UNIVERSITI TEKNOLOGI MARA

BPA-INDUCED MOLECULAR ALTERATION AND BRAIN DEFORMITIES: OLIGOMERIC PROANTHOCYANIDINS INTERVENTION STUDY

FATIN NAZIHA BINTI OSMAN

Dissertation submitted in partial fulfillment of the requirements for the degree of
Master of Science

Faculty of Applied Sciences

May 2018

ABSTRACT

This study was conducted to assess the effects of Oligomeric Proanthocyanidins (OPC) supplementation on behavioural characteristics and neurogenesis in Bisphenol-A (BPA)-induced rats. Thirty-Six Adult *Sprague Dawley* male rats were divided into six groups: Normal, BPA (200 mg/kg b.wt. BPA), OPC10 (10 μg/kg b.wt. OPC), OPC20 (20 μg/kg b.wt. OPC), BPA+OPC10 (200 mg/kg b.wt. BPA + 10 μg/kg b.wt. OPC) and BPA+OPC20 (200 mg/kg b.wt. BPA + 20 μg/kg b.wt. OPC) treated groups. Rats from the respective groups were treated accordingly with the respective dosages, daily, for 21 consecutive days via oral route. Novel Object Discrimination (NOD) test was performed 24 hours after the final administration. The rats were sacrificed after the completion of NOD test. Brains were collected and fixed in 10% formalin, sectioned, stained using cresyl violet staining and examined to assess neurogenesis in hippocampus. Blood was collected and stored at -80°C for chromosome microarray analysis. During NOD test, Normal, OPC10, OPC20, BPA+OPC10 and BPA+OPC20 groups had a significantly longer novel object exploration time as compared to familiar object ($p<0.05$). Similarly, BPA group had no significant difference between novel and familiar object exploration time ($p>0.05$). Results from NOD test suggested that BPA may cause impairment in the rat's ability to remember the previously encountered familiar object while OPC treatment may ameliorate the effect of BPA towards object recognition memory. A significantly thicker dentate gyrus in BPA+OPC10 group ($p<0.05$) than BPA group suggested that OPC may improve neurogenesis in BPA-induced rats. BPA+OPC10 group also has lesser neuronal cell death as compared to BPA group, suggesting that OPC treatment may able to reduce neuronal cell damage. Microarray analysis revealed a significant change in the expression of genes. Overall, these results suggested that OPC may has the ability to reduce memory impairment, improve neurogenesis, reduce neuronal cell damage and alleviate DNA damage in BPA-induced rats.

ACKNOWLEDGEMENT

In the name of Allah, the Most Beneficent and Most Merciful. All praises belong to Allah, the Lord of the whole universe, Peace be upon prophet Muhammad, his family and companions. Alhamdulillah for his endless blessings for granted me with strength and endurance to complete this study successfully.

"Nothing worth having comes easy". I have been living my life as a master student with this quote in my mind. Master's life is a journey of independence, cherishing life when the going gets harder with a consistent remembrance to finish what I have already started. It is easier said than done but I am grateful that along the pathway in pursuing precious knowledge, I am not alone in this journey.

With that, I would like to express my endless gratitude to my supervisor, Mrs. Fatin Nadzirah binti Zakaria, for her kindness, assistance and patience during the whole process of finishing this thesis and making this a reality. Not to forget, Mr. Razif bin Dasiman for his guidance and directions. I would like to thank Dr. Nooraain binti Hashim for her generous ideas and counsels as well as her sacrifices for assisting AS730 students throughout this course. She is truly an inspiration for us to never stop pursuing our dreams and never give up hope.

Above all, I would like to express my greatest appreciation towards my parents, Osman bin Hamzah and and to my one and only brother, Anwar Farid bin Osman, for their patience and never-ending support along the journey. Thank you for your kind words and valuable advices that enable me to keep going. This master's degree is a gift to all of you.

I would like to thank my research partner, Siti Aishah binti Zulazlan for her assistance during this whole journey, for which I can never repay for her good deeds. I would like to thank Muhamad Zulhusni Abdul Wahab for his patience and guidance which allows me to complete my lab work and assist me with research-related documentations. Not to forget, I also like to thank Mohamad Tarmizi bin Omar for his help during our animal study.

I want to thank my friends, Nurul Husna bt. Md. Jani, Nursirhan Athirah bt. Sharkawi and Nor Atiqah binti Jusril for their moral support, kindness and existence, especially when I need them the most. And endless thanks to my fellow batch-mates in AS730 for making this journey a colourful and wonderful one.

Lastly, I would like to thank everyone from various institutions (Faculty of Applied Sciences UiTM Shah Alam, Faculty of Health Sciences and Faculty of Medical Lab Technology, UiTM Puncak Alam, Neoscience Sdn. Bhd.) who had been there along the journey. Thank you to everyone that had been giving me endless support to help me meet the finish line.

TABLE OF CONTENT

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Environment has the ability to alter the genetic composition not only in humans but also in other species (Hanson & Skinner, 2016). The rapid growth of industrialisation especially among developed countries causes an immense introduction of new chemicals and compounds that have the ability to substitute those which are only found in natural resources. However, these compounds may have unstable, unique chemical arrangements and can cause health problems once consumed or exposed to humans. In fact, a growing number of research recently showed that these environmental contaminants can cause transgenerational epigenetic diseases. A wide range of exogenous contaminants being used as pesticides, fertilisers, heavy metals and industrial gases which can cause epigenetic diseases, where most of the studies that had been conducted, revolves around the epigenetic mechanism via DNA methylation (Bacarelli & Bollati, 2009).

Epigenetics can be defined as the change in the expression of gene caused by the alteration in the DNA by epigenetic marks and not because of the change in the DNA sequences itself (Fernández-santiago & Ezquerra, 2016). The epigenetic marks can be passed down from one generation to another which can cause a stable change in the gene expression of the descendants, a condition known as transgenerational epigenetic inheritance. This causes the descendants who are unexposed to the contaminant to experience changes in their gene expression which is inherited from their progenitors that are exposed to those contaminants (Haque *et al.*, 2016).

Endocrine disrupting chemicals such as phthalates and Bisphenol-A (BPA) are among components being recently studied by researchers. These substances can cause epigenetic inheritance due to its ubiquitous presence in the environment in which their structures and mechanisms resemble those of natural hormones (estrogen) in human body.

BPA is used as one of the important compounds in making plastics, thermal papers, dental sealants and epoxy resins used to line food storage containers and water bottles (Mileva *et al.*, 2014; Sadowski *et al.*, 2014; Singh & Li, 2012). Consumers can