Prev. Nutr. Food Sci. 2020;25(3):272-279
https://doi.org/10.3746/pnf.2020.25.3.272
pISSN 2287-1098 · eISSN 2287-8602

Fermented Non-Digestible Fraction of Andean Berry (*Vaccinium meridionale* Swartz) Juice Induces Apoptosis in Colon Adenocarcinoma Cells

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ABSTRACT: *Vaccinium meridionale* Swartz, known as Andean berry, has a high content of anthocyanins, phenolic acids, and other flavonoids due to their putative anticancer activity. However, after consumption, the structures and function of these molecules may be altered. The purpose of this study was to evaluate the pro-apoptotic effect of fermented non-digestible fraction (FNDF) of Andean berry juice (ABJ) on colon adenocarcinoma HT29 cells. HT29 cells were treated by FNDF-ABJ obtained by *in vitro* gastrointestinal fermentation. We determined the proapoptotic capacity by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays, oxidative stress by analyzing superoxide dismutase and catalase activity, lipid peroxidation by measuring 8-iso-prostaglandin F2α, and measured lactate dehydrogenase. Our results show that FNDF-ABJ inhibited cell growth [lethal dose 50(%)=26% v/v]. In addition, FNDF-ABJ increased the number of TUNEL positive cells 2-fold compared with untreated cells without altering the release of lactate dehydrogenase. However, superoxide dismutase activity was reduced in HT29 cells treated with FNDF-ABJ, catalase activity was not affected and 8-iso-prostaglandin F2α levels were increased. These results support that the anti-proliferative effects of FNDF-ABJ on HT29 cells can be explained by apoptotic mechanisms.

Keywords: apoptosis, colon cancer, oxidative stress, *Vaccinium*

INTRODUCTION

The genus *Vaccinium* includes blue mulberries, black mulberries, and blueberries, among others. These fruits are a source of polyphenolic compounds, including phenolic acids (chlorogenic acid and hydroxycinnamic acid), flavonoids (anthocyanins; responsible for their pigmentation), flavonols, and tannins (proanthocyanidins and ellagitannins). The content of these polyphenols vary according to genetic factors (i.e. the variety), cultivation methods, soil, climate, and harvest time (Seeram et al., 2004; Seeram et al., 2006; Burdulis et al., 2009). *Vaccinium meridionale* Swartz is a native Colombian berry commonly known as "Mortiño" or "Agraz" that belongs to Ericaceae family. This fruit is considered a potential functional food because it has a high content of phenolic compounds and anthocyanins, which have an antioxidant capacity similar to or greater than those of other species of the same genus (Gaviria et al., 2009; Garzón et al., 2010).

Anticancer activity of polyphenolic compounds in berries has been reported in *in vitro* studies using colon adenocarcinoma cell lines (Seeram et al., 2004; Seeram et al., 2006; Stoner et al., 2007; Gaviria et al., 2009; Garzón et al., 2010; Zu et al., 2010), and *in vivo* studies using preclinical colorectal cancer models (CRC) (Lala et al., 2006; González-Barrio et al., 2011). These studies have shown that polyphenolic compounds increase the amount of apoptotic cells by 40% to 90% and reduce the number of paraneoplastic lesions by 45% to 89%. Recently, it was reported that treatment of colon adenocarcinoma SW480 cells with the fermented non-digestible fraction (FNDF) of Andean berry juice (ABJ) has an antiproliferative effect, and is associated with an half maximal inhibitory concentration (IC_{50}) value of 8% v/v (Agudelo et al., 2018). In

Received 16 April 2020; Accepted 16 May 2020; Published online 30 September 2020

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addition, ABJ treatment increased caspase 3 activity in a time-dependent manner, and increased expression of the proapoptotic proteins cleaved caspase 3, cleaved poly (ADP-ribose) polymerase-1, p53, and total benzamide adenine dinucleotide by 1.6- to 2.0-fold (Agudelo et al., 2017).

Most studies examining the anticancer activity of polyphenols in food use pure extracts or fractions that are evaluated using *in vitro* assays. As a consequence, only a few studies have used gastrointestinal conditions to evaluate the nutraceutical properties of anthocyanins and phenolic compounds from *Vaccinium* gender species in digested food (Seeram et al., 2004; Seeram et al., 2006; Burdulis et al., 2009). Gastrointestinal digestion models are valuable tools that represent physiological conditions (pH, temperature, and enzymatic activity) (Bermúdez-Soto et al., 2007; Cilla et al., 2008; Correa-Betanzo et al., 2014; Stanisavljević et al., 2015). Therefore, our research group have explored beneficial effects of colonic extracts derived from ABJ digestion in both *in vivo* and *in vitro* CRC models (Agudelo et al., 2018). The catabolized compounds from ABJ resulted in a mixture of biologically active compounds that are able to reach the colonic mucosa, and which display chemopreventive effects against CRC (Bermúdez-Soto et al., 2007; de Kok et al., 2008; Del Rio et al., 2010).

Thus, further studies are necessary to gain insight into the intimate mechanisms involved in the antiproliferative effects of digested ABJ as promissory dietary chemopreventive agents on colon adenocarcinoma cells. The objective this study was to determine the proapoptotic and oxidative stress effects of the FNDF-ABJ on HT29 human colon adenocarcinoma cells.

MATERIALS AND METHODS

Reagents

Pentobarbital sodium salt, casein peptone, HCl, NaCl, KCl, $MgSO_4$, KH_2PO_4, $NaHCO_3$, $CaCl_2$, NaOH, pepsin, pancreatin, Tween salts, hematin, glucose, crystal violet, formaldehyde, and ethanol were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Animals (14 male Wistar rats, 6～8 weeks of age) were provided by Instituto de Neurobiología (Universidad Nacional Autónoma de México, Campus Juriquilla, Queretaro, Mexico). All procedures that involved animals were approved by the Universidad Autónoma de Querétaro Human and Animal Internal Committee (approval code: CBQ17/094), and complied with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. HT-29 cells were purchased from the American Type Culture Collection (ATCC). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), antibiotic-antimycotic, trypsin, and ethylenediaminetetraacetic acid (EDTA) were purchased from Gibco or Thermo Fisher Scientific, Inc. (Waltham, MA, USA).

Plant material

Fresh ripe berries of *V. meridionale* Swartz (Andean berry) were harvested from the Municipality of Retiro (Antioquia, Colombia), at an altitude of 2,175 m and 16°C in May, 2015. The berries were washed, selected, disinfected (using sodium hypochlorite 100 ppm), dried, and centrifuged (for 2 min at 2,500 rpm). The berries were then freeze-dried in a vacuum chamber under pressure (0.427 +0.5 mm Hg; temperature of −50°C) and the powder was stored at room temperature in a polyethylene terephthalate-aluminum package to protect from light.

Preparation of ABJ

ABJ was prepared as described previously (Franco Tobón et al., 2016). Briefly, 30 g of the freeze-dried powder of Andean berry was dissolved in sterile water. Sucrose was added to obtain an 11.1°Brix juice with an acidity of 4.33 mg citric acid/mL at pH 3.06. The juice was homogenized in a sonicator (42 kHz, 135 W; Branson 3510, Branson, Danbury, CT, USA) at different time intervals (15, 30, 45, and 60 min) at room temperature. Samples were aliquoted and stored at −70°C protected from light until use.

In vitro gastrointestinal digestion

To simulate human physiological conditions and obtain the non-digestible fraction (NDF)-ABJ, we followed the procedure described by Campos-Vega et al. (2015). Briefly, after signing informed consent, four healthy volunteers chewed 25 mL of ABJ 15 times for 15 s. The resulting product was expectorated into a beaker containing 5 mL of distilled water. Suspensions of each sample were mixed, and aliquots (10 mL) were adjusted to pH 2.0 using HCl (150 mM). Pepsin (0.055 g, Sigma-Aldrich Co.) dissolved in 0.94 mL of 20 mM HCl was added to each sample and samples were incubated for 2 h at 37°C to obtain the gastric solution.

The intestinal extract was prepared by dissolving 3 mg of gall ox and 2.6 mg of pancreatin (Sigma-Aldrich Co.) in 5 mL of Krebs-Ringer buffer (118 mM of NaCl, 4.7 mM of KCl, 1.2 mM of $MgSO_4$, 1.2 mM of KH_2PO_4, 25 mM of $NaHCO_3$, 11 mM of glucose, and 2.5 mM of $CaCl_2$, pH 6.8). Five milliliters of this solution was added to each sample and the suspension was transferred into an everted rat gut sac.

For the preparation of the everted gut sacs, male Wistar rats (body weight 250～300 g, n=7 per experiment) were fasted overnight (16～20 h) with water *ad libitum* and then anesthetized with pentobarbital sodium (60 mg/kg, injected intraperitoneally). The small intestine (20～25 cm of the proximal rat jejunum) was exposed by

a midline abdominal incision and cut into 6 cm length segments. Segments were then everted and filled with 1 mL of the Krebs-Ringer buffer containing gall ox and pancreatin. The everted sacs were placed into the gastric solution (15 mL) and incubated in an oscillating water bath (80 cycles per min, 37°C) for 2 h. The sample on the outside of the everted sac was considered to be NDF-ABJ. Two complete gastrointestinal digestions were carried out in triplicate, with human saliva, prepared with distilled water following the same steps as for the sample, used as a blank.

In vitro colonic fermentation

FNDF-ABJ was obtained following the procedure previously described by Campos-Vega et al. (2009), with slight modifications. Fecal samples were obtained from healthy donors with no previous history of gastrointestinal conditions, were of a normal weight and who had not undergone antibiotics therapy in the last three months. For the experiment, 15 mL sterile conic tubes were filled with 9 mL of sterile basal culture medium containing peptone 2 g/L of water, 2 g/L of yeast extract, 0.1 g/L of NaCl, 0.04 g/L of K_2HPO_4, 0.04 g/L of KH_2PO_4, 0.01 g/L of $MgSO_4 \cdot 7H_2O$, 0.01 g/L of $CaCl_2 \cdot 2H_2O$, 2 g/L of $NaHCO_3$, 0.5 g/L of cysteine HCl, 0.5 g/L of bile salts, 2 mL of Tween 80, and 0.2 g of hematin (diluted in 5 mL of NaOH). Tubes were sealed and gasified with H_2-CO_2-N_2 [10:10:80 (v/v/v)] gas to maintain anaerobic conditions for 24 h. Human gut fecal samples were prepared by mixing 3 g of homogenized fresh stools with 27 mL of 0.1 M sodium phosphate buffer, pH 7. Tubes containing medium were inoculated with 1 mL of the prepared fecal samples and either sample (1 mL liquid and 1 g of solid sample) or 100 mg raffinose as positive control. The pH at time zero was recorded prior to placing the samples in a water bath at 37°C, and pH was assessed at 6, 12, and 24 h. These fermented extracts were centrifuged (Hermle Z323 K, Hermle Labortechnik GmbH, Wehingen, Germany) at 4,500 rpm for 15 min at 4°C. The supernatant was transferred into 1.5 mL microcentrifuge tubes and stored at −70°C until cell assays.

Prior to the cell assays, we calculated the polyphenolic and mono-oligosaccharide composition of the FNDF-ABJ (Agudelo et al., 2018). The FNDF contained gallic acid (132.21±0.03 mg/g), chlorogenic acid (87.76±0.06 mg/g), caffeic acid (2.08±0.002 mg/g), ellagic acid (33.19±0.09 mg/g), rutin (15.62±2.43 mg/g), raffinose (1.13±0.05 mg/g), stachyose (0.65±0.03 mg/g), and xylose (97.77±0.31 mg/g).

Cell viability assays and determination of LC50

HT-29 cells were purchased from the ATCC. Cells were grown and maintained in DMEM supplemented with 10% FBS (Gibco BRL, Grand Island, NY, USA) and 1% of antibiotic-antimycotic (Gibco BRL) in a humidified atmosphere at a temperature of 37°C with 5% CO_2. Cells were trypsinized (trypsin/EDTA solution 0.05/0.02%) and sub-cultured in 24-well plates (5×10^4 cells/well).

Cell viability was assessed through crystal violet assays following the procedure described by Feoktistova et al. (2016) with slight modifications. Briefly, HT29 cells were seeded in 96-well culture plates (2×10^3 cells/well) in a humidified atmosphere (37°C, 5% CO_2). After 24 h, cells were exposed to different concentrations of FNDF-ABJ (1, 2, 5, 10, 20, 30, and 40% v/v) and incubated for 24 h. After the incubation, adherent cells were washed twice with phosphate buffer saline (PBS) and incubated for 10 min at 37°C in 50 μL of crystal violet staining solution (0.5% w/w crystal violet, 4% formaldehyde, 30% ethanol, and 0.17% NaCl). Cells were washed with tap water and dried for 1 h at 50°C. Stained cells were then dissolved in 33% v/v acetic acid and the absorbance was measured in a microplate reader (Thermo Fisher Scientific, Inc.) at 570 nm. The concentration able to kill 50% of cells (LC_{50}) was calculated with GraphPad Prism software (v. 6.0). The absorbance of non-treated cells (control group) was considered to correspond to 100% viability and the percentage of inhibition was calculated as follows:

$$\text{Inhibition (\% of crystal violet)}: [1-(ODt-ODc)]\times100$$

where ODt is the optical density (OD) of treated cells and ODc is the optical density of control cells.

Lactate dehydrogenase (LDH) cytotoxicity assays

LDH assays were used to determine the release of LDH from HT29 cells treated with FNDF-ABJ at the IC_{50} dose for 48 h, and thus cell damage (Lala et al., 2006). The experiments were carried out following the manufacturer's protocol (lactate dehydrogenase activity assay kit (MAK 066) (Sigma-Aldrich Co.) and as described in Fotakis and Timbrell (2006). Briefly, following exposure to FNDF-ABJ at the IC_{50} dose, culture medium was aspirated and centrifuged, and then aliquots were mixed with the reagent. Absorbance was recorded at 450 nm and the LDH activity in the medium was determined following the kit protocol.

Apoptosis quantification

The terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling method (TUNEL) was used to quantify the pro-apoptotic effects of FNDF-ABJ. Briefly, HT29 cells were treated with FNDF-ABJ at the IC_{50} dose for 24 h. Untreated cells treated with 0.05% dimethyl sulfoxide (v/v) were used as the negative control. To detect cells undergoing apoptosis, the *in situ* cell death detection kit (Roche Molecular Biochemicals, Basel, Switzerland) was used according to the manufacturer's instruction.

Briefly, treated cells were fixed using 4% paraformaldehyde/PBS (pH 7.4) and then washed twice with PBS. Cells were then incubated with blocking solution (3% H_2O_2 in methanol) for 10 min, rinsed with PBS and then permeabilized using 0.1% Triton X-100 in 0.1% sodium citrate. The enzyme solution and label solution reaction mixture were added to label the fragmented DNA and the mixture was incubated at 37°C for 1h. Digital images of apoptotic cells [fluorescein isothiocyanate (FITC)-labeled TUNEL-positive cells] were randomly selected under a fluorescence microscope.

Oxidative stress assays

***Determination of HT29 superoxide dismutase (SOD) activity*:** The HT29 cells exposed to FNDF-ABJ at the IC_{50} dose were homogenized to give a final concentration of 50 mg/mL in cold 1× lysis buffer (10 mM of Tris, pH 7.5, 150 mM of NaCl, and 0.1 mM of EDTA), centrifuged at 12,000 *g* for 10 min and the supernatant was collected for analysis. The OxiSelect™ SOD activity assay kit (Cells Biolabs, San Diego, CA, USA) was used to measure SOD activity. Briefly, 20 μL of sample, 5 μL of xanthine solution, 5 μL of chromogen solution, 5 μL of 10× SOD assay buffer and 50 μL of deionized water were added (total volume of 90 μL) to a 96-well microplate. Then, 10 μL of pre-diluted 1× xanthine oxidase solution was added to each well. Samples were mixed well and incubated for 1 h at 37°C. The absorbance was determined using a spectrophotometric microplate reader at 490 nm.

***Catalase (CAT) activity assays*:** HT29 cells exposed to FNDF-ABJ at the IC_{50} dose were homogenized in cold PBS with 1 mM EDTA to give a final concentration of 50 mg/mL, centrifuged at 10,000 *g* for 15 min at 4°C and the supernatant was collected. The OxiSelect™ catalase activity assay kit (Cells Biolabs) was used to measure catalase activity. Briefly, 20 μL of the diluted catalase standard or sample and 50 μL of the H_2O_2 working solution (12 mM) were added to a 96 well microplate. Samples were mixed well and incubated for 1 min. Reactions were stopped by adding 50 μL of the catalase quencher into each well and mixed thoroughly. Five microliters of each reaction was then transferred into fresh wells, and 250 μL of the chromogenic working solution was added to each. Samples were then mixed and incubated for 60 min. Absorbance was measured using a spectrophotometric microplate reader at 520 nm.

***Measurement of lipid peroxidation in treated HT29 cells*:** The F2-isoprostanes are a recently described class of prostaglandins formed by free radical-mediated lipid peroxidation (McDougall et al., 2005). Thus, the OxiSelect™ 8-iso-prostaglandin F2 (PGF2) kit is a competitive enzyme-linked immunosorbent assay (ELISA) for rapid detection and quantification of 8-iso-PGF2. The quantity of 8-iso-PGF2 in samples was determined by comparing the absorbance at 450 nm with that of a known 8-iso-PGF2 standard curve. The enzyme immunoassay ELISA kit OxiSelect™ 8-iso-PGF2α (Cell Biolabs) was used to determine lipid peroxidation of cell lysates treated with FNDF-ABJ at the IC_{50} dose, following the manufacturer's instructions.

Statistical analysis

All data were presented as mean±standard deviation (SD) from at least two independent experiments, in triplicate. Significant differences between groups were evaluated by ANOVA and Dunnett's multiple comparisons post-hoc test to determine the significance of statistical differences between data at the level of $P<0.05$, using GraphPad Prism (ver. 6.0) for Windows (GraphPad Software, San Diego, CA, USA).

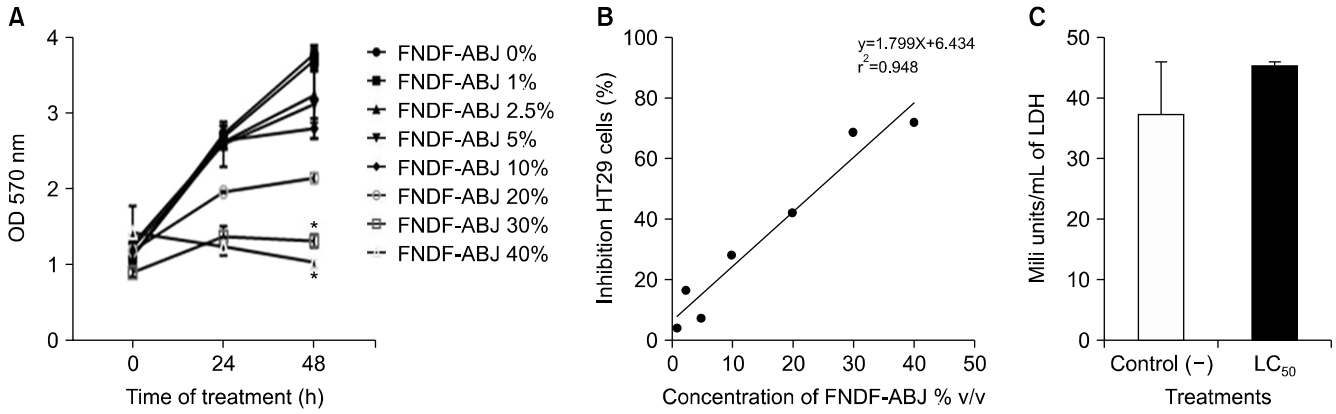

Fig. 1. (A) Antiproliferative effects of different concentrations of the fermented non-digestible fraction (FNDF) of Andean berry juice (ABJ) on the HT29 cell growth after treatment for 24 and 48 h. Data are mean±SD of two independent experiments. Differences between groups were assessed using ANOVA followed by Dunnett's multiple comparison post-hoc test. The asterisk indicates a significant difference between non-treated cells and treated (*$P<0.05$). OD, optical density. (B) Inhibitory activity of FNDF-ABJ on HT29 cells viability. (C) Release of lactate dehydrogenase (LDH) from HT29 cells treated with FNDF-ABJ. Differences between groups were assessed using unpaired *t*-tests.

RESULTS

Effect of FNDF-ABJ on HT29 cell growth

HT29 cell growth following treatment with different concentrations of FNDF-ABJ (1～40%) for 24 and 48 h was presented as OD of cell proteins at 570 nm (Fig. 1A). After treatment with FNDF-ABJ, the OD of HT29 cell proteins was reduced by 2.01 to 71.6% compared with that of untreated cells. The inhibitory effect on cell viability was significantly increased ($P<0.05$) with increasing concentrations (1～40%) of FNDF-ABJ (Fig. 1B). The LC_{50} value calculated from the linear-regression between percent inhibition and concentration was 24.69%.

LDH activity

To determine the membrane integrity of HT29 cells after treatment with FNDF-ABJ at the LC_{50} dose for 48 h, the amount of LDH in the culture media was assessed. Treatment with FNDF-ABJ induced increased release of LDH in HT29 cells compared with untreated cells (Fig. 1C). Loss of LDH from HT29 cells was 1.7-fold greater than from untreated cells (control group) but the amounts did not significantly differ.

Effect of FNDF-ABJ on HT29 cell apoptosis

We next assessed the effect of FNDF-ABJ on inducing apoptosis as a mechanism for inhibiting HT29 cell vitality. Cells were treated with FNDF-ABJ at the LC_{50} dose for 48 h and analyzed using TUNEL assays. Treated cells exhibited green fluorescence indicative of apoptotic cell death (FITC-labeled TUNEL-positive cells) whereas untreated HT29 cells did not exhibit a fluorescence signal ($P<0.0001$) (Fig. 2).

Levels of 8-isoprostane

Lipid peroxidation was evaluated through measuring levels of 8-isoprotane. Treatment of cells with FNDF-ABJ at the LC_{50} dose for 48 h, significantly decreased 8-isopro-

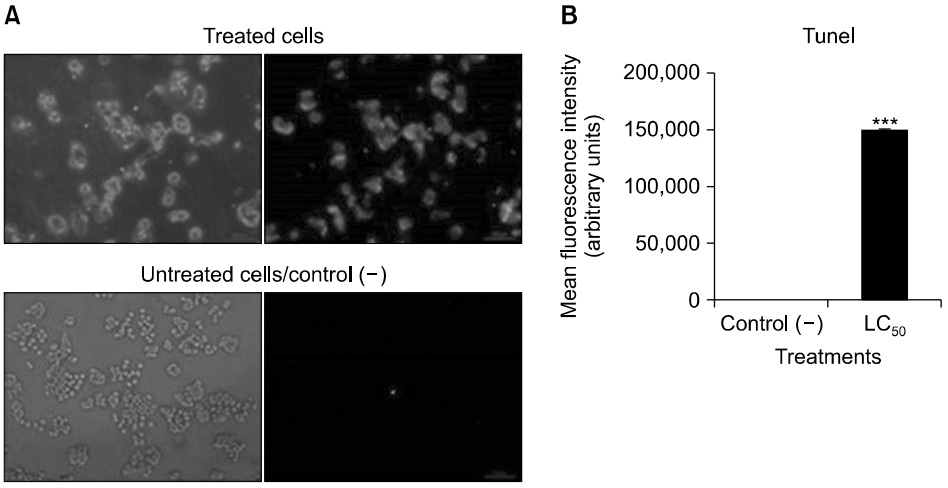

Fig. 2. Apoptotic effect of the fermented non-digestible fraction (FNDF) of Andean berry juice (ABJ) at the LC_{50} dose after 48 h of treatment. (A) Photomicrographs of HT29 cells treated with FNDF-ABJ at the IC_{50} dose for 48 h and control cells. Apoptotic cells can be seen as green cells stained with the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay regent fluorescein isothiocyanate (FITC). (B) Histogram of DNA fragmentation. Fragmentation is presented as relative intensity (arbitrary units) of fluorescence for treated cells compared with control cells. Bars represent mean±SD (n=5). ***$P<0.0001$.

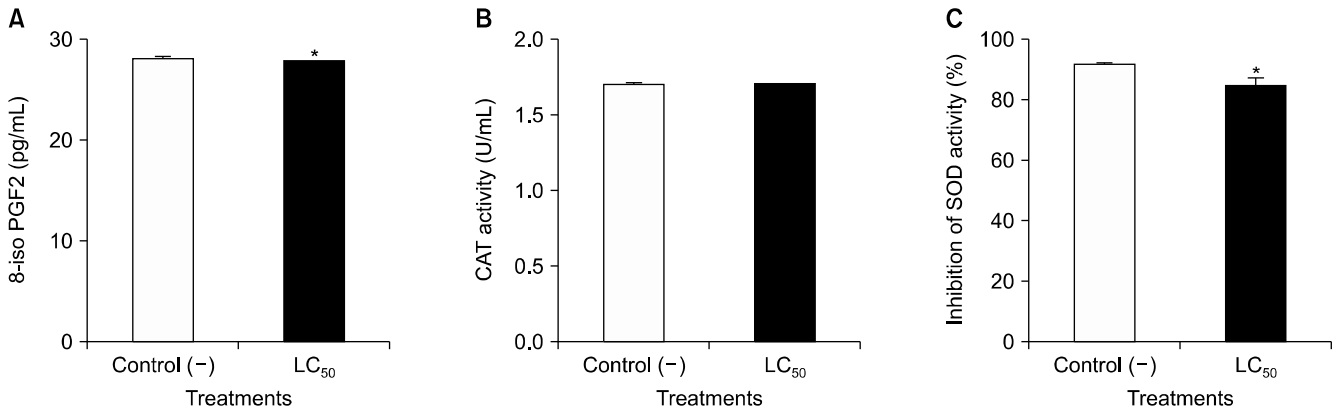

Fig. 3. Effect of FNDF-ABJ at the LC_{50} dose on oxidative stress in HT29 cells after 48 h of treatment. (A) Level of 8-iso-prostaglandin F2α (PGF2 α), (B) catalase (CAT) activity, and (C) percentage of superoxide dismutase (SOD) activity. Data are mean±SD of three independent experiments, analyzed by unpaired *t*-tests. *$P<0.05$.

stane concentrations from 28.2±0.052 pg/mL (untreated cells) to 27.9±0.0009 pg/mL (P=0.0042) (Fig. 3A).

CAT and SOD activity

Following treatment of HT29 cells with FNDF-ABJ at the LC_{50} dose for 48 h, it was not possible to detect significant differences in catalase activity compared with untreated cells (P=0.0866) (Fig. 3B). However, the activity of SOD in HT29 cells was significantly reduced by 7.8% (P=0.0252) compared with in untreated cells (Fig. 3C).

DISCUSSION

In this study, we show that ABJ maintains its antiproliferative and proapoptotic activities after gastrointestinal digestion (FNDF-ABJ), including after colonic fermentation. This results suggest that gastrointestinal digestion does not affect the antiproliferative activity of phytochemicals present in FNDF-ABJ on colon adenocarcinoma HT29 cells. The same trend has been observed in other studies evaluating non-digested (Agudelo et al., 2017) and digested ABJ (Agudelo et al., 2018). These events were associated with an increase in the pro-oxidative intracellular environment that favors DNA damage and, consequently, apoptosis. Therefore, these data highlight the anticarcinogenic potential of ABJ in colorectal cancer chemoprevention.

The value of this study is in the evaluation of a complete food matrix rich in phenolic compounds (i.e. ABJ subjected to *in vitro* gastrointestinal digestion). Several reports have shown production of polyphenolic metabolites following colonic fermentation. For instance, colonic fermentation of Chilean currants (*Ribes* spp.) has been shown to produce chlorogenic acid (710 mg/g) (Burgos-Edwards et al., 2018), whereas *in vitro* colonic fermentation of blueberry (*Vaccinium ashei*) phenolics has been shown to produce gallic acid (15.82～18.70 mg/g) (Russell et al., 2010).

FNDF-ABJ inhibited HT29 cell viability in a dose-dependent manner. However, analysis of LDH levels in the extracellular medium showed that FNDF-ABJ did not induce significantly increased release of LDH from HT29 cells compared with control cells. However, FNDF-ABJ treatment induced DNA fragmentation, as identified using TUNEL assays. This indicates that treatment with FNDF-ABJ induces cell apoptosis without inducing loss of membrane integrity. During apoptosis, it is expected that cell membranes remain intact. This is considered a non-inflammatory characteristic of apoptotic cells to avoid the inflammation, as apoptotic cells are cleared by phagocytes, preventing release of proinflammatory mediators such as interleukin-1 and tumor necrosis factor-α from macrophages (Patel et al., 2006).

Consistent with our results, Brown et al. (2012) reported that digested and fermented extracts of raspberries, strawberries, and blackcurrants (0～50 μg/mL gallic acid equivalents) showed cytotoxic (P<0.05) and antigenotoxic activity (P<0.001), and decreased the percentage of tail DNA to approximately 30%. Moreover, after 24 h, invasion of HT29 cells were significantly reduced by 40% (P<0.01) compared with the respective control group.

Similarly, Coates et al. (2007) showed that raspberry extract available to the colon (extracts treated with a procedure that mimicked the physiochemical conditions of the upper gastrointestinal tract) significantly decreased the population of HT29 cells and significantly protected against DNA damage induced by hydrogen peroxide in HT29 cells. Thus, the above data together with our findings indicate that FNDF-ABJ or whole berries fruits that are in direct contact with the colonic epithelium may have an antiproliferative and proapoptotic effect *in vivo* on colon adenocarcinoma cells.

8-Iso-PGF2α is a lipid membrane peroxidation product of arachidonic acid that has been identified as the most useful biomarker of oxidative damage (van't Erve et al., 2017). In our study, FNDF-ABJ reduced significantly levels of 8-iso-PGF2 in HT29 cells, which suggests that FNDF-ABJ acts by interfering with mechanisms of oxidative stress. Therefore, the reduction of oxidative stress in human colon adenocarcinoma cells grade II may play a critical role in chemoprevention of colorectal cancer at a secondary level (Khan et al., 2008). However, the ability of FNDF-ABJ to reduce oxidative stress did not involve the activity of the antioxidant enzymes catalase and superoxide dismutase, suggesting that FNDF-ABJ induces apoptosis in a pro-oxidant intracellular environment.

Oxidative stress is associated with the three stages of carcinogenesis: initiation, promotion, and progression (Chikara et al., 2018). Thus, the antioxidant blocking action of dietary phytochemicals is important in the prevention cancer. However, these compounds have been recognized as agents able to induce reactive oxygen species (ROS) and are able to produce high levels of ROS, which is deleterious to cancer cells (Wang and Yi, 2008). For example, apple procyanidins trigger apoptosis of SW620 colon cancer cells by increasing ROS production, which leads to mitochondrial disruption and activation of cytochrome c and caspase-9 and caspase-3 (Maldonado-Celis et al., 2008; Maldonado-Celis et al., 2009). This was confirmed by using a specific inhibitor of the polyamine catabolic pathway (MDL 72527) that inhibited ROS generated by apple procyanidins treatment of human colon cancer-derived metastatic cells and exerted a protective effect on mitochondrial function (Maldonado-Celis et al., 2008; Maldonado-Celis et al., 2009). Taking into consideration the observed pro-oxidant and pro-apoptotic mechanism induced by FNDF-ABJ on HT29 cells, we might

infer FNDF-ABJ induces intrinsic apoptotic cell death involving mitochondrial damage. As a consequence, FNDF-ABJ increases levels of ROS, activates caspase-3 and triggers overall proapoptotic process.

In conclusion, the treatment of human colon adenocarcinoma HT29 cells with FNDF-ABJ induced apoptosis, as shown by fragmentation of DNA. Apoptosis was induced by intracellular oxidative stress as FNDF-ABJ reduced SOD activity but had no effect on catalase activity. However, additional *in vitro* and *in vivo* studies are required to characterize the proapoptotic mechanisms responsible for the observed results. These findings highlight the chemopreventive potential of Andean berry juice against colon cancer.

ACKNOWLEDGEMENTS

This work was supported by COLCIENCIAS Project number 58580 through the Program Ecosistema Científico under Grant FP44842-211-2018; and Escuela de Nutrición y Dietética de la Universidad de Antioquia through the Program Estrategia de Sostenibilidad de Grupos Escuela de Nutrición y Dietética 2018∼2020 under Grant number 106. Authors Ivan Luzardo-Ocampo and Angélica Hernández-Arriaga were supported by a scholarship from the Consejo Nacional de Ciencia y Tecnología (CONACYT). The authors would like to thank M.Sc. Kenia Vázquez Sánchez for her support in the preparation of samples.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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