EFFECTS OF HARUAN PRESSURIZED IN WATER EXTRACT ON SK-N-SH HUMAN NEUROBLASTOMA CELL LINE IN VITRO

BUHARI IBRAHIM

FPSK(m) 24
EFFECTS OF HARUAN PRESSURIZED IN WATER EXTRACT ON SK-N-SH HUMAN NEUROBLASTOMA CELL LINE IN VITRO

By

BUHARI IBRAHIM

Thesis submitted to the School of Graduate Studies, University Putra Malaysia in Fulfilment of the Requirement for the Degree of Master of Science

June 2015
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DEDICATION

This work is dedicated to:

Almighty Allah who made this research work possible,
My parents,
My wife and son, and
My Supervisor Prof. Dr. Abdul Manan Mat Jais
For the efficient support they gave me throughout the time of the study.
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfilment of
the Requirement for the Degree of Master of Science

EFFECTS OF HARUAN PRESSURIZED IN WATER EXTRACT ON SK-N-SH
HUMAN NEUROBLASTOMA CELL LINE IN VITRO

By

BUHARI IBRAHIM

June 2015

Chairman: Professor Abdul Manan Mat Jais, PhD
Faculty: Medicine and Health Sciences

Effects of haruan pressurized in water extract (HPIWE) on SK-N-SH neuroblastoma
cell line was studied in vitro. MTT assay, phase contrast microscopy and flow
cytometry were employed to evaluate the effects of HPIWE on SK-N-SH cells. To start
with, MTT assay was run to observe the effect of the haruan extract on cell viability of
SK-N-SH human neuroblastoma cells. The cells were treated with various
concentrations of the HPIWE to undertake a first step in screening the extract for its
cytotoxic effects. The result showed the cell viability of 68.8% at the highest
concentration of 100 µg/ml, indicating that HPIWE possesses no cytotoxic effect
because no IC\textsubscript{50} could be determined. Another MTT assay was run with 250 µg/ml of
the extract as highest concentration to observe if increasing the concentration will
produce better result. However, the result indicates about 72% cell viability of the SK-
N-SH cells. Therefore, since 100 µg/ml produces less viability, it was adopted for
subsequent experiments. This concentration was then used to study the effects HPIWE
on cellular morphology of the SK-N-SH cells using phase contrast microscope. No
alteration of cellular morphology was observed on SK-N-SH cells as compared to the
control cells which did not receive treatment. Based on the MTT assay and the
morphological studies by phase contrast, there were no observable effects of the
HPIWE on SK-N-SH cells. Our study further search for the apoptotic effects of the
extract using ApoBrdU \textit{in situ} DNA fragmentation assay kit followed by quantification
of the DNA strand break using flow cytometry. One-way ANOVA test was used to
compare the difference between the treated cells and the control cells (untreated cancer
cells). The analysis showed that the HPIWE induced apoptosis in a time dependent
manner and significant value (*p< 0.05) was obtained when compared with the control.
The result confirmed that the HPIWE induced apoptosis on the SK-N-SH human
neuroblastoma cancer cells. However, the amount of cells that underwent apoptosis
were less than 50% of the total cells, meaning that even from the flow cytometry result,
no IC\textsubscript{50} could be generated. Therefore, the result of flow cytometry could be correlated
with the MTT assay result, because in both assays, over 30% of cell death was
recorded. However, the overall result of the study showed that HPIWE was not an
effective anti-proliferative agent on SK-N-SH cells. Therefore, further studies should
target the use of non-polar haruan extract for cancer related studies. Also angiogenesis
and cell migration assay should be used to demonstrate the effect of HPIWE on brain
cancer and or other cancer cell lines.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN ESTRAK HARUAN BERTEKANAN DALAM AIR KE ATAS GARIS SEL NEUROBLASTOMA SK-N-SH MANUSIA IN VITRO

Oleh

BUHARI IBRAHIM

Jun 2015

Pengerusi: Profesor Abdul Manan Mat Jais, PhD
Fakulti: Perubatan dan Sains Kesihatan

penghijrahan sel boleh digunakan untuk menunjukkan kesan HPIWE kepada kanser otak dan atau garis sel kanser lain.
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All thanks be to almighty Allah who gave me the talent, health, wisdom and strength to complete the research work.

Special thanks to my supervisor Professor Dr. Abdul Manan Mat Jais, who taught me scientific research, and gave me substantial advice. May the almighty Allah continue to guide and support him in all his endeavours.

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Hearty thanks to all my friends, and roommates for their brotherly support throughout my stay in University Putra Malaysia. May God reward you abundantly.

Finally, my sincere gratitude goes to my parents Mallam Ibrahim Muhammad Ibbi and Aishatu Muhammad Khamisu for their spiritual, moral and financial support throughout the course of my study. May almighty Allah rewards you with Jannatul Firdaus and protect you throughout your life.
I certify that a Thesis Examination Committee has met on 29 June 2015 to conduct the final examination of Buhari Ibrahim on his thesis entitled "Effects of Haruan Pressurized in Water Extract on Sk-N-Sh Human Neuroblastoma Cell Line In Vitro" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia (P.U.(A) 106) 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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<td>Percent</td>
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<tr>
<td>ºC</td>
<td>Degree Celsius</td>
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<td>ABU</td>
<td>Ahmadu Bello University</td>
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<td>AECS</td>
<td>Aqueous extract of <em>Channa striatus</em></td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>AO</td>
<td>Acridin Orange</td>
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<td>ATCC</td>
<td>American Type Culture Collection</td>
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<tr>
<td>BrdUTP</td>
<td>Bromodeoxyuridine triphosphate</td>
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<tr>
<td>Cm</td>
<td>Centimetre</td>
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<tr>
<td>CO2</td>
<td>Carbon dioxide</td>
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<tr>
<td>COX-2</td>
<td>Cyclo-oxygenase</td>
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<tr>
<td>DDH20</td>
<td>Deionized distilled water</td>
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<td>DHA</td>
<td>Deicosahexaenoic acid</td>
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<td>DMSO</td>
<td>Dimethylsulfoxide</td>
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<td>DNA</td>
<td>Deoxyribonucleotide acid</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediamine tetra-acetic acid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>EPA</td>
<td>Ecosapentaenoic acid</td>
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<tr>
<td>FA</td>
<td>Fatty acid</td>
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<tr>
<td>FACS</td>
<td>Fluorescence-activated cell sorting</td>
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<td>FBS</td>
<td>Fetal bovine serum</td>
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<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
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<td>HL-60</td>
<td>promyelocytic leukaemia</td>
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<td>Haruan Pressurized in water extract</td>
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<td>Hours</td>
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<td>human colon adenocarcinoma</td>
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<td>IC50</td>
<td>Inhibition concentration for 50% of cell population</td>
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<td>INRG</td>
<td>International neuroblastoma risk group</td>
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<tr>
<td>KCl</td>
<td>Potassium chloride</td>
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<td>KH2PO4</td>
<td>Potassium dihydrogen phosphate</td>
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<td>LECS</td>
<td>Lipid extract of <em>Channa striatus</em></td>
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<td>MEM</td>
<td>Eagle’s Minimum essential Medium</td>
</tr>
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<td>mL</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>Millimetre</td>
</tr>
<tr>
<td>MTT</td>
<td>Microculture tetrazolium</td>
</tr>
<tr>
<td>NFKB</td>
<td>Nuclear Factor Kappa Beta</td>
</tr>
<tr>
<td>NYSC</td>
<td>National youth service corps</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PDECGF</td>
<td>Platelet-Derived Endothelial Cell Growth Factor</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet drive growth factor</td>
</tr>
<tr>
<td>PGE2</td>
<td>Prostaglandin-E2</td>
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<td>pH</td>
<td>Hydrogen ion concentration</td>
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<td>PhD</td>
<td>Doctor of philosophy</td>
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<tr>
<td>PUFA</td>
<td>Polynsaturated fatty acids</td>
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<tr>
<td>RNase</td>
<td>Ribonuclease</td>
</tr>
<tr>
<td>Rpm</td>
<td>Rotation per minute</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<td>TdT</td>
<td>Terminal deoxynucleotidyl Transferase</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
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<td>TUNEL</td>
<td>Terminal deoxyribonucleotide Transferase-mediated dUTP Nick-End Labelling</td>
</tr>
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<td>UPM</td>
<td>Universiti Putra Malaysia</td>
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<tr>
<td>VEGF</td>
<td>Endothelial growth factor</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight per volume</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>μL</td>
<td>Microlitre</td>
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CHAPTER 1

INTRODUCTION

Cancer is one of the major causes of death globally. The World Health Organization (WHO) estimated the sum of 14 million new cancer cases and about 8.2 million deaths due to cancer in the year 2012, and it was said the death record could hit 13.1 million by the year 2030. About 60% of total cancer cases in the world occur in Asia, Africa, and Central and South America. These regions were said to account for 70% of total cancer death in the world (World Cancer Report, 2014). According to the International Agency for Research on Cancer (IARC) Globocan of the World Health Organisation (WHO), in 2012, the number of cancer incidence and cancer death stood at 37,000 and 21,700 respectively.

Brain cancer is the second most popular cancer among children after leukaemia (Siegel et al., 2013). Brain tumour may be categorised into primary and secondary tumours. Primary tumour originated from the brain tissue, whereas secondary tumour metastasizes from other tissues (Villarreal-Garza. et al., 2013). Even though brain cancer was termed the third most cause of death in adult under the age of 34 years, more of these tumours show up after the age of 50 years (Castro et al., 2003).

Neuroblastoma is the prevailing solid malignant tumour in infancy and childhood. Two-thirds of the tumour metastasize from other organs (Modak and Cheung, 2010). Despite the fact that neuroblastoma accounts for only 8% of all childhood malignant tumours, 15% of childhood cancer deaths are due to it (Castel et al., 2013). Treatment of neuroblastoma includes induction of high dose radiotherapy, chemotherapy, stem-cell transplantation and surgical removal of the tumour (Navarra et al., 2014). Although, some level of improvement was accomplished using the earlier mention methods of treating brain malignancy, a number of patients with high risk of neuroblastoma do not respond to the conventional therapy, or the symptoms appear to come back later (Castel et al., 2013). Therefore, screening of natural products that can cure this disease may give an alternative treatment with no or less side effects.

Natural products derived from marine animals such as, coral, sponges, microorganisms and fishes was shown to possess anti-inflammatory, anti-viral and anti-cancer effects. It was reported that 74.8% of currently used anti-cancer drugs such as taxanes, vinca alkaloids and camptothecin class of compounds are sourced from natural products (Newman and Cragg, 2012).

Haruan fish, found in many tropical countries like Malaysia, Indonesia, and Thailand is widely known as a source of food and traditional medicinal products since pre-historic time (Abdul Manan, 2007). Preliminary bioactive component (Zakaria et al., 2007) include proteins, peptides, glycoproteins, fat, and minerals (Abdul Manan, 2007). Amino acids; histidine, lysine, aspartate and arginine are reported to produce antioxidant effect (Dahlan-daud et al., 2010; Frankel, 1998). The amino acids as well as the fatty acids content of the fish could give a tremendous effect on cancer treatment. An earlier report by Nur Syamsiah (2012) suggested the cytotoxic and anti-proliferative effects different fractions of haruan traditional extract (HTE) on colon-rectal and Leukaemia cancers. Also, antioxidant effects of aqueous extract of Channa
striatus (AECS) was demonstrated using 2,2-diphenyl-picrylhydrazyl (DPPH) assay, azino-bis(3 ethylbenzothiazoline-6 sulphonic acid (ABTS) and ferric reducing ability of power (FRAP) (Radzak et al., 2014). The authors were able to correlate the antioxidant effects of AECS with cytotoxicity assay on in human liver hepatoma HepG2 cells by the MTT assay.

Therefore, this study evaluated the anti-proliferative effect of HPIWE on SK-N-SH neuroblastoma, brain cancer cell in vitro.

1.1 Problem Statement

Neuroblastoma is a solid brain tumour that accounts for more than 15% of childhood cancer death. However, conventional treatment of the tumour using chemotherapy, radiotherapy and surgery is associated with toxicity, drug resistance and screening effect of blood brain barrier. Search for potential agent from natural product with less side effects will be a welcome development.

1.2 Justification of the study

HPIWE showed the anti-proliferative effects on human colon adenocarcinoma (HT-29) and Human promyelocytic leukaemia (HL-60). The inhibitory concentration (IC$_{50}$) value of 78.0 ± 0.28 µg/ml and 44.0 ± 0.37 µg/ml was found in the two cells respectively. However, the potential of the HPIWE on SK-N-SH neuroblastoma cells have not been studied. Because our interest was on cancer that affects neuronal cells, SK-N-SH cell was used throughout the study.

1.3 Research Questions

a) Could HPIWE inhibit proliferation of SK-N-SH neuroblastoma cell line in vitro?

b) Will there be any change in the morphological appearance of SK-N-SH when treated with HPIWE?

c) Could HPIWE induce apoptosis on SK-N-SH neuroblastoma cells when observed under Flow cytometry?

1.4 Hypothesis

HPIWE inhibit proliferation of SK-N-SH neuroblastoma via apoptosis.

1.5 Main Objectives

To determine the effects of the HPIWE on human neuroblastoma cell line.
1.5.1 Specific Objectives

1. To determine the anti-proliferative effects of the HPIWE on SK-N-SH neuroblastoma cells in vitro using MTT assay.
2. To study the morphological features of SK-N-SH neuroblastoma cells treated with HPIWE using phase contrast microscope.
3. To determine the apoptotic effect of the HPIWE on SK-N-SH neuroblastoma cells by flow cytometry.
REFERENCES


Joardar, A. (2013). Omega-6 fatty acids have a negative impact on the proliferation and survival of three different neural cell lines. *International Journal of Advanced Research, 1*(10), 77–82.


