

Research Article

Neuroprotective Effects of Açai (*Euterpe oleracea* Mart.) against Rotenone *In Vitro* Exposure

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Neuropsychiatric diseases, such as bipolar disorder (BD) and schizophrenia (SCZ), have a very complex pathophysiology. Several current studies describe an association between psychiatric illness and mitochondrial dysfunction and consequent cellular modifications, including lipid, protein, and DNA damage, caused by cellular oxidative stress. *Euterpe oleracea* (açai) is a powerful antioxidant fruit. Açai is an Amazonian palm fruit primarily found in the lowlands of the Amazonian rainforest, particularly in the floodplains of the Amazon River. Given this proposed association, this study analyzed the potential *in vitro* neuropharmacological effect of *Euterpe oleracea* (açai) extract in the modulation of mitochondrial function and oxidative metabolism. SH-SY5Y cells were treated with rotenone to induce mitochondrial complex I dysfunction and before and after we exposed the cells to açai extract at 5 µg/mL. Treated and untreated cells were then analyzed by spectrophotometric, fluorescent, immunological, and molecular assays. The results showed that açai extract can potentially increase protein amount and enzyme activity of mitochondrial complex I, mainly through NDUFS7 and NDUFS8 overexpression. Açai extract was also able to decrease cell reactive oxygen species levels and lipid peroxidation. We thus suggest açai as a potential candidate for drug development and a possible alternative BD therapy.

1. Introduction

Neuropsychiatric diseases are an important problem in public health around the world [1, 2]. Bipolar disorder (BD) is a chronic mental illness that causes significant impairment in life quality and is an important cause of disability in young people [3, 4]. The prevalence of BD in the world is around 3%, and it can affect populations independently

of socioeconomic status or nationality [3]. Patients with BD present recurrent episodes of mania and depression, but the etiology of the disease is still not completely clear. Usually subjects with BD have a genetic component that interacts with the environment to develop the disease [5]. Some evidence suggests the significant role of mitochondria in BD [6–8]. Current research has demonstrated that BD is associated with mitochondrial complex I deficiency and it

can decrease ATP production and increase reactive oxygen species (ROS) levels. Consequently, the cells present oxidative stress followed by different cell damage, including lipid peroxidation, protein oxidation, and DNA damage [9, 10]. The brain is one of the tissues most affected by mitochondrial dysfunction due to its high sensitivity to oxidative stress and energy demands for normal neurotransmission [11, 12].

Currently, the pharmacotherapy for BD involves mood stabilizers and second-generation antipsychotics [13–15] whose side effects are proportional to the duration of treatment and dose of medication [4], and these side effects are the main cause of treatment interruption. The prolonged use of different antipsychotic medications has been related to the incidence of metabolic dysfunctions, such as obesity, dyslipidemia, high blood pressure, and increased glycaemia levels [1, 4]. Lithium, the main mood stabilizer used in BD therapy [2], acts directly on cell mitochondria. Hou et al. [16] showed that lithium is able to protect dopaminergic cells against mitochondrial complex I deficiency induced by rotenone exposition; however, lithium can also cause side effects, including memory disorders, renal dysfunction [17–19], and metabolic diseases such as diabetes and hypothyroidism [4]. Therapy for BD is also still limited, because the results obtained from drug development studies are usually unsatisfactory or unsafe, and the action mechanism of some medications is still unclear [2]. Functional foods and natural product studies that could possibly improve mitochondrial function might thus help subjects with BD to recover their quality of life and decrease the extensive burden of this disease.

This is the case of *Euterpe oleracea*, an Amazonian Brazilian fruit popularly known as açai [20]. Açai presents several bioactive molecules with different bioactive properties, including antioxidant, anti-inflammatory, and analgesic activities, and it is also able to modulate calcium homeostasis and autophagy on brain cells [21–26]. Açai's biological effects are related to its chemical matrix, which includes numerous phytochemicals components such as flavonoids [27]. These molecules can neutralize ROS by itself and/or inactivate molecules with prooxidant capacity [28–30]. It is also known that flavonoids have potent anti-inflammatory effects [31, 32]. In an *in vitro* study, Xie et al. [33] demonstrated that velutin, found in açai fruit, is able to decrease proinflammatory cytokines as tumor necrosis factor- (TNF-) alpha and interleukin- (IL-) 6 in macrophage cells. Another flavonoid found in açai fruit is apigenin [34]. Apigenin has been identified as a neuroprotective biomolecule against Alzheimer's disease [35], Parkinson disease [36], and ischemic injury [37, 38]. Since açai is a rich source of flavonoids and other compounds with bioactive power we hypothesize that freeze-dried hydroalcoholic açai extract might have positive effects against neuropsychiatric diseases as BD.

On this basis, we developed a study of açai freeze-dried hydroalcoholic extract effects on neuronal-like cells (SH-SY5Y) with mitochondrial complex I deficiency. The main objective of this research was to analyze whether *Euterpe oleracea* extract is able to prevent and/or reverse mitochondrial dysfunction induced by rotenone exposure and also protect against cell imbalance consequences. This study could open

new exploratory means of drug development and targets of therapy for BD.

2. Materials and Methods

2.1. *Euterpe oleracea* Extract and Quantification of Compounds. Fresh açai fruits were obtained from a harvesting area in Manaus city, Amazonas state. To prevent any change in the fruit quality and properties of the components, they were frozen immediately after the fruit harvest and kept at -20°C . The frozen fruits were transported to the Biogenomics Laboratory at the Federal University of Santa Maria. The fruits were confirmed to be *Euterpe oleracea* by a specialist in plant ecology and botany. A freeze-dried hydroalcoholic extract of açai was obtained. First of all the açai fruits were manually macerated to remove the seeds. Then the skin and pulp were placed in ethanol (Neon® commercial-03467; Sao Paulo, SP, Brazil) 70% (70% absolute ethanol:30% distilled water; v:v) for 21 days at a concentration of 300 mg/mL. After the period of extraction, the material was filtered and the liquid part was lyophilized after ethanol removal. Freeze-dried hydroalcoholic extract powder was then conducted to compounds quantification and characterization.

To determine the main molecules present in açai hydroalcoholic extract as a chemical matrix we performed the analysis through high performance liquid chromatography (HPLC-DAD) using the Shimadzu Prominence Auto Sampler (SIL-20A) system (Shimadzu, Kyoto, Japan). The freeze-dried hydroalcoholic extract was analyzed following the protocol reported by Klimaczewski et al. [39] at 15 mg/mL. The standards used in this analysis included formic acid, gallic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, catechin, and epicatechin purchased from Merck (Darmstadt, Germany), chrysin, luteolin, apigenin, orientin, and vitexin, acquired from Sigma Chemical Co. (St. Louis, MO, USA), and cyanidin 3-*O*-glucoside acquired from ChromaDex (Irvine, CA, USA). We calculated the limit of detection (LOD) and the limit of quantification (LOQ) based on the standard deviation of response and the slope using three independent curves of analysis, as described by Abbas et al. [40].

2.2. Cell Culture and Treatments. Neuronal-like cells SH-SY5Y were obtained from the American Type Culture Collection (ATCC® CRL-2266™; Manassas, VA, USA) and cultured in DMEM/F12 medium (Gibco® Thermo Fisher-11320033; Mississauga, ON, Canada) supplemented with 10% of fetal bovine serum (FBS) (Gibco® Thermo Fisher-12484028; Mississauga, ON, Canada) and 1% penicillin (100 U/mL)/streptomycin (100 mg/mL) (Gibco® Thermo Fisher-15140122; Mississauga, ON, Canada). Cells were cultured until there was an ideal confluence and number of cells to perform all the treatments and experiments at 37°C in 5% CO_2 and 95% O_2 in a humidified environment.

The treatments were performed to measure the capacity of açai freeze-dried hydroalcoholic extract to prevent and/or reverse mitochondrial complex I deficiency and the possible damage caused by this cellular imbalance. To induce mitochondrial complex I deficiency we exposed the cells

to rotenone in different concentrations (5, 15, and 30 nM) during 24 h incubation as described by Kim et al. [41]. Before and after rotenone exposure treatment was performed with hydroalcoholic lyophilized açai extract at optimal cells culture conditions.

2.3. Cell Viability Assay. To select the most effective concentration of açai freeze-dried hydroalcoholic extract in SH-SY5Y cells we measured cell viability response by XTT (tetrazolium salt; Sigma-Aldrich-x4251; St. Louis, MO, USA) assay, following the manufacturer's instructions. The cells were exposed to different concentrations of açai freeze-dried hydroalcoholic extract (0.001 µg/mL–1000 µg/mL) under different periods of incubation (24, 48, and 72 hours). The most effective freeze-dried extract concentration and time of incubation treatment were selected calculating the EC50 to perform all the other assays of this research, adding açai freeze-dried hydroalcoholic extract before or after rotenone exposure.

2.4. Scanning Confocal Microscopy Analysis of Cell Morphology. SH-SY5Y cells treated with rotenone and/or açai freeze-dried hydroalcoholic extract were placed under sterile glass cover lids (VWR Collection-2441; Radnor, PA, USA) in 6-well cell culture plates with 7.5×10^4 cells/well overnight to evaluate cell morphology. Cells were treated with rotenone and/or açai freeze-dried hydroalcoholic extract according to the experimental designs performed for cell viability analysis. After all treatments the cells were fixed with ethanol:acetic acid (3:1, v:v). Images were acquired using a fluorescence microscope (Nikon Eclipse Ti-U; Mississauga, ON, Canada) and a high sensitivity QImaging camera, model Retiga™ 1300 (QImaging Scientific cameras; Surrey, BC, Canada) at 20x magnification.

2.5. Analysis of Human Mitochondrial Oxidative Phosphorylation (OXPHOS). The oxidative phosphorylation of neuronal-like cells previously treated with rotenone and/or açai freeze-dried hydroalcoholic extract for both research elements (prevent and reverse) was measured through a human oxidative phosphorylation magnetic bead panel (Millipore, H0XPSMAG-16K; Toronto, ON, Canada) following the manufacturer's instructions. In this assay we analyzed all the mitochondrial complexes (I, II, III, IV, and V) simultaneously in an individual well reaction per treatment in triplicate. Results were obtained using the high precision machine Megapix® (Luminex Corporation xMAP Technology; Toronto, ON, Canada).

2.6. Mitochondrial Complex I Enzyme Activity. We measured mitochondrial NADH: ubiquinone oxidoreductase complex (mitochondrial complex I) activity using a mitochondrial complex I enzyme activity kit (Abcam-ab-109721; Cambridge, UK) following the manufacturer's instructions. This is a colorimetric assay based on enzyme immunocapture through the oxidation of NADH to NAD⁺.

2.7. Protein Expression of Mitochondrial Complex I Subunits. To analyze the effect of açai on the amount of protein in the mitochondrial complex I Q module subunits NDUFS7 and NDUFS8 and also in N module subunits NDUFV1 and NDUFV2, four very important subunits associated with complex I activity, we performed western blot analysis according to a protocol reported by Andreatza et al. [42]. The cell lysate of each treatment was loaded on to 12% polyacrylamide (Sigma-Aldrich-M7279; St. Louis, MO, USA) gels and transferred to polyvinylidene difluoride (PVDF) membranes (GE Healthcare-10600023; Little Chalfont, UK). The membranes were stained with Memcode reversible stain (Thermo Fisher-23227; Mississauga, ON, Canada) according to the manufacturer's protocol. After a blocking process (1h, 5% BSA) the membranes were incubated with primary anti-NDUFS7, anti-NDUFS8, anti-NDUFV1, or anti-NDUFV2 (Santa Cruz Biotechnology-98644; Dallas, USA) (Abcam-ab96123; Cambridge, UK) rabbit polyclonal antibodies (1:1000). After 90 minutes of incubation we added the secondary anti-rabbit (1:1000). Membranes were incubated with ECL western blotting substrate (Thermo Fisher-32106; Mississauga, ON, Canada) and analyzed by acquiring images using Versa Doc equipment 5000 MP (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada) and Quantity One Analysis software version 4.6.9 (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada). Sample intensity was normalized by beta-actin protein levels.

2.8. Gene Expression of Mitochondrial Complex I Subunits Genes. To complement mitochondrial complex I analysis, we measured NDUFS7, NDUFS8, NDUFV1, and NDUFV2 gene expression using qRT-PCR. Initially the RNA was extracted from treated cells using Trizol® reagent (Thermo Fischer-15586026; Mississauga, ON, Canada). After RNA quantification through a NanoDrop™ 1000 Spectrophotometer System® (Thermo Scientific, Wilmington, DE, USA), RNA was converted to complementary DNA (cDNA) using a QuantiTect® Reverse Transcription Kit (Qiagen-205311; Toronto, ON, Canada). Real-time PCR was performed using NDUFS7, NDUFS8, NDUFV1, or NDUFV2 QuantiTect® Primers (Qiagen-cat. QT0045850; Toronto, ON, Canada) and QuantiFast® SYBR® Green PCR Kit (Qiagen-cat. 204054; Toronto, ON, Canada) on CFX 96™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Beta-actin was used as the housekeeping gene to normalize the gene expression of all samples.

2.9. Total Levels of ROS Measurement. Total levels of ROS were determined in treated SH-SY5Y cells using 2,7 dichlorodihydrofluorescein diacetate (DCFH-DA) assay, as described by Costa et al. [43]. DCFH-DA (Sigma-Aldrich-D6883; St. Louis, MO, USA) is a nonfluorescent compound that is deacetylated by mitochondrial esterase enzymes to DCFH which reacts with ROS molecules and becomes DCF, a fluorescent compound. Fluorescence was measured at an excitation wavelength of 488 nm and an emission wavelength of 525 nm.

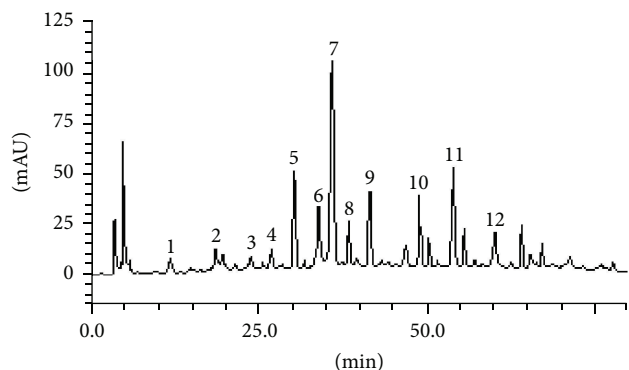


FIGURE 1: Representative high performance liquid chromatography profile of *Euterpe oleracea* freeze-dried hydroalcoholic extract. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), *p*-coumaric acid (peak 5), epicatechin (peak 6), orientin (peak 7), vitexin (peak 8), cyanidin-3-*O*-glucoside (peak 9), luteolin (peak 10), apigenin (peak 11), and chrysin (peak 12).

2.10. Lipid Peroxidation Analysis. Lipid peroxidation was analyzed using thiobarbituric acid reactive substances (TBARS) kit assay (Cayman Chemical-700870; Ann Arbor, MI, USA), following the instructions described by the manufacturer. The malondialdehyde (MDA)/thiobarbituric acid reaction was determined measuring the absorbance at 535 nm.

2.11. Statistical Analysis. The data was first transformed to percentages against a negative control group. Results from dose-response curves were statistically analyzed using one-way ANOVA, and other results were analyzed using two-way ANOVA analysis of variance. Both were followed by a Tukey *post hoc* test, using Graphpad prism software, version 5.0 (Graphpad Prism software, 2015; San Diego, CA, USA). Results of $p < 0.05$ were considered significant.

3. Results

Twelve molecules were quantified in the açai freeze-dried hydroalcoholic extract: gallic acid (retention time, $R_t = 10.27$ min; peak 1), catechin ($R_t = 17.83$ min; peak 2), chlorogenic acid ($R_t = 23.91$ min; peak 3), caffeic acid ($R_t = 26.11$ min; peak 4), *p*-coumaric acid ($R_t = 29.97$ min; peak 5), epicatechin ($R_t = 34.08$ min; peak 6), orientin ($R_t = 35.14$ min; peak 7), vitexin ($R_t = 37.41$ min; peak 8), cyanidin-3-*O*-glucoside ($R_t = 41.65$ min; peak 9), luteolin ($R_t = 48.25$ min; peak 10), apigenin ($R_t = 54.13$ min; peak 11), and chrysin ($R_t = 60.27$ min; peak 12) (Figure 1 and Table 1). The three most concentrated molecules found in the extract were orientin (8.05 ± 0.03), *p*-coumaric acid (3.52 ± 0.01), and apigenin (3.49 ± 0.01).

The cell viability response of neuronal-like SH-SY5Y cells to different concentrations of açai freeze-dried hydroalcoholic extract was analyzed (Figure 2). Initially, the cell response to rotenone treatments at 5, 15, and 30 nM was also measured to confirm the toxicity of these concentrations. Cell morphology and rate of proliferation were also

TABLE 1: Components of hydroalcoholic *Euterpe oleracea* freeze-dried hydroalcoholic extract.

Compounds	<i>Euterpe oleracea</i>		LOD $\mu\text{g/mL}$	LOQ $\mu\text{g/mL}$
	Fruits (mg/g)	%		
Gallic acid	0.73 ± 0.01^a	0.07	0.011	0.036
Catechin	0.75 ± 0.03^a	0.07	0.028	0.093
Chlorogenic acid	0.41 ± 0.01^b	0.04	0.023	0.075
Caffeic acid	0.76 ± 0.01^a	0.07	0.008	0.026
<i>p</i> -Coumaric acid	3.52 ± 0.01^c	0.35	0.031	0.102
Epicatechin	2.37 ± 0.02^d	0.23	0.024	0.079
Orientin	8.05 ± 0.03^e	0.80	0.016	0.053
Vitexin	2.19 ± 0.01^f	0.21	0.015	0.048
Cyanidin-3- <i>O</i> -glucoside	2.62 ± 0.01^g	0.26	0.009	0.029
Luteolin	2.57 ± 0.02^g	0.25	0.021	0.070
Apigenin	3.49 ± 0.01^c	0.34	0.027	0.089
Chrysin	1.83 ± 0.01^h	0.18	0.013	0.043

Açai freeze-dried hydroalcoholic extract compound determination. Results are expressed as mean \pm standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.05$.

analyzed under rotenone and/or açai freeze-dried hydroalcoholic extract treatments. As expected, rotenone treatments decreased cell viability in a dose-dependent way with a rate of mortality about 24% at 5 nM, 26% at 15 nM, and 43% at 30 nM of rotenone (Figure 2(a)). On the other hand, there was a hormetic response of neuronal-like cells under açai extract concentrations mainly after 48 and 72 h of treatment (Figures 2(c) and 2(d)) increasing the cell viability rate from 0,005 $\mu\text{g/mL}$ until 100 $\mu\text{g/mL}$ of açai freeze-dried extract. The açai extract significantly increased cell viability, mainly after 48 h of incubation, compared to the negative control. We determined the EC50 of açai freeze-dried hydroalcoholic extract for this dose-response curve, and the value obtained was 5 $\mu\text{g/mL}$. All other experiments were performed using this specific concentration and period of incubation for the açai freeze-dried extract.

Scanning confocal microscopy analysis showed that cell morphology was preserved even at rotenone treatments; however cell proliferation decreased considerably at these conditions of exposition (Figures 2(g), 2(h), and 2(i)). The concomitant açai freeze-dried hydroalcoholic extract exposition at 5 $\mu\text{g/mL}$ (Figure 2(f)) retained cell morphology and recovered the proliferation of cells exposed to different concentrations of rotenone (Figures 2(j)–2(o)) similarly to the final negative control (Figure 2(p)). In both experimental designs (prevent and reverse) açai extract was able to stimulate cell proliferation compared to the negative control, neutralizing the rotenone effect.

The capacity of açai to prevent and/or reverse mitochondrial function was evaluated from these results. The OXPHOS analysis showed a decreased protein expression for mitochondrial complex I and açai freeze-dried hydroalcoholic extract presented an increased potential. Açai supplementation at 5 $\mu\text{g/mL}$ also improved the mitochondrial complex I proteins of neuronal-like cells in both experimental designs, mainly preventing the rotenone effects of

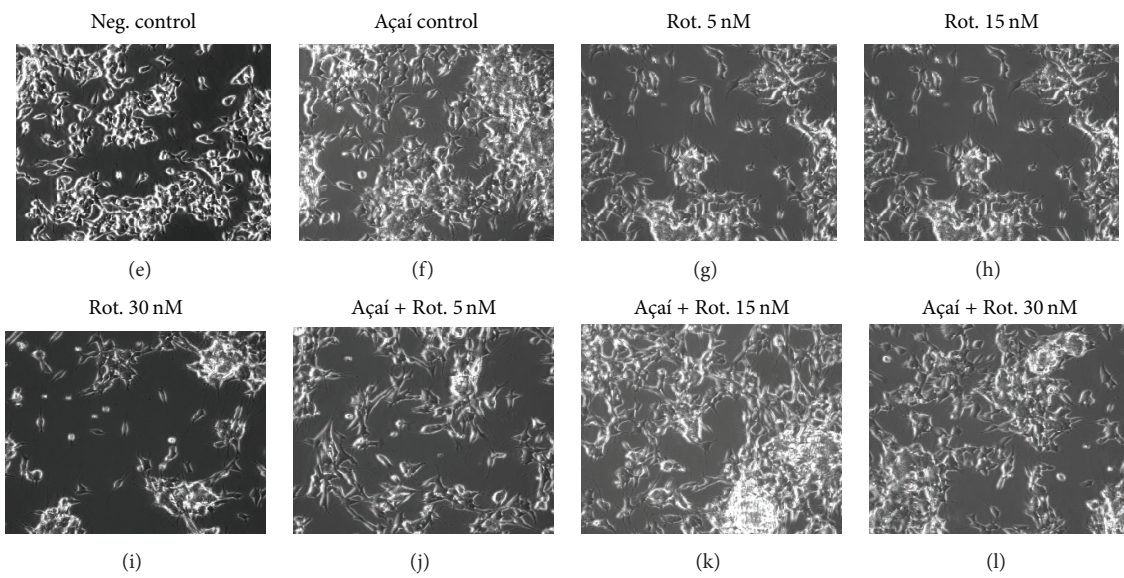
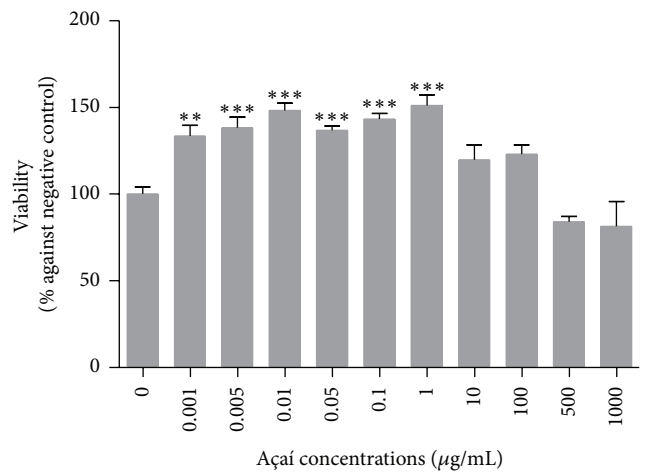
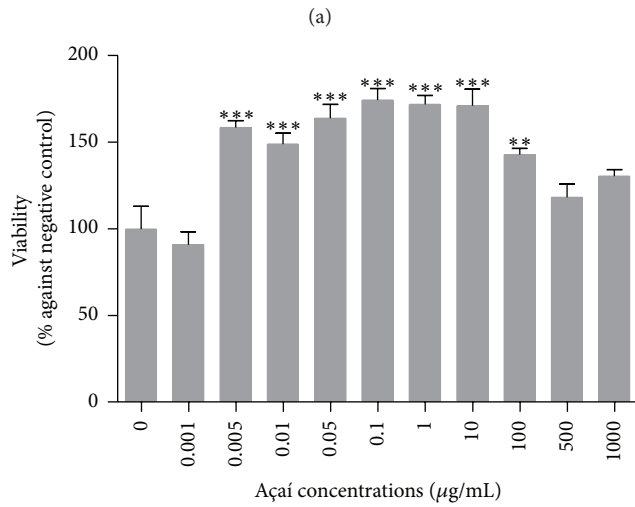
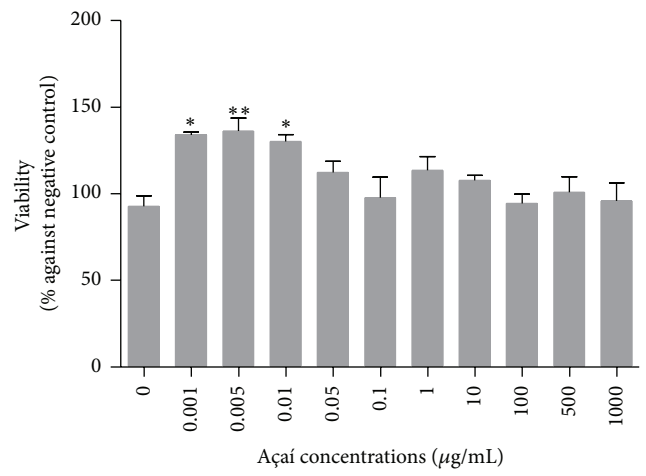
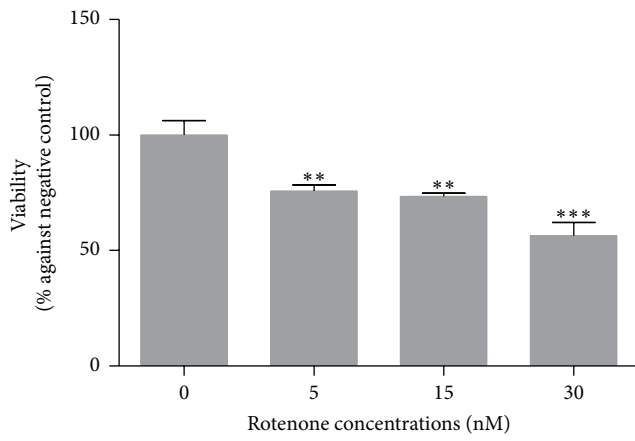


FIGURE 2: Continued.

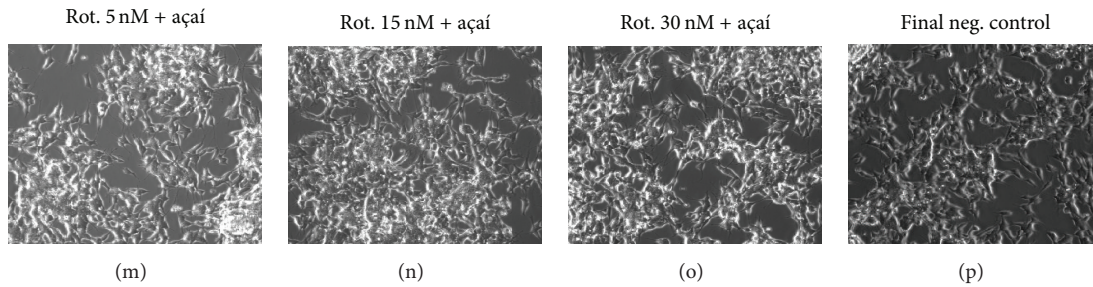


FIGURE 2: Cell viability measurements. (a) SH-SY5Y cells exposure to 5, 15, and 30 nM of rotenone during 24 h showing significant cell mortality for all concentrations; (b, c, and d) SH-SY5Y cells exposure to different concentrations of açai freeze-dried hydroalcoholic extract during 24, 48, and 72 h showing a hormetic cell response; (e–p) microscopic scanning of SH-SY5Y cells exposure to rotenone and/or açai freeze-dried hydroalcoholic extract (5 $\mu\text{g}/\text{mL}$) showing the cytotoxic effect of rotenone and the protective action of açai freeze-dried hydroalcoholic extract. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

5 and 15 nM (Figure 3(a)) and reversing those of 15 and 30 nM (Figure 3(b)). On the other hand, we observed the opposite response for mitochondrial complexes II and III under rotenone treatments and in this case, açai freeze-dried hydroalcoholic extract proved to cause protein expression in similar conditions to the negative control at 15 and 30 nM of rotenone for both experimental designs (Figures 3(c), 3(d), 3(e), and 3(f)). No significant results were observed for mitochondrial complexes IV and V (Figures 3(g), 3(h), 3(i), and 3(j)).

Mitochondrial complex I enzyme activity presented a decreased dose-dependent activity under rotenone treatments and an increased enzyme action under açai exposure. Açai proved to be able to improve the enzyme activity of this complex under preventative and reverse conditions, especially before and after rotenone 15 nM (Figures 3(k) and 3(l)).

Açai freeze-dried hydroalcoholic extract modulated NDUFS7, NDUFS8, and NDUFV2 protein expression differently, as shown in Figure 4. Rotenone significantly decreased all the subunits tested in this study at all concentrations unless NDUFV1. On the other hand, açai extract improved protein expression tested in at least one design of the experiment and in one concentration of rotenone for NDUFS7, NDUFS8, and NDUFV2. qRT-PCR for those mitochondrial complex I subunits showed a similar profile for gene and protein expression for NDUFS7 and NDUFS8 (Figures 4(i), 4(j), 4(k), and 4(l)); however, açai extract modified gene expression differently to protein expression finds, with significant improvements in gene expression for NDUFV1 (Figures 4(m) and 4(n)), which was not observed with western blot analysis.

While rotenone treatments increased ROS total levels at SH-SY5Y cells in a dose-dependent way, açai freeze-dried hydroalcoholic extract at 5 $\mu\text{g}/\text{mL}$ considerably reduced ROS production for all rotenone concentrations in both experimental designs (Figures 5(a) and 5(b)), showing important antioxidant activity. As a consequence of oxidative stress, rotenone also increased lipid peroxidation tested by TBARS assay; however, as previously expected açai freeze-dried hydroalcoholic extract was also able to decrease lipid peroxidation rates in both experimental designs (Figures 5(c)

and 4(d)), normalizing this imbalance compared to rotenone concentrations and negative control.

4. Discussion

The present *in vitro* study described the important protective effects of açai in neuronal-like SH-SY5Y cells exposed to rotenone that caused mitochondrial dopaminergic dysfunction. The açai protection involved a reversion of mitochondrial complex I dysfunction and oxidative stress caused by rotenone (Figure 6).

It is currently known that BD is associated with mitochondrial dysfunction, especially at complex I, and this abnormality has several cell oxidative consequences [8, 9, 44]. Since the pharmacotherapy used for BD is neither fully effective nor safe, it is a necessity to search for new alternative therapies. The results described here indicate that the chemical matrix found in açai fruit could be a potential candidate for improving the function of mitochondrial complex I and subsequently improving neuropsychiatric BD symptoms [21–23].

Açai freeze-dried hydroalcoholic extract showed the presence of different important compounds with known biological effects, mainly orientin, *p*-coumaric acid, and apigenin. Orientin is known to be an important phenolic compound found in different fruits. This molecule has significant antioxidant, anti-inflammatory, and neuroprotective effects [45–48]. In a study performed by An et al. [49], orientin demonstrated significant antioxidant activity and neural ultrastructure improvement in aged mice. The *p*-coumaric acid is another phenolic compound with considerable antioxidant capacity [50]; however, apigenin also demonstrates antioxidant activity as an important neuroprotective competence due to its ability to cross the blood-brain barrier [51]. Previous studies have shown that apigenin has no toxic effects, even at high doses [52].

It is still unknown whether açai could cross the blood-brain barrier by itself; however, there are some studies describing its neuroprotective effects. In work developed by Poulouse et al. [24], for example, açai was able to attenuate calcium dysregulation in rodent brain cells and to modulate

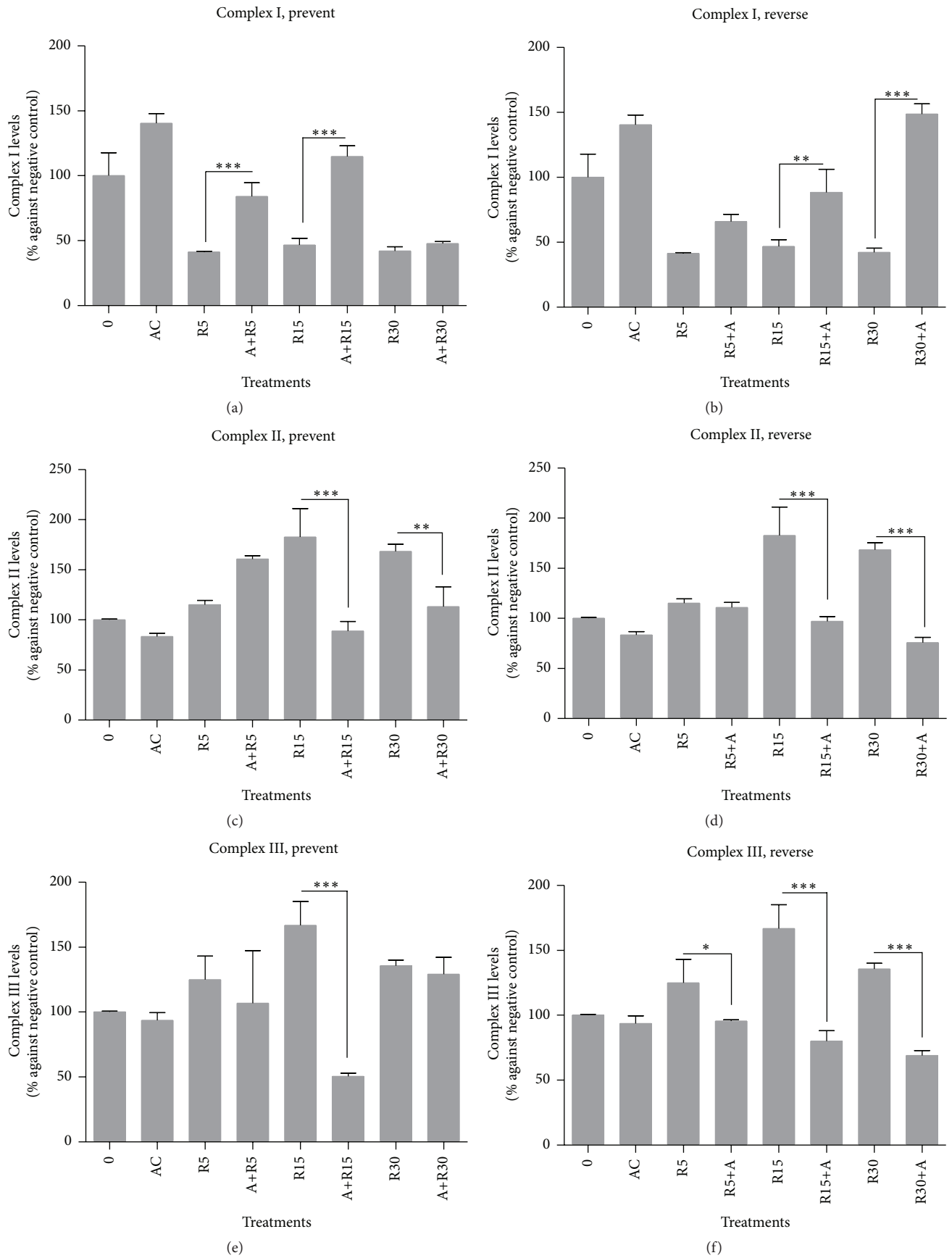


FIGURE 3: Continued.

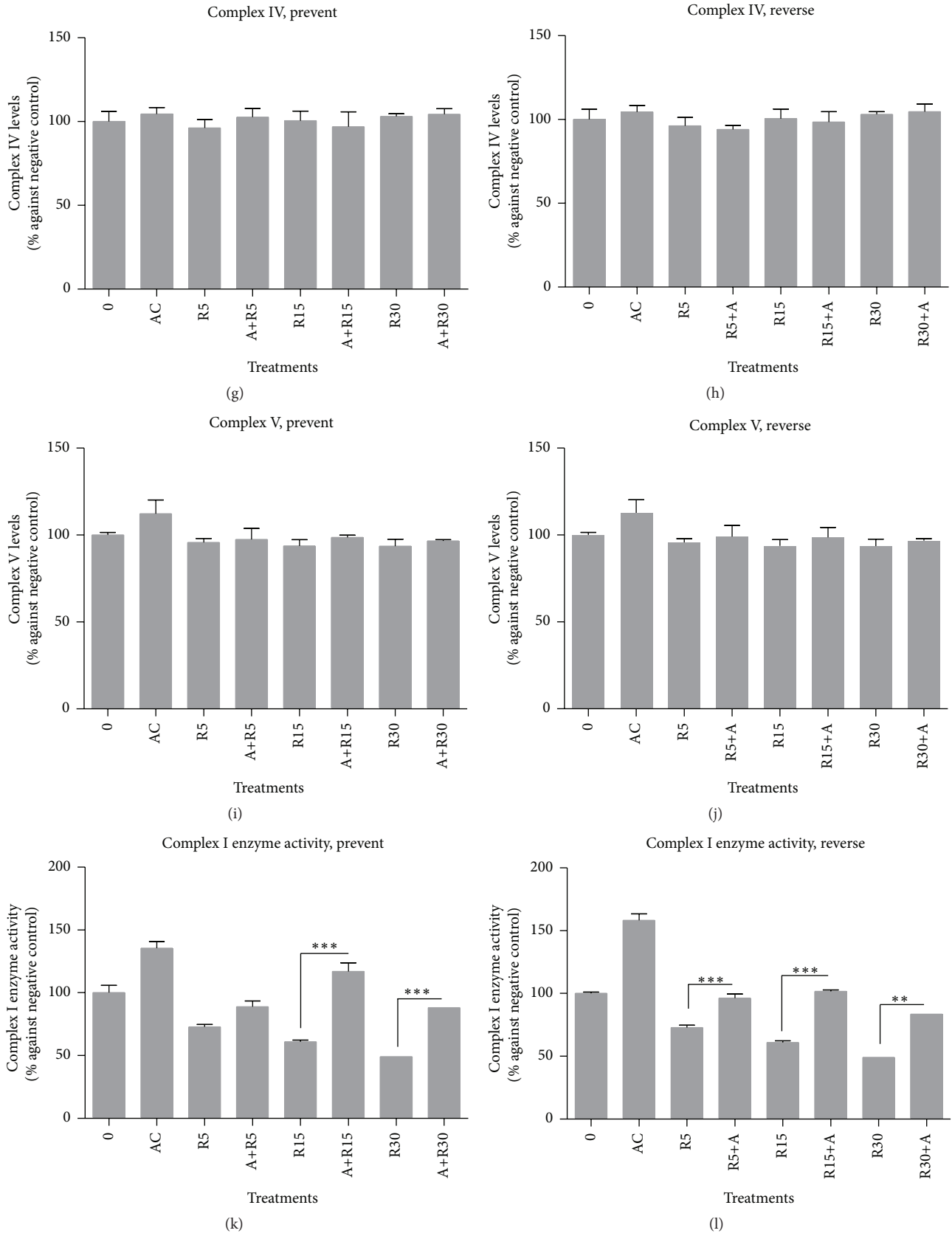


FIGURE 3: Cellular oxidative phosphorylation pathway measurements. (a–j) Rotenone exposition decreased mitochondrial complex I and increased mitochondrial complexes II and III, and açai freeze-dried hydroalcoholic extract normalized the protein expressions. (k and l) Rotenone decreased mitochondrial complex I enzyme activity and açai freeze-dried hydroalcoholic extract was able to increase enzyme activity in both designs of experiment. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

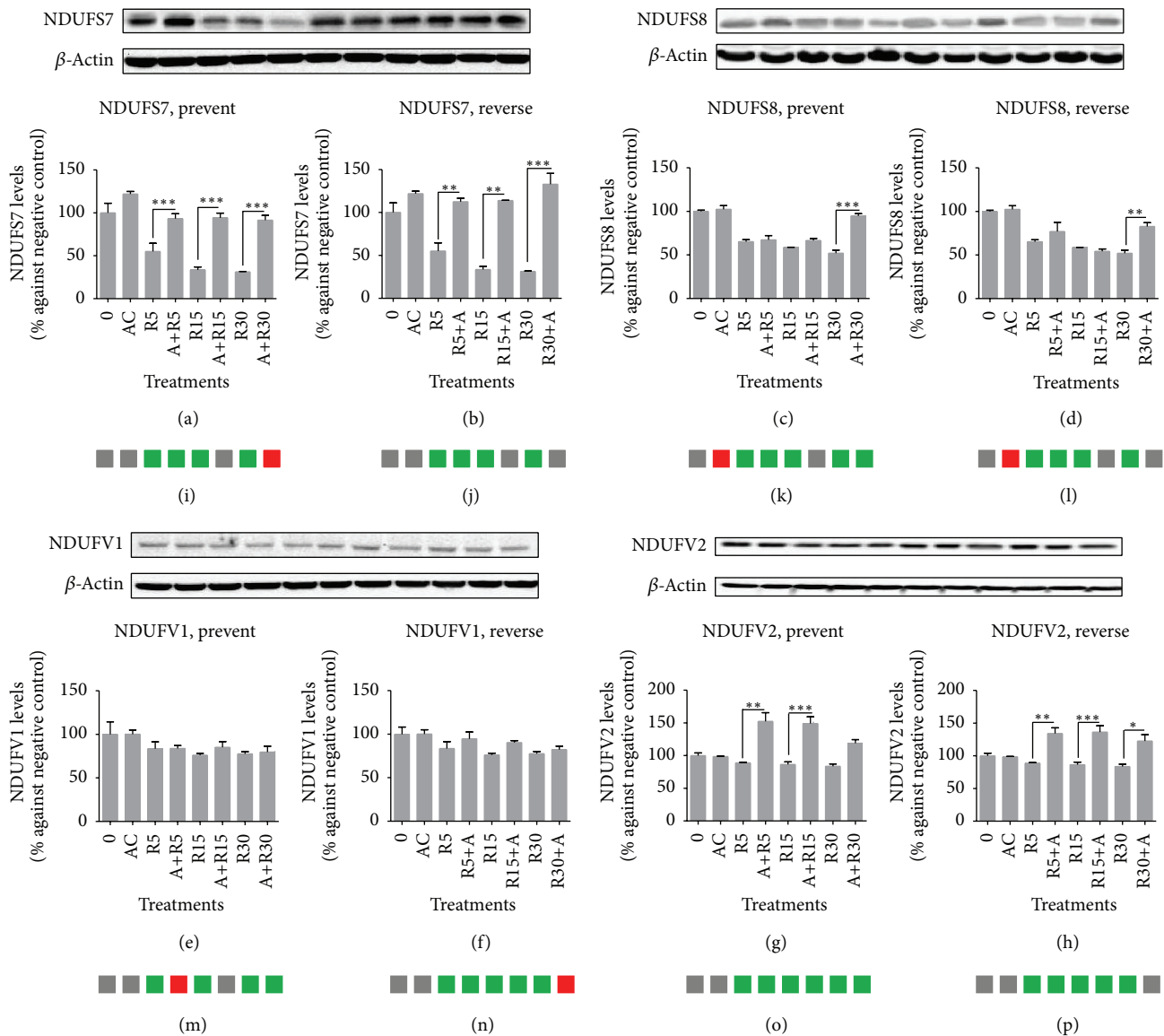


FIGURE 4: Mitochondrial complex I subunits analysis by western blot and qRT-PCR assays. The order of treatment for all the blot images: 0, Rot. 5, Rot. 15, Rot. 30, A+R5, A+R15, A+R30, R5+A, R15+A, and R30+A; (a and b) NDUFV2 protein expression analysis; (c and d) NDUFV1 protein expression analysis; (e and f) NDUFV2 protein expression analysis; (g and h) NDUFV1 protein expression analysis; (i and j) NDUFV2 gene expression analysis; (k and l) NDUFV1 gene expression analysis; (m and n) NDUFV2 gene expression analysis; (o and p) NDUFV1 gene expression analysis. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

cell autophagy. Moreover, it is already known that different bioactive compounds, especially flavonoids, can cross the blood-brain barrier [53–55]. In this sense, considering that the chemical matrix of açai is composed of different flavonoids, perhaps these molecules are able to reach the brain. It is necessary to perform *in vivo* studies to test this hypothesis.

As expected, rotenone decreased SH-SY5Y cell viability in a dose-responsive way as a cytotoxic effect of this chemical, corroborating the finds of Kim et al. [41]. Açai freeze-dried hydroalcoholic extract increased cell viability measured by XTT assay in a hormetic response. The best rate of cell proliferation was observed at 48 h of incubation and the EC50 for

this period of exposition was 5 $\mu\text{g}/\text{mL}$. In a study performed by Wong et al. [56] using rat pheochromocytoma cells, açai freeze-dried hydroalcoholic extract showed significant neuronal protection against beta-amyloid peptide exposure, increasing cell viability, especially at 5 and 50 $\mu\text{g}/\text{mL}$ of açai freeze-dried hydroalcoholic extract, also supporting our finds.

Rotenone is known to be a reagent able to directly inhibit mitochondrial complex I [57]. In a complex I dysfunction the mitochondria electron transport chain will try to maintain energy production by using complexes II and III which explains their protein overexpression found in this study by OXPHOS assay. Proving an affinity between açai freeze-dried

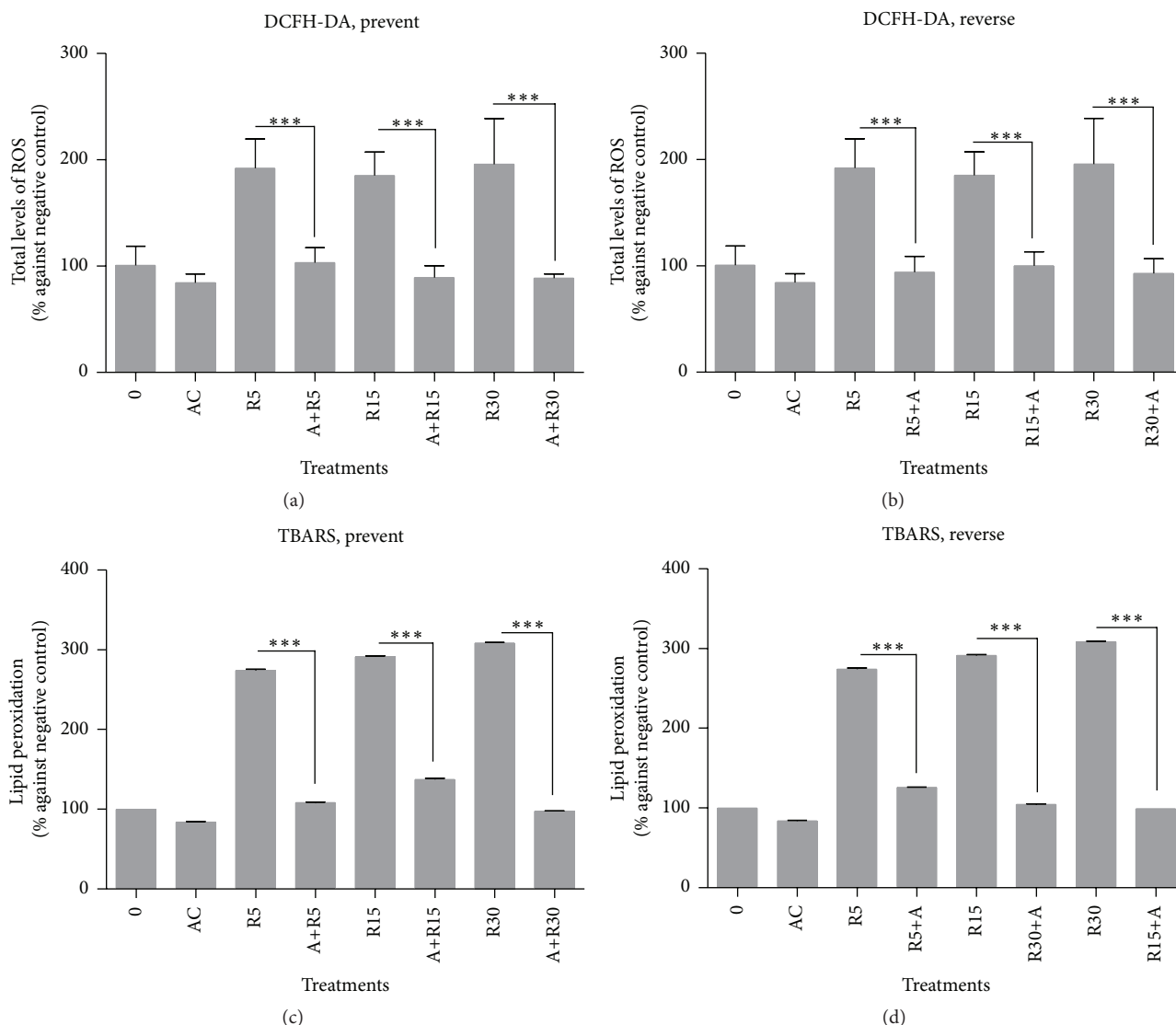


FIGURE 5: Cell oxidative metabolism biomarker measurements. (a and b) Total levels of ROs measured by DCFH-DA assay; (c and d) lipid peroxidation measured by TBARS assay. While rotenone increased ROs levels in a dose-dependent way and also lipid peroxidation, açai freeze-dried hydroalcoholic extract was able to decrease both biomarkers compared to the negative control. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

hydroalcoholic extract and mitochondrial complex I we also observed the significant effect of our extract against rotenone-induced dysfunction, showing not only a protein expression improvement but also a mitochondrial complex I enzymatic activity enhancement. Açai 5 $\mu\text{g}/\text{mL}$ exposition was able to recover mitochondrial complex II and III protein expression, recovering the mitochondrial electron transport chain function. Our results thus suggest a pharmacological effect of açai freeze-dried hydroalcoholic extract on mitochondrial complex I.

The effects of açai on the prevention and reversion of mitochondrial complex I dysfunction have been not associated with other plants or pharmacological drugs. For example, in a study developed with rat E18 cortical neurons, Scola et al. [58] showed that lithium at 0.75 mM was able to increase complex I activity compared to rotenone treatments;

however lithium treatment was unable to recover 100% of the activity of this complex compared to the negative control. Our findings demonstrate that açai can increase mitochondrial complex I activity under rotenone-induced mitochondrial dysfunction and also recover 100% or more of the complex I activity, compared to the negative control, renormalizing mitochondrial function.

We measured NDUFS7, NDUFS8, NDUFV1, and NDUFV2 protein and gene expression at SH-SY5Y cell after rotenone and/or açai treatments. The results showed a significant decrease in their expressions with rotenone exposure; however, açai increased these expressions in some experimental designs and rotenone concentrations. The main complex I subunit in which açai presented a pharmacological effect of prevention and reversion of rotenone effects was NDUFS7, measured by both western

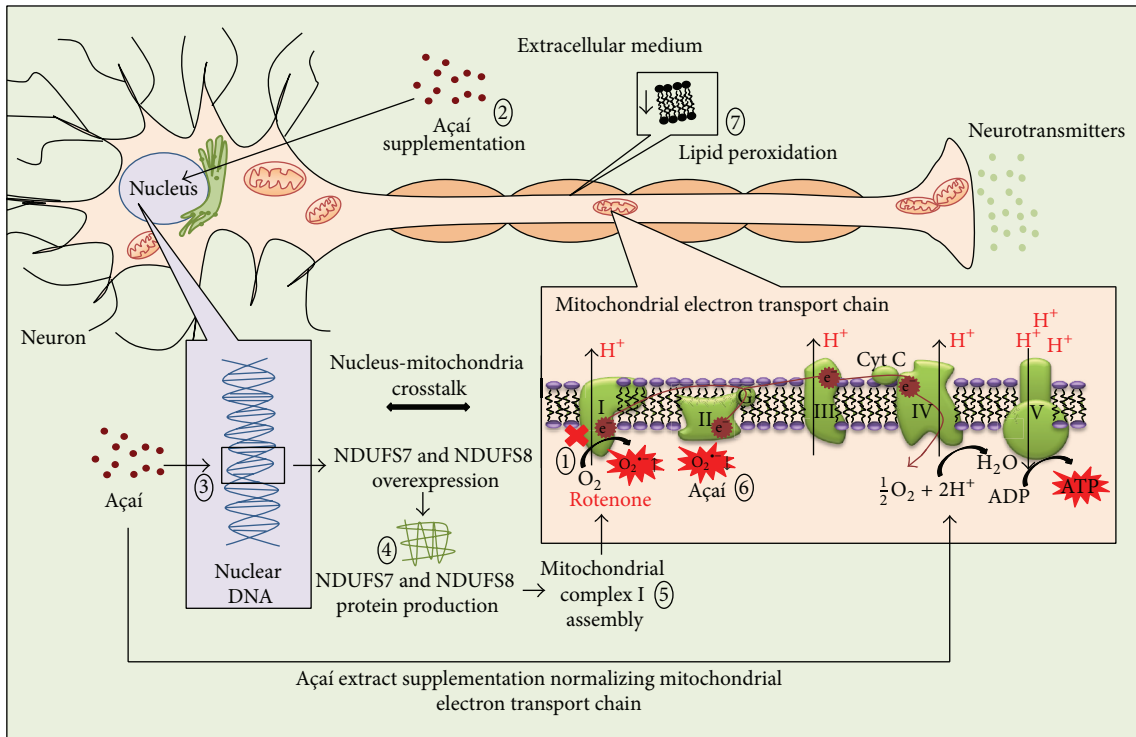


FIGURE 6: Protective effects of açai supplementation in neuron-like cells SH-SY5Y exposed to rotenone. ① Rotenone causing mitochondrial complex I dysfunction, increasing superoxide production, and decreasing ATP synthesis; ② açai freeze-dried hydroalcoholic extract supplementation acting at cell nucleus; ③ açai freeze-dried hydroalcoholic extract increasing NDUFS7 and NDUFS8 gene expression in particular; ④ NDUFS7 and NDUFS8 protein production in response to the gene overexpression; ⑤ mitochondrial complex I assembly by new protein subunits; ⑥ renormalization of the mitochondrial electron transport chain, decreasing the oxidative stress, and normalizing ATP synthesis. ⑦ Decreased lipid peroxidation in consequence of the oxidative metabolism balance recovery.

blot and qRT-PCR. It is already known that rotenone is able to deactivate mitochondrial complex I electron transfer to ubiquinone, meaning mitochondrial complex I Q module (formed by NDUFS7, NDUFS8, NDUFS2, and NDUFS3 subunits) inactivation. Rotenone can also impair complex I transcriptions through direct effects and by induction of the oxidative stress that causes DNA damage and affects the translocation of important transcription factors [58, 59]. Our results showed that açai has the capacity to improve the gene expression of different nuclear genes. It is also already known that açai can modulate the gene expression of antioxidant enzymes, including SOD, CAT, and GPx [58]; however, the way in which açai is able to modulate gene expression positively has not yet been determined. One potential hypothesis is that açai could increase the transcription factor involved in nuclear gene expression located at promoter regions, such as NRF2, by flavone interaction and activation [60]. The results described here suggest that açai freeze-dried hydroalcoholic extract acts mainly at the complex I Q module under mitochondrial dysfunction, especially at the NDUFS7 subunit, and this effect is associated with its gene overexpression.

Although rotenone treatments were able to increase oxidative stress by elevation of ROS production and lipid peroxidation, açai freeze-dried hydroalcoholic extract was able to decrease the levels of both biomarkers for all rotenone

concentrations. These results were found in both experimental designs. We postulate that açai extract can act as an antioxidant agent under mitochondrial complex I deficiency, recovering mitochondrial function and neutralizing cell damage caused by oxidative imbalance.

It is also important to emphasize that açai did not demonstrate any *in vitro* neurotoxic effect in SH-SY5Y cells. *Annona muricata* fruit, for example, has an important neurotoxic effect because of its chemical matrix which includes polyketides specific to Annonaceae. These metabolites are the most potent mitochondrial complex I inhibitor found in nature, inducing neuronal death [61]. Otherwise, in this study, açai modulated positively different cellular aspects preserving neuronal viability.

All the results noted in this study are based on *in vitro* experiments. Due to methodological limitations associated with *in vitro* studies, complementary studies using *in vivo* experimental models need to be performed to confirm the evidence described here.

5. Conclusions

There are many studies that describe the different activities of plant extracts; however, the effects of fruits and other functional foods on mitochondrial function have not yet been well evaluated. Our results showed that açai hydroalcoholic

extract has an important affinity to mitochondrial complex I. Açai is able to recover the mitochondrial electron transport chain function of neuronal-like cells under mitochondrial complex I dysfunction mainly through overexpression of important nuclear mitochondrial complex I subunits genes and improvement in their proteomic expression. Our findings suggest that açai freeze-dried hydroalcoholic extract has a significant pharmacological capacity. Açai could be a new alternative for drug development research for neuropsychiatric diseases, as BD, mainly as a result of the isolated chemical matrix of this fruit. It is important to note that all findings described here are limited to *in vitro* studies and they may be confirmed by an *in vivo* experimental model in a future study.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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