**Compounds from Ilex paraguariensis extracts confer antioxidant effects in the brains of rats subjected to chronic immobilization stress**

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<td>Colpo, Ana; Universidade Federal do Pampa de Lima, Maria Eduarda; Universidade Federal do Pampa - Campus Uruguaiana Maya-López, Marisol; Instituto Nacional de Neurologia y Neurocirugía Manuel Velasco Suarez Rosa, Hemerson; Universidade Federal do Pampa - Campus Uruguaiana Márquez-Curiel, Cristina; Instituto Nacional de Neurologia y Neurocirugía Manuel Velasco Suarez Galván-Arzate, Sonia; Instituto Nacional de Neurologia y Neurocirugía Manuel Velasco Suarez Santamaría, Abel; Instituto Nacional de Neurologia y Neurocirugía Manuel Velasco Suarez Folmer, Vanderlei; Universidade Federal do Pampa - Campus Uruguaiana</td>
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Original research

Compounds from *Ilex paraguariensis* extracts confer antioxidant effects in the brains of rats subjected to chronic immobilization stress

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Abstract

Immobilization induces oxidative damage to the brain. *Ilex paraguariensis* extracts (Mate) and chlorogenic acid (CGA), its major natural compound, exert protective effects against reactive oxygen species (ROS) formation. Here, the effects of Mate and CGA on oxidative damage induced by chronic immobilization stress (CIS) in cortex (CTX), hippocampus (HIP) and striatum (STR) were investigated. For CIS, animals were immobilized during 6 h every day for 21 consecutive days. Rats received Mate or CGA daily by intra-gastric gavage 30 min before every restraint session. Endpoints of oxidative stress (levels of lipid peroxidation, protein carbonylation, and reduced (GSH) and oxidized (GSSG) forms of glutathione) were evaluated following CIS. While CIS increased oxidized lipids and carbonyl levels in all brain regions, CGA (and Mate in a lesser extent) attenuated lipid and protein oxidation as compared to control groups. GSH/GSSG balance showed a tendency to increase in all regions in response to stress and antioxidants. Taken together, our results support a protective role of dietary antioxidants against the neuronal consequences of stress.

Keywords *Yerba mate*; Chlorogenic acid; Immobilization stress; Oxidative damage; Antioxidant defense; Natural neuroprotective strategies.
Introduction

Chronic stress affects the functions of the nervous, endocrine and immune systems (Halliwell and Gutteride 2007). Stress elevates corticosterone (CORT) secretion, causing mitochondrial dysfunction induced by a prolonged decrease in the mitochondrial membrane potential (Takahashi et al. 2002), which results in excitotoxicity and reactive oxygen species (ROS) formation (Hansson et al. 2008). In addition, the increased levels of glucocorticoids produced during stress may affect the whole antioxidant capacity of the Central Nervous System (Şahin and Gümüşlü 2007). In line with this, restraint stress creates a neurotoxic environment in the brain, increasing the susceptibility of neuronal cells to suffer metabolic alterations and death through the activation of deleterious signaling pathways (Gerecke et al. 2013). Stress induced by immobilization is related with oxidative damage to lipids, proteins and DNA in the brain (Liu et al. 1996), possibly associated with mitochondrial dysfunction, disruption of energy metabolism, neuronal damage, impaired neurogenesis and induction of signaling events during apoptotic cell death (Grizzell et al. 2014), all accompanied by ROS production.

ROS and antioxidants have been the focus of a considerable number of current investigations. Polyphenols and other compounds from natural products have been described as major antioxidants. In this regard, Ilex paraguariensis extracts particularly rich in bioactive compounds, are known to possess various redox modulatory properties, the majority of which are associated with defense against ROS (Lima et al. 2014; Colpo et al. 2016).

Yerba mate is the main product obtained from branches and leaves of the Ilex paraguariensis tree. This product is used to make a traditional beverage consumed in South America, named “Chimarrão” in Brazil, “Mate” in Argentina and Uruguay, and
“Tererê” in Paraguay (Bracesco et al. 2011). The predominant compound of yerba mate extracts is chlorogenic acid (CGA, 3-O-caffeoylquinic acid) (Bracesco et al. 2011; Colpo et al. 2016). Recent evidence suggests that CGA may produce beneficial effects related to antioxidant activity (Gul et al. 2016; Liang and Kitts 2016). Additional evidence indicates that both yerba mate crude extracts and CGA can produce neuroprotective effects associated primarily to their activities as antioxidants. Gul et al. (2016) demonstrated that CGA protected rat cortical slices against H_2O_2-induced alterations on oxidative stress parameters. In turn, Lee et al. (2012) showed that CGA reduced brain damage, blood-brain barrier damage and brain edema by stimulating radical scavenging activity.

Regarding crude extracts, Ilex paraguariensis extracts have demonstrated the ability to prevent memory deficits in rats (Colpo et al. 2007; Santos et al. 2015), and a capacity to reduce the frequency of seizures, minimizing the neuronal damage associated with their periodic occurrence (Branco Cdos et al. 2013). Moreover, Cittadini et al. (2015) reported that this plant could be a source of chemopreventive agents to combat oxidative stress-related neurodegenerative pathologies because of its redox effects in brain regions.

Despite of the aforementioned evidence, the role of yerba mate crude extracts and CGA in modulating the oxidative stress-induced alterations in the brain remains unexplored. Thus, in this work we investigated whether yerba mate crude extracts and CGA may exert protective effects in the brains of rats submitted to CIS, and if these effects are due to their antioxidants capacities.
Materials and methods

Reagents and extracts preparation

*Yerba mate* was purchased in a popular market in Uruguay. The aqueous extract was prepared as an infusion of *Ilex paraguariensis* (Aquifoliaceae) at a 200 mg/ml concentration. The infusion was prepared with 10 ml of ultrapure water at 85°C in 2 g of *yerba mate* for 10 min. The extract infusion was named simply “Mate”. Chlorogenic acid (5.6 mM) was diluted in saline solution 0.9% (in accordance with the manufacturer’s instructions). Thiobarbituric acid (TBA), HEPES, malondialdehyde (MDA), sucrose, and CGA were all obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other reagents were obtained as reagent-grade from other well-known commercial sources.

Animals

Thirty-nine adult male Wistar adult rats (225–250 g), which were not littermates, were obtained from the vivarium of the Instituto Nacional de Neurología y Neurocirugía (INNN- Mexico). Upon arrival at the INNN vivarium, the animals were housed in polycarbonate cages and had free access to food (rodent Chow 5001; PMI Feeds Inc., Richmond, IN, U.S.A.), water, and controlled conditions of temperature (25 ± 3 °C), humidity (50 ± 10%), and a 12:12-h light-dark schedule. Rats were given four days to acclimate to the animal housing room after their arrival, and before any experimental manipulations took place. After the acclimation period, all animals were randomly assigned to one of six groups (n=6/group). All procedures were carried out in accordance to the guidelines established by the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health, and following the recommendations of the Ministry of Health of Mexico.
Chronic immobilization stress protocol and compounds administration design

Six experimental groups (n=6 rats per group randomly selected) were designed: Water, Water+CIS (Chronic Immobilization Stress), Mate, Mate+CIS, CGA, CGA+CIS. The groups submitted to CIS were immobilized daily during 6 h (from 8:00 am to 2:00 pm) for 21 consecutive days in a glass device with the following dimensions: 7 x 6 x 18 cm (Colín-González et al. 2015). To avoid the animals to adapt in the glass device, different daily stressing stimuli were applied for two hours during the restraint period; they included cold water basement, inclination of the box, hot water basement, ice, dark and light (all applied external to the glass device). Groups that were not submitted to CIS were isolated daily in acrylic cages (30 x 30 x 20 cm) during 6 h for 21 days. The four groups were deprived of food and water during the 6 h isolation-period. All groups received treatments by intra-gastric gavage, 30 min before immobilization or isolation for the same 21 days. The treatment dose was determined according to the body weight of the animal (1.5 µl/g for vehicle and mate; 0.5 µl/g for CGA) (Brasil 1996, 2004; Andrade et al. 2012; Lee et al. 2012). An additional small control group (n=3) was subjected to random stressors but not being immobilized; since these animals did not show changes in body weight, neither exhibited signs of anxiety, they only served to assess that random stressors per se exerted slight or no effects in control rats (data not represented).

At the end of the chronic stress procedure (21 days), all animals were euthanized by decapitation, their brains were extracted, and the cortex (CTX, as frontal cortex), hippocampus (HIP, as CA3) and striatum (STR, as caudate-putamen) were immediately dissected out. Tissue samples were frozen immediately after collected and kept frozen (-20°C) until analysis.

Body weight
The body weight of animals was estimated individually using an analytical balance. This procedure was carried out every three days since the beginning of the experiment. In order to analyse the variations in this parameter, at the end of the treatment the final weight was subtracted from the initial weight.

**Assay of lipid peroxidation**

The assay to detect thiobarbituric acid-reactive substances (TBA-RS) was used for the determination of the levels of lipid peroxidation in tissue samples, according to a previous report (García et al. 2014). The principle of this technique is based on the reaction of TBA with oxidized lipids to form malonaldehyde (MDA) as a product of peroxidation, as well as other products. Results were corrected by the protein content in samples and expressed as nmol of TBA-reactive substances (TBAR-RS) per mg protein.

**Assay of protein carbonylation**

Hydrazone formation after reaction with DNPH was calculated as an index of protein carbonylation (Colín-González et al. 2013). The levels of proteins containing carbonyl groups were expressed as nmol of DNPH per mg of protein, using the molar absorption coefficient for DNPH (22,000 M⁻¹ cm⁻¹). The total amount of protein was obtained through the estimation of the optical density at 280 nm in blank probes prepared in parallel. Results were expressed as the percent of increase vs. the control value.

**Determination of oxidized and reduced glutathione concentrations**

For measurement of GSH and GSSG levels, we used a method previously described by Galván-Arzate et al. (2005). The GSH and GSSG levels were determined in homogenates from the brain regions. Their fluorescent signals were recorded in a luminescence spectrometer at 420 nm of emission and 350 nm of excitation wavelengths (Perkin-Elmer LS50B equipment). For GSH levels, final results were expressed as nMol GSH per mg of protein. For GSSG levels, data were expressed as
nMol GSSG per mg of protein. The reduced/oxidized glutathione (GSH/GSSG) ratio was also calculated and expressed.

**Statistical analysis**

Data are presented as mean values ± one standard deviation (SD). All data were analyzed by two-way ANOVA followed by Tukey’s or Bonferroni’s tests, with a minimum level of significance of $p \leq 0.05$. The GraphPad Software, Inc. was used to create the artworks.
Results

Mate and chlorogenic acid prevented the chronic immobilization-induced body weight loss in stressed rats

Fig. 1 shows the changes in body weight of animals during the entire period of treatment in the different experimental groups. The body weight of each rat was recorded during the entire period of protocol involving immobilization. In rats subjected to Water+CIS (21 days), the weight loss was significant. The mean value obtained between the initial and final weights showed a loss of 16 grams in this experimental group (p≤0.001). In the groups submitted to CIS and treated with Mate or CGA there was no weight loss as compared to control group (p<0.01 vs. CIS group).

Mate and chlorogenic acid prevented the chronic immobilization-induced changes in parameters of oxidative stress in rat brain regions

Lipid peroxidation

Results of TBA-RS formation in the cortex, hippocampus and striatum of control and stressed animals are presented in Figs. 2a, 2b, and 2c, respectively. In the cortex, CIS increased the levels of lipid peroxidation in the Water+CIS treated group by 35% (p≤0.001), and in the Mate+CIS treated group in 21.6% (p≤0.05), above their corresponding control groups. In the CIS+CGA treated group, there was no change in TBA-RS formation (4.33%) as compared to the control group (Fig. 2a).

Fig. 2b depicts the levels of TBA-RS formation in the hippocampus. There was a significant increase in this parameter in the Water+CIS treated group (p≤0.01), in which the level of lipid peroxidation was 98 % above the control group. Mate and CGA
significantly reduced the CIS-induced TBA-RS formation by 16 % and 8 %, respectively (p≤0.001).

The effect of CIS on the striatal levels of TBA-RS formation is showed in Fig. 2c. In this brain region, lipid peroxidation was found significantly increased in the Water+CIS (p≤0.01) and Mate+CIS (p≤0.05) treated groups, which corresponded to 33 % and 40 % above their corresponding controls. CGA was able to maintain the TBA-RS formation similar to control values.

When comparing the data of all groups submitted to CIS, it was found that, in the CTX and HIP, lipid peroxidation was significantly lower in the Mate- and the CGA-treated groups than the control (Water) treated group. In the STR, only CGA presented a significant response against stress. The statistical significance derived from the analysis of these data was represented by the letter “c” in Fig. 2a, 2b and 2c.

Protein carbonylation

The statistical analysis revealed that CIS condition produced a significant increase in protein oxidation in all three regions studied (Figs. 3a, 3b and 3c): using water as control, it was found that the protein carbonylation levels were increased by CIS in CTX, HIP and STR (p≤0.001). Mate was able to counteract the effects of CIS in the hippocampus (p≤0.05), whereas in the cortex and the striatum, this effect was not observed. In contrast, CGA was capable of inhibiting oxidative damage to proteins, as the carbonyl protein rate was similar to control in all the brain regions tested.

When comparing all groups submitted to CIS, the CGA-treated group exhibited lower levels of protein carbonylation in relation to the Water (control)-treated group in cortex (p≤0.01), hippocampus (p≤0.05) and striatum (p≤0.001). Mate significantly decreased the protein oxidation in the hippocampus (p≤0.05).

Glutathione (GSH/GSSG) balance
Table 1 shows the changes in GSH, GSSG and GSH/GSSG balance in control and treated groups. Except for the significant increase of GSH levels in the cortex of the CIS group compared with control group (p<0.05), no other changes in GSH, GSSG of GSH/GSSG values were found among groups, although the three regions analyzed showed marked tendencies to increase the GSH/GSSG balance in CIS, Mate+CIS and CGA+CIS conditions, suggesting that GSH defense is activated under stressing conditions in an attempt to counteract oxidative stimuli.
Discussion

In this report, we have evaluated for the first time the effects of *yerba mate* extract, and its major compound CGA, on stress induced by immobilization in rats. Particular emphasis was given to oxidative stress and the redox status of the brain. Our results confirm that CIS produces oxidative damage to specific brain regions, affecting important functions of the CNS.

Noteworthy, it is known that many factors may modify the responses to stress during chronic immobilization conditions, including the predictability to stressors. In this regard, Thakur et al. (2015) suggested that chronic unpredictable stress responses, and its unexpected nature, prevent the development of adaptation and coping strategies, thus accounting for a better model of CIS. Because of this, in the present study we choose to apply different types of stressors in our stress model.

Chronic stress is associated with dysregulation of energy homeostasis; previous reports have demonstrated that chronic exposure to restraint stress reduces the body weight of rodents (Gamaro et al. 2003; Flak et al. 2011). Our study confirms that CIS was responsible for a significant weight loss, while mate and CGA were able to reduce the alterations associated to the stress mechanisms, thus maintaining homeostasis and limiting the weight loss.

The factors associated to these positive effects remain unclear; however, it is known that physical and psychological stressors cause oxidative damage by inducing an imbalance between the pro-oxidant and antioxidant status (Zafir and Banu 2009). Stress is known to induce alterations in the energetic metabolism, consequently leading to the formation of ROS, such as superoxide radical, from the mitochondrial electron transport chain.
transport chain (Du et al. 2009). The role of polyphenols in the animals’ body weight gain can be associated, at least in part, with the improvement in the redox status and an improved systemic response to the induced stress. However, we need to extend this knowledge to fully understand the action of the compounds from mate in food intake-related genes, as well as in the regulation of the stress response through corticosterone.

With respect to the brain sensitivity to alterations in redox status, this condition occurs mainly because the brain is an important oxygen consumer. However, the presence of excitatory amino acids, its modest capacity to activate antioxidant defence systems, and many other factors, are pointed out as relevant for its increased susceptibility to oxidative cell damage (Halliwell and Gutteride 2007). Moreover, neuronal cells are vulnerable to the attack of ROS mainly because neuronal membranes are rich in polyunsaturated fatty acids (PUFA) (Friedman 2010). Disorders of cellular metabolic homeostasis resulting from enhanced levels of ROS, glucose or reactive carbonyl compounds, cause modifications on cellular components, particularly proteins (Ambrożewicz and Bielawska 2016).

Commonly, oxidative damage is manifested as an increase in lipid peroxidation, DNA base oxidation products, and oxidative protein damage (Halliwell 2001), and these events have been increasingly associated to neurodegenerative disorders (Chen et al. 2012). For instance, the lipid peroxidation end-product 4-hydroxy-2,3-nonenal (HNE) is cytotoxic to neurons and impairs the function of membranes, including the neuronal glucose transport and use involving the inactivation α-ketoglutarate dehydrogenase (an enzyme with relevant activity in the cycle of tricarboxylic acids) (Bruce-Keller et al. 1998; McLain et al. 2011). In turn, ROS-mediated protein oxidation produces secondary damage to other biomolecules, for example by raising the intracellular Ca\(^{2+}\) levels (Halliwell and Gutteride 2007).
Responses to immobilization stress result in the over-production of free radicals, leading to lipid peroxidation in cell membranes (Şahin and Gümüşlü 2007). The present study clearly demonstrates the CIS-induced oxidative damage in CTX, HIP and STR, evidenced by the increase of TBA-RS and carbonyl protein levels. In contrast, treatment of animals with CGA resulted in a decrease in these parameters, in comparison to the untreated stressed animals. Mate, in turn, was able to reduce the oxidative damage to lipids only in HIP. This finding suggests that CGA is more efficient as an isolated antioxidant than Mate, to counteract oxidative damage in the brain in this restraint stress model.

Natural antioxidants prevent the ROS generation, oxidation of proteins, and lipid peroxidation, thus acting as upstream therapeutic barriers to oxidative stress (Chen et al. 2012). Results of this study showed that hippocampal cells display better responses to the stimulus produced by yerba mate crude extracts on antioxidant activity than cortex and striatum. In turn, these differences could be due to intrinsic characteristics of the hippocampal cells, especially regarding their properties to use external antioxidant sources. These characteristics deserve further and detailed investigation.

In addition, some studies have reported yerba mate to possess neuroprotective properties, such as antidepressant-like (Ludka et al. 2016), anxiolytic and stimulant (Santos et al. 2015) effects, as well as attenuation of dyskinesia and memory dysfunction (Colpo et al. 2007; Costa et al. 2015), and reduction of the frequency of pentylenetetrazol-induced seizures (Branco Cdos et al. 2013). These effects emphasize the therapeutic potential of this natural product.

CGA is a free radical and metal scavenger and represents the major component in Ilex paraguariensis extracts (Colpo et al. 2016). In the brain, CGA has shown to improve the spatial learning and memory (Han et al. 2010), also exerting anti-amnesic
activity via inhibition of acetylcholinesterase and malondialdehyde (MDA) in the hippocampus and frontal cortex (Kwon et al. 2010). CGA also reduces brain and blood-brain barrier (BBB) damages, as well as brain edema, by radical scavenging activity and inhibitory effects on metalloproteinases (Lee et al. 2012). The present report highlights the protective effects of CGA in different brain regions from rats submitted to CIS. Of note, the brain concentration of bioactive compounds uptake is limited, although it is known that, usually, metabolites are accumulated at levels around 0.4 nmol/g tissue (El-Mohsen et al. 2006). Numerous mechanisms, such as free radical scavenging, metal chelation and the modulation of enzyme activities, have been proposed to explain the positive impact of polyphenols in the brain (Schaffer et al. 2012). Furthermore, Halliwell (2006) observed that the sufficient supply of the CNS with antioxidants is of prime importance because of the brain’s vulnerability to oxidative and nitrosative stress.

Antioxidant enzymes play important roles in the cerebral cellular defense against oxidative damage, and are able to lower the risk of some neurological disorders (Halliwell and Gutteride 2007). Reduced glutathione (GSH) is involved in many metabolic processes and prevent protein-SH groups from oxidation and cross-linking (Halliwell and Gutteride 2007). Moreover, GSH plays a fundamental role in the detoxification of ROS, which is critical for the normal function of the brain (Hirrlinger et al. 2002). During oxidative stress, GSSG and glutathione S-conjugates are generated, being GSSG/2GSH described as an important indicator of the intracellular redox environment (Khramtsov and Gillies 2014). Our results show that CIS produced an increase in GSH levels only in CTX (p≤0.05), and this change can be interpreted as a more adaptive/compensatory response to the oxidative damage in course. The same
interpretation can be adopted for the tendency induced by CIS, CIS+Mate and CIS+CGA conditions to increase the GSH/GSSG balance in the three regions studied.

Consistent with a cytoprotective role, low GSH levels decrease cellular antioxidant capacity, as occurs in various neurodegenerative disorders. In turn, elevated GSH levels and GSH/GSSG balance increase antioxidant capacity and resistance to oxidative stress (Ballatori et al. 2009). The mechanisms by which GSH is increased remain unclear since they might be due to a variety of factors, including increased transcriptional activity of proteins used to synthesize GSH, increased translational activity, decreased degradation of GSH, increased reduction of GSSG, and increased transport of precursors (Johnson et al. 2012).

There is now enough collected evidence to claim beneficial effects of antioxidants in prolonged stress and unbalanced redox present in neurodegenerative disorders, such as Alzheimer’s (AD) and Parkinson’s (PD) diseases. For instance, Zaidi and Banu (2004) showed that Vitamin E was effective in restoring antioxidant systems in the brain tissue of animals submitted to restraint stress. In addition, in the hippocampus, S-allyl cysteine (SAC), the most abundant organosulfur molecule found in aged garlic extracts, exerted a modulatory role on antioxidant responses in acute restraint stress (Colín-González et al. 2015). Moreover, Hong et al. (2014) demonstrated that Rooibos tea prevents lipid peroxidation, restores stress-induced protein degradation, and regulates GSH metabolism. Our findings are in agreement with all these reports. Since the beneficial effects exerted by Mate and CGA are assumed to be due to their properties as polyphenols, then the transfer of hydrogen atoms, single electron transfer and metal chelation are likely to be part of their protective effects observed here (Leopoldini et al. 2011). Furthermore, Ríos-Hoyo et al. (2014) observed that polyphenols could evoke
beneficial effects acting through the activation of metabolic pathways, a consideration that shall be investigated for the model developed here in a near future.

To conclude, in the present study, we evidenced a pro-oxidant action of CIS in rat brain regions. It was also demonstrated that mate and CGA presented antioxidant capacity in this stress model. Because ROS are involved in chronic stress and degenerative disorders, the knowledge about natural compounds counteracting the harmful effects of these disorders is an important contribution.
Ethical approval

The care and handling of all animals was carried out in accordance with the guidelines of the Guide of the National Institutes of Health for the Care and Use of Laboratory Animals NOM-062-ZOO 1999, as well as the Local Committee of Bioethics of the Instituto Nacional de Neurología and Neurocirugía (Mexico) established upon approval of the Ministry of Health of Mexico.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors’ contributions

AC Colpo, ME de Lima, M Maya-López, H Rosa and S Galván-Arzate, all contributed to project development, data collection and analysis. AC Colpo and A Santamaria contributed to writing of the manuscript. AC Colpo, V Folmer and A Santamaria designed the whole project. All authors have approved the final manuscript.

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References


Friedman, J. 2010. Oxidative stress and free radical damage in neurology (why is the Nervous System vulnerable to oxidative stress?). Springer New York, pp. 19-27.


Table 1. Status of glutathione balance in control and Mate- or CGA-treated groups after 21 days of CIS.

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<th>Parameters</th>
<th>Water</th>
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<th>Mate+CIS</th>
<th>CGA</th>
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<td>GSH (nmol/mg protein)</td>
<td>0.57±0.03</td>
<td>0.92±0.28*</td>
<td>0.50±0.03</td>
<td>0.48±0.09</td>
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<td>GSSG (nmol/mg protein)</td>
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<td>2.32±0.79</td>
<td>1.58±0.30</td>
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Table 1. GSH: reduced glutathione; GSSG: glutathione disulfide. Values are expressed as mean ± S.E.M. of 4-6 animals per group. *Significantly different of control (p≤0.05); two-way ANOVA followed by Bonferroni’s test.
Figure captions

**Fig. 1** Body weight changes in rats exposed to CIS (6 h per day for 21 days). Experimental groups designed: Water/Water+CIS (1,5 µl/g), Mate/Mate+CIS (1,5 µl/g), CGA/CGA+CIS (0,5 µl/g). Every recording was made 5 minutes before the immobilization and/or isolation of animals. Data represent the means ± SEM (n=4-6). Two-way ANOVA + Tukey’s test; a=Water/Water+CIS; b=Water+CIS/Mate+CIS; c=Water+CIS/CGA+CIS. *p≤0.05, **p≤0.01, ***p≤0.001.

**Fig. 2** Effect of Mate (1,5 µl/g) and CGA (0,5 µl/g) on CIS-induced levels of lipid peroxidation in the cortex (a), hippocampus (b) and striatum (c) of rats. Animals were subjected to immobilization (stress) or isolation (control) during 6 h for 21 days. At the end of treatments, the brain regions were collected. Results are presented as mean values ± S.E.M of n=4-6 rats per group, analyzed by two-way ANOVA followed by Tukey’s test. a=Water/Water+CIS, Mate/Mate+CIS, CGA/CGA+CIS; b=Water/Mate, Water/CGA, CGA/Mate; c=Water+CIS/Mate+CIS, Water+CIS/CGA+CIS, CGA+CIS/Mate+CIS. *p≤0.05, **p≤0.01, ***p≤0.001.

**Fig. 3** Effect of Mate (1,5 µl/g) and CGA (0,5 µl/g) on CIS-induced levels of protein carbonylation in the cortex (a), hippocampus (b) and striatum (c) of rats. Animals were subjected to immobilization (stress) or isolation (control) during 6 h for 21 days. At the end of treatments, the brain regions were collected. Data are presented as mean values ± S.E.M of n=4-6 rats per group, analyzed by two-way ANOVA followed by Tukey’s test. a=Water/Water+CIS, Mate/Mate+CIS, CGA/CGA+CIS; b=Water/Mate, Water/CGA, CGA/Mate; c=Water+CIS/Mate+CIS, Water+CIS/CGA+CIS, CGA+CIS/Mate+CIS. *p≤0.05, **p≤0.01, ***p≤0.001.
Figure 1. JPD

Average Body Weight (g)

Treatments

Water
Water+CIS
Mate
Mate+CIS
CGA
CGA+CIS

a*** b** c**
Figure 3

(a) 

(b) 

(c) 

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