2011 International Conference on Environmental, Biomedical and Biotechnology
IPCBEE vol.16 (2011) © (2011)IACSIT Press, Singapoore

Palm Puree : Potential Neuroprotective Effect from *Elaeis guineensis* Jacq. Fresh Fruit Bunch

Nordalilah Rosni [1], Zainon Mohd Noor [1] and Mazatulikhma Mat Zain [2]

[1] Faculty of Applied Sciences, Universiti Teknologi MARA Malaysia
[2] Institute of Science, Universiti Teknologi MARA Malaysia

Abstract. Palm Puree is a new food product derived from the squeezed oil palm fruitlets and was prepared from fresh fruit bunches. The objective of the present study was to investigate the effects of Palm Puree as neuroprotective agents against hydrogen peroxide (H_2O_2)-induced toxicity on human neuroblastoma SH-SY5Y cell line thus producing an *in vitro* model for neurotoxicity or neuroprotection in experimental brain research. Neuronal damage was quantitatively assessed using cytotoxicity assay kit from Promega Corp. (USA) and live neurons were counted by measuring the production of coloured formazan. Results showed that the Palm Puree exerted neuroprotective effects in a good dose-dependent manner which probably maybe due to them containing tocopherol and tocotrienols.

Keywords: Palm Puree, Neuroprotection, hydrogen peroxide, SH-SY5Y cell line.

1. Introduction

Neuroprotection is the effort to maintain the highest possible intactness of intercellular communication in the brain resulting in overall undisturbed function. In brief, it is the protection of neuronal function [1]. The understanding of the toxicity of the Palm Puree is crucial in order to identify and develop more effective means of neuroprotection. Neurodegenerative diseases are characterized by death of neuron cells in the central nervous system (CNS) which includes Parkinson's disease, Alzheimer's disease, Huntington's disease and others. Parkinson's disease is typically considered an aging-related neurodegenerative disorder characterized by degeneration of the nigrostriatal system [2]. Alzheimer's disease is a devastating neurodegenerative disorder characterized by progressive memory loss and dementia [3]. Central nervous system (CNS) involves the brain and the spinal cord that connects millions of neuron cells that controls feelings, perceptions, movements and others. Therefore, the brain should be protected from any prolong stress that could possibly lead to neurodegenerative diseases as mentioned earlier [4]. Neurodegenerative diseases were also related to the presence of reactive oxygen species or ROS. These include oxygen ions, free radicals, and peroxides (H_2O_2) which are being constantly generated in organisms. Cellular antioxidants act in concert to detoxify these species but, when the balance is disrupted, a condition referred to as oxidative stress exists [5]. If oxidative stress persists, oxidative damage to critical biomolecules accumulates and eventually results in several biological effects ranging from alterations in signal transduction and gene expression to mitogenesis, transformation, mutagenesis and cell death [6,7]. Antioxidants such as vitamin E (found abundant in Palm Puree) and vitamin C were shown to inhibit the chain reaction of free radicals on lipid, protein and DNA molecules in Alzheimer's patients [8,9]. Many studies have showed that vitamin E possesses significant inhibiting oxidative damage [10,16]. Each of the vitamin E isoforms has a different biopotency. Tocopherol is generally considered to be the most important form [11]. However, tocotrienols supplementations are proved to reduce blood levels of lipid peroxides with an improved blood flow in patients with carotid atherosclerosis [12]. Several reports that tocotrienols possess non-antioxidant functions which contribute to their cholesterol-lowering, anticarcinogenic and cytoprotective properties [13,14].

2. Materials and Methods

2.1. Neuroblastoma SH-SY5Y Cell line

The human neuroblastoma cell line (SH-SY5Y) used in this study was a gift from Mazatulikhma Mat Zain of Tissue Culture Laboratory, Institute of Science, UiTM Malaysia. Cells were treated with retinoic

acid (RA), at a final concentration of 10μM, for its ability to enhance the differentiation during the first 5 days of incubation to become neuronal-phenotypic cells. The cell line was cultured in complete culture media (CCM) of EMEM-F12 supplemented with 2 mM glutamine (Sigma, USA), 1% non-essential amino acid (PAA Laboratories GmbH, Austria), 50 μg/mL gentamicin (PAA Laboratories GmbH, Austria) and 10% heat activated fetal bovine serum (PAA Laboratories GmbH, Austria) in a humidified atmosphere containing 5% CO_2 at 37°C. The media was changed after 3 days with fresh RA and cells were ready for testing at day 6. The cells were plated at a density of 2 x 10^4 cells per mL.

2.2. Sample Preparation

The Palm Puree was weighed 10 mg individually and mixed with 1 mL ethanol in a sterile microcentrifuge tube. These tubes content were mixed using a vortex and kept at 4°C as stock samples. These samples were diluted to these concentrations; 100 μg/mL. 10 μg/mL, 1 μg/mL, 100 ng/mL, 10 ng/mL and 1 ng/mL and were prepared fresh prior to experiment.

2.3. Determination of IC_{50} for Hydrogen Peroxide

The final concentrations ranges from 1 nM to 100 mM for dilutions of hydrogen peroxide (H_2O_2) in CCM were prepared fresh prior to each experiment. Next, 10 μL of H_2O_2 was added into each well in a 96-well plate and incubated in a humidified incubator at 37°C, 5% CO_2 for an hour. Cell viability was measured using a light-sensitive MTS Assay [3(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide], (CellTiter $96_{Aqueous}$ Non-Radioactive Cell Proliferation Assay, Promega, USA). Dehydrogenase enzymes found in metabolically active cells reduce the MTS compound into a formazan product thus the amount of coloured formazan product is proportional to the number of viable cells. 20 μL of MTS was added into each well and the quantity of formazan present was determined by measuring the absorbance at 490 nm using GloMax Integrated Multidetection System by Promega, USA.

2.4. Analysis of Neurotoxicity Properties

The samples dilutions were made fresh prior to experiment. Palm Puree was added into each well in a 96-well plate with the final concentrations of 100 μg/mL, 10 μg/mL, 1 μg/mL, 100 ng/mL, 10 ng/mL and 1 ng/mL. The plate was agitated gently and incubated in a humidified incubator at 37°C, 5% CO_2 for 24 hours. Cell viability was measured as absorbance at 490 nm with MTS Assay using GloMax (Promega, USA).

2.5. Analysis of Neuroprotection Properties

Differentiated SH-SY5Y cells were incubated with the IC_{50} value of H_2O_2 (100 μM) for an hour which indicates only 50% of the cells were viable. Afterwards, the cells were treated with the Palm Puree samples prepared in concentrations ranges between 100 μg/mL to 1 ng/mL for 24 hours incubation in CO_2 at 37°C. Cell viability was assessed using CellTiter $96_{Aqueous}$ Non-Radioactive Cell Proliferation Assay (MTS, Promega, USA).

2.6. Statistical Analysis

The results were presented as the analysis of variance (ANOVA) followed by Dunnet's test for post hoc multiple comparisons test where *p*-values lower than 0.05 were considered as statistically significant using GraphPad Prism 5 software. The data were expressed as mean ± S.E.M.

3. Results and Discussion

3.1. Determination of IC_{50} for Hydrogen Peroxide

In this study, the IC_{50} was carried out using hydrogen peroxide (H_2O_2) with series of dilutions at 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, 100 μM, 1 mM, 10 mM and 100 mM. The IC_{50} value is 100 μM which can be seen in Fig. 1, was determined by MTS assay. The assay was based on the quantity of formazan produced by the reaction of tetrazolium salt (MTS). Dehydrogenase enzymes found in metabolically active cells reduce the MTS compound into a formazan product that is soluble in the complete culture media. The amount of coloured formazan product is proportional to the number of viable cells. From the graph, it can be observed that the cell lines number decreases as the concentration increases. This showed that the hydrogen peroxide gave oxidative stress environment in the well in a dose-dependent manner [15]. The concentration of 100 μM

as IC_{50} was in accordance to the finding of Whittermore [16]. The finding showed that this concentration was able to induce apoptotic cell death of primary neuronal culture within 3 hours of incubation and exposure to H_2O_2.

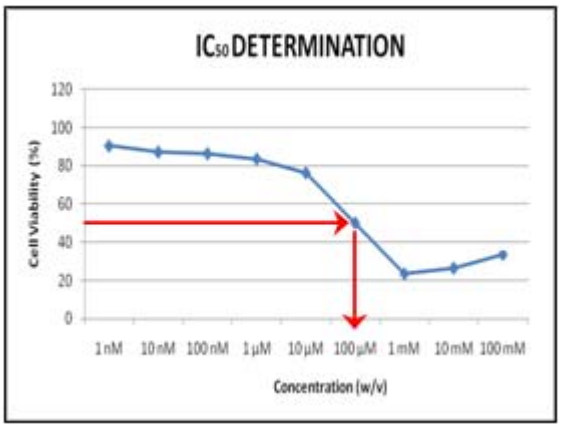

Fig. 1 Concentration of H_2O_2 ranges from 1 nM up to 100mM that caused 50% cell viable (IC_{50}) as measured by MTS Assay. The IC_{50} value concentration as shown is at 100 µM. The control is measured as 100% viability.

3.2. Analysis of Neurotoxicity Properties

Human neuroblastoma cell lines SH-SY5Y was exposed to a series of concentrations from Palm Puree samples ranging from 1 ng/mL to 100 µg/mL for 24 hours. Before that, the cells were induced with 10 µL of 100 µM (IC_{50} concentration) for an hour in a humidified atmosphere containing 5% CO_2 at 37°C. Neurotoxicity test was carried out with the objective mainly to determine whether the Palm Puree sample was toxic to the cells or otherwise. The histogram below (Fig. 2) showed the concentration-response pattern of cytotoxicity obtained by exposure to samples measured by MTS reduction. From the figure, the sample displayed no toxic effect towards the cells and yet, the cells still proliferate at the highest concentration tested. These results are promising as a preliminary testing since the Palm Puree is being developed as a new health food product which is hoped to ascertain consumers' health and benefits. One way ANOVA showed moderate significant effect of 1 µg/mL concentration ($P<0.05$) as compared to the others. Meanwhile, 10 µg/mL and 100 µg/mL of concentrations have less significance and the rest are not significantly different indicating lower sensitivity towards the experiment.

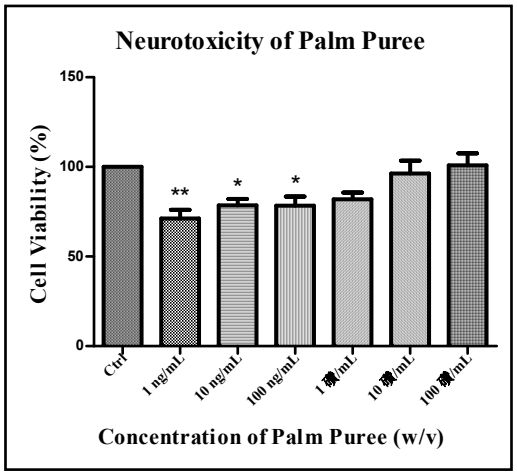

Fig. 2 Neurotoxicity test of Palm Puree towards neuron cells as measured by MTS assay after 24 hours of incubation in a humidified incubator with 5% CO_2 at 37°C. Protective effect can be seen in the concentration range of 1-100 ng/mL. Data is presented as mean±SEM, n=3. * indicates less significant difference. ** indicates moderate significant difference.

3.3. Analysis of Neuroprotection Properties

Neuroprotection may be defined as the effort to maintain the highest possible intactness of cellular interactions/intercellular communication in the brain resulting in an overall undisturbed function. In this experiment, the cells were exposed to hydrogen peroxide at 100 μM (IC_{50} value) for an hour of incubation and later being exposed to the sample dilutions. As shown in Fig. 3, most of the concentrations have high significant difference ($P<0.05$) except for 1 ng/mL of concentration.

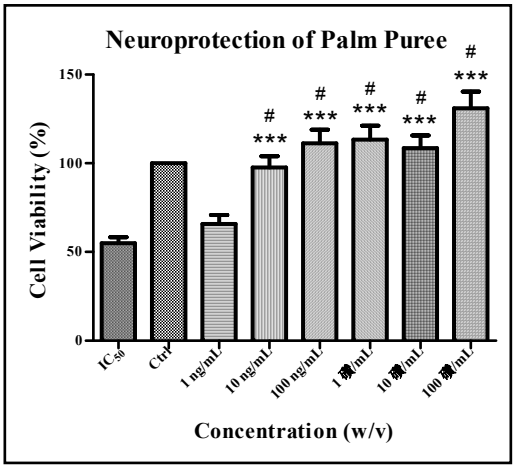

Fig. 3 Neuroprotection test of Palm Puree towards neuron cells as measured by MTS assay after 24 hours of incubation in a humidified incubator with 5% CO_2 at 37°C. Earlier, the cells were induced with 100 μM of H_2O_2 for an hour before treatment with sample. Protective effect can be seen in the concentration range of 1-100 ng/mL. Data is presented as mean±SEM, n=3. Dunnet's MC post hoc test for each treatment indicated difference ($p<0.05$) compared to control (*) or compared to IC_{50} (#).

The Palm Puree samples gave promising neuroprotective effects on SH-SY5Y cell lines in a dose-dependent manner as the cell viability percentage increases with increasing concentration. The results indicated that the Palm Puree gave higher percentage of cell viability as compared to control. The cells showed the most significant viability at 100 μg/mL which might demonstrate proliferation as a result of the cells utilizing the sample instead of just protecting the cells from hydrogen peroxide. Palm Puree as a whole contains high amount of vitamin E, in both tocopherol and tocotrienols forms. Tocotrienols in its four isoforms namely, α-, β-, γ- and δ-tocotrienols, and several previous studies have demonstrated that δ-tocotrienol exerts the most potent antiproliferative activity among tocotrienols isoforms [11,14] because it promotes greater accumulation in the cells which might be the reason for this effect [12,13]. These have somewhat proved the Palm Pure exerts neuroprotective effects at low concentration (>1 ng/mL) against H_2O_2-induced oxidative stress in human neuroblastoma SH-SY5Y cell line. Musalmah *et al.* [17] also demonstrated another method by treating the cells with sample for an hour followed by inducing with hydrogen peroxide for 24 hours. This resulted in 30% reduction in viable cell number from positive control when incubated with 100 μL of γ-tocotrienol and measured using MTS assay.

3.4. Conclusion

In conclusion, Palm Puree extracts possessed neuroprotective effects towards H_2O_2-induced neuronal cell line. It is beneficial to further study the neuroprotective potential as it showed no toxic effects towards the cells which proves it is safe for human consumption. The effects were seen highest at 100 μg/mL concentration which can be considered and commercialized as potential neuroprotective drugs. It is recommended to identify cell death using LDH assay and caspases activity which lead to apoptosis using commercial apoptosis kit in order to validate and produce better results.

4. Acknowledgements

Authors wish to thank Institute of Science, UiTM Malaysia for technical support. The study was supported by the grant (100-RMI.P37/7/2005) provided by Sime Darby Plantation Sdn. Bhd.

5. References

[1] E. Ehrenreich, and A.L. Siren. Neuroprotection – What does it mean? What means do we have?. *Eur.Arch. Psychiatry Clin. Neurosci.* 2001, **251**: 149-151.

[2] K.L. Leenders, and W.H. Oertel. Parkinson's disease: Clinical signs and symptoms, neural mechanisms, positron emission tomography, and therapeutic interventions. *Neural. Plast.* 2001, **8**: 99-110.

[3] P.N. Lacor, M.C. Buniel, L. Chang, S.J. Fernandez, Y. Gong, K.L. Viola, M.P. Lambert, P.T. Velasco, E.H. Bigio, C.E. Finch, G.A. Krafft, and W.L. Klein. Synaptic targeting by Alzheimer's-related amyloid beta oligomers. *J. Neuroscience*. 2004, **24**: 10191-10200.

[4] N.A. Campbell, and J.B. Reese. Biology. *Benjamin Cummings*. 2002, **6**: 1040-1053.

[5] D.M. Skovronsky, M. Virginia, Y. Lee, and J.Q. Trojanoski. Neurodegenerative diseases: New concepts of pathogenesis and their therapeutic implications. *Ann. Rev. Path. Mech.*2006, **1**: 151-170.

[6] C.R. Hunt, J.E. Sim, S.J. Sullivan, T. Featherstone, W. Golden, C.V. Kapp-herr, R.A. Hock, R.A. Gomez, A.J. Parsian, and D.R. Spitz. Genomic instability and catalase gene amplification induced by chronic exposure to oxidative stress. *Cancer Research*. 1998, **58**: 3986-3992.

[7] E.M. Mills, K. Takeda, Z.X. Yu, V. Ferrans, Y. Katagiri, H. Jiang, M.C. Lavigne, T.L. Leto, and G. Guroff. Nerve growth factor prevents the increase in superoxide produced by epidermal growth factor in PC12 cells. *J. Biol. Chem.*1998, **273**: 22165-22168.

[8] R.J. Mark, E.M. Blanc, and M.P. Mattson. Amyloid beta-peptide and oxidative cellular injury in Alzheimer's disease. *Mol. Neurobiol.* 1996, **12**: 211-224.

[9] N. Chandrasekharan. Changing concepts in lipid nutrition in health and disease. *Medical Journal of Malaysia*. 1999, **54**(3): 408-427.

[10] B.J. Weimann, and H. Weiser. Functions of vitamin E in reproduction and in prostacyclin and immunoglobulin synthesis in rats. *Am. J. Clin. Nutr.*1991, **53**: 1056S-1060S.

[11] K.S. Chandan, K. Savita, and R. Sashwati. Tocotrienols in health and disease: The other half of the natural vitamin E family. *Molecular Aspects of Medicine*. 2007, **28**: 692-728.

[12] A.C. Tomeo, M. Gellar, T.R. Watkins, A. Gapor, and M.L. Bierenbaum. Antioxidant effects of tocotrienols in patients with hyperlipidemia and carotid stenosis. *Lipid.* 1995, **30**: 1179-1183.

[13] Y. Bermudez, S. Ahmadi, N.E. Lowell, and P.A. Kruk. Vitamin E suppresses telomerase activity in ovarian cancer cells. *Cancer Detection and Protection*. 2007, **31**: 119-128.

[14] K. Nesaretnam, J.L. Eu, R. Karin, and C.L. Lestie. Effect of a carotene concentrates on the growth of human breast cancer cells and pS2 gene expression. *Toxicology*. 2000, **151**: 117-126.

[15] M. Encinas, M. Iglesias, Y. Liu, H. Wang, A. Muhaisen, V. Cena, C. Gallego, and J.X. Comella. Sequential treatment of SH-SY5Y cells with retinoic acid and brain-derived neurotrophic factor gives rise to fully differentiated, neurotrophic factor-dependent, human neuron-like cells. *Journal of Neurochemistry*. 2000, **75**: 991-1003.

[16] R. Whittermore, D.T. Loo, and C.W. Cotman. A detailed analysis of hydrogen peroxide-induced cell death in primary neuronal culture. *Neurosciences*. 1995, **67**: 921-932.

[17] M. Musalmah, T.S. Mian, M.T. Gapor, and W.Z.W. Ngah. Comparative effects of α-tocopherol and γ-tocotrienol against hydrogen peroxide induced apoptosis on primary-cultured astrocytes. *Journal of the Neurological Sciences*. 2006, **243**: 5-12.