Prev. Nutr. Food Sci. 2022;27(4):436-447
https://doi.org/10.3746/pnf.2022.27.4.436
ISSN 2287-8602

Inhibition of Cell Proliferation and Induction of Cell Cycle Arrest in Colon Cancer Cells by Lyophilized Mango (*Mangifera indica* L.) Pulp Extract

Gustavo Argenor Lozano-Casabianca[1], Sandra Sulay Arango-Varela[2], Johanny Aguillón-Osma[3], María Alejandra Llano-Ramírez[2], and María Elena Maldonado-Celis[1]

[1]School of Nutrition and Dietetics, University of Antioquia, Medellín 050010, Colombia
[2]Faculty of Exact and Applied Sciences, Instituto Tecnológico Metropolitano (ITM)-Institución Universitaria, Medellín 050034, Colombia
[3]Faculty of Science of Education, The University of Quindío, Armenia 630004, Colombia

ABSTRACT: The present study evaluated the antiproliferative capacity and possible cell death mechanisms of lyophilized mango pulp extract (LMPE), applied to human colon cancer cells (SW480) and their metastasis-derived counterparts (SW620). The total phenolic content of LMPE was estimated by the Folin-Ciocalteu method. Three assays were employed to determine its antioxidant capacity: ferric-reducing antioxidant power, oxygen radical absorbance capacity, and 2,2-diphenyl-1-picrylhydrazyl. Furthermore, the antiproliferative activity of LMPE was assessed by sulforhodamine B, clonogenic, and Ki-67 assays. Flow cytometry was employed to examine the cell cycle, production of intracellular reactive oxygen species (ROS), cell-surface phosphatidylserine, and change in mitochondrial membrane potential. LMPE exhibited a high level of total phenolic content and antioxidant activity. The mean maximal inhibitory concentration values of LMPE at 48 h of exposure were 43 and 29 mg/mL for SW480 and SW620, respectively. In the SW480 and SW620 cell lines, LMPE at 50 mg/mL and 48 h of exposure induced an increase in intracellular ROS, cell cycle arrest in the G2/M phase, and probably, apoptotic processes without mitochondrial depolarization. LMPE had an antiproliferative capacity against the human colorectal cancer cell lines SW480 and SW620. These results highlight the chemopreventive potential of LMPE in colorectal cancer treatments.

Keywords: apoptosis, cell cycle, colon cancer, *Mangifera indica* L.

INTRODUCTION

Although there have been advances in the prevention and treatment of colorectal cancer (CRC), its incidence and mortality rates remain significantly high. In 2020, the GLOBOCAN database estimated more than 1.9 million new cases of CRC and 935,000 mortalities worldwide (Sung et al., 2021). Some risk factors for CRC are age, genotype, body height, and family history related to cancer (Wong et al., 2019). In addition, some modifiable risk factors for the disease are obesity, sedentary lifestyle, and frequent consumption of processed meats (Pointet and Taieb, 2017). Also, according to the World Cancer Research Fund Network and the American Institute for Cancer Research (2018), there is limited evidence of a relationship between low fruit ingest and increased risk of CRC.

There are several treatments according to the stage of CRC and the condition of the patient, including surgery, radiotherapy, and medication on the diagnosis. However, medication can have unfavorable effects such as toxicity related to adverse reactions, insufficient specificity for tumor cells, and the development of drug resistance. Therefore, it affects normal cells in the blood, mucous membranes (digestive tract, oral cavity, bladder, etc.), and hair follicles. These side effects impact the quality of life of the patient (Costea et al., 2018). The diagnosis of CRC usually occurs at advanced stages, and the treatment fails to be effective due to aggressive disease progression (Saif and Chu, 2010). Therefore, the exploration of new chemopreventive agents that eliminate malignant cells without affecting healthy tissues is a potentially beneficial and

Received 2 August 2022; Revised 14 September 2022; Accepted 16 September 2022; Published online 31 December 2022

Correspondence to Gustavo Argenor Lozano-Casabianca, E-mail: gustavo.lozano@udea.edu.co
Author information: Gustavo Argenor Lozano-Casabianca (Professor), Sandra Sulay Arango-Varela (Professor), Johanny Aguillón-Osma (Professor), María Alejandra Llano-Ramírez (Graduate Student), María Elena Maldonado-Celis (Professor)

complementary strategy to control its development and progression (Martín et al., 2016).

Several studies have shown that the mango fruit (*Mangifera indica* L.) contains bioactive compounds with potential chemopreventive capacity against CRC. Noratto et al. (2010) found that polyphenolic extracts of the Ataulfo mango variety had cytotoxic activity in SW480 cell line. Velderrain-Rodríguez et al. (2018) employed an *in vitro* model and demonstrated that phenolic compounds in the peel of the Ataulfo mango have potential antiproliferative capacity in the colon cancer cell line LS180. Similarly, Corrales-Bernal et al. (2014b) established that an aqueous extract of mango pulp had chemopreventive action in the SW480 cells and decreased the formation of azoxymethane-induced aberrant crypt foci (ACF) of CRC in a murine animal model. Furthermore, Boateng et al. (2007) showed in Fischer 344 rats that intake of mango juice at 20% for 9 weeks reduced the frequency of azoxymethane-induced ACF by 83%.

Mango pulp can be consumed fresh but can be preserved using several dehydration techniques, such as hot air, microwaving, and lyophilization. Izli et al. (2017) displayed that lyophilization protected the color, phenolic content, and antioxidant capacity of mango pulp. Furthermore, some studies have indicated the chemopreventive potential of berry (Stoner et al., 2007), açaí (Fragoso et al., 2018), and black raspberries (Harris et al., 2001) lyophilizates against CRC.

The colon cancer cell line (SW480) and its metastasis-derived counterpart (SW620) constitute unique models to investigate the stages of CRC progression (Hewitt et al., 2000). The present study evaluated the antiproliferative capacity and possible cell death mechanisms of lyophilized mango pulp extract (LMPE) applied to adenocarcinoma cells (SW480) and their metastasis-derived counterparts (SW620).

MATERIALS AND METHODS

Vegetal material

Commercial lyophilized pulp of mango (cv. Yulima) was procured from Grupo Fontus S.AS (Bogotá, Colombia).

Preparation of the LMPE

The commercial lyophilizate was dissolved in sterile distilled water for 24 h at 37°C with continuous shaking at 180 rpm. Later, the mixture was filtered using sterile gauze. The extract obtained was lyophilized again in a vacuum chamber at −50°C and pressure of 0.427±0.5 mm Hg. The LMPE was stored at −20°C and protected from light until further use.

Chemical characterization

The LMPE was reconstituted in distilled water at 1 mg/mL for subsequent chemical characterization.

***Determination of total phenolic content*:** The Folin-Ciocalteu method was used to determine the total phenolic content in the LMPE (Muñoz-Bernal et al., 2017). The results were expressed as mg gallic acid equivalent (GAE) per gram of LMPE (mg GAE/g LMPE).

***Determination of total flavonoid content*:** Total flavonoid content in the LMPE was determined by reaction with aluminum chloride in a colorimetric assay. Catechin was used as the standard, and the results were expressed in mg catechin equivalent (CAE) per gram of LMPE (mg CAE/g LMPE) (Armentano et al., 2015).

***Determination of total tannin content*:** Total tannins content in the LMPE was determined by oxidation reaction with ferric chloride. The results were expressed in mg tannic acid equivalent (TAE) per gram of LMPE (mg TAE/g LMPE) (Armentano et al., 2015).

***Determination of total polysaccharide content*:** The total polysaccharide content in the LMPE was established by the phenol-sulfuric acid method (López-Legarda et al., 2017). The results were expressed as mg glucose equivalent (GE) per gram of LMPE (mg GE/g LMPE).

Antioxidant activity analysis

***Determination of ferric-reducing antioxidant power (FRAP)*:** This assay was performed according to the protocol described by Benzie and Strain (1996). The results were expressed as micromole Trolox equivalent (TE) per gram of LMPE (μmol TE/g LMPE).

***Determination of oxygen radical absorbance capacity (ORAC)*:** The methodology reported by Ou et al. (2001) was followed with some modifications (Zapata et al., 2014). The results were expressed as μmol TE/g LMPE.

***Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH)*:** This determination was performed according to the protocol described by Garzón et al. (2012). The results were expressed as μmol TE/g LMPE.

Cell culture and treatments

SW480 and SW620 cell lines were obtained from the European Collection of Authenticated Cell Cultures (Salisbury, UK). The skin fibroblasts (nonmalignant cells) from discarded biopsy samples were donated by the University of Antioquia, Colombia. Briefly, cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 25 mM glucose, 2 mM L-glutamine, 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 1% nonessential amino acids (Invitrogen, Carlsbad, CA, USA). The cells were incubated at 37°C in a humidified atmosphere with 5% CO_2.

Further, 0, 10, 20, 25, 30, 40, and 50 mg LMPE/mL solutions were prepared in DMEM supplemented with

25 mM glucose, 2 mM L-glutamine, 3% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, 1% nonessential amino acids, 10 μg/mL insulin, 5 μg/mL transferrin, and 5 ng/mL selenium for the experiments. The mixture was sonicated for 20 min at 42 kHz and later centrifuged at 5,000 rpm for 20 min. The supernatant was collected in a fresh tube by filtering through a 0.2 μm membrane. Finally, the solutions were stored in the dark at −20°C until further use. The cells were treated with LMPE at different concentrations, and the untreated cells (0 mg LMPE/mL) were used as control.

Cytotoxic activity assay

Briefly, 20,000 cells per well were seeded and incubated for 24 h. Later, the cells were treated for 48 h with different concentrations (10, 20, 30, 40, and 50 mg/mL) of LMPE and maintained at 37°C and 5% CO_2. After treatment, cytotoxic activity was assessed with sulforhodamine B (SRB) according to the method described by Vichai and Kirtikara (2006). The level of inhibition of cell viability was calculated as follows:

$$\text{Viability inhibition} = 1 - \frac{A_t}{A_c} \times 100$$

where A_t is the absorbance of the treated cells, and A_c is the absorbance of the control cells (untreated, 0 mg LMPE/mL).

In addition, a linear regression was used to calculate the mean maximal inhibitory concentration (IC_{50}). The selectivity index (SI) was calculated from the IC_{50} ratio in nonmalignant versus cancer cells using the following equation:

$$SI = \frac{IC_{50}\ \text{Skin fibroblasts (nonmalignant cells)}}{IC_{50}\ \text{Cancer cells}}$$

SI indicates the selectivity of the LMPE to the tested cells. An SI >1 is more cytotoxic to tumor cells than to nonmalignant cells, whereas an SI <1, is less cytotoxic to tumor cells compared to nonmalignant cells.

Antiproliferative assays

To evaluate the antiproliferative effect of the LMPE on SW480 and SW620 cell lines, three assays were performed, i.e., SRB; clonogenic; and Ki-67.

***Colorimetric assay with SRB*:** This assay was performed according to the protocol described by Gossé et al. (2005). Briefly, 2,500 cells/well were seeded and incubated at 37°C in a 5% CO_2 environment. After 24 h of establishment, the cells were treated with different concentrations (10, 20, 30, 40, and 50 mg/mL) of LMPE, and the cultures were incubated for different periods (0, 24, 48, and 72 h). Later, the cell culture was stopped by adding 50% (v/v) trichloroacetic acid, and cellular proteins were determined by staining with 0.4% SRB (w/v).

***Clonogenic assay*:** It was performed according to the method described by Franken et al. (2006). Briefly, 200 cells/well were seeded and incubated at 37°C in a 5% CO_2 environment. After 24 h of establishment, the cells were treated for 48 h with 25 and 50 mg/mL concentrations of LMPE. After treatment time, the cells were incubated for 7 days at 37°C in a 5% CO_2 environment. Cells were then fixed with Carnoy's solution (ethanol-acetic acid ratio 3:1) and stained with crystal violet (0.5% w/v). The number of colonies formed was counted using colonies of 50 or more cells as inclusion criteria. The results were presented as absolute cloning efficiency (ACE) and relative cloning efficiency (RCE) and were determined using the following equations:

$$\text{ACE} = \frac{\text{Number of colonies}}{\text{Number of inoculated cells}} \times 100$$

$$\text{RCE} = \frac{\text{ACE of each treatment}}{\text{ACE of the control}} \times 100$$

***Ki-67 assay*:** In this assay, 20,000 cells were seeded and incubated for 24 h. Later, the cells were treated for 48 h with different concentrations (10, 30, and 50 mg/mL) of LMPE, and maintained at 37°C in a 5% CO_2 environment. Ki-67 concentrations in the cell culture supernatant were determined after the treatment using a quantitative enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems Inc., Minneapolis, MN, USA) following the manufacturer's instructions. The absorbance of each well was determined at 450 nm in the microplate reader. The concentration of human Ki-67 in the samples was calculated by comparing the absorbance of the samples with a standard curve.

Assays to evaluate cell death mechanisms

Four assays were performed to explore the possible cell death mechanisms in cell lines as a consequence of the treatment with the LMPE: cell cycle analysis, detection of cell-surface phosphatidylserine (PS), change in mitochondrial membrane potential ($\Delta\psi m$), and intracellular ROS production.

***Cell cycle analysis*:** This assay was performed according to the method proposed by Nicoletti et al. (1991). Briefly, 1×10^6 cells per culture dish were maintained at 37°C with 5% CO_2. After incubation for 24 h, the cells were treated for 48 h with the LMPE at 50 mg/mL. The cells were harvested using trypsin. They were fixed with 96% ethanol (4°C) and then washed and resuspended in phosphate-buffered saline (PBS). Later, 1 μL of propidium iodide (PI, Thermo Fisher Scientific, Waltham, MA, USA) was added per 200 μL of cells resuspended in PBS, and

RNAse was added at a concentration of 250 μg/mL. The culture was maintained for 30 min at 37°C in the dark. The data of at least 10,000 events per sample were collected by flow cytometry and analyzed using the FlowJo™ Software (version 7.6.2, Becton, Dickinson and Company, Ashland, OR, USA).

***Detection of cell-surface PS*:** PS present on the membrane surface of the cell was detected by its binding to annexin V-fluorescein isothiocyanate (FITC) (Demchenko, 2013). Simultaneous PI staining was applied to determine plasma membrane integrity. Briefly, cells (1×10^6) were seeded and treated as described for cell cycle analysis. The cells were harvested using trypsin. Later, cells were washed, resuspended in PBS, and incubated with annexin V-FITC (Becton, Dickinson and Company) and PI (Thermo Fisher Scientific) for 15 min at 37°C in the dark. 1.5 μL of annexin V-FITC was added for each 100 μL of resuspended cell solution in PBS. The cultures were incubated for 10 min at room temperature (RT) (20±1°C) in the dark. Subsequently, 2 mL of 1× binding buffer was added, and the mixture was centrifuged at 400 *g* for 5 min at RT. The supernatant was discarded, and the cells were resuspended in 200 μL of 1× binding buffer and 1 μL of PI. The data of at least 10,000 events per sample were collected by flow cytometry and analyzed using FlowJo™ Software (Becton, Dickinson and Company).

***Change in the mitochondrial membrane potential*:** The dye 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6$) was used to determine changes in the mitochondrial membrane potential ($\Delta\psi m$) (Maldonado-Celis et al., 2009). Simultaneous PI staining was applied to determine plasma membrane integrity. Briefly, cells (1×10^6) were seeded and treated as described for cell cycle analysis. The cells were harvested using trypsin and later washed, and resuspended in PBS. Later, the cells were incubated with $DiOC_6$ (Thermo Fisher Scientific) at a final concentration of 700 nM and PI (1 μL per 200 μL of cells resuspended in PBS) at 37°C for 30 min. The data of at least 10,000 events per sample were collected by flow cytometry and analyzed using FlowJo™ Software (Becton, Dickinson and Company).

***Intracellular ROS production*:** The intracellular ROS production was determined according to the method proposed by Maldonado-Celis et al. (2009). Briefly, 1×10^6 cells per culture dish were maintained at 37°C with 5% CO_2. After incubation for 24 h, the cells were treated for 48 h with different concentrations (10, 30, and 50 mg/mL) of LMPE. The cells were harvested using trypsin and resuspended in PBS containing 5 μM of 5 (and 6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (mixed isomers; Invitrogen). The cells were incubated for 30 min at RT in the dark. Fluorescence was detected by flow cytometry with an FL-1 filter (530 nm). The data of at least 10,000 events per sample were collected by flow cytometry and analyzed using FlowJo™ Software.

Statistical analysis

To determine the cytotoxic and antiproliferative effect of the LMPE using SRB staining, comparative parametric statistical tests were performed. The differences between the means of the treatments and the control group were analyzed by Dunnett's post-hoc test. The nonparametric Kruskal-Wallis test, using Dunn's post-hoc test was employed to analyze the clonogenic assay and the Ki-67 assay. Student's *t*-tests were conducted to compare cell groups in terms of the cell cycle, cell-surface PS, mitochondrial membrane potential change ($\Delta\psi m$), and intracellular ROS production. In all cases, a $P<0.05$ was considered statistically significant. GraphPad Prism 8.0 statistical software (GraphPad Software, San Diego, CA, USA) was used for the data analysis. All the assays were conducted at least in triplicate.

RESULTS

Chemical characterization and antioxidant activity of LMPE

Table 1 shows the total content of phenolic compounds, flavonoids, tannins, and polysaccharides present in the LMPE, and its antioxidant capacity measured in terms of FRAP, ORAC, and DPPH.

Cytotoxicity effect

The cytotoxic effect of the LMPE was evaluated by employing a colorimetric assay with SRB, as previously described. Fig. 1A shows that the treatment has a cytotoxic effect in SW480 cells from 10 mg LMPE/mL ($P<0.001$) with a directly proportional relationship between LMPE concentration and viability inhibition. Fig. 1B shows that the viability inhibition in SW620 cells begins at 10 mg LMPE/mL ($P<0.001$) and increases evidently at 30 mg LMPE/mL and above. In skin fibroblasts (nonmalignant

Table 1. Chemical characterization and antioxidant activity of LMPE

Parameter	
Total phenolic compounds (mg GAE/g LMPE)	69.3±15.6
Total flavonoids (mg CAE/g LMPE)	8.7±2.3
Total tannins (mg TAE/g LMPE)	21.7±1.7
Total polysaccharides (mg GE/g LMPE)	633.1±66.7
FRAP (μmol TE/g LMPE)	627.9±17.3
ORAC (μmol TE/g LMPE)	866.1±3.5
DPPH (μmol TE/g LMPE)	576.2±65.9

Data are presented as mean±SD (n=3).
LMPE, lyophilized mango pulp extract; GAE, gallic acid equivalents; CAE, catechin equivalents; TAE, tannic acid equivalents; GE, glucose equivalents; FRAP, ferric reducing antioxidant power; TE, Trolox equivalent; ORAC, oxygen radical absorbance capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

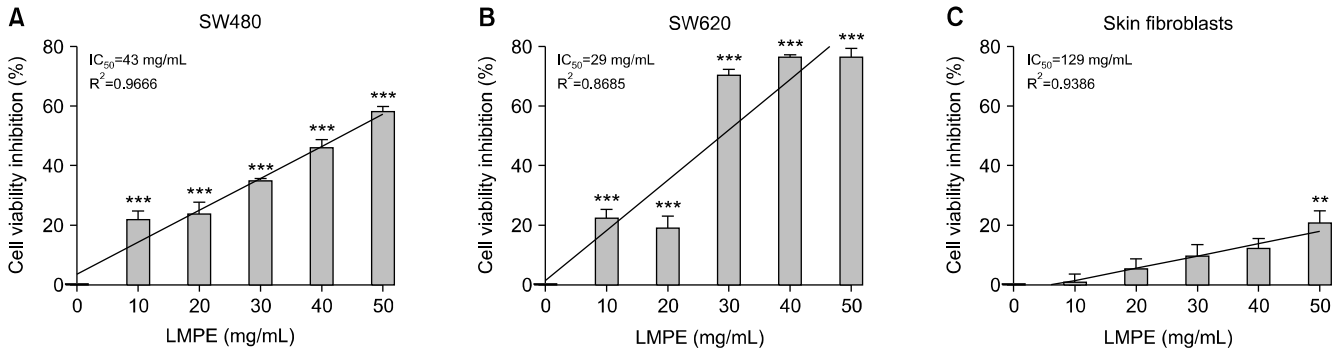

Fig. 1. Cytotoxicity of the lyophilized mango pulp extract (LMPE) in SW480 (A), SW620 (B), and skin fibroblasts (nonmalignant) (C) cells. Cells were exposed to different concentrations of the treatment (10, 20, 30, 40, and 50 mg LMPE/mL) for 48 h. Untreated cells (0 mg LMPE/mL) were used as control. Data are presented as mean±SEM (n=5). ANOVA test was conducted to compare cell groups. The differences between the means of the treatments and the control group were analyzed by Dunnett's post-hoc test. Significant differences between control and treated cells at $^{**}P<0.01$ and $^{***}P<0.001$.

cells), the inhibition increases with extract concentration, however, statistical significance was achieved at 50 mg LMPE/mL ($P<0.01$) (Fig. 1C). The IC_{50} values of the LMPE at 48 h of exposure for SW480, SW620, and skin fibroblasts were 43, 29, and 129 mg/mL, respectively. Additionally, the SI of the LMPE in the SW480 and SW620 cell lines was 3 and 4.5, respectively. This indicates that LMPE was cytotoxic and selective for SW480 and SW620 cell lines regarding nonmalignant skin fibroblast cells.

Antiproliferative effect

***Colorimetric assay with SRB*:** Fig. 2 shows the inhibition of cell proliferation in both cell lines increased in a directly proportional manner as a function of extract concentration and exposure time. An increase in the cell viability inhibition (compared to the control) was observed in SW480 cells treated for 24 h starting at 20 mg LMPE/mL ($P<0.001$); and, after 48 and 72 h, it was found from 10 mg LMPE/mL ($P<0.001$). Similarly, an increase in the cell viability inhibition was observed at 30 mg LMPE/mL ($P<0.05$) in SW620 cells treated for 24 h; and after 48 and 72 h, it increased from 20 and 10 mg LMPE/mL ($P<0.001$), respectively.

***Clonogenic assay*:** The capacity of SW480 and SW620 cell lines to form colonies after treatment was evaluated. Table 2 shows the total inhibition of colony-forming ability ($P<0.05$) in SW480 and SW620 cells at concentrations of 25 and 50 mg LMPE/mL at 48 h of exposure.

***Ki-67 assay*:** The Ki-67 expression was measured in the cell culture supernate using a quantitative ELISA Kit. Fig. 3A shows the downregulation of Ki-67 expression in the SW480 line treated for 48 h with 30 and 50 mg LMPE/mL (compared to control cells). In SW620 cells, the expression of Ki-67 decreased with the exposure to the LMPE for 48 h, however, it did not reach significant differences (Fig. 3B). These results indicate that the LMPE

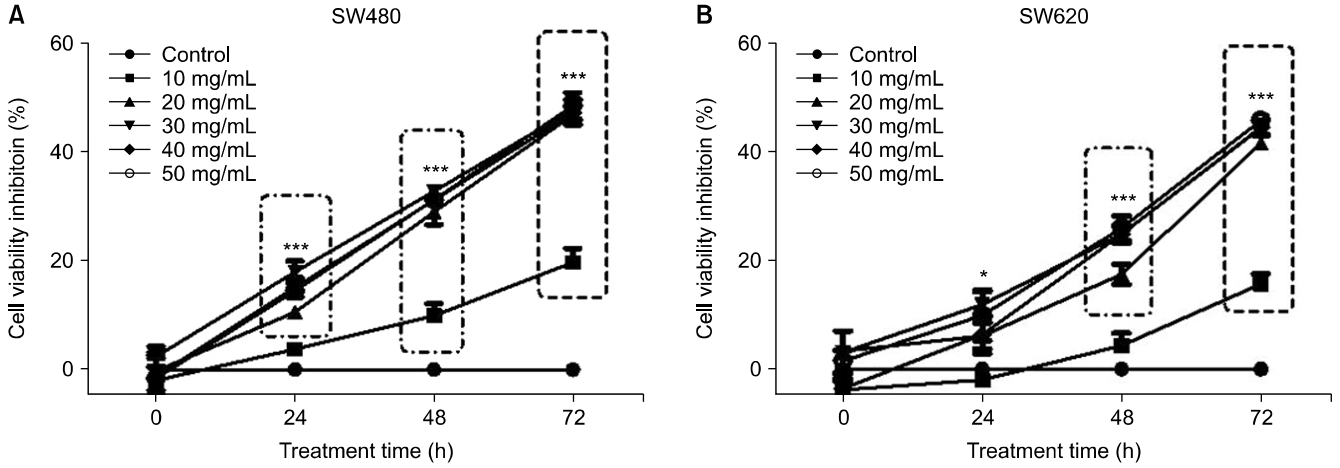

Fig. 2. Effect of the lyophilized mango pulp extract (LMPE) on cell proliferation in SW480 (A) and SW620 (B) cells. Cells were exposed to 10, 20, 30, 40, and 50 mg LMPE/mL for 0, 24, 48, and 72 h. Untreated cells (0 mg LMPE/mL) were used as control. Data are presented as mean±SEM (n=5). ANOVA test was conducted to compare groups. The differences between the means of the treatments and the control group were analyzed by Dunnett's post-hoc test. Significant differences between control and treated cells at $^{*}P<0.05$ and $^{***}P<0.001$. Rectangles cluster several concentrations of LMPE with the same significant difference from the control.

Table 2. Effect of LMPE on the colony-forming ability of SW480 and SW620 cells (unit: %)

Concentration (mg LMPE/mL)	SW480		SW620	
	ACE	RCE	ACE	RCE
0 (control)	20.5 (14.5~26.0)	100.0	8 (5.5~9.5)	100.0
25	0.0 (0.0~0.0)*	0.0	0 (0.0~0.0)*	0.0
50	0.0 (0.0~0.0)*	0.0	0 (0.0~0.0)*	0.0

Cells were exposed to 25 and 50 mg LMPE/mL for 48 h. The Kruskal-Wallis test was performed to compare groups and the differences between the medians of the treatments and the control group were analyzed by Dunn's post-hoc test.
Data are presented as median (interquartile range) (n=3).
Significant differences between control and treated cells at $^{*}P<0.05$.
LMPE, lyophilized mango pulp extract; ACE, absolute cloning efficiency; RCE, relative cloning efficiency.

has an antiproliferative effect on both cell lines.

Cell death mechanisms

***Cell cycle analysis*:** After the treatment with 50 mg LMPE/mL for 48 h, the cell cycle phases of SW480 and SW620 cells were examined (Fig. 4A). Compared to the controls, the percentage of cells in the G2/M phase increased 3.8-fold ($P<0.05$) in the SW480 line (Fig. 4B). Similarly, Fig. 4C shows a 2.7-fold ($P<0.01$) increase in the SW620 line. The LMPE induced cell cycle arrest in the G2/M phase in both lines. In addition, the percentage of cells in the G0/G1 phase decreased in both cell lines, indicating an increase in the number of dead or dying cells.

***Detection of cell-surface PS*:** Apoptosis is related to the localization of PS in the outer monolayer of the cell membrane. After 48 h of treatment with 50 mg LMPE/mL, PS was identified here using annexin V-FITC. Simultaneous PI staining was applied to determine plasma membrane integrity. Fig. 5A depicts the fluorescence intensity of these two fluorochromes in SW480 and SW620 cells. Fig. 5B and 5C display the quantification of fluorescence intensity in SW480 cells and SW620 cells, correspondingly. The combined annexin V-FITC/PI staining enables the identification of necrotic cells (Q1), late apoptotic cells (Q2), early apoptotic cells (Q3), and viable cells (Q4). Treated SW480 and SW620 cells displayed a 39% and 37% increase, respectively, in the Q3 quadrant compared to controls ($P<0.001$). This quadrant contains cells with a greater presence of PS on their surface but without damage to their plasma membrane, i.e., cells in early apoptosis. These results suggest that their cell death mechanism may be associated with apoptosis.

***Change in the mitochondrial membrane potential ($\Delta\psi m$)*:** Flow cytometry was implemented with the fluorescent probe $DiOC_6$ to evaluate the effect of the treatment with 50 mg LMPE/mL for 48 h on mitochondrial membrane potential ($\Delta\psi m$). The decrease in the dye in mitochondria is related to a reduction in $\Delta\psi m$ (Kataoka et al., 2005). Simultaneous PI staining was applied to determine plasma membrane integrity. Fig. 6A shows the fluorescence intensity of these two fluorochromes in SW480 and SW620 cell lines. Fig. 6B and 6C show the quantification of the fluorescence intensity in SW480 and SW620 cell lines, correspondingly. The combined $DiOC_6$/PI staining enables to identify dead cells with mitochondrial depolarization (Q1), dead cells with mitochondrial polarization (Q2), cells with membrane integrity and mitochondrial polarization (Q3), and cells with membrane integrity and mitochondrial depolarization (Q4). The treatment reduced the percentage of SW480 and SW620 cells located in the Q3 quadrant by 12.7% ($P<0.01$) and 14% ($P<0.05$), respectively, compared to the controls. In the Q2 quadrant, the percentage of SW480 cells increased by 12% ($P<0.001$), and that of SW620, by 14% ($P<0.01$). These re-

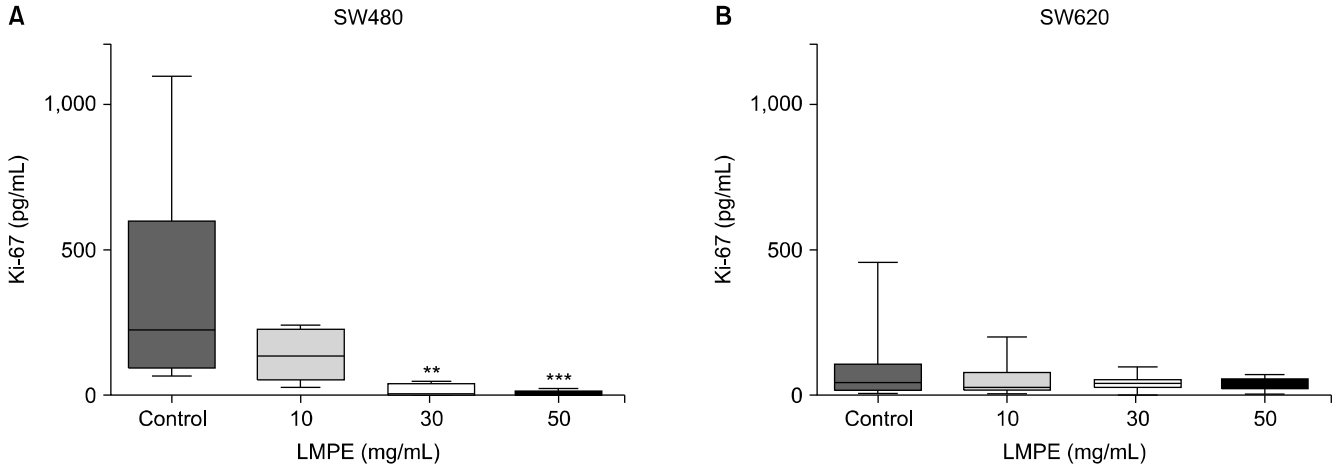

Fig. 3. Effect of the lyophilized mango pulp extract (LMPE) on Ki-67 expression in SW480 (A) and SW620 (B) cells. Cells were exposed to different concentrations of the treatment (10, 30, and 50 mg LMPE/mL) for 48 h. Untreated cells (0 mg LMPE/mL) were used as control. Data are presented as median (interquartile range) (n=5). The Kruskal–Wallis test was performed to compare groups. The differences between the medians of the treatments and the control group were analyzed by Dunn's post-hoc test. Significant differences between control and treated cells at $^{**}P<0.01$ and $^{***}P<0.001$.

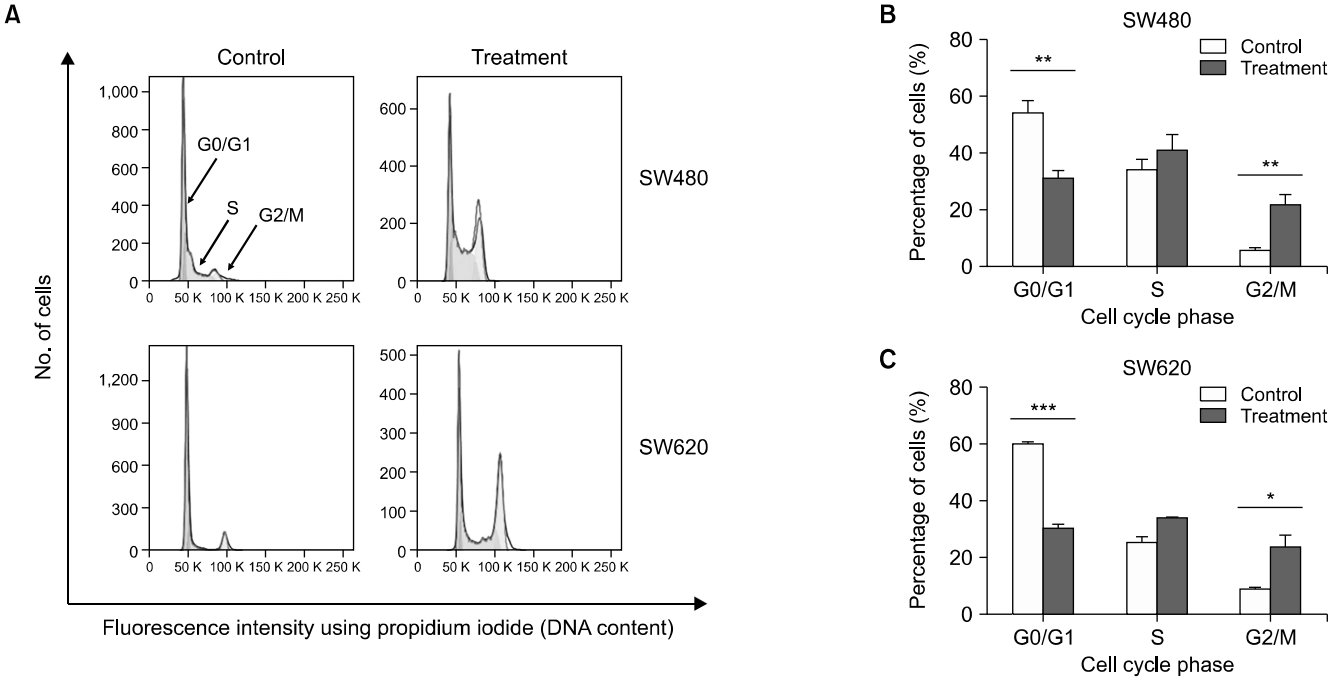

Fig. 4. (A) Effect of the lyophilized mango pulp extract (LMPE) on the cell cycle of SW480 and SW620 cell lines. Representative histograms of fluorescence intensity (which indicates the cell cycle phase) were created using flow cytometry. Cell percentages as classified by cycle phase in SW480 (B) and SW620 (C). Cells were exposed to 50 mg LMPE/mL for 48 h. Untreated cells (0 mg LMPE/mL) were used as control. Data are presented as mean±SEM (n=3). Significant differences between control and treated cells at $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ by Student's *t*-test.

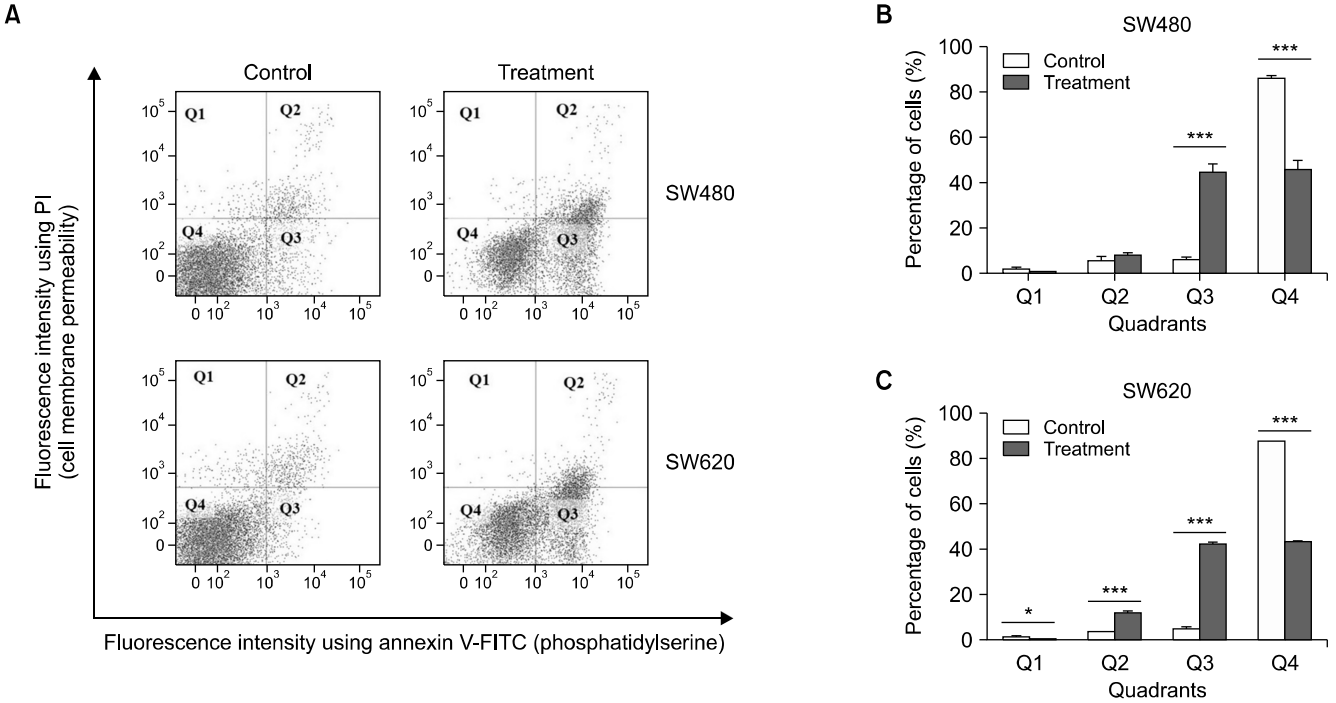

Fig. 5. (A) Representative dot charts annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining (detected by flow cytometry) in SW480 and SW620 cells. Quantification of cells classified by quadrants in SW480 (B) and SW620 (C). Cells were exposed to 50 mg lyophilized mango pulp extract (LMPE)/mL for 48 h. Untreated cells (0 mg LMPE/mL) were used as control. Four quadrants represent necrotic cells (Q1), late apoptotic cells (Q2), early apoptotic cells (Q3), and viable cells (Q4). Data are presented as mean±SEM (n=3). Significant differences between the control and treated cells quadrants at $^{*}P<0.05$ and $^{***}P<0.001$ by Student's *t*-test.

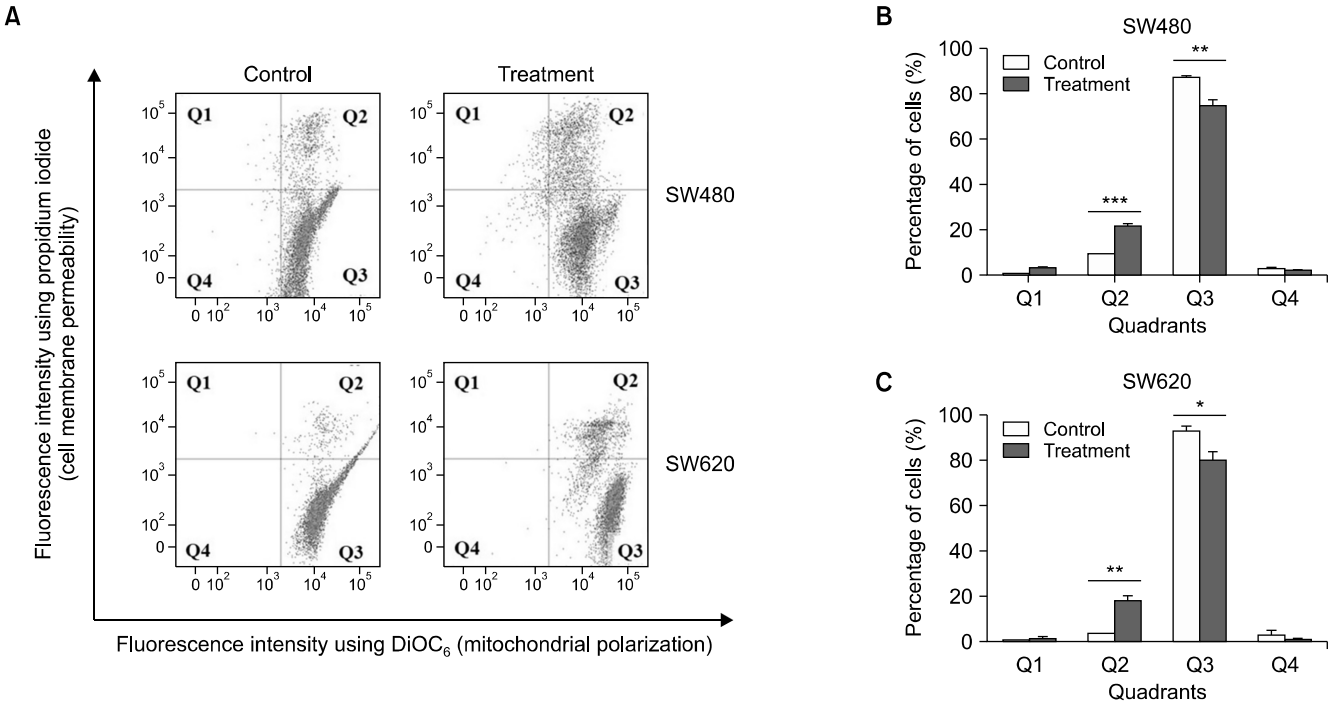

Fig. 6. (A) Representative dot charts of $DiOC_6$/propidium iodide staining (detected by flow cytometry) in SW480 and SW620 cells. Quantification of cells classified by quadrants in SW480 (B) and SW620 (C). Cells were exposed to 50 mg lyophilized mango pulp extract (LMPE)/mL for 48 h. Untreated cells (0 mg LMPE/mL) were used as control. Four quadrants represent dead cells with mitochondrial depolarization (Q1), dead cells with mitochondrial polarization (Q2), cells with membrane integrity and mitochondrial polarization (Q3), and cells with membrane integrity and mitochondrial depolarization (Q4). Data are presented as mean±SEM (n=3). Significant differences between the control and treated cells quadrants at $^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$ by Student's *t*-test.

sults, along with those presented in Fig. 5, suggest that the cell death caused by the LMPE treatment may be associated with cell apoptotic processes without mitochondrial depolarization.

***Intracellular ROS production*:** Several studies have shown that, by producing ROS, chemopreventive compounds can cause cell cycle arrest and apoptosis of cancer cells. Moreover, in the process of apoptosis, the mitochondrial electron transport chain can be disrupted, leading to the production of ROS, such as hydroxyl radicals, and H_2O_2 (Maldonado-Celis et al., 2009). Therefore, this study evaluated whether different doses of the treatment, i.e., 10, 30, and 50 mg LMPE/mL could induce intracellular ROS production in SW480 and SW620 cells. Fig. 7 presents representative histograms (created using flow cytometry) of the intracellular ROS production in control and treated cells. The intracellular ROS production increased in the cells treated with 10, 30, and 50 mg LMPE/mL for 48 h of exposure—SW480 ($P<0.01$) and SW620 ($P<0.05$)—compared to their untreated counterparts.

DISCUSSION

The present study demonstrated that LMPE can inhibit the proliferation of SW480 cells and their metastasis-derived counterparts, i.e., SW620 for the first time. Previous studies had reported the ability of mango pulp to inhibit SW480 cell proliferation (Noratto et al., 2010; Corrales-Bernal et al., 2014b). However, none of them had evaluated metastatic colon cancer lines such as SW620.

The chemical characterization of the LMPE showed a higher content of total phenolic compounds (69.3±15.6 mg GAE/g) than that reported by the *Corporación Colombiana de Investigación Agropecuaria* (Colombian Corporation for Agricultural Research) for Colombian mango pulp (between 0.5 and 2 mg GAE/g) (Lozano et al., 2010). Furthermore, the phenolic content was significantly higher than that reported for the pulp of ripe and green mangoes of the Chinese Irwin variety, which ranged between 26.9±3.76 and 27.8±2.21 mg GAE/g, respectively (Kim et al., 2010). Several phenolic compounds identified in mango fruit have been shown to inhibit cancer cell proliferation and induce apoptosis (Noratto et al., 2010; Ramos, 2007; Pan et al., 2014; Darvin et al., 2015; Velderrain-Rodríguez et al., 2018). Gallic acid (a phenolic acid) demonstrated antiproliferative activity in LS180 colon adenocarcinoma cells (Velderrain-Rodríguez et al., 2018). Mangiferin, a polyphenol of the xanthone group have shown an antiproliferative effect against PC3 prostate cancer malignant cells (Li et al., 2016) and CNE2 nasopharyngeal cancer cells by inducing apoptosis (Pan et al., 2014). Quercetin, a flavonoid identified in mango fruits, has inhibited the proliferation of HCT-116 and HT-29 colon

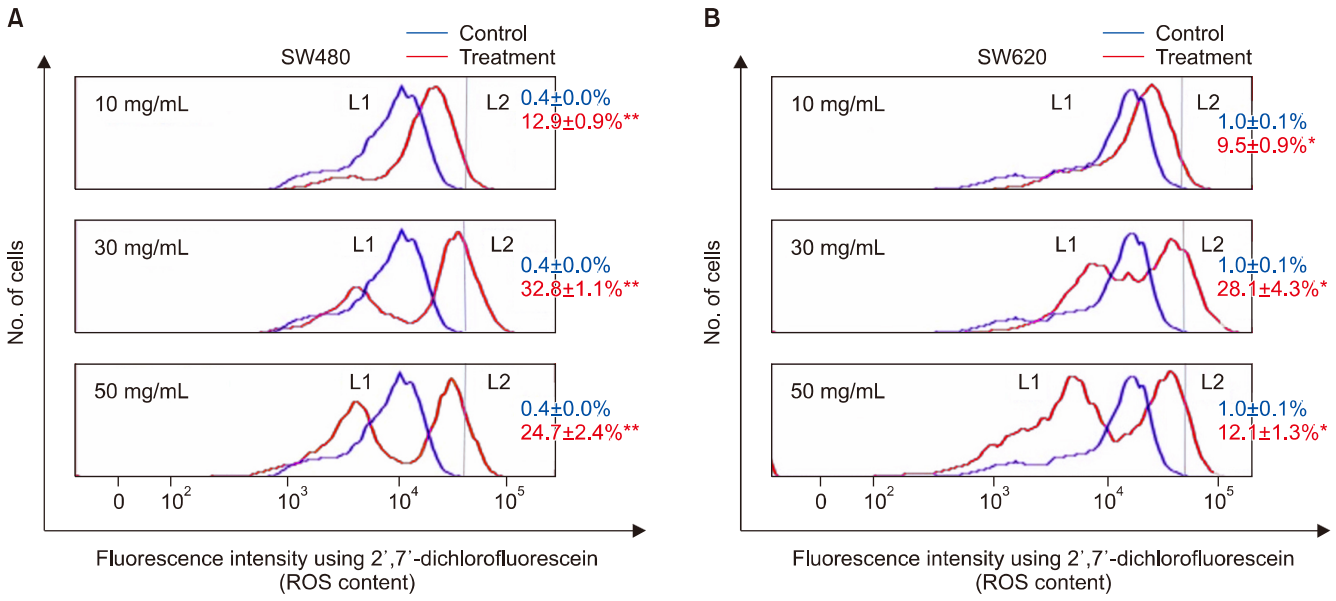

Fig. 7. Effect of the lyophilized mango pulp extract (LMPE) on intracellular reactive oxygen species (ROS) production in SW480 (A) and SW620 (B) cell lines. Cells were exposed to 10, 30, and 50 mg LMPE/mL for 48 h. Untreated cells (0 mg LMPE/mL) were used as control. Representative histograms of fluorescence intensity (which indicates intracellular ROS production) were created using flow cytometry. Two rectangles represent cells with lower production of ROS (L1), and cells with higher production of ROS (L2). Data are presented as mean±SEM (n=3). Significant differences between the control and treated cells of rectangle (L2) at $^{*}P<0.05$ and $^{**}P<0.01$ by Student's *t*-test.

malignant cells. Furthermore, quercetin has induced apoptosis in human cancer cell lines such as HSC-3 cells, submandibular gland HSG carcinoma cells, and promyelocytic HL60 leukemia (Ramos, 2007). Tannic acid has been reported to have an apoptotic effect on YD-38 gingival cancer cells (Darvin et al., 2015). Therefore, LMPE may have potential antiproliferative capacity against SW 480 cells and their metastasis-derived counterparts, i.e. SW620, due to its high content of phenolic compounds.

The results of this study showed the antioxidant capacity of the LMPE. In particular, the DPPH assay proved its capacity to reduce free radicals (576.2±65.9 μmol TE/g), showing values higher than those reported in mango pulp by Kuskoski et al. (2005) (13.7±0.4 μmol TE/g) and those found by Corrales-Bernal et al. (2014a) in green and semi-ripe mangoes (ranging between 55.23±2.21 and 64.71±1.67 μmol TE/g). The free radical scavenging activity of the LMPE measured by the ORAC method (866.1 ±3.5 μmol TE/g) was greater than those described by Ma et al. (2011) in eight mango varieties (ranging from 3,248 ±589 to 8,545±828 μmol TE/100 g), and by Corrales-Bernal et al. (2014a) (maximum 22.09±0.25 μmol TE/g). Nevertheless, the FRAP method determined that the capability of the LMPE to reduce Fe^{3+} to Fe^{2+} (627.9±17.3 μmol TE/g) was lower than that reported by Ma et al. (2011), which ranged from 910,421±123,407 to 6,769,478±241,990 μmol TE/100 g. Therefore, the results suggest that LMPE has an important antioxidant capacity, which could be associated with its high phenolic content (Zapata et al., 2014).

In this study, LMPE showed antiproliferative activity in SW480 and SW620 cells. SRB staining results revealed that LMPE has a dose-dependent and time-dependent antiproliferative effect that is selective regarding nonmalignant skin fibroblast cells. In addition, LMPE at concentrations of 25 and 50 mg LMPE/mL for 48 h induced reproductive cell death (i.e., loss of the ability to reproduce "unlimitedly") in SW480 and SW620 cell lines. The Ki-67 protein is present during all of the cell cycle's active phases (G1, S, G2, and M), but it is lacking in cells that are at rest (G0) (Li et al., 2015). Hence, it has been used as a marker of cell proliferation (Obaid et al., 2018). In this study, the treatment of SW480 cells with 30 and 50 mg LMPE/mL resulted in a substantial decrease in the Ki-67 protein. Although treated SW620 cells showed a trend toward decreased Ki-67 expression, they did not reach significant differences. These results confirm the antiproliferative capacity of the extract. Noratto et al. (2010) found that after 48 h, exposure to a polyphenol extract (5 mg GAE/L) of Ataulfo mango pulp reduced the proliferation of SW480 cells by about 72% while remaining non-cancerous colonic myofibroblast CCD-18Co cells unaffected. Similarly, Corrales-Bernal et al. (2014b) exhibited that, in SW480 cells exposed for 72 h to aqueous extracts of mango at 50 and 100 μg/mL, growth inhibition was induced by 15.7% and 21.6%, respectively.

The cytotoxic effects of LMPE in SW480 and SW620 cell lines were established in this study, and some of its cell death mechanisms were also analyzed. The results suggest that LMPE at 50 mg/mL and 48 h of exposure arrests the cell cycle of SW480 and SW620 in the G2/M phase. Several anticancer agents have been studied to in-

terfere with the G2/M phase and generate arrest in cancer cells through different mechanisms (Lagunas et al., 2014). Mangiferin had an antiproliferative effect on the nasopharyngeal carcinoma cell line CNE2 (Pan et al., 2014) and HL60 promyelocytic leukemia cells by blocking at the G2/M phase (Peng et al., 2015). Similarly, Noratto et al. (2010) demonstrated that polyphenolic extracts of mango at a concentration of 10 mg GAE/L and 24 h of exposure caused cell cycle arrest in the G2/M phase on SW480 cells. Therefore, the cytostatic effect could help to explain the antiproliferative action of LMPE on SW480 and SW 620 cells.

It is known that cell cycle arrest may be related to apoptosis. This hypothesis was tested in this study using annexin V-FITC assays, which demonstrated that LMPE at 50 mg/mL and 48 h of exposure induced PS externalization to SW480 and SW620 cells, suggesting apoptosis-associated cell death. Apoptosis is a mechanism involved in the chemopreventive properties of the compounds present in the mango fruit, according to earlier studies. Mangiferin promoted apoptosis in different kinds of cell lines such as PC3 human prostate cancer (Li et al., 2016), CNE2 nasopharyngeal carcinoma (Pan et al., 2014), and ovarian adenocarcinoma (Zou et al., 2017). Studies by Noratto et al. (2010) and Lauricella et al. (2019) specifically demonstrated the apoptotic capacity of mango extracts in the context of CRC.

This study aimed to explore a relationship between the antiproliferative effect of LMPE on oxidative status and mitochondrial damage. The results showed that in SW480 and SW620 cells exposed to LMPE, intracellular ROS production increased without mitochondrial depolarization. It is therefore possible that, despite its antioxidant capacity (which was evidenced in the ORAC, DPPH, and FRAP assays), LMPE can increase intracellular ROS production in SW480 and SW620 cells. This could be explained by the high concentration of phenolic compounds contained in LMPE, which may have antioxidant effects on normal cells but cause pro-oxidant damage in cancer cells (Fernando et al., 2019). It has been documented that, under certain conditions, phenolic compounds can function as pro-oxidants, such as elevated levels of redox-active transition metals present in many cancer cells (León-González et al., 2015). Therefore, LMPE could have a dual effect on the cellular redox state by promoting an antioxidant action in normal (nonmalignant) cells but acting as a pro-oxidant in SW480 and SW620 cells. It is well documented that oxidative conditions can cause DNA damage leading to a response such as cell cycle arrest and apoptosis observed in SW480 and SW620 cells (Shi et al., 2021). It is yet unclear why some pro-oxidants might selectively destroy cancer cells, although it may have something to do with the fact that tumor cells can produce more hydrogen peroxide (H_2O_2) than healthy cells. Thus, manipulation of redox homeostasis in cancer cells is a possible strategy to attack them (León-González et al., 2015). However, future studies should further investigate molecular biomarkers to understand the signaling pathways involved in the antiproliferative capacity of LMPE against SW480 and SW620 cells.

In conclusion, LMPE is a food matrix with antiproliferative capacity against colon adenocarcinoma cells (SW480) and their metastasis-derived counterparts (SW620). The cell death mechanisms involved in said capacity include increased intracellular ROS production, cell cycle arrest in the G2/M phase, and, possibly, apoptotic processes without mitochondrial depolarization. These results highlight the chemopreventive potential of LMPE in CRC treatments.

ACKNOWLEDGEMENTS

The staff of the Laboratorio de Ciencias Biomédicas at the Instituto Tecnológico Metropolitano in Medelln, Colombia, has our sincere gratitude.

FUNDING

This study was funded by Ministerio de Ciencia Tecnología e Innovación; Ministerio de Educación Nacional; Ministerio de Comercio, Industria y Turismo; and ICETEX, Colombia, through the Scientific Ecosystem component of the Colombia Científica program (Alianza NanoBioCancer code FP44842-211-2018, Project No. 58580).

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: GALC, MEMC. Analysis and interpretation: all authors. Data collection: GALC, JAO, MALR. Writing the article: GALC, MEMC, SSAV. Critical revision of the article: all authors. Final approval of the article: all authors. Statistical analysis: GALC. Overall responsibility: GALC, MEMC. Obtained funding: MEMC.

REFERENCES

Armentano MF, Bisaccia F, Miglionico R, Russo D, Nolfi N, Carmosino M, et al. Antioxidant and proapoptotic activities of *Sclerocarya birrea* [(A. Rich.) Hochst.] methanolic root extract on the hepatocellular carcinoma cell line HepG2. Biomed Res Int.

2015. 2015:561589. https://doi.org/10.1155/2015/561589
Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996. 239:70-76.
Boateng J, Verghese M, Shackelford L, Walker LT, Khatiwada J, Ogutu S, et al. Selected fruits reduce azoxymethane (AOM)-induced aberrant crypt foci (ACF) in Fisher 344 male rats. Food Chem Toxicol. 2007. 45:725-732.
Corrales-Bernal A, Maldonado ME, Urango LA, Franco MC, Rojano BA. Sugar mango (*Mangifera indica*), variety from Colombia: antioxidant, nutritional and sensorial characteristics. Rev Chil Nutr. 2014a. 41:312-318.
Corrales-Bernal A, Urango LA, Rojano B, Maldonado ME. *In vitro* and *in vivo* effects of mango pulp (*Mangifera indica cv*. Azucar) in colon carcinogenesis. Arch Latinoam Nutr. 2014b. 64:16-23.
Costea T, Hudiță A, Ciolac OA, Gălățeanu B, Ginghină O, Costache M, et al. Chemoprevention of colorectal cancer by dietary compounds. Int J Mol Sci. 2018. 19:3787. https://doi.org/10.3390/ijms19123787
Darvin P, Baeg SJ, Joung YH, Sp N, Kang DY, Byun HJ, et al. Tannic acid inhibits the Jak2/STAT3 pathway and induces G1/S arrest and mitochondrial apoptosis in YD-38 gingival cancer cells. Int J Oncol. 2015. 47:1111-1120.
Demchenko AP. Beyond annexin V: fluorescence response of cellular membranes to apoptosis. Cytotechnology. 2013. 65:157-172.
Fernando W, Rupasinghe HPV, Hoskin DW. Dietary phytochemicals with anti-oxidant and pro-oxidant activities: A double-edged sword in relation to adjuvant chemotherapy and radiotherapy?. Cancer Lett. 2019. 452:168-177.
Fragoso MF, Romualdo GR, Vanderveer LA, Franco-Barraza J, Cukierman E, Clapper ML, et al. Lyophilized açaí pulp (*Euterpe oleracea* Mart) attenuates colitis-associated colon carcinogenesis while its main anthocyanin has the potential to affect the motility of colon cancer cells. Food Chem Toxicol. 2018. 121:237-245.
Franken NA, Rodermond HM, Stap J, Haveman J, van Bree C. Clonogenic assay of cells *in vitro*. Nat Protoc. 2006. 1:2315-2319.
Garzón MAG, Rojano BA, Guerrero CA. Inhibition of poliphenoloxidase from bananas (*cavendish*) by the use of some isoespintanol derivatives. Corporación Universitaria Lasallista, Caldas, Colombia. 2012. p 193-248.
Gossé F, Guyot S, Roussi S, Lobstein A, Fischer B, Seiler N, et al. Chemopreventive properties of apple procyanidins on human colon cancer-derived metastatic SW620 cells and in a rat model of colon carcinogenesis. Carcinogenesis. 2005. 26:1291-1295.
Harris GK, Gupta A, Nines RG, Kresty LA, Habib SG, Frankel WL, et al. Effects of lyophilized black raspberries on azoxymethane-induced colon cancer and 8-hydroxy-2'-deoxyguanosine levels in the Fischer 344 rat. Nutr Cancer. 2001. 40:125-133.
Hewitt RE, McMarlin A, Kleiner D, Wersto R, Martin P, Tsokos M, et al. Validation of a model of colon cancer progression. J Pathol. 2000. 192:446-454.
Izli N, Izli G, Taskin O. Influence of different drying techniques on drying parameters of mango. Food Sci Technol. 2017. 37:604-612.
Kataoka M, Fukura Y, Shinohara Y, Baba Y. Analysis of mitochondrial membrane potential in the cells by microchip flow cytometry. Electrophoresis. 2005. 26:3025-3031.
Kim H, Moon JY, Kim H, Lee DS, Cho M, Choi HK, et al. Antioxidant and antiproliferative activities of mango (*Mangifera indica* L.) flesh and peel. Food Chem. 2010. 121:429-436.
Kuskoski EM, Asuero AG, Troncoso AM, Mancini-Filho J, Fett R. Application of various chemical methods to determine antioxidant activity in fruit pulp. Ciênc Tecnol Aliment. 2005. 25:726-732.
Lagunas Cruz MC, Valle Mendiola A, Soto Cruz I. Cell cycle: regulation mechanism. Vertientes Rev Espec Cienc Salud. 2014. 17:98-107.
Lauricella M, Lo Galbo V, Cernigliaro C, Maggio A, Palumbo Piccionello A, Calvaruso G, et al. The anti-cancer effect of *Mangifera indica* L. peel extract is associated to γH2AX-mediated apoptosis in colon cancer cells. Antioxidants. 2019. 8:422. https://doi.org/10.3390/antiox8100422
León-González AJ, Auger C, Schini-Kerth VB. Pro-oxidant activity of polyphenols and its implication on cancer chemoprevention and chemotherapy. Biochem Pharmacol. 2015. 98:371-380.
Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep. 2015. 11:1566-1572.
Li M, Ma H, Yang L, Li P. Mangiferin inhibition of proliferation and induction of apoptosis in human prostate cancer cells is correlated with downregulation of B-cell lymphoma-2 and upregulation of microRNA-182. Oncol Lett. 2016. 11:817-822.
López-Legarda X, Taramuel-Gallardo A, Arboleda-Echavarría C, Segura-Sánchez F, Restrepo-Betancur LF. Comparison of methods using sulfuric acid for determination of total sugars. Rev Cub Quim. 2017. 29:180-198.
Lozano JG, Sandoval AP, Forero F, Floriano JA, Salamanca G, Bernal EJA, et al. Quality attributes of the Creole mango for the agroindustry. Corporación Colombiana de Investigación Agropecuaria, Bogotá, Colombia. 2010. p 48.
Ma X, Wu H, Liu L, Yao Q, Wang S, Zhan R, et al. Polyphenolic compounds and antioxidant properties in mango fruits. Sci Hortic. 2011. 129:102-107.
Maldonado-Celis ME, Bousserouel S, Gossé F, Minker C, Lobstein A, Raul F. Differential induction of apoptosis by apple procyanidins in TRAIL-sensitive human colon tumor cells and derived TRAIL-resistant metastatic cells. J Cancer Mol. 2009. 5:21-30.
Martín MA, Goya L, Ramos S. Preventive effects of cocoa and cocoa antioxidants in colon cancer. Diseases. 2016. 4:6. https://doi.org/10.3390/diseases4010006
Muñoz-Bernal ÓA, Torres-Aguirre GA, Núñez-Gastélum JA, de la Rosa LA, Rodrigo-García J, Ayala-Zavala JF, et al. New approach to the interaction between Folin-Ciocalteu reactive and sugars during the quantification of total phenols. TIP Rev Esp Cienc Quim Biol. 2017. 20:23-28.
Nicoletti I, Migliorati G, Pagliacci MC, Grignani F, Riccardi C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. J Immunol Methods. 1991. 139:271-279.
Noratto GD, Bertoldi MC, Krenek K, Talcott ST, Stringheta PC, Mertens-Talcott SU. Anticarcinogenic effects of polyphenolics from mango (*Mangifera indica*) varieties. J Agric Food Chem. 2010. 58:4104-4112.
Obaid RF, Al-Khafaji YARK, Obied HN, AL-Jibouri SA. Comparison between antitumor activity of live-attenuated measles virus and cisplatin on Ki-67 expression of colon cancer cell line (SW-480) *in vitro*. Med J Babylon. 2018. 15:74-77.
Ou B, Hampsch-Woodill M, Prior RL. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J Agric Food Chem. 2001. 49:4619-4626.
Pan LL, Wang AY, Huang YQ, Luo Y, Ling M. Mangiferin induces apoptosis by regulating Bcl-2 and Bax expression in the CNE2 nasopharyngeal carcinoma cell line. Asian Pac J Cancer Prev. 2014. 15:7065-7068.
Peng ZG, Yao YB, Yang J, Tang YL, Huang X. Mangiferin induces cell cycle arrest at G2/M phase through ATR-Chk1 pathway in HL-60 leukemia cells. Genet Mol Res. 2015. 14:4989-5002.
Pointet AL, Taieb J. Cáncer de colon. EMC. 2017. 21:1-7.
Ramos S. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. J Nutr Biochem. 2007. 18:

427-442.
Saif MW, Chu E. Biology of colorectal cancer. Cancer J. 2010. 16: 196-201.
Shi T, van Soest DMK, Polderman PE, Burgering BMT, Dansen TB. DNA damage and oxidant stress activate p53 through differential upstream signaling pathways. Free Radic Biol Med. 2021. 172:298-311.
Stoner GD, Wang LS, Zikri N, Chen T, Hecht SS, Huang C, et al. Cancer prevention with freeze-dried berries and berry components. Semin Cancer Biol. 2007. 17:403-410.
Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021. 71:209-249.
Velderrain-Rodríguez GR, Torres-Moreno H, Villegas-Ochoa MA, Ayala-Zavala JF, Robles-Zepeda RE, Wall-Medrano A, et al. Gallic acid content and an antioxidant mechanism are responsible for the antiproliferative activity of 'Ataulfo' mango peel on LS 180 cells. Molecules. 2018. 23:695. https://doi.org/10.3390/molecules23030695
Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc. 2006. 1:1112-1116.
World Cancer Research Fund Network, American Institute for Cancer Research. Wholegrains, vegetables and fruit and the risk of cancer. 2018 [cited 2021 Jul 29]. Available from: https://www.wcrf.org/wp-content/uploads/2020/12/Wholegrains-veg-and-fruit.pdf
Wong MC, Ding H, Wang J, Chan PS, Huang J. Prevalence and risk factors of colorectal cancer in Asia. Intest Res. 2019. 17:317-329.
Zapata S, Piedrahita AM, Rojano B. Oxygen radical absorbance capacity (ORAC) and phenolic content of fruits and vegetables from Colombia. Perspect Nut Hum. 2014. 16:25-36.
Zou B, Wang H, Liu Y, Qi P, Lei T, Sun M, et al. Mangiferin induces apoptosis in human ovarian adenocarcinoma OVCAR3 cells via the regulation of Notch3. Oncol Rep. 2017. 38:1431-1441.