Cytotoxic effect and apoptotic activity of Parmentiera edulis DC. hexane extract on the breast cancer cell line MDA-MB-231

Cynthia Carolina Estanislao Gómeza, Cynthia Ordaz Pichar晓得, Eduardo San Martín Martínezb, Nury Pérez Hernándezc, Guillermo Pérez Ishiwaraa, María del Consuelo Gómez Garcíaa


INTRODUCTION

The worldwide use of natural products including medicinal plants has become increasingly important in primary health care. Natural products are excellent alternatives for therapeutics, particularly in developing countries, because of their relatively well-established safety profile (Graham et al., 2000; Da Rocha et al., 2001; Moura and Silva, 2002; Cragg and Newman 2005a,b). Phytochemicals isolated from herbs have emerged as a new and promising source of anticancer remedies, or as adjuvants for chemotherapeutic drugs, to enhance their efficacy and decrease side effects (De Vita et al., 2001; Balunas and Kinghorn, 2005; Newman and Cragg, 2007; Newman, 2008; Bailly, 2009). Cancer is responsible for approximately 25% of deaths in developed countries and 15% of all deaths worldwide (Forlay et al., 2013). Specifically, breast cancer is the leading cause of cancer-related death in women worldwide suggesting that further improvement in therapy is needed because the treatments are currently based on a combination of surgery, radiation, hormonal therapy and chemotherapy.

However, to date, there have been no completely successful intervention options developed as such, new treatments derived from traditional medicine, medicinal chemistry and rational drug design are necessary. Mexico is a country with extensive flora diversity, and the traditional use of various medicinal plants is common across the country (Laza et al., 2003).

Many people use medicinal plants to treat different diseases, including infections, cancer and multiple types of inflammation. Parmentiera edulis DC is a medicinal tree from Mexico that grows up to 5 meters tall and belongs to the Bignoniaceae family.

It grows at an altitude range of 2 – 2240 meters above sea level in the northern central mountain region of Tamaulipas and is commonly called “cuajilote” (Pennington and Sarukán, 2005; Perez et al., 1998; Rzedowsky and Rzedowsky, 2001). People in the local community use an infusion of fruits but other parts of the plant as the bark are also used to treat diabetes, gastroenteritis, flu, cough and fever in addition to breast cancer (Perez et al., 1998, Hernandez-Galicia et al., 2002; Perez et al., 2000; Sorela 2006).
To date only the hypoglycemic activity of *P. edulis* fruit has been reported (Perez et al., 2000). Therefore, the medicinal properties of this plant against other diseases as cancer have been no studied. Therefore, the main aim of this study was to evaluate the cytotoxic effect of different extracts of *P. edulis* on breast carcinoma cells (MDA-MB-231 cell line) and determine the mechanisms by which they induce cell death. Our findings revealed that the hexane extract inhibited the growth of MDA-MB-231 cells but did not affect the MCF10A epithelial breast cells or lymphocyte primary culture. The immunohistochemical analysis showed that this extract induced apoptosis via the mitochondrial intrinsic pathway. These combined observations suggest that the hexane extract from *P. edulis* shows potential anti-cancer activity in breast cancer cells.

**MATERIAL AND METHODS**

**Plant material**

*P. edulis* stem bark was collected from Gomez Farias, Tamaulipas in the spring of 2009. The plant was identified as *P. edulis* D. C. by Maria Patricia Jacquez Rios of the Facultad de Estudios Superiores Iztacala (FESIztac), UNAM, Mexico. A representative specimen (number 1766) is available at the institute’s Iztac Herbarium.

**Preparation of *P. edulis* extracts**

The bark of *P. edulis* was cut into small pieces, and 25 g were extracted with 250 ml of water, acetone or hexane for 96 h at room temperature. Subsequently, each extract was filtered and concentrated using a rotary evaporator under vacuum conditions to obtain a final residue. The yield of the dried residue for the water, acetone and hexane extract was 25, 2 and 9 percent, respectively. Each extract was dissolved in 0.1% DMSO and diluted with DMEM (Dulbecco’s Modified Eagle Medium, Gibco) media to the desired final concentration. Stock solutions were stored at -20°C.

The *P. edulis* extracts used for experimentation ranged in concentration from 25 to 350 μg/ml.

**Phytochemical screening of the extracts of *P. edulis***

The chemical constituents in the different extracts were screened using chemical protocols and methodologies, as previously described (Shrivastava et al., 2013). Plant extracts were screened for the presence of sugars, carbohydrates, glycosides, flavonoids, tannins, coumarins, alkaloids, saponins, sesquiterpenes and quinones.

**Cell culture**

MDA-MB-231, a breast adenocarcinoma cell line, was cultured in DMEM, supplemented with 5% fetal bovine serum (Invitrogen). MCF10A, an epithelial mammary cell line, was cultured in DMEM-F12 supplemented with 10% fetal bovine serum, 100 mg/ml EGF, 100 mg/ml insulin and 1 mg/ml hydrocortisone. Cells were maintained in 75 cm² flasks in a humidified incubator at 37°C with 5% CO₂ (Debnath et al., 2003).

Furthermore, human peripheral blood lymphocytes were isolated and cultured as described previously (Hofman et al., 1982). Briefly, after diluting blood with PBS, the lymphocytes were isolated by centrifugation over a lymphocyte-specific density gradient using a Hystopaque-1077 solution (Sigma) for 30 min at 400 g at room temperature. The layer containing mononuclear cells was then aspirated, and isolated cells were washed twice with PBS. The cells were suspended in complete RPMI media (Invitrogen) with 10% fetal bovine serum.

**Cytotoxicity assay: 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay**

The cytotoxicity effects of the different extracts on MDA-MB-231, MCF10A and lymphocytes were determined using an MTT assay as previously described (Youn et al; 2009). Briefly, the cells were plated (7000 cells/well) onto a 96-well plate. Following a 24 h incubation and attachment, the plating medium was removed and replaced with fresh medium with or without extract treatment. The cells were treated with media alone, or different concentrations (25-1000 μg/ml) of aqueous, acetonic or hexane plant extracts for 24, 48 and 72 h at 37°C. Paclitaxel (0.25 μg/ml) was used as a positive control. After incubation, 20 μl of MTT solution (5 mg/ml) was added to each well, and the plates were incubated at 37°C for 4 h. Subsequently, the supernatant was removed and 100 μl of DMSO were added to each well to solubilize the formazan crystals. Absorbance was quantified at 570 nm using an ELISA reader (Labsystem Multiskan Ms). Experiments were performed in triplicate. The results are expressed as the percentage of cell proliferation with respect to the media-treated controls. GraphPad Prism 5.0 software was used to calculate the IC₅₀ values (Loyola and Vazquez, 2006). Also, selective index (SI) value was calculated as the ratio of IC₅₀ of cancer cells to the IC₅₀ of normal cells.

**Cell Morphology Analysis**

Cells were stained with hematoxylin and eosin to qualitatively analyze their morphological characteristics (Stander et al., 2009). MDA-MB-231 cells (1x10⁴) were seeded on 6-well plates. After 24 h of incubation, the medium was removed and the cells were treated with hexane extract (94.63 ± 1.63 μg/ml), Paclitaxel (0.25 μg/ml) or media alone for 6, 12, 24, 48 and 72 h. After incubation, the cells were washed in PBS and fixed using a 4% paraformaldehyde solution. The cells were then stained with hematoxylin and eosin and analyzed under a light microscope (Nikon Eclipse TE300).

**Annexin V-FITC binding assay**

Apoptotic cells were detected using an Annexin V-FITC Apoptosis Detection Kit (Biovision) following the manufacturer’s instructions. Briefly, MDA-MB-231 cells (1x10⁴) were seeded and allowed to proliferate for 24 h. The medium was then removed and cells were treated with medium alone, Paclitaxel (0.25 μg/ml) or hexane extract (94.63 ± 1.63 μg/ml). After 6, 12, 24 and 48 h of treatment, cells were harvested by trypsinization, centrifuged,
washed in PBS and re-suspended in binding buffer. Subsequently, 5 µl of Annexin V-FITC and 5 µl of propidium iodide were added, and the samples were incubated at room temperature for 5 min. Cells were then analyzed using flow cytometry (FACScan, Beckton Dickinson Cytometer).

Terminal dUTP nick end labeling (TUNEL)

A TUNEL assay was also used to detect apoptosis by analyzing DNA fragmentation with the In Situ Cell Death Detection Kit AP (Roche) according to the manufacturer’s instructions. Briefly, MDA-MB-231 cells (1x10^5) were cultured on cover slips for 24 h; after incubation, the cells were treated with hexane extract (94.63 ± 1.63 µg/ml), Paclitaxel (0.25 µg/ml) or media alone for 6, 12, 24 and 48 h. The cells were washed with PBS, fixed with 4% paraformaldehyde solution, and immersed in permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) for 30 min at 4°C and washed with PBS. The cells were then incubated in kit’s solutions A and B for 60 min at 37°C in the dark and finally washed in PBS and examined under a fluorescence microscope (Nikon diaphot 200) supported with a laser scanning confocal imaging system (MCRR 1024).

Western blotting analysis

MDA-MB-231 cells were cultured (1x10^5) for 24 h. After incubation, the cells were treated with medium alone, Paclitaxel (0.25 µg/ml) or hexane extract for 6, 12, 24 and 48 h. The cells were then washed with PBS, harvested by trypsinization, centrifuged, and the pellet was re-suspended in ice-cold lysis buffer (50 mM/L Tris- HCl, 150 mM/L NaCl, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 1 mmol/L phenylmethylsulfonyl fluoride, 100 µg/L leupeptin, and 2 µg/L aprotinin) containing a protease inhibitor cocktail.

The protein lysates were centrifuged at 13,300 rpm for 5 min at 4°C to remove insoluble material and the protein concentration in the supernatants was determined using the Bradford protein quantification assay. Protein lysates were subjected to electrophoresis on 15% SDS-polyacrylamide gels and transferred to 0.45 µm nitrocellulose membranes. The membranes were stained with 0.2% Ponceau S red to ensure equal protein loading and transfer. After blocking the membranes with 5% milk in PBS-Tween, they were incubated with primary rabbit polyclonal antibodies targeting procaspase -3 (1:200, Santa Cruz, CA), procaspase-8 (1:200, Santa Cruz, CA), procaspase-9 (1:200, Santa Cruz, CA) or β-actin (1:2000, Santa Cruz, CA) as a positive control overnight at 4°C. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibody (1:2000), and the immunocomplexes were visualized by ECL western blotting detection reagents. All experiments were performed in triplicate.

Statistical analysis

All data are expressed as the means ± S.E. One-way ANOVA followed by Tukey’s tests were used to compare all groups to each other. For all tests, p < 0.05 was considered significant.

RESULTS AND DISCUSSION

The development of new therapeutic strategies, such as integrative and complementary medicine, for breast cancer treatment is greatly needed. Medicinal plants are very important in folk and traditional medicine across many countries. Specifically in Mexico, plants have been utilized in traditional medicine since pre-Hispanic times (Alonso-Castro et al., 2011; Waizel, 2012). P. edulis is a plant that has been used to treat cancer in the different communities of Tamaulipas, Mexico. However, the bioactivity of P. edulis extracts has not been established. Thus, we investigated the cytotoxic activity of different P. edulis extracts against breast cancer cells by assessing both cellular and molecular changes.

Effects of P. edulis hexane extract on the MDA-MB-231 breast cancer cell line

The cytotoxicity activity of P. edulis extracts against MDA-MB-231 breast carcinoma cells was evaluated using MTT assays. To this end, the metastatic cell line MDA-MB-231 was cultured with medium alone, DMSO 0.2% (negative control), Paclitaxel (0.25 µg/ml; positive control) or aqueous, acetone hexane plant extracts at different concentrations (25-350 µg/ml) for 24, 48 and 72 h.

The viability of negative control cells was considered the 100% mark, whereas the viability of cells exposed to the positive control, Paclitaxel, decreased by 35.25 % after 72 h of treatment. The aqueous extract did not show any cytotoxic activity at any concentration or at any time, while the acetone extract inhibited only 26 % of cell proliferation after 72 h of incubation (data not shown). In contrast, cytotoxic effect was most pronounced when cells were treated with the hexane extract (Fig. 1). The viability of cancer cells treated with 25 or 50 µg/ml of hexane extract for 72 h was reduced by 19.55% and 25.95%, respectively. Moreover, the decreases in viability were reduced by 94.2% when treated with the highest concentration, 350 µg/ml of extract after 72 h. Therefore, the hexane extract showed both a time and dose-dependent inhibition of MDA-MB-231 cell proliferation. The IC50 value obtained for hexane extract was 94.63 ± 1.63 µg/ml.

Because the hexane extract showed the most pronounced effect in initial experiments, additional cell types, such as a primary lymphocyte culture and the normal MCF10A epithelial mammary cell line, were treated with different concentrations of the hexane extract (25-1000 µg/ml) for varying durations (24, 48 and 72 h) to ensure that the effect of the hexane extract was specific to cancer cells. Interestingly, both cell lines were significantly less sensitive to P. edulis extracts, showing IC50 values of 926.8 ± 2.8 (MCF10A) and 864.5 ± 2.9 µg/ml (lymphocyte culture) (data not shown). On the other hand, the selective index value of hexane extract against MDA-MB-231 and MCF10A cells was 0.10.
This finding indicated that normal cells were less sensitive to the cytotoxicity of *P. edulis* hexane extract, probably due to membrane or cytoskeleton proteins, genetic changes, motility or cell surface differences between cancer and normal cells (Agus et al., 2013). These results indicated that the cytotoxic components present in the hexane extract of *P. edulis* showed a selective and specific cytotoxic action against tumor cells. Based on these results, the hexane extract was chosen for further investigation in subsequent experiments.

**Morphological analyses of MDA-MB-231 cells treated with hexane extract**

To investigate if the cytotoxicity induced by the *P. edulis* hexane extract was produced by a specific apoptosis or necrosis process, the cells were exposed to hexane extract and were, then stained with H&E and analyzed under a light microscope. Untreated cells appeared to be elongated, forming a confluent colony and attaching to the plate (Fig. 2A). In contrast, important changes in the morphology and density of cells were observed after treatment with 94.63 µg/ml of hexane extract for 6, 12, 24 and 48 h (Fig. 2C-F). After 6 h of treatment, changes, such as cell size reduction and loss of colony formation were observed. These cellular changes increased over time, and other morphological characteristic changes of apoptosis, such as chromatin condensation and the formation of apoptotic bodies, were also observed (Fig. 2D-F). After 48 h of treatment, the cells appeared rounded and detached, with clear membrane blebbing and presence of apoptotic bodies. Similar morphological characteristics were observed in the cells exposed to Paclitaxel, in which the apoptotic process has been previously described (Fig. 2B) (Ofir et al., 2002).

**Cell death analyses by Annexin V-FITC binding assay**

To confirm and quantify apoptosis, MDA-MB-231 cells were treated with hexane extract, incubated with Annexin V–FITC/PI and then analyzed by flow cytometry (Fig. 3). These assays revealed the percentage of apoptotic and necrotic cells observed under different treatment conditions. Untreated cells showed a high percentage of viable cells (97.0 %) and a low percentage of cell death (3 %) (Fig. 3A); in contrast, cells treated with Paclitaxel demonstrated a viability of 4.9 %, with 7.8 % of cells that were necrotic and 87.3% that were apoptotic (Fig. 3B). Moreover, the number of viable cells decreased after treatment with 94.63 µg/ml of hexane extract for 6, 12, 24 and 48 h, while the corresponding percentage of apoptotic cells increased in a time-dependent manner (Fig. 3C-G). These findings indicated that the cell death due to the hexane extract was primarily the result of apoptosis, resulting in 50.15% dead cells after 48 h of treatment. These observations were consistent with the previously detected morphological changes. Our results indicated that the hexane extract mainly activated apoptosis rather than necrosis. Necrosis is characterized by cytosolic swelling, early loss of plasma-membrane integrity and organelle breakdown (Festjens et al., 2006). Anticancer drugs, such as cisplatin or Paclitaxel reportedly induce two different pathways of cell death, necrosis and apoptosis, in different proportions (Liao and Lieu 2005; Gonzalez et al., 2001). The assays indicated that the hexane extract of *P. edulis* promoted apoptotic cell death and decreased the proliferation of MDA-MB-231 cells.

**Terminal dUTP nick end labeling (TUNEL)**

To evaluate DNA fragmentation, a TUNEL assay was performed in MDA-MB-231 cells treated with 94.63 µg/ml of *P. edulis* extract for various times (6, 12, 24, 48 and 72 h). The results revealed that cells treated with the hexane extract displayed activation of DNA fragmentation in a time-dependent manner (Fig. 4D-H), as TUNEL staining increased over the time compared with the negative control (Fig 4A). Furthermore, apoptotic cell death was observed in MDA-MB-231 cells after DNAse or Paclitaxel treatment (Fig. 4B-C, respectively). Combined with the data from the microscopy and Annexin V-FITC assays, these findings show that *P. edulis* hexane extract specifically inhibited the proliferation of human breast cancer cells, primarily by inducing apoptosis rather than necrosis, while no effect was observed in normal MCF10A cells (data not shown).
Fig. 2: Hematoxylin and eosin staining of the MDA-MB-231 cell line. A, Control cells with medium alone; B, cells treated with 0.25 µg/ml of Paclitaxel; C, D, E and F, cells treated with 94.63 µg/ml hexane extract for 6, 12, 24 and 48 h, respectively (all at 40 x magnification). Cells exposed to hexane extract showed important cellular changes that increased with the treatment period. These changes, such as size reduction, membrane blebbing and apoptotic bodies, are evident 48 h after treatment. These observations were consistent with the cellular viability detected by the MTT assays.

Fig. 3: Flow cytometry analyses of MDA-MB-231 cancer cells stained with Annexin V-FITC/PI. A, cells treated with medium alone; B, cells exposed to 0.25 µg/ml Paclitaxel; C, D, E and F, cells treated with 94.63 µg/ml hexane extract for 6, 12, 24 and 48 h, respectively; G, The percentage of dead cells (apoptosis and necrosis) in the control cells and cells treated with hexane extract for differing times. The percentage of cell death is represented as the mean values ± SE for at least three experiments. Significance was analyzed using a one-way ANOVA followed by Tukey’s test.

Fig. 4: TUNEL assays of the MDA-MB-231 cell line. A, Negative control cells; B, Cells treated with DNase (3000 U/ml); C, cells treated with Paclitaxel 0.25 µg/ml; D, E, F and G, cells exposed to 94.63 µg/ml of P. edulis hexane extract for 6, 12, 24, 48 and 72 h, respectively (all at 63 x magnification). Arrows indicate the nucleus of the apoptotic cells.
Western blotting analysis

Apoptosis can be divided into two pathways, the intrinsic and extrinsic pathways, which involve the activation of particular caspases that are constitutively expressed during the process (Kuribayashi et al., 2006). To elucidate the possible mechanism of cell death activation, a western blot analysis was performed to evaluate the protein expression of procaspase 3, 8 and 9 (Fig. 5).

Fig. 5: Effect of P. edulis hexane extract on expression of procaspase 3, 8 and 9. MDA-MB-231 breast cancer cells were exposed to the hexane extract at 94.63 μg/ml for 6, 12, 24, 48 and 72 h.

MDA-MB-231 cells were exposed to hexane extract for different times (6, 12, 24, 48 and 72 h) prior to blotting; the results revealed that expression of procaspase 8 (55 kDa inactive protein form) did not change between the negative control cells and those exposed to hexane extract, indicating that the extrinsic apoptosis pathway had not been activated.

In contrast, the expression of procaspase 9 (46 kDa inactive protein form) decreased in cells treated with hexane extract, and was no longer detectable after 24 h, suggesting that the protein had been activated early in treatment.

The expression of procaspase 3 (32 kDa inactive protein form) decreased, starting at 6 h through 72 h post-treatment, while the cleaved form (17 kDa active form) was also detected by 6 h post-treatment; similar results were observed for cells exposed to Paclitaxel. These experiments indicated that the intrinsic apoptosis pathway was activated as a result of the expression and activation of caspases 3 and 9 in response to P. edulis hexane extract exposure. Caspases are proteases that serve as central components of a proteolytic system in the apoptotic process. Caspase 3 is a critical executioner of apoptosis and one of the enzymes responsible for the proteolytic activation of different proteins involved in programmed cell death (Kuribayashi et al., 2006). Additionally, the same activation pathway has been described in studies using other types of plant extracts, including those from Chrysanthemum indicum (Zong-Fang, et al., 2009), Uncaria tormentosa (De Martino et al., 2006) and Cordyceps militaris, among others (Jin et al., 2008).

Phytochemical screening of P. edulis hexane extract

Preliminary phytochemical screening of different P. edulis extracts was then carried out to detect metabolites using standard chemical reagents (Table 1). Our results showed that P. edulis hexane extract is strongly cytotoxic for MDA-MB-231 breast cancer cells, an effect that may be due to the presence of various phytochemicals.

Table 1: Phytochemical screening of different P. edulis extracts.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Test</th>
<th>Acetone Extract</th>
<th>Hexane Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars and Carbohydrates</td>
<td>Fehling’s test, Benedict’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Baljet’s test, Pyridine</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda test, Alkaline reagent test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Gelatin solution, Ferric chloride test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>KOH - ethanol solution</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s reagent, Wagner’s reagent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids and Terpenes</td>
<td>Liebermann - Buchard test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sesquiterpenes</td>
<td>Hydroxylation</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test, Rosenthaler</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quinones</td>
<td>Bornträger test</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ (Present) – (Absent)

However, traditional medicine utilizes an aqueous infusion. Thus, the differences observed in the cytotoxic activity on breast cancer cells between the hexane and aqueous extracts may be due to the extraction method performed, which enriched the concentrations of different bioactive compounds in the hexane extract, such as terpenes and quinones. The preliminary phytochemical characterization of the hexane extract from P. edulis showed the presence of terpenes, quinones and coumarins, which could be involved in the induction of programmed cell death in the MDA-MB-231 cells.

The identification of metabolites in the hexane extract is currently in progress and will allow us to associate the cytotoxic effect with specific chemical components. Interestingly, different types of extracts obtained from trees of the Bignoniaceae family contain similar metabolite profiles (Daniel 2006). Monoterpenes, such as geraniol and limonene, induce 80% tumor regression in murine models of breast cancer (Gould, 1997), while coumarins, such as esculetin, daphnetin and scopoletin, exert strong cytotoxic effects on HL60 and MCF-7 cell lines, inducing morphological changes and activating apoptosis (Chu et al., 2001; Jimenez-Orozco et al., 2011; Kim et al., 2005; Finn et al., 2002). Moreover, quinones, such as doxorubicin, mitomycin c and β-lapachol, are cytotoxic to various cancer cell lines. Quinones can accept electrons and improve the intracellular production of free radicals and reactive oxygen species in the mitochondria. This action induces cellular stress and activates apoptosis (Esteves-Souza et al., 2007).

CONCLUSION

P. edulis, a plant used in traditional medicine in Mexico (State of Tamaulipas), showed specific antiproliferative activity
against the breast cancer cell line MDA-MB-231, and activated apoptosis via the intrinsic pathway. Further investigation is underway to identify and characterize the precise compounds in this extract, which could provide a very promising lead for new therapeutic drug.

AKNOWLEDGEMENTS

This work was supported by the CONACYT project 114028 and by SIP (IPN) projects SIP 20101474, SIP 20113658 and SIP 20120468. We would like to thank Dr. Mario A. Rodríguez and Dra. Patricia Talamas Rohana of the Confocal Microscopy Area from the Departamento de Infectómica y Patogénesis Molecular, CINVESTAV.

REFERENCES


Festjens N, Vanden BT, Vandenabeeke P. Necrosis, a well-orchestrated form of cell demise: signalling cascades, important mediators and concomitant immune response. Biochimica et Biophysica Acta, 2006; 1757:1371-1387.


Kuribayashi K, Mayes PA, El-Deiry WS. What are caspases 3 and 7 doing upstream of the mitochondria? Cancer Biol Ther, 2006; 5; 763–765.


Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 Years. Journal of Natural Products, 2007; 70, 461-477.


Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 Years. Journal of Natural Products, 2007; 70, 461-477.


How to cite this article: