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Chronic acrolein exposure in Wistar rats: The effects of guarana extracts

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ABSTRACT

Previous studies have reported that acrolein, may exert harmful effects on the brain. However, information regarding the neuroprotective properties of guarana against acrolein is not available. Due to the lack of research, we initiated the current study to investigate the effects of guarana extracts on acrolein-induced toxicity in the liver and the central nervous system of Wistar Rats. Twelve groups of 60 days old Wistar rats treated with guarana extracts (150, 250, and 350 mg/kg/day) for 8 weeks, were challenged with acrolein (2.5 mg/kg/day). Several parameters associated with oxidative damage to the brain and hepatic function, as well as behavior were evaluated. All tested concentrations of guarana extracts exerted protective effects against acrolein induced damage. No hepatic and oxidative damages or behavioral changes were observed in guarana control groups. To the best of our knowledge, this is the first study of its kind and therefore a milestone in this field.

1. Introduction

Acrolein (ACR), the IUPAC name of which is 2-propenal, is a highly electrophilic α, β-unsaturated aldehyde. It is a colorless to yellowish flammable liquid with an irritating odor at room temperature. Acrolein is used as an indispensable intermediate in the industrial-scale production of many organic chemicals/materials (Faroon, Roney, Taylor, & Ashizawa, 2008a). In addition to amounts released through various industrial processes, its environmental presence is principally attributed to incomplete combustion of organic matter, including petrol, coal, plastics, wood material, and tobacco. ACR is mainly found in food items, such as cocoa beans, chocolates, liquor, and fried potatoes, or in volatiles generated during the thermal treatment of animal or vegetable fat at high temperatures (Ewert, Granvogl, & Schieberle, 2014; Faroon, Roney, Taylor, & Ashizawa, 2008b). Furthermore, ACR is regarded as an endogenous product of myeloperoxidase-mediated degradation of threonine, amine oxidase-mediated degradation of spermine and spermidine (O'Brien, Siraki, & Shangari, 2005) during the metabolism of alkylating oxazaphosphorines (anticancer drugs), and free radical-initiated lipid peroxidation of polyunsaturated fatty acids in cell membranes (Pan, Kaneko, Ushio, & Ohshima, 2005; Uchida, 1999).

ACR has been suggested as the strongest electrophile among all α, β-unsaturated aldehydes. Therefore, its strong toxicity is attributed to extremely high reactivity towards biological nucleophiles (Burcham,

Abbreviations: ACR, acrolein; ALE, Advanced Lipid Peroxidation End Product; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BSA, Bovine Serum Albumine; GGT, Gamma Glutamyl transferase; GO, glyoxal; GSH, reduced glutatione; GPx, glutathione peroxidase; GST, Glutathione Transferase; LDH, Lactate Dehydrogenase; MGO, methylglyoxal; MDA, malondialdehyde; NAC, N-Acetyl-cysteine; OFT, Open Field Test; PYC, pycnogenol; ROS, Reactive Oxygen Species; SH, Reduced Thiols; TBARS, thiobarbituric acid reactive species

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Kaminskas, Tan, & Pyke, 2008). Due to its solubility in water, alcohol and diethyl ether, ACR easily migrates across cell membranes via passive diffusion (Stevens & Maier, 2008). Extensive studies have been conducted to establish the toxicological profile of ACR in cells, tissues and animals. In general, ACR exerts its toxic effects through the disruption of protein/DNA function by direct addition, lowering of intracellular glutathione (GSH) levels and interference in cell signaling pathways, with particular reference to signaling in redox pathways (Stevens & Maier, 2008).

Kingdom Plantae is considered one of the largest and most diverse sources of bioactive molecules. Plants used in folk medicine have provided a basis for the discovery and characterization of several drugs that are currently being used clinically. The worldwide use of folk medicinal plants is significant. World Health Organization (WHO) data indicate that approximately 80% of the world's population uses herbal plants to relieve diverse painful or unpleasant symptoms. In addition, several plants are usually consumed *in natura* or as dietary supplements.

Polyphenols have been investigated as direct trapping agents of dicarbonyls (Glyoxal - GO and Methyl Glyoxal - MGO); (Beretta, Furlanetto, Regazzoni, Zarrella, & Facino, 2008; Lo, Li, Tan, & Pan, 2006; Shao, Bai, He, & Ho, 2008). Similar polyphenols have also been documented as effective trapping agents of ACR (Pochernich, Lange, Sultana, & Butterfiled, 2011). In a screening of 21 natural polyphenols, it was found that nine (in concentrations ranging 0.5–1 mM) directly reduced ACR activity by 27.4–99.6% under simulated physiological conditions of 0.01 M PBS, pH 7.4, 37 °C and 90 min of incubation. These 9 effective polyphenols were epicatechin, epigallocatechin, epicatechin-3-gallate, epigallocatechin-3-gallate, theaflavin, theaflavin-3,3-digallate, cyanomaclurin, phloretin and phloridzin. Of these, phloretin was the most powerful scavenger. Evaluation of the relationship between its structure and activity showed that a phloroglucinol moiety, usually termed an A ring, was shared among all effective scavengers. This suggested the importance of this scaffold for trapping ACR. Furthermore, the substitution of any hydroxyl group in the A ring weakened electrophilic aromatic substitution, thereby lowering the trapping effect (Pochernich et al., 2011).

Guarana (*P. cupana*), a bushy plant found in the rainforest of the amazon basin, is cultured using its caffeine-polyphenol rich-seeds (Smith, Cappai, & Barnham, 2007), which constitute the most physiologically active ingredient in many energy drinks. The US Food and Drug Administration (FDA) also consider guarana a safe dietary supplement (Duchan, Patel, & Feucht, 2010). Despite being considered a safe supplement, not much information is available regarding its bioactive compounds and their biological properties. Although most guarana bioactivity is attributed to the caffeine content of the extract, a growing number of scientific studies indicate that several other biologically active components may be at play. In contrast, it has not been established whether these different properties are due to caffeine alone or whether other compounds present in guarana seeds may be involved. In a previous characterization study, we identified catechin, epicatechin and epicatechin gallate as the main polyphenols (flavan-3-ols) present in guarana powder (Bittencourt et al., 2014). All these polyphenols are known to be powerful antioxidants. Furthermore, the combined synergistic effect of all components of the extract, as represented by the synergic effect of polyphenol and xanthine contents, may have to be taken into consideration.

In fact, previous studies have reported the anti-bacterial (Basile et al., 2005), antioxidant (Bittencourt et al., 2013, 2014; Portella et al., 2013; Yamaguti-Sasaki et al., 2007; Zeidán-Chuliá et al., 2013), thermogenic (Subbiah & Yunker, 2008), chemo-preventive and anti-mutagenic (Fukumasu et al., 2006) activities of guarana extracts. Previously, we have demonstrated that acrolein is able to induce oxidative stress and loss of viability in a neuronal-like model of SHSY-5Y cells (Bittencourt et al., 2014). Furthermore, other groups have demonstrated that acrolein is capable of inducing oxidative damage in *in vitro* cell lines as well as in the central nervous system (CNS) of a rat model *in vivo*, leading to cognitive impairment (Huang et al., 2013). Evidently, chronic acrolein exposure is associated with neurodegenerative diseases, in which oxidative or nitrosative stress play a pivotal role, as seen in the onset and progression of Alzheimer's Disease (AD) and Parkinson's Disease (PD); (Huang et al., 2013; Mizoi et al., 2014; Sultana, Perluigi, & Butterfield, 2013; Yoshida et al., 2015). In this context, supplementation with antioxidants such as vitamin E, ascorbic acid, omegas and glutathione precursors has been extensively studied during the last few decades. Previously, our research group has also demonstrated the protective effects of guarana against acrolein-mediated cytotoxicity and oxidative stress in the neuronal-like SHSY-5Y cell line (Lo et al., 2006). Due to the intrinsic capability of producing a variety of antioxidant compounds and mixtures through secondary metabolism, natural foods have been considered as promising alternatives for the modulation or attenuation of oxidative stress and associated deleterious effects related to chronic diseases and exposure to environmental pollutants (Pochernich et al., 2011; Zhu, Sun, Jiang, Chen, & Wang, 2011).

It is evident that a range of guarana biological activities remains to be investigated. However, the current study aimed to provide important information regarding the effect of guarana dietary supplements against acrolein-mediated neurotoxicity in an *in vivo* experimental model. We performed several biochemical analyses of oxidative parameters in 4 different regions of the CNS of Wistar rats, and conducted hepatic toxicity and behavioral analyses as well, in order to better understand the protective effects exerted by guarana.

2. Material and methods

The study protocol (project number 25776) was reviewed and approved by the Ethics Committee on Animal Research of the Federal University of Rio Grande do Sul – Porto Alegre/Brazil. All experimental procedures were performed in accordance with the recommendations of the Brazilian Society of Laboratory Animal Science (SBCAL-COBEA) and the US National Institute of Health (U. S. National Institute of Health, Guide for the Care and Use of Laboratory Animals 8th Edition, 2011).

2.1. Chemicals

Guarana (*P. cupana* Mart.) extract powder was obtained from Lifar Ltda. (Porto Alegre, RS, Brazil). All other chemicals were purchased from Sigma-Aldrich©, St. Louis, MO, USA.

2.2. Animals

Because 60 day old male Wistar rats (*Rattus norvergicus*) exhibited responses that were very similar to those exhibited by young adults, which were probably the best responses to acrolein-induced toxicity in this subpopulation, 60 d old rats were selected as a suitable experimental model. Experimental animals were obtained from our breeding colony and kept in groups of 4–5 in polypropylene cages (approximately 41 × 34 × 16 cm) with wire bar lids and pine shaving as bedding (Maravalha Rossa Ltda., Porto União, SC, Brazil) under standard conditions of a 12 h light–dark cycle (lights off at 7:00 PM), constant temperature (22 ± 4 °C) and humidity (30–70%), with free access to water and food *ad libitum*.

The Rats were randomly assigned to 12 experimental groups and treated for 8 weeks via orogastric gavage.

2.3. Experimental groups and treatments

The 12 groups consisted of 7 groups as follows: 0.9% saline (control group, n = 12), acrolein 2,5 mg/kg (n = 10), guarana 150 mg/kg (n = 12), 250 mg/kg (n = 12), 350 mg/kg (n = 12), caffeine 14 mg/kg (n = 12), and N-Acetyl-cysteine (NAC) 50 mg/kg (n = 12); and another 5 groups, which received the same treatment plus acrolein 2,5

mg/kg, as follows: guarana 150 mg/kg plus acrolein (n = 12), 250 mg/kg plus acrolein (n = 12), 350 mg/kg plus acrolein (n = 12), caffeine 14 mg/kg plus acrolein (n = 12), and N-Acetyl-cysteine (NAC) 50 mg/kg plus acrolein (n = 12).

The following events are considered to be noteworthy: (i) 2 animals from acrolein and NAC plus acrolein groups died during the 8 week treatment period; (ii) groups treated with acrolein plus guarana, caffeine or NAC, received these pre-treatments prior to acrolein at 8 AM and (iii) the caffeine and other methylxanthines content, polyphenols of guarana extract were determined by HPLC in our previous study (Bittencourt et al., 2014).

Acrolein, guarana, caffeine and N-acetyl-cysteine were prepared daily in 0.9% saline, away from light and heat. Animals were weighed weekly, and 0.5 mL was the maximum volume used for gavage. Animals were exposed to acrolein at the beginning of the dark phase (8:00 PM) as the compound is best absorbed during this period. Appropriate action was taken to minimize pain and discomfort to animals. Due it volatility, acrolein was freshly dissolved in saline 0.9% prepared daily and used within 30 min. Previous study estimated the maximal human daily unsaturated aldehyde consumption to be 5 mg/kg/day (Wang et al., 2008). Based on these estimates, and with the intent of using acrolein as a representative unsaturated aldehyde, we tested the chronic effects of 2.5 mg/kg/day. Acrolein, representing 50% of the expected overall unsaturated aldehyde intake. Animals were gavage-fed with acrolein (in 500 μL saline 0.9%, n = 12) daily for 8 weeks (Huang et al., 2013). It is important to mention that up to 3 mg/kg/day the animal survival rate are about 1 day (Wang et al., 2008).

2.4. Behavioral parameters

The Open Field Test (OFT) is widely used to elucidate drug-induced behavior in rodents; it allows simultaneous assessment of general locomotor activity, novel environment exploration, and anxiety-related behavior (Buccafusco, 2009). OFT was conducted during the light phase of the last day of treatment (between 8:00 and 11:00 AM) in an 80 cm sq. arena with 40 cm high walls. During the test, all animals were individually and gently placed on the apparatus and allowed to freely explore it for 5 min and returned to their home cages. All tests were recorded with ANY-maze Video tracking Software (Stoelting Co., Wood Dale, IL, USA), wherein a number of conventional and etiological parameters were collected during each session. The total distance travelled was analyzed as a parameter of general locomotor activity. Activity time and number of rearing were analyzed as parameters of novel environment exploration. Time and the distance travelled in the periphery area, the time and distance travelled in the center area, and number of entries into the center area (entries into the center of the apparatus by placing all 4 paws) were analyzed as parameters for novel environmental exploration and anxiety-related behavior. Freezing time, number of periphery area returns (enters the center area with just the front paws and returns to the periphery) were analyzed as parameters of anxiety-related behavior.

2.5. Biochemical analyses

Animals were euthanized through decapitation 24 h after administering the last treatment. Immediately following decapitation, blood was collected in BD Vacutainer® rapid serum tubes and then centrifuged (1000*g*, 5 min), serum was collected and hepatic enzymes activity was determined, according item 2.5.1.

The liver, the cerebral cortex, hypothalamus, hippocampus, and striatum of each animal were separated, through dissection on ice, and immediately stored at −80 °C. For analytical purposes, tissues were homogenized in 50 mM PBS at a pH of 7.4 and centrifuged to remove cell debris (12,000*g*, 10 min), following which the supernatant was collected. All results were normalized for protein content using BSA as the standard.

Determination of sample protein contents was performed using a BioRad© Protein Assay kit.

2.5.1. Hepatic parameters

Activity of the hepatic enzymes, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Lactate Dehydrogenase (LDH) and Gamma Glutamyl transferase (GGT) in the serum, were determined using commercial kits (Labtest®) according manufacturer's instructions.

2.5.2. Redox status in biomolecules (proteic GSH, non-proteic GSH and lipids)

Total reduced thiol (SH) content was estimated according to the overall redox status of the cellular environment. Briefly, samples were mixed in a slightly alkaline medium with 10 mM 5,5-dithiobis-2-nitrobenzoic acid prepared in ethanol. SH content was determined after 60 min by observing absorbance at 412 nm and results were expressed as μmol SH/mg protein. To determine the GSH (reduced glutathione) content, the sample was deproteinized with 10% trichloroacetic acid and centrifuged at 10,000*g* for 10 min, following which the supernatant was mixed with 10 mM 5,5-dithiobis-2-nitrobenzoic acid prepared in ethanol. GSH content was determined after 60 min as absorbance at 412 nm and results were expressed as μmol GSH/mg protein. Formation of thiobarbituric acid reactive species (TBARS) was measured as an index of lipid peroxidation. The purpose of this assay is to evaluate the production of malondialdehyde (MDA), which means TBARS is an indirect indicative of lipid damage. Briefly, samples were deproteinized with 10% trichloroacetic acid and the supernatant was heated with 0.67% thiobarbituric acid for 25 min. TBARS level was determined by absorbance at 532 nm and results were expressed as nmol TBARS/mg protein.

2.5.3. Statistical analysis

All Biochemical data were assessed for underlying distributions through the Kolmogorov-Smirnov test. Parametric data were analyzed using one-way or two-way analysis of variance (ANOVA) followed by an adequate post-hoc test. Body weight was analyzed using repeated measures two-way ANOVA followed by the Bonferroni's post hoc test. Supplementation and animal age were considered analysis factors. Biochemical and behavioral data were analyzed using one-way ANOVA followed by Tukey's post hoc test. All data were analyzed using GraphPad Prism Software v.7.0 (GraphPad Software Inc, San Diego, CA, USA). Results were expressed as the mean ± SEM. Statistical significance was set at $p \leq 0.05$.

3. Results

3.1. Guarana attenuates acrolein-induced body weight (BW) loss

Chronic administration of 150, 250 and 350 mg/kg/day guarana, 14 mg/kg/day of caffeine or 50 mg/kg/day of NAC *per se*, did not cause body weight loss in Wistar Rats when compared to the control. However, acrolein induced significant body weight loss compared to the control, and this loss was significantly attenuated in animals simultaneously treated with guarana, caffeine or NAC plus acrolein (n = 12) and N-Acetyl-cysteine (NAC); (Tables 1 and 2).

Experimental animals treated with acrolein for 8 weeks differed from the control and other treatment groups. No difference in body weight was observed in experimental animals treated with *P. cupana*, CAF or NAC compared to the control group. Results are expressed as the mean ± SD (n = 12 for animals/control, *P. cupana*, CAF and NAC; n = 10 for ACR group). Different letters denote significant differences ($p < 0.05$; Two-way ANOVA; Bonferroni's post hoc test). ***a.*** Statistical difference from control, ***b.*** Statistical difference from *P. cupana*, CAF and NAC administered alone.

Results are expressed as the mean ± SD (n = 12 animals; *P. cupana*, CAF plus ACR and NAC plus ACR; n = 10). Different letters

Table 1
Body weight of *P. cupana*, CAF and NAC administered alone versus control and ACR in Wistar rats.

	Control	ACR	*P. cupana extracts*			CAF	NAC
mg/kg/day	0	2,5	150	250	350	14	50
N	12	10	12	12	12	12	12
week 1	243 ± 10	253 ± 8	250 ± 13	255 ± 9	248 ± 15	246 ± 10	256 ± 11
week 2	278 ± 8	257 ± 9***ab***	276 ± 14	281 ± 10	287 ± 8	275 ± 9	280 ± 12
week 3	303 ± 11	258 ± 8***ab***	296 ± 11	292 ± 11	290 ± 9	290 ± 8	296 ± 6
week 4	318 ± 12	255 ± 9***ab***	316 ± 17	312 ± 12	305 ± 10	312 ± 11	313 ± 10
week 5	339 ± 11	250 ± 10***ab***	329 ± 10	328 ± 17	322 ± 11	324 ± 13	330 ± 10
week 6	353 ± 8	248 ± 10***ab***	348 ± 9	349 ± 11	342 ± 14	345 ± 14	349 ± 9
week 7	387 ± 11	241 ± 10***ab***	374 ± 11	377 ± 8	380 ± 9	376 ± 17	379 ± 12
week 8	402 ± 9	238 ± 11***ab***	398 ± 14	394 ± 10	392 ± 10	391 ± 11	380 ± 9

denote significant differences ($p < 0.05$, Two-way ANOVA, Bonferroni's post hoc test. ***a.*** Different from control, ***c.*** Different from ACR group.

3.2. Guarana prevents acrolein-induced hepatic damage

Hepatic enzymes is the first parameter which must be investigated when a drug or compound is suspected of inducing toxicity. Acrolein significantly increased all tested serum transaminases, while simultaneous treatment with guarana or caffeine was effective in reducing these enzymes at all tested doses (Fig. 1; $p \leq 0.01$) as compared to the acrolein group. Notably, neither guarana nor caffeine nor NAC administered alone caused any increase in tested serum transaminases compared to the saline group (Fig. 1).

3.3. Guarana, caffeine and NAC attenuated acrolein-induced damage in redox biomolecules

Chronic treatment with acrolein induced oxidative damage in the livers of Wistar rats by increasing MDA and decreasing SH and GSH levels, respectively, when compared to the control group (Fig. 2, $p \leq 0.0001$). Treatment with guarana decreased MDA, followed by an increase in GSH and total SH levels compared to the acrolein groups ($p \leq 0.05$). Caffeine and NAC groups also showed protective effects against acrolein-induced oxidative damage ($p \leq 0.05$; Fig. 2). At the same time, guarana, caffeine and NAC at tested doses, did not cause any oxidative damage (Fig. 2).

It is known that acrolein causes oxidative damage to the CNS. In the acrolein group, all tested regions exhibited high MDA and low SH and GSH levels respectively, indicating significant oxidative damage when compared to the control group ($p \leq 0.05$; Fig. 3), and many of these regions were known to be involved in neurodegenerative diseases. All guarana doses decreased oxidative damage induced by acrolein, mainly in the hippocampus, hypothalamus and cortex ($p \leq 0.01$; Fig. 3). Caffeine also exerted protective effects as compared to the acrolein groups ($p \leq 0.01$; Fig. 3). All tested guarana, Caffeine and NAC treatments, administered alone, did not exert oxidative damage, as compared to the control group (Fig. 3).

3.4. Effects of guarana and caffeine in behavioral parameters

Chronic administering of acrolein impaired all exploratory and anxiety-related behavior of Wistar rats ($p \leq 0.05$; Fig. 4) as compared to the control. Animals treated with guarana and caffeine exhibited a significant improvement in exploratory activity, while there was a decrease in anxiety-related behavior ($p \leq 0.05$; Fig. 4) when compared to those in the group treated with acrolein. Caffeine and NAC group also showed significant improvements in behavioral parameters when compared to the acrolein group (Fig. 4). Guarana, Caffeine or NAC, administered alone, did not alter such behavior, when compared to the control (Fig. 4).

4. Discussion

The current study investigated the protective effect induced by commercial extracts of guarana on acrolein-induced toxicity. Chronic administration of 150, 250 or 350 mg/kg/day of guarana, 14 mg/kg/day of caffeine, or 50 mg/kg/day of NAC attenuated acrolein-induced loss of body weight (Table 1) as well as hepato- and neuro-toxicity (Figs. 1–3). Body weight and hepatic enzymes are frequently used as a marker of drug toxicity. ALT, AST, LDH and GGT are expressed by hepatocytes; ALT and LDH is secreted into the cytoplasm, whereas AST is mainly produced in the mitochondria of hepatocytes and finally GGT are located in cell plasma membrane. Damage to cells due to hepatitis, myocarditis, and pancreatitis induces ALT, LDH and GGT to enter the bloodstream leading to an increase of these enzymes beyond reference values. However, during severe damage, AST also enters the bloodstream (Ahmed, Abdelrahman, & Salama, 2017; Pan, Long, Yi, & Zhao, 2018). Thus, a significant increase in ALT, LDH, AST and GGT levels indicates liver damage (Maksymchuk, Shysh, Rosohatska, & Chashchyn, 2017). The analysis of these parameters indicated that commercial guarana extracts attenuated the toxic effects induced by acrolein. There

Table 2
Body weight of experimental animals treated with *P. cupana*, CAF and NAC plus ACR, versus the control and ACR groups (Table 1) in Wistar rats.

	P. cupana extracts plus ACR			CAF plus ACR	NAC plus ACR
mg/kg/day	150	250	350	14	50
N	12	12	12	12	10
week 1	248 ± 8	250 ± 9	255 ± 9	250 ± 10	258 ± 17
week 2	270 ± 9	263 ± 8	271 ± 10	264 ± 11	269 ± 12
week 3	276 ± 8***ac***	275 ± 8***ac***	276 ± 9***ac***	280 ± 9***ac***	278 ± 10***ac***
week 4	282 ± 9***ac***	277 ± 11***ac***	286 ± 10***ac***	291 ± 10***ac***	290 ± 12***ac***
week 5	290 ± 10***ac***	291 ± 11***ac***	295 ± 11***ac***	297 ± 9***ac***	299 ± 11***ac***
week 6	300 ± 9***ac***	304 ± 10***ac***	303 ± 9***ac***	308 ± 11***ac***	310 ± 12***ac***
week 7	309 ± 12***ac***	312 ± 8***ac***	308 ± 10***ac***	314 ± 10***ac***	316 ± 10***ac***
week 8	315 ± 10***ac***	320 ± 11***ac***	315 ± 11***ac***	322 ± 11***ac***	320 ± 10***ac***

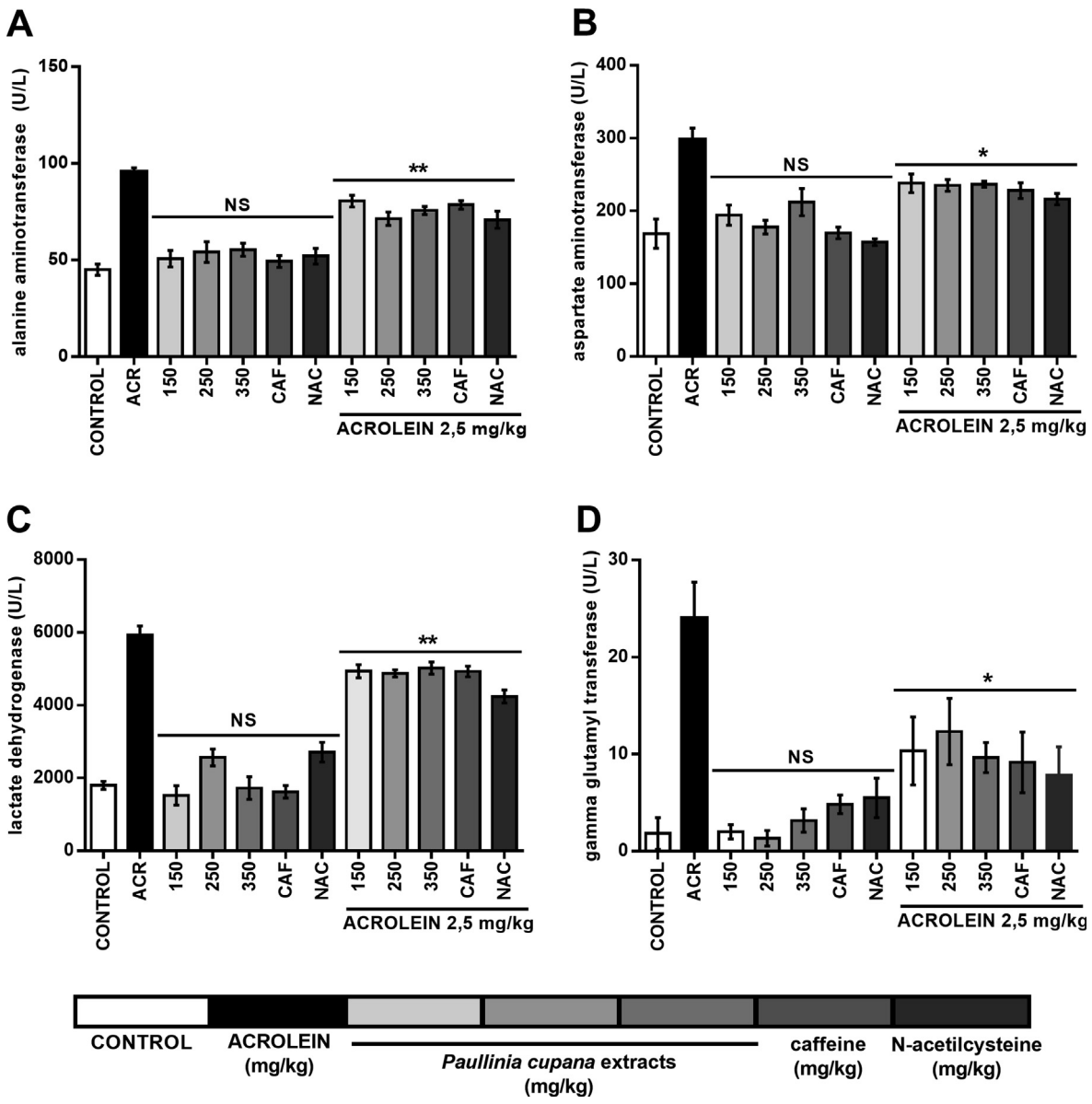

Fig. 1. Hepatic enzymes were analyzed to evaluate ACR-induced hepatotoxicity. (A) Alanine aminotransferase, (B) Aspartate aminotransferase, (C) Lactate Dehydrogenase, and (D) Gamma Glutamyl Transferase. Results are expressed as the mean ± SEM. **NS** denotes no difference related to the control. * and ** symbols denote significant changes related to the ACR group ($p < 0.05$ and $p < 0.01$ respectively, one-way ANOVA Tukey's post hoc test).

is evidence that phenolic content (catechin, epicatechin, epicatechin and tanins) and caffeine, it major compound, are the main responsible for the observed effects (Fukumasu et al., 2006; Kleber Silveira et al., 2018). One reason could be pointed out for acrolein-iduced body weight loss is the disruption of tight junctions proteins and endoplasmic reticulum stress-mediated cell death (due severe oxidative damage) leading to loss of integrity in intestinal epithelium, inflammatory response inducing mucositis (Chen et al., 2017; Thomsen, Clarke, & Vitetta, 2018). This scenario causes pain to the animal and prevents appropriate digestion and absorption of nutrients and thus losing body weight. In this direction, polyphenols and caffeine (both contained in guarana extracts) are powerful acrolein scavengers, protecting this way the stomach and gut mucosa.

ROS production is a natural phenomenon that occurs in physiological processes. ROS may be increased in response to certain cytotoxic agents, by way of downstream signaling pathways (Naoi et al., 2005). Excessive ROS production may be toxic, causing membrane damage and activating cell death related pathways through protein oxidation, lipid peroxidation and DNA damage (Akbar et al., 2016; Ansari et al., 2006). Oxidative stress is associated with neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), which deplete the antioxidant system and increase lipid peroxidation products (Padurariu et al., 2013). ACR is one such lipid peroxidation product (ALE). ACR may induce oxidative stress (Park et al., 2005), by reacting with proteins, phospholipids, and DNA, forming stable Michael adducts (Faroon et al., 2008a, 2008b). This compound plays an important role in the development of oxidative damage and, consequently, in the pathogenesis of certain diseases, such as AD. The mechanism(s) by which acrolein causes oxidative damage and neurotoxicity are not well defined, but increasing evidence indicates that this alkenal primarily binds and depletes cellular nucleophiles, such as reduced glutathione (GSH), lipoic acid and thioredoxin (Padurariu et al., 2013). Acrolein attacks free thiol (SH) groups of cysteine residues, γ-amino groups of lysine residues and histidine residues, resulting in an acrolein-amino acid adduct, which impairs the function of selected proteins by introducing a carbonyl group (Pocernich & Butterfield, 2012). It is estimated that humans are exposed to approximately 2.5–7 mg/kg/day of ACR due to environmental factors (Huang et al., 2013). For this reason,

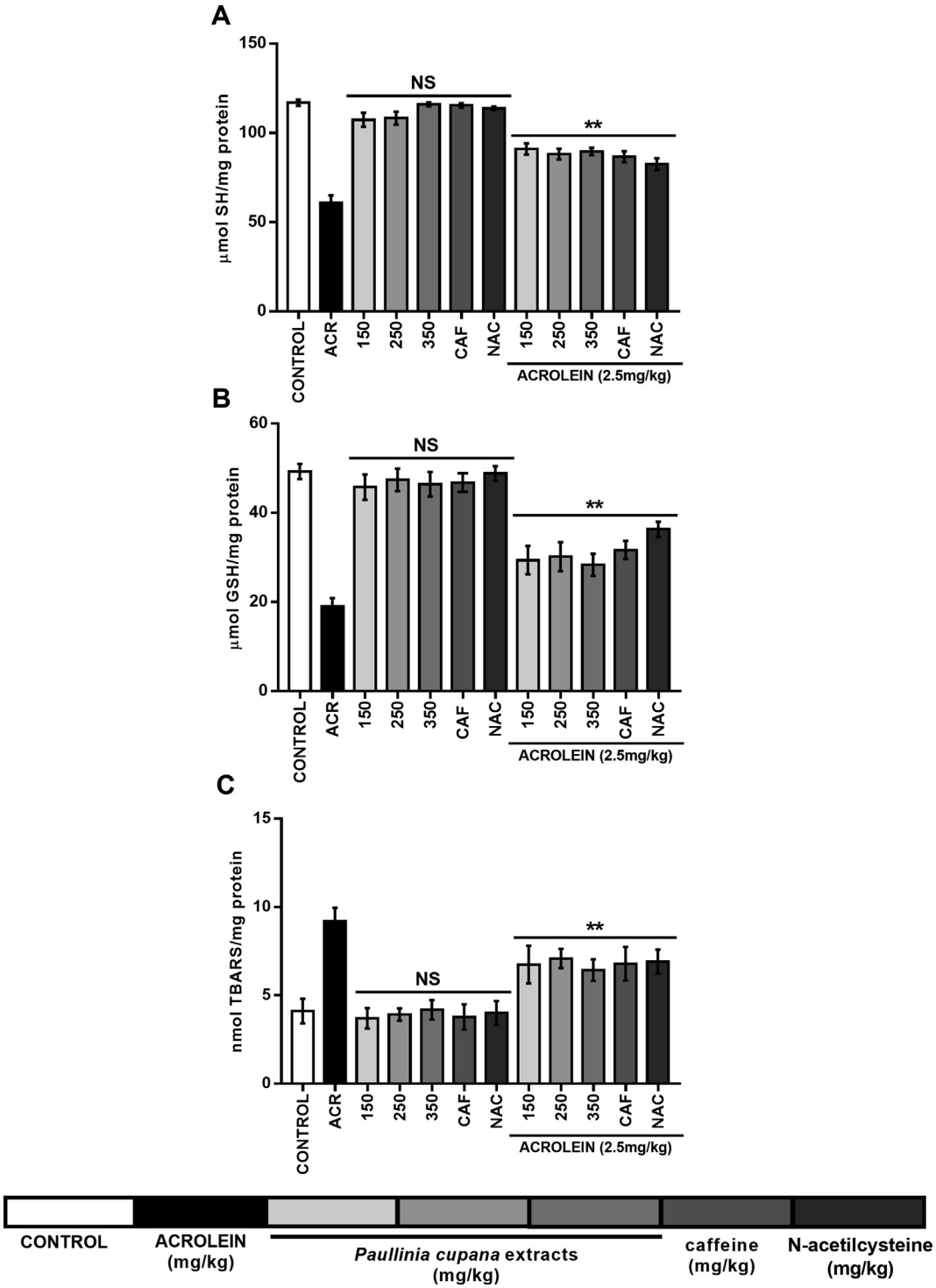

Fig. 2. Redox profile of biomolecules in the liver. (A) Total reduced thiol content, (B) reduced glutathione content, and (C) thiobarbituric acid-reactive species level. Results are expressed as the mean ± SEM. **NS** denotes no difference related to control. * and ** symbols denote significant changes related to the ACR group ($p < 0.05$ and $p < 0.01$, respectively; one-way ANOVA; Tukey's post hoc test).

Wistar rats were subjected to chronic administering of 2.5 mg/kg/day of ACR for 8 weeks. This led to an increase in lipid peroxidation, a decrease in GSH, a reduction of SH in the liver and an altering of CNS associated behavioral patterns in the form of a prevalence of anxiety/depression and decreased locomotor activity, which are all symptoms of AD (Alzheimer's Association, 2015).

The ubiquitous existence of natural polyphenolic substances in many plants makes these compounds good candidates for promoting human health. Polyphenolic compounds were traditionally used as antioxidants as well as chelators of free radicals (such as oxygen ions and peroxides) and metal ions (Ansari et al., 2006). Recent emergence of polyphenols as ACR scavengers has focused interest on the complicated interaction between polyphenols and the cascade of intermediates formed during lipid peroxidation, as well as its end products, such as α, β-unsaturated aldehydes. This suggests multiple roles for polyphenols in human health management. Amazon guarana is a polyphenol-rich plant that may exert many beneficial effects on human health, but, its neuroprotective properties against acrolein-induced toxicity remain

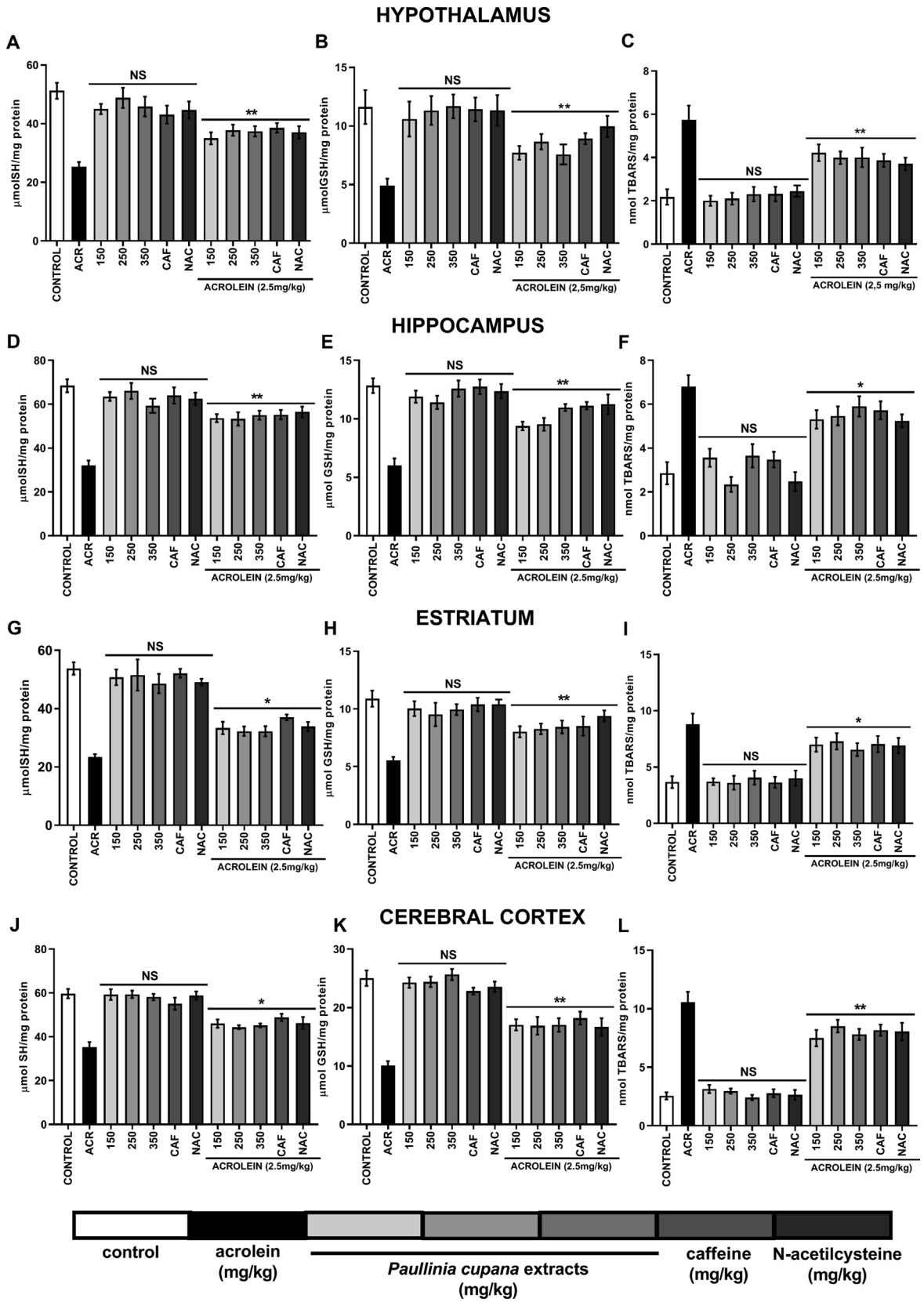

Fig. 3. Redox profile of biomolecules in four regions of the CNS. (A) Total reduced thiol content, (B) reduced glutathione content, and (C) thiobarbituric acid-reactive species level in the hypothalamus. (D) Total reduced thiol content, (E) reduced glutathione content, and (F) thiobarbituric acid-reactive species level in the hippocampus. (G) Total reduced thiol content, (H) reduced glutathione content, and (I) thiobarbituric acid-reactive species level in the striatum. (J) Total reduced thiol content, (K) reduced glutathione content, and (L) thiobarbituric acid-reactive species level in the cerebral cortex. Results are expressed as the mean ± SEM. **NS** denotes no difference related to the control. * and ** symbols denote significant changes related to the ACR group ($p < 0.05$ and $p < 0.01$, respectively, one-way ANOVA; Tukey's post hoc test).

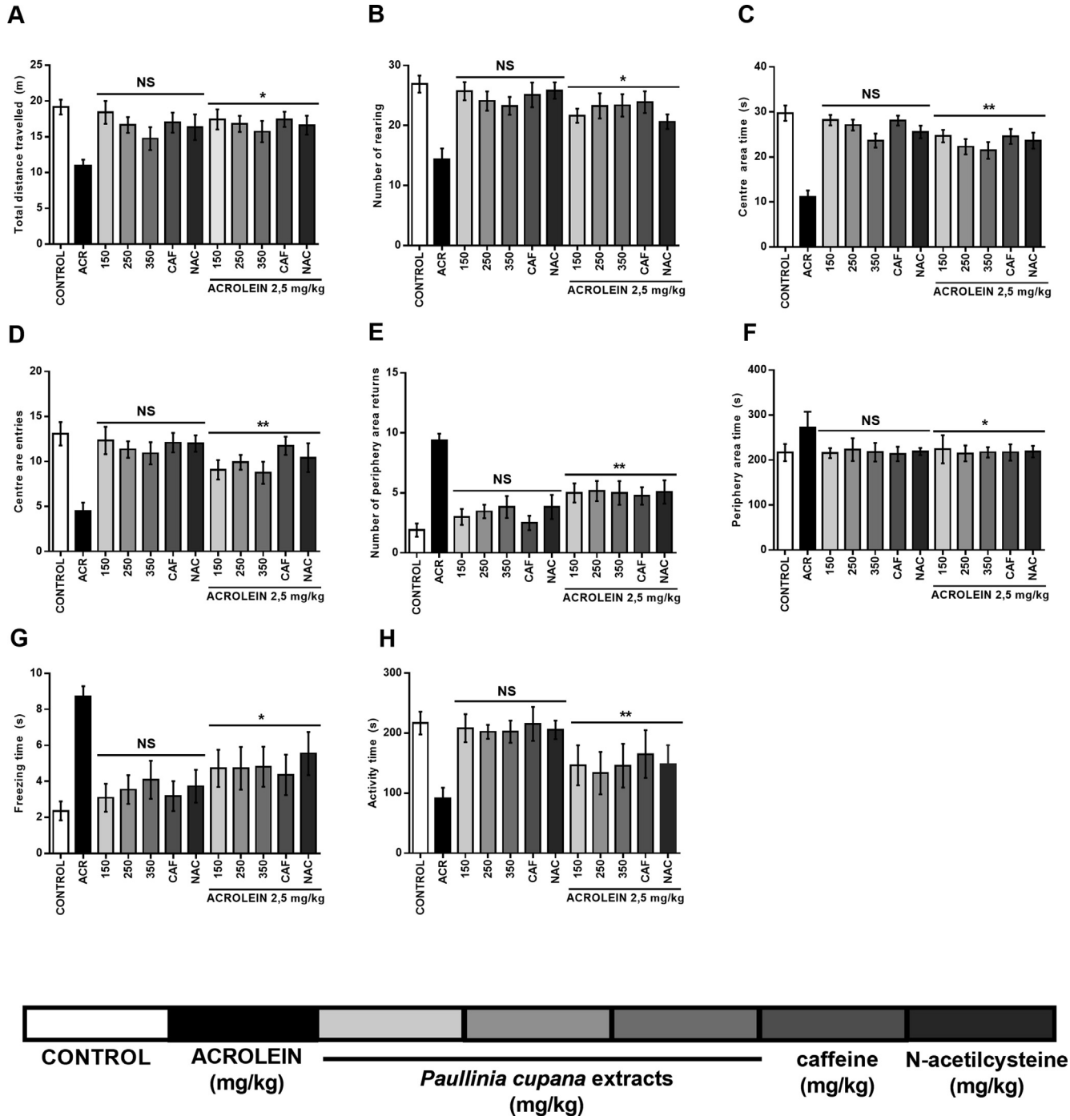

Fig. 4. Classical and ethological parameters observed in the open field test (OFT) after 8 weeks of treatment with ACR, *P. cupana*, CAF, and NAC alone or *P. cupana,* CAF, and NAC plus ACR. Each animal freely explored the OFT apparatus for 5 min and (A) total distance traveled, (B) number of rearing, (C) centre area time, (D) centre area entries, (E) number of periphery area returns, (F) periphery area time, (G) freezing time and (H) activity time. Results are expressed as the mean ± SEM. NS denote no difference related to the control. * and ** symbols denote significant changes related to the ACR group ($p < 0.05$ and $p < 0.01$ respectively; one-way ANOVA; Tukey's post hoc test).

unclarified.

The current study demonstrated that chronic treatment with the commercial extract of guarana (*P. cupana*) seeds (150, 250 and 350 mg/kg/day), significantly reduced oxidative damage induced by acrolein in rat brain and liver, in addition to reducing acrolein-induced hepatotoxicity and neurotoxicity (Figs. 1–3). All tested doses of the extract decreased lipid peroxidation and increased total thiol and GSH contents in all assessed regions of the brain and liver (Fig. 3). Animals treated with caffeine, in concentrations equivalent to that of the highest guarana dose, also exhibited significantly decreased oxidative damage in the brain and liver, as well as attenuated hepatotoxicity and neurotoxicity (Fig. 3). Caffeinated beverages, such as guarana, are widely consumed worldwide. Their molecules all consist of a caffeine-catechin matrix having varied concentrations and chemical structures. Several studies have suggested that these food beverages may exert beneficial biological activities, such as anti-inflammatory properties, on chronic conditions (Fraga, Croft, Kennedye, & Tomás-Barberán, 2019). A study performed by Alves et al., 2019 analyzed four extracts obtained from

caffeinated plants (*Coffee arabica*, *Camelia sinensis*, *Ilex paraguariensis* and *Paullinia cupana* – guarana) and showed that those extracts are able to improve the inflammatory response, attenuating this way tissue damage (Algarve et al., 2019; Alves et al., 2019). Acrolein is known as a potent proinflammatory agent that chronically leads to degenerative diseases such as, cancer, neurodegenerative diseases (Huang et al., 2013; Zong et al., 2018) and noteworthy; there is considerable amount of evidences supporting our hypothesis that protective effects of guarana is mediated by methylxanthine and polyphenolic content that scavenge acrolein directly or improve antioxidant and immune defense by modulating related signaling pathways (Alves et al., 2019; Algarve et al., 2019; Huang et al., 2013; Pochernich et al., 2011; Ansari et al., 2006; Ansari, Keller, & Scheff, 2008).

Regarding the behavioral parameters of Wistar rats, results showed that acrolein decreased their exploratory behavior and increased anxiety like-behavior. However, animals co-treated with acrolein and guarana extract, or acrolein and caffeine, showed a reduction in the loss of exploratory behavior and anxiety-like behavior, as compared to those in the acrolein group. These results suggested that the guarana commercial extract shows potential for preventing acrolein induced neurotoxicity.

We hypothesized that the mechanism underlying such protective capacity may be associated with the high antioxidant potential of guarana which contains compounds with radical scavenging activity. It is of significant importance that purine alkaloids, especially caffeine, which are constituents of *P. cupana* (Bittencourt et al., 2014), exhibit antioxidant properties that may lead to neuroprotective activities. Animals treated with caffeine, in concentrations equivalent to the highest dose of guarana extract, exhibited significantly decreased oxidative damage in the brain and liver, as well as attenuated hepatotoxicity and neurotoxicity and improved behavioral parameters compared to those in the acrolein group (Figs. 3 and 4).

In rats, green tea attenuated acute lung injury induced by boiling oil smoke, which contains ACR as the main aldehyde, by decreasing both oxidative stress and the synthesis of pro-apoptotic proteins (Zhang, Li, Li, & Ren, 2011).

Reportedly, some polyphenols lowered cellular toxicity of ACR in several cell cultures. Our research group observed the protective effects of amazon guarana extract against acrolein-induced toxicity in the neuronal like SHSY-5Y cell line. In this study, guarana decreased ROS production in a dose-dependent manner, thereby increasing cell viability and decreasing oxidative stress (Bittencourt et al., 2014). Using the same cell model, it was shown that pycnogenol (PYC), a combination of polyphenols extracted from *Pinus maritima*, which decreased ROS, restored GSH levels and modulated NADPH oxidase in *Bacopa mineira* extracts (BM), was also effective against acrolein induced toxicity in SHSY-5Y cell cultures (Ansari et al., 2006). In addition to reducing ROS generation, it influenced mitochondrial membrane potential, modulated the expression of several proteins involved in cellular redox state regulation, including NF-kappa B, Sirt-1, ERK1/2 and p66Shc, and decreased ACR induced toxicity (Singh, Murthy, & Ramassamy, 2010). Furthermore, *Scutellaria baicalensis* extract decreased the toxic effects induced by ACR on endothelial cells by increasing GSH and the expression of many enzymes related to GSH metabolism, including Glutathione peroxidase (GPx) and Glutathione Transferase (GST) (Zhang et al., 2011).

Considered together, our data suggest that the commercial extract of guarana reduced the production of MDA, increased GSH and reduced total thiol in all CNS regions, thereby improving behavioral parameters of the acrolein co-treated animals. The data further showed that caffeine, the main methylxanthine, played an important role in this process. Guarana contains several classes of natural compounds (Yamaguti-Sasaki et al., 2007) which may show great potential against toxicity induced by ACR, but at different levels of reactivity. Efficacy of the extract against ACR toxicity may be a function of the direct interaction between these compounds and ACR, which modulates enzymatic and non-enzymatic redox defense systems and detoxification enzyme complexes induced by ACR or the guarana extract. The authors postulate that a possible explanation for the increase in GSH and decrease in total thiol groups (SH) may be that the guarana extract induces a direct reduction in excessive oxidative stress caused by ACR, through the scavenging activity of reactive species resulting from the action of this aldehyde. We believe that the guarana extract modulates the activity of enzymes related to GSH metabolism and increases conjugation of GSH with ACR, as well as the activity of enzymes involved in the metabolism of carbonyl compounds.

As demonstrated in our previous paper (Bittencourt et al., 2014), the potential of guarana extract as an ACR trapping agent indicates the possibility of using guarana as a promising alternative for preventing the initiation and progress of diseases of which are induced by ACR.

Ethics statement

The Ethics Committee on Animal Research of the Federal University of Rio Grande do Sul reviewed and approved the study protocol (project number 25776). All experimental procedures were performed in accordance with the recommendations of the Brazilian Society of Laboratory Animal Science (SBCAL-COBEA) and the US National Institute of Health (U. S. National Institute of Health, Guide for the Care and Use of Laboratory Animals 8th Edition, 2011).

CRediT authorship contribution statement

Leonardo da Silva Bittencourt: Conceptualization, Data curation, Methodology, Formal analysis, Writing - original draft. **Carlos Eduardo Schnorr:** Validation, Methodology. **Daniela Copetti Santos:** Investigation. **Diana Carolina Rostirolla:** Methodology. **Karla Suzana Moresco:** Methodology. **Pedro Ozório:** Methodology. **Moara Rodrigues Mingori:** Methodology. **Luana Heinfarth:** Methodology. **Daniel Pens Gelain:** Visualization, Resources. **José Cláudio Fonseca Moreira:** Writing - review & editing, Supervision, Funding acquisition, Project administration.

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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.

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