

Synergistic effect of curcumin, piperine and resveratrol in MCF-7 and MDA-MB-231 breast cancer cells.Betina Schmidt¹, Carlos Luan A Passos¹, Christian Ferreira¹, Jerson Lima da Silva², Eliane Fialho^{1*}¹Department of Basic and Experimental Nutrition, Josué de Castro Nutrition Institute, Federal University of Rio de Janeiro, Rio de Janeiro, RJ 21941-590, Brazil²Institute of Medical Biochemistry, Federal University of Rio de Janeiro, Rio de Janeiro, RJ 21941-590, Brazil**Abstract**

Breast cancer is the type of cancer that kills more women in the world and the conventional cancer treatment has high costs and many side effects. The bioactive compounds from diet alone or in combination with others substances may be a potential source for the prevention and treatment of breast cancer. The aim was to investigate the effect of curcumin, piperine and resveratrol alone and associated in MCF-7 and MDA-MB-231 breast cancer cells culture. Curcumin, piperine and resveratrol showed cytotoxicity for MCF-7 and MDA-MB-231 cells with IC₅₀ values of 24.50 μM, 94.50 μM and 131.00 μM, and 23.30 μM, 276.00 μM and 306.00 μM, respectively. The combination of curcumin+piperine, curcumin+resveratrol, piperine+resveratrol showed synergy in MCF-7 cells, and piperine+resveratrol in MDA-MB-231 cells. We conclude that the combination of bioactive compounds presents in diet as curcumin, piperine and resveratrol is better than used in isolated forms, and these results may be useful in future clinical trials with breast cancer.

Keywords: Breast cancer cells, Resveratrol, Piperine, Curcumin, Synergism.

Accepted on July 06, 2020

Introduction

Cancer is one of the leading causes of death worldwide. According to WHO in 2015, there were 8.8 million deaths and breast cancer contributed 571.000 deaths. Breast cancer is the type of cancer that kills more women in the world, particularly in low- and middle-income countries [1]. Current treatments for cancer include cytotoxic chemotherapy. However, some limitations are observed as high toxicity, in which it implies many adverse effects, but contributes to the survival of the patient [2].

Previous studies have demonstrated that bioactive compounds are a promising source to search for novel drugs for the treatment of various diseases. Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-hepta-diene-3,5-dione) is a lipophilic phenolic compound extracted from the rhizome of turmeric and possess various biological effects such antioxidant, anti-inflammatory, antimicrobial and anticancer [3-5]. Piperine {1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine} is an alkaloid found in the fruits of *Piper nigrum L.* and possess various biological effects such as antioxidant, anti-inflammatory and anticancer [6-9]. Resveratrol (3,4',5-trihydroxy-trans-stilbene) is a naturally occurring polyphenol found in several edible natural products, including grapes, berries, peanuts and in a number of herbal medicines [10]. Besides has been shown to possess antioxidant effect, anti-

inflammatory, anti-*Leishmania amazonensis* and as well chemoprevention of cancer [11-14]. The structures of these molecules are shown in the Figure 1.

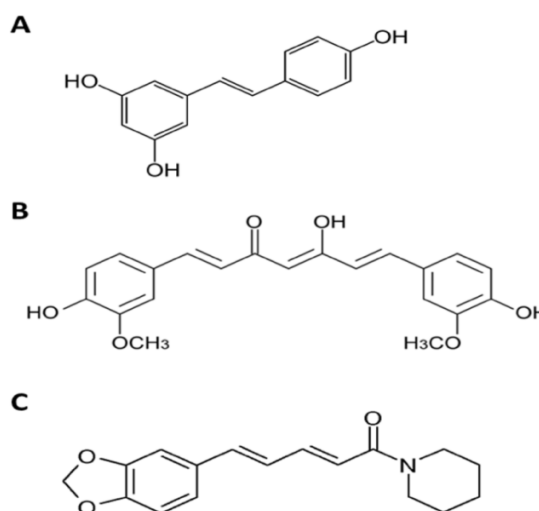


Figure 1. Chemical structure of resveratrol (A), curcumin (B) and piperine (C).

The bioactive compounds from diet alone or in combination with other substances may be a potential source for the prevention and treatment of breast cancer [15]. Rai et al. [16] demonstrated that resveratrol synergistically induced the chemotherapeutic potential of doxorubicin by *in vitro* and *in vivo* model of breast cancer. The combination of resveratrol, quercetin and catechin was able to reduce the primary tumor in the *in vivo* model of breast cancer [17]. Here, we report the synergistic activity of curcumin+piperine, curcumin+resveratrol and piperine+resveratrol on estrogen receptor-positive breast cancer cells (MCF-7) and triple negative breast cancer cells (MDA-MB-231).

Material and Methods

Reagents

Unless otherwise stated, all reagents were of analytical grade from Sigma-Aldrich (St. Louis, MO, EUA).

Cell culture

Monolayer cultures of human breast carcinoma MCF-7 and MDA-MB-231 cells were obtained from American Type Culture Collection (ATCC) and were grown in DMEM supplemented with 10% FBS, 100 units/mL of penicillin, 100 µg/mL of streptomycin and 5 µg/mL of insulin; the cells were kept at 37°C in a humidified atmosphere of % CO₂ in air. When the cultures reached 70%-80% confluence, the cells were treated with various concentrations of resveratrol, piperine and curcumin or both compounds in associations for 24 hours.

Cell viability assay

The cell viability assay was performed using MTT assay [13]. After treatment, the cells were washed with phosphate buffered saline (PBS) and then incubated for 3 h in 0.5 mL of MTT solution (0.5 mg/mL of PBS) at 37°C in 5% CO₂ in an incubator, and then 500 µL isopropanol was added to each well to dissolve the resulting formazan crystals. The absorbance was measured at a wavelength of 570 nm. The results are expressed in percentage of viable cells compared to untreated control.

Combination index (CI)

After cell viability analysis, determination of IC₅₀ was determined by the method described by Chou using the CalcSyn (Biosoft) software 2.0. For the analysis of the compounds used in association the combination index equation was used, where: (D)1 and (D)2 are the doses of two compounds used in combination and (Dx)1 and (Dx)2 are the doses of two compounds used alone. For the analysis of the combination, CI<1, CI=1 and CI>1 indicated, respectively, synergic, additive and antagonistic effects.

$$CI = ((D)1/(Dx)1) + ((D)2/(Dx)2)$$

Statistical analysis

IC₅₀ were calculated according to a nonlinear regression using a second-order polynomial equation, with 95% confidence intervals, using Graph Pad Prism 5.0 software. The results were expressed as the IC₅₀ standard error of the mean (SEM). The data were analysed by Student's t test when comparing two groups or by one-way analysis of variance (ANOVA) for more than two groups. p values of 0.05 were considered significant.

Results

Curcumin, piperine or resveratrol treatments on cellular viability of MCF-7 and MDA-MB-231 cells

In the present study, we investigated the cytotoxicity in the presence of different concentrations of compounds (curcumin, piperine or resveratrol) on MCF-7 cells evaluated by MTT method. As shown in Figure 2A, resveratrol, curcumin and piperine presented a dose-dependent cytotoxic effect on MCF-7 cells. In order to evaluate the effect of the compounds on other breast cancer cell line, we performed the MTT viability assay on MDA-MB-231 cells. As shown in Figure 2B, resveratrol, curcumin and piperine also presented a dose-dependent cytotoxic effect on MDA-MB-231 cells; however curcumin showed greater inhibition (56.1%) from the concentration of 25 µM than resveratrol and piperine. Our results showed that curcumin, piperine or resveratrol reduced MCF-7 and MDA-MB-231 cells viability after 24 hours of treatment, with IC₅₀ values of 24.50 µM, 94.50 µM and 131.00 µM; and 23.30 µM, 276.00 µM and 306.00 µM, respectively (Table 1).

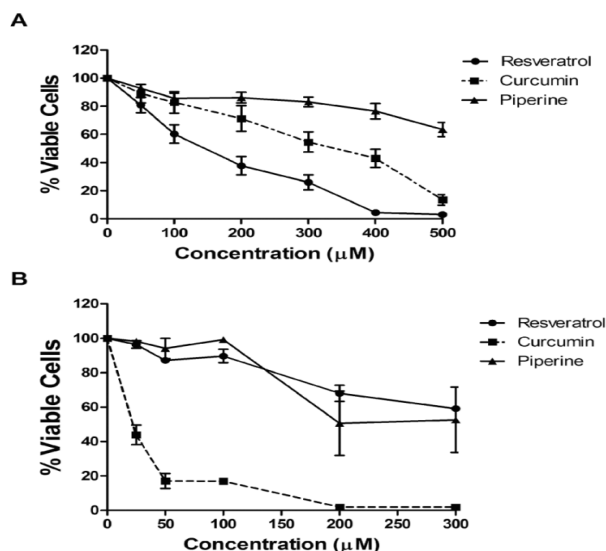


Figure 2. The effects of resveratrol, curcumin or piperine on the cell viability of MCF-7 and MDA-MB-231 cells. MCF-7 (A) and MDA-MB-231 (B) cells were treated for 24 hours with different concentrations of resveratrol, curcumin or piperine, and the cell viability was then determined by the MTT assay. The results are representative of three experiments performed in triplicate \pm SEM. * p <0.05, ** p <0.001, *** p <0.0001 in relation of control.

Table 1. Analysis of IC₅₀ values obtained after treatment with various concentrations of curcumin, piperine or resveratrol for 24 hours.

Bioactive compounds	IC ₅₀ MCF-7	IC ₅₀ MDA-MB-231
Curcumin	24.50 μM	23.30 μM
Piperine	94.50 μM	276.00 μM
Resveratrol	131.00 μM	306.00 μM

Effect of curcumin, piperine and resveratrol in association on cellular viability of MCF-7 and MDA-MB-231 cells

After observing the effect of isolated compounds and calculating their IC₅₀ values, the compounds were associated in pairs. For this, the concentrations used for curcumin in MCF-7 cells were 3.06, 6.12, 12.25, 18.37, 24.50 and 30.62 μM, with association of 5.91, 11.81, 23.62, 35.44, 47.25 and 59.06 μM of piperine or 8.19, 16.37, 32.75, 49.12, 65.50 and 81.87 μM of resveratrol. The association between the compounds reduced the cell viability in a dose-dependent manner (Figures 3A and 3B). In the same way, the association of 11.81, 23.62, 47.25, 70.87, 94.50 and 118.12 μM of piperine with 8.19, 16.37, 32.75, 49.12, 65.50 and 81.87 μM of resveratrol also reduced the cell viability in a dose-dependent manner (Figure 3C). The association of 5.8, 11.6, 17.5, 23.3 and 29.1 μM of curcumin with 69.0, 138.0, 207.0, 276.0 and 345.0 μM of piperine or 76.5, 153.0, 229.5, 306.0 and 382.5 μM of resveratrol reduced the cell viability of MDA-MB-231 cells in a dose-dependent manner (Figures 3D and 3E). Besides, the association of 69.0, 138.0, 207.0, 276.0 and 345.0 μM of piperine with 76.5, 153.0, 229.5, 306.0 and 382.5 μM of resveratrol reduced the cell viability in a dose-dependent manner (Figure 3F).

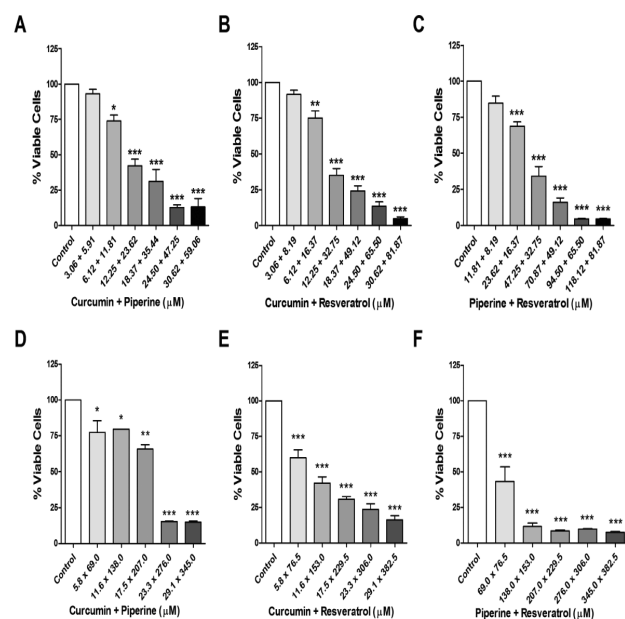


Figure 3. The effects of the association of bioactive compounds in breast cancer cells MCF-7 and MDA-MB-231. The cells were treated with different concentrations of curcumin plus piperine (A and D), curcumin plus resveratrol (B and E) and piperine plus resveratrol (C and F) for 24 hours, and the cell viability was determined by MTT assay. The percentage of viable cells was calculated as the ratio of treated cells to control cells. Data represent the mean ± SEM of three independent experiments. * p<0.05, ** p<0.01, *** p<0.0001, in relation of control.

Combination index of curcumin, piperine and resveratrol

An important strategy for the treatment of many types of cancer is a combination therapy. Therefore, we assessed whether compounds could be having an additive, synergistic or antagonistic effect one with other, using the CalcSyn (Biosoft) software. Thus, in MCF-7 cells the combination index values for curcumin+piperine, curcumin+resveratrol, piperine+resveratrol were 0.6666, 0.5862 and 0.4759, respectively, that indicates synergism (Table 2). On the other hand, in the MDA-MB-231 cells the combination index values for curcumin+piperine and curcumin+resveratrol were 4.2564 and 2.5907, respectively, which indicates antagonism. The combination index values for piperine+resveratrol were 0.2126 and indicate synergism; this result was 2.24-times lower than that of MCF-7 cells (Table 2).

Table 2. Combination index analysis of the associations of curcumin, piperine and resveratrol in MCF-7 and MDA-MB-231 cells.

Bioactive compounds	Cells	Combination index	Description
Curcumin+Piperine	MCF-7	0.6666	Synergism
Curcumin+Resveratrol	MCF-7	0.5862	Synergism
Piperine+Resveratrol	MCF-7	0.4759	Synergism
Curcumin+Piperine	MDA-MB-231	4.2564	Antagonism
Curcumin+Resveratrol	MDA-MB-231	2.5907	Antagonism
Piperine+Resveratrol	MDA-MB-231	0.2126	Synergism

Discussion

Natural products play an important role in the treatment of several pathologies, among them cancer [18]. The use of combination of natural products and chemotherapeutic agents, in reduced concentrations, is associated with a decrease in toxic effects and an improvement in treatment efficacy [19,20]. The inhibitory effect of curcumin, piperine or resveratrol treatments has been described on several types of tumour cells. Previously, we have reported that resveratrol in association with melphalan enhanced the cytotoxic effects on MCF-7 and MDA-MB-231 cells *in vitro*, with 1.67-time more pronounced

effect on MCF-7 cells [13]. Patel and colleagues [21] demonstrated that the combination of curcumin and citral, a lemon essential oil, induced death by apoptosis with activation of the p53 protein in MCF-7 and MDA-MB-231 cells. Bayet-Robert and colleagues [22] performed a clinical phase I dose escalation trial of combination docetaxel chemotherapy with curcumin in advanced and metastatic breast cancer patients, and showed that combination of curcumin plus docetaxel has antitumor activity and a superior response rate in comparison to monotherapy with docetaxel. The combination of piperine and paclitaxel produces synergistic effects in ovarian adenocarcinomas SKOV-3 cells, through loss of membrane integrity of mitochondria leading the cells to apoptosis [23].

We determine the IC₅₀ of curcumin, piperine and resveratrol for MCF-7 and MDA-MB-231 cells, which were 24.50 μM, 94.50 μM and 131.00 μM, and 23.3 μM, 276.00 μM and 306.00 μM, respectively. In the literature, there is IC₅₀s values of curcumin, established for SHI-1 monocytic leukemia cells and HT-29 colon cancer cells, which was of 14.13 μM and 50.0 μM, respectively [24,25]. Besides, for piperine the IC₅₀s values in melanoma cells were: 221.0 μM in SK-MEL-28 cells, 200.0 μM in B16-F0 cells, 225.0 μM in A375 cells and 250.0 μM in ASPC-1 cells [26]. Likewise for resveratrol, the IC₅₀s values were 120.0 μM and 370.0 μM for MCF-7 and MDA-MB-231 breast cancer cells, respectively, and 127.0 μM in MGC803 gastric cancer cells [13,27].

Drug association therapy has several important advantages as reduced dosages and treatment duration, lower treatment costs and decrease drug resistance development, besides the associations of natural products in cancer are fundamental, because they act in inhibition of cell growth, viability, invasion, migration and autophagy, among others [19]. Our data showed for the first time that the combination of curcumin + piperine, curcumin resveratrol and piperine + resveratrol has a synergistic effect against MCF-7 cells, and piperine + resveratrol has a synergistic effect against MDA-MB-231 breast cancer cells. Shoba and colleagues [28], described that piperine increases the serum concentration and the oral bioavailability of curcumin in rats and humans. The curcumin has also been shown to act synergistically with piperine in the suppression of hepatocellular carcinoma in rats, and this effect might be owing to increased bioavailability of curcumin in the presence of piperine [29]. Recently, the combination of resveratrol and curcumin has been shown to induce apoptosis in MCF-10A-Tr cells (cigarette smoke condensate-transformed breast epithelial cell line) through p21Waf1/Cip1 mediated inhibition of the Hedgehog-Gli cascade [30,31].

Conclusion

In conclusion, we can affirm that the combination of bioactive compounds present in diet as curcumin, piperine and resveratrol, may be useful as possible application in future clinical trials with breast cancer.

Acknowledgements

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico, <http://www.cnpq.br>; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, <http://www.capes.gov.br>; Fundação do Câncer, [HTTP://www.cancer.org.br](http://www.cancer.org.br), and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro, <http://www.faperj.br>. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

B.S., C.L.A.P., C.F., J.L.S. and E.F. designed and analyzed all experiments, B.S., C.L.A.P., and C.F. performed experiments. All authors reviewed the manuscript.

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*Correspondence to

Eliane Fialho

Department of Basic and Experimental Nutrition

Federal University of Rio de Janeiro

Rio de Janeiro

Brazil