the repeated-dose toxicity test, the EPA was administered OR daily to male and female Wistar rats at doses of 30, 150, and 300 mg/kg/day/90 days. Their behavior, mortality, weight changes, laboratory tests, and the weights and histopathology of organs were evaluated. No rats died during the tests. Males dosed at 150 or 300 mg/kg gained weight more slowly and lost kidney weight (absolute and relative weights, compared to the control group). Hematological and biochemical tests showed few changes, differing somewhat between males and females; the histopathological evaluation indicated no significant changes. These results indicate that the EPA fraction of guaraná caused no toxicity in rats at the smallest dose evaluated (30 mg/kg). No other species was evaluated.

1. Introduction

Guaraná, also known by the native Americans as "uaraná", grows naturally in the Maués region of the Amazon basin. Guaraná seeds are widely used in pharmaceuticals and foods as a tonic, a stimulant of the central nervous system, and an ingredient of beverages (Henman, 1982; Angelo et al., 2008).

Pharmacological studies have shown, in addition to the known stimulant effects, antioxidant properties *in vitro* (Mattei et al., 1998; Yamaguti-Sasaki et al., 2007) and augmentation of learning and memory (Espínola et al., 1997; Otobone et al., 2005). However, similar beneficial effects on cognition in healthy human volunteers were not observed (Galduróz and Carlini, 1994).

Mattei et al. (1998) evaluated young and old rats in several protocols, such as various signs after chronic administration (urinary settling frequency, piloerection, motor activity, etc.), time of sleep induced by pentobarbital, weight change after 12 months of treatment, mortality, and histopathology parameters; and found no significant changes from the control groups. *In vitro* studies have shown that guaraná has genotoxic and mutagenic effects in rats (Santamaria et al., 1998; Fonseca et al., 1994).

Guaraná is currently listed in the official Brazilian Pharmacopoeia (Farmacopéia Brasileira, 2000). The pharmaceutical industry uses the seeds in the production of medicines, and the food industry uses them mainly in soft drinks and food supplements (Ortega et al., 1989). From the semipurified fraction (EPA), several compounds have been isolated and identified, including caffeine, catechin, epicatechin, and procyanidins B2, B3, and B4 (Antonelli-Ushirobira et al., 2007). In addition to these compounds, Yamaguti-Sasaki et al. (2007) isolated and identified *ent*-epicatechin and procyanidins B1, A2, and C1.

Recently, our research group showed that the crude extract (EBPC) and semipurified fraction (EPA) of guaraná cause antidepressant effects in animal models after prolonged administration (Otobone et al., 2007). These observations led to the filing of a patent application and to tests of the acute and subchronic toxicity of the semipurified extract, including analyses of the hematology, biochemistry, and histopathology of various animal organs.

2. Materials and methods

2.1. Preparation of the extract

An extract was prepared from guaraná seeds (1000 g) with acetone:water (7:3; v/v) extractor liquid, and after the organic solvent was removed, the remaining solid material was lyophilized (EBPC – patent pending PI0006638-9). The semipurified,

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lyophilized extracts were obtained from the EBPC: 158 g of the lyophilized extract was partitioned with ethyl acetate (10×, 5 L), resulting in an ethyl-acetate fraction (EPA: 44 g) (patent pending PI0006638-9). The EPA was solubilized in distilled water immediately before administration.

2.2. Animals

Forty-eight Wistar rats of each sex and 100 male Swiss mice were obtained from the Main Animal Facility of the Universidade Estadual de Maringá (UEM), and kept under controlled temperature (22 ± 2 °C) and a 12 h light/dark cycle controlled via an automatic timer. A pelleted diet (Nuvital® for laboratory rats and mice, Nuvilab Ltd., Curitiba, PR, Brazil) and tap water were provided *ad libitum*. The UEM Animal Ethics Committee approved the experimental procedure (protocol No. 039/2003).

2.3. Acute toxicological evaluation (LD_{50})

The test procedures were carried out in accordance with the "Guia para a realização de estudos de toxicidade pré-clínica de fitoterápicos" (Brasil, 2004). The mice were divided into 10 experimental groups of 10 animals each, to form two treatment groups and two control groups. The treatment groups received a single oral dose (OR, gavage) (5.0, 2.5, or 1.0 g/kg; acute administration) of EPA, and also intraperitoneally (ip) in doses of 2.5, 1.5, 1.0, 0.5, or 0.1 g/kg. The two control groups received water (OR and ip). The animals were observed immediately after the administration and then daily for 14 days, and were weighed on the 1st, 7th, and 14th day. At the end of the experiment, they were euthanized, and the kidneys, heart, liver, and lungs were removed and weighed, and evaluated for possible macroscopic changes or abnormalities. The ip route for administration was selected according to the Brazilian regulation for this test (Brasil, 2004). Large numbers of animals were used in the acute study for DL_{50}. For this study, mice were used because a Brazilian regulation requires only behavioral evaluations, and not biochemical and hematological evaluations for the acute test (Brasil, 2004). We therefore used mice rather than rats, as have several other investigators (Rebecca et al., 2002; Li et al., 2010). Through this means, the approval by the Animal Ethics Committee was facilitated, in addition to the economic considerations.

2.4. 90-Day subchronic study

Male and female rats were weighed and divided into groups by sex. The mean weights of males and females are listed in Table 1. They were divided into a control group and three test groups of 12 animals each, and maintained in groups of 4 in labeled polypropylene cages. Each animal received daily, for a period of 90 days, administration of water (control group) or EPA at doses of 30, 150, and 300 mg/kg (OR, gavage). The behavior of the animals was observed daily, and they were weighed weekly. After 60 days of treatment, the animals were submitted to the open-field test and were gently manipulated in order to collect urine for physical, chemical, and sedimentoscopic tests. At the end of the experiment (90 days), the animals were euthanized, and their blood was collected for hematological and biochemical analyses.

2.5. Open-field test

The open-field test followed the method described by Carlini et al. (1986).

Table 1
90-Day subchronic study: body-weight changes of male rats treated with EPA.[a]

Dose (mg/kg/day)	Control	30	150	300
1	247.00 ± 25.0	246.67 ± 18.4	246.45 ± 18.6	247.89 ± 19.2
7	306.49 ± 23.5	300.48 ± 18.5	292.31 ± 29.2	291.12 ± 26.2
14	324.83 ± 27.0	319.09 ± 19.6	289.36 ± 78.4	306.31 ± 26.7
21	346.19 ± 28.6	340.75 ± 23.2	318.75 ± 29.9*	318.45 ± 24.8*
28	355.57 ± 29.2	351.40 ± 22.3	344.79 ± 22.6	331.87 ± 24.5*
35	368.91 ± 28.9	365.91 ± 21.8	347.12 ± 28.3	337.22 ± 36.9*
42	384.12 ± 29.2	374.91 ± 27.2	357.29 ± 29.7*	353.00 ± 23.8*
49	392.71 ± 30.8	385.75 ± 26.9	366.91 ± 26.5*	361.56 ± 24.8*
56	393.05 ± 29.6	390.43 ± 26.7	371.46 ± 29.3*	365.06 ± 25.9*
63	404.58 ± 32.1	400.91 ± 25.2	376.35 ± 31.4*	370.29 ± 27.4*
70	399.19 ± 31.7	406.55 ± 25.8	381.69 ± 33.4	363.82 ± 30.3*
77	406.38 ± 30.3	404.81 ± 30.1	383.83 ± 30.2	366.47 ± 34.4*
84	413.19 ± 30.4	411.40 ± 28.8	391.97 ± 31.0	377.61 ± 34.4*
90	419.74 ± 30.2	415.59 ± 30.3	394.48 ± 30.0*	379.66 ± 35.3*

* Indicates significant difference at $P < 0.05$ level compared to the control group.
[a] Values are presented as means ± SD (g).

2.6. Laboratory tests

Laboratory tests for urine (physical, chemical, and sedimentoscopy) and blood (blood glucose; total cholesterol; triglycerides; urea; uric acid; creatinine; glutamic-pyruvic transaminase (GPT); direct, indirect, and total bilirubin; albumin; amylase; and alkaline phosphatase) were performed.

One blood sample was placed in test tubes containing EDTA as an anticoagulant, and another in a dry tube for separation of serum. The biochemical tests were performed with commercial kits (Labtest and Biodiagnóstica) through enzymatic–colorimetric, colorimetric, or kinetic reactions. The blood was placed in an automated counter (Cell-Dyn, Abbott Laboratories), and a differential count was performed after the smear was stained with Leishman dye. The partial examination of urine used reactive strips (Multistix, Bayer Diagnostics), and the positive reactions on the strips were confirmed by qualitative biochemical reactions. Bacterioscopy was performed by Gram-staining of the sediment obtained after centrifugation.

The blood collection and the laboratory tests followed a sealed numerical protocol. This effectively blinded the biochemical work, so that the technicians had no prior knowledge of which group was being evaluated.

2.7. Histopathological analysis

Histopathological analysis was performed on 10 randomly selected rats in each experimental group, totaling 80 animals. The animals were dissected using the routine described in the literature (Mikel, 1994). Heart, kidneys, liver, and lungs were harvested for analysis. The tissues were preserved in 10% neutral-buffered formalin, embedded in paraffin, sectioned at approximately 5 mm, stained with hematoxylin and eosin, and examined with an optical microscope.

2.8. Statistical analysis

The statistical analysis compared the treated groups with the negative control group, using the STATISTICA program (version 5.0). The data are presented as mean ± SD. We applied the one-way analysis of variance (ANOVA) to the toxicological tests (acute and subchronic), followed by Duncan's test. The frequencies of histopathological events were evaluated by Fisher's exact test. The level of significance accepted was $P < 0.05$ for all experiments. The LD_{50} was assessed by the method proposed by Miller and Tainter (1944).

2.9. Legal regulation

The parameters set for this study followed those established by ANVISA (National Health Surveillance Agency, Brazil) Resolução RE No. 90 (Brasil, 2004).

3. Results

3.1. Acute toxicological evaluation

The acute tests with EPA produced mortality in 50% of the population at doses of 5.0 and 2.5 g/kg (OR) and 2.5, 1.5, and 1.0 g/kg (ip); the LD_{50} was 1.769 ± 0.242 g/kg OR and 0.593 ± 0.097 g/kg ip. No changes in the behavior of the surviving animals were observed over the 14-day duration of the experiment.

The animals showed no significant differences in body weight between the control and treated groups. Similarly, the macroscopic analyses of the organs revealed no changes. The only significant change observed in the organ weights was a decrease of weight of the lungs of mice treated with a dose of 0.1 g/kg.

3.2. 90-Day subchronic study

3.2.1. Evolution of animal weight and organ weights

No animals died during the 90 days of treatment. There was, however, a decrease in body weight of males treated with 150 and 300 mg/kg of EPA from day 21 to day 90 (Table 1), compared to the control group. The weights of females did not change significantly. As mentioned above, the animals were fed *ad libitum*; all the groups consumed substantial amounts of feed, although these amounts were not measured.

A decrease in right and left kidney weight in males at the doses of 150 and 300 mg/kg was demonstrated for both the absolute and relative weights, compared to the control group. The data for absolute weight were 150 (1.18 ± 0.10; 1.19 ± 0.09) and 300

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(1.18 ± 0.08; 1.20 ± 0.08) mg/kg, and for relative weight were 150 (0.30 ± 0.01; 0.30 ± 0.02) and 300 (0.31 ± 0.03; 0.31 ± 0.03) mg/kg. Males treated with a dose of 150 mg/kg also showed a decrease in absolute and relative weight of the lungs (1.91 ± 0.32; 0.48 ± 0.08, respectively). Females, however, showed no significant changes in any of the organs evaluated.

3.2.2. Open-field test

The results showed a significant increase in movement and rearing of males treated with doses of 150 and 300 mg/kg. All the groups of females treated with EPA also showed significant increases in movement, but only the dose of 150 mg/kg produced a significant increase in rearing ($P \leqslant 0.05$) (data not shown).

3.2.3. Laboratory tests

The urinary parameters examined did not differ significantly between the treated and control groups (data not shown).

Blood leukocytes were significantly reduced in both males and females at a dose of 150 mg/kg (5.8 ± 1.3; 5.2 ± 0.7, respectively), and in males only at a dose of 300 mg/kg (6.1 ± 0.6), compared with the control group. The biochemical parameters of blood showed several slight but significant changes in both males and females at all three doses, compared with the control group.

The males showed increases levels of alkaline phosphatase (143.8 ± 32.9; 163.1 ± 54.5; 173.9 ± 30.4) and glutamic-pyruvic transaminase (GPT) (50.6 ± 3.7; 52.8 ± 10.6; 49.5 ± 8.8) in all the treated groups. In females, only those treated with 150 mg/kg (238.9 ± 58.8) showed changes in the levels of alkaline phosphatase, which increased in relation to the control group.

All the groups of treated females showed changes in the biochemical values in the tests for urea (62.7 ± 9.1; 61.8 ± 2.7; 63.3 ± 7.2), glycemia (59.7 ± 6.9; 65.3 ± 15.2; 68.2 ± 12.8), and triglycerides (139.9 ± 26.8; 113.3 ± 21.6; 105.6 ± 24.2); and those treated with the doses of 150 mg/kg (134.5 ± 10.2) and 300 mg/kg (138.4 ± 12.7) showed changes in the levels of amylase. These levels were increased in relation to the control group, except for triglycerides which decreased.

3.2.4. Histopathological analyses

Macroscopic analysis of the lungs did not reveal any abnormality that would justify their microscopic evaluation. The histopathological analyses of liver and kidneys showed no histological changes indicating abnormalities.

4. Discussion

This study investigated the possibility of subchronic toxicity of a semipurified extract from guaraná seed (EPA). In the acute toxicity test (LD_{50}), although a group of animals treated with a dose of 0.1 g/kg showed a decrease in weight of the lungs, this did not compromise their development. This decrease probably resulted from biological variability occurring in this group, since no decrease in lung weight was observed at higher doses, and there were no changes in their body weight or behavior.

Subchronic toxicity studies have provided information on health risks, when a drug is administered, especially orally, for shorter or longer periods (30 or 90 days) (Brasil, 2004). The repeated-dose toxicity test conducted on animals for 90 days of treatment with EPA, provided results for the target organs and the effects of the cumulative-fraction test. For the 90-day subchronic treatment test, provisions must be made for a series of laboratory examinations that require a sufficient volume of blood to perform. In this case, rats, because they have a body volume nearly 10 times larger than mice, better satisfy this requirement.

The increase or decrease in body weight of an animal may indicate important physiological changes, such as liver or hormonal, or failure to absorb components such as proteins, amino acids, and others. Females showed little change in weight, and this factor was not statistically significant nor did it affect the development of the organs. Male rats, however, showed a significant decrease in body weight, which was reflected in the weight of some organs at the end of the treatment. These data may indicate a possible toxicity of the product, but since these events were not equivalent in both sexes, they may also indicate a greater susceptibility of male rats. The food consumption was not determined.

In the open-field test, the locomotor activity of the animal may indicate the activity of a stimulant or sedative drug. The observed increases in movement may indicate that the animals were stimulated. This may be related to the presence of caffeine in the EPA fraction (Antonelli-Ushirobira et al., 2007; Yamaguti-Sasaki et al., 2007), since methylxanthines are associated with stimulation of the central nervous system (CNS). An interesting aspect is the lack of apparent stimulation of males by the lowest dose, 30 mg/kg, because the open-field test provided the same environmental conditions for both sexes. Therefore, the significant changes in movement by all females treated, including at the lowest dose, may be related to a greater propensity of females for stimulation of the CNS.

Mendes and Carlini (2002) conducted pre-clinical toxicology studies in male rats, where the groups received extracts of coffee at concentrations of 100, 200, and 400 mg/kg and caffeine at a dose of 20 mg/kg, for 180 days. There was no increase in movement of animals treated with caffeine in the open-field test. According to Mendes and Carlini (2002), this result may indicate that other compounds than caffeine in the coffee extract may have exerted some stimulation on the CNS. Similarly, the test performed with guaraná can be interpreted as indicating the presence of other substances in the EPA that act on the CNS. Otobone et al. (2007) showed that the crude extract (EBPC) and semipurified fraction (EPA) reduced immobility time in the forced-swim test, indicating an antidepressant activity. The presence of phenolic compounds, especially tannins, isolated and identified by Antonelli-Ushirobira et al. (2007) and Yamaguti-Sasaki et al. (2007), may be related to this activity, since these compounds are found in the largest quantity in the EPA fraction. Furthermore, the presence of caffeine by itself is insufficient to explain all the popular uses of guaraná.

In the blood of females treated with a dose of 150 mg/kg, the number of leukocytes, although statistically significant, was close to the minimum level of normal biological variation. For males, the leukocyte levels were above the normal minimum, at doses of 150 and 300 mg/kg (Wolford et al., 1986). These data, however, may indicate a tendency to leukopenia, which may signify toxicity and/or greater susceptibility of males. The other hematological levels found in the groups of males and females showed no toxicologically significant changes, being within the normal ranges.

In the biochemical tests, compared with the control group, males had altered levels of alkaline phosphatase and glutamic-pyruvic transaminase (GPT) at all doses evaluated. However, females showed changes in the levels of alkaline phosphatase only in animals treated with a dose of 150 mg/kg, and the levels of GPT showed no changes. High levels of alkaline phosphatase occur in bone disorders such as rickets, and in liver diseases such as cirrhosis and acute viral hepatitis. GPT is also present at high levels in liver diseases (Lima et al., 2001). These biochemical changes occurred in males, and although the levels remained within the normal range, they may suggest a change in the liver. However, this evidence of possible liver alteration should be considered with caution, since the histopathological analysis of the organ and also the serum levels of total bilirubin showed no significant changes from those in the control group. Fukumasu et al. (2006a,b) showed

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that guaraná protects the liver against DEN-induced DNA damage in mice.

All females treated with EPA showed changes in the levels of urea, glucose, and triglycerides. In females treated with doses of 150 and 300 mg/kg, there were changes in the levels of amylase. These values were high in relation to the control group, except for triglycerides, which decreased. Amylase can increase in certain conditions, especially in acute pancreatitis (Lima et al., 2001). The high values for amylase compared to the control group may indicate changes in the animals' intestine. However, in the dissected animals, no evidence of steatonecrosis was observed in the pancreatic tissue or the serous layer of the bowel.

The changes in blood glucose and triglyceride levels in females (Wolford et al., 1986), although statistically significant, have no biological importance. The same occurred in the males, in which glucose levels decreased at doses of 30 and 150 mg/kg, again indicating a probable greater biological susceptibility of this sex.

Physiologically, increases in blood urea are due to decreased renal function, decreased liver function, or a hyperprotein diet (Lima et al., 2001). The levels of urea in females were high compared with those reported in the literature; however, these changes were not statistically different between the groups. Even the control groups of both sexes showed increased levels compared with the literature (Wolford et al., 1986).

Renal dysfunction can be assessed by concurrent measurements of urea and creatinine. For both males and females, the creatinine levels remained normal (Wolford et al., 1986), reducing the likelihood of kidney problems. Reinforcing these data, the histopathological analysis of kidneys revealed no abnormality in the organ. Thus, the most likely hypothesis for the increase in the amounts of urea, is a decrease in liver function. The biochemical evidence of liver function showed considerable changes in both groups of animals, although these were more significant in males than in females.

The histopathological evaluation of organs did not show changes that could establish the dose–response-dependent relationship to treatment with the EPA test fraction.

Based on these results, we can conclude that after 90 days of treatment, the animals showed biochemical changes indicating that the liver is the target organ in case of possible toxicity of the EPA fraction of guaraná, especially in males, at doses of 150 and 300 mg/kg. The results also indicate a possible greater biological susceptibility of males. However, these doses are about 50 and 100 times the effective dose assessed by Otobone et al. (2005), respectively.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fct.2010.04.013.

References

Angelo, P.C., Nunes-Silva, C.G., Brígido, M.M., Azevedo, J.S.N., Assunção, E.N., Sousa, A.R.B., Patrício, F.J.B., Rego, M.M., Peixoto, J.C.C., Oliveira Jr., W.P., Freitas, D.V., Almeida, E.R.P., Viana, A.M.H.A., Souza, A.F.P.N., Andrade, E.V., Acosta, P.O.A., Batista, J.S., Walter, M.E.M.T., Leomil, L., Anjos, D.A.S., Coimbra, R.C.M., Barbosa, M.H.N., Honda, E., Pereira, S.S., Silva, A., Pereira, J.O., Silva, M.L., Marins, M., Holanda, F.J., Abreu, R.M.M., Pando, S.C., Gonçalves, J.F.C., Carvalho, M.L., Leal-Mesquita, E.R.R.B.P., Silveira, M.A., Batista, W.C., Atroch, A.L., França, S.C., Porto, J.I.R., Schneider, M.P.C., Astolfi Filho, S., 2008. Guaraná (*Paullinia cupana* var. *sorbilis*), an anciently consumed stimulant from that Amazon rain forest: the seeded-fruit transcriptome. Plant Cell Rep. 27, 117–124.

Antonelli-Ushirobira, T.M., Yamaguti, E., Uemura, L.M., Nakamura, C.V., Dias Filho, B.P., Palazzo de Mello, J.C., 2007. Chemical and microbiological study of extract from seeds of guaraná (*Paullinia cupana* var. *sorbilis*). Acta Farm. Bonaerense 26, 5–9.

Brasil, 2004. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução RE n° 90 de 16.3.2004. Guia para a realização de estudos de toxicidade pré-clínica de fitoterápicos. Diário Oficial da União.

Carlini, E.A., Contar, J.D.P., Silva-Filho, A.R., Silveira-Filho, N.G., Frochtengarten, M.L., Bueno, O.F.A., 1986. Pharmacology of lemongrass (*Cymbopogon citratus* Stapf). I. Effects of teas prepared from the leaves on laboratory animals. J. Ethnopharmacol. 17, 37–64.

Espínola, E.B., Dias, R.F., Mattei, R., Carlini, E.A., 1997. Pharmacological activity of guarana (*Paullinia cupana* Mart.) in laboratory animals. J. Ethnopharmacol. 55, 223–229.

Farmacopéia Brasileira, 2000. fourth ed. Editora Atheneu, São Paulo.

Fonseca, C.A.S., Leal, J., Costa, S.S., Leitão, A.C., 1994. Genotoxic and mutagenic effects of guaraná (*Paullinia cupana*) in prokaryotic organisms. Mutat. Res. 321, 165–173.

Fukumasu, H., Avanzo, J.L., Heidor, R., Silva, T.C., Atroch, A., Moreno, F.S., Dagli, M.L.Z., 2006a. Protective effects of guarana (*Paullinia cupana* Mart var. *sorbilis*) against DEN-induced DNA damage on mouse liver. Food Chem. Toxicol. 44, 862–867.

Fukumasu, H., Silva, T.C., Avanzo, J.L., Lima, C.E., Mackowiak, I.I., Atroch, A., Spinosa, H.S., Moreno, F.S., Dagli, M.L.Z., 2006b. Chemopreventive effects of *Paullinia cupana* Mart var. *sorbilis*, the guaraná, on mouse hepatocarcinogenesis. Cancer Lett. 233, 158–164.

Galduróz, J.C.F., Carlini, E.A., 1994. Acute effects of the *Paullinia cupana*, "Guaraná" on the cognition of normal volunteers. Rev. Paul. Med. 3, 607–611.

Henman, A.R., 1982. Guaraná (*Paullinia cupana* var. *sorbilis*): ecological and social perspectives on an economic plant of the central Amazon basin. J. Ethnopharmacol. 6, 311–338.

Li, C.-Y., Devappa, R.K., Liu, J.-X., Lv, J.M., Makkar, H.P.S., Becker, K., 2010. Toxicity of *Jatropha curcas* phorbol esters in mice. Food Chem. Toxicol. 48, 620–625.

Lima, A.O., Soares, J.B., Greco, J.B., Galizzi, J., Cançado, J.R., 2001. Métodos de laboratório aplicados à clínica. Técnica e Interpretação, Editora Guanabara Koogan. Rio de Janeiro, Brazil.

Mattei, R., Dias, R.F., Espínola, E.B., Carlini, E.A., Barros, S.B.M., 1998. Guaraná (*Paullinia cupana*): toxic behavioral effects in laboratory animals and antioxidant activity in vitro. J. Ethnopharmacol. 60, 111–116.

Mendes, F.R., Carlini, E.A., 2002. Toxicologia pré-clínica do extrato de café BST 0101: estudos iniciais em roedores. In: Simpósio de plantas medicinais do Brasil, 17 Cuiabá. Resumos.Cuiabá.

Mikel, U.V., 1994. Advanced Laboratory Methods in Histology and Pathology. Armed Forces Institute of Pathology American Registry of Pathology, Washington, DC.

Miller, L.C., Tainter, M.L., 1944. Estimations of the ED_{50} and its error by means of log-probit graphic paper. Proc. Soc. Exp. Biol. Med. 57, 261–264.

Ortega, G.G., Schenkel, E.P., Athayde, M.L., Mentz, L.A., 1989. Brasilianische Phytotherapeutika. Ihre rolle im Arzneimittelmarkt. Dtsch. Apoth. Ztg. 129, 1847–1848.

Otobone, F.J., Sanches, A.C., Nagae, R.L., Martins, J.V.C., Obici, S., Mello, J.C.P., Audi, E.A., 2005. Effect of crude extract and its semi purified constituents from guaraná seeds [*Paullinia cupara* var. *sorbilis* (Mart.) Ducke] on cognitive performance in Morris water maze in rats. Braz. Arch. Biol. Technol. 48, 723–728.

Otobone, F.J., Sanches, A.C.C., Nagae, R., Martins, J.V.C., Sela, V.R., Mello, J.C.P., Audi, E.A., 2007. Effect of lyophilized extracts from guaraná seeds [*Paullinia cupara* var. *sorbilis* (Mart.) Ducke] on behavioral profiles in rats. Phytother. Res. 21, 531–535.

Rebecca, M.A., Ishii-Iwamoto, E.L., Grespan, R., Cuman, R.K.N., Caparroz-Assef, S.M., de Mello, J.C.P., Bersani-Amado, C.A., 2002. Toxicological studies on *Stryphnodendron adstringens*. J. Ethnopharmacol. 83, 101–104.

Santamaria, A., Lopez, A., Diaz, M.M., Munoz-Mingarro, D., Pozuelo, J.M., 1998. Evaluation of the toxicity of guaraná with in vitro bioassays. Ecotoxicol. Environ. Saf. 39, 164–167.

Wolford, S.T., Schroer, R.A., Gohs, F.X., Gallo, P.P., Brodeck, M., Falk, H.B., Ruhren, R., 1986. Reference range data base for serum chemistry and hematology values in laboratory animals. J. Toxicol. Environ. Health 18, 161–188.

Yamaguti-Sasaki, E., Ito, L.A., Canteli, V.C.D., Antonelli-Ushirobira, T.M., Ueda-Nakamura, T., Dias Filho, B.P., Nakamura, C.V., Mello, J.C.P., 2007. Antioxidant capacity and in vitro prevention of dental plaque formation by extracts and condensed tannins of *Paullinia cupana*. Molecules 12, 1950–1963.

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