
RESEARCH ARTICLE

Induction of Caspase-9, Biochemical Assessment and Morphological Changes Caused by Apoptosis in Cancer Cells Treated with Goniothalamin Extracted from *Goniothalamus macrophyllus*

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Abstract

Goniothalamin, a natural compound extracted from *Goniothalamus* sp. belonging to the Annonaceae family, possesses anticancer properties towards several tumor cell lines. This study focused on apoptosis induction by goniothalamin (GTN) in the Hela cervical cancer cell line. Cell growth inhibition was measured by MTT assay and the IC₅₀ value of goniothalamin was 3.2±0.72 µg/ml. Morphological changes and biochemical processes associated with apoptosis were evident on phase contrast microscopy and fluorescence microscopy. DNA fragmentation, DNA damage, caspase-9 activation and a large increase in the sub-G1 and S cell cycle phases confirmed the occurrence of apoptosis in a time-dependent manner. It could be concluded that goniothalamin show a promising cytotoxicity effect against cervical cancer cells (Hela) and the cell death mode induced by goniothalamin was apoptosis.

Keywords: Goniothalamin - hela cervical cancer cell line - fluorescence microscopy - DNA fragmentation - DNA damage

Introduction

Currently, almost 510,000 cases of cervical cancer are reported each year with nearly 80% of the cases are reported in the developing countries; 68,000 in Africa, 77,000 in Latin America, and 245,000 in Asia. In Malaysia, cervical cancer is the most common gynecological malignancy and the second most prevalent cause of death among female cancer patients (Zvavahera et al., 1995). Through the recent development of technologies, cervical cancer patients can be treated by surgery, radiotherapy, chemotherapy and immunotherapy. However, the side effect caused by this treatment is severe and affect their quality of the life (WHO, 2012).

The side effects from the cancer therapy are that the treatment can also damage healthy cells and tissue. Apoptosis is the cell death mode that can be seen in multicellular organisms which kills the specific cells and removed it through phagocytosis for the benefit of the organisms. It involved biochemical and physical changes in the cytoplasm, nucleus and plasma membrane. Due to it programmed death mode, the apoptotic bodies which undergo physiological changes in the plasma membrane will enable the recognition of the apoptotic bodies by the phagocytic cells. Since the apoptotic bodies are surrounded by intact plasma membrane, apoptosis usually occur without the leakage of the cell content and without inflammation (Lawen, 2003).

Several medicinal plants had been used traditionally for the prevention and treatment of cancer (Madhuri and Govind, 2009). According to the latest reports, there are about 1200 species of plants in Malaysia that potentially had pharmaceutical or medicinal value (Khatijah, 2006). *Goniothalamus* is one of the medicinal plants that contain substance which possess the ability to control growth of cancerous cell through increasing the cancerous cell death without give the inflammatory effect to the normal cells (Ali et al., 1997; Nasir et al., 2004).

This plant is commonly found in Malaysia and Thailand is from the family Announce. *Goniothalamin*
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is a biologically active styrylpyrone derivative isolated from various Goniothalamus sp. Styryl-lactones is the active compound that able to kill the cancerous cell. Currently, there are approximately 100 styryl-lactones either discovered from natural products or synthetic analogs. These compounds have been demonstrated to be cytotoxic with prefential killing of cancer cells (Lin and Pihie, 2003; Chen et al., 2005; De Fátima et al., 2006; Al-Qubaisi et al., 2011).

Goniothalamin had been able to induce cytotoxicity in a variety of cancer cell lines including gastric (HGC-27), kidney (768-0), breast carcinomas (MCF-7, T47D and MDA-MB-231) and leukemia (HL-60, Jurkat and CEM-SS) (Chen et al., 2005; Rajab et al., 2005; Inayat-Hussain et al., 2010). Goniothalamin has been proved to be only cytotoxic to ovarian cancer cell line (Caov-3) as happened in tamoxifen or taxol treated cells (Lin and Pihie, 2003). In addition, goni oathalamin showed lower toxicity to normal liver Chang cell line as compared to doxorubicin (chemotherapy drug) (Al-Qubaisi et al., 2011). It also represents a novel class of anticancer drug that induces apoptosis in in Hepatoellular carcinoma (Kuo et al., 2011). Goniothalamin is a promising antitumor agent against cancerous cell lines (Wattanapiromsakul et al., 2005). On the other hand a study by Vendramini-Costa et al., 2010 demonstrated that the antiproliferative activity of goni oathalamin in some solid tumor experimental model with no evidence of toxic effects in the animals after single and repeated doses.

This study is carried out to detect apoptosis accrue in Hela cervical cell line induced by goniothalamin via observing the morphological and biochemical features and to study the mechanism of GTN toxicity on Hela cells particularly on the mode of cell death, cell proliferation, DNA damage and caspases activity.

Materials and Methods

Goniothalamin extract. Dried and powdered root (500g) of Goniothalamus macrophyllus were extracted with dichloromethane and concentrated. 50g of brown resin was subjected to silica gel chromatography with gradient of hexane/ethyl acetate (8:2) which gave goniothalamin 5g (colorless crystal), structurally confirmed by comparing of hexane/ethyl acetate (8:2) which gave goniothalamin 5g.

Morphological assessment

Phase Contrast Microscopy. Hela cells at concentration of 1x10^5 cell/ml in 2 ml culture medium containing 10% FBS was seeded into 6 wells plate (NuncIon TM, Nunc) and was treated with Goniothalamin at IC_{50}. Then, the plates incubated in an atmosphere of 5%CO_{2} at 37°C. The morphological changes were observed after 72h by using a phase contrast microscope.

Quantification of Apoptosis Using Propidium Iodide and Acridine Orange Double Staining. Hela cells were quantified using propidium iodide (PI) and acridine-orange (AO) double staining according to standard procedures and examine under fluorescence microscope (Leica attached with Q-Floro Software) (Mishell et al., 1980; Ali, 2011). Hela cells were seeded in 6-well plate and incubated at 37°C in 5%CO_{2} atmosphere. Twenty-four hours later, the medium in each well was removed and replaced with Goniothalamin at IC_{50} concentration dissolved in the culture medium and incubated at 37°C in 5%CO_{2} atmosphere for 72 h. After the incubation period, detached cells in the medium were collected and added back to trypsinized adherent cells. The cell suspensions were washed with PBS and then incubated with 5µl of acridine orange (10µg/ml) and 5µl propidium iodide (10µg/ml) at a ratio of 1:1 in 1ml of cells and centrifuged at 1000rpm/5min. A volume of 10µl of pellet was pipetted on a slide before putting on the cover slip. Within 30 min, the slide was analyzed using fluorescent microscope (Leica, Germany). Each experiment was assayed three times (n=3). Viable, apoptotic and necrotic cells was quantified in a population of 200 cells. The results were expressed as a proportion of the total number of the cells examined. In apoptotic bodies, blebbing of plasma membrane and condensation of chromatin were seen. In contrast, necrotic cells fluorescent red after propidium iodide staining with tiny fraction of chromatin dispersed around the nucleus. This assay provides a useful quantitative evaluation (n=3).
Biochemical assessment

DNA Fragmentation Assay. Cells at a concentration of 5x10^5 cells/ml were seeded into 6-well plate (Nunclon™, Denmark) in 2ml culture medium with a concentration of IC_{50} value of goniotalamin. Some wells were left without treatment to be used as a control. After the 72h of incubation, detached cells in the medium were collected and added back to trypsinised adherent cells. The cells were spun down and the pellet was washed with PBS twice. The DNA extraction from treated and untreated cells was carried out according to the protocol of a kit for Blood and Cultured Cells from QIAGEN.

Single-cell gel electrophoresis Comet assay. The Comet assay was performed under alkaline conditions using the method adapted from Singh et al. (1980) as described previously by Gedik et al. (1992) and Collins et al. (1997). In brief, Hela cells with goniotalamin treatment were suspended in 1% low melting point agarose in PBS, pH 7.4, at 37°C, and 100 µl were pipetted onto a frosted glass microscope slide pre-coated with an 100 µl layer of 1% normal melting point agarose. The agarose was allowed to set on ice for 10 min and the slide immersed in lysis solution (2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris, NaOH to pH 10.0, and 1% Triton X-100) at 4°C for 1h to remove the cellular proteins. Slides were then placed in an electrophoresis tank containing 0.3 M NaOH and 1 mM Na2EDTA pH>12 for 30 min before electrophoresis at 25 V (1 V/cm, 300 mA) for 30 min at an ambient temperature of 4°C. The slides were then washed 3 times for 5 min, each with 0.4 M Tris-HCl, pH 7.5, at 4°C before staining with 5 µg/ml DAPI. The scoring for DNA damage was based on the length of the formed tail which represents the migration of DNA with strand breakage during electrophoresis following treatment (Chan et al., 2006). The images were captured using fluorescence microscope.

Caspases activity assay. The apoptosis assay, employing the caspase kits, was performed according to supplier’s protocol (Promega, USA). Briefly, Hela cells were plated in a 96 well plate at 5x10^4 cell/ml and treated with IC_{50} concentration of Goniothalamin in triplicate. Luminescence emission spectra were measured for the samples. Untreated cells were used as a negative control. This procedure was done to detect caspases 3/7, 8 and 9.

Quantification of Early apoptotic cells using fluorescent conjugation of Annexin V/PI double staining. HeLa cells at a concentration of 1x10^6 cell/ml were seeded into the 6-well plate and treated with IC_{50} concentration of Goniotalamin. After 24, 48 and 72h incubation, the cells were detached and stained by using PE Annexin V Apoptosis Detection Kit I. All samples were read by the flow cytometer.

Effects of goniotalamin on Cell Cycle Distribution. HeLa cells at a concentration of 1x10^6 cells/ml were seeded into 6-well plate in 2 ml culture medium with a concentration of IC_{50} value of goniotalamin and were incubated at 37°C in an atmosphere of 5%CO_{2} for 24, 48 and 72 h. Some wells were left with no treatment to be used as a control. After the incubation period, the cultured cells were harvested using trypsin and centrifuged. After incubation, the cells were detached and stained by using the Cycle TEST TM PLUS DNA Reagent Kit. Cell cycle was read using the Cell Quest software within 3 h.

Flow cytometry (Annexin V/PI double staining): HeLa cells at a concentration of 1x10^6 cell/ml were seeded into the 6-well plate and treated with IC_{50} concentration of Goniotalamin. After 24, 48 and 72h incubation, the cells were detached and stained by using PE Annexin V Apoptosis Detection Kit I. All samples were read by the flow cytometer.

Statistical analysis

Data was expressed as mean±SE. Statistical analysis was performed with Student’s t-test for data from AO/PI staining assay, Comet assay and Caspase activity assay. Differences were considered significant at p=0.05.

Results

Cell proliferation assessment

MTT cytotoxicity assay: Cytotoxicity of goniotalamin was evaluated using MTT assay. As shown as in Figure 1, the IC_{50} value of goniotalamin concentration that reduced the growth of treated cells by 50% compared to untreated cells was 3.2±0.72 µg/ml. Table 1 determined the percentage of cell viability after treatment with different concentrations of goniotalamin, cell viability decreased with the increasing of goniotalamin concentration.

Trypan blue exclusion assay: Based on the results of the trypan blue dye exclusion assay, increasing goniotalamine exposure time had a significant effect on Hela cells viability. Treatment of Hela cells with IC_{50} and IC_{50} concentrations of goniotalamine (3.2 and 0.9µg/ml) resulted in a dose-dependent inhibition of cell growth. As observed in Figure 2, at high concentration IC_{50} cell viability had reduced from 78% at 24h to 61% and 55% at

![Figure 1. Percentage of Hela Cell Viability Against Goniotalamin Concentration.](image)

**Table 1. Percentage of Hela Cells Viability Treated with Goniotalamin in Different Concentrations**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>% Cell viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>25.79867</td>
</tr>
<tr>
<td>15</td>
<td>36.01634</td>
</tr>
<tr>
<td>7.5</td>
<td>44.7142</td>
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<tr>
<td>3.75</td>
<td>47.11773</td>
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<tr>
<td>1.875</td>
<td>60.99233</td>
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<tr>
<td>0.9375</td>
<td>75.09763</td>
</tr>
<tr>
<td>0.46875</td>
<td>79.23344</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>
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Figure 2. Trypan Blue Exclusion Assay. The percentage of viable cells in HeLa cell population after treatment with different concentrations of Goniothalamin at various time intervals.

Figure 3. Phase Contrast Microscope. A) HeLa cells without any treatment after 72 h; B) Hela Cells Treated with IC_{50} of Goniothalamin Compound after 72h. Appearance of membrane cell blebs (arrow) and also has some part of cell membranes disrupted. The decreasing number of cells signified that cell death had occurred (Magnification 200x).

Figure 4. Cells Under Fluorescent Microscope after Staining with AO/PI Dye. A) Untreated cells stained bright green fluorescence with intact membrane and nucleus; B) Cells treated at IC_{50} concentration of goniothalamin after 72h, cells appear with membrane blebbing (Magnification 400x).

48h and 72h, respectively. Whereas, at IC_{25}, cell viability reduced from 87% at 24h to 78% and 66% at 48h and 72h, respectively. Compared with the untreated cells, there were no significant differences in cell viability with time.

Morphological assessment of HeLa cell

Phase Contrast Microscopy. Morphological study of HeLa cell treated with goniothalamin compound was observed using the Phase Contrast Microscopy. The characteristics of apoptosis can be recognized by the presence of cell membrane blebbing, condensation of nuclear and fragmentation. Furthermore, the cells in culture lost contact with adjacent cells. Besides that, apoptotic bodies and oval masses of cytoplasm, which were smaller than the origin cells were observed in the plasma membrane. The cytoplasmic organelles became compact from plasma membrane as observed in a small portion of the cell. These morphological changes were found to characteristics of apoptotic cell death (Figure 3).

Quantification of Apoptosis Using Propidium Iodide and Acridine Orange Double Staining. Fluorescent microscope was conducted to study of morphological changes of cell death mode induced by goniothalamin. Acridine orange (AO) and propidium iodide (PI) staining was used. Viable cells displayed green fluorescence with the appearance of circular cell with intact DNA and nucleus give a round and green nuclei. The early apoptotic cells have fragmented DNA which gives several green colored nuclei and cell blebbing. Late apoptotic and necrotic cell’s DNA would be fragmented and stained orange and red (Figure 4). Besides the study of morphological changes, the percentage of viable, apoptotic and necrotic cells plotted as a graph in Figure 5. The percentage of apoptotic cells in untreated cells slightly increased from 0.67% after 24h to 5.33% and 7% after 48 and 72h, respectively (Figure 5). Whereas, cells treated with goniothalamin at IC_{50} concentration, the percentage with the appearance of circular cell with intact DNA and nucleus give a round and green nuclei. The early apoptotic cells have fragmented DNA which gives several green colored nuclei and cell blebbing. Late apoptotic and necrotic cell’s DNA would be fragmented and stained orange and red (Figure 4). Besides the study of morphological changes, the percentage of viable, apoptotic and necrotic cells plotted as a graph in Figure 5. The percentage of apoptotic cells in untreated cells slightly increased from 0.67% after 24h to 5.33% and 7% after 48 and 72h, respectively (Figure 5). Whereas, cells treated with goniothalamin at IC_{50} concentration, the percentage...
of apoptotic cells increased rapidly from 37% after 24h to 53% and 63% after 48 and 72h, respectively.

**Biochemical assessment**

DNA Fragmentation Assay. The DNA ladder assay is generally accepted as specific for apoptosis because it detects oligonucleosomal cleavage rather than artificial DNA cleavage or necrosis. The DNA of Hela cells treated for 24, 48 and 72h with goniothalamin was extracted and analyzed by agarose gel electrophoresis. Untreated cells contained only high-molecular-weight genomic DNA. The inoculation of goniothalamin on Hela cells showed the characteristic pattern of nucleosomal ladderings specific to apoptosis which was visible as faint bands on the gel (Figure 6). Goniothalamin produce DNA fragments of low molecular weight consisting of multimers of 180-200 base pairs on Hela cell line.

Single-cell gel electrophoresis Comet assay. In this study, the alkaline comet assay was carried out to determine the DNA damage occurrence in Hela cells induced by goniothalamin. As shown in Figure 6 the DNA damage in Hela cells treated with goniothalamin increased in a concentration dependent manner. The percentage of total DNA damage induced by GN was 88% and 100% at IC$_{10}$ and IC$_{25}$ concentrations, respectively (Figure 7 and 8). As shown in Figure 6 and 7 untreated cells showed 100% cells with intact DNA (score 0). However, there was a significant decrease (p<0.05) in the percentage of cells with intact DNA (score 0) following treatment with IC$_{10}$ and IC$_{25}$ where the percentage was 12 and 0%, respectively. On the other hand, there was a significant increase in percentage of cells with damaged DNA following treatment with IC$_{10}$ GN, with 60, 24, 2 and 2% cells at scores 1, 2, 3 and 4, respectively. More cells with damaged DNA were observed in Hela cells treated with GN treatment at IC$_{25}$ where the percentage was 34, 48, 12 and 6% at scores 1, 2, 3 and 4, respectively. There was a significant difference between treated (IC$_{10}$ and IC$_{25}$) and untreated cells (p<0.05).

Activation of the cellular level of caspase, 3/7, 8 and 9. Figure 8 shows the luminescence intensity values for caspase 3/7, 8 and 9. The chart shows a much higher luminescent intensity values of caspase 9 for Hela cells treated with goniothalamin (2.6×10$^5$) and (10×10$^5$) for 6h and 24h, respectively, compare with untreated cells (2.5×10$^5$). As shown in Figure 9 goniothalamin significantly (p<0.05) stimulated caspase 9 and there was no increase in caspase 3/7 and 8.

Quantification of Early apoptotic cells using fluorescent conjugation of Annexin V/PI double staining. Apoptotic cells exclude all dyes which are in use for cell viability assays, such as PI, while necrotic cells do not. In cells with a damaged cell membrane PI induces a red fluorescence on the DNA, whilst it is excluded by cells with a preserved cytoplasm membrane. Hence during the initial phase of apoptosis, the cells are still able to exclude PI and therefore do not show any red fluorescence signal, similar to that of living cells. Table 2 showed the results of Annexin V/PI flow cytometry of HeLa cells after treatment with IC$_{10}$ value of goniolahtamin. Untreated cell was found to be 6.4% of HeLa cells population while after 24h post-treatment apoptotic cells increased to 26.45%.

Effects of goniothalamin on Cell Cycle Distribution. The state of HeLa cells was monitored by flow cytometry after propidium iodide staining nuclei. The treatment resulted in a loss of cells in G1 and G2/M phases of the cycle accompanied with a large increase in the sub-G1 and S phases. Goniothalamin induced time-dependent increase in the proportion of sub-G1 population. Apoptosis peak (sub-G1) was found in untreated cells with small percentage for about 2.44 and 6.62 for 24 and 48 hrs, respectively. The sub-G1 population in treated cells were 5.91 and 27.99 for 24 and 48 hrs, respectively. This GNT-induced cell cycle perturbation concurred with the results of the previous assay to suggest that HeLa cells undergo apoptosis more extensively With increasing in...
time. On the other hand DNA histogram showed that gonoithalamin increased the population of cells at S phase in a time-dependent manner (Figure 9). The S population increased significantly from 6.17% and 8.53% in the untreated cells to 23.17% and 25.92% in cells treated with IC_{50} gonoithalamin for 24 and 48h, respectively. DNA histogram showed that gonoithalamin induce cell cycle arrest in S phase (Figure 10).

Discussion

Apoptosis is a regulated cell death process which leads to the complete cell destruction. These changes occur through characterized pathway including cell surface signalling, changes in morphology and caspase activation which lead to the DNA condensation and fragmentation. Most study had been carried out to determine the qualitative analysis of apoptosis in cervical cancer treated with anticancer compound. In this study we showed that gonoithalamin decreases the human cervical cancer cells (Hela) viability via apoptosis. The cell viability decreased by gonoithalamin was assessed by MTT Cytotoxicity Assay and Trypan Blue Exclusion Assay. Furthermore, the apoptosis induced by gonoithalamin was confirmed by Propidium Iodide and Acridine Orange Double Staining, caspase-9 activation, DNA fragmentation.

In this study, gonoithalamin isolated from Goniothalamus sp has indicated significant growth inhibition in Hela human cervical cancer cell line at low concentration of IC_{50} values (3.2±0.72 µg/ml). These results conduced to other studies investigated the cytotoxic effect of Gonoithalamin towards human breast cancer, vascular smooth muscle cells (VSMCs), Jurkat leukemia cells, HL-60 leukemia cells, Chinese hamster ovary (CHO) and hepatoblastoma HepG2 cells (Ali et al., 1997; Pihie et al., 1998; Inayat-Hussain et al., 1999; 2003; Chen et al., 2005; 2006; Nasir et al., 2004; Al-Qubaisi et al., 2011).

Based on the results of the trypan blue dye exclusion assay, Treatment of Hela cells with gonoithalamin resulted in a dose- and time-dependent inhibition of cell growth rate. This observation was consistent with MTT results. It appears that Hela cells reacted differently when exposure to gonoithalamin in the trypan blue dye exclusion assay. Cells count results indicated that viability of cells treated with gonoithalamin was significantly inhibited while no obvious inhibition was detected in untreated cells. This may be contributed to by changes in the cell membrane triggered by the high gonoithalamin concentrations, which may increase the uptake of trypan blue dye and the subsequent membrane alteration may have gradually resulted in cell death. Some evidence has indicated that gonoithalamin exposure can alter the membrane properties (Inayat-Hussain et al., 2003).

Cell death can occur by two distinct processes: apoptosis and necrosis (Muneesh and Vishva, 1995). Apoptosis is natural phenomenon that occur in organism to eliminate a cell from organism without causing any inflammatory effect. The typical Morphological features of apoptosis in Hela cells after gonoithalamin treatment observed through Phase Contrast Microscopy, and characterized by the presence of shrunken cell with blebbing, nuclear condensation and fragmentation. Whereas the untreated Hela cells also showed a high confluence of monolayer cells compared to treated cells. This finding indicates that apoptosis had occurred on this method was previously done by Akira et al. (2003) on human colorectal Adenocarcinoma cells and Dandan et al. (2006) on Hela cells (Murakami et al., 2003; Dandan et al., 2006).

The apoptotic features were confirmed and the percentage of apoptotic cells was determined from at least 300 counted cells observed under fluorescent microscope. The calculation of apoptotic cells is described as the percentage of apoptotic cells and apoptotic bodies within the overall population of cells. The percentage of apoptotic cells and the graph showed that the percentage of apoptotic cells treated with gonoithalamin was increasing among the time. These distinctive morphological features form the basis of some of the most widely used techniques for the identification and quantification of apoptosis, and thus morphologic description using Phase Contrast microscopy and fluorescence microscopy remains one of the best ways to define apoptosis (Doonan and Cotter, 2008).

One of the most common methods used to confirm apoptosis occurred on cells is identification of DNA laddering. This is the biochemical hallmark of apoptosis with the fragmentation of the genomic DNA, an irreversible event that commits the cell to die but this method is qualitative rather than quantitative (Cohen et al., 1992). In this study we reported that gonoithalamin stimulate DNA fragmentation characteristic of apoptosis in Hela cell line at 24, 48 and 72h post-treatment. DNA fragments reveal, upon agarose gel electrophoresis, a distinctive ladder pattern consisting of multiples of an approximately 180 base pairs subunit. DNA ladder formation is observed only when the extent of oligonucleosomal cleavage is prominent. Internucleosomal cleavage of DNA is likely to be in the later phase of apoptotic process (Cohen et al., 1992; Oberhammer et al., 1993; Gooch and Yee, 1999). In most cell types, the biochemical characteristics of apoptotic response include activation of endogenous calcium and magnesium dependent endonucleases, leading to fragmentation of the chromosomal DNA. Initially, the DNA fragments are large (50-300 kb) but are later digested to oligonucleosomal size (multimers of 180-200 bp). The formation of this distinct DNA ladder is considered to be a biochemical hallmark of apoptosis (Cohen, 1993).

Comet analysis of somatic cells electrophoresed under alkaline conditions measures single DNA strand breaks, whereas neutral conditions measure double strand breaks. However, many forms of structural damage can be converted to strand breaks during cell preparation and electrophoresis (Collins et al., 1997; Olive, 1999). In this study, we report on the increased DNA strand breaks in Hela cells after exposure to gonoithalamin. Comet assay confirms the observation that islet DNA is a target for cytotoxic levels of gonoithalamin. In Untreated samples, the cell’s nuclei remained unchanged but when exposed to gonoithalamin a significant increase of DNA damage was observed. Low doses of gonoithalamin (IC_{10}) caused...

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DOI:http://dx.doi.org/10.7314/APJCP.2013.14.11.6273

The activity of the caspase-3/7 should be in the equivalent treatment with GTN at IC_{50}. However, there was no significant increase in the activity of caspase-3/7 after 24 hours of treatment (p>0.05).

In 2004, Tawa et al. studied reported that in contrast to pro-enzyme, caspase-3 is difficult to detect once activated in HeLa cells due to its rapid degradation. Previous study also showed similar result in which caspase-3 activity in GTN treated cells was increased up to 6 hours (1.68-folds) before a steady decline onwards (Chan et al., 2010). The short-lived increase of caspase-3 activity was further supported with detection by immunoblotting analysis or Western blotting at 4 and 6 hours (Chan et al., 2010). Caspase-3/7 activity may also be detected if the experiment time-interval is planned on 2, 4, 6, 12, and 24 hours.

However, GTN-induced HeLa apoptosis did not involved caspase-8 activation. Although there was a small significant increase of caspase-8 activity in HeLa cells at 6 and 24 hours of treatment (p<0.05). The significant increase of caspase-8 activity was probably due to the cell naturally undergoes apoptosis at senescence stage through extrinsic and intrinsic pathway with the activation of caspase-8 and -9 respectively. Therefore, the small increase of caspase-8 activity was detected in HeLa cells at 6 and 24 hours of treatment.

In comparison, caspase-9 activity was detected 4.5-folds and 16-folds higher than caspase-8 activity at 6 and 24 hours of treatment respectively. However, there was no significant increase of caspase-9 activity at 6 hours of treatment (p>0.05). This may due to high activity of caspase-9 was detected only in untreated cells. Furthermore, there was a very high significant increase of caspase-9 activity (5.2-folds) at 24 hours after treatment of GTN (p<0.05). This showed that GTN was able to induce apoptosis in HeLa cells through the intrinsic pathway with the activation of caspase-9 as an initiator caspase.

Previous study reported that the sequential activation of caspase-9 but not caspase-8 leading to the downstream caspase-3 cleavage was observed in GTN-treated CASMCs (Coronary Artery Smooth Muscle Cells) (Chan et al., 2010). It has also been reported that GTN induced apoptosis in HL-60 and Jurkat cells via mitochondrial pathway (Izat-Hussain et al., 2003; 2010). Thus, this findings suggested that the insignificant role of caspase-8 as an initiator caspase. Caspase-8 is not essential in GTN-induced apoptosis in HeLa cells.

Overall, these data presented here reveal that the cytotoxic effect of Goniothalamin towards the cervical cancer cells via apoptosis.

Acknowledgements

This research was funded by Research Grant from Universiti Sultan Sultan Zainal Abidin (UniSZA), Malaysia.

References