Ayahuasca prevents oxidative stress in a rat model of depression elicited by unpredictable chronic mild stress

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ABSTRACT

Background: Depression is a highly disabling common mental disorder and, due to its multifactorial nature, the development of effective therapies is challenging and of great relevance. Ayahuasca (AYA), an entheogenic traditional beverage, has emerged as an alternative for antidepressant treatment, however, AYA preclinical and clinical trials are still incipient.
Objectives: This investigation aimed to evaluate some behavioral and biochemical effects of AYA subchronic administration in rats submitted to a model of depression elicited by unpredictable chronic mild stress (UCMS).
Methods: 500 mg/kg of AYA was administered in adult male rats once a day for 15 days before submitting the animals to UCMS. Anhedonia-like and locomotion behavior, lipid peroxidation, antioxidant enzyme activities, and sulfhydryl/nitrite content were evaluated.
Results: AYA intake failed to prevent anhedonia-like behavior. Locomotion was not altered by AYA consumption or by the experimental condition. UCMS increased TBARS and nitrites levels, decreased the levels of catalase in the cerebral cortex and of Sulfhydryl in the hippocampus. AYA treatment counteracted these biochemical alterations but did not display any alterations in non-stressed rats.
Conclusions: Taken together, results indicate an adaptogenic antioxidant molecular mechanism of AYA in relation to depression induced by stress.

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Keywords: depression; ayahuasca; unpredictable chronic mild stress; oxidative stress.

INTRODUCTION

Ayahuasca (AYA) is the Quechua name of a South American psychotropic plant tea used traditionally by indigenous populations of the Amazon region as part of spiritual ceremonies, for medicinal and social purposes[1,2]. Usually, the beverage consists of a blend of two plants: *Banisteriopsis caapi* and *Psychotria viridis*. The *Banisteriopsis caapi* is abundant in β-carbolines (harmine, tetrahydroharmine, and harmaline), which monoaminoxidase inhibiting effects is widely documented[1]. The *Psychotria viridis* contains considerable amounts of the hallucinogenic compound N, N-dimethyltryptamine (DMT), a 5-HT1A/2A/2C agonist[3,5]. Convergent evidence signpost to the antidepressant property of AYA use[4-7].

Depression is a common and highly disabling syndrome with a prevalence rate of approximately 10% of the world population[8]. Depressive and other mood disorders have long been pharmacologically treated, however, due to its multifactorial nature – which involves the articulation of biological, environmental, and biographic factors[9] – no remarkable improvement has been

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achieved with currently available therapies.[10] The identification of effective pharmacotherapies are challenging[10,11] and necessary.

Stressful and harmful experiences articulated with oxidative stress have been systematically implicated in the neuropsychopathology of depression[11-13]. Oxidative stress results from an unevenness between oxidant production and antioxidant mechanisms that causes oxidative damage, such as lipid peroxidation and DNA oxidation[14]. It contributes to the pathogenesis of depression through changes in brain structures, such as modification in the hippocampus and the pituitary[15].

While evidence shows that antidepressant treatment response is associated with changes in oxidative and inflammatory markers[16], β-carboline components of AYA – such as Harmine – seem to increases superoxide dismutase (SOD) and catalase (CAT) activities and to decrease lipid and protein oxidation[17]. Also, DMT seems to rise serotonin neurotransmission, which may reduce lipid peroxidation and improve antioxidant status[4].

The unpredictable chronic mild stress (UCMS) model is an established translationally-relevant rodent paradigm used to induce depressive-like behaviors – such as increased immobility and decreased sucrose consumption/anhedonia – and to evaluate the antidepressant property of specific substances[18]. UCMS consists of chronic exposure of rodents to diverse randomly scheduled, low-intensity environmental, and social stressors.

Rodents submitted to stress tend to display a hyperactive adrenergic sympathetic adrenal medulla system and HPA axis, which regulates glucocorticoid function.[19] Stress also affects metabolism and feeding behavior[20]. Depending on the severity, duration, and type of stressor, a decrease in food intake with weight loss is expected[21]. Chronic stressors also cause a decrease in the rewarding properties of a variety of unconditioned stimuli and substances, such as decreased consumption of sweet solutions[22].

The current study aimed to advance in the understanding of the AYA as an alternative antidepressant therapy. We evaluated the sub-chronic effects of AYA administration on some behavioral and oxidative stress parameters associated with depression in rats submitted to UCMS. Two main hypotheses motivated this study: first, AYA treatment would prevent the behavioral and biochemical alteration induced by the UCMS paradigm, and second, AYA treatment would not alter the oxidative parameters in the non-stressed rats, reveling its adaptogenic property.

METHODS

Preparation of AYA

AYA solution (3L) was donated to our research group by a Santo Daime Church in March 2017. The preparation followed the traditional procedure: approximately 50% of *Banisteriopsis caapi* and 50% of *Psychotria viridis* leaves were boiled to concentration into the water for several hours. After cooled down, the tea was stored in a refrigerator in bottles. The AYA sample was lyophilized and immediately stored at -80ºC in an Ultra freezer. All experiments were performed using the lyophilized AYA and its main psychoactive compounds were characterized.

Characterization of the main psychoactive compounds in AYA

AYA alkaloids extraction

An aliquot of 0.5 mL of AYA was added to 3.0 mL of borate buffer solution (pH 9.0). Diphenhydramine was used as an internal standard. C18 solid phase extraction cartridges were conditioned with 2.0 mL of methanol, 1.0 mL of deionized water, and 2.0 mL of borate buffer solution (pH 9.0). The sample solution was then transferred to the cartridges. Washing was performed with 1.0 mL of acetonitrile: Water solution (1: 9). After drying for 7 min under vacuum the elution was performed with 2.0 mL of methanol. From the eluate, 2μL was injected into the gas chromatography with nitrogen phosphorous detector (GC-NPD).[26]

Reagents

Boric acid, methanol, and acetonitrile were obtained from Merck (Darmstadt, Germany). Classic Sep-Pack® C18 cartridges (360 mg) were purchased from Waters (Milford, Massachusetts, USA). Tetrahydroharmine was synthesized according to the procedure described by Callaway *et al.* (1996). DMT (1 mg/mL) was obtained from Cerilliant Co (Round Rock, Texas, USA).

GC-NPD analysis

GC-NPD analysis of DMT, harmine, harmaline, and tetrahydroharmine were performed on an Agilent Technologies model 6890 gas chromatograph equipped with a nitrogen-phosphorus detector and a 7683 series automatic injector (Agilent Technologies, Little Falls, DE, USA). Chromatographic separation was achieved on an HP Ultra-2 fused silica capillary column (25 m × 0.2mm × 0.33 μm) with ultra-pure nitrogen as the entrainment gas at a constant flow of 1.0 mL/min. Injections (2 μL) were performed in the splitless mode. The injector and detector temperatures were 280°C. The oven temperature was maintained at 70°C for 1 min and programmed to ramp at 30°C/min. After attaining 120°C, the oven was ramped at 20°C/min to 300°C and maintained at 300°C for 4 min. Analytes were identified based on the comparison of retention times relative to the corresponding internal standard analyzed during the same run. The quantification was based on the ratio of the integrated peak area of the analytes to that of the internal standard. The final results were multiplied by 100 to compensate for the dilution[23]. Diphenhydramine, harmine (hydrochloride), and harmaline (hydrochloride) were obtained from Sigma Co. (St Louis, MO, USA).

Animals

Thirty-six adult male *Wistar* rats (60 days old; 250–300g body weight) were provided by the Universidade Federal de Pelotas. The animals were housed in groups of four rats per cage in a room with a constant temperature of 22 ±1°C, and a 12-h light/dark cycle (lights on at 7:00 a.m.). The rats had free access to food (standard rat chow) and water, except for the stressed group, which received no food or water during the period when the stressor (water and food deprivation) was applied. Body weights were measured at the beginning and the end of the treatments. Animal care protocols followed the official governmental guidelines in compliance with the Institutes of Health guidelines for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and were previously submitted to the Ethics Committee for Animal Experimentation of UFPel (CEEA) and approved under the number 6936.

AYA treatment and UCMS model development

First, the animals were randomly divided into two groups: Those receiving vehicle (water) and those treated with AYA. The AYA (500mg/kg) was administrated via gavage once per day for 15 days. After 15 days of treatment with AYA, the animals were subdivided into two groups: control and stress. These procedures LED to four comparative groups: Control, AYA, Stress, and AYA-Stress (Figure 1).

The UCMS protocol was adapted from Gamaro and colleagues[21] and was applied for 30 days. The individual stressors and the lengths of their application each day are listed in Table 1. The following stressors were used: a) 24 h of food deprivation; b) 24 h of water

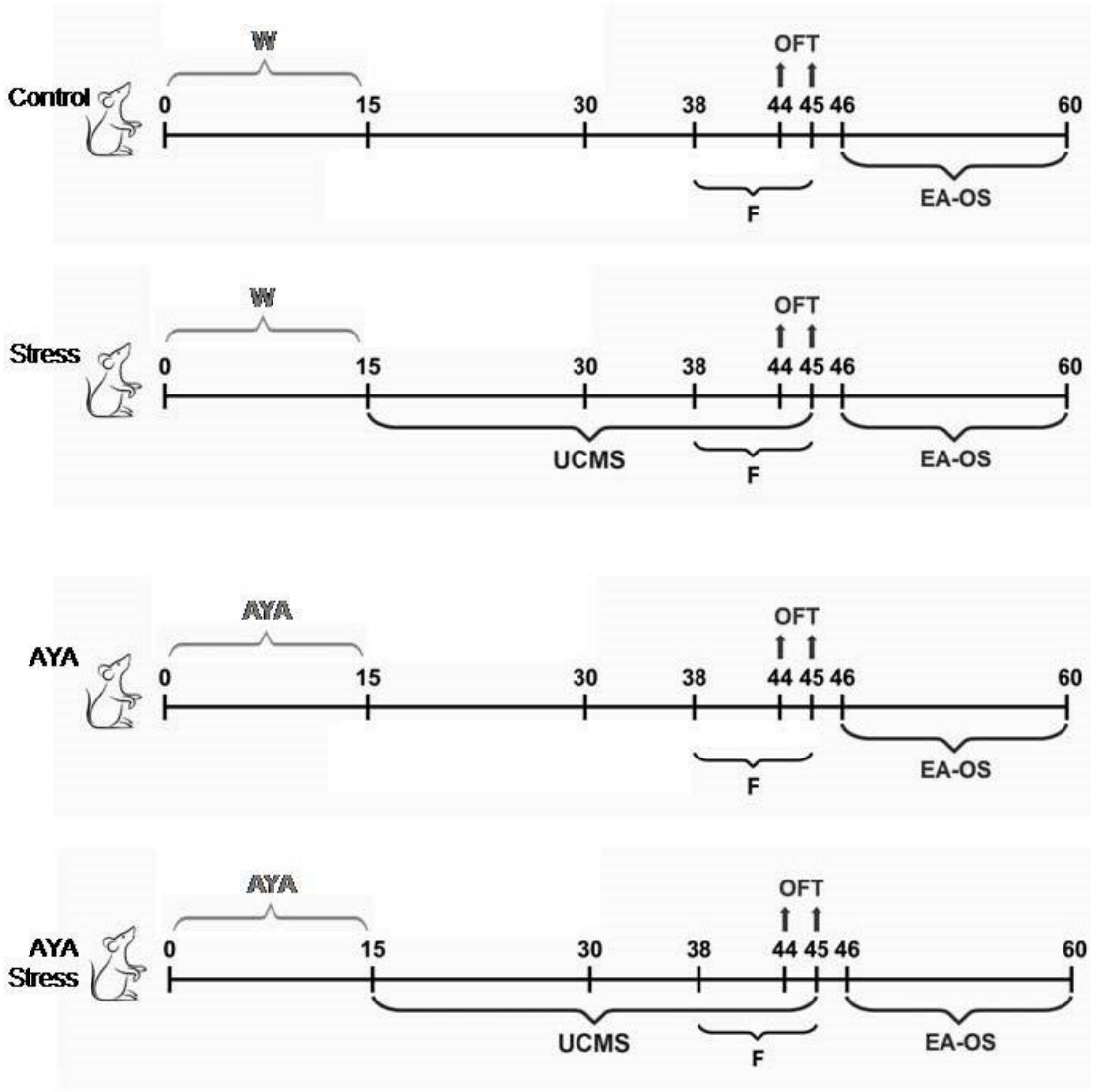

Figure 1

deprivation; c) 1 to 3 h of restraint, as described below; d) noise; e) forced swimming for 10 or 15 min, as described below; f) flashing light for 120 to 210 min; g) isolation (2 to 3 days) in inclined cages.

Restraint stress was imposed by placing the animal in an immobilized 25 ×7 cm plastic cylinder with an A1-cm hole at the far end, facilitated air exchange within the restraining device. Forced swimming test was performed by placing the animal in a plastic tank measuring 50 ×47 ×40 cm with 30 cm of water at 23± 2°C. Exposure to flashing light was performed by placing the animal in a 50-cm-high, 40 ×60 cm open field with brown plywood sides and a frontal glass wall. A 40 W lamp, flashing at a frequency of 60 flashes per minute, was used as the light stimulus. To impose noise stress, animals were exposed for 60 to 138 min to a 100 dBA/4h/d white noise amplifier (40 W) connected to a loudspeaker located above the animal cage. To induce isolation stress, the animal was placed in a cage with dimensions of 30 ×20 ×13 cm, isolated from other animals in a separate room for 60 to 180 min. Cage inclination stress was induced by positioning the housing cage at an inclined angle of 45º ffrom 120 min to 20 hrs. Water and food deprivation stress was induced by restricting access to water and food for up to 24h.

Behavioral tests

Consumption of sweet food

After 30 days of treatment, the consumption of sweet food was measured in all groups.[21] The animals were placed in a light-colored rectangular box made of wood (40 cm × 15 cm × 20 cm) with a glass ceiling. Ten *Froot loops* cereal pellets (Kellogg's® - pellets of wheat, cornstarch, and sucrose) were placed in one extremity of the box. Animals were subjected to five 3-min trials, once a day, to become familiarized with this food. After habituation, the animals were exposed to a test session when the number of ingested pellets was measured. A protocol was established so that when the animal consumed a portion of the cereal pellets (e.g., 1/3 or 1/4), the fraction was recorded.

Open-field test

Locomotor behavior was monitored using an open-field apparatus.[2] The apparatus consisted of a wooden box measuring 40×60×50 cm. The floor was divided into 12 equal squares and located in a sound-proof room. Animals were positioned in the central quadrant and allowed to freely explore the apparatus. During a 5-min session, the

exploratory behavior was recorded, and the following parameters were analyzed: crossings, (total and inner), grooming, and rearing. The apparatus was cleaned with a 10% alcohol solution and dried after each animal session.

Biochemical assay

Tissue preparation

Animals were killed 24hours after the last stress session to minimize its immediate effects on neurotransmitters' metabolism. After a cardiac puncture following an overdose of isoflurane, a subsequent decapitation was performed. The cerebral cortex, hippocampus, and adrenal glands were dissected and weighed. The adrenal gland weight was evaluated as an indirect indicator of HPA axis activation. The brain structures were stored at -80ºC. Afterward, the cerebral cortex and hippocampus were homogenized in 20 mM sodium phosphate buffer containing 140 mMKCl (pH 7.4) and centrifuged at 3,500 rpm for 10 min at 4ºC. The supernatant was used to evaluate the parameters of oxidative stress. The protein content was quantified by the method of Lowry and coleagues[24] using bovine serum albumin as a standard.

Determination of lipid peroxidation

Lipid peroxidation was estimated by measuring TBARS and determined according to the method described by Ohkawa and colleagues.[25] Briefly, the tissue supernatant was mixed with 15% trichloroacetic acid and 0.8% thiobarbituric acid and heated in a boiling water bath for 60 min. TBARS concentrations were determined by measuring the absorbance at 535 nm and reported as nmol TBARS/mg protein.

SOD activity

The total SOD activity was measured by the method of Misra and Fridovich[26]. This method is based on the inhibition of superoxide-dependent adrenaline auto-oxidation in a spectrophotometer adjusted to 480 nm. One SOD activity unit was defined as the enzyme amount required to cause 50% inhibition of adrenaline autoxidation. The specific activity of SOD was reported as units/mg protein (U/mg of protein).

CAT activity

CAT activity was determined based on H_2O_2 consumption and the absorbances detected using a spectrophotometer at 240nm at time intervals of 7s for 56 s. One CAT unit decomposes 1 µmol of H_2O_2 per mg protein in 1 min[27]. After calibration of the equipment, 1 mL of 25% phosphate buffer was placed in a quartz cuvette. After the apparatus was reset, the buffer was discarded. Afterward, 955 µL of phosphate buffer was added to the cuvette with 10 µL of the sample, at a concentration of 0.8 µg/µL. After 10 s, the device was zeroed and the value displayed was used as the baseline value. Afterward, 35 µL of H_2O_2 was added to start the reading curve. The experiments were performed in duplicate.

Total sulfhydryl content

The method is based on the reduction of DTNB by thiols, which in turn is oxidized (disulfide), generating a yellow derivative (TNB) whose absorption is measured spectrophotometrically at 412 nm[27]. Briefly, homogenates were added to PBS buffer, pH 7.4, containing EDTA. The reaction was initiated by the addition of 5,5'-dithio-*bis* (2-nitrobenzoic acid) (DTNB). Results were reported as nmol TNB per mg of protein.

Nitrite levels assay

The supernatants were subjected to a colorimetric reaction with the Griess reagent[28]. For this reaction, 50 µLof sulfanilamide in 5% phosphoric acid was added to 50 µL of the supernatant. Afterward, the samples were incubated at room temperature for 10 min. Subsequently, the samples were mixed with 100µLof Griess reagent (0.1% N-(1-naphthyl) ethylenediaminedihydrochloride) and incubated for 10 min in a dark room. The absorbance was measured at 540nm, and the amount of nitrite was expressed as µmol of nitrite per mg of protein.

Statistical analysis

Data are shown as mean±SEM and analyzed using two-way ANOVA, followed by Tukey's multiple range test when indicated. All analyses were performed using GraphPad Prism 5 software and the significance level was set at a *p*-value lower than 0.05.

RESULTS

Characterization of main psychoactive compounds in AYA

The AYA dosage (500mg/kg) used in this experiment had the following concentrations of psychoactive alkaloids: 0.28mg/kg DMT, i.e., 0.056% (w/w); 0.70mg/kg tetrahydroharmine, i.e., 0.14% (w/w); 0.13mg/kg of harmaline, i.e., 0.026% (w/w); and 0.57mg/kg of harmine, i.e., 0.114% (w/w).

Validation of the UCMS model

The UCMS led to a significant decrease in pellet consumption (2.00±0.98) when compared with the control groups (5.57±0.83). AYA treatment had no effect (5.05±0.80) and was unable to prevent the reduction of sweet food consumption (anhedonia-like behavior) after UCMS exposure (1.94±0.71; Figure 2A).

The relationship between adrenal weight and rat weight was increased in stress and stress AYA groups (15.90±0.44 and 16.7±0.59, respectively) when compared with the control group (13.30±0.71), while AYA had no effect on this parameter (14.80±0.98; Figure 2B).

Locomotor activity

The locomotor activity in the open field test showed no differences between groups in the parameters of the number of line crossings (Figure 3A; 63.89±7.55 control; 68.55±8.03 stress; 61.22±7.09 AYA; 69.06±3.39 AYA-Stress), grooming (Figure 3B; 0.89±0.31 control; 1.33±0.29 stress;1.22±0.49 AYA; 2.44±1.01 AYA-Stress), and rearing (Fig 3C; 12.78±1.27 control; 15.44±1.47 stress; 13.22±2.14 AYA; 16.44±2.05 AYA-Stress). Both stressed and control animals exhibited a similar number of entries to the center of the arena (0.25±0.13 and 0.73±0.32, respectively; P >0.05).

TBARS analysis

TBARS in hippocampus and cortex cerebral were analyzed to measure lipid peroxidation (Figure 4). The analysis in the hippocampus showed no differences between groups (3.64±0.56 AYA; 3.64±0.91 stress; 4.13±0.83 stress AYA, see Figure 4A) when compared with the control group (3.24±0.32). In the cerebral cortex, an increase in lipid peroxidation was observed in the stress group (3.1690±0.23) when compared with the control one (2.2670±0.14). Furthermore, the AYA and AYA stress groups were similar to control animals (2.8300±0.26 and 2.6810±0.19, respectively), as shown in Figure 4B.

Antioxidant enzymes

In hippocampal samples, an increase in SOD activity in the AYA stress group (30.34±2.06) (Figure 5A) was observed in relation to the control group (21.43±1.58). SOD activity in the AYA stress group showed an increase concerning to the stress group (23.41±0.80). The AYA group showed no differences compared with the control animals (26.24±2.25). In the cerebral cortex samples (Figure 5B),

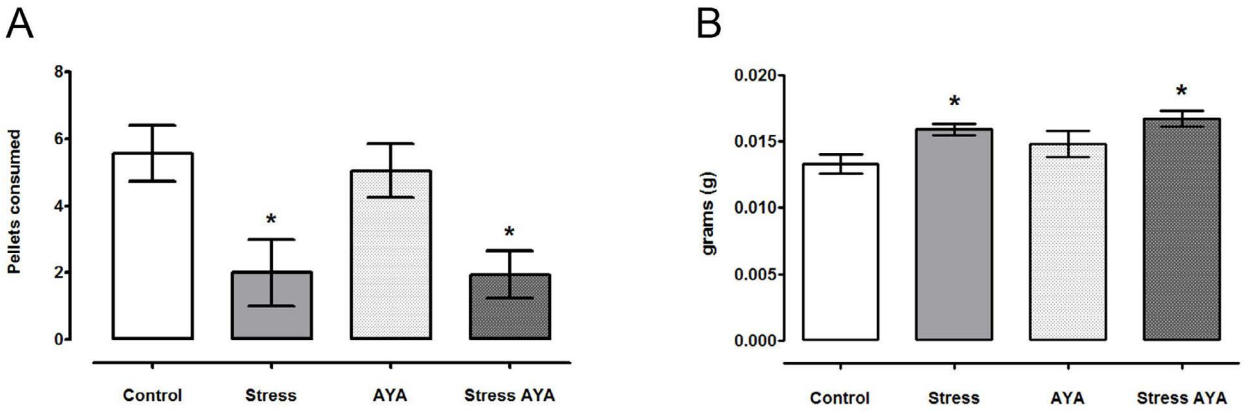

Figure 2

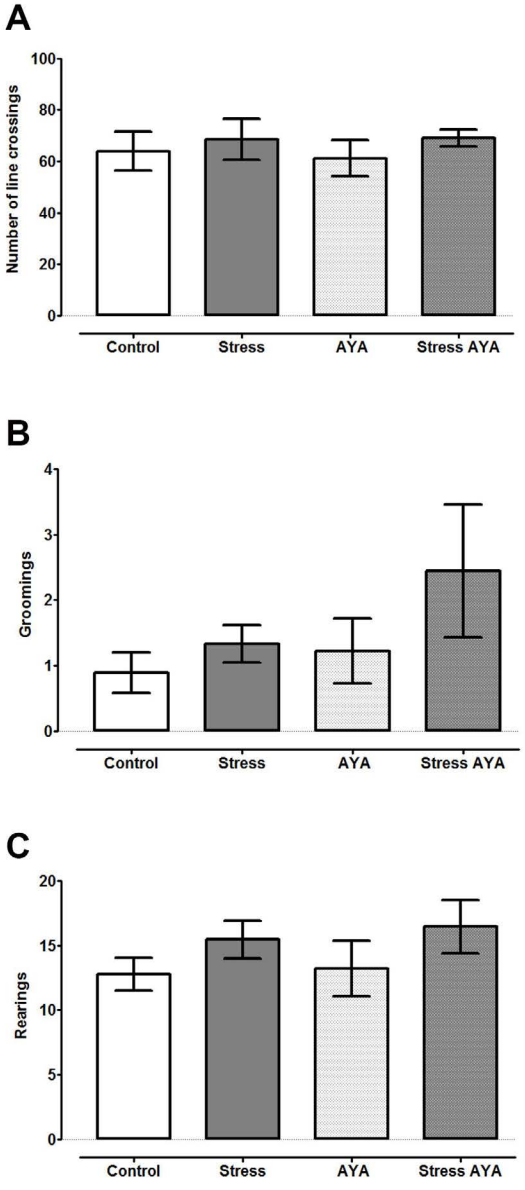

Figure 3

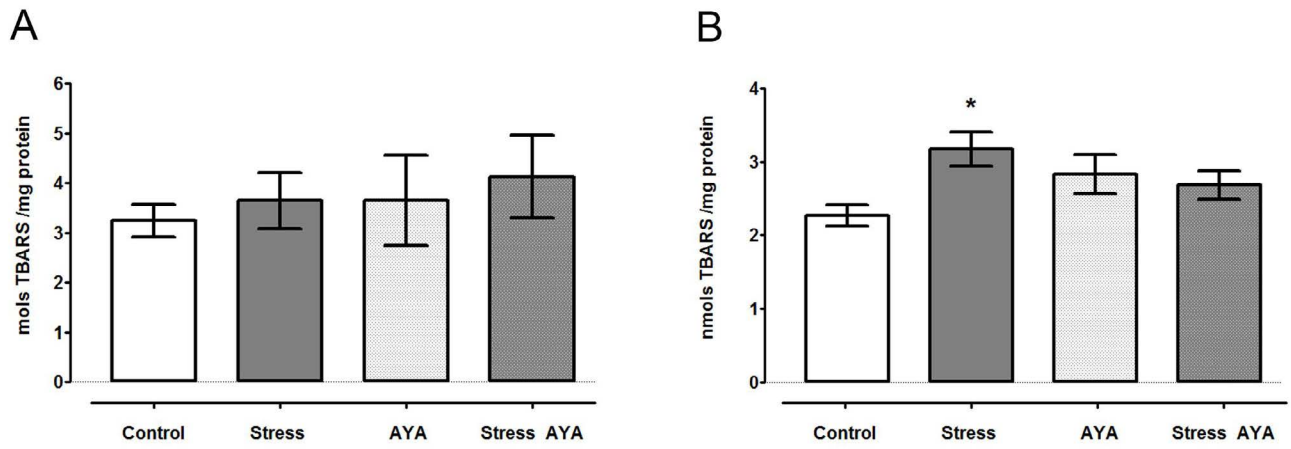

Figure 4

SOD activity showed a decrease in the stress group (15.62±0.66) when compared with the control group (17.52±1.43). AYA and stress AYA groups did not show differences in SOD activity (16.61±0.93 and 17.10±1.16, respectively) in relation to the control group.

CAT activity was decreased in the cerebral cortex of the stress group (1.26±0.14) in relation to the control group (1.90±0.21), this decrease was also reduced (1.49±0.15) in stressed animals treated with AYA. The AYA treatment alone (1.78±0.13) produced no differences in comparison with the control animals. No differences were observed in the hippocampus of all groups tested (2.11±0.16 control; 2.19±0.40 stress; 2.12±0.13AYA; and 2.16±0.25 stress AYA; Figures 5C and 5D).

Sulfhydryl activity

Figures 6A and 6B, respectively, depict levels of sulfhydryl in the hippocampus and cerebral cortex. A decrease in sulfhydryl activity was observed in the hippocampus of the stress group (55.99±6.94) when compared with the control group (72.58±2.81). AYA and stress-AYA groups did not differ from the control group (78.23±3.66 and 71.29±3.50, respectively). No difference was observed in sulfhydryl activities in the cerebral cortex among groups (87.53±3.39control; 93.39±7.01 stress; 80.56±2.37 AYA; and 81.75±2.80 stress AYA).

Nitrites level

The levels of nitrites in the hippocampus and the cerebral cortex was also evaluated (Figures 6C and 6D, respectively). In the cerebral cortex, there was a significant increase of nitrite activity in the stress (9.55±0.50) and AYA (10.42±0.83) groups in relation to the control group (7.46±0.34). The AYA stress group (8.19±0.40) was not different from the control group. No difference was observed in the hippocampus (10.22±2.29 control; 6.76±0.58 stress; 7.66±0.65 AYA; and 9.04±2.25 stress AYA).

DISCUSSION

The current study aimed to investigate some effects of AYA sub-chronic administration on behavioral and oxidative stress parameters in rats submitted to UCMS, a naturalistic paradigm of depression in rats. Inspired by scientific findings[4-7], we conjectured that AYA treatment would prevent both the behavioral and oxidative parameters of depression induced by UCMS and that AYA would not interfere with the oxidative parameters in the non-stressed animals. Our results partially corroborated these hypotheses: AYA treatment reversed oxidative parameters induced by unpredictable stress, did not alter oxidative markers in the non-stressed animals, however, was unable to prevent anhedonia-like behavior.

As expected[21], a reduction in the sucrose consumption (anhedonia-like behavior) and deleterious changes in oxidative parameters were observed in the rats submitted to UCSM. These results corroborate the UCMS as a valid model of depression in rats.

The administration of (isolated) harmine (1.67mg/300g of body weight in primates, and 1 ml/kg in humans) reversed the anhedonia-like behavior induced by UCMS in rats as reported elsewhere[6,29,30]. In contrast, our protocol failed to prevent anhedonia-like behavior. This difference may be accounted for differences in methodological procedures: (1) we administered an AYA preparation (which contains other psychoactive substances along with harmine), (2) harmine concentration in our AYA sample was smaller than the one reported in the aforementioned studies (500mg/kg of AYA that contained 0.57mg/kg of harmine), and (3) our animals were submitted to a different depression-like model. Thus, comparisons between our investigation and the cited study reports should be done cautiously.

Animals exposed to chronic stress may exhibit an increase in the HPA axis activation and corticosterone secretion, a hypertrophy of the adrenal gland, and an increase in the adrenal weight/body weight ratios[21]. Da Silva and colleagues observed in their study that only one dose AYA (1.67 mL/ 300 g) decreased depression-like behaviors, restored body weight and fecal cortisol to baseline levels[6]. Besides that, another study using an oral dose of AYA (1.0 mg DMT/kg body weight) induced significantly higher cortisol levels in the relationship to de control group[5]. Also, Fortunato and colleagues[30] observed that CMS in rats procedure induced an increase of adrenal gland weight in stressed (control-saline) compared with non-stressed. Interestingly, the treatment with harmine restored the weight of the adrenal gland to a normal range in the stressed-group.[30]

Convergently, our results showed that the ratio of adrenal and body weight of both stress and AYA-stress groups increased in comparison to the control group. Thus, AYA failed to prevent the increase of adrenal/body weight ratio, indicating that it may have no or a limited effect upon the HPA axis.

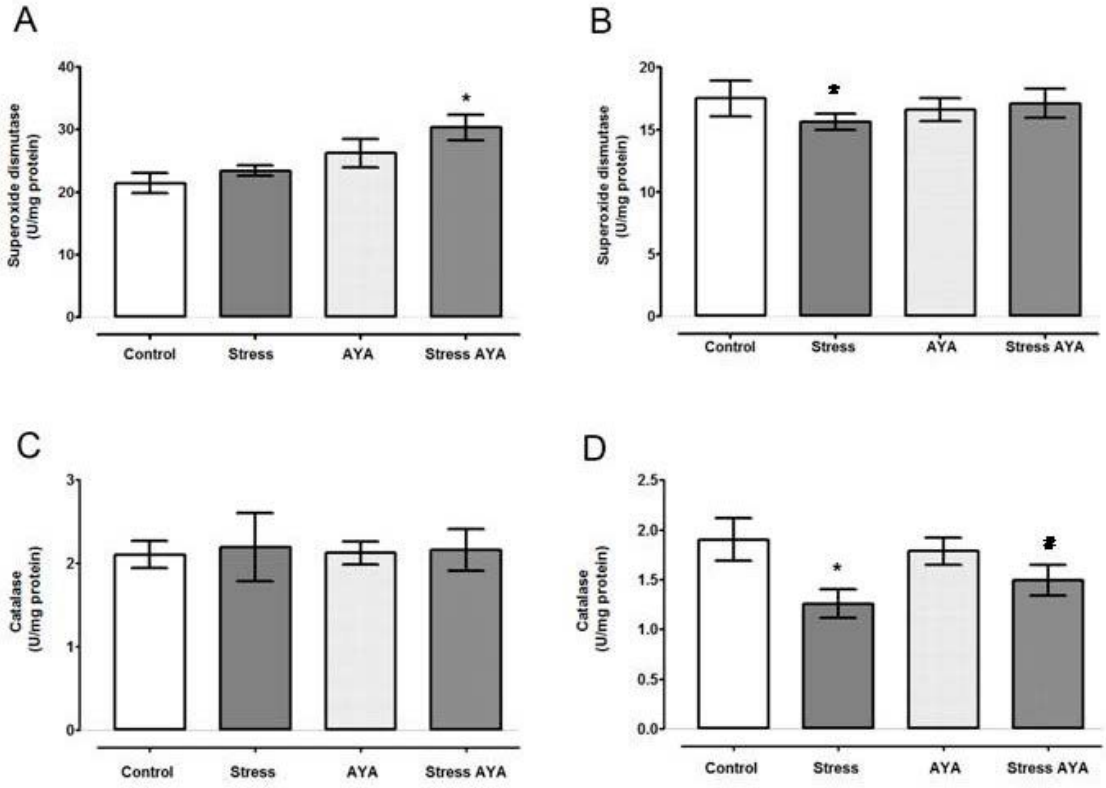

Figure 5

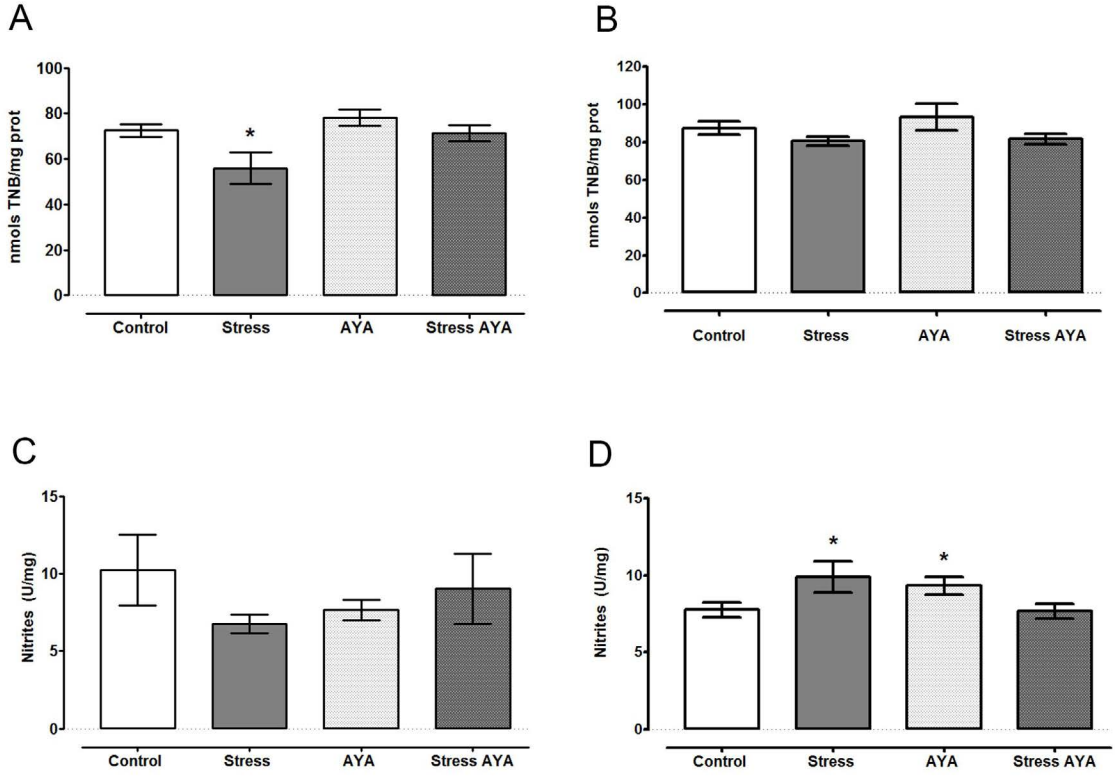

Figure 6

Inconsistent data have been reported concerning the locomotor activity of rats in an UCMS depression-like model. Correa-Netto and colleagues[31] did not observe any locomotion changes in the open field test in adult mice after a 4-weeks long AYA chronic treatment when compared to a control group. Xie and colleagues[32] reported changes in line crossings and time spent in the outer zone, nevertheless, Fortunato and colleagues[30] and Abelaira and coleagues[29] observed no alterations in open field test parameters (rearing and crossings) of rats in a UCMS model and under harmine treatment. Our UCMS protocol (with or without AYA treatment) did not produce any locomotion alteration. More studies are required to establish reliable locomotion parameters for rodents in relation to both: AYA treatments and the UCMS paradigm.

Oxidative damage in the brain is considered to be one of the main neurochemical mechanisms involved in the pathogenesis of depression[12,15]. Similarly to Lucca and colleagues[33], our findings show that UCMS increased lipoperoxidation (TBARS levels) in the cerebral cortex, but not in the hippocampus. Gupta and colleagues[34] also found increased TBARS levels in whole brain samples of mice induced to depression by corticosterone. The discrepancy of lipoperoxidation in distinct brain regions may be partially explained by the differential amount of oxidizable lipids available in the cerebral cortex and the hippocampus[35]. Also, our results show that the cerebral cortex may be more susceptible to UCMS induced oxidative stress[30].

The observed UCMS-induced increases in TBARS levels in the cerebral cortex of the stress group were accompanied by a reduction in the CAT antioxidant activity. This is partially coherent with studies that show (1) a reduction in CAT activity in all stages of cortex and hippocampus development and all brain tissues of rats submitted to depression-like models induced by maternal deprivation[17] or UCMS[36], respectively. Contrariwise, we did not find any changes in hippocampal samples from the stress group.

It was also observed that an increase in hippocampal SOD levels occurred in the stress AYA group when compared with the control group. This data showed that only when combined – the stress and the AYA treatments, changes are expected to occur to this enzyme activity. In the cerebral cortex, there was no change in SOD activity after all treatments. Kumar and Mondal[37] reported that there was a decrease in SOD in the hippocampus of mice that underwent UCMS. Besides, in a maternal deprivation model, performed to mimic depression, it was observed that SOD activity decreased at all stages of development of the cerebral cortex and hippocampus[17].

Réus and collegues[17] reported an increase in SOD activity in the hippocampus and prefrontal cortex of animals that undergo UCMS and were treated by harmine (5 mg/kg).

Our study has important methodological differences, such as the use of AYA of 500 mg/kg animal body weight (which contained 0.57mg/kg of harmine, besides other components) and not a fraction of the drink (5, 10 and 15 mg/kg) as performed in the study by Réus and colleagues[17]. Moreover, we use a lot more cortical tissue (cerebral cortex vs. prefrontal cortex) when compared to Réus and colleagues[17]. Therefore, direct comparisons are restrict and further studies are needed to clarify those relatioships.

We also examined sulfhydryl and nitrite levels after AYA and UCMS treatments and observed that sulfhydryl levels decreased in the hippocampus of the animals of the stress group in relation to the control group. Unfortunately, to date, there are no reports in the literature on the evaluation of sulfhydryl in animals exposed to AYA, making our data on this subject pioneering. Reduced glutathione (GSH) is one of the most important agents of the cellular antioxidant defense system and the main eliminator of free radicals in the brain. The GSH levels have been used as a marker of cellular health and deficits are indicative of cell injury[38]. The SH group determines the reducing ability of GSH. Protein thiols exert non-enzymatic antioxidant effects by maintaining the stability of the intracellular environment and eliminating free radicals and ROS[39]. The endoplasmic reticulum thiol flux in the brains of mice with depression phenotypes were found to be decreased after two-photon imaging analysis[40]. Thus, a decrease in thiol content may be associated with depression in rodents, as our results showed.

Nitrite can be considered to be an indirect measure of the production of nitric oxide, which induces hyper-nitrosylation, causing progressive brain damage[15]. We observed that the levels of nitrites in the hippocampus of all treated rats did not change. However, in the cerebral cortex, an increase in nitrite levels was observed in the stress and AYA groups when compared with the control group. Our results are coherent with those reported in this literature[15]. The antidepressant and anxiolytic potential of *Acacia hydaspica* extract showed decreased levels of nitrites in the stress group in relation to the control group[36]. Mice submitted to an UCMS model of depression induced by corticosterone presented high levels of brain nitrite.[31]

The current investigation has strengths and limitations worth mentioning. To the best of our knowledge, this is the first study that examined some behavioral and biochemical effects of AYA in rats submitted to UCMS. Also, because the UCMS is an animal model with recognized translational value to understand depression mechanisms and antidepressant therapeutics, our results advanced in the understanding of some neuromechanisms that may account for AYA therapeutic effects in humans. Moreover, it is possible to conjecture that AYA frequent users may display a more favorable oxidative stress status and, consequently, may respond better to antidepressant drugs. However, more studies are indeed required to advance in the understanding of neuromechanisms associated with the antidepressant therapeutics of AYA.

CONCLUSION

Our UCMS protocol was successful in inducing anhedonia-like behavior and coherent changes in oxidative stress parameters. The subchronic AYA treatment was unable to avert anhedonia however, it prevented oxidative stress from the occurrence. These findings indicate that AYA therapeutics may be related to the inhibition of the oxidative stress component of depression. Furthermore, AYA treatment alone did not interfere with the oxidative parameters assessed in this research, which may be interpreted as a sign of the adaptogenic potential of AYA.

C**ontributions:** GdePC, JXS, and GDG designed the methodology, analyzed the data, and wrote the manuscript; JXS and CPF collected behavioral data; JXS and MSPS performed oxidative stress analyses. GdeOS and MY quantified ayahuasca alkaloids; RMS contributed with infrastructure and reagents, and HWdeC acquired the ayahuasca, wrote the manuscript, revised and submitted its final version.

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Conflicts of Interest: none.

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