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RESEARCH ARTICLE

Genoprotective and hepatoprotective effects of Guarana (*Paullinia cupana* Mart. var. *sorbilis*) on CCl_4-induced liver damage in rats

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Abstract

Context: Several biological effects of *Paullinia cupana* (guarana) have been demonstrated, but little information is available on its effects on the liver. *Objective*: The current study was designed to evaluate the hepatoprotective and genoprotective effects of powder seeds from guarana on CCl_4-induced liver injury in rats. *Materials and methods*: Male Wistar rats were pretreated with guarana powder (100, 300 and 600 mg/kg) or silymarin 100 mg/kg daily for 14 days before treatment with a single dose of CCl_4 (50% CCl_4, 1 mL/kg, intraperitoneally). *Results*: The treatment with CCl_4 significantly increased the serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In addition, CCl_4 increased the DNA damage index in hepatocytes. Guarana in all concentrations was effective in decreasing the ALT and AST activities when compared with the CCl_4-treated group. The treatment with guarana decreased DNA damage index when compared with the CCl_4-treated group. In addition, the DNA damage index showed a significant positive correlation with AST and ALT. *Discussion and conclusion*: These results indicate that the guarana has hepatoprotective activity and prevents the DNA strand breakage in the CCl_4-induced liver damage in rats.

Keywords

Genoprotection, liver, medicinal plants, *Paullinia cupanna*

History

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Introduction

The liver is the largest and most complex organ in the body, and it is responsible for a large variety of exocrine and endocrine functions involving mainly synthesis, storage and metabolism. Liver injury can be caused by toxic chemicals, drugs and virus infiltration from ingestion or infection (Lee et al., 2008). The risk of liver toxicity is increased by the high exposure to environmental toxins, pesticides and frequent use of chemotherapy (Orhan et al., 2003). The lack of effective modern medications to treat acute and chronic liver injury (Subramoniam et al., 1999) has led to research into the hepatoprotective activity of numerous medicinal plants using various experimental models. Carbon tetrachloride (CCl_4) is a potent, lipid-soluble hepatotoxic agent which is widely used as a model for screening hepatoprotectors (Wang et al., 2008). The administration of CCl_4 to rats is an accepted experimental model to produce damage to the liver (Janakat & Al-Merie, 2002). Liver injuries induced by CCl_4 are mediated through the formation of reactive intermediates such as trichloromethyl radical ($CCl_3^{\bullet}$) and its derivative trichloromethyl peroxyl radical ($CCl_3OO^{\bullet}$) generated by cytochrome P-450 of liver microsomes. These free radicals are thought to react with membrane lipids leading to their peroxidation (Recknagel et al., 1989).

Paullinia cupana Mart. var. *sorbilis* (Sapindaceae), popularly called guarana, is a plant originated in Brazil that has been used for centuries as a stimulant by the Saturê-Mauê, which is an indigenous tribe living in the Amazon region (Henman, 1982). Approximately 70% of the production of guarana is used by the industry of soft and energy drinks, and the other 30% becomes guarana powder for direct consumption in capsules or dilution in water, or it serves as a raw material for the pharmaceutical and cosmetics industries (Schimpl et al., 2013). The popular motivations for consuming guarana extracts as dietary supplements are weight loss, energy boost, improvement of fitness and sexual performance, and increase of cognitive capacity (O'Dea, 2003; Oliveira et al., 2005; Ray et al., 2005). Guarana use is valued mainly for its stimulant property because of its high content of caffeine, which can be up to 6% in the seeds (Schimpl et al., 2013). Several biological effects of guarana have been

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demonstrated such as antioxidant activity (Basile et al., 2005; Mattei et al., 1998), antimicrobial effects (Da Fonseca et al., 1994; Pinheiro et al., 1987; Yamaguti-Sasaki et al., 2007), anticarcinogenic properties (Fukumasu et al., 2006; Leite et al., 2010), improvement in cognitive performance (Espínola et al., 1997; Kennedy et al., 2004), antidepressive effects (Campos et al., 2005; Otobone et al., 2007) and enhanced weight loss (Boozer et al., 2001; Opala et al., 2006). Recently, our team has reported that habitual guarana ingestion contributes positively to the prevention of various metabolic disorders in elderly (Costa Krewer et al., 2011). Although there are studies reporting different biological activities of guarana, little information is available on its effects on the liver, especially in the hepatotoxicity model induced by CCl_4. Therefore, considering the growing consumption of guarana by the population and its potential benefits, the aim of this study was to evaluate the hepatoprotective and genoprotective effects of powder seeds from guarana on CCl_4-induced liver injury in rats.

Materials and methods

Chemicals

Silymarin was purchased from the Sigma Chemicals Co. (St. Louis, MO). CCl_4 purchased from Vetec Química (Rio de Janeiro, Brazil). Griess reagent was composed of mixture of sulphanilamide 2%, N-(1-naphthyl)ethylenediamine (NED) 0.2% and orthophosphoric acid in distillated/deionized water. The reduction of nitrate to nitrite was performed with the vanadium (III) chloride (VCl_3) 0.08%. Sulphanilamime, NED, VCl_3, phosphate buffered saline (PBS) and agarose were purchased from Sigma Chemical Co. (St. Louis, MO), and orthophosphoric acid was purchased from Vetec Química (Rio de Janeiro, Brazil). Commercial kits for the measurement of albumin, creatinine, glucose, total cholesterol, urea and activities of AST and ALT were purchased from Bioclin Quibasa (Belo Horizonte-MG, Brazil).

Paullinia cupana powder

Paullinia cupana Mart. var. *sorbilis* powder seeds was supplied by EMBRAPA, Amazônia Ocidental (Agropecuary Research Brazilian Enterprise) and was conserved dry and protected from light at −20 °C until administration. The solution administered to the animals was prepared on the day of use by diluting the guarana powder seeds in water. The determination of the guarana bioactive compounds was previously performed, and it was estimated that guarana presents 12.240 mg/g caffeine, 6.733 mg/g theobromine, 4.336 mg/g total catechins and 16 mg/g of condensed tannin (Bittencourt et al., 2013).

Animals and treatments

Adult male Wistar rats (280–320 g) from Central Animal House of the Federal University of Santa Maria (UFSM) were used. The animals were housed in cages and kept on a 12 h light/dark cycle, at room temperature of 22 ± 2 °C, with free access to food and water for 1 week before and during the experiments. It was housed one animal per each cage. The present study has been approved by the Institutional Ethics Committee on Animal Use (protocol number 027/2012). The animals were randomly divided into six groups of 5–8 rats each. Group I ($n = 8$) served as normal control and received orally vehicle (water) daily for a period of 14 days. Group II ($n = 5$) served as CCl_4 control and also received orally vehicle daily for a period of 14 days. Group III ($n = 6$) served as hepatoprotective control and received silymarin (100 mg/kg) daily for a period of 14 days. Group IV ($n = 8$), group V ($n = 8$) and group VI ($n = 7$) received, orally, the guarana powder dissolved in water at doses of 100, 300 and 600 mg/kg, respectively, daily for a period of 14 days. On day 14, one hour after treatment, animals of groups II, III, IV, V and VI were injected with a single intraperitoneally (i.p.) dose of carbon tetrachloride (1 ml/kg, 50% CCl_4 in olive oil) to induce hepatotoxicity. This methodology was carried out as previously described (Porchezhian et al., 2005) with slight modifications.

Blood collection and biochemical measurements

Blood samples were obtained by collecting retro-orbital 24 h after the end of the indicated treatments into tubes without anticoagulant. The samples were centrifuged for 15 min at 2800 rpm, and serum was used to measure the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, as well as the levels of albumin, glucose, total cholesterol, creatinine, urea and nitrite/nitrate (NO_x). Serum levels of albumin, creatinine, glucose, total cholesterol, urea and activities of AST and ALT were analyzed using standard techniques (Bioclin Quibasa, Belo Horizonte-MG, Brazil) on Cobas MIRA® automated analyzer (Roche Diagnostics, Basel, Switzerland). NO_x was measured by the modified Griess method using the Cobas Mira automated analyzer as previously described (Tatsch et al., 2011).

Assessment of genoprotective effect by comet assay

The alkaline comet assay was performed as described by Singh et al. (1988) in accordance with the general guidelines for the use of the comet assay (Hartmann et al., 2003; Tice et al., 2000). The samples of liver were rapidly removed to perform the comet assay. Hepatocytes were suspended in 0.7% low-melting-point agarose and PBS at 37 °C and placed on microscopic slides with a layer of 1% agarose. The slides were immersed in lysis solution at 4 °C for 1 h and followed by electrophoresis at 25 V, 300 mA, for 40 min at steady temperature. The slides were then silver-stained as described by Nadin et al. (2001). All steps, from sample collection to electrophoresis, were conducted under yellow light to minimize the possibility of cellular DNA damage. One hundred cells (50 cells from each of the two replicate slides) were selected and analyzed. Cells were visually scored according to tail length and received scores from 0 (no migration) to 4 (maximal migration). Therefore, the damage index for cells ranged from 0 (all cells with no migration) to 400 (all cells with maximal migration). Slides were analyzed under blind conditions by at least two different individuals.

Statistical analysis

Results are presented as mean ± standard error of mean (S.E.M). One-way analysis of variance (ANOVA) followed by

Table 1. Effects of guarana pretreatment on serum biochemical parameters of the study animals.

Parameters	Control	CCl_4	Silymarin + CCl_4	G100 + CCl_4	G300 + CCl_4	G600 + CCl_4
AST (U/L)	146 ± 11	3349 ± 1098[a]	394 ± 92*	842 ± 136*	479 ± 115*	668 ± 181*
ALT (U/L)	68 ± 3	2510 ± 1013[a]	208 ± 50*	556 ± 94*	427 ± 122*	427 ± 125*
Glucose (mmol/L)	10.0 ± 0.7	8.2 ± 0.7	11.0 ± 0.7	10.1 ± 1.1	9.4 ± 0.4	9.8 ± 0.9
Total cholesterol (mmol/L)	1.8 ± 0.1	1.7 ± 0.2	1.7 ± 0.2	1.7 ± 0.1	1.5 ± 0.1	1.7 ± 0.2
Urea (mmol/L)	8.3 ± 0.3	9.5 ± 0.5	10.8 ± 0.5	10.3 ± 0.8	11.4 ± 0.8	11.0 ± 1.2
Creatinine (μmol/L)	26.5 ± 2.6	30.9 ± 2.6	28.3 ± 2.6	30.0 ± 3.5	30.9 ± 2.6	27.4 ± 3.5
Albumin (g/L)	34 ± 1	34 ± 2	35 ± 2	34 ± 2	34 ± 2	33 ± 2
NO_x (μmol/L)	74 ± 29	52 ± 19	60 ± 32	77 ± 24	48 ± 23	89 ± 33

Values are mean and S.E.M. Differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.
[a]$p < 0.001$ compare with the control group.
*$p < 0.01$ compare with the CCl_4 group.

Tukey's multiple comparison test was used to determine statistical differences between the groups. Pearson correlation was assessed to evaluate the association between the variables. Statistical significance was assumed at $p < 0.05$.

Results

Effects of guarana pretreatment on serum biochemical parameters of the study animals are summarized in Table 1. There was a significant increase of serum ALT and AST activities in CCl_4 group when compared to the control group ($p < 0.001$). Pre-treatment with silymarin and guarana in all concentrations used in this experiment significantly decreased AST and ALT activities ($p < 0.01$). No significant difference was observed for glucose, total cholesterol, triglycerides, albumin, urea, creatinine or NO_x between the groups. CCl_4 group presented significantly higher DNA damage index than the control group, as shown in Figure 1. The pretreatment with guarana powder provided a significant decrease in the rate of DNA damage when compared with the CCl_4 group. In addition, the DNA damage index showed significant positive correlations with AST ($r = 0.43$, $p = 0.0053$) and ALT ($r = 0.39$, $p = 0.0120$).

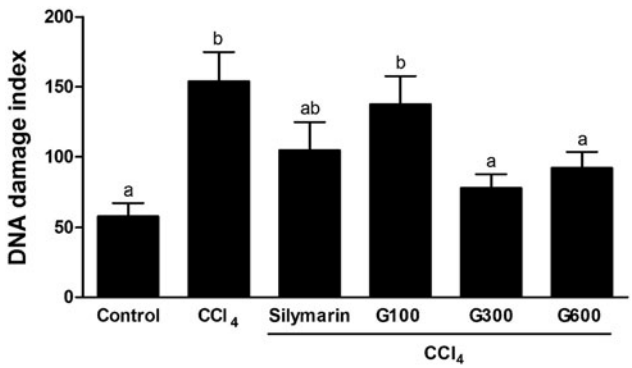

Figure 1. Effect of pretreatment with silymarin 100 mg/kg (silymarin), guarana 100 mg/kg (G100), guarana 300 mg/kg (G300) and guarana 600 mg/kg (G600) on CCl_4-induced DNA strand breakage. Values are expressed as mean and S.E.M. of 5–8 rats. Different letters indicate significant statistical differences ($p < 0.05$) between the groups.

Discussion

Hepatotoxicity induced by CCl_4 is the most commonly used model system for the screening of hepatoprotective activity of plant extracts and drugs (Ray et al., 2005). In this study, we reported at first time the hepatoprotective and genoprotective effects of guarana in CCl_4-hepatotoxicity induced in rats. In addition, we observed similar hepatoprotective effects of guarana in comparison with sylimarin, which is a well-documented hepatoprotective agent. We used an experimental model of CCl_4-induced acute hepatotoxicity in rats because this chemical is a potent hepatotoxin and a single exposure can rapidly lead to severe hepatic necrosis and steatosis (Brautbar et al., 2002; Recknagel et al., 1989). It is well known that chemical agents, such as CCl_4, produce hepatic injury causing large increases in both ALT and AST activities (Weber et al., 2003). These enzymes are employed in the evaluation of hepatic disorders and an increase in these enzyme activities reflects acute liver damage and hepatocellular disorders. Since these enzymes are cytoplasmic in nature, upon liver injury, these enzymes enter into the circulatory system due to altered permeability of the membrane (Zimmerman & Seeff, 1970). In the present study, a single dose intraperitoneally of CCl_4 caused an accentuated elevation in ALT and AST activities in serum, indicating that liver is susceptible to CCl_4-induced toxicity. In contrast, the increased levels of these markers were significantly inhibited by the pretreatment with guarana in all concentrations. The AST activity in the groups pre-treated with guarana was 4–7 times lower than in the CCl_4 group, whereas the ALT activity was 4–6 times lower. We postulate that the guarana may stabilize the hepatic cellular membrane and protect the hepatocytes against toxic effects of CCl_4, which may decrease the rate of leakage of the enzymes into the bloodstream.

Previous studies have reported that oxidative stress plays an essential role in the hepatic injury mediated by CCl_4 (Lin et al., 2008; Recknagel et al., 1989; Weber et al., 2003).

Despite the guarana has recognized antioxidant activities *in vitro*, some results regarding its protective activity seem to be contradictory. In an interesting study carried out by Zeidán-Chuliá et al. (2013), guarana showed concentration-dependent nonenzymatic antioxidant potential, decreased the basal levels of free radical generation, and reduced both superoxide dismutase (SOD) and catalase (CAT) activities in human neuronal SH-SY5Y cells. However, guarana-treated cells developed signs of neurite degeneration, and the neurotoxicological effects were exerted in part by disruption

of redox homeostasis. In a study conducted by our research group (Bittencourt et al., 2013), guarana was also able to modulate the activity of some enzymes as SOD and CAT. In addition to antioxidant activity, guarana presented effects on NO modulation in fibroblast NIH-3T3 cells exposed to sodium nitroprusside. However, in another report of our group (Costa Krewer et al., 2011), we carried out a study on 637 elderly individuals classified as either those who habitually drank guarana or those who never drank guarana, and there were no significant differences in NO metabolites between the groups. For this reason, despite the guarana present antioxidant activity and seems to modulate NO *in vitro*, some results of *in vivo* studies are different and appear to be contradictory. For this reason, further investigations are required to elucidate other mechanisms that may be involved in these pathways.

Oxidative stress not only damages cellular proteins and lipids, but also significantly affects DNA and generates various base modifications (Collins et al., 1993; Vanderauwera et al., 2011). This is of special importance because its accumulation and inheritance generations results in altered genotypes and functionally deficient cells (Bohr et al., 1998; Hamilton et al., 2001). In the present study, we carried out the comet assay to determine the possible protective role of the guarana against CCl_4-induced DNA damage. This test is widely used to evaluate the genotoxic potential of chemicals and environmental contaminants that can induce oxidative stress leading to hepatic injury (Das et al., 2007). The results obtained with the comet assay in the present study show that the administration of a single dose of CCl_4 promoted a significant increase in the DNA damage index when compared to the control group. In addition, the pretreatment with guarana 300 mg/kg significantly decreased DNA damage index when compared with CCl_4 group, demonstrating the genoproctetive effect of guarana. Furthermore, we also observed that the pretreatment with guarana in other concentrations showed a potential to prevent DNA damage, although not significantly. An interesting study carried out by Fukumasu et al. (2006) reported the protective effect of guarana against N-nitrosodiethylamine (DEN)-induced DNA damage in mouse liver. The experimental model used in the study by Fukumasu et al. is different from our study, but both studies have confirmed the genoprotective activity of guarana. Fukumasu et al. (2006) showed that guarana treatment presented a 52.54% reduction in comet image length when animals were exposed to DEN. In the present study, we demonstrated that guarana treatment decreased the DNA damage index by up to 49.5%, confirming the genoprotective activity of guarana. In addition, we also observed that the DNA damage index was positively correlated with AST and ALT activities, suggesting that CCl_4 promotes the DNA breakage as well as the release of enzymes from hepatocytes.

Several studies have reported that guarana shows a number of beneficial effects probably because this plant is a source of bioactive substances with multifaceted activity. Guarana is rich in methylxanthines such as caffeine, theobromine and theophylline, and contains tannins, saponins, cathechins, epicatechins, proanthocyanidols as well as trace concentrations of many other compounds (Belliardo et al., 1985). Studies *in vitro* have evidenced the antioxidant effects of guarana (Basile et al., 2005; Mattei et al., 1998; Yamaguti-Sasaki et al., 2007) probably due to a high concentration of polyphenols, such as tannins (Mattei et al., 1998). We infer that the hepatoprotective effects of guarana observed in this study may be attributed to the action of several bioactive compounds present in guarana. Thus, we suggest that these effects may have been caused by the action of the joint or isolated bioactive compounds in guarana.

An interesting result that has to be discussed is that the genoprotective effects of guarana were not dose-dependent. Treatment with 300 mg/kg of guarana led to a decrease of 49.5% in the DNA damage index, whereas treatment with 600 mg/kg led to a decrease of 40.3% in the DNA damage index. These apparent discrepant results can be associated with hormetic effects related with doses tested here. The magnitude of the response is a measure of biological plasticity, within a broad range of biological contexts (Calabrese, 2013), and hormetic dose-response is quite common and highly generalizable by biological model, endpoint and chemical class (Calabrese, 2006). In these terms, it is possible that some molecules can present a range of concentration without any biological effect or an intermediary range of concentrations that are effective on the biological effect. Furthermore, biological effect can be effectively lost or cause some toxic response in the presence of higher concentrations.

There are some limitations in this study. It was not checked the use of guarana in animals not treated with CCl_4 for the comparison of baseline levels of the measured parameters, and it was not tested the isolate bioactive compounds of guarana. In addition, it was not measured the 8-hydroxy-2′-deoxyguanosine (8-OHdG) and other oxidative stress parameters in order to provide a general view of the redox status in each group. For this reason, it is not possible to determine the exact mechanism of action of guarana in this study.

Conclusions

In summary, this study demonstrates that the guarana has hepatoprotective activity and prevents the DNA strand breakage in CCl_4-induced liver damage in rats. Guarana is a well-known and widespread plant used popularly and new reports assessing other biological activities are of interest. In this context, additional studies are required to investigate other activities of guarana, such as the effects of its isolated compounds in different models.

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Declaration of interest

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References

Basile A, Ferrara L, Pezzo MD, et al. (2005). Antibacterial and antioxidant activities of ethanol extract from *Paullinia cupana*. J Ethnopharmacol 102:32–36.

Belliardo F, Martelli A, Valle MG. (1985). HPLC determination of caffeine and theophylline in *Paullinia cupana* Kunth (guaraná) and *Cola* spp. samples. Z Lebensm Unters Forsch 1180:398–401.

Bittencourt LS, Machado DC, Machado MM, et al. (2013). The protective effects of guaraná extract (*Paullinia cupana*) on fibroblast NIH-3T3 cells exposed to sodium nitroprusside. Food Chem Toxicol 53:119–125.

Bohr V, Anson RM, Mazur S, Dianov G. (1998). Oxidative DNA damage processing and changes with aging. Toxicol Lett 103:47–52.

Boozer CN, Nasser JA, Heymsfield SB, et al. (2001). An herbal supplement containing Ma Huang-Guaraná for weight loss: a randomized, double-blind trial. Int J Obes Relat Metab Disord 25: 316–324.

Brautbar N, Williams J. (2002). Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms. Int J Hyg Environ Health 205:479–491.

Calabrese EJ. (2006). The failure of dose-response models to predict low dose effects: a major challenge for biomedical, toxicological and aging research. Biogerontology 7:119–122.

Calabrese EJ. (2013). Hormetic mechanisms. Crit Rev Toxicol 43: 580–606.

Campos AR, Barros AI, Albuquerque FA, et al. (2005). Acute effects of guarana (*Paullinia cupana* Mart.) on mouse behaviour in forced swimming and open field tests. Phytother Res 19:441–443.

Collins AR, Duthie SJ, Dobson VL. (1993). Direct enzymic detection of endogenous oxidative base damage in human lymphocyte DNA. Carcinogenesis 14:1733–1735.

Costa Krewer C, Ribeiro EE, et al. (2011). Habitual intake of guaraná and metabolic morbidities: an epidemiological study of an elderly Amazonian population. Phytother Res 25:1367–1374.

Da Fonseca CA, Leal J, Costa SS, Leitão AC. (1994). Genotoxic and mutagenic effects of guaraná (*Paullinia cupana*) in prokaryotic organisms. Mutat Res 321:165–173.

Das RK, Hossain SU, Bhattacharya S. (2007). Protective effect of diphenylmethyl selenocyanate against CCl_4-induced hepatic injury. J Appl Toxicol 27:527–537.

Espinola EB, Dias RF, Mattei R, Carlini EA. (1997). Pharmacological activity of guaraná (*Paullinia cupana* Mart.) in laboratory animals. J Ethnopharmacol 55:223–229.

Fukumasu H, Avanzo JL, Heidor R, et al. (2006). Proctetive effects of guarana (*Paullinia cupana* Mart. var *sorbilis*) against DEN-induced DNA damage on mouse liver. Food Chem Toxicol 44:862–867.

Hamilton ML, Van Remmen H, Drake JA, et al. (2001). Does oxidative damage to DNA increase with age. Proc Natl Acad Sci USA 98: 10469–10474.

Hartmann A, Agurell E, Beevers C, et al. (2003). Recommendations for conducting the in vivo alkaline comet assay: 4th International Comet Assay Workshop. Mutagenesis 18:45–51.

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

Janakat S, Al-Merie H. (2002). Optimization of the dose and route of injection, and characterisation of the time course of carbon tetrachloride-induced hepatotoxicity in the rat. J Pharmacol Toxicol Methods 48:41–44.

Kennedy DO, Haskell CF, Wesnes KA, Scholey AB. (2004). Improved cognitive performance in human volunteers following administration of guaraná (*Paullinia cupana*) extract: comparison and interaction with *Panax ginseng*. Pharmacol Biochem Behav 79:401–411.

Lee SH, Heo SI, Li L, et al. (2008). Antioxidant and hepatoprotective activities of *Cirsium setidens* NAKAI against CCl_4-induced liver damage. Am J Chin Med 36:107–114.

Leite RP, Wada RS, Monteiro JC, et al. (2010). Protective effect of guaraná (*Paullinia cupana* var. *sorbilis*) pretreatment on cadmium-induced damages in adult Wistar testis. Biol Trace Elem Res 141: 262–274.

Lin HM, Tseng HC, Wang CJ, et al. (2008). Hepatoprotective effects of *Solanum nigrum* Linn. extract against CCl_4-induced oxidative damage in rats. Chem Biol Interact 171:283–293.

Mattei R, Dias RF, Espínola EB, et al. (1998). Guarana (*Paullinia cupana*): toxic behavioral effects in laboratory animals and antioxidant activity in vitro. J Ethnopharmacol 60:111–116.

Nadin S, Vargas-Roig L, Ciocca D. (2001). A silver staining method for single-cell gel assay. J Histochem Cytochem 49:1183–1186.

O'Dea JA. (2003). Consumption of nutritional supplements among adolescents: usage and perceived benefits. Health Educ Res 18: 98–107.

Oliveira CH, Moraes ME, Moraes MO, et al. (2005). Clinical toxicology study of an herbal medicinal extract of *Paullinia cupana*, *Trichilia catigua*, *Ptychopetalum olacoides* and *Zingiber officinale* (Catuama) in healthy volunteers. Phytother Res 19:54–57.

Opala T, Rzymski P, Pischel I, et al. (2006). Efficacy of 12 weeks supplementation of a botanical extract-based weight loss formula on body weight, body composition and blood chemistry in healthy, overweight subjects – a randomized double-blind placebo-controlled clinical trial. Eur J Med Res 11:343–350.

Orhan DD, Aslan M, Aktay G, et al. (2003). Evaluation of hepatoprotective effect of *Gentiana olivieri* herbs on subacute administration and isolation of active principle. Life Sci 72: 2273–2283.

Otobone FJ, Sanches AC, Nagae R, et al. (2007). Effect of lyophilized extracts from guaraná seeds [*Paullinia cupana* var. *sorbilis* (Mart.) Ducke] on behavioral profiles in rats. Phytother Res 21:531–535.

Pinheiro CE, de Oliveira SS, da Silva SM, et al. (1987). Effect of guaraná and *Stévia rebaudiana* Bertoni (leaves) extracts, and stevioside, on the fermentation and synthesis of extracellular insoluble polysaccharides of dental plaque. Rev Odontol Univ Sao Paulo 1:9–13.

Porchezhian E, Ansari SH. (2005). Hepatoprotective activity of *Abutilon indicum* on experimental liver damage in rats. Phytomedicine 12: 62–64.

Ray S, Phadke S, Patel C, et al. (2005). Short-term and long-term in vivo exposure to an ephedra and caffeine containing metabolic nutrition system does not induce cardiotoxicity in B6C3F1 mice. Arch Toxicol 79:330–340.

Recknagel RO, Glende Jr EA, Dolak JA, Waller RL. (1989). Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther 43:139–154.

Schimpl FC, da Silva JF, Gonçalves JFC, Mazzafera P. (2013). Guarana: revisiting a highly caffeinated plant from the Amazon. J Ethnopharmacol 150:14–31.

Singh NP, McCoy MT, Tice RR, Schneider EL. (1988). A simple technique for quantification of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191.

Subramoniam A, Pushpangadan P. (1999). Development of phytomedicines for liver disease. Indian J Pharmacol 31:166–175.

Tatsch E, Bochi GV, Pereira RS, et al. (2011). A simple and inexpensive automated technique for measurement of serum nitrite/nitrate. Clin Biochem 44:348–350.

Tice RR, Agurell E, Anderson D, et al. (2000). Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 35:206–221.

Vanderauwera S, Suzuki N, Miller G, et al. (2011). Extranuclear protection of chromosomal DNA from oxidative stress. Proc Natl Acad Sci USA 108:1711–1716.

Wang T, Sun NL, Zhang WD, et al. (2008). Protective effect of dehydrocavidine on carbon tetrachloride-induced acute hepatotoxicity in rats. J Ethnopharmacol 117:300–308.

Weber LW, Boll M, Stampfl A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol 33:105–136.

Yamaguti-Sasaki E, Ito LA, Canteli VC, et al. (2007). Antioxidant capacity and *in vitro* prevention of dental plaque formation by extracts and condensed tannins of *Paullinia cupana*. Molecules 12: 1950–1963.

Zeidán-Chuliá F, Gelain DP, Kolling EA, et al. (2013). Major components of energy drinks (caffeine, taurine, and guarana) exert cytotoxic effects on human neuronal SH-SY5Y cells by decreasing reactive oxygen species production. Oxid Med Cell Longev 2013: 791795.

Zimmerman HJ, Seeff LB. (1970). Enzymes in hepatic disease. In: Goodly EL, ed. Diagnostic enzymology. Philadelphia: Lea & Febiger, 1–38.