Evaluation on Hot/Cold Drug Property of Açai (Euterpe oleracea Mart.) by Cytological Study Methods

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Abstract

Objective: To determine the hot/cold of South American Açai (Euterpe oleracea Mart.). Materials and Methods: 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was performed to compare the influence of Açai and phellodendron bark (Cortex Phellodendri Chinensis) on the growth and proliferation of HepG2 and Hep3B cell strains. The morphological changes of the two cell strains treated with Açai and C. Phellodendri were observed using an inverted phase contrast microscope. Trypan blue dye exclusion assay was used to compare and analyze the toxicity effects of Açai. The content of nicotinamide adenine dinucleotide (NADH) and the ratio of NADH/NAD⁺ of the treated cells were detected using a spectrophotometer to determine the influence of Açai to the energy metabolism of the two cell strains.

Results: Within the range of measured concentrations, both Açai and C. Phellodendri displayed separately the stronger inhibitory effects on cell growth, proliferation, and energy metabolism of the two cell strains. By MTT assay, Açai showed significant (P < 0.05) or highly significant (P < 0.01) inhibitory effects on cell proliferation within the concentration range of 0.25–40 μg/mL, which was similar to the effects of C. Phellodendri. From the results of microscopic examination, the morphological of lower cell density, attenuated granularity, and more outstretched cells in irregular polygonal shape could be observed in the cells treated with Açai, which was also similar to that of C. Phellodendri. In trypan blue staining assay, all concentrations of Açai showed negligible toxicity effects, exactly as that of C. Phellodendri. Açai showed a highly significant effect of decreasing the content of intracellular NADH as well as the ratio of NADH/NAD⁺ of the treated cells, which was also similar to that of C. Phellodendri. Conclusion: The effect of Açai on HepG2 and Hep3B strains just as C. Phellodendri can reduce energy metabolism and inhibit the growth and proliferation. All performance characteristics of two cell strains treated with Açai belong to that characteristic of cold property drug. From the study results, we can deduce that the drug property of Açai is cold.

Keywords: Açai (Euterpe oleracea Mart.), hot/cold drug property, cytological study

INTRODUCTION

Açai is widely noted for its multi-purpose and health benefits, especially, its high quality nutritional value and alternative medicinal properties. Actually, Açai was used as a daily food for people and as an herbal plant in the Brazilian Amazon region with a history of hundreds of years. Many published reports claimed that Açai possessed the potential therapeutic activities, for instance, antioxidant, anti-inflammatory, immune system modulation, antihyperlipidemic, hypoglycemic, hepatoprotectant and antiradiation and so forth. Anyway, it possesses the basic features of Chinese medicinal herbs.

In the past 10 years, with the increase communication of personnel, Açai was introduced into China and was approved as a new resource food in 2013. However, at present, there are few domestic studies about South American Açai. The published reports are mainly focused on antioxidation, protecting against alcoholic...
hepatic injury and radiation injury.[5-7] Although it had been reported that Açaí has superior medicinal value, it is unprecedented to integrate this exotic natural medicinal resource into Chinese herb resources. However, so-called Chinese Materia Medica, its main characteristics, which is also different from other ethnic medicines, is that their clinical uses should completely follow the guidance of the fundamental theory of traditional Chinese medicine (TCM). Hence, we should first determine the drug properties of Açaí before that Açaí is introduced into practice. Anyway, the problem that Açaí is attributed to cold, cool, warm, and hot property is a primary problem and should be resolved before practical application.

In the past decades, as for the identification of cold and hot characteristic of TCM herbs, the main evidence laid on the efficacy characteristic results of pharmacology studies. However, with the further development of scientific methods and technology, the identification and study methods of cold and hot drug properties of herbs are becoming more diversified.[8] For better understanding of Açaí drug properties, Lin-Yuan et al.[9] conducted an in-depth study on pharmacy efficacy related with cold and hot characteristic through animal in vivo experiments. Anyway, our study intended to reveal Açaí drug properties from another perspective. Actually, in this study, through applying some methods of cytological biology, we respectively observed the influence on the growth and proliferation of HepG2 and Hep3B cell strains when treated with Açaí and Cortex Phellodendri Chinensis and detected the changes of the intercellular energy metabolism. In addition, on the basis of the tested results, Açaí was taken further analogy analysis with that of C. Phellodendri Chinensis to investigate and verify the cold/hot drug property. The study can provide more scientific data for the identification of Açaí drug property and also an important complementary for present research.

**Materials and Methods**

**Cell lines and chemical reagents**

Human hepatic carcinoma HepG2 was donated by the Guangdong Provincial Centre for Disease Control and Prevention, and human hepatic carcinoma Hep3B was donated by the College of Pharmacy of Jinan University. All were cultured at 37°C in a humidity controlled atmosphere containing 5% CO₂ in Dulbecco’s modified Eagle medium (DMEM) supplemented with sodium bicarbonate (2.2%, w/v), L-glutamine (0.03%, w/v), fetal bovine serum (FBS, 10%), penicillin (100 µg/mL), and streptomycin (100 µg/mL). Dimethyl sulfoxide (DMSO, MP Biomedicals LLC), DMEM (Thermo-Fisher Biochemicals, Beijing Inc., China), FBS (Hangzhou Sijiqing Bioengineering Materials Inc., China), 0.25% trypsin-EDTA (Gibco, USA), 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich), trypsin blue stain (Sigma-Aldrich, USA), penicillin-streptomycin double antibiotic (Beijing Puboxin Biotechnology Inc., China), and coenzyme I NAD (H) kit (NJJCBIO, China).

**Drugs and tested materials**

Açaí powder was produced from Para States, Brazil, which was provided by Jian-Jun Zhang, Professor of Beijing University of Chinese Medicine. C. Phellodendri Chinensis was purchased from Guangdong Kangmei Pharmaceutical Co., Ltd. Açaí powder was weighed precisely and then was dissolved in 10 mL Milli-Q water per gram and thoroughly mixed under room temperature. After the solution was centrifuged at 10,000 rpm by a high-speed refrigerated centrifuge (FC18R, Guangzhou Fangtong Biotechnology Inc., China) for 30 min at 4°C, the supernatant was collected. Subsequently, the precipitation resuspended in appropriate volume Milli-Q water was then centrifuged again as described previously. Finally, before vacuum freeze-drying process, the collected supernatant was prefreeze at −80°C for 24 h, and then was transferred to a vacuum freeze dryer (FD8-6P, Gold Sim International, USA). Forty-eight hours later, all freeze-dried powder was collected, sealed, and stored at room temperature. In the same way, C. Phellodendri Chinensis was accurately weighed, and then, 10 times volume Milli-Q water was added into it at the ratio of mass to volume and immersed for 1 h. C. Phellodendri Chinensis was decocted with water for three times, and the decoction lasted for 30 min after boiled each time. The decoction of C. Phellodendri was collected and the residues were discarded. Subsequently, the combined decoction was concentrated following by centrifuging at 10,000 rpm for 30 min at 4°C. Finally, the supernatant was collected. The rest manipulation procedures were the same as that of Açaí processing.

**Cell resuscitation and inoculation**

Human hepatic carcinoma HepG2 and Hep3B cell lines were resuscitated and inoculated in 9 cm diameter culture plates, respectively, and allowed to attach at 37°C in a humidified air with 5% CO₂ (Thermo HERAcell 240i, USA) for 24 h. When the cells reached to 80% confluence, the culture medium was discarded. After harvested, cells were washed with phosphate buffer saline (PBS) twice followed by centrifuging at 300 ×g for 5 min, and then cells were resuspended in the fresh culture medium and inoculated separately in three 9 cm plates. Medium was exchanged once every 24 h. After 48 h, the growth states and morphological changes of HepG2 cells were observed with an inverted microscope (OLYMPUS IX51, Japan). By this way, HepG2 and Hep3B cell lines continue to be passed through five generations until these cells grew stable.

**Cell growth and proliferation assay**

The viability of cancer cells was evaluated by MTT assay. Briefly, we first precisely weighed 100 mg of the freeze-dried powder of Açaí and C. Phellodendri Chinensis, respectively, then dissolved them separately in 10 mL cell culture medium with ultrasonic for 10 min at 35 kHz, and filtered through a 0.22-µm filtration membrane to prepare the tested mother solutions of a final concentration of 10 mg/mL. After these mother solutions were sterilized, they were subpackaged separately into 1 mL sterile centrifuge tubes and marked.
Second, 10 mg/mL solutions of Açaí and *C. Phellodendri* were separately diluted with media by concentration gradient method into a serial of concentration of the tested solutions, including 100, 40, 20, 10, 5, 2, 1, 0.5, and 0.25 μg/mL.

The cells were inoculated in 96-well culture plates at the density of $1 \times 10^3$/well with complete medium containing 10% fetal calf serum in a final volume of 0.2 mL. Cells were incubated at 37°C for at least 24 h to allow optimal attachment. Afterward, cells were treated, respectively, with specific concentrations of Açaí (0.25, 0.5, 1, 2, 5, 10, 20, 40, and 100 μg/mL) and *C. Phellodendri* (0.25, 0.5, 1, 2, 5, 10, 20, 40, 100 μg/mL) and incubated in a humidity-controlled 5% CO$_2$ incubator at 37°C for 48 h. Medium was exchanged once every 24 h. Cell control group and blank group were set up in the same manner, with six parallel wells each group. Cell survival was assessed by directly adding 22 μL of 5 mg/mL MTT to 0.2 mL medium. After the cells were incubated at 37°C for 4 h, medium in each well was discarded. Subsequently, the formazan precipitate was dissolved in 150 μL DMSO per well and completely mixed with shaker. Afterward, the optical density of each well was detected at 490 nm wavelength by iMark microplate reader (Thermo Varioskan Flash, USA). Cell proliferation inhibition rate is calculated by the formula. This experiment was repeated at least three times.

**Morphological analysis**

For morphological observation, human hepatic carcinoma HepG2 and Hep3B cells in logarithmic growth phase were seeded in a 96-well plate at the density of $1 \times 10^3$/well with 0.2 mL complete medium containing 10% fetal calf serum. Cells were allowed to attach at 37°C for 24 h. Then, the cells were treated, respectively, with Açaí and *C. Phellodendri* at a specific concentration. Each group has six parallel wells. Cells were incubated in a humidified 5% CO$_2$ incubator at 37°C for 48 h. Medium was exchanged once every 24 h. After 48 h, the growth states and morphological changes of HepG2 and Hep3B cells were observed under 10-fold objective lens with an inverted microscope and taken photographs (OLYMPUS IX51, Japan).

**Dye exclusion assay**

Trypan blue is a kind dye of cell activity. The trypan blue dye exclusion test was commonly used to detect the integrity of cell membranes and the viability of cells. When cells are damaged or dead, trypan blue dye can penetrate the denatured cell membrane and further bind to the disintegrated DNA to color it. On the contrary, the living cells prevent trypan blue dye from entering their interior. Hence, this test can be used to judge whether the cells are alive or not.

As described previously, human hepatic carcinoma HepG2 and Hep3B cell lines were inoculated 96-well culture plates at the density of $1 \times 10^4$/well with 0.2 mL complete medium containing 10% fetal calf serum. Cells were allowed to attach at 37°C for 24 h. Afterward, cells were treated with specific concentration Açaí and *C. Phellodendri*, respectively. Cell control group was set in the same way. Each group has six parallel wells. Then, cells were incubated in a humidified incubator with 5% CO$_2$ at 37°C for 48 h. Medium was exchanged once every 24 h. In the end, all media were discarded and 20 μL 0.4% trypsin blue staining solution was added into cells. Cells were cultured in a humidified incubator with 5% CO$_2$ at 37°C for 5 min. After that, the changes of the quantities and growth states of the dyed HepG2 and Hep3B cells were observed and analyzed under 10-fold objective lens of an inverted microscope and photographed (OLYMPUS IX51, Japan).

**Analysis of cellular energy metabolism**

Cellular energy metabolism reflects the function state of cells. To confirm the cold/hot drug property of Açaí, the analysis of cellular energy metabolism was conducted. First, 10 mg/mL solutions of the Açaí and *C. Phellodendri* were separately diluted with media by concentration gradient method into a serial concentration of the tested solutions, including 100, 50, and 25 μg/mL. When human hepatic carcinoma HepG2 and Hep3B cell lines were in logarithmic growth phase, the culture medium was discarded and cells were digested with 2–3 mL 0.25% trypsin-EDTA solution at 37°C for 5 min. After that, the suspension of cells was harvested by centrifuging at 300 x g for 10 min. The cells were washed with PBS twice followed by centrifuging at 300 x g for 10 min. Subsequently, cells were resuspended in appropriate volume medium after the supernatant was discarded. The cell density was adjusted to $1 \times 10^5$ L$^{-1}$ with cell culture medium. After mixed thoroughly, the cells were inoculated induplicate at $3 \times 10^4$/dish density in 9 cm diameter culture plates and allowed to attach at 37°C for 24 h. Afterward, medium was exchanged as described previously; cells were treated with specific concentrations of Açaí (100, 50, and 25 μg/mL) and *C. Phellodendri* (100, 50, and 25 μg/mL), respectively. Cell control group was set up in the same way. Then, the cells were incubated in a humidified incubator with 5% CO$_2$ at 37°C for 24 h.

Finally, the cells were collected by centrifugation and disrupted with ultrasonication (JY92-IIIN, Ningbo Scientz Biotechnology, China). Nicotinamide adenine dinucleotide (NADH) and NAD$^+$ were extracted separately from HepG2 and Hep3B cells using coenzyme I NAD(H) kit (NJJCBIO, China) according to the manufacturer’s instructions. The contents of NADH and NAD$^+$ were measured using spectrophotometry method and presented in nmol/10$^4$ cells. The ratio of NADH/NAD$^+$ was calculated from content values of NADH and NAD$^+$ in HepG2 and Hep3B cells. The experiment was repeated at least three times.

**Statistical analysis**

Quantitative data were presented as the mean ± standard deviation (SD). The SPSS Statistical Software Package (SPSS Inc. Chicago, IL, USA) for Windows Version 20.0 was employed for one-way ANOVA analysis followed by the LSD test for multiple comparisons when the variance is homogeneous, if not, by Tamhane’s T2 (M) and Dunnnett’s T3 test. $P$ levels of significance were set at <0.05. GraphPad.
Prism 5 software (GraphPad Software, Inc, San Diego, CA, USA) was used for plotting. NIS-Elements F/Viewer software (Tengrant Inc, China) was using for image processing and analysis. For cell proliferation and cellular energy metabolism experiments, data were representative of at least three independent experiments.

Results

Cell growth and proliferation assay

We have tested the effects of specific concentration Açaí and C. Phellodendri (0.25, 0.5, 1, 2, 5, 10, 20, 40, and 100 μg/mL) on the growth and proliferation of the tested human hepatic carcinoma cells lines [Figure 1 and Table 1].

As shown in Tables 1 and 2, just as the effects of Cortex Phellodendri to HepG2 and Hep3B cell, all tested concentration of Açaí presented stronger inhibitory effect on the growth and proliferation of HepG2 and Hep3B cell strains to some extent. In general, the inhibition activities of both of Açaí and C. Phellodendri showed positive dose–effect relationship. Actually, during the concentration range of 0–10 μg/mL, the dose–effect curve diagrams of Açaí to HepG2 cell strains displayed V-shaped changes, when compared to that of C. Phellodendri. Further analysis revealed that Açaí had very significant anti-proliferation effects against HepG2 cell line at the concentration of <0.5 μg/mL or >10 μg/mL, when compared with the blank control group (P < 0.05, P < 0.01). However, for another cell strain Hep3B, the antiproliferation effects of Açaí that is significantly different from the blank control group is at the concentration of <1 μg/mL or >5 μg/mL (P < 0.05, P < 0.01). As shown in Figures 1 and 2, the growth inhibitory effect of higher dose Açaí on HepG2 and Hep3B cells significantly increased with the increase of concentration of Açaí; it keeps consistent with that of C. Phellodendri. It is obvious that the effects of Açaí against HepG2 and Hep3B cell lines are similar and exhibit inhibition effects. Moreover, this performance is in accordance with the theory opinion that Jian-Li et al. proposed on the identification of cold/hot drug properties, that is whether at high doses or low doses, all herbs with cold and cool drug property can produce the efficacy of inhibiting cells growth and proliferation. Based on our experiment results, we conclude that the drug property of Açaí belongs to cool or cold characteristic.

Effect of Açaí on the morphology of cells

To reflect more directly the growth inhibitory effect, we observed the morphological changes of HepG2 and Hep3B cells after

![Figure 1: Dose-response curves of Açaí when HepG2 cells were treated for two day. Two curves represent the dose-response curve of Açaí, C. Phellodendri on HepG2 cell. The error bars represent mean± standard deviation (three replicates). The horizontal axis represents the concentration of Açaí and C. Phellodendri, the vertical axis represents the inhibition rate](Image)

![Figure 2: Dose-response curves of Açaí when Hep3B cells were treated for two day. Two curves represent the dose-response curve of Açaí, C. Phellodendri on Hep3B cell. The error bars represent mean± standard deviation (three replicates). The horizontal axis represents the concentration of Açaí and C. Phellodendri, the vertical axis represents the inhibition rate](Image)

**Table 1: Effect of Açaí on HepG2 cell proliferation (x±s, n=3)**

<table>
<thead>
<tr>
<th>Concentrations (μg/mL)</th>
<th>Blank control group</th>
<th>Positive control group (Cortex phellodendri)</th>
<th>Açaí tested group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Inhibition rate (%)</td>
<td>A</td>
<td>Inhibition rate (%)</td>
</tr>
<tr>
<td>0</td>
<td>1.25±0.142</td>
<td>-</td>
<td>1.178±0.078</td>
</tr>
<tr>
<td>0.25</td>
<td>-</td>
<td>1.171±0.075</td>
<td>4.23±0.32</td>
</tr>
<tr>
<td>0.5</td>
<td>-</td>
<td>1.154±0.092</td>
<td>5.85±0.24</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>1.141±0.101</td>
<td>7.10±1.65</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>1.112±0.070</td>
<td>9.58±1.03</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>1.019±0.143</td>
<td>18.61±2.35</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>0.995±0.163</td>
<td>20.97±2.15</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>0.964±0.128*</td>
<td>23.38±3.02</td>
</tr>
</tbody>
</table>

*P<0.05 versus control group. **P<0.01 versus control group. The inhibition rates of HepG2 cells are means of three independent experiments (n=3, mean±SEM). SEM: Standard error of mean.
treated with Açaí [Figures 3 and 4], because the morphological changes of cells may reflect the growth state. As a result, for blank control groups, the untreated HepG2 and Hep3B cells displayed a high confluence of monolayer cells and flourished in the logarithmic growth phase, which had obvious and typical characteristics of strong growth such as intercellular tight connections, clear membrane, full cytoplasm, and good refractive performance. Most of cells looked like quasi-circular shape. However, due to contact inhibition caused by flourishing growth, the debris of dead and granular-shaped folded cells was detached from the plate bottom and scattered around, especially for Hep3B cells. In contrast, for cells treated with C. Phellodendri, the growth number was apparently less than that of blank control groups, and the morphological characteristics of slow growth were clearly displayed that includes cell-to-cell conjuction reduction in a dose-dependent manner; almost all cells grew and expanded into flat irregular polygons with weak graininess that may due to enough space for growth. Moreover, the debris of dead and suspended cells is less, and the morphological of...
Toxicity effects of Açaí on HepG2 and Hep3B cells

To further investigate whether Açaí led to cells damaged or dead, the activity of HepG2 and Hep3B cells treated with Açaí was detected with trypan blue dye exclusion method. Living cells are not stained into blue, while dead cells are dyed into light blue. As shown in Figures 5 and 6, it is very evident that the number of light blue cells dyed was less than that of blank control group, after HepG2 and Hep3B cells were treated with 20 and 40 μg/mL C. Phellodendri for 48 h, respectively, followed by trypan blue staining for 5 min. Moreover, the blue cells dyed were granular-shaped exfoliated cells. Similarly, HepG2 and Hep3B cells treated with 20 and 40 μg/mL Açaí almost rarely were stained. The results suggested that Açaí had no obvious toxicity effect on HepG2 and Hep3B cell lines within the set concentrations range.

Effects of Açaí on cellular energy metabolism

The content of NADH and the ratio of NADH/NAD+ can reflect the level of glycolysis and tricarboxylic acid cycle in cells. Hence, the detection of NADH content and NADH/NAD+ ratio of cells can be used to evaluate the change of energy metabolism and to further understand the function state of cells. The results can be seen from Tables 3 and 4, Figures 7 and 8 that 100, 50 and 25μg/mL Açaí can down-regulate the NADH content and the NADH/NAD+ ratio of HepG2 and Hep3B cells, basically, the ratio of NADH/NAD+ of HepG2 and Hep3B cells significantly decreased, in comparison with blank control groups (P<0.05, P<0.01). We can see that Açaí has inhibition effects on the NADH content and NADH/NAD+ ratio of HepG2 and Hep3B cell lines in a concentration-dependent manner; the depression effects were very significant when Açaí was in a higher concentration. The results suggested that the level of glycolysis and tricarboxylic acid cycle in cells treated with Açaí became weaken, and the level of energy metabolism decreased. Hence, we can infer that Açaí can lower cells the oxygen consumption and inhibit the growth and proliferation.

![Figure 5: Microscopic appearance of trypan blue-stained of HepG2 cell treated. The morphological changes of HepG2 cell were observed with microscopy after incubated with Açaí and C. Phellodendri for 48 h respectively at 20μg/mL and 40μg/mL concentrations (×100). The blue cells dyed with granular-shaped are visible in the control cells, but almost none in the treated cells](image)

![Figure 6: Microscopic appearance of trypan blue-stained of Hep3B cell treated. The morphological changes of Hep3B cell were observed with microscopy after incubated with Açaí and C. Phellodendri for 48 h respectively at 20μg/mL and 40μg/mL concentrations (×100). The blue cells dyed with granular-shaped are visible in the control cells, but almost none in the treated cells](image)
Evaluation on hot/cold drug property of Chinese herbs by cytological study methods is also based on their efficacy characteristics. Anyway, cells as research material, there are many advantages that include minor differences between groups and better homogeneity and reproducibility, and it is fit for cytological study methods to combine with many other methods such as the expression of protein and gene and thermodynamics (energy) measure. Meanwhile, it can overcome the disadvantage such as individual differences between different animals, indicators fluctuating largely, and long experiment period.

Jian-li Liu et al. applied earlier cytological study methods to analyze the hot/cold drug properties of Chinese herbs, in particular, to analyze unknown drug property herbal plants including those which were from other ethnic regions. The determination to the drug properties is based mainly upon the drug efficacy activities on cell level, such as the influence of herbal plants on the proliferation and growth states of cells. Furthermore, they put forward the identification criteria for hot/cold drug properties, that is, hot property herbs can promote the growth, proliferation, and metabolism of cells in a low concentration, while at a high concentration, it will produce cellular toxicity effects. Those characteristics are different from cold property herbs which can exert an inhibitory effect on cells at a low concentration without obvious cytotoxicity.[10] Anyway, until now, nobody still studied the hot/cold drug property of Açaí with cytological study methods. Therefore, this study intended to understand the hot/cold drug property of Açaí from another perspective by the cytological model study methods.[10,11]

**Discussion**

Most botanical drugs contain natural pigment components, so the color of their aqueous solution usually looks like dark brown. Similarly, the aqueous solution of Açaí extracts also present brown color though that the aqueous extraction of Açaí had been treated in advance.

For the reason, we investigated the most suitable concentration of Açaí for these studies to prevent from the color of Açaí to interfere the optical density values detected. In the choice of the tested concentration of Açaí, we analyzed the concentration range of 0.25–40.00 μg/mL, and found that the color of Açaí solution is very light within the range of 0.25–40 μg/mL and can completely reduce the experimental error caused by color difference from drug residue in the adherent cell layer.[12] In addition, the false-positive results caused by the cytotoxicity effects due to too high concentration of hot property drug can be eliminated, if we take the lower concentration range of 0.25–40 μg/mL.[10] The results of trypan blue dye exclusion tests also confirm that the growth and proliferation inhibition of 0.25–40 μg/mL Açaí on HepG2 and Hep3B cells was not caused by the cytotoxicity effects of Açaí.

Based on Açaí’s traditional application, it was deduced that the drug property of Açaí should belong to the cold or cool characteristic.[4] Lin-Yuan et al. established deficiency-hot model and deficiency-cold models of SD rats by intramuscular injecting, respectively, hydrocortisone and dexamethasone sodium phosphate for 21 days. After successful establishment of these models and drug administration, some serum biochemical indexes that can reflect rats body about hot production and metabolic changes were detected, including endocrine hormones (T3, T4, and rT3), cyclic nucleotide (cAMP and cGMP), and metabolic markers (TP, UA, TC, TG, and ALB).
On the basis of these results combined with the changes of body weight, hair color, mind state, autonomic activity, cold and hot resistance, and defecation, and compared to the ejection of *C. Phellodendri*, they analyzed the hot/cold drug property of Açaí with the methods including the similar comparison method and the heterogeneous contradiction counter-evidenced method and further verified the above-deduced opinion. They concluded that the drug property of Açaí is inclined to be cold.[9]

Subsequently, Wang et al. also established the same rat models as Lin-Yuan’s and analyzed the hot/cold drug property of Açaí through detecting the cyclic nucleotides, neurotransmitters, and the relevant enzymes, which neurotransmitters include dopamine, norepinephrine, and dopamine beta-hydroxylase, and investigating the changes of immune molecules including IgG, IgM, complement C3, and complement C4 in the serum of rat, as well as the changes of vital signs. Moreover, Wang et al. also studied the capability of Açaí regulating and improving nervous system, immune system, and the second messenger between the two systems. Anyway, they likewise demonstrated that the drug property of Açaí is cold.[10]

The chemical components and their fingerprint of a Chinese herb usually happened large changes if the extract methods of the same Chinese herb is different. However, the chemical components of Chinese herb act as the basis for Chinese herb to exert the efficacy and keep tightly connection with its hot/cold drug properties.[14] Hence, the extraction methods can influence the drug properties of Chinese herb. In this study, the tested solutions of Açaí were prepared with water as a solvent without through any processing, and this extraction method keep consistent with traditional method of decoction. Therefore, the tested Açaí in the study can better retain the components of Açaí, and the hot/cold drug property of the tested solutions of Açaí can represent that of South America Açaí in the cytological study. In addition, the positive control *C. Phellodendri* in this study took the same conditions as that of Açaí, including the prepared method, the tested concentration range and the operating procedures. It is well known that *C. Phellodendri* was recognized as a cold property drug in the theory of TCM and *C. Phellodendri* possess bitter flavor and cold property. In this study, *C. Phellodendri* also exhibited a significant inhibition of the growth and proliferation on HepG2 and Hep3B cell lines in a dose-dependent manner. The growth morphology changes and the extent of trypan blue dye exclusion of both cell lines after treated with *C. Phellodendri* also completely embodied the function effect characteristics of cold property drug.[10] Correspondingly, the effects of Açaí were identical on the influence of cell proliferation inhibition, growth morphology changes, and trypan blue dye exclusion reaction with that of *C. Phellodendri* and also equally accorded with the characteristics of cold property drugs.[10] This study confirmed that the drug property of Açaí was cold and there were not obvious toxicity effects. In brief, the results in this study are completely consistent with the conclusion deduced from traditional practice and the study results of model animal experiments described previously.[4,9,13]

In addition, the changes of hot and energy production in the body are an important reflection of hot/cold drug property of herbs. Hot property herbs produce body excitation function and facilitate the energy metabolism and hot production. On the contrary, cold property herbs produce the opposite function.[15] As for cellular energy metabolism, the results indicated that the contents of NADH and the ratio values of NADH/NAD⁺ significantly declined when two kinds of cell models were treated with Açaí. This also suggested that the functional activity and energy metabolism of the treated cell lines actually decreased.

NADH is the reduced state of the biofunctional molecule NAD (coenzyme I) that produced from the glycolysis and tricarboxylic acid cycle. Acting as an electron donor, NADH further participates not only in the electron transport of the respiratory chain in the body energy metabolism process but also in the important link of oxidative phosphorylation generating ATP.[15] The content of NADH can reflect indirectly the intensity of the glycolysis and tricarboxylic acid cycle and the activity of the cellular energy metabolism because oxidative phosphorylation is a main process of the hot production and energy production for eukaryotes. However, the ratio values of NADH/NAD⁺ can more effectively reflect the situation of intracellular energy metabolism because they are free from the restrictions of the content difference of NADH. Hence, these tests can determine the cold drug property of Açaí.  

**Conclusion**

In this study, with homogeneous comparison methods, we investigated the effects of Açaí on the growth and proliferation of two human hepatic carcinoma cell lines HepG2 and Hep3B and independently analyzed its possible mechanism on the influence of the energy metabolism of two cell strains. It was deemed through comprehensive analysis that Açaí has the cold property. Therefore, in terms of Açaí integrated into Chinese herb, we thought that Açaí can be used to therapy and alleviate hot syndrome diseases in the way of alone use or compatibility use under the guidance of TCM principle. However, practical application of Açaí demands to be summed up constantly and verified further in clinical practice.

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**Conflicts of interest**

There are no conflicts of interest.
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