Toxicology Reports 14 (2025) 101946

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Preventive treatment with guarana powder (*Paullinia cupana*) mitigates acute paracetamol-induced hepatotoxicity by modulating oxidative stress

Clécia Dias Teixeira [a], Priscila Oliveira Barbosa [b], Wanderson Geraldo Lima [c], Gustavo Silveira Breguez [a], Miliane Martins de Andrade Fagundes [a], Daniela Caldeira Costa [c], Cintia Lopes de Brito Magalhães [c], Joana Ferreira Amaral [a], Melina Oliveira de Souza [a,*]

[a] Post-Graduate Program in Health and Nutrition (PPGSN), Nutrition School, Federal University of Ouro Preto, Ouro Preto, Minas Gerais, Brazil
[b] Department of Gynecology and Obstetrics, Ribeirao Preto Medical School, University of São Paulo, Ribeirao Preto, São Paulo, Brazil
[c] Department of Biological Sciences (DECBI), Institute of Exact and Biological Sciences, Federal University of Ouro Preto, Minas Gerais, Brazil

ARTICLE INFO

Handling Editor: Prof. L.H. Lash

Keywords:
Guarana
Hepatotoxicity
Liver injury
Oxidative stress
Paullinia cupana
Paracetamol

ABSTRACT

Liver damage caused by high doses of paracetamol is a global public health concern. Consequently, therapeutic strategies are being explored to prevent this damage. The bioactive compounds present in fruits have shown promise in protecting against disorders associated with paracetamol-induced liver damage. This study assessed the preventive effects of guarana powder on redox status in a rat model of acute hepatotoxicity induced by a toxic dose of paracetamol. Male Wistar rats were divided into four groups: control (C), guarana (G), paracetamol (P), and guarana + paracetamol (GP). Animals in groups G and GP received 300 mg/kg guarana powder daily for seven days. Hepatotoxicity was induced in the P and GP groups by a single dose of 3 g/kg paracetamol on the last day. Paracetamol effectively induced liver damage and oxidative stress in group P animals. Preventive treatment with guarana significantly mitigated this damage and prevented the serum elevation of ALT, AST, and ALP by 44 %, 29 %, and 24 %, respectively. It also prevented a 133 % increase in the necrotic liver area in GP animals compared to the P. Guarana treatment, which prevented reductions in glutathione levels, modulated antioxidant enzyme (SOD and CAT) expression and activity, and protein carbonylation, while enhancing the total antioxidant capacity. Our results suggest that preventive treatment with guarana can attenuate oxidative damage, modulate antioxidant defense gene expression, and protect against paracetamol-induced hepatotoxicity in rats, highlighting guarana powder as a potential therapeutic agent to prevent liver damage induced by high doses of paracetamol.

1. Introduction

Medication-induced liver injury is a substantial factor contributing to acute liver failure (ALF) globally, with an estimated incidence of more than one per million [9]. Notably, paracetamol, also known as acetaminophen, which is commonly used by the general population, emerges as a major medication associated with the development of ALF, exerting a significant impact across regions such as the United States of America (USA), South America, Australia, the United Kingdom, and Sweden [9,36].

When paracetamol is administered, approximately 5–10 % is metabolized by cytochrome P450 enzymes, leading to the formation of N-acetyl-p-benzoquinone-imine (NAPQI), a highly reactive and hepatotoxic metabolite [35]. Under normal conditions and at therapeutic doses, NAPQI promptly conjugates with glutathione (GSH) to form metabolites that are excreted in the urine [38]. However, in cases of toxic doses of paracetamol, excessive NAPQI production can lead to GSH depletion, resulting in excess NAPQI binding predominantly to cellular proteins, particularly in the mitochondria, triggering the generation of reactive species and promoting oxidative stress, which can culminate in liver dysfunction [6].

The liver contains an endogenous antioxidant defense system that is essential for protecting it from damage induced by reactive species and for neutralizing their effects [18]. This defense system comprises enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR). SOD facilitates the

* Correspondence to: Laboratory of Nutritional Biochemistry and Molecular Biology, Department of Food, School of Nutrition, Federal University of Ouro Preto, Ouro Preto, Minas Gerais 35402-145, Brazil.
E-mail address: melina@ufop.edu.br (M.O. de Souza).

https://doi.org/10.1016/j.toxrep.2025.101946
Received 8 October 2024; Received in revised form 21 January 2025; Accepted 31 January 2025
Available online 3 February 2025

dismutation of superoxide anion (O_2^-) molecules into hydrogen peroxide (H_2O_2), with the Cu/ZnSOD isoform functioning in the cytoplasm and the MnSOD isoform functioning in the mitochondria. In contrast, CAT facilitated the breakdown of H_2O_2 into water and oxygen. GPx also contributes to H_2O_2 degradation by oxidizing GSH, which is continuously replenished by GR [18]. However, as the production of reactive species increases with excessive doses of paracetamol, these substances can overwhelm the body's antioxidant defense system [38] resulting in damage to cellular components, such as proteins and lipids, further compromising the effectiveness of antioxidant enzymes and exacerbating oxidative stress and damage to the liver [19]. Some studies have explored the potential bioactive compounds found in fruits, particularly polyphenols, which are known for their beneficial effects in combating hepatic oxidative stress in cases of paracetamol intoxication [16,31,8]. However, the use of isolated compounds does not always translate into beneficial effects, making it essential to evaluate the whole fruit as it contains a complex mixture of bioactive compounds that may work synergistically.

Guarana, commonly consumed in powder form, has gained attention for its potential therapeutic effects owing to its bioactive composition. Guarana is marked by its abundant methylxanthines, primarily caffeine, known for its potential action on the central nervous system, as well as theobromine and theophylline. Guarana also contains polyphenols – primarily catechins and epicatechins – with potent antioxidant and anti-inflammatory activities [10,37], contributing to protection against oxidative stress, a key mechanism in liver damage. Despite its promising bioactive composition, there is a scarcity of studies investigating its health benefits, mainly involving its antioxidant capacity in animal models, as recently illustrated in a systematic review [10]. Therefore, our study aimed to evaluate the preventive effects of guarana powder treatment on redox status in a rat model of acute hepatotoxicity induced by a toxic dose of paracetamol. We propose that guarana powder protects against the severe complications resulting from paracetamol-induced hepatotoxicity.

2. Materials and methods

2.1. Guarana powder

Guarana powder used in this study was obtained from Embrapa Amazônia Ocidental, aliquoted, stored in opaque packaging, and refrigerated. Guarana powder was registered in the National System for Management of Genetic Heritage and Associated Traditional Knowledge (SISGEN) under registration number AB4ACC4, in accordance with Brazilian Law No. 13.123/2015.

The polyphenol content was measured using the Folin–Ciocalteu reagent [29], yielding 4459.41 ± 88.23 mg GAE/100 g. Antioxidant capacity was evaluated through ABTS (386.87 μM ET/g) [13], DPPH (IC50 of 146.6 g guarana/g DPPH), [14], FRAP (224.79 ± 0.68 μM ET/g) [12] and ORAC (221.10 ± 12.53 μM ET/g) [7] assays, following established protocols.

2.2. Experimental design

The study protocol was approved by the Animal Use Ethics Committee (CEUA) of the Federal University of Ouro Preto (UFOP); (Protocol No. 3488300122). Thirty-two Wistar male albino rats, Rattus norvegicus, weighing approximately 180–200 g and aged between 45 and 50 days, were obtained from the Animal Science Center (CCA) at UFOP. The animals were divided into four experimental groups (n = 8 per group): the control group (C), which received only filtered water via orogastric gavage, used as the vehicle for guarana powder and paracetamol solution; the guarana group (G), which received a daily dose of 300 mg/kg guarana powder solution via orogastric gavage for seven consecutive days [21]; the paracetamol group (P), which was subjected to hepatotoxicity induced by a single dose of 3 g/kg paracetamol solution via orogastric gavage for seven consecutive days [1,20,35]; and the guarana + paracetamol group (GP), which received both the daily guarana powder solution and paracetamol via orogastric gavage to induce hepatotoxicity. The guarana powder was prepared daily, adjusted according to the body mass of the animals in each group, and diluted in filtered water, then homogenized using a magnetic stirrer. Paracetamol was administered as a single dose to groups P and GP to induce hepatotoxicity (Lot 22C62A; Prati, Donaduzzi & Cia Ltda, Paraná, Brazil).

All animals were housed under controlled conditions, including a 12-hour light/dark cycle and a temperature of 24 ± 2 ºC, with *ad libitum* access to food and water. Daily food intake and body mass were monitored to adjust the dose of guarana powder. Twenty-four hours after paracetamol administration, the animals were anaesthetized with isoflurane and euthanized by guillotine decapitation. The experimental design is shown in Fig. 1.

2.3. Food consumption and murinometric parameters

Food intake was calculated by subtracting the amount of diet offered the day before from the amount remaining on the following day. The food efficiency coefficient (FEC) and weight gain coefficient per caloric intake (WGCCI) were calculated as previously described [30]. Body mass gain was calculated by subtracting the final weights from initial weights, and the naso-anal length was measured to calculate the Lee index [22,30].

2.4. Biochemical and inflammatory analysis

After euthanasia, blood was collected and centrifuged at 2000 × g for 15 min to obtain serum. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using Labtest Diagnostic kits (Lagoa Santa, Minas Gerais, Brazil), while alkaline phosphatase (ALP) activity was assessed with Bioclin Quibasa kit (Química Básica Ltd. (Belo Horizonte, Minas Gerais, Brazil).

For quantification of TNF approximately 100 mg of liver tissue was homogenized in 1 mL of ice-cold PBS (1 mM) containing 10 μL of Halt Protease Inhibitor (Thermo Fisher Scientific, Rockford, U.S.A.) for 30 s, followed by 1 min of rest on ice, repeated three times. After homogenization, samples were centrifuged at 9300 × g for 10 minutes at 4ºC, and the supernatants were collected for analysis. TNF level was quantified using the commercial ELISA kit from Invitrogen™ (Thermo Fischer Scientific, Carlsbad, CA; Catalog number: ERA57RB). Results were expressed as pg/mL.

2.5. Histological analysis

The livers were collected at euthanasia, washed in cold PBS, and weighed. One lobe from each animal was separated for histological analysis and fixed in 10 % buffered formalin solution. The sections were then processed by undergoing dehydrated in an increasing series of alcohol (70–100 %) and diaphanized using xylene before being sealed in paraffin. Using a semi-automatic microtome (Leica Biosystems, Nussloch, Germany), 4 μm sections were made and placed on histological slides. Sections were then blushed using the hematoxylin and eosin (H&E) technique and photomicrographed at 40x magnification using a digital camera coupled to a Leica DM5000B Digital Optical Microscope (Leica Biosystems, Wetzlar, Germany). Photomicrographs of random locations were verified using ImageJ software (National Institutes of Health, Bethesda, MD, USA), where the injured areas of each animal were added together.

2.6. Sample preparation and redox status analysis

2.6.1. Sample preparation

Liver extracts were prepared as described previously [34]. Approximately 100 mg of liver tissue was homogenized in 1 mL ice-cold

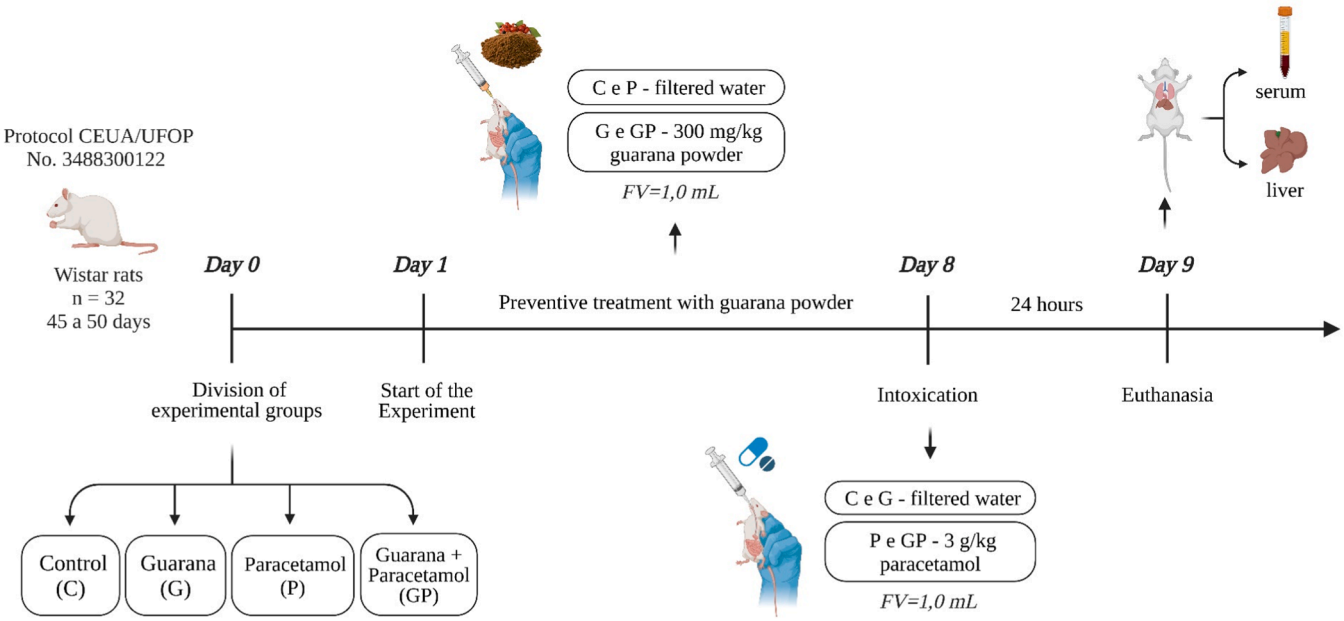

Fig. 1. Summary of the experimental design carried out in the present study.The experimental groups were divided into C, the control group, which received the standard Nuvilab diet; G, the guarana group that received the standard Nuvilab diet + 300 mg/kg of guarana powder by orogastric gavage, once a day for 7 days; P, the paracetamol group, in which intoxication was induced by a single dose of 3 g/kg of paracetamol via orogastric gavage on the 8th day of the experiment; GP, the guarana + paracetamol group, which received standard Nuvilab diet + 300 mg/kg of guarana powder by orogastric gavage, once a day for 7 days and intoxication was induced by a single dose of 3 g/kg of paracetamol by orogastric gavage on the 8th day of the experiment. FV, final volume administered; n, number of animals; CEUA/UFOP, Ethics Committee on the Use of Animals of the Federal University of Ouro Preto. (Created with Biorender.com).

phosphate buffer (50 mM potassium phosphate, 0.5 mM EDTA, pH 7.2) containing 10 μL of Halt Protease Inhibitor (Thermo Fisher Scientific, Rockford, U.S.A.). The homogenization was repeated three times, followed by centrifugation at 9300 × g for 10 minutes at 4ºC, and the supernatants were collected for oxidative stress analysis. Total protein concentration was determined using Lowry method [26].

2.6.2. Analysis of oxidative damage markers

Lipid peroxidation in the liver was assessed by quantifying malondialdehyde (MDA) [15], and liver protein damage was evaluated by measuring protein carbonyl concentrations [23]. Results were expressed as nmol/mg total protein.

2.6.3. Glutathione system assessment

Glutathione levels were quantified using an Invitrogen™ colorimetric detection kit (Thermo Fischer Scientific, Carlsbad, CA; Catalog number: EIAGSHC), measuring total (tGSH) and oxidized (GSSG) forms. Reduced glutathione form (GSH) was calculated by subtracting GSSG from tGSH. The results were expressed in μM.

2.6.4. Antioxidant enzyme activity

The activities of the antioxidant enzymes Superoxide Dismutase (SOD; Catalog number: EIASODC) and catalase (CAT; Catalog number: EIACATC) were determined using commercial kits from Invitrogen™ (Thermo Fisher Scientific, Carlsbad, CA, USA). Results were expressed as U/mL.

2.7. Total antioxidant capacity

2.7.1. Sample preparation

Approximately 100 mg of liver tissue was homogenized in 1 mL of ice-cold PBS (1 mM) for 30 s, followed by 1 min of rest on ice, repeated three times. After homogenization, samples were centrifuged at 9300 × g for 10 minutes at 4ºC, and the supernatants were collected for analysis. Total protein concentration was determined using the Lowry method [26].

2.7.2. Total antioxidant capacity (TAC)

To measure serum and liver TAC, a commercial *QuantiChrom™ Antioxidant Assay Kit* (DTAC-100) (BioAssay Systems, CA, USA) was used following the manufacturer's technical guidelines. Results were expressed in μM trolox.

2.8. Gene expression of antioxidant enzymes

Total RNA was extracted using the SV Total RNA Isolation System (Promega Corporation, Madison, WI, USA), and RNA purity was assessed via A260/280 ratio using a NanoDrop Lite Spectrophotometer (Thermo Fischer Scientific). Complementary DNA (cDNA) was synthesized using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Lithuania, Europe) following the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was performed with SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA). Amplification was conducted using the ABI 7500 Real-Time PCR Instrument (Applied Biosystems). Gene expression was normalized to β-2 microglobulin, with reactions run in duplicate, with each reaction including a negative control with water instead of cDNA. The primers sequences for the target genes are provided in the supplementary material (Table S1). Data analysis was performed using the comparative gene expression quantification method, where the ΔCt (Detection Cycle – "Cycle threshold") values of the target gene were normalized by the value of the normalizing gene.

2.9. Statistical analysis

The sample size was calculated based on the expected change in serum AST enzyme activity [35], using ANOVA with 90 % powder and 0.05 significance level, using the statistical website of the Faculty of Sciences at UNESP-BAURU.

Statistical analyses were conducted using GraphPad Prism® version 9.5 (GraphPad Software Inc., San Diego, CA). Data distribution was assessed using the Shapiro-Wilk test, confirming normality. Subsequently, a two-way ANOVA was followed by the Bonferroni post-hoc test

to assess differences between groups. Statistical significance was set at $p < 0.05$. The results are expressed as mean ± standard deviation (SD).

3. Results

3.1. Effect of preventive treatment with guarana powder on food consumption and murinometric parameters in rats intoxicated with paracetamol

Food intake was monitored daily; however, a significant change was observed only after intoxication, indicating the effects of preventive treatment ($p = 0.035$) and intoxication ($p < 0.001$) (Table 1). Animals in the P group exhibited a 24 % reduction in food intake compared with those in the C group ($p < 0.001$), whereas those in the GP group showed a 26 % reduction compared with those in the G group ($p < 0.001$). Neither FEC nor WGCCI exhibited statistically significant effects (Table 1).

No statistically significant changes were observed in the initial and final body masses of the experimental groups, body mass gain, or Lee index. Regarding liver weight, only the effect of intoxication was observed ($p = 0.0049$), although this difference was not evident in the post-test. In terms of the relative weight of the liver, a significant intoxication effect was observed ($p < 0.001$). Animals in the P group exhibited an 8 % reduction in the relative weight of the organ compared with the C group ($p < 0.05$), whereas animals in the GP group demonstrated a 11 % reduction compared with the G group ($p = 0.007$). Table 1 presents the results of the study.

3.2. Preventive treatment with guarana powder prevents liver damage in rats intoxicated with paracetamol

The experimental model used in this study induced hepatotoxicity by administering a toxic dose of paracetamol, which is known to cause liver damage. Serum ALT enzyme activity (Fig. 2A) was significantly affected b intoxication ($p < 0.001$), preventive treatment ($p < 0.001$), and their interactions ($p < 0.001$). ALT activity increased by 144 % in animals in group P compared to those in group C ($p < 0.001$). Preventive treatment with guarana attenuated this increase by 44 % in animals in the GP group compared with those in the P group ($p < 0.001$).

Similarly, serum AST enzyme activity (Fig. 2B) was significantly affected by intoxication ($p < 0.001$), preventive treatment ($p = 0.002$), and their interactions ($p < 0.001$). Animals in the P group demonstrated a 104 % increase in AST activity compared to those in the C group ($p < 0.001$). However, preventive treatment with guarana powder mitigated this increase by 29 % in animals in the GP group compared with those in the P group ($p < 0.001$).

Regarding serum ALP enzyme activity (Fig. 2C), there were significant effects of intoxication ($p < 0.001$), preventive treatment ($p = 0.03$), and their interaction ($p = 0.001$). Animals in the P group exhibited a 52 % increase in ALP activity compared to those in the C group ($p < 0.001$). Conversely, preventive treatment with guarana powder reduced this increase by 24 % in the GP group ($p < 0.001$).

Hepatic concentration TNF (Fig. 2D) were significantly influenced by intoxication ($p < 0.001$) and effect of interaction ($p < 0.001$). Animals in the P group exhibited 110 % increase in TNF concentration compared to those in the C group ($p < 0.001$) and animals in the GP group exhibited increase 31 % compared G group ($p = 0.001$). However, preventive treatment with guarana powder mitigated this increase by 20 % in animals in the GP group compared with those in the P group ($p < 0.001$).

Histopathological analyses were performed to confirm liver damage. Photomicrographs of animals from groups C (Fig. 3A) and G (Fig. 3B) showed normal morphology. However, animals in the P group (Fig. 3C) exhibited visible damage to the hepatic parenchyma morphology. Conversely, animals from the GP group (Fig. 3D) showed less damage than those from the P group, confirming the potential effect of guarana in preventing liver damage.

To confirm hepatocellular death, the necrotic area was quantified (Fig. 3E) in the different groups, showing a significant effect of intoxication ($p < 0.001$), preventive treatment ($p = 0.002$), and their interaction ($p = 0.002$). Paracetamol intoxication led to an increase in the necrotic area in the liver tissue of the P group animals by 97 % (P versus C; $p < 0.001$) and 92 % in the GP group (GP versus G; $p < 0.001$). Moreover, preventive treatment with guarana powder demonstrated 133 % prevention of this increase in the GP group (GP versus P; $p < 0.001$).

3.3. Preventive treatment with guarana powder prevents protein carbonylation in rats intoxicated with paracetamol

To elucidate the effect of preventive treatment with guarana powder on paracetamol-induced hepatotoxicity in redox processes, several oxidative stress biomarkers were analyzed, including carbonylated proteins and MDA (Fig. 4).

Concerning protein damage, assessed by carbonylated protein concentration in the liver (Fig. 4A), significant effects of intoxication ($p < 0.001$), preventive treatment ($p < 0.001$), and their interaction ($p < 0.001$) were observed. Animals in group P exhibited higher concentrations of carbonylated proteins than those in group C (114 %; $p < 0.001$). However, preventive treatment with guarana powder

Table 1
Effect of preventive treatment with guarana powder on food consumption and murinometric parameters.

Variables	Experimental group				p-value		
	C (n = 8)	G (n = 8)	P (n = 8)	GP (n = 8)	Intoxication	Preventive treatment	Interaction
Food intake parameters							
Food intake after intoxication (g)	27.75 ± 3.06	26.00 ± 2.73	21.13 ± 1.43$^{\#*}$	19.25 ± 1.58$^{\#*}$	< 0.001	0.035	0.939
FEC	0.30 ± 0.04	0.30 ± 0.07	0.29 ± 0.04	0.27 ± 0.03	0.156	0.568	0.535
WGCCI	0.09 ± 0.01	0.09 ± 0.02	0.09 ± 0.01	0.09 ± 0.009	0.156	0.569	0.535
Murinometric parameters							
Initial body weight (g)	182.0 ± 13.42	183.0 ± 11.11	184.0 ± 16.19	182.3 ± 12.26	0.896	0.937	0.773
Final body weight (g)	233.5 ± 18.09	232.9 ± 19.21	231.5 ± 19.69	224.0 ± 16.97	0.413	0.540	0.604
Body mass gain (g)	51.50 ± 6.52	49.88 ± 11.29	47.50 ± 6.91	41.75 ± 6.99	0.045	0.212	0.481
Lee index (g/cm3)	0.304 ± 0.007	0.307 ± 0.008	0.309 ± 0.012	0.306 ± 0.009	0.582	0.937	0.387
Liver weight (g)	10.47 ± 1.16	10.40 ± 1.24	9.52 ± 1.13	8.95 ± 0.87	0.004	0.426	0.520
Relative liver weight	0.045 ± 0.003	0.045 ± 0.003	0.041 ± 0.003$^{\#}$	0.039 ± 0.002$^{\#*}$	0.469	< 0.001	0.592

Legend: $p < 0.05$: $^{\#}$ versus C and *versus G; C, control group; G, guarana group; P, paracetamol group; GP, guarana + paracetamol group; FEC, food efficiency coefficient; WGCCI, weight gain coefficient per caloric intake. The experimental groups were divided into C, the control group, which received standard Nuvilab diet; G, the guarana group, which received standard Nuvilab diet + 300 mg/kg of guarana powder by oral gavage once a day for 7 days; P, the paracetamol group, which was induced with intoxication by a single dose of 3 g/kg of paracetamol by oral gavage on the 8th day of the experiment; and GP, the guarana + paracetamol group, which received standard Nuvilab diet + 300 mg/kg of guarana powder by oral gavage once a day for 7 days and was induced with intoxication by a single dose of 3 g/kg of paracetamol by oral gavage on the 8th day of the experiment. Statistical significance was set at $p < 0.05$. Results are presented as mean ± standard deviation.

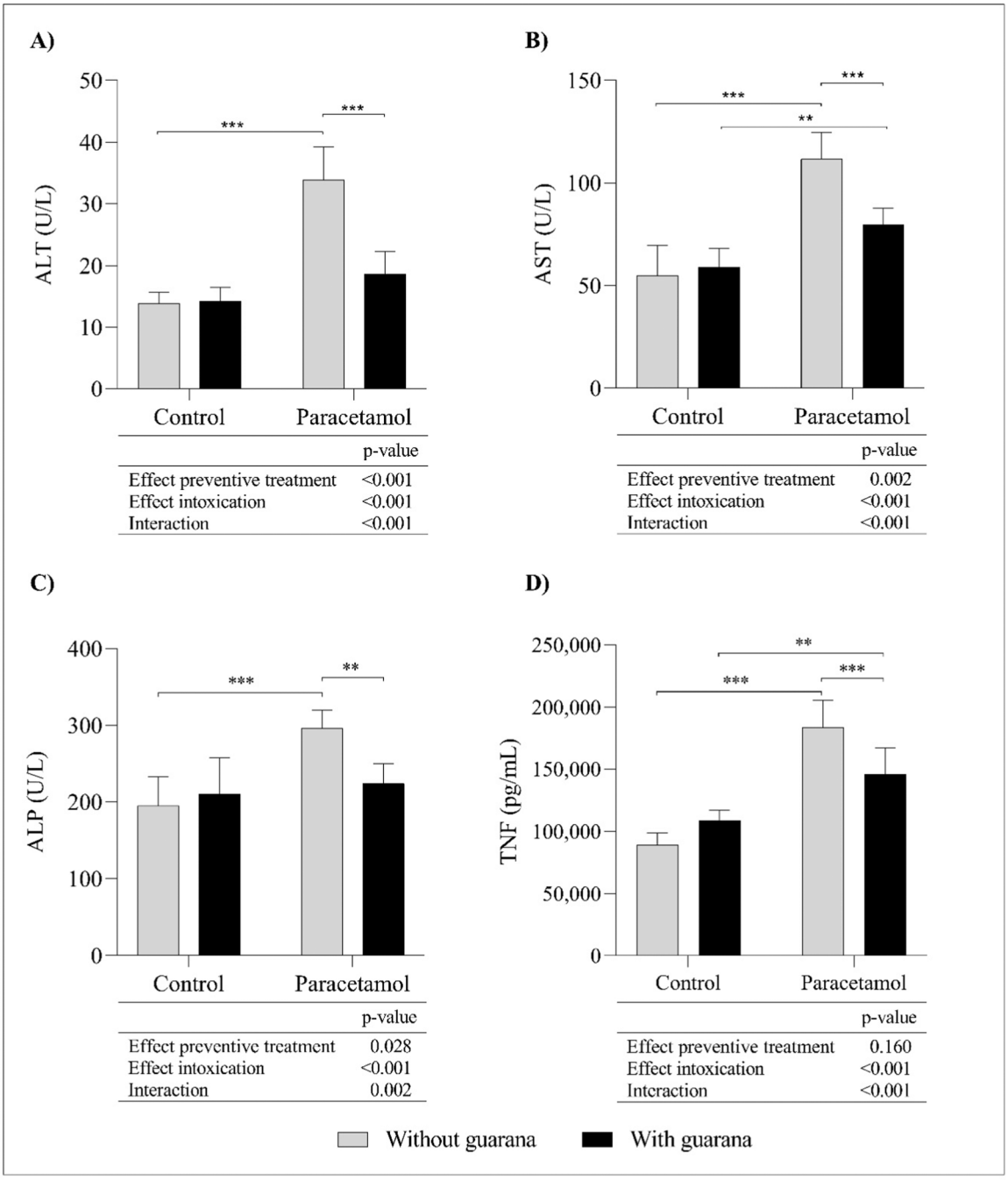

Fig. 2. The effect of preventive treatment with guarana powder and intoxication on hepatic function parameters and inflammation.A) ALT activity, B) AST activity, C) ALP activity and D) TNF concentration. Statistical significance was set at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Results are presented as mean ± standard deviation. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TNF, tumor necrosis factor.

mitigated protein damage in the GP group compared to that in the P group (35 %; $p < 0.001$).

The concentration of MDA (Fig. 4B), an indicator of lipid damage, was also evaluated. A significant effect of intoxication ($p < 0.001$) was observed, particularly in group P, where the concentration was 169 % higher than that in group C ($p < 0.001$). Additionally, animals in the GP group had a concentration that was 113 % higher than that in the G group ($p < 0.001$).

3.4. Preventive treatment with guarana powder prevents dysregulation of the glutathione system in rats intoxicated with paracetamol

GSH is an essential component of the antioxidant defense system and is directly involved in the elimination of toxic metabolites resulting from paracetamol metabolism. Therefore, the concentrations were analyzed (Fig. 5). The total GSH concentration (tGSH) (Fig. 5A) was significantly affected by intoxication ($p < 0.001$), preventive treatment ($p = 0.001$), and their interactions ($p < 0.001$). A 67 % reduction was observed in the P group compared to the C group ($p < 0.001$), and a 28 % reduction was observed in the GP group compared to the G group ($p < 0.001$). Preventive treatment with guarana powder demonstrated a 103 %

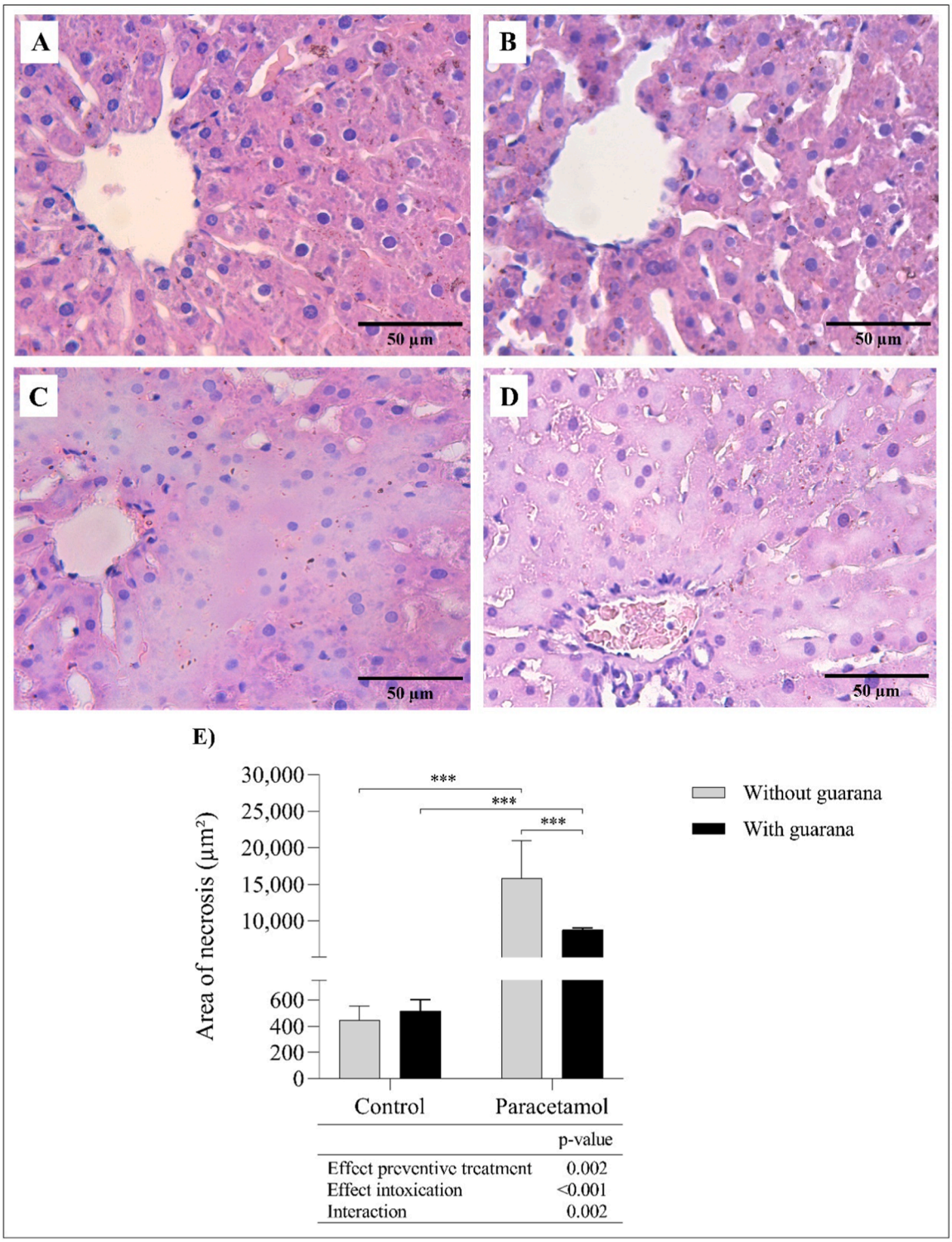

Fig. 3. Effect of preventive treatment with guarana powder and intoxication on hepatic parenchyma. A) Control group; B) Guarana group; C) Paracetamol group; D) Guarana + Paracetamol group; E) Area of necrosis in μm^2. Statistical significance was set at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Results are presented as mean ± standard deviation.

preventive effect on this parameter in the GP group compared to that in the P group ($p < 0.001$).

Analyzing GSH in the reduced (GSH) and oxidized (GSSG) forms of GSH demonstrated similar results. The GSH concentration (Fig. 5B) showed significant effects of intoxication ($p < 0.001$), preventive treatment ($p < 0.05$), and their interaction ($p = 0.001$). The intoxicated animals (P) exhibited a 63 % lower concentration than the C group ($p < 0.001$). Moreover, preventive treatment with powder guarana prevented this decrease by 119 % in the GP group compared to that in the P group ($p = 0.004$). Similar effects were observed for GSSG (Fig. 5C), with significant effects of intoxication ($p < 0.001$), preventive treatment ($p < 0.001$), and their interaction ($p < 0.001$). The intoxicated animals showed a 75 % lower concentration than group C ($p < 0.001$), and the GP group exhibited a 40 % lower concentration

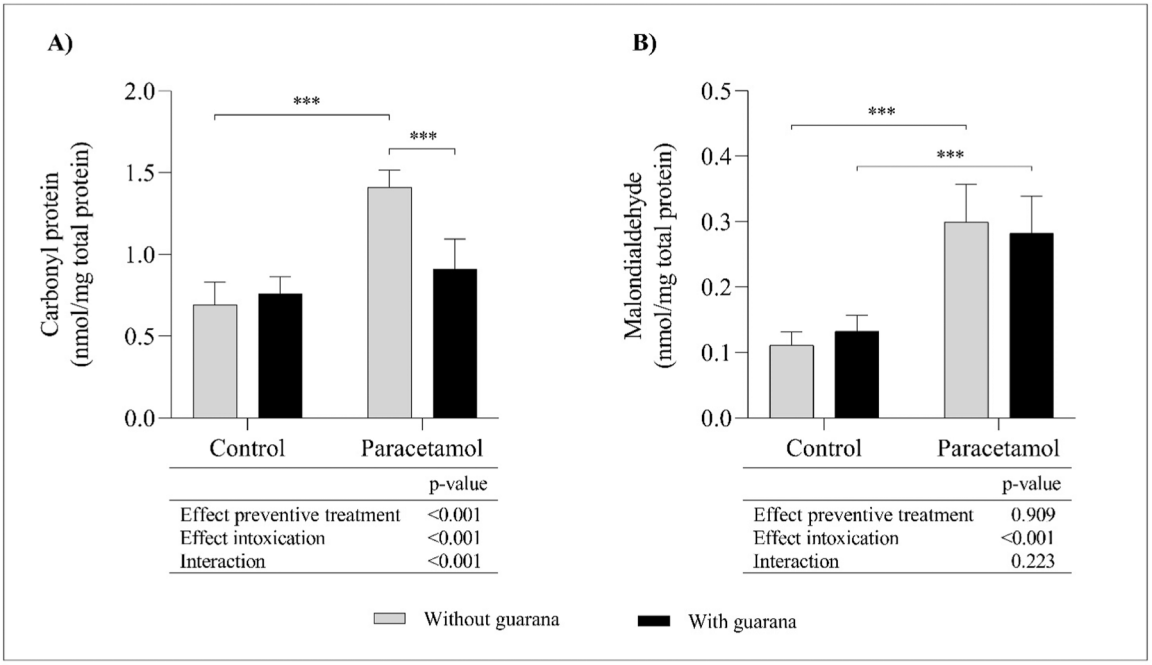

Fig. 4. The effect of preventive treatment with guarana powder and intoxication on biomarkers of oxidative stress. A) Concentration of carbonyl protein; B) Concentration of malondialdehyde. Statistical significance was set at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Results are presented as mean ± standard deviation.

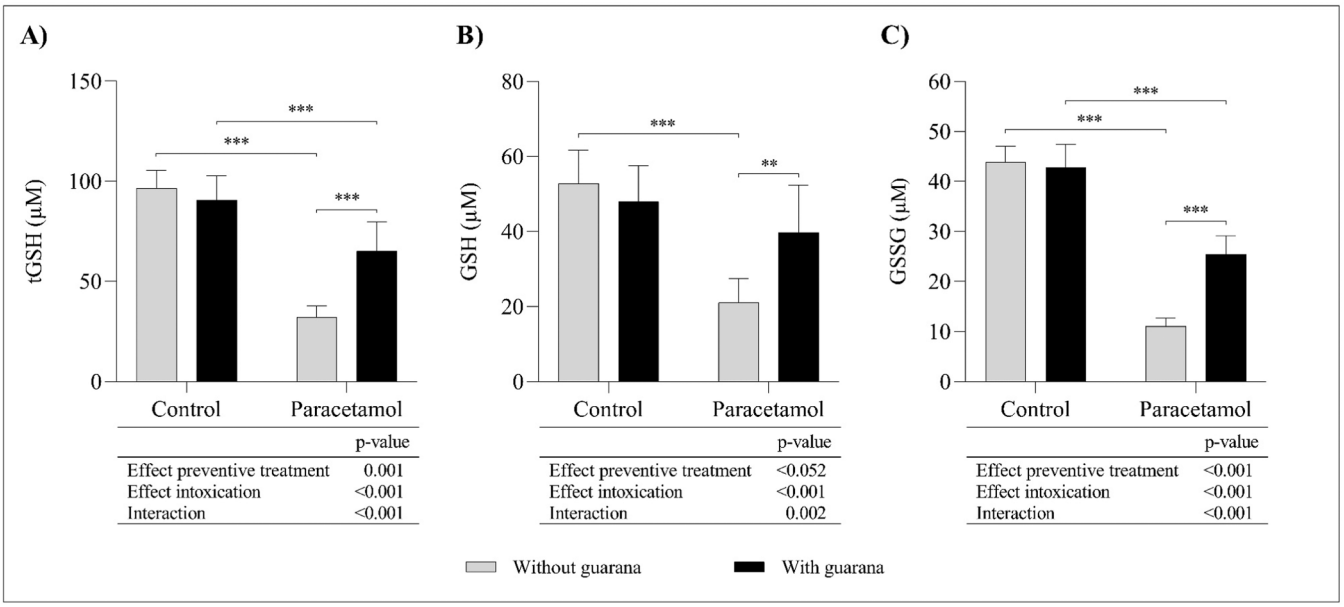

Fig. 5. Effect of preventive treatment with guarana powder and intoxication on the glutathione system. A) GSH concentration; B) GSH concentration; C) GSSG concentration. tGSH, total glutathione; GSH, reduced glutathione; GSSG, oxidized glutathione. Statistical significance was set at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Results are presented as mean ± standard deviation.

than the G group ($p < 0.001$). Prevention of this increase by 130 % was observed in the GP group compared to the P group ($p < 0.001$).

3.5. *Preventive treatment with guarana powder mitigates imbalance in antioxidant defense system in paracetamol-intoxicated rats*

Antioxidant defense was evaluated by analyzing the key enzymes *Sod*, *Cat*, and *Gpx* in the liver. The *ZnSod* enzyme expression ratio (Fig. 6A) showed a significant effect on toxicity ($p < 0.001$). Animals in the P group exhibited a 1.55-fold change in the expression ratio compared to those in the C group ($p = 0.003$), whereas those in the GP group displayed a 1.52-fold higher expression ratio compared to those in the G group ($p = 0.005$). Regarding the *MnSod* isoform expression ratio (Fig. 6B), significant effects of intoxication ($p < 0.001$), preventive treatment ($p = 0.029$), and their interactions ($p < 0.001$) were observed. Animals in the P group showed a 1.94-fold increase compared to those in the C group ($p < 0.001$). Preventive guarana powder treatment mitigated this increase by 1.43-fold in the GP group compared to that in the P group ($p < 0.001$). Analysis of *Sod* enzyme activity (Fig. 6C) revealed a significant effect of intoxication ($p < 0.001$), preventive

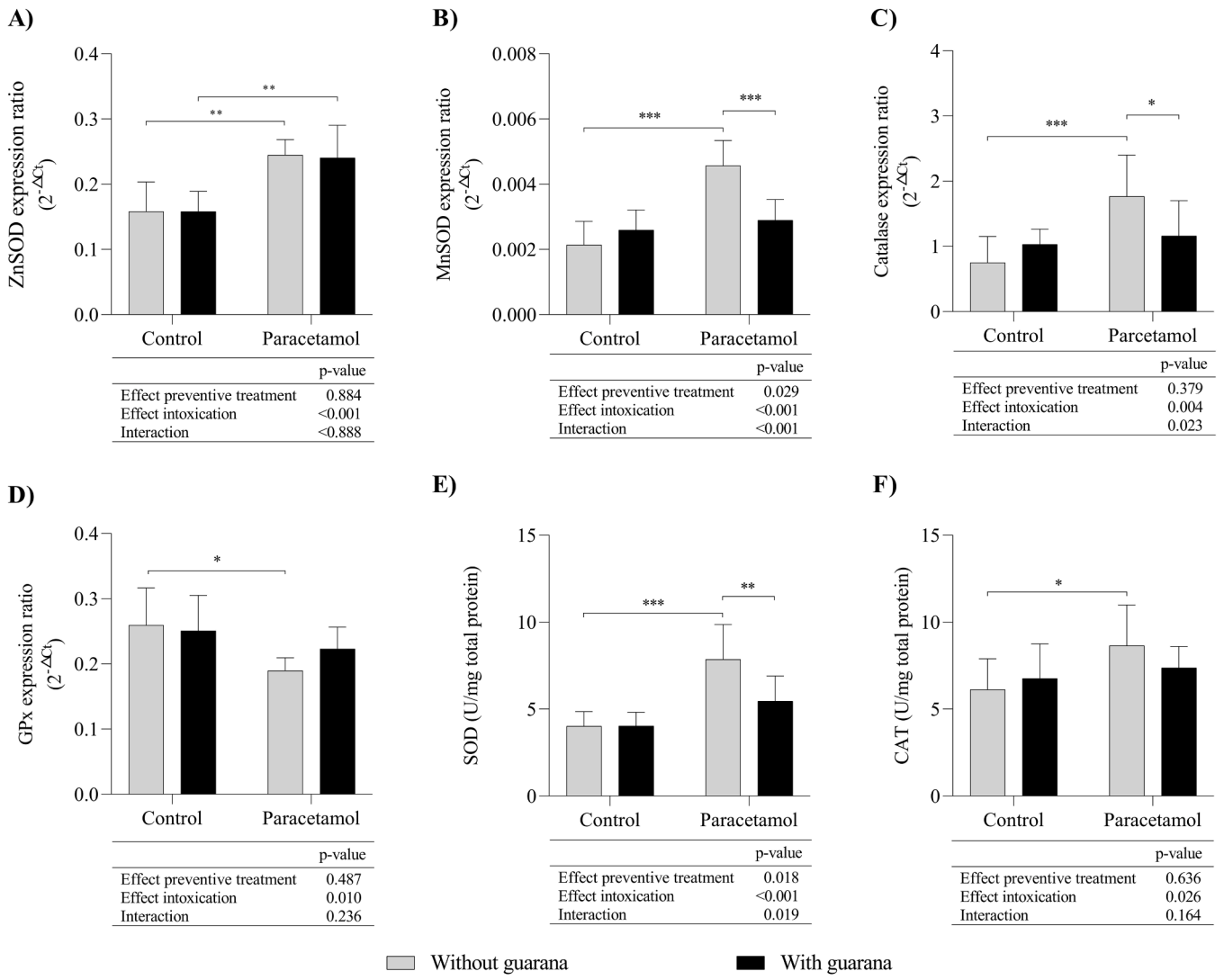

Fig. 6. Effect of preventive treatment with guarana powder and intoxication on antioxidant enzymes. A) ZnSod expression ratio; B) MnSod expression ratio; C) superoxide dismutase activity in U/mg total protein; D) catalase expression ratio; E) catalase activity in U/mg total protein; F) glutathione peroxidase. Statistical significance was set at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Results are presented as mean ± standard deviation.

treatment ($p = 0.018$), and their interaction ($p = 0.019$). Animals in the P group showed a 96 % increase compared to the C group ($p < 0.001$), with preventive guarana powder treatment preventing a 31 % increase in the GP group relative to the P group ($p = 0.009$).

The *Cat* enzyme expression ratio (Fig. 6D) was significantly affected by intoxication ($p = 0.023$) and the interaction ($p = 0.004$). The P group exhibited a 2.09-fold change compared to the C group ($p < 0.001$). Preventive guarana powder treatment reduced this increase by 1.21-fold (GP vs. P; $p = 0.048$). Concerning *Cat* activity (Fig. 6E), only intoxication had a significant effect ($p < 0.001$), with a 33 % increase observed in the P group compared to the C group ($p = 0.027$).

Lastly, the *Gpx* enzyme expression ratio (Fig. 6F) showed a significant effect on intoxication ($p = 0.010$), indicating a 1.37-fold change in expression in group P compared to that in group C ($p = 0.023$).

3.6. *Preventive treatment with guarana powder prevents the reduction of total antioxidant capacity in rats intoxicated with paracetamol*

Evaluating total antioxidant capacity is a crucial factor in determining an organism's ability to withstand oxidative stress. Therefore, we examined the impact of guarana intervention on total antioxidant capacity to elucidate its effectiveness in combating stress-induced oxidative damage (Fig. 7). Concerning the serum total antioxidant capacity (Fig. 7A), a significant effect was observed for preventive treatment ($p < 0.001$), indicating that the animals in the G group showed an increase of 53 % in this parameter (G vs. C; $p < 0.001$), whereas the GP group exhibited an increase of 31 % (GP versus P; $p = 0.003$).

In the evaluation of hepatic total antioxidant capacity (Fig. 7B), significant effects were observed for intoxication ($p = 0.01$), preventive treatment ($p = 0.03$), and their interaction ($p < 0.001$). Animals in the P group exhibited a 21 % decrease in total antioxidant capacity compared to those in the C group ($p = 0.010$). However, preventive treatment with guarana powder effectively prevented this reduction by 21 % in animals in the GP group compared the those in the P group ($p < 0.001$).

4. Discussion

Guarana is rich in bioactive compounds, mainly polyphenols [27, 28], with significant hepatoprotective potential and in vitro antioxidant capacity. Our study investigated the effects of preventive treatment with guarana powder on acute paracetamol-induced hepatotoxicity in rats through the modulation of oxidative stress. We observed that a toxic dose of paracetamol resulted in hepatic damage, which was prevented in animals administered guarana. Guarana powder demonstrated a

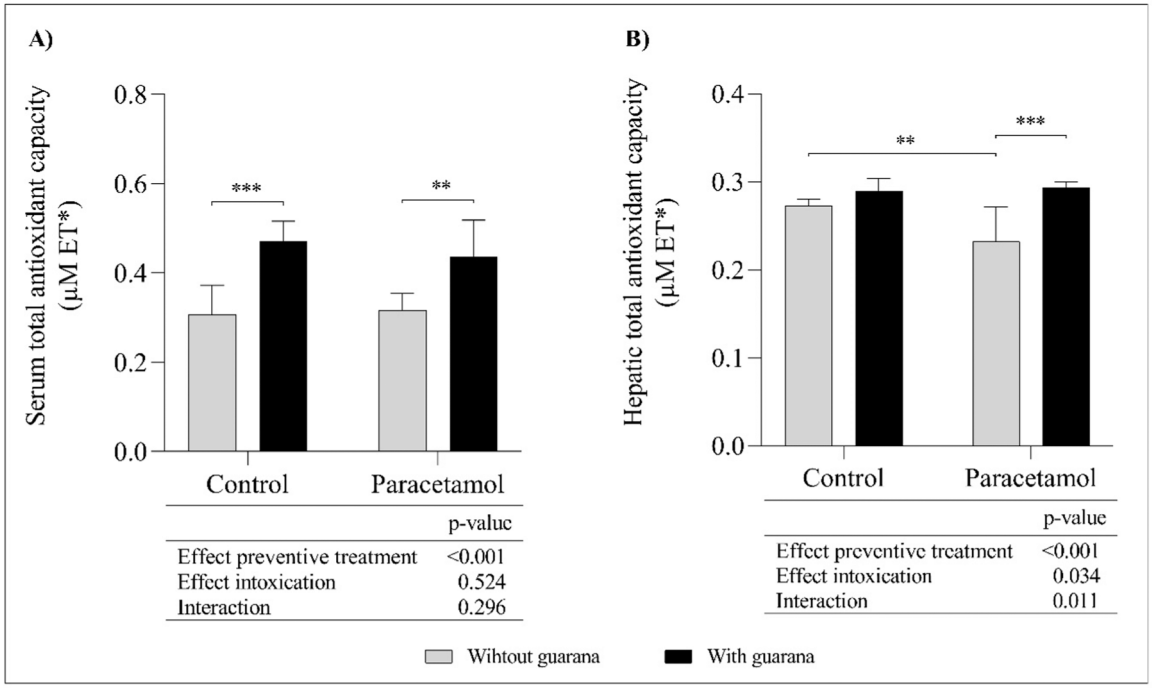

Fig. 7. Effect of preventive treatment with guarana powder and intoxication on total antioxidant capacity. A) Serum TEAC in μM; B) Hepatic TEAC in μM. Statistical significance was set at $p < 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Results are presented as mean ± standard deviation. TEAC, Trolox Equivalent Antioxidant Capacity.

protective effect by attenuating damage to proteins, modulating the glutathione system, and increasing antioxidant defense, leading to an overall enhancement in systemic and hepatic total antioxidant capacity.

It is widely documented in the literature that the ingestion of toxic doses of paracetamol is associated with the development of hepatotoxicity [27,28], being a medication significantly related to the increased incidence of ALF in the global population [9,36]. Among the mechanisms underlying ALF is oxidative stress, which disrupts cellular homeostasis and leads to hepatocytes death. These changes manifest as alterations in the activity of liver enzymes, used as biomarkers for liver damage [4]. In our study, we observed a significant increase in the serum activities of ALT, AST, and ALP in animals in paracetamol group, corroborating biochemical evidence of liver injury. Furthermore, to complement the analysis of liver damage induced by the toxic dose of paracetamol, was evaluated the key inflammatory mediator TNF, known for its role in triggering inflammation and promoting the progression of hepatotoxicity [6]. The results showed a significant increase in TNF concentrations in the P group, whereas guarana powder treatment effectively prevented this elevation. Histological analysis further supported our findings, revealing extensive hepatic necrosis in paracetamol-intoxicated animals, consistent with reports in the literature [38,5,6].

Hepatotoxicity induced by paracetamol is primarily attributed to the exacerbated production of the metabolite NAPQI, which triggers depletes glutathione reserves and generates oxidative stress. This imbalance leads to the formation of reactive species capable of damaging cellular components such as lipids and proteins [19,38]. In our study, intoxicated animals exhibited lipid and protein damage. Protein carbonylation, a crucial factor in paracetamol-induced hepatotoxicity, was significantly prevented in animals pretreated with guarana powder. This protective effect can be attributed to the polyphenols in guarana, which are known for their antioxidant and metal-chelating properties, mitigating oxidative stress and protein damage [11,18,33].

We also investigated the potential protective effects of guarana powder against oxidative stress induced by paracetamol, focusing on its impact on the GSH system. GSH present in the liver stands out as a crucial antioxidant in maintaining the balance of redox processes and in the detoxification of toxic liver metabolites, such as NAPQI [25,32]. Paracetamol poisoning resulted in a significant reduction in GSH concentration in the liver. However, guarana powder effectively prevented the reduction of GSH, reinforcing the hypothesis that its polyphenols help maintain redox balance and the integrity of the glutathione system.

Polyphenols have been shown to interact positively with CYP450 enzymes, which are responsible for converting paracetamol to NAPQI, [2,11]. Through the action on CYP450, polyphenols can reduce the production of NAPQI, thus alleviating oxidative stress and minimizing the need for glutathione to detoxify this harmful component. As a result, the reduced burden on the glutathione system helps maintain its availability for other cellular processes, which could explain the preserved glutathione levels observed in the guarana-pretreated group. Furthermore, catechins may increase phase II enzyme expression, further promoting detoxification of paracetamol and reducing NAPQI formation [3,24].

Oxidative stress plays a key role in paracetamol toxicity by triggering the activation of the Nrf2 pathway and its downstream targets, including antioxidant enzymes, such as SOD and CAT, which are responsible for maintaining the redox balance [4,38]. We observed an increase in the expression and activity of these antioxidant enzymes in animals exclusively exposed to paracetamol. Interestingly, this included significant upregulation of *MnSod* expression, a mitochondrial enzyme critical for combating oxidative damage. Conversely, animals pretreated with guarana did not increase *MnSod* expression, suggesting a mitigating effect of guarana on mitochondrial dysfunction, which is the primary target of excessive NAPQI binding [4,27].

In addition, animals treated with guarana powder showed reduced expression of *Cat*, consistent with our other findings. These results reinforce that capacity of guarana powder mitigates the reactive oxygen species (ROS) generated during paracetamol intoxication. The catechins and epicatechins in guarana powder, which contain hydroxyl groups, are capable of donating electrons to ROS under oxidative stress conditions, thereby stabilizing and reducing ROS levels [3]. Consequently, the decrease in SOD activity observed after preventive treatment with

guarana powder is likely correlated with reduced ROS levels, which may help explain the subsequent decrease in *Cat* enzyme expression.

Guarana is known for its high caffeine content, one of the most studied compounds in this fruit [16]. However, other bioactive compounds, such as catechins, and epicatechins [17], may also can act synergistically, enhancing its overall antioxidant capacity. In our study, we observed a significant increase in total antioxidant capacity in both the liver and serum of animals treated with guarana powder, supporting the idea that these compounds work together to mitigate liver damage by reducing oxidative stress, further establishing guarana as an important source of antioxidants.

To the best of our knowledge, this is the first study to assess guarana powder in the context of paracetamol toxicity, a global public health concern. One of the strengths of our study was the evaluation of the holistic effects of guarana powder, considering all its bioactive compounds rather than isolating specific components. Additionally, administration of the powder via orogastric gavage ensured precise dosing and enhanced experimental control. While our study yielded promising preventive results, it has limitations, such as the limited number of factors investigated. Specifically, we did not evaluate the CYP450 pathway, apoptosis, or the mitochondrial dysfunction. Future studies should explore these aspects to fully understand the potential of guarana powder. Moreover, the investigation of its impact on inflammation, considering the potential acute inflammatory components associated with paracetamol intoxication, warrants attention in future studies.

5. Conclusion

Overall, our study demonstrates that preventive treatment with guarana powder effectively mitigates acute paracetamol-induced hepatotoxicity in rats. This protection was primarily linked to guarana ability to modulate oxidative stress, as evidenced by the reduction in protein carbonylation, maintenance of the glutathione system, and an increase in total antioxidant capacity. Furthermore, guarana powder prevents the increase of enzymes associated with oxidative stress, suggesting its role in maintaining redox homeostasis. Our findings underscore the potential of guarana powder as a natural therapeutic agent for managing liver damage associated with oxidative stress.

Funding

This work was supported by Universidade Federal de Ouro Preto (UFOP), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001, the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG APQ- 01156–22) and the Pró-Reitoria de Pesquisa, Pós-Graduação e Inovação (PROPPI-UFOP). POB was supported by the São Paulo Research Foundation, Brazil (FAPESP), grant numbers 21/12010–7 and 23/07589–1.

CRediT authorship contribution statement

Fagundes Miliane Martins de Andrade: Writing – review & editing, Methodology. **Costa Daniela Caldeira:** Writing – review & editing, Resources, Methodology. **Lima Wanderson Geraldo:** Writing – review & editing, Resources, Methodology, Formal analysis. **Breguez Gustavo Silveira:** Writing – review & editing, Methodology. **Teixeira Clécia Dias:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Barbosa Priscila Oliveira:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Amaral Joana Ferreira:** Writing – review & editing, Supervision, Resources. **Souza Melina Oliveira de:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization. **de Brito Magalhães Cintia Lopes:** Writing – review & editing, Resources, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the Associação de Agricultores Familiares da Comunidade Ribeirinha e Tradicional de Jatuarana, part of Embrapa Occidental Amazon, for donating the guarana powder; the CCA UFOP for providing the animals (FINEP and FAPEMIG project APQ-02511–22); Quênia de Castro for handling the gavage; Tamara Anastácio, Camila dos Santos, Paloma Gonçalves, Carol Constantino, Mariana Vidigal, Míriam Silva, and Isabela de Deus for their assistance with animal handling; and Marília Menegato for support in the gene expression technique. We would like to thank Editage (www.editage.com.br) for English language editing.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2025.101946.

Data availability

Data will be made available on request.

References

[1] P.P. Barros, G.H. Silva, G.M.S. da, Gonçalves, J.C. Oliveira, L.G. Pagnan, L. Arco-e-Flexa, Hepatoprotective effect of quercetin pretreatment against paracetamol-induced liver damage and partial hepatectomy in rats, Braz. Arch. Biol. Technol. (2017) *60*, https://doi.org/10.1590/1678-4324-2016160138.

[2] L. Basheer, Z. Kerem, Interactions between CYP3A4 and dietary polyphenols, Oxid. Med. Cell. Longev. 2015 (2015) 854015, https://doi.org/10.1155/2015/854015.

[3] J. Bernatoniene, D.M. Kopustinskiene, The role of catechins in cellular responses to oxidative stress, Mol. A J. Synth. Chem. Nat. Prod. Chem. 23 (4) (2018) 965, https://doi.org/10.3390/molecules23040965.

[4] A.S. Chidiac, N.A. Buckley, F. Noghrehchi, R. Cairns, Paracetamol (acetaminophen) overdose and hepatotoxicity: mechanism, treatment, prevention measures, and estimates of burden of disease, Expert Opin. Drug Metab. Toxicol. 19 (5) (2023) 297–317, https://doi.org/10.1080/17425255.2023.2223959.

[5] A.M. Coelho, I.F. Queiroz, W.G. Lima, A. Talvani, L.O. Perucci, M. Oliveira de Souza, D.C. Costa, Temporal analysis of paracetamol-induced hepatotoxicity, Drug Chem. Toxicol. 46 (3) (2023) 472–481, https://doi.org/10.1080/01480545.2022.2052891.

[6] A.M. Coelho, I.F. Queiroz, L.O. Perucci, M.O. de Souza, W.G. Lima, A. Talvani, D. C. Costa, Piperine as therapeutic agent in paracetamol-induced hepatotoxicity in mice, Pharmaceutics 14 (9) (2022) 1800, https://doi.org/10.3390/pharmaceutics14091800.

[7] A. Dávalos, C. Gómez-Cordovés, B. Bartolomé, Extending applicability of the oxygen radical absorbance capacity (ORAC−Fluorescein) assay, J. Agric. Food Chem. 52 (1) (2004) 48–54, https://doi.org/10.1021/jf0305231.

[8] A.C.N. de Moraes, C.B.V. de Andrade, I.P.R. Ramos, M.L. Dias, C.M.P. Batista, C. F. Pimentel, J.J. de Carvalho, R.C. dos S. Goldenberg, Resveratrol promotes liver regeneration in drug-induced liver disease in mice, Food Res. Int. 142 (2021) 110185, https://doi.org/10.1016/j.foodres.2021.110185.

[9] H. Devarbhavi, S.K. Asrani, J.P. Arab, Y.A. Nartey, E. Pose, P.S. Kamath, Global burden of liver disease: 2023 update, J. Hepatol. 79 (2) (2023) 516–537, https://doi.org/10.1016/j.jhep.2023.03.017.

[10] C. Dias Teixeira, P. Oliveira Barbosa, M. Oliveira de Souza, Effects of guarana (Paullinia cupana) powder on obesity-associated diseases in animal models: a systematic review, J. Funct. Foods 112 (2024) 105944, https://doi.org/10.1016/j.jff.2023.105944.

[11] A. Duda-Chodak, T. Tarko, Possible side effects of polyphenols and their interactions with medicines, Molecules 28 (6) (2023) 2536, https://doi.org/10.3390/molecules28062536.

[12] EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. (2006). Metodologia científica: Determinação da atividade antioxidante total em frutas pelo método de redução do ferro (FRAP). 2006. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/664098/metodologia-cientifica-determinacao-da-atividade-antioxidante-total-em-frutas-pelo-metodo-de-reducao-do-ferro-frap.

[13] EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. (2007a). Metodologia científica: Determinação da atividade antioxidante total em frutas pela captura do radical livre ABTSo+. 2007. https://www.embrapa.br/busca-de-publicacoes/-/p

ublicacao/426954/metodologia-cientifica-determinacao-da-atividade-antioxidante-total-em-frutas-pela-captura-do-radical-livre-abts.
[14] EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. (2007b). Metodologia científica: Determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. 2007. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/426953/metodologia-cientifica-determinacao-da-atividade-antioxidante-total-em-frutas-pela-captura-do-radical-livre-dpph.
[15] H. Esterbauer, K.H. Cheeseman, Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal, Methods Enzymol. 186 (1990) 407–421, https://doi.org/10.1016/0076-6879(90)86134-h.
[16] D.F. Gonçalves, C.C. Tassi, G.P. Amaral, S.T. Stefanello, C.L. Dalla Corte, F. A. Soares, T. Posser, Jeferson L. Franco, N.R. Carvalho, Effects of caffeine on brain antioxidant status and mitochondrial respiration in acetaminophen-intoxicated mice, Toxicol. Res. 9 (5) (2020) 726–734, https://doi.org/10.1093/toxres/tfaa075.
[17] B. Gottwalt, P. Tadi, Methylxanthines. Em *StatPearls.*, StatPearls Publishing, 2022 http://www.ncbi.nlm.nih.gov/books/NBK559165/.
[18] O.M. Ighodaro, O.A. Akinloye, First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid, Alex. J. Med. 54 (4) (2018) 287–293, https://doi.org/10.1016/j.ajme.2017.09.001.
[19] H. Jaeschke, T.R. Knight, M.L. Bajt, The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity, Toxicol. Lett. 144 (3) (2003) 279–288, https://doi.org/10.1016/S0378-4274(03)00239-X.
[20] P. Kaur, R. Shergill, R.G. Mehta, B. Singh, S. Arora, Biofunctional significance of multi-herbal combination against paracetamol-induced hepatotoxicity in Wistar rats, Environ. Sci. Pollut. Res. Int. 28 (43) (2021) 61021–61046, https://doi.org/10.1007/s11356-021-15019-6.
[21] H. Kober, E. Tatsch, V.D. Torbitz, L.P. Cargnin, M.B. Sangoi, G.V. Bochi, A.R.H. da Silva, F. Barbisan, E.E. Ribeiro, I.B.M. da Cruz, R.N. Moresco, Genoprotective and hepatoprotective effects of Guarana (Paullinia cupana Mart. Var. Sorbilis) on CCl4-induced liver damage in rats, Drug Chem. Toxicol. 39 (1) (2016) 48–52, https://doi.org/10.3109/01480545.2015.1020546.
[22] M.O. Lee, Determination of the surface area of the white rat with its application to the expression of metabolic results, Am. J. Physiol. -Leg. Content 89 (1) (1929) 24–33, https://doi.org/10.1152/ajplegacy.1929.89.1.24.
[23] R.L. Levine, D. Garland, C.N. Oliver, A. Amici, I. Climent, A.G. Lenz, B.W. Ahn, S. Shaltiel, E.R. Stadtman, Determination of carbonyl content in oxidatively modified proteins, Methods Enzymol. 186 (1990) 464–478, https://doi.org/10.1016/0076-6879(90)86141-h.
[24] X. Li, S. Li, M. Chen, J. Wang, B. Xie, Z. Sun, −)-Epigallocatechin-3-gallate (EGCG) inhibits starch digestion and improves glucose homeostasis through direct or indirect activation of PXR/CAR-mediated phase II metabolism in diabetic mice, Food Funct. 9 (9) (2018) 4651–4663, https://doi.org/10.1039/C8FO01293H.
[25] T. Lőrincz, A. Szarka, The determination of hepatic glutathione at tissue and subcellular level, J. Pharmacol. Toxicol. Methods 88 (2017) 32–39, https://doi.org/10.1016/j.vascn.2017.05.004.
[26] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1) (1951) 265–275.
[27] G. Luo, L. Huang, Z. Zhang, The molecular mechanisms of acetaminophen-induced hepatotoxicity and its potential therapeutic targets, Exp. Biol. Med. 248 (5) (2023) 412–424, https://doi.org/10.1177/15353702221147563.
[28] L.L. Mazaleuskaya, K. Sangkuhl, C.F. Thorn, G.A. FitzGerald, R.B. Altman, T. E. Klein, PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses, Pharm. Genom. 25 (8) (2015) 416–426, https://doi.org/10.1097/FPC.0000000000000150.
[29] M.B. Medina, Simple and rapid method for the analysis of phenolic compounds in beverages and grains, J. Agric. Food Chem. 59 (5) (2011) 1565–1571, https://doi.org/10.1021/jf103711c.
[30] C. Nery, S. da, I.L. Pinheiro, G. Muniz, S. de, D.A.A. Vasconcelos, S.P. de de, França, E. do Nascimento, Medidas murinométricas e eficiência alimentar em ratos provenientes de ninhadas reduzidas na lactação e submetidos ou não ao exercício de natação, Rev. Bras. De. Med. do Esport. 17 (2011) 49–55, https://doi.org/10.1590/S1517-86922011000100010.
[31] Z. Papackova, M. Heczkova, H. Dankova, E. Sticova, A. Lodererova, L. Bartonova, M. Poruba, M. Cahova, Silymarin prevents acetaminophen-induced hepatotoxicity in mice, PLoS ONE 13 (1) (2018) e0191353, https://doi.org/10.1371/journal.pone.0191353.
[32] A. Ramachandran, H. Jaeschke, Mechanisms of acetaminophen hepatotoxicity and their translation to the human pathophysiology, J. Clin. Transl. Res. 3 (1) (2017) 157–169.
[33] A. Ramachandran, H. Jaeschke, Oxidant stress and acetaminophen hepatotoxicity: mechanism-based drug development, Antioxid. Redox Signal. 35 (9) (2021) 718–733, https://doi.org/10.1089/ars.2021.0102.
[34] G.R. Ramos-Vasconcelos, M. Hermes-Lima, Hypometabolism, antioxidant defenses and free radical metabolism in the pulmonate land snail Helix aspersa, J. Exp. Biol. 206 (Pt 4) (2003) 675–685, https://doi.org/10.1242/jeb.00124.
[35] E. Sinaga, A. Fitrayadi, A. Asrori, S.E. Rahayu, S. Suprihatin, V.D. Prasasty, Hepatoprotective effect of Pandanus odoratissimus seed extracts on paracetamol-induced rats, Pharm. Biol. 59 (1) (2021) 31–39, https://doi.org/10.1080/13880209.2020.1865408.
[36] N.J. Thusius, M. Romanowicz, J.M. Bostwick, Intentional or inadvertent acetaminophen overdose—how lethal it really is? Psychosomatics 60 (6) (2019) 574–581, https://doi.org/10.1016/j.psym.2019.05.001.
[37] E.A.F.S. Torres, A.C.D.C. Pinaffi-Langley, M.S. Figueira, K.S. Cordeiro, L.D. Negrão, M.J. Soares, C.P. da Silva, M.C.Z. Alfino, G.R. Sampaio, A.C. de Camargo, Effects of the consumption of guarana on human health: a narrative review, in: Comprehensive Reviews in Food Science and Food Safety, 21, Medline, 2022, pp. 272–295, https://doi.org/10.1111/1541-4337.12862.
[38] M. Yan, Y. Huo, S. Yin, H. Hu, Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions, Redox Biol. 17 (2018) 274–283, https://doi.org/10.1016/j.redox.2018.04.019.