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GUARANA (*Paullinia cupana*) INCREASES MESENCHYMAL STROMAL CELLS POLARITY, VIABILITY AND ANTIOXIDANT PROPERTIES.

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Trabalho de Conclusão de curso apresentado como requisito parcial para obtenção do título de Bacharel em Ciências Biológicas na Universidade Federal do Rio Grande do Sul.

Orientadora: Prof. Dra. Ana Helena da Rosa Paz

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GUARANA (*Paullinia cupana*) INCREASES MESENCHYMAL STROMAL CELLS POLARITY, VIABILITY AND ANTIOXIDANT PROPERTIES.

Short title: Guarana effects on MSC

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Abstract

Background: Guarana (*Paullinia cupana*) is known for its antioxidant, cicatricial effects having caffeine as the main component of the extract. Mesenchymal stromal cells (MSCs) have therapeutic potential for their ability to differentiate, immunomodulate and migrate to injured tissues, and such effects are potentiated when cells are activated. The objective was to evaluate the effects of guarana and caffeine on MSCs. *Methods:* After the initial characterization, MSCs were treated with *P. cupana* (10, 100 and 1000 μg/mL) or caffeine (0.4, 4 and 40 μg/mL) for 24 h and cell morphology, cell cycle, autophagy, viability, antioxidant potential, nuclear regularity, mitochondrial membrane potential and proliferation were evaluated. *Results*: MSCs treatment with guarana 1000 μg/mL promoted an increase in cell polarity ($p<0.0001$), viability ($p<0.001$) and antioxidant potential ($p<0.05$), while reduced the levels of autophagy ($p<0.05$). MSCs treated with guarana 100 and 1000 μg/mL showed decrease of cell proliferation ($p<0.0001$), and guarana 100 μg/mL promoted a lower cell area ($p<0.05$). None of the treatments affects the cell cycle of MSCs. *Discussion:* The present study shows in vitro evidence that guarana could be a promising alternative for activating mesenchymal stromal cells in order to promote better cellular products for future clinical therapies.

Key Words: guarana extract, *Paullinia cupana*, MSC, mesenchymal stem cell, cell therapy, priming, cell activation.

Abbreviations:

MSC: mesenchymal stem cells

DMEM: Dulbecco’s modified Eagle’s medium

FBS: fetal bovine serum

PBS: phosphate buffered saline

PD: population doubling

SOD: dismutase superoxide

NO: nitric oxide

NMA: nuclear morphometric analysis

SH: reduced thiol

Introduction

Guarana (*Paullinia cupana*) is a native plant to the central Amazon basin known for its stimulating and medicinal properties. Roasted seed extracts have been used as medicinal beverages for centuries by indigenous communities [1]. Seeds are the commercially useful part of the plant due to its high content of caffeine purine alkaloid (1,3,7-trimethylxanthine) to which its stimulant property is attributed. Despite the large variation in seed's caffeine content (varies from 2.5 to 6%) the values still high when compared to any other species, including coffee (*Coffea arabica*), tea (*Camellia sinensis*) and yerba mate (*Ilex paraguariensis*) [1, 2]. The extract of guarana seed is composed mainly of caffeine (34.19 mg/g of extract), theobromine (0.14 mg/g), catechin (3.76 mg/g) and epicatechin (4.05 mg/g) [3].

Previous investigations describe antidepressant, antimicrobial, anti-platelet aggregation, antioxidants and cardioprotective effects [4–6]. The antioxidant effects of guarana have been evaluated in NIH-3T3 cell line where guarana decreased cell mortality, lipid peroxidation, DNA damage and cell oxidative stress as well as increased the dismutase superoxide (SOD) levels demonstrating the effects on nitric oxide (NO) metabolism in situations with higher cellular NO levels [7]. Also, an increase in the non-enzymatic antioxidant potential in the SH-SY5Y human neuronal cell line [8], and a dose-dependent increase in DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was demonstrated [9].

In addition, guarana supplementation was shown to improve and accelerate the healing process of fractures in animal models [10] and affect the cell cycle of pre-neoplastic and highly metastatic phenotypes demonstrating the therapeutic potential of *P. cupana* in the treatment of cancer in experimental models.

Mesenchymal stromal cells (MSCs) are being investigated as a potential alternative for cellular therapy because of their characteristics of self-renewal, differentiation and mainly for their paracrine immunomodulatory and immunosuppressive effect [11]. MSCs from embryonic attachments are immature cells, with lower risk of mutations and with superior cellular activity, including increased differentiation [12], homing, graft potency and lower immunogenicity [13]. Da Silva et al. (2012) demonstrated the effects of cell therapy in neuroprotection, tissue regeneration, inflammation and induction to growth factors [14]. Moreover, trials demonstrate that the mechanisms of action of mesenchymal stromal cells are associated with the release of

paracrine factors that act by mediating inflammatory processes and helping to reduce the damage generated by oxidative stress [15].

In recent years, different approaches to enhance the recruitment of MSCs and also their anti-inflammatory capabilities are attracting significant interest. In this way, the activation of MSCs with cytokines, growth factors, hypoxia, pharmacological drugs, biomaterials among other different culture conditions, mechanisms also known as activation or "priming", are being extensively investigated in order to generate cellular products with greater potential for the different clinical applications [16].

Because previous investigation has described several biological properties of guarana in cell lines, we studied here the effects of guarana and caffeine on mesenchymal stromal cells searching for the possibility of use this extract as a priming approach for cell therapy.

Methods

Isolation, characterization and culture of MSCs

MSCs from human chorionic membrane were isolated according to Araújo et al. (2017) [11]. Cells were characterized by immunophenotypic analysis and in vitro differentiation in mesodermal lines (chondrocytes, adipocytes and osteocytes).

MSCs were cultivated until reaching 70-80% confluency in Dulbecco's Modified Eagle Medium (DMEM-Gibco) supplemented with 10% Fetal Bovine Serum (FBS-Gibco), 1% Penicillin-Streptomycin (PS-Gibco) and 2 mM L-glutamine (Gibco), maintained in incubator at 37°C, 5% CO^2 and 95% humidity.

Treatments

Guarana (*P. cupana*) powder was obtained from Lifar Ltd. (Porto Alegre, RS, Brazil). The stock solution consisted of 10 mg/mL of extract in DMEM supplemented with 10% FBS and 1% PS and incubated at 37°C for 15 minutes. The guarana working solutions were 10, 100 and 1000 µg of guarana/mL DMEM 10% FBS.

Since caffeine is the major component of guarana extract, we used caffeine (0.4, 4 and 40 µg/mL) that corresponds to its concentration in guarana working solutions. The caffeine stock solution - anhydrous, 99% (Sigma) was prepared with 1 mg of caffeine diluted in 1 mL of DMEM 10% FBS.

Experimental design in vitro

The effects of *P. cupana* 10, 100 and 1000 µg/mL were analyzed, as well as their caffeine proportions corresponding to 0.4, 4 and 40 µg/mL, according to Da Silva Bittencourt et al. (2014) [3]. The control group consists of MSCs cultured with DMEM 10% FBS. All groups were cultivated in an incubator at 37°C, 5% CO^2 and 95% humidity for 24 h and the cell passages used were between P3-P7.

Area, cell polarity and nuclear morphology

MSCs were plated ($4x10^4$ cells/well) into coverslips in a 12 well plate. After the treatment period, samples were washed with phosphate buffered saline (PBS - Laborclin) and fixed with 4% paraformaldehyde solution + 4% saccharose for 15 minutes at room temperature. After washing with PBS, the membranes were permeabilized with 0.3% Tween (Sigma-Aldrich) in PBS for 10 minutes, washed and then blocked with FBS (1:10) for 1 hour at room temperature. Subsequently, cells were labeled with rhodamine-phalloidin (Invitrogen-Termo Fisher) for 1 hour at 4°C. Nuclei were stained with DAPI (Sigma-Aldrich) for fluorescence microscopy analysis.

To assess cell polarity, the polarity index was calculated as the length of the major migration axis (parallel to the membrane protrusion) divided by the length of the perpendicular axis that intersects the center of the cell nucleus [11,17]. A total of 100–150 cells were analyzed in each condition.

A nuclear morphometric analysis (NMA) was performed as described by Filippi-Chiela et al. (2012) [18] Images were taken from a total of 300–400 nuclei, obtained from random fields. Images were analyzed using Image Pro-Plus 6.0, for the acquisition of the nuclear area and the parameters of nuclear irregularity (roundness, aspect, radius ratio and area/box, which are grouped in an index, named the nuclear irregularity index (NII). Nuclear Morphometric Analysis (NMA) tool were used in order to quantify senescence, mitosis, apoptosis and mitotic catastrophe.

Cell viability (MTT)

In a 96-well plate MSCs ($1x10^4$/well) were seeded and exposed to the treatment conditions. After 24 h, treatment medium was changed to standard medium containing 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) at the final concentration of 0.5 mg/mL. At the end of the experiment, the medium was

removed, and DMSO was added. The absorbances of 570 nm and 690 nm were read using a spectrophotometer (Spectramax, Hexis).

Mitochondrial membrane potential (JC-1)

In 24-well plates, $4x10^4$ MSCs were seeded. After the 24 h treatment period, cells were washed with PBS, detached with a trypsin/EDTA solution and a labeling mix containing DMEM 10% FBS and tetraethylbenzimidazolylcarbocyanine iodide (JC-1 - ab113850) was added. After incubating for 20 minutes at 37°C, PBS was added, and the samples were analyzed on Attune flow cytometer (ThermoFisher Scientific).

Antioxidant Potential

To evaluate the antioxidant potential, measurement of total SH (reduced thiol) groups of proteins and other thiol compounds was performed. In a 6-well $30x10^4$ MSC/well were plated. After treatment, cells were detached, resuspended in PBS and centrifuged at 3000 g for 10 minutes at 4°C, then the supernatant was used for the determination of total SH groups as described by Kleber Silveira et al. (2018) [19]. Briefly 30- to 80 ug aliquot was diluted in phosphate-buffered saline and reacted with 10 mM 5,5′-dithiobis-(2-nitrobenzoic acid). After 60 min of incubation at room temperature (25°C), the absorbance was read using a spectrophotometer 412 nm. Results are expressed as mmol–SH groups/mg protein.

Cell proliferation (population doubling)

MSCs were plated at a density of $4x10^4$ cells/well in a 24-well plate and treated. On days 3 and 6 cells were detached, counted using a Neubauer's chamber and re-seeded at the same initial concentration (4×10^4 cells/well). To obtain the population doubling (*PD)* values, the initial and final values of cells in each time interval was plotted into a formula: $PD = (\log N(t)-\log N(t0))/\log 2$, where N(t) was the number of cells per well at the time of trypsinization after 3 and 6 days and N(t0) was the number of cells initially plated.

Autophagy and cell cycle characteristics

To analyze autophagy, $4x10^4$ cells were seeded per well in 24-well plates. After the treatment period, the cells were washed with PBS, trypsinized and then each sample was resuspended in a labeling mix containing 400 µl of DMEM 10% FBS + 0.6 µL acridine

orange (Sigma-Aldrich). After, the plates were incubated for 15 minutes at room temperature, protected from light and analyzed on the Attune flow cytometer (ThermoFisher Scientific).

For cell cycle analysis, after treatment, cells were washed with PBS, trypsinized and centrifuged at 1200 rpm for 10 minutes. After that cells were resuspended in 0.5 µg/ml propidium iodide (Sigma-Aldrich), 0.1% Triton x-100 and 0.1% sodium citrate solution, agitated and finally incubated for 15 minutes at 4°C in the dark. Finally, the nuclei were analyzed on the flow cytometer.

Statistical analysis

The parameters of interest were compared using analysis of variance (ANOVA) and multiple comparisons with Tukey, Kruskal-Wallis and Dunn's. Analysis was performed in statistical program GraphPad Prism software version 6 (San Diego, California, USA) with mean ± standard deviation (SD). Results were considered significant if $p<0.05$.

Results

Characterization of MSC from chorionic membrane

Chorion-derived MSC from human placenta with stable fibroblast-like phenotype were isolated by adherence separation and characterized by flow cytometry and differentiation assays. Cells expressed MSC markers CD73 (99.2 ± 0.6), CD90 (96.6 ± 0.4), CD105 (92.8 ± 7.0), CD29 (97.1 ± 0.5), CD44 (96.0 ± 2.3), and lacked expression of CD45-CD34-CD19-CD11b-HLA-DR (0.2 ± 0.1), CD14 (0.4 ± 0.3), CD3 (0.3 ± 0.3) and HLA-G (1.6 ± 0.6). MSCs readily differentiated into osteocytes, adipocytes and chondrocytes after incubation in appropriate differentiation media for 14–28 days.

Cell characteristics: morphology, size, polarity and regularity nuclear

Mesenchymal stromal cells maintained their characteristic fibroblastic morphology (figure 1-A) after treatment. Regarding cell area G100 and C4 groups presented significantly lower area (67.99 ± 12.84 μ^2 and 74.71 ± 4.87 μ^2 respectively) when compared to control (162.3 ± 29.49 μ^2) and C40 groups (152.7 ± 12.30 μ^2) (figure 1-B).

As shown in figure 1-C, cells treated with guarana 1000µg/mL showed the most elongated morphology, presenting the higher polarity index (5,120 ± 0,2224), ($p < 0.0001$), while cells of C0.4 group presented lowest polarity index (2,705 ± 0,1318).

Nuclear morphology provides information on possible cellular changes such as senescence and apoptosis. After NMA analysis, no biologically relevant modifications were observed in the cell nucleus among different treatment groups as demonstrated in figure 1-D.

Cell viability - MTT

Viability of treated cells under guarana or caffeine effects was measured and the results are presented in figure 2-A. MTT assay demonstrated the treatment with guarana at 1000µg/mL concentration provides significantly higher viability (1,491±0,0379) in relation to all groups analyzed ($p<0.001$).

Mitochondrial membrane potential - JC-1

After demonstrating that *P. cupana* induced greater cell viability (measured by mitochondrial conversion of MTT), mitochondrial membrane potential was evaluated by flow cytometry. Due to low mitochondrial membrane potential JC-1 yields green fluorescence however when mitochondria present high membrane potential the dye yields red. The red/green fluorescence ratio was used to determine the mitochondrial function of cells after treatments (figure 2-B). Both groups, guarana (G100 and G1000) or caffeine (C0.4, C4 and C40) promoted an increase in mitochondrial membrane potential being significantly more polarized $p=0,0016$ and $p=0,002$, respectively, than the control.

Antioxidant potential

The process of oxidative stress occurs naturally in cells, however, it is cause of cell death and is regarded as one of the causes of aging being involved in almost every body process. Our results demonstrated, that treatment with guarana 1000 µg/mL increased the number of reduced thiols $p=0.0224$ in (figure 3). This may provide cells protective effects against oxidative changes of proteins.

Cell proliferation - population doubling (PD)

The proliferation assay demonstrated that guarana at 100 and 1000 μg/mL promoted lower PD values ($p<0.0001$) compared to the control group (figure 4-A). It was also observed that the G100 and G1000 group obtained lower PD when compared to G10 ($p<0.01$) demonstrating a dose dependent response. Caffeine treated cells proliferate in a manner similar to control.

Autophagy and Cell cycle

MSCs treated with guarana 1000 μg/mL presented, statistically ($p<0.05$), lower levels of autophagy (1,320 ± 0,4704) in relation to control (6,530 ± 0,7182) (figure 4-B). Moreover MSCs cell cycle was analyzed using flow cytometry and showed no difference among all groups. Treatments with guarana and caffeine did not change the percentage of cells that were in the G0/G1, S or G2-M phases ($p<0.05$) when compared to the control group (figure 4-C).

Discussion

The therapeutic potential of MSC is related to lineage-specific differentiation, immune system modulation, secretion of bioactive factors and to their antioxidant properties. These properties can be tightly regulated by biological, biochemical, and biophysical factors which are capable of enhance MSC therapeutic potential [20]. Guarana presents high content of polyphenols and caffeine and has demonstrated strong antioxidant properties in human neuronal-like cells (SH-SY5Y) [8] and fibroblasts (NIH-3T3) [7]. In this context, here we evaluate MSC viability, cell and nuclear morphometry, cell polarity, cell cycle, autophagy, mitochondrial membrane potential as well as antioxidant properties in the presence of guarana extract aiming to apply this natural product as a MSC priming agent.

Regarding morphometric analysis, our data demonstrate that cells treated with guarana and caffeine (100 and 4 μg/mL, respectively) revealed smaller sizes compared to control group. A recent work from Oja et al. (2018) evaluated bone marrow MSC and observed that an increase in cell size is associated with the expression of p16 and the activity of β-galactosidase demonstrating that morphological changes, correlates with biochemical senescence markers [22]. The same work declares that MSCs at early passages are small and spindle-shaped, however Oja did not observe a reduction in cell

area. Nevertheless, to date there is not yet a consensus about critical MSC size, and whether cell size is influenced by tissue source and culture conditions [23].

Cell polarity results from the internal organization of the cell and is a key step for the induction of cell motility. Cell migration depends on the activation of several signaling pathways that result in the break of cell asymmetry, where a more elongated morphology is usually associated with a better mesenchymal cell migratory performance process [21,24]. Using a polarity index distribution, we demonstrated that 24 hours of guarana treatment indicated a more elongated cell shape, while caffeine resulted in a more rounded morphology. Schneider et al. (2015) demonstrated, using time-lapse microscopy, that random migration corroborates with polarity index data, once elongated shaped cells presented a significant increase in cell speed and spatial trajectory when compared to MSCs with rounded morphology [17]. Also Geiβler et al. (2012) observed that chronological and *in vitro* aging causes round-shaped MSCs and diminished migration potential along with a decrease in the expression of genes associated with cytoskeletal organization [26]. Considering that the homing process is a key step for MSCs to participate in tissue repair and immunomodulation, the modification on polarity index promoted by guarana could affect cellular migration possibly contributing to cell therapy.

Changes in cell viability could result in a diminished number of cells and a low effectiveness of cell therapy. We demonstrated here that guarana and caffeine in the studied concentrations did not impair the viability of human derived MSCs. Nuclear morphometry can indicate several cell fates such as early apoptosis and senescence, which may compromise MSC quality for therapeutic purposes. No biologically relevant difference was observed in nuclear morphometry or cell cycle indicating that treatments studied here are not toxic for MSCs. Zeidán-Chuliá et al. (2013) however reported that guarana 12.5-50 mg/mL and its combinations with caffeine promoted signs of apoptosis, including membrane blebbing, cell shrinkage, and cleaved caspase-3 positivity on human neuronal SH-SY5Y cells [8]. On the other hand corroborating with our data Da Silva Bittencourt et al. (2014) showed that guarana (10, 100, and 1000 μg/mL) actually improved induced toxicity on the same cell line demonstrating that different concentrations could influence biologically effects [3]. Also protective effects were observed on NIH-3T3 fibroblasts [7].

Interestingly we observed that cells cultured in the presence of guarana 1000 μg/mL presented lower population doubling values compared to the control group which may be associated with the presence of tannins [27]. However this scenario of lower

proliferation is also observed when MSCs are primed with interferon a very powerful priming agent knowing for induce a strong immunomodulatory phenotype with high IDO activity, PD-L1 expression and potential to inhibit lymphocyte proliferation [28,29]. Nevertheless, flow cytometry showed no effects in MSCs cell cycle, indicating no MSC cycle arrest in the concentrations studied. These results are interesting once that *P. cupana* can be used as a source of bioactive activating substances, generating cellular products with lower potential of tumorigenesis after transplantation [30,31].

Mitochondria are the main energy-producing organelles and play a key role in the regulation of cellular bioactivity [32], and their transmembrane potential is eventually lost after significant cellular damage [33]. Lu et al. (2018) has observed improvements in mitochondrial membrane potential of activated MSCs and this was associated with superior therapeutic effects [34]. Here we demonstrate that guarana (100 and 1000 ug) as well as caffeine not only preserved mitochondrial membrane potential but also promote a significant hyperpolarization. Actually, guarana (1000 ug/mL) promoted an increase in cell viability thought MTT assay which is a mitochondrial dehydrogenase presented in the living cells. Recently Bortolin et al. (2019) demonstrated that guarana was able to induce mitochondrial biogenesis suggesting AMPK activation on brown adipose tissue [35]. This is especially interesting because it is known that MSC acts also through the transfer of healthy organelles (including mitochondria) to the host cells [36].

Autophagy is critical for maintenance of bioenergetics and cell viability in vitro, and also plays an essential role in survival of the whole organism in vivo. Its depletion inhibits autophagosome formation by interacting with phosphatidic acid on endosomes, thereby preventing endosomal development into autophagosomes [37]. In vitro, normally human mesenchymal stem cells exhibited a high level of constitutive autophagy but it can be up or downregulated in stress or differentiation [38]. We observed that guaraná 1000 ug/ml significantly reduced autophagy levels. Remarkably De Witte et al. (2018) recently demonstrated that priming MSC with IFN promotes the metilation of HS1BP3 a gene implicated in the regulation of MSC autophagy [39].

Finally, we show that higher concentration of guarana extract provides higher levels of reduced thiols, demonstrating the *P. cupana* antioxidant effects observed in cell lines are also present on MSC [3,7]. This effect may be associated with the presence of caffeine, theobromine, tannins, catechins and epicatechins [40]. However, here the treatment with proportional caffeine concentration were similar to the effects observed on the control group, confirming the need for mix of compounds, which are present in

guarana, in order to promote higher levels of antioxidant potential. Such cellular characteristic can help reduce apoptosis levels among other damages present in induced oxidative stress [41].

Conclusion

In conclusion, the present study shows in vitro evidence that guarana could be a promising alternative for activating mesenchymal stromal cells in order to promote better cellular products for future clinical therapies.

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Disclosure of Interest:

The authors declare no conflicts of interest.

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Figure Legends

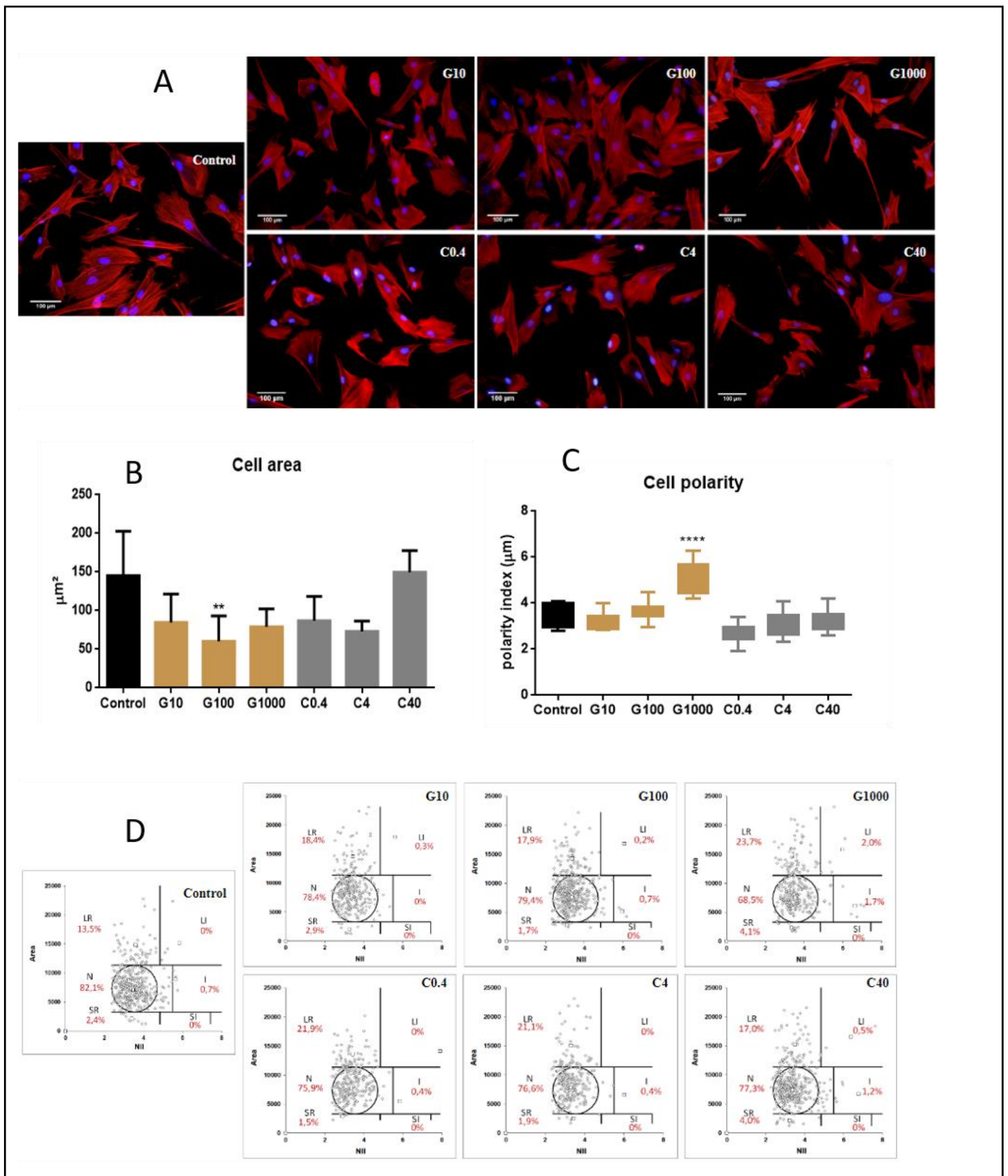
Figure 1. MSCs morphometry (A) Human MSCs morphology after 24 h treatment with guarana (*P. cupana*) or caffeine. Rhodamine-phalloidin and DAPI staining. (B) Cell area: G100 and C4 cells were significantly lower **$p<0.001$ *$p<0.05$. (C) Cell polarity index: G1000 presented the most elongated cells (****$p<0.0001$), while C0.4 cells showed the opposite (*$p< 0.05$). (D) Nuclear morphometric analysis. Numbers indicate the percentage of nuclei in each category (N normal, S mitotic, SR apoptotic, SI small and irregular, LR senescent, LI large and irregular, I irregular). Analysis was performed using one-way ANOVA and Tukey's post-hoc multi-comparative test. Data are presented as mean ± standard error (SE). G: guarana 10, 100 and 1000 µg/ml; C: caffeine 0.4, 4 and 40µg/mL.

Figure 2: Cell viability. (A) MTT assay of human mesenchymal after 24h of guarana or caffeine treatment. **$p< 0.001$ between G1000 and all other groups. (B) Mithocondrial membrane potential of human mesenchymal stromal cells using mean values of intensity (red/green) ratio quantification of JC-1. **$p=0.0016$ between G100 and G1000 and ## $p=0.002$ regarding C0.4, C4 and C40 comparing to control. Data are presented as mean ± standard error (SE). Analysis was performed using one-way ANOVA and Tukey's post hoc multi-comparative test

Figure 3: Antioxidant Potential. Quantity of thiols reduced per mg of protein after 24 hours of treatment with guarana (*P. cupana*) or caffeine. G: guarana 10, 100 and 1000 µg/ml; C: caffeine 0.4, 4 and 40µg/mL. Data are presented as mean ± standard error (SE). The analysis was performed using one-way ANOVA and Tukey's post-hoc multi-comparative test. *$p=0.0224$ between G1000 and the C4 and C40 groups.

Figure 4. MSC Growth Kinetics. (A) Number of Population Doubling after 24h of treatments with guarana (*P. cupana*) or caffeine. *$p<0.01$ between G1000 and control, G10 and C0.4 groups; #$p<0.01$ between G100 and the control, G10, C0.4, C4 and C40 groups. (B) Percentage of autophagic cells stained with acridine orange after 24h of guarana or caffeine treatments. * $p<0.05$ between G1000 and control. (C) MSC cell cycle after 24h of guarana (*P. cupana*) or caffeine treatment. Data are presented as mean ± standard error (SE). Analysis was performed using one-way ANOVA and Tukey's post hoc multi-comparative test.

FIGURE 1



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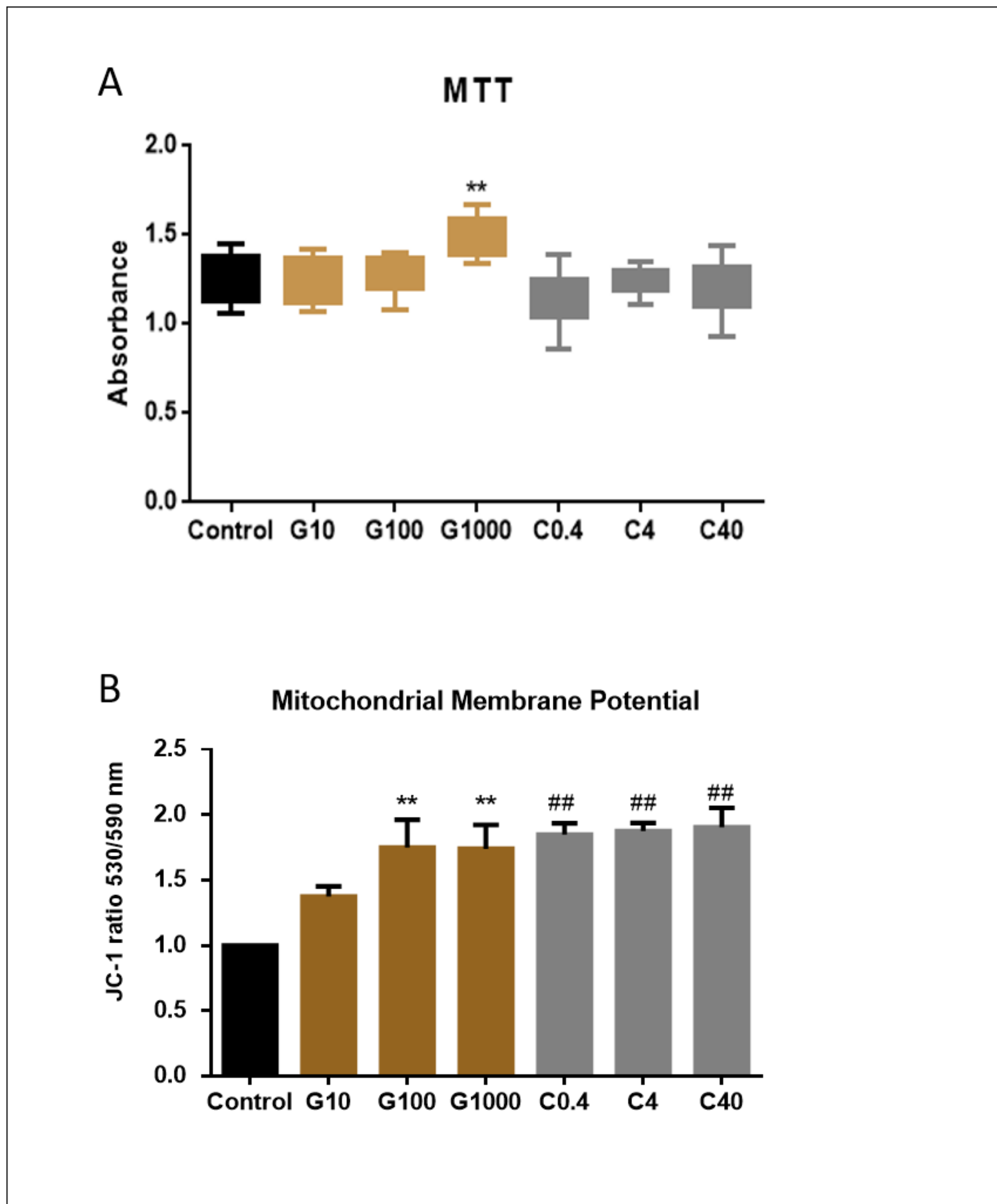


FIGURE 3

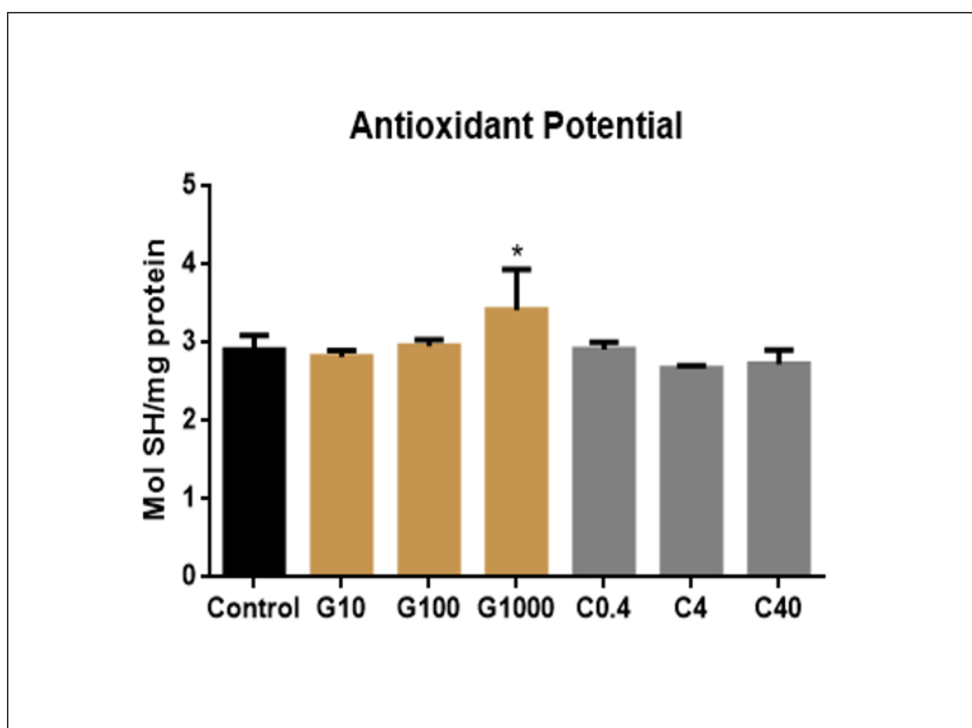
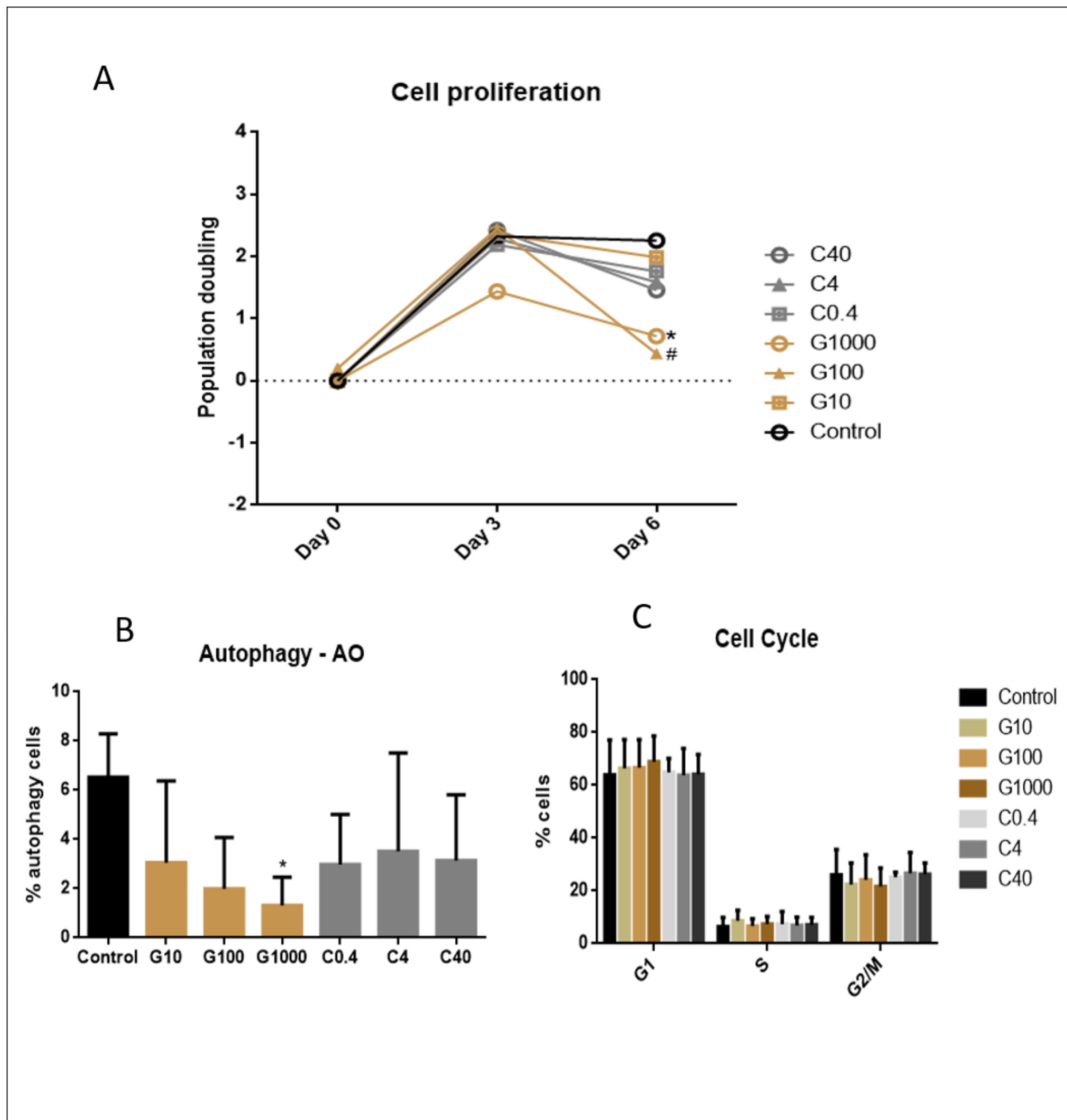


FIGURE 4



ANEXO - Regras da Revista Cytotherapy

CYTOTHERAPY

The official journal of the International Society for Cell & Gene Therapy (ISCT®)

AUTHOR INFORMATION PACK

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DESCRIPTION

Cytotherapy, the official journal of the International Society for Cell & Gene Therapy (ISCT®), publishes novel and innovative results from high quality scientific and clinical studies in the fields of cell and gene therapy. Studies evaluating the potency of experimental cell and gene therapies in clinically relevant animal models of disease and describing important advances in cell/gene-based product manufacturing and validation are welcomed. Results of clinical studies evaluating the safety and efficacy of cell and gene therapies in early and late phase trails are also of interest. In addition to Short Reports and Full-Length Articles, the journal also accepts Editorials addressing emerging trends and potential controversies in the field, and Review articles summarizing bodies of work that have made lasting impacts in the field.

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