ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Toxicity Mechanism of Triphenyltin (IV) Butylphenyldithiocarbamate on Acute Lymphoblastic Cells, Jurkat E6.1.

[1*]Normah Awang, [1]Nur Shuhada Abd Kadir, [2]Nurul Farahana Kamaludin, and [1]Kok Meng Chan.

[1]Environmental Health and Industrial Safety Programme, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.
[2]Biomedical Science Programme, School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia .

ABSTRACT

Organotin(IV) derivatives have emerged as potential metallopharmaceuticals due to their efficacy to induce cytotoxicity in various types of cancerous cell lines. Triphenyltin(IV) butylphenyldithiocarbamate (TFBF), a novel compound has been found to exhibit primary apoptosis in Jurkat E6.1 cells with IC_{50} of 0.4 µM. In this study, the role of reactive oxygen species (ROS) and loss of mitochondrial membrane potential (Δψm) in Jurkat E6.1 cells were assessed along with activation of caspase-3. The measurement of ROS and the loss of Δψm were conducted using dihydroethidium (HE) staining assay and tetramethylrhodamine ethyl ester (TMRE) staining assay, respectively. The cells were treated in the time series from ½ h up to 4 h prior to flow cytometric quantification. Caspase-3 activation from 1 h up to 6 h was evaluated using Caspase-Glo® Luminescence Kit. The results showed that there was an early increase of positive-HE stained cells as early as 1 h of treatment as compared to the negative control. Increased level of ROS led to the loss of Δψm. Activated caspase-3 was significant at 4 h of treatment. In conclusion, TFBF-induced apoptosis in Jurkat E6.1 cells via early production of ROS with loss of Δψm finally leads to caspase-3 activation.

Keywords: Cytotoxicity, Apoptosis, Organotin(IV), Dithiocarbamate, Jurkat E6.1

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Leukemia is a disease characterized by a rapid increase in the number of immature blood cells in the bone marrow. This event will result in high numbers of abnormal white blood cells called blasts or leukemia cells (Stankovic & Marston 2008; Manisha 2012). One of the four types of leukemia is acute lymphoblastic leukemia (ALL), which includes a group of neoplasms of lymphoid precursor cells that morphologically resemble B-cells and T-cells (Mihaela 2009). Most patients suffering from this type of leukemia are children, and a small group of adults also suffer from this disease (Pui et al. 2008).

The treatment for ALL is almost 80% effective for pediatric patients (Pui et al. 2008). Among the common chemotherapeutic agents in the treatment are glucocorticoid, vincristine, L-asparaginase, and anthracycline. However, ALL patients who recover would suffer from health effects usually associated with cardiac and vascular diseases (Christian et al. 2011). Currently, many researches have been done to treat ALL. Many syntheses and applications of metal-based drugs have also been studied to search for new anticancer agents (Awang et al., 2012).

New compounds including various metal-based compounds have been actively synthesized to evaluate their potential as anticancer agents (Awang et al. 2011). As organotin compounds have strong cytotoxicity, they have caught many attention among researchers (Awang et al. 2011).

Previous study by Kamaludin et al. (2013) shows that triphenyltin(IV) butylphenyldithiocarbamate (TFBF) has high cytotoxic effects on Jurkat E6.1 cells. The IC_{50} value obtained was 0.4 µM upon 24 h of exposure. However, the action mechanism of TFBF in the cells was not elucidated in the study. According to Pizzaro et al. (2010), the exact mechanisms of action of organotin compounds are still unrevealed although the capability of these compounds to induce apoptotic cell death is frequently reported. Therefore, this study was carried out to determine the mechanism of action of TFBF upon treatment in Jurkat E6.1 cells, which are clone cells for human ALL. This study was a continuation from the previous research done by Kamaludin et al. (2013) that demonstrated a high cytotoxicity of TFBF in Jurkat E6.1 cells.

MATERIALS AND METHODS

Compounds

Triphenyltin(IV) butylphenyldithiocarbamate (TFBF) was synthesized at the School of Chemical Sciences and Food Technology, Faculty of Science and Technology, UKM Bangi.

The stock solution of TFBF (20 mM) was prepared by dissolving 0.0115 g of TFBF into 1 mL of DMSO. The stock solution was kept at −20 °C. During the treatment, the stock solution was diluted with medium to get a final concentration of 100 µM.

Two compounds namely menadione (MENA) and goniothalamin (GN) were chosen as positive controls. The stock solution of MENA (25 mM) was prepared by dissolving 0.0172 mg of MENA in 1 mL of DMSO. Meanwhile, an amount of 10 mg of GN was dissolved in 1 mL of DMSO to prepare a 50 mM stock of GN. Both stock solutions were kept at −20 °C.

Cell Culture

Jurkat E6.1 human leukemic cell line was obtained from the American Type Culture Collection (ATCC). The cells were maintained in Roswell Park Memorial Institute 1640 (RPMI-1640) medium (GIBCO, USA) enriched with 10% fetal bovine serum (FBS) (GIBCO, USA). The cell line was maintained at 37 °C in 5% CO_2 atmosphere according to the recommended protocols by ATCC.

Annexin V-FITC/PI Assay

The Jurkat E6.1 cells (2×10^6 cells/mL) were treated with 0.4 µM TFBF for 24 h. After 24 h, 1,000 µL of cells was harvested and transferred to a microcentrifuge tube. The sample was centrifuged at 200 *g* for 5 min at 4 °C. Then, the supernatant was discarded, and 500 µL of cold PBS was added to the sample prior to the

centrifugation for 5 min. The supernatant was discarded. Next, 150 µL of Annexin V binding buffer and 2.5 µL of Annexin V-FITC were added to the sample in a dark condition. The sample was incubated for 15 min at room temperature. Then, an amount of 10 µL propidium iodide (50 µg/mL) was added to the sample and incubated for 2 min at room temperature. After incubation, an amount of 350 µL of Annexin V binding buffer was added to the sample and transferred to a falcon tube. Finally, the sample was analyzed using BD FACSCanto II flow cytometer.

Dihydroethidium Assay (HE Assay)

This assay was done according to the method described by Chan et al. (2010) with some modifications. Cell enumeration was conducted in advance to ensure sufficient cell concentration during the test. Cell concentration required for this treatment was 2×10^6 cells/mL. The concentration of 100 µM TFBF was obtained using 20 mM of TFBF stock solution. Each well that contained cells was treated with TFBF at a concentration of 0.4 µM. The cells were treated in 6-well plates. Each well contained 2 mL of cells and media.

MENA was used as a positive control for verification purposes, while the untreated cells were used as a negative control. The cells were treated for 4 h, 2 h, 1 h, and ½ h. Cells with MENA were treated for ½ h. After 4 h of treatment, a total of 1 mL of cells was harvested in microcentrifuge tubes, prior to centrifugation at 2500 rpm for 5 min at 4 °C. After centrifugation, the supernatant was discarded.

Next, 1 mL of unenriched RPMI 1640 growth medium and 1 µL of HE were added to the pellets in a dark condition. The pellets were incubated for 15 min at a temperature of 37 °C. Then, the pellets were centrifuged at 2500 rpm for 5 min at 4 °C. Supernatant was removed, and the pellets were washed with 1 mL of cold PBS and recentrifuged. After centrifugation, the supernatant was removed, and 500 µL of cold PBS was added. Finally, the cells were transferred into falcon tubes and analyzed using BD FACSCanto II flow cytometer.

Tetramethylrhodamine Ethyl Ester Assay (TMRE Assay)

The method was carried out according to the method described by Chan et al. (2010) with some modifications. The cell concentration used in this treatment was 2×10^6 cells/mL. As a positive control and for the purpose of verification of the test results, MENA at a concentration of 25 µM was exposed to the cells for 2 h. On the other hand, the untreated cells were used as a negative control. Each cell excluding the controls was treated with 0.4 µM of TFBF. The cells were exposed to the TFBF for 4 h, 2 h, 1 h, and ½ h, respectively.

Upon treatment completion, 1 mL of cells in each treatment time was harvested in the microcentrifuge tubes prior to the centrifugation at 2500 rpm for 5 min at 4 °C. The supernatant was removed, and the pellets were added with 1 mL of unenriched RPMI-1640 growth medium and 1 µL of TMRE (50 mM) in a dark condition. The cells were incubated for 15 min at 37 °C. Next, the cells were centrifuged at 2,500 rpm for 5 min at 4 °C. The supernatant was removed, and the pellets were washed with 1 mL of cold PBS prior to the centrifugation. The supernatant was removed, and 500 µL of cold PBS was added to the pellets. Finally, the samples were transferred into the falcon tubes and analyzed using BD FACSCanto II flow cytometer.

Caspase-3 Activation Luminescent Assay

Caspase-Glo® reagent was prepared by dissolving 10 mg of substrate Caspase-Glo® in 10 mL Caspase-Glo® buffer. The reagent solution was thawed at a room temperature and homogenized before use.

The cells with the concentration of 2×10^6 cells/mL were treated with TFBF by incubating them simultaneously with control. Positive control used was 50 µM of GN. The positive control was treated for 4 h. The cells were treated in a 6-well plate. Each well contained 2 mL of cells and media. The cells were treated at intervals of 6 h, 4 h, 2 h, and 1 h.

After 4 h, the cells were recalculated before being transferred into a 96-well plate. The cell concentration needed was 4×10^5 cells/mL in 50 µL for each well. The cells were inserted into the well, and complete media was added to fill up to 50 µL.

Then, Caspase-Glo® reagent active caspase-3 was thawed at a room temperature and added to each well equally. The plate was then shaken using orbital shaker for 30 s, and the plate was wrapped with aluminum foil and incubated at room temperature for 1 h. After 1 h, the plate was inserted into the GloMax Multi Detection System (Promega) machine for the analysis of signal luminescence, and data were expressed as the percentage of active caspase-3.

Morphological Observation

Cells were seeded into a sterile 6-well plate at a density of 2×10^6 cells/mL. Then, the cells were treated with TFBF (0.4 µM) for 1 h, 2 h, 4 h, and 6 h. Finally, the cells were observed under light inverted microscope at 200× magnification.

Statistical Analysis

The data were expressed as the mean ± standard error of mean (SEM). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 21.0 by employing one-way ANOVA. The data were considered statistically significant when $p<0.05$.

RESULTS

Annexin V-FITC/PI

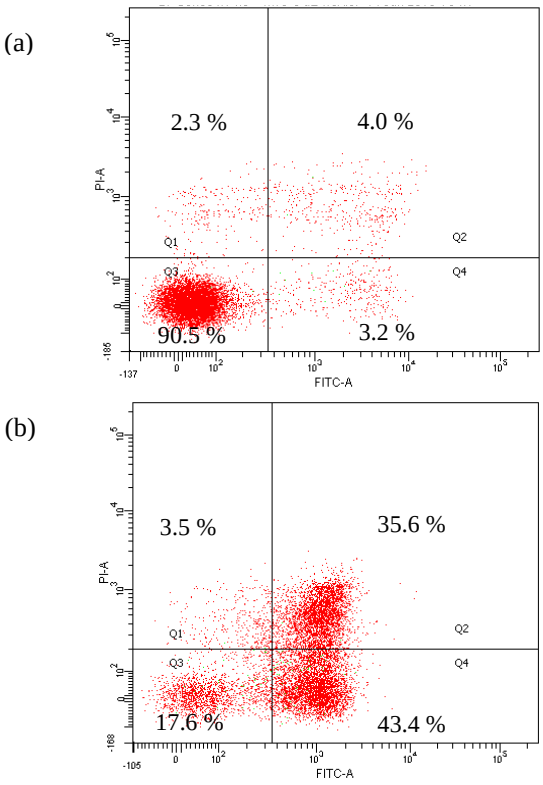


Figure 3.1. Percentage of Jurkat E6.1 cells measured using flow cytometer. (a) Untreated cells and (b) cells treated with 0.4 µM TFBF for 24 h. Almost 80% of TFBF-induced Jurkat cells were quantified as apoptosis.

Indicators:

Q1: PI+ / Annexin V– (necrotic cells)
Q2: PI+ / Annexin V+ (late apoptotic cells)
Q3: PI– / Annexin V– (viable cells)
Q4: PI– / Annexin V+ (early apoptotic cells)

ISSN: 0975-8585

TFBF was able to induce almost 80% of cells to die via apoptosis at 0.4 µM within 24 h of treatment (see Fig. 3.1). The percentage of viable cells (Q3) significantly decreased from 90.5% to 17.6%. On the other hand, the apoptotic cells (Q2+Q4) increased significantly from 7.2% to 79.0%. The percentage of necrotic cells for treated and untreated cells was not much different.

HE Assay

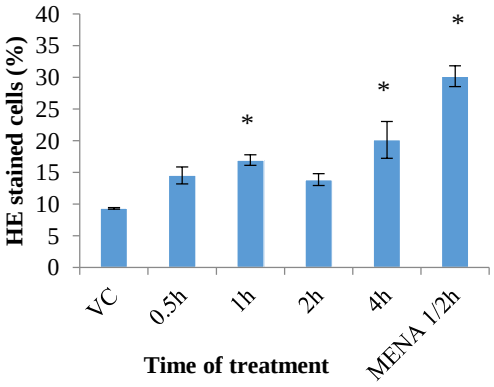


Figure 3.2: Percentage of cells positively stained with HE upon treatment with 0.4 µM of TFBF for ½ h, 1 h, 2 h, and 4 h. Average values are expressed in mean ± standard error of mean (SEM).

Fig. 3.2 shows the percentage of the population of cells positively stained with HE against duration of treatment (h). VC refers to the negative control, while MENA refers to the positive control treated with 25 µM of MENA. In the assay, the percentage of cells positively stained with HE indicated the percentage of reactive oxygen species (ROS) in cells. In general, the induction of TFBF in Jurkat E6.1 cells at ½ h and 1 h of treatment increased the intracellular ROS. However, at 2 h of treatment, the ROS decreased and picked up after 4 h of treatment. As compared to the negative control, there was no significant increase of ROS with 14.5% ± 1.322 and 13.9% ± 0.917 at ½ h and 2 h, respectively. However, there was a significant difference ($p<0.05$) at 1 h (16.9% ± 0.837) and 4 h (20.1% ± 2.904) treatments compared to the negative control.

TMRE Assay

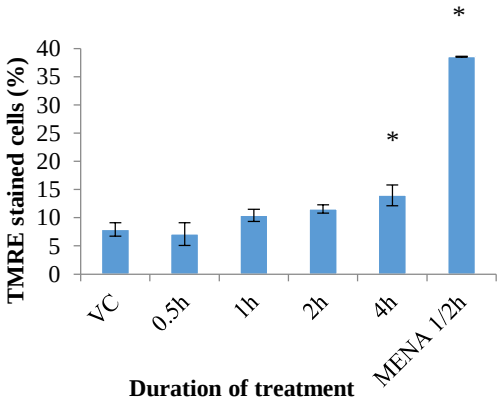


Figure 3.3: The loss of Δψm in Jurkat E6.1 cells after treatment with 0.4 µM of TFBF for ½ h, 1 h, 2 h, and 4 h. Average values are expressed in mean ± standard error of mean (SEM).

Fig. 3.3 shows the percentage of Δψm loss against duration of treatment (h). In general, there was a little increase in the percentage of population with Δψm loss from ½ h to 4 h. Based on one-way ANOVA result, there was no significant decrease of Δψm loss in all duration of treatment except for 4 h, which showed a significant decrease of Δψm loss as compared to the negative control.

ISSN: 0975-8585

Caspase-3 Activation Luminescent Assay

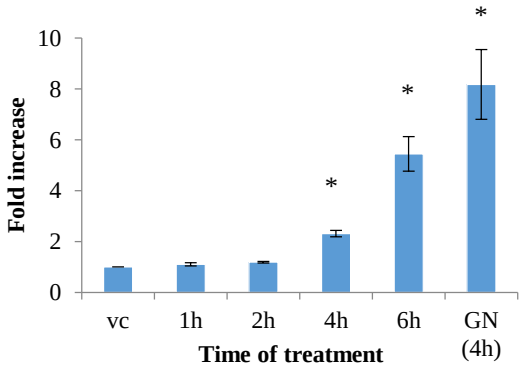
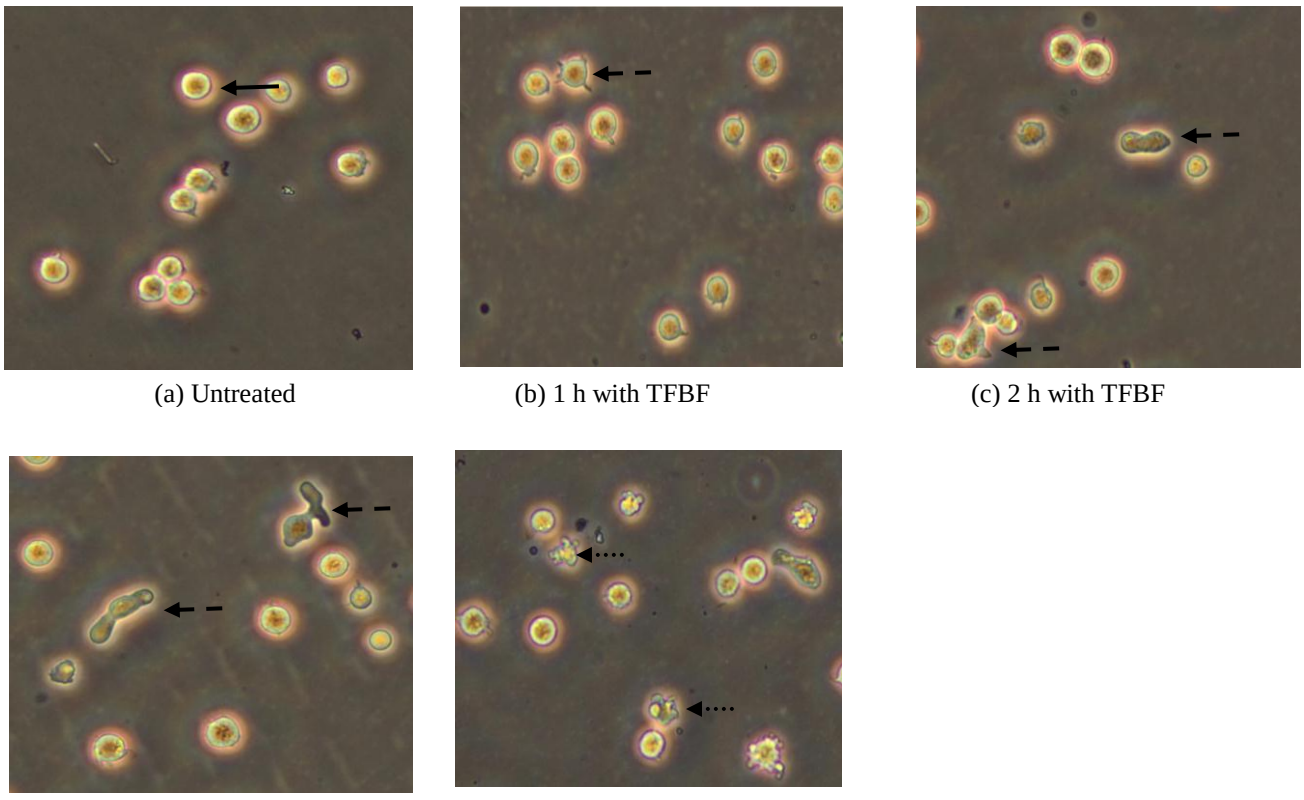


Figure 3.4: The fold of increase of caspase-3 in Jurkat E6.1 cells after treatment with 0.4 µM of TFBF for 1 h, 2 h, 4 h, and 6 h. Average values are expressed in mean ± standard error of mean (SEM).

Fig 3.4 shows the activation of caspase-3 in Jurkat cells upon treatment with TFBF up to 6 h. VC refers to the negative control, while GN refers to the positive control. There was a significant increase of caspase-3 activation at 4 h and 6 h of treatment. Two-fold and five-fold increases were recorded at 4 h and 6 h, respectively. One-way ANOVA analysis showed that the activation of caspase-3 was significant at 4 h (2.34 ± 0.124) and 6 h (5.4 ± 0.684) compared to the negative control.

Morphological Observation



(a) Untreated (b) 1 h with TFBF (c) 2 h with TFBF

(d) 4 h with TFBF (e) 6 h with TFBF

Figure 3.5: Morphological observation on Jurkat E6.1 cells upon treatment with TFBF (0.4 µM) at 4 time points (200× magnification). (a) Untreated cells; (b) 1 h with TFBF; (c) 2 h with TFBF; (d) 4 h with TFBF; and (e) 6 h with TFBF. The compound induced morphological changes including membrane blebbing as early as 1 h of treatment and cell lysis was observed at 6 h of treatment in Jurkat E6.1 cells.

Indicator:

Normal-shaped cell | Elongated, blebbing cell | Lysed cell

DISCUSSION

Organotin compounds are metal-based inorganic compounds that are increasingly studied by researchers around the world. Organotin compounds are synthesized and tested for their ability as new anticancer agents in the development of anticancer drugs (Awang et al. 2014). Kamaludin et al. (2013) reported that TFBF is a novel compound with a high cytotoxicity in Jurkat E6.1 cells. The compound requires a low micromolar dose (0.4 µM) to inhibit the proliferation of half Jurkat cells' population within 24 h. In the present study, it was found that around 80% of the cells were quantified as apoptosis upon treatment for 24 h at 0.4 µM (see Fig. 3.1). The evaluation of the toxicity mechanism exerted by this compound was done by determining the upstream targets of TFBF in Jurkat E6.1 cells. These upstream targets of TFBF include the involvement of ROS and mitochondria to induce apoptosis in the cells.

The process of apoptosis is initiated by the presence of the internal and external stimuli including oxidative stress (Rajesh et al. 2009). Many studies have shown that the intracellular oxidative stress is very common in cells treated with organotin (Alama et al. 2009). In the present study, the TFBF also demonstrated an increasing trend of ROS production in Jurkat cells within ½ h and significantly increased at 1 h of treatment (see Fig. 3.2). However, a slight decrease of ROS was detected at 2 h. The ROS tended to increase significantly up to 20.1% at 4 h. Principally, oxidative stress occurs when the number of ROS produced is beyond the ability of antioxidants to neutralize the ROS (Haufel & Bauer 2001; Paul et al. 2012). In this study, the induction of TFBF in cells was suggested to cause the generation of intracellular ROS. This finding was indicated by the increasing trend of ROS at the earlier time points namely from ½ h to 1 h. The antioxidant mechanism system typically the glutathione (GSH) was activated upon the occurrence of the excessive intracellular ROS (Seung et al. 2001). The GSH will metabolize the ROS by reducing the hydrogen peroxide to water and oxygen (Bindoli et al. 2008). Therefore, we suggested that the slight decrease of ROS at 2 h might be due to the activation of GSH in the Jurkat cells. However, the amount of GSH generated in cells was insufficient to neutralize the excessive ROS produced, hence significantly increased the amount of intracellular ROS at 4 h. Such situation was demonstrated by Inayat-Hussain et al. (2010) whereby the increasing amount of ROS in GN induced-Jurkat cells resulted from the low level of GSH.

The intracellular ROS production is a common phenomenon in a cell's normal condition whereby the metabolism processes by certain organelles including endoplasmic reticulum and mitochondria will produce ROS (Assim & Reem 2012). However, the excessive generation of intracellular ROS might cause some interruptions in cells' or organelles' normal mechanism. Thus, the increasing amount of ROS generation in TFBF-induced Jurkat cells might lead to certain disruption including the loss of the mitochondrial membrane potential, $\Delta\psi m$ (Gottlieb et al. 2003; Pellerito et al. 2010). In this study, the percentage of cells positively stained with TMRE was in time-dependent manner (see Fig. 3.3), with an increasing trend from ½ h to 4 h. The significant amount of $\Delta\psi m$ loss was detected at 4 h of treatment. As compared to VC, a slight decrease of $\Delta\psi m$ was recorded at 1 h. This decrease of $\Delta\psi m$ was in parallel to the increasing amount of intracellular ROS within ½ h to 1 h. The mitochondrion plays a vital role as it regulates the release of important apoptotic proteins from its intermembrane space (Suen et al. 2008). Therefore, the decline in mitochondrial membrane potential might indicate the early event that occurs in the process of apoptosis (Grubb & Lawen 2003). Therefore, we suggested that the presence of excessive ROS was responsible to reduce the mitochondrial membrane's integrity, thus leading to the $\Delta\psi m$ loss and the release of cytochrome *c*. The continuous production of ROS and the loss of $\Delta\psi m$ may activate apoptosis (Kong et al. 2000; Paul 2010).

Therefore, caspase 3 activation assay was conducted in this study to confirm the occurrence of apoptosis event (Brian et al. 2002; Seung et al. 2001). Caspase 3 activation is often regarded as a sign of apoptosis (Judith et al. 2004). Its function is important in several cellular processes including the removal of cells and the formation of apoptotic bodies (Alan & Reiner 1999; Gerald 1997). In this study, the percentage of caspase 3 activation significantly increased at 4 h and 6 h of treatment (see Fig. 3.4). Two-fold and five-fold increases were measured at 4 h and 6 h of treatment, respectively. The activation of caspase 3 with a significant difference from VC was regarded as a sign of apoptosis occurrence in TFBF induced-Jurkat cells.

Apart from that, the changes on the cells' morphology were also observed. Some of the features of apoptosis were cell shrinkage, membrane blebbing, nuclear chromatin condensation, and formation of apoptotic bodies (Charles & Michael, 2014; Pellerito et al. 2006; Saraste et al. 2000). The TFBF induced-Jurkat cells showed some characteristics of cells undergoing apoptosis including membrane blebbing and elongated cells. A slight membrane blebbing was observed as early as 1 h of treatment, whereby the elongated cells were clearly observed at 4 h (see Fig. 3.5). Meanwhile, some cells already lysed at 6 h. A similar morphological change was also observed in HT-29 human colorectal carcinoma cells induced by triphenyltin(IV) ethylphenyldithiocarbamate compound (Awang et al. 2014).

CONCLUSION

In conclusion, the triphenyltin(IV) butylphenyldithiocarbamate (TFBF) compound showed a good cytotoxicity in Jurkat E6.1 cells as the compound induced apoptosis at a micromolar dose. The ROS production was suggested as an important factor that disrupted the normal mitochondrial function, consequently caused apoptosis. This compound warranted further studies to ensure its potential as a new anticancer agent.

ACKNOWLEDGEMENT

We would like to thank the Ministry of Higher Education for the fundamental research grant scheme (FRGS/2/2013/SKK10/UKM/02/1) to support this research. We would also like to thank the Biomedical Science Program and Environmental Health and Industrial Safety Program, Faculty of Health Sciences, Universiti Kebangsaan Malaysia for the laboratory assistance and technical support.

REFERENCES

[1] Alama, A., Tasso, B., Novelli, F. & Sparatore, F. 2009. Organometallic compounds in oncology: implications of novel organotins as antitumor agents. *Drug Discovery Today* 14: 500-508.

[2] Alan, G.P. & Reiner, U.J. 1999. Emerging roles of caspase-3 in apoptosis. *Cell Death and Differentiation.* 6 : 99-104

[3] Assim, A.A., & Reem, M.S. 2012. Reactive Oxygen Species in Health and Disease. *Journal of Biomedicine and Biotechnology*

[4] Awang, N., Baba, I., Abd Aziz, Y.F., & M. Yamin, B. 2005. Sintesis, Pencirian dan Aktiviti Biologi Sebatian Trifeniltin(IV) ditiokarbamat. *Proceeding of The 6th ITB-UKM Joint Seminar on Chemistry.*

[5] Awang, N., Kamaludin, N.F., Barnard, E., Hamid, A., Norfadilah, R., & Azhar, A.H. 2011. Cytotoxic and Genotoxic effect of Triphenyltin(IV) Benzylisopropyldithiocarbamate in Thymoma Murine Cell Line (WEHI 7.2). *Research Journal of Chemistry and Environment* 15(2)

[6] Awang, N., Kamaludin, N.F. & Ghazali, A.R. 2011. Cytotoxic Effect of Organotin(IV) Benzylisoproprylditiocarbamate Compounds On Chang Liver Cell and Hepatocarcinoma HepG2 Cell. *Pakistan Journal of Biological Sciences* 14(15) : 768-774.

[7] Awang, N, Kamaludin, N.F., Hamid, A., Mokhtar, N.W.N. & Rajab, N.F. 2012. Cytotoxic of Triphenyltin(IV) Methyl- and Ethylisopropyldithiocarbamate Compounds in Chronic Myelogenus Leukemia Cell Line (K562). *Pakistan Journal of Biological Sciences* 15(17) : 833-838

[8] Awang, N., A.Aziz, Z., Kamaludin, N.F., & Chan, K.M. 2014. Cytotoxicity and Mode of Cell Death Induced by Triphenyltin(IV) Compoounds in Vitro. *Online Journal of Biological Science* 14 (2):84-93

[9] Bindoli, A., Fukuto, J.M., Forman, H.J. 2008. Thiol chemistry in peroxidase catalysis and redox signaling. *Antioxid Redox Signal* 10 : 1549-64

[10] Brian, S.C., & Rick, G.S. 2002. Cisplatin-Induced Renal Cell Apoptosis: Caspase 3- Dependent and – Independent Pathways. *The Journal of Pharmacology and Experimental Therapeutics* 302:8-17

[11] Chan, K.M., Rajab, N.F., Siegel, D., Din, L.B., Ross, D., Inayat-Hussain, S.M. 2010. Goniothalamin Induces Coronary Artery Smooth Muscle Cells Apoptosis: The p53-Dependent Caspase-2 Activation Pathway. *Toxicology Sciences* 116: 533- 548

[12] Charles, E.C.Jr & Michael, R.R. 2014. Organotin polymers as Anticancer and antiviral Agents. *Journal of Organometallic Chemistry* 751: 67-82

[13] Gerald M.Cohen. 1997. Caspases : the executioners of apoptosis. *Biochem J* 326 : 1-16

[14] Grub, J.D.Ly., R., Grubb & Lawen. 2003. The mitochondrial membrane potential in apoptosis. *Apoptosis.* 8 : 115-128

[15] Gottlieb, E., Armour, S.M., Harris, MH., & Thompson, C.B. 2003. Mitochondrial membrane potential regulates matrix configuration and cytochrome C release during apoptosis. *Cell death and Differentiaton* 10 : 709-717

[16] Haufel, T. & Bauer, G. 2001. Transformation of rat fibroblast by TGF-beta and EGF induce sensitivity for intracellular induction of apoptosis. *Anticancer Res.* 21 : 2617-2628

[17] Inayat-Hussain, S.H., Chan, K.M., Rajab, N.F., Din, L.B., Chow, S.C., Kizilors, A., Farzaneh, F., & Williams, G.T. 2010. Goniothalamin-induced Oxidative Stress, DNA Damage and Apoptosis via Caspase-2 Independent and Bcl-2 Independent Pathways in Jurkat T-Cells. *Toxicology Letters* 193:108-114

[18] Judith, H.W., Caroline, D., Jean-Claude, M., & Dominic, J. 2004. Role of mitochondrial membrane permeabilization in apoptosis and cancer. *Oncogene* 23 : 2850-2860

[19] Kamaludin, N.F., Awang, N., Baba, I., Hamid, A., & Chan, K.M. 2013. Synthesis, Characterization and Crytsal Structure of Organotin(IV) N-Butyl-N- Phenyldithocarbamate Compounds and their Cytotoxicity in Human Leukemia Cell Lines. *Pakistan Journal of Biological Sciences* 16(1) : 12-21

[20] Kong, Q., Beel, J.A., Lillehei, K.O. 2000. A threshold concept for cancer therapy. *Medicine Hypotheses* 55 : 405-409

[21] Manisha Pokharel. 2012. Leukemia : A Review Article. *International Journal of Advanced Research in Pharmaceuticals & Bio Sciences* 2 : 397-407

[22] Mihaela Onciu. 2009. Acute Lymphoblastic Leukemia. *Hematol Oncol Clin N Am* 23 : 655-674

[23] Paul, H. 2010. An Introduction to Reactive Oxygen Species : Measurement of ROS in Cells.http://www.biotek.com/assets/tech_resources/ROS%20Application%20Guide.pdf [21 March 2014]

[24] Pellerito, C., Nagy, L., Pellerito, L., & Szorcsik. A. 2006. Biological Activity Studies on Organotin(IV) Complexes and Parent Compounds. *Journal of Orgnometallic Chemistry* 691 : 1733-1747

[25] Pellerito, C., Prinzivalli, C., Pellerito, O., & Guiliano, M. 2010. Organotin(iv) Complexes with n-acetylcysteine Possessing Antitumoral activity, Porcess for their production and their use. http://www.google.com/patents/WO2010015590A1?cl=en [31 Januari 2014]

[26] Pizzaro, A.M., Habtemariam, A., Sadler, P.J. 2010. Activation Mechanisms for Organometallic Anticancer Complexes. *Top Organomet Chem* 32 : 21-56

[27] Pui, C.H., Robinson, L.L., & Thomas, A.L. 2008. Acute Lymphoblastic Leukaemia. *Lancet* 371 : 1030-43

[28] Rajesh, P.R., Richa & Rajeshwar, P.S. 2009. Apoptosis : Molecular Mechanisms and Pathogenicity. *EXCLI Journal* 8 : 155-181

[29] Saraste, A. & Pulkki, K. 2000. Morphologic and Biochemical Hallmarks of Apoptosis. *Cardiovascular Research* 45: 528-537

[30] Sartaj, T., & Claudio, P. 2006. Chemical and biotechnological developments in organotin cancer chemotherapy. *Journal of Organometallic Chemistry* 691 : 1761-1766

[31] Seung, H.J., Mi, K.S., Ho, J.K., Su, M.L., In, H.S., Ypng, O.K., Young, S.L., Kyu, S.J., Won, B.K., Jeen, W.P., Bypung, J.S., & Tae, L.H. 2001. Control of Mitochondrial Redoz Balance and Cellular

[32] Defense against Oxidative Damage by Mitochindrial NADP+-dependent Isocitrate Dehydrogenase. *The Journal of Biological Chemistry* 276:16168-16176

[33] Stankovic, T., & Marston, E. 2008. Molecular Mechanisms Involved in Chemoresistance in Paediatric Acute Lymphoblastic Leukemia. *BIBLID* 136 : 187-192

[34] Suen, D.F., Norris, K.L., & Youle, J. 2008. Mitochondrial dynamics and apoptosis. *Genes and Development* 22 : 1577-1590