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Fruit Extract from A Sechium edule Hybrid Induce Apoptosis in Leukaemic Cell Lines but not in Normal Cells

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The antiproliferative potential of a crude extract from the chayote hybrid H-837-07-GISEM and its potential for apoptosis induction were assessed in leukaemic cell lines and normal mouse bone marrow mononuclear cells (BM-MNCs). The extract strongly inhibited the proliferation of the P388, J774, and WEHI-3 cell lines (with an IC₅₀ below 1.3 μg·mL⁻¹), reduced cell viability, and induced apoptotic body production, phosphatidylserine translocation, and DNA fragmentation. However, the extract had no effect on BM-MNCs. We postulate that these properties make the extract a good candidate for an anti-tumour agent for clinical use.

INTRODUCTION

Acute myeloid leukaemia (AML) is a neoplastic hematological malignancy characterized by an increase in the number of blast cells in the bone marrow. This increase is due to a blockade in cell differentiation and apoptotic cell death (1). Treatments for AML include chemotherapy, bone marrow transplant, immunotherapy, and molecular target therapy (2). Many of these treatments have secondary effects and severely impair the patient’s quality of life (3). Furthermore, because of the lack of treatment specificity and the development of resistance to anti-neoplastic agents in some leukaemic cells (4), the 5-yr survival rates remain low (45% in young patients and 10% in elderly patients) (5-7). For this reason, several new strategies are being developed, especially those that fulfill the criteria for an ideal therapeutic agent: selective killing of cancer cells with minimal toxicity in normal tissues (8).

Mexico is the principal center of chayote diversity [Sechium edule (Jacq.) Sw.] (9). Studies of the chayote fruit and leaves have reported their diuretic, anti-inflamatory, and hypotensive activities (10-12). Furthermore, this plant aids in eliminating kidney stones, promotes ulcer healing, and relieves intestinal and cutaneous inflammation (9). S. edule contains peroxidases, sterols, alkaloids, saponins, phenols, polyphenols, flavonoids, and cucurbitacins (9,13-15). There are several chayote varieties in Mexico. Of these, 8 varieties have...
been registered in the Mexican National Catalogue of Plant Varieties (CNVV in Spanish), which is managed by the Mexican National Seed Inspection and Certification Service (SNICS in Spanish) (16). Fruit extracts from each of these 8 varieties have antiproliferative activity in tumor cell lines (17). Recently, a hybrid referred to as H-387-07-GISEM® was generated from Sechium edule varietal grup wild type II chayote (18). This hybrid was registered at the CNVV-SNICS by the Mexican Interdisciplinary Research on Sechium edule (GISEM in Spanish). It is unknown whether this hybrid is similar to its parental varieties with respect to anti-proliferative activity in leukaemic or normal cells. Therefore, the present study assessed the antiproliferative activity of the H-387-07-GISEM® hybrid in the murine leukaemic macrophage cell lines P388 and J774, the myelomonocytic leukaemia cell line WEHI-3, and normal murine bone marrow cells.

MATERIALS AND METHODS

Plant Material and Extraction

H-387-07-GISEM® hybrid fruits at horticultural maturity were used in this study. These fruits were obtained from a mesophyll mountain forest with an altitude of 1340 m, 80 CNVV 85% ambient humidity, well-drained, slightly acidic soil (pH 6.5) that was rich in humus (19), a mean annual precipitation of 2000 mm, up to a 12-h photoperiod with respect to flower bud initiation, and a temperature of 20°C. The fruits were cut into small pieces and dried for 3 days at 50°C until they reached 10% humidity. The fruits were then reduced to a standard 2-mm particle size using no. 10 mesh. Subsequently, 800 g of this plant material was subjected to discontinuous extraction in methanol (99.8%, ACS; Merck, NJ) for 48 h at room temperature (20°C ± 2). The tissue residue was removed, and the solvent was evaporated under vacuum (Rotavapor R-114; Büchi, Switzerland) until a crude extract that was free of organic solvent was obtained (20).

Extract Dilution

A total of 71.2 mg of alcoholic crude extract was solubilized in 40 µL of absolute ethanol and 960 µL of phosphate-buffered saline (PBS; Sigma, St. Louis, MO). The mixture was centrifuged at 500 g for 5 min (Hermle-Labortecnik, Germany), and the supernatant was subsequently filtered and stored at 4°C.

Cell Line Maintenance

Assays were conducted with the murine leukaemic cell lines P388 (macrophagic), J774 (monocytic), and WEHI-3 (myelomonocytic). All cell lines were purchased from the American Type Collection Culture (ATCC; The Global Bioresource Center, Manassas, VA). The cell lines were maintained in continuous proliferation in a CO2 incubator (Thermo Forma Scientific, Marietta, GA) at 37°C with 5% CO2 and 95% humidity. The cells were grown in Iscove’s Modified Dulbecco’s Medium (IMDM) (Gibco BRL, Grand Island, NY) supplemented with 10% foetal bovine serum (FBS) (Gibco BRL, Grand Island, NY), which was previously inactivated at 56°C, and re-seeded every 48 h (21).

Assessment of Antiproliferative Activity in Leukaemic Cell Lines

The P388, J774, and WEHI-3 cell lines were grown in 96-well plates at initial densities of 2, 1.8, and 0.3 × 104 cells·mL⁻¹, respectively, with or without the addition of different concentrations of the hybrid extract. In all case we used 0 µg.L⁻¹ (20ul/well of 1:5000 ethanol/PBS) as vehicle or control. As a positive control for the inhibition of cell proliferation, 5 µM cytarabine (Ara-C) was used as an antineoplastic agent. After 72 h in culture, the cells were fixed with 1% glutaraldehyde for 1 h, and a crystal violet dye solution was added (Sigma, St. Louis, MO) to label the nuclei (22) and quantify the cell number using a plate reader at 570 nm (Spectra; Tecan, Austria) (23). The data were plotted, and the IC50 was obtained using a linear regression equation.

Assessment of Antiproliferative Activity in Normal Bone Marrow Cells

Clinically healthy, 2- to 3-month-old female mice of the CD1 strain were sacrificed by cervical dislocation to obtain their femurs. Whole bone marrow cells were harvested by flushing the marrow content with IMDM supplemented with 10% FBS. Subsequently, the mononuclear cells of bone marrow (BM-MNCs) were separated using a density gradient of Ficoll-Paque at 1.077 g·mL⁻¹ (Sigma, St. Louis, MO). These BM-MNCs were cultured at a density of 1 × 10⁵ cells·mL⁻¹ in 96-well plates (Corning Costar, Cambridge, MA) using IMDM supplemented with 10% FBS and 5% horse serum (Gibco BRL, Grand Island, NY); recombinant murine interleukin-3 (eBiobioence, San Diego, CA) was used as a cell proliferation inducer. After 48 h in culture, the cells were either stimulated with different doses of extract or left untreated as a negative control. The cells were then harvested, and cell proliferation was assessed.

Viability Assessment

The cells were maintained under the aforementioned conditions and stimulated with the IC50 dose that was determined for each cell line or the BM-MNCs. After the indicated culture times, cell viability was measured using the trypan blue dye exclusion test (Sigma, St. Louis, MO) (24,25).

Apoptosis Detection

The P388, J774, and WEHI-3 cell lines were cultured, and apoptosis was determined by either assessing the formation of
apoptotic bodies via Giemsa staining, the presence of phosphatidylserine via annexin V staining, or DNA fragmentation analysis.

**Apoptotic Bodies**

Cells treated with their respective IC₅₀ doses were fixed with absolute methanol, stained with Giemsa (Sigma, St. Louis, MO) and examined under a BH-2 light microscope (Olympus, Tokyo, Japan) at 100× magnification. Morphological changes, such as decreased cell volume, chromatin condensation, abnormal cell membranes, and chromatin condensation, were indicative of apoptosis (26,27). A total of 200 cells were counted for each experiment, and the presence of apoptotic bodies was evaluated.

**Annexin V Staining**

Translocation of phosphatidylserine molecules from the inner to the outer layer of the cell membrane was detected with an annexin V-FITC kit (BD Biosciences, San Jose, CA). This phenomenon is representative of early apoptotic stages (28–31). Briefly, the cells were washed and incubated with FITC-labelled annexin V for 15 min, and the samples were analyzed by flow cytometry (FACSARia II; BD Biosciences, San Jose, CA).

**DNA Fragmentation**

Oligonucleosomal DNA fragments in multiples of 180 to 200 base pairs are indicative of cells undergoing late-stage apoptosis, and this fragmentation is visible by electrophoresis (32). The P388, J774, and WEHI-3 cell lines as well as the BM-MNCs were incubated either with or without the hybrid extract or Ara-C. Subsequently, 2 × 10⁶ cells were incubated in 300 μL of lysis buffer containing proteinase K (Promega, Madison, WI) at 37°C for 4 h. After lysis, 7.5 μL of RNase (Sigma, St. Louis, MO) was added, and the samples were incubated for 1 h at 37°C. The DNA was extracted using 300 μL of a chloroform-phenol-isomyl alcohol mixture, precipitated with cold isopropanol, and hydrated in Tris-EDTA (TE) buffer. The DNA extract was separated on a 1.5% agarose gel (UltraPure™, Invitrogen, Carlsbad, CA) in Tris-borate-EDTA (TBE) buffer using an electrophoresis chamber (Gibco BRL, Grand Island, NY) at 100 V. Finally, the gel was analyzed using a transilluminator (Foto/Eclipse™, FotoDyne, Hartland, WI), and the DNA fragments were evaluated.

**Statistical Analysis**

The data are presented as the mean ± SD of at least 3 independent experiments performed in triplicate. Variance analysis with a significance level of $P \leq 0.05$ and Tukey’s test were applied to all data sets, with extract and concentration as variables. SAS® version 9.0 software (SAS Institute, Cary, NC) was used.

**RESULTS**

A Fruit Extract from the Hybrid H-387-07-GISEm® Shows a 500-Fold Greater Inhibition of P388 Cell Proliferation Compared with Fruit Extracts from its Progenitors

It was recently shown that fruit extracts from the bernynano® and wild type *Sechium edule* varieties inhibit the proliferation of tumor cell lines (17). In this work we cultured P388 cells in the presence of extracts from these varieties at 0, 40, 200, 400, 1200, and 2500 μg·mL⁻¹ for 72 h and observed a dose-dependent growth inhibition (Fig. 1).

An extract from the hybrid H-387-07-GISEm®, which was obtained from crossing bernynano® and wild type *Sechium edule*, was cultured with P388 cells in doses of 0, 0.07, 0.15, 0.3, 0.6, 1.2, 2.5, and 5.0 μg·mL⁻¹. We also observed a significant dose-dependent inhibition of proliferation with this extract (Fig. 2). Surprisingly, the extract from the hybrid showed a 500-fold greater inhibition of P388 cell growth compared with the extracts from either of its parental types.

A Fruit Extract from the Hybrid H-387-07-GISEm® Inhibits the Proliferation of the J744 and WEHI Leukaemic Cell Lines but Has Less Activity on Normal Bone Marrow Cells

To evaluate whether the inhibitory effect found on P388 cells by the H-387-07-GISEm® extract can be extrapolated to other leukaemic cell lines, the same concentrations were added to cultures of the J774 and WEHI-3 cell lines, which are derived from monocytic and myelomonocytic leukaemia, respectively. Our results indicate that this extract also reduces the proliferation of J774 and WEHI-3 cells in a dose-dependent manner (Fig. 2). We determined that the J774 and

**FIG. 1.** Proliferation of the P388 leukaemic cell line after 72 h of exposure to 0, 40, 200, 400, 1200, and 2400 μg·mL⁻¹ of fruit extracts from the bernynano® and wild type *Sechium edule* varieties. Each value is the mean ± SD of 3 separate experiments performed in triplicate. *Significant difference relative to the vehicle (0 μg·mL⁻¹) (Tukey's test, $P \leq 0.05$).
WEHI-3 cells were far more sensitive to the extract compared with the P388 cells. In fact, the proliferation of the WEHI-3 cells was completely inhibited at 1.2 μg·mL⁻¹, which was similar to the effect of the positive control, 1.2 μg·mL⁻¹ Ara-C, an antineoplastic drug that is commonly used in research and as a therapy for AML patients (33). When normal bone marrow cells were cultured with the different doses of the extract, their proliferation was also inhibited; however, this inhibition was less than that observed in the leukaemic cell lines (Fig. 2).

We calculated the IC₅₀ values for the hybrid extract using the P388, J774, WEHI-3 and normal bone marrow cells with the aim of using these values to evaluate apoptosis, and we obtained values of 1.3, 0.6, 0.5, and 2.5 μg·mL⁻¹, respectively. When we used these concentrations to evaluate cell viability, we found that only the normal bone marrow cells were unaffected by the extract, whereas the cell lines were affected to various extents. The viability of the P388 cells was the most affected, with 25% non-viable cells (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>IC₅₀ (μg·mL⁻¹)</th>
<th>Viability (%)</th>
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</thead>
<tbody>
<tr>
<td>P388</td>
<td>1.38 ± 0.1</td>
</tr>
<tr>
<td>J774</td>
<td>0.51 ± 0.02</td>
</tr>
<tr>
<td>WEHI-3</td>
<td>0.51 ± 0.09</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>2.56 ± 0.11</td>
</tr>
</tbody>
</table>

*Significant difference relative to the vehicle (0 μg·mL⁻¹). Tukey’s test, P ≤ 0.05.

All of which were similar to those obtained with the positive control Ara-C (Fig. 3A). We found that only 13% of the J774 cells showed apoptotic bodies; however, this number was more than 50% in the P388 and WEHI-3 cells, which was similar to the results obtained with Ara-C (Fig. 3B), a classic inducer of apoptotic bodies (35, 36).

Once apoptotic bodies were detected, we subsequently evaluated the presence of phosphatidylserine, a marker of early apoptosis, in the cytoplasmic membranes of these cells. Our results showed that a significant number (50%) of the cells were positive for this marker, indicating the presence of apoptosis (Fig. 4A). Interestingly, there was no increase in the number of phosphatidylserine-positive cells when normal bone marrow cells were cultured with the hybrid extract at their IC₅₀ value. Our results thus indicate that the hybrid extract inhibits the proliferation of bone marrow cells but does not induce apoptosis in these cells as it does in leukaemic cells (Fig. 4A).

DNA fragmentation is considered to occur during late-stage apoptosis (32, 37). To establish whether the H-387-07-GiseM hybrid extract could induce this type of DNA fragmentation, we cultured P388, J774, WEHI-3, and normal bone marrow cells at their respective IC₅₀ values for 72 h. We used Ara-C as a positive control and a culture without inducers as a negative control. Our results show that all of the extract-treated cell lines underwent DNA fragmentation similar to that induced by Ara-C, whereas the extract did not induce fragmentation in the bone marrow cells (Fig. 4B).

### DISCUSSION

Many plants in Mexico are used in traditional medicine, and some of them, such as chayote (S. edule Jacq.) Sw. are used to treat cancer (38). We recently showed the antiproliferative effect of extracts from several chayote varieties on tumor cell lines (17). In this work, we demonstrated that a fruit extract of the chayote hybrid H-387-07-GiseM inhibits the proliferation of the leukaemic macrophage cell line P388, and this inhibition is 500-fold greater than that of the extracts from its parental varieties. This activity was also observed in monocytic (J774) and myelomonocytic (WEHI-3) leukaemic cell
SECHIUM EDULE HYBRID INDUCE APOPTOSIS IN LEUKAEMIC CELLS

A

P388

J774

WEHI-3

B

Apoptotic body (%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P388</th>
<th>J774</th>
<th>WEHI-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>0.5 ± 0.71</td>
<td>0 ± 0</td>
<td>0.5 ± 0.71</td>
</tr>
<tr>
<td>H-837-07-GISeM</td>
<td>65 ± 9.90*</td>
<td>13 ± 4.2*</td>
<td>53 ± 9.4*</td>
</tr>
<tr>
<td>Ara-C</td>
<td>51 ± 6.36*</td>
<td>72 ± 4.2*</td>
<td>61.5 ± 8.2*</td>
</tr>
</tbody>
</table>

FIG. 3. Apoptotic bodies in the P388, J774, and WEHI-3 cell lines treated with or without extract. A: Apoptotic bodies analysed by light microscopy at 100x magnification. 1: Control cells; 2: cells treated with their respective H-387-07-GISeM IC₅₀ dose; 3: cells treated with 5 μM Ara-C as a positive control for the induction of apoptosis. † Indicates apoptotic bodies. B: Percentage of apoptotic bodies. *Significant difference relative to the vehicle (0 μg.mL⁻¹) (Tukey’s test, P ≤ 0.05).

A

[Graph showing percentage of annexin V-FITC-positive cells]

B

[Graph showing DNA fragmentation analysis]

FIG. 4. Induction of apoptosis in the P388, J774, and WEHI-3 leukaemic cell lines and mononucleated bone marrow cells (BM-MNCs) treated with or without the H-387-07-GISeM extract (IC₅₀ dose) or Ara-C. A: Percentage of annexin V-FITC-positive cells treated with their respective IC₅₀ dose of the H-387-07-GISeM hybrid extract. B: DNA fragmentation analysed by agarose gel electrophoresis. bp, molecular weight marker ((0000-250); 1: control cells; 2: cells treated with 5 μM Ara-C; 3: cells treated with their respective IC₅₀ dose of the H-387-07-GISeM hybrid extract. *Significant difference relative to the vehicle (0 μg.mL⁻¹) (Tukey’s test, P ≤ 0.05).
lines. Interestingly, although the extract had a somewhat lower inhibitory activity against BM-MNCs, it did not induce cellular death as it did in leukemic cells. This finding indicates that this extract has a specific effect on leukemic cells. We also observed that this specific induction of cell death in leukemic cells is apoptotic in nature. In fact, the hybrid extract induced the formation of apoptotic bodies in the J774, P388, and WEHI-3 cell lines to a level similar to that of Ara-C, which is a classic inducer of apoptotic bodies (36,37). We also detected phosphatidylserine translocation to the cytoplasmic membrane of these cells, which is an early apoptotic event (32), and we observed DNA fragmentation later on. Once again, we did not detect any increase in these apoptotic characteristics when the hybrid extract was used on BMMNCs, and considering that AML is a necrotic hematological malignancy characterized by a blockade in cell differentiation and apoptotic cell death (1), the chayote hybrid extract could prove to be useful for the induction of apoptosis in malignant cells, sparing their normal equivalents in the marrow of patients with AML.

Natural compounds, such as crude extracts, and pure molecules are considered to have anticancer therapeutic value when their IC_{50} values are below 20 μg·mL^{-1} and 4 μg·mL^{-1}, respectively (39). Thus, the fact that our hybrid extract presented IC_{50} values of less than 1.3 μg·mL^{-1} in several leukemic cell lines indicates its possible therapeutic application. The IC_{50} value of less than 1.3 μg·mL^{-1} compares well with other published natural products that presented significantly higher values; these include Terminalia catappa L., which inhibits the proliferation of a human lung carcinoma cell line (A549) at concentrations greater than 100 μg·mL^{-1} (40), phenolic extracts from Pinus massoianana Lamb., which inhibit the growth of breast cancer cell lines (MCF-7 and HELF) at concentrations ranging from 3.5 to 500 μg·mL^{-1} (41), and leaf extracts from Polyalthia longifolia (Sonnn.) Thw., which show anticancer potential in the human colon cancer cell line SW-620 with an IC_{50} of 6.1 μg·mL^{-1} (42).

The inability of the hybrid extract to induce apoptotic death in BM-MNCs endows it with an additional advantage over other plant-derived compounds used in leukemia therapy that are also toxic to normal cells, such as vinca alkaloids, epipodophyllotoxins, taxanes, camptothecins, homoharringtonine, and ellipticine (4,43–45). Selective cytotoxicity toward tumor cells has already been shown for parthenolide, a sesquiterpene lactone that induces apoptotic cell death in leukemic cells but not in normal cells (46). Here, we have shown that an H-387-07-GISEm extract has a similar activity Ara-C and also exhibits the features of an ideal therapeutic agent (i.e., it selectively kills cancer cells and has minimal toxicity in normal tissues) (8).

Characterization of the phytochemical content of the hybrid crude extract is needed to determine its possible anticancer components. It is possible that the cucurbitacins or flavonoids is involved, because both exert antitumor activity (47–51) and both are present in S. edule (14, 9). Nevertheless, care should be taken because its biological activity could be due to a combination of different molecules with anti-cancer properties. In fact, recent studies suggest that crude extracts have a greater anti-tumour activity than their isolated active ingredients. Furthermore, the extract could also act as an adjuvant to classical antineoplastic compounds while decreasing their secondary effects (52).

In conclusion, a crude extract from the H-387-07-GISEm hybrid inhibits the proliferation of the P388, J774, and WEHI-3 leukemic cell lines with 500-fold greater efficacy than that of the extracts from either of its parental species, with an IC_{50} below 1.3 μg·mL^{-1}; in comparison, its IC_{50} is greater than 2.5 μg·mL^{-1} in normal bone marrow cells. This decrease in proliferation was due to the induction of apoptotic cell death in all 3 leukemic cell lines, which was not observed in normal bone marrow cells. Therefore, this extract has good potential for use in leukemia treatment because it can selectively kill cancer cells with minimal toxic effects on their normal counterparts.

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REFERENCES


