



Purification and differential biological effects of ginger-derived substances on normal and tumor cell lines

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ARTICLE INFO

Article history:

Received 30 March 2012

Accepted 16 July 2012

Available online 20 July 2012

Keywords:

Zingiber officinale

HPLC

Gingerols

Ginger

Cell proliferation

Cancer

MDA-MB-231

ABSTRACT

This study describes an optimization of [6]-, [8]- and [10]-gingerol isolation and purification in semi-preparative HPLC scale and their anti-proliferative activity. The gingerols purification was carried out in HPLC system using a Luna-C₁₈ and the best mobile phase evaluated was MeOH/H₂O (75:25, v/v). This new methodology for the gingerols isolation was very effective, since considerable amounts (in the range of milligrams) with a good purity degree (~98%) were achieved in 30 min of chromatographic run. [6]-, [8]- and [10]-Gingerol purified by this methodology inhibited the proliferation of MDA-MB-231 tumor cell line with IC₅₀ of 666.2 ± 134.6 μM, 135.6 ± 22.6 μM and 12.1 ± 0.3 μM, respectively. These substances also inhibited human fibroblasts (HF) cell proliferation, however in concentrations starting from 500 μM. In conclusion, our results demonstrate an optimization of gingerols isolation and their specific anti-proliferative activities against tumor cells, suggesting their use as important models for drug design in an attempt to develop new compounds with fewer side effects when compared to conventional chemotherapy.

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1. Introduction

Several population-based studies indicate that people in Asian countries have a much lower risk of different cancer types such as colon, gastrointestinal, prostate and breast, when compared to their Western counterparts. It is widely claimed that constituents of their diet such as ginger, garlic, soy, curcumin, onion, tomatoes, cruciferous vegetables, chillies, and green tea contribute to that lower incidence [1]. Therefore, there is increasing interest in naturally occurring cancer chemopreventive agents.

Ginger (*Zingiber officinale* Roscoe) is widely used worldwide as a food, spice and herb [2]. Together with [6]-gingerol, [8]- and [10]-gingerol are the major pungent constituents in the ginger oleoresin

from fresh rhizome, being [6]-gingerol the most abundant. Gingerols comprise a series of homologue substances differentiated by the length of their alkyl chains with [6]-, [8]- and [10]-gingerol having 10, 12 and 14 carbons in their unbranched alkyl chains, respectively [3].

Regarding [6]-gingerol a variety of pharmacological characteristics was already described, including analgesic, antipyretic, cardiotoxic, hypothermia inducing and cancer preventing effects [4–6]. However, the effects on tumor cell proliferation for [8]- and [10]-gingerol are still scarce.

Breast cancer is the third most frequent cancer in the world and one of the most common malignant diseases in women worldwide. In developing countries, it is the second highest cause of death in women after cervical cancer [7]. To treat breast and many other cancer types, chemotherapy is one of the most extensively studied methods. However, its efficacy and safety remain a primary concern as well as its toxicity and other side effects [8,9]. Another reason for concern regarding this method is the development of chemotherapy resistance, which is a major obstacle to the effective treatment of many tumor types, including breast cancer [8]. Tumor cells are found to adopt multiple mechanisms to resist drugs, such as decreased uptake, and/or enhanced efflux and altered drug

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metabolism. Alteration in drug targets, activation of detoxification systems, enhanced DNA repair ability, and inhibition of apoptosis are also cancer cell strategies to resist against chemotherapy drugs [10].

Many anticancer therapies can temporarily stop tumor growth; it means that, in most cases, the effect is not permanent. In this way, there is a significant need for new agents with low susceptibility to common drug resistance mechanisms in order to improve response rates and potentially prolong patient's survival. Approximately 30% of the women diagnosed with early-stage disease progress to metastatic breast cancer. To these women, therapeutic options are still limited. Current recommendations for first-line chemotherapy include anthracycline-based and taxanes treatments (paclitaxel and docetaxel) [8]. Taxanes – which are taxol derivatives that were first described as microtubule stabilizing agents [11] and then had their antineoplastic effects confirmed [12,13] – are the first example of natural product derivatives used in antitumor therapy. Therefore, the search for new natural products that may be used as an additional alternative to the chemotherapy in an attempt to develop more effective drugs with fewer side effects is of great interest [14]. Based on this assumption it is important to develop an effective method for the isolation of these compounds.

The aim of this work was to search a faster, efficient and productive procedure for isolation of [6]-, [8]- and [10]-gingerol by reverse phase HPLC operating under overloading conditions and to test their anti-proliferative activity on MDA-MB-231 breast cancer tumor line and normal human fibroblasts (HF), a non tumor cell line. The results presented in this study showed a more specific anti-proliferative activity for [8]- and [10]-gingerol against breast cancer compared to HF and suggest their use as important models for anti-cancer therapy in an attempt to develop new drugs with fewer side effects when compared to conventional chemotherapy.

2. Materials and methods

2.1. Chemicals

[6]-, [8]- and [10]-Gingerol were isolated from the ethanol extract of the ginger rhizome (purchased locally) by liquid chromatography. The structures were elucidated by ¹H NMR using a Bruker DRX200 instrument (Bruker, USA), operating at 200 MHz for ¹H spectra, with TMS as internal standard, GC-MS on a Shimadzu QP 5000 and comparison with literature data. The purity was determined by elemental analysis on an EA 1108, CHNS-O (Fisons). HPLC grade acetonitrile and methanol were purchased from JTBaker Company (Netherlands). Water was deionised. All other analytical grade reagents were from Sigma-Aldrich (St. Louis, MO, USA).

2.2. [6]-, [8]- and [10]-Gingerol isolation

The fresh ginger rhizome was thin sliced, dried at 40 °C and ground to powder. The powder was extracted with ethanol for 72 h, three times. The solvent was evaporated under reduced pressure at 40 °C. The extraction of ginger (0.4 kg dry weight) yielded 21.2 g of crude extract (5.3%). The extract was stored at -4 °C for the later fractionation.

In order to isolate [6]-, [8]- and [10]-gingerol, dry ginger rhizome crude ethanol extract was chromatographed on SiO₂ (70–230 mesh) column with bed height 20 cm (20 cm × 8 cm i.d.), using initially *n*-hexane (1 L), to eliminate the lipids. After, *n*-hexane/ethyl acetate 50:50 (v/v, 2.5 L) was used as eluent resulting in 5 fractions (A1–5), where the fractions A1, A2, A3 and A4 (68.9%, 14.6 g), rich in [6]-, [8]- and [10]-gingerols, were joined resulting in the fraction A1–4. Part of this fraction (5.5 g) was re-chromatographed on SiO₂ column (28 cm × 5 cm i.d.) using

n-hexane/ethyl acetate 60:40 (v/v, 2.4 L). Forty eight fractions (50 mL each) were collected and combined after monitoring by TLC resulting in six sub-fractions with the following volumes: 250 mL (B1), 400 mL (B2), 150 mL (B3), 800 mL (B4), 200 mL (B5) and 600 mL (B6). The gingerols were the main constituent on the fraction B4 (800 mL, 2.8 g, yellow oil).

Analytical and semi-preparative HPLC were carried out using a Shimadzu SCL-10AVP system with an SPD-10AVP UV-vis detector with a flow cell analytical or flow cell preparative, according with the HPLC mode (analytical or semi-preparative), and a Shimadzu LC-6AD pump. The isolation optimization was carried out on a Luna-C₁₈ column (250 mm × 4.6 mm i.d., 10 μm, homemade, UFSCar, São Carlos, SP, Brazil) and the column was maintained at 25 °C, the detection wavelength was set at 282 nm and the flow rate at 1.0 mL/min. The mobile phases consisted of isocratic binary mixtures (v/v) of MeOH/H₂O (80:20; 75:25; 70:30) and ACN/H₂O (67:33). Forty microlitres of methanol containing 0.156 mg, 5 mg, 7.5 mg, 15 mg and 30 mg of B4 sample were injected. In the column scale-up reverse phase semi-preparative, a Luna-C₁₈ column (300 mm × 10 mm i.d., 10 μm, homemade, UFSCar, São Carlos, SP, Brazil) was used. This column was maintained at 25 °C, the detection wavelength was set at 254 nm (lower absorbance wavelength for the gingerols) and the flow rate at 6.0 mL/min. The best mobile phase was MeOH/H₂O 75:25 (v/v) and the scale-up factor was calculated using Eq. (1) below, where *S*, *R*, *L*, *P*, *A* are scale-up factor, column radius, column length, semi-preparative and analytical columns, respectively. The maximum amount of fraction B4 injected in the semi-preparative column was 170.1 mg diluted to final volume of 400 μL.

$$S = \frac{R_P^2 \times L_P}{R_A^2 \times L_A} \quad (1)$$

2.3. Cell line and culture

MDA-MB-231 human breast tumor and HF cell lines, obtained from ATCC (Manassas, VA, USA), were maintained at 37 °C in 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM, Cultilab – Campinas; SP, Brazil) containing 10% of FBS (fetal bovine serum), penicillin (100 U/mL), streptomycin (100 mg/mL) and L-glutamine (2 mM). Cell cultures and experiments were conducted in a humidified environment with 5% CO₂ at 37 °C.

2.4. Proliferation assays

Cell lines were prepared at a concentration of 5 × 10⁴ cells/200 μL, in appropriate medium with 10% of FBS, and plated on sterile 96-well plates for 5 h. After cell adhesion to the plates, the DMEM medium was replaced by DMEM medium without FBS and cells were maintained for 14–18 h at 37 °C and 5% CO₂ in a cell culture incubator. This procedure, known as starvation, was used to synchronize the cell cycle. After starvation, the culture medium was removed from the wells and a new one, supplemented with 10% of FBS, containing different concentrations of the tested compounds was added to the wells. The cells were incubated for 24 and 48 h under the same conditions as described above. Cell proliferation assay was performed in comparison to the wells where the vehicle compound (2.5% DMSO) was added instead of the tested compounds. After incubation, the culture medium of each well was removed and a solution containing MTT (0.5 mg/mL) was added (100 μL/well). The plates were then kept at 37 °C for 4 h and the formed crystals were dissolved in isopropyl alcohol. The absorbance was read on an ELISA plate reader at a wavelength of 595 nm. Doxorubicin was used as a positive control for inhibition of cell proliferation [15].

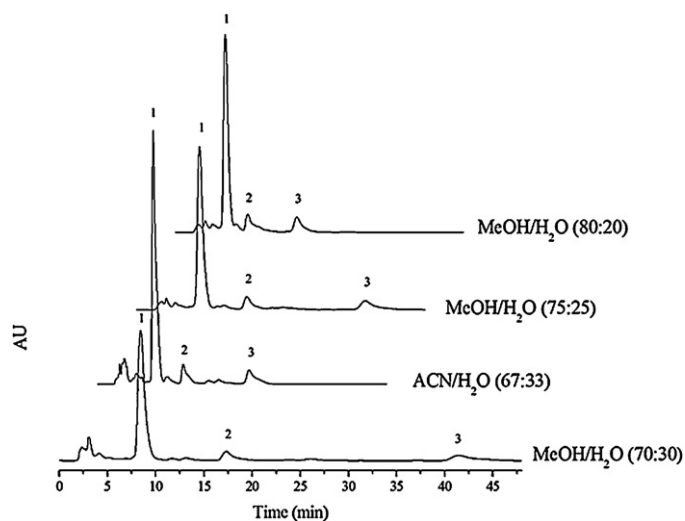


Fig. 1. Chromatograms of fraction enriched in [6]-gingerol (peak 1), [8]-gingerol (peak 2) and [10]-gingerol (peak 3), obtained from HPLC in analytical scale. The fraction was injected in a C_{18} (250 mm \times 4.6 mm i.d., 10 μ m) column maintained at 25 °C with MeOH/H₂O 80:20; MeOH/H₂O 75:25; ACN/H₂O 67:23 and MeOH/H₂O 70:30, UV detection at 282 nm and flow rate at 1.0 mL/min. The injection volume was 40 μ L.

2.5. Statistical analysis

Each experiment was repeated three times in triplicate and a standard error mean was calculated. Shapiro–Wilk's W test was used to verify data normality. As normal distribution was present, the results were compared statistically with a two-way analysis of variance (ANOVA). Since the ANOVA tests showed significant differences (acceptable P level < 0.05), Bonferroni's significant difference *post hoc* analyses were performed to determine differences between simple and grouped main-effect means, respectively. The data were analyzed by using Statistica software (version 8.0; Stat Soft Inc., Tulsa, USA) and IC_{50} calculations were made using Hill's equation in the Origin software (version 8.5; OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. [6]-, [8]- and [10]-Gingerol isolation

Four different conditions were carried out on analytical C_{18} column HPLC to achieve the best separation of [6]-, [8]- and [10]-gingerol. The best separation condition was achieved using MeOH/H₂O 75:25 (v/v), where a good separation of three gingerols in a single run of 30 min was obtained (Fig. 1).

The injection volume (400 μ L) was larger than the usual size of an injection volume and also it was not the one that was calculated by the scale up formula. This occurred because of the sample viscosity, which would be very high if 170.1 mg were diluted in 230 μ L (calculated volume) that would impair the chromatographic separation. For column overloading with the sample, increasing amounts of fraction B4 (0.156 mg, 5 mg, 7.5 mg, 15 mg and 30 mg diluted to final volume 40 μ L) were injected in a C_{18} analytical column. Using the detector at 282 nm electronic saturation was observed when 5 mg of sample was injected, although the peaks were still visible for isolation of gingerols until 15 mg. With the injection of 30 mg of sample, the electronic saturation hindered the visualization of peaks closer to peak 1 (Fig. 2). To solve this problem, on the semi-preparative mode, the detector was set at 254 nm and in this condition it was possible to identify the three gingerols present in the fraction.

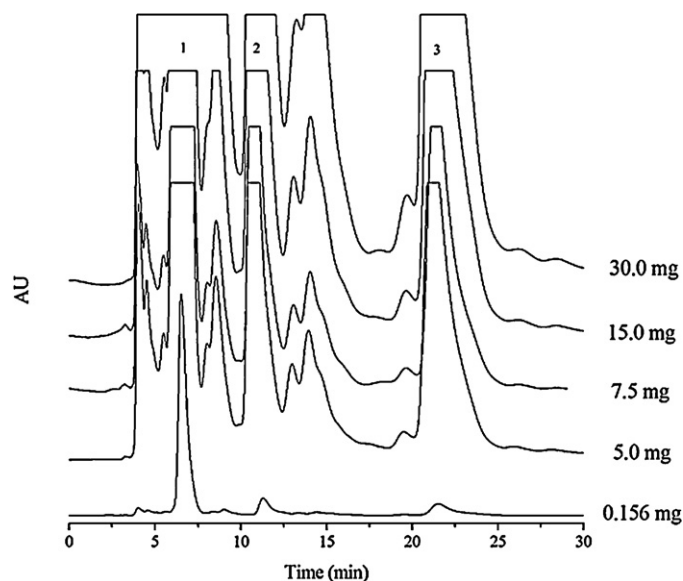


Fig. 2. Overload chromatograms of fraction enriched in [6]-gingerol (peak 1), [8]-gingerol (peak 2) and [10]-gingerol (peak 3), obtained from HPLC in analytical scale. The fraction was injected in a C_{18} (250 mm \times 4.6 mm i.d., 10 μ m) column maintained at 25 °C with MeOH/H₂O 75:25, UV detection at 282 nm and flow rate at 1.0 mL/min. The injected masses were 0.156 mg, 5 mg, 7.5 mg, 15 mg and 30 mg. The injection volume was 40 μ L.

Using Eq. (1) for the determination of scale-up factor, the value 5.67 was obtained, consequently the maximum amount of B4 fraction that could be injected in a semi-preparative column was 170.1 mg (Fig. 3). However, to make the adjustment of the flow rate easier we decided to use 6.0 mL/min instead of 5.67 mL/min, and by working in this condition the results obtained were satisfactory.

The mass of each gingerol obtained from 170.1 mg of fraction B4 was: 94 mg ([6]-gingerol), 22 mg ([8]-gingerol) and 17 mg ([10]-gingerol). The purity of gingerols was calculated based on the elemental analysis of each gingerol. The purification yields based on the fresh ginger weight were 1027, 241 and 187 ppm for [6]-, [8]- and [10]-gingerol, respectively. The purity obtained was 94.4, 99 and 97.3% for [6]-, [8]- and [10]-gingerol, respectively.

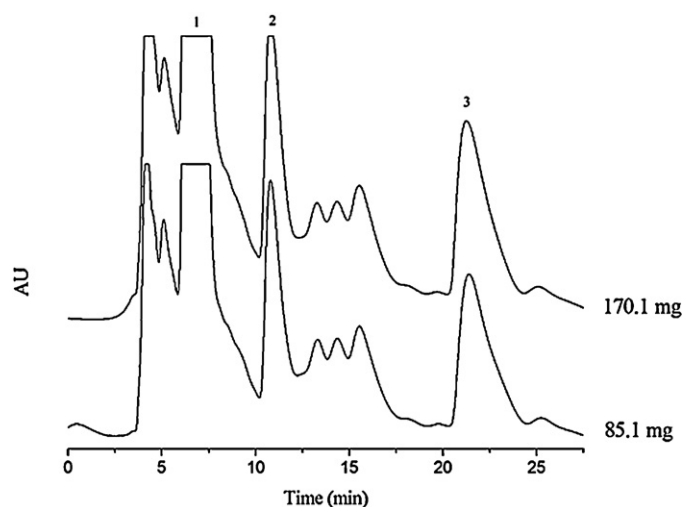


Fig. 3. Scale-up chromatograms of fraction enriched in [6]-gingerol (peak 1), [8]-gingerol (peak 2) and [10]-gingerol (peak 3), obtained from HPLC in analytical scale. The fraction was injected in a C_{18} (300 mm \times 10 mm i.d., 10 μ m) column maintained at 25 °C with MeOH/H₂O 75:25, UV detection at 254 nm and flow rate at 6.0 mL/min. The injected masses were 85.1 mg, 170.1 mg. The injection volume was 400 μ L.

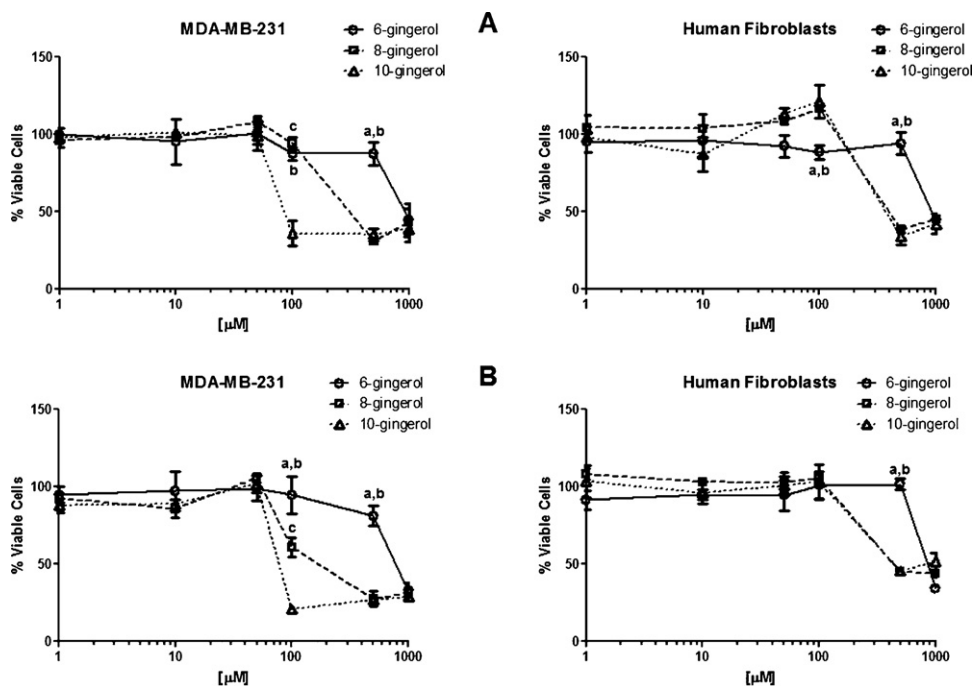


Fig. 4. Effects of [6]-, [8]- and [10]-gingerol on MDA-MB-231 and Human Fibroblasts cell proliferation. MDA-MB-231 tumor cells or Human Fibroblasts were plated at a density of 5×10^4 cells/200 μ L in a 96-well plate containing DMEM medium with 10% of FBS (fetal bovine serum) for 5 h. After cell adhesion to the plates, the DMEM medium was replaced by DMEM medium without FBS and cells were maintained for 14–18 h at 37 °C and 5% CO₂ in a cell culture incubator. This procedure, named starvation, was used to synchronize the cell cycle. After starvation the monolayers were incubated with different concentrations of [6]-, [8]- or [10]-gingerol in DMEM medium, supplemented with 10% of FBS. The cells were incubated for 24 (A) or 48 (B) hours. Viable cells were estimated by the MTT assay. Results are mean \pm SD of three independent assays in triplicate (a = [6]-gingerol vs. [8]-gingerol $P < 0.05$; b = [6]-gingerol vs. [10]-gingerol $P < 0.01$; c = [8]-gingerol vs. [10]-gingerol $P < 0.001$).

3.2. Identification of [6], [8] and [10]-gingerol

The gingerols were identified based on the ¹H NMR and MS spectra data compared with literature [16,17].

3.2.1. [6]-Gingerol

Yellow oil; C₁₇H₂₆O₄. MS m/z 294 [M]⁺ (9), 276 (4), 205 (8), 194 (13), 179 (4), 150 (26), 137 (100). ¹H NMR (200 MHz, CDCl₃): δ 0.88 (t, 3H, t, $J = 6.0$ Hz, H-10), 1.22–1.48 (8H, m, H-6 to H-9), 2.47–2.90 (2H, m, H-4), 2.78 (4H, m, H-1, H-2), 3.85 (3H, s, –OCH₃), 4.02 (1H, q, H-5), 6.64 (1H, dd, $J = 8.0, 2.0$ Hz, H-6'), 6.67 (1H, d, $J = 2.0$ Hz, H-2'), 6.82 (1H, d, $J = 8.0$ Hz, H-5').

3.2.2. [8]-Gingerol

Brown oil; C₁₉H₃₀O₄. MS m/z 322 [M]⁺ (3), 304 (5), 205 (9), 194 (23), 179 (2), 150 (13), 137 (100). ¹H NMR (200 MHz, CDCl₃): δ 0.88 (t, 3H, t, $J = 6.0$ Hz, H-10), 1.22–1.48 (12H, m, H-6 to H-11), 2.47–2.90 (2H, m, H-4), 2.78 (4H, m, H-1, H-2), 3.86 (3H, s, –OCH₃), 4.02 (1H, q, H-5), 6.64 (1H, dd, $J = 8.0, 2.0$ Hz, H-6'), 6.67 (1H, d, $J = 2.0$ Hz, H-2'), 6.82 (1H, d, $J = 8.0$ Hz, H-5').

3.2.3. [10]-Gingerol

Colorless powder; C₂₁H₃₄O₄. MS m/z 351.2 [M+1]⁺, m/z 373.3 [M+23]⁺, 389.2 [M+39]⁺. ¹H NMR (200 MHz, CDCl₃): δ 0.88 (t, 3H, t, $J = 6.0$ Hz, H-10), 1.22–1.48 (14H, m, H-6 to H-13), 2.47–2.90 (2H, m, H-4), 2.78 (4H, m, H-1, H-2), 3.87 (3H, s, –OCH₃), 4.02 (1H, q, H-5), 6.64 (1H, dd, $J = 8.0, 2.0$ Hz, H-6'), 6.67 (1H, d, $J = 2.0$ Hz, H-2'), 6.82 (1H, d, $J = 8.0$ Hz, H-5').

3.3. Effects of [8] and [10]-gingerol on normal and tumor cell proliferation

The effects of isolated compounds, [6]-, [8]-, and [10]-gingerol, were compared to their effects on HF, a normal (non tumor) cell

line (Figs. 4A and B and 5). In these assays, the controls were made by incubating the cells in a normal medium, supplemented with 10% of FBS (+FBS), in a medium without FBS (–FBS), adding DMSO (final concentration of 2.5%) to the medium supplemented with 10% of FBS (DMSO) and finally, in doxorubicin (1.5 μ M), a compound used in breast cancer chemotherapy, as a positive control for cell proliferation inhibition [15].

The results showed that [8]-gingerol affects MDA-MB-231 cell proliferation after 24 h of incubation, with 500 and 1000 μ M inhibiting approximately 75% and 50% of tumor cell proliferation, respectively (Fig. 4A and B). After 48 h of incubation with [8]-gingerol 100 μ M there was approximately 60% of MDA-MB-231 cell proliferation inhibition (Fig. 4B). At the same incubation period with [8]-gingerol 500 and 1000 μ M, MDA-MB-231 inhibition remained in a rate of 75% compared to control cells (+FBS). [8]-Gingerol 1, 10 and 50 μ M had no effects on MDA-MB-231 cell proliferation (Fig. 4A and B).

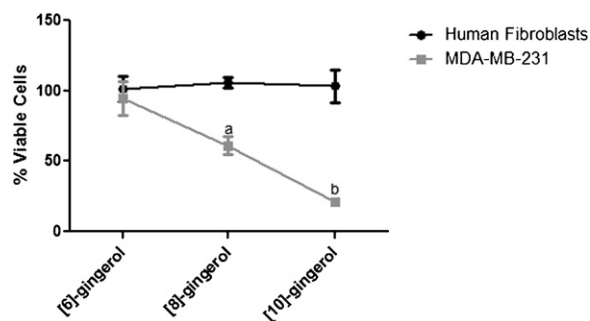


Fig. 5. Differential effects of [6]-, [8]- and [10]-gingerol 100 μ M on MDA-MB-231 and Human Fibroblasts cell proliferation after 48 h of incubation. Results are mean \pm SD of three independent assays in triplicate (a = MDA-MB-231 vs. HF $P < 0.01$; b = MDA-MB-231 vs. HF $P < 0.001$).

For [10]-gingerol, a concentration of 100 μM was significantly effective in inhibiting MDA-MB-231 cell proliferation at 24 h of incubation in a rate of 75%, compared to cells seeded on normal medium (+FBS). The same pattern was observed after 48 h of [10]-gingerol incubation; there was a cell proliferation inhibition of about 90% (Fig. 4B).

HF were used in order to compare the effects of both [6]-, [8]- and [10]-gingerol in a non tumor cell line (Figs. 4A and B and 5). No significant differences in MDA-MB-231 cell proliferation using 1, 10, 50 and 100 μM for [6]-, [8]- and [10]-gingerol in 24 or 48 h of incubation were verified. However, 500 and 1000 μM concentrations were effective in this inhibition, but in an approximate rate of only 50% (Fig. 4A and B).

The differential effects of [8]- and [10]-gingerol 100 μM after 48 h of incubation with HF and MDA-MB-231 tumor cell line are demonstrated in Fig. 5. These compounds were more effective in inhibiting tumor cell proliferation (MDA-MB-231) when compared to normal cell proliferation (HF). The IC_{50} values for [6]-, [8]- and [10]-gingerol on MDA-MB-231 cell proliferation were calculated. The results showed that these compounds inhibited the proliferation of MDA-MB-231 tumor cell line with IC_{50} of $666.2 \pm 134.6 \mu\text{M}$; $135.6 \pm 22.6 \mu\text{M}$ and $12.1 \pm 0.3 \mu\text{M}$, respectively. These substances also inhibit HF cell proliferation, nevertheless, we were not able to calculate the IC_{50} values for this cell line, since the initial concentration that had an effect was 500 μM for [6]-, [8] and [10]-gingerol.

4. Discussion

Studies of isolation and purification of gingerols are scarce in the literature. Considering the interesting biological activities and the potential of these compounds to the medical area it is imperative to develop new methodologies to achieve an optimization of gingerol separation and purification. The isolation of gingerols ([6]-, [8]- and [10]-gingerol) described in this work was achieved satisfactorily, once it was possible to obtain relatively high mass and a good degree of gingerols purity. To our knowledge, only one work about isolation of gingerols using HPLC system was published [18]. In this work, the authors isolated the major gingerols from the crude dichloromethane extract of ginger rhizome by normal phase HPLC. A low purity degree (calculated by the percentage of peak area) for both, [8]-gingerol (86%) and [10]-gingerol (84%) was achieved, while for the [6]-gingerol the purity was 99%. The purity degree obtained for each gingerol does not represent the real one, because HPLC system using UV detection does not guarantee the absence of other components in the sample. In a recent work, the isolation of [6]-, [8]- and [10]-gingerol was carried out using counter-current chromatography (CCC). In this study gingerols were obtained with high purity degree (99%), however the time of chromatographic run was very length (240 min) [19]. In another work regarding isolation of gingerols from crude extract of ginger rhizome using CCC the time of chromatographic run was again very extensive, and the purity was about 98% [20].

Electronic saturation is a factor that considerable limits the increasing of injected mass in the semi-preparative scale. In our work, this factor had limited injections of samples with higher masses, even at a wavelength of low absorption of gingerols (254 nm) (Fig. 3). However, the gingerols were isolated in significant quantities and with a good purity degree. To avoid the electronic saturation we could use either a less sensitive flow cell or make the adjustment of the wavelength well off the lambda max. We have not tried a less sensitive cell, however all the experiment conditions were tested in the lower absorption wavelength for the gingerols, and we still obtained electronic saturation. It may be possible to use other detectors even though we have only used UV.

The effects of [6]-gingerol on tumor cell proliferation are already well documented [5,21–23], however, the effects of [8]- and [10]-gingerol on tumor cells are still scarce. In fact, among all the ginger constituents, much more attention has been given to [6]-gingerol in order to explore its pharmacological properties and action mechanisms. In this sense, besides [6]-gingerol effects, those of [8]- and [10]-gingerol on *in vitro* tumor cell proliferation using MDA-MB-231 and HF cell lines were also investigated.

The overall results suggest that the higher the alkyl chain, the greater the effectiveness of gingerol in inhibiting tumor cell proliferation, probably due to the manner in which these substances enter the cells through their plasma membrane. In general, with increasing alkyl chain lengths, there is an increase in lipophilic character ($\log P$) of compounds, and this can be quantified by the partition coefficient (P) in an n-octanol/water system. It is also known that the higher the $\log P$ value, to a certain extent, the higher will be the permeation in biological membranes [24,25]. This can be extended to compounds such as gingerols, which represent a homologous series of substances.

Similar effects of gingerols and other ginger-derived substances were already described in the literature. Tjendraputra et al. [26] demonstrated that the inhibition of COX-2 by ginger constituents and synthetic analogs was structure-dependent. The lipophilicity and the functional group substitution on the alkyl side chain, as well as the aromatic moiety were the main aspects influencing COX-2 inhibition. Similar correlations between the side-chain length of gingerols and their activities of apoptosis induction and neuronal cells protection from β -amyloid insult have already been demonstrated [27,28].

The cytotoxic effect of [8]- and [10]-gingerol has been investigated by a few authors [27,29]. Kim and coworkers [29] have tested the effects of [8] and [10]-gingerol in a variety of human tumor cells lines including A-549 (lung cancer), SK-OV-3 (ovarian cancer), SK-MEL-2 (skin cancer) and HCT15 (colon cancer). They found an IC_{50} ranging from approximately 4.52 $\mu\text{g}/\text{mL}$ (13 μM) to 12.57 $\mu\text{g}/\text{mL}$ (40 μM), with [10]-gingerol being more effective than [8]-gingerol in decreasing the viability of these tumor cell lines [29]. Wei and coworkers [27] found an IC_{50} of $87.9 \pm 3.5 \mu\text{M}$ and $56.5 \pm 6.0 \mu\text{M}$ for [8]- and [10]-gingerol, respectively, in the inhibition of HL-60 proliferation, a human leukemia tumor cell line. These data are in accordance with the findings in this work on MDA-MB-231 cells, revealing the same activity dependence on the chain length size pattern.

Kuo and collaborators [30] working with SW480 cells, a lineage of colorectal cancer, observed that [10]-gingerol caused a significant concentration-dependent and sustained $[\text{Ca}^{2+}]_i$ rise, suggesting that this elevation can alter cellular functions, leading to cell death. They found a cytotoxic effect of [10]-gingerol 50, 75 and 100 μM [30]. It is important to notice that the methodology in the present study was different, even though, similar effects were found in MDA-MB-231 tumor cell line.

Our findings demonstrate a specificity of [8]- and [10]-gingerol on the inhibition of breast tumor cell proliferation when compared to non tumor human fibroblast cell line. The findings of other authors corroborate with our results. Kazi et al. [31] described the effects of antibiotics derived from β -lactam, lactam 1 and 12 on normal and tumor cells. They show that lactam 1 selectively induces apoptosis in human leukemic Jurkat T, but not in the non transformed, immortalized human natural killer cells. Furthermore, the authors demonstrated that lactam 12 induces apoptosis selectively in Jurkat T and simian virus 40-transformed, but not in non transformed NK and parental normal fibroblast cells. The authors propose that these N-thiolated β -lactams act by inducing DNA damage that leads to apoptosis preferentially in cancer and transformed over normal/non transformed cells. Another example includes pancratistatin, a natural compound isolated from the

spider lily, which is an efficient inducer of apoptosis in human lymphoma (Jurkat) cells, with minimal effect on normal nucleated blood cells. The authors discuss that this selective effect could be due to the expression of the Fas receptors, which are found to be greater in blood lymphoma cells than normal blood cells. Furthermore, in fast-dividing cancer cells, the plasma membrane tends to run short of lipids, perhaps changing the fluidity of the membrane [32]. Ovadje et al. [33] demonstrate that dandelion root extract is capable of inducing apoptosis at low concentrations specifically in cancer cells with no toxicity to peripheral blood mononuclear cells (PBMCs). Finally, Mathen et al. [34] working with extracts from *Calotropis gigantea*, the giant milkweed, traditionally used for the treatment of cancer and in Ayurvedic medicine and *Poeciloceris pictus*, an orthopteran insect, which feeds on *C. gigantea*, demonstrated that both the insect and the plant extracts induce concentration-dependent apoptosis and differentiate between human cancer cells and normal cells and exhibit species specificity.

The chemopreventive mechanisms of gingerols are not well understood however, are thought to involve proapoptotic [27,35–37], antioxidant [38], anti-inflammatory [6,39–41], and anti-angiogenic [42] activities. Further investigation will be done in order to determine the mechanisms by which [8]- and [10]-gingerol act inhibiting with more specificity the MDA-MB-231 cell proliferation.

5. Conclusion

In conclusion, this work presents a new methodology for the isolation of gingerols, which proved to be effective, once considerable amounts with a good purity degree in a relatively short time of chromatographic run were achieved from these important bioactive compounds, mainly [8]-gingerol (99%) and [10]-gingerol (97.3%). This is the first report on the separation of these compounds with such a purity degree using HPLC semi-preparative scale. Optimizations in the separation process regarding column overloading can still be made so that the electron saturation be not reached, which can be achieved by using other less sensitive detectors. The gingerols isolated through this methodology, demonstrate an anti-proliferative activity of [8]- and [10]-gingerol specifically on MDA-MB-231 breast cancer cells when compared to HF normal cells. As far as we know, this is the first report regarding a specific cell effect of ginger derivatives. The results also suggest the use of [8]- and [10]-gingerol as important models for anti-cancer therapy and drug design in an attempt to develop new compounds with fewer side effects when compared to conventional chemotherapy.

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