UNIVERSITI TEKNOLOGI MARA

THE EFFECTS OF PRENATAL BISPHENOL A EXPOSURE ON THE EXPRESSION OF MIRNA AND NMDA RECEPTOR SUBUNITS IN LEARNING AND MEMORY FUNCTION

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Thesis submitted in fulfilment
of the requirements for the degree of
Doctor of Philosophy
(Medicine)

Faculty of Medicine

October 2024

ABSTRACT

Bisphenol A (BPA) is one of the most widely used chemicals in the production of plastic and epoxy resin. BPA reportedly can be leached and migrate into the foodstuff and the environment, potentially posing a health hazard to humans. Early life exposure to BPA reportedly causes cognitive impairment in young children. The effects are believed to be derived from epigenetic modification since the early neurodevelopmental stage. In the hippocampus, miR-19a and miR-539 are recognized to regulate the N-methyl-D Aspartate (NMDA) receptor subunits, GluN2A and GluN2B. However, the impact of BPA-induced miRNA alteration remains elusive. This study was done to investigate the effects of prenatal BPA exposure on the expression of microRNA (miRNA) and NMDA receptor subunits in male rat hippocampus as well as its memory retention ability. Pregnant Sprague Dawley rats were orally exposed to 5 mg/kg/day of BPA with 0.5% Tween 80 in RO water from pregnancy day 1 to day 21. Whereas, the control group were without BPA. The pregnant rats were subjected to cesarean section or spontaneous delivery. At GD21, cesarean section was performed and the placenta and fetus hippocampus were dissected. The remaining mother that undergoes spontaneous delivery, the male pups were raised until postnatal day 7, 14, 21 (PND7, 14 and 21) and adolescent day 35 (AD35) where their hippocampus was collected. The expression of miR-19a, miR-539, GRIN2A, and GRIN2B in the hippocampus was measured by qRT-PCR. The level of ERα and ERβ in placenta and synaptic GluN2A, GluN2B, PSD-95 and synapsin I in the hippocampus were measured in ELISA. The extrasynaptic GluN2A and GluN2B were next measured in the Western Blot. At AD35, neurobehavioral tests were also assessed to evaluate the anxiety-related behavior and memory retention ability of the rat. The findings showed that the levels of ERα ($p<0.05$) and ERβ ($p<0.05$) were significantly increased in the placenta. In the hippocampus, prenatal BPA exposure at 5 mg/kg/day significantly reduced the expression of miR-19a ($p<0.001$), miR-539 ($p<0.01$), GRIN2A ($p<0.001$) and GRIN2B ($p<0.001$) at all stages of age. The levels of synaptic and extrasynaptic GluN2A ($p<0.01$) and GluN2B ($p<0.001$, $p<0.01$), PSD-95 ($p<0.05$) and synapsin I ($p<0.05$) were also significantly reduced when reaching adolescent age. Consequently, the adolescent male rats experienced anxiety-related behavior and impairments in fear and spatial memory. These findings suggest that the increment of ERα and ERβ in the placenta of a BPA-exposed pregnany rats may increase the susceptibility of fetus to the free BPA. In the hippocampus, the changes in miR-19a and miR-539 expression were postulated to influence the expression of GRIN2A and GRIN2B along the development. Further, the decrement of GluN2A, GluN2B, PSD-95 and synapsin I suggested to cause learning and memory impairment. In conclusion, BPA-induced miRNA alteration in regulating NMDA receptor subunits suggested to increase the susceptibility of the offspring to have impairment in learning and memory function. These findings suggest that miR-19a and miR-539 as epigenetic markers, may act as future therapeutic targets in highlighting the disruption in hippocampal function at an early stage of life.

ACKNOWLEDGEMENT

In the name of Allah, the most gracious and merciful, thank you for giving me the gift of life and for allowing me to experience this journey. Thank you for His blessing for giving me the strength to complete my PhD study.

I am grateful and sincerely thankful to my wonderful supervisor, Associate Professor Dr. Rosfaiizah Siran for her endless support and guidance throughout my study. Her passion and dedication in neuroscience study have taught me a lot to be a better researcher. Thank you for being caring, supportive and helpful in numerous ways. May Allah SWT reward her immensely. My gratitude and appreciation also go to my co-supervisors, Associate Professor Dr Siti Hamimah Sheikh Abd Kadir and Dr. Andrean Husin for their invaluable knowledge and assistance they had given to me.

My ultimate thanks are dedicated to my beloved mother my beloved father my siblings
for their endless support and prayer. Also, huge thanks to my husband,
who has provided me his endless support, motivation and prayers until the end of this journey. To my in-laws, thank you for your support and care. Without my family, I wouldn't be who I am today. May Allah shower them with endless blessings in this dunya & akhirah. My warm and heartfelt thanks to my friends for their help and support and also for every single moment of joy and sorrow we cherished together. Thank you for the wonderful friendship and I hope everyone will be successful in their careers.

Lastly, I would like to express my appreciation to the Faculty of Medicine, UiTM for giving me the opportunity to enroll in this doctorate study. Thank you to the staff of the Institute of Medical Molecular and Biotechnology (IMMB), Laboratory Animals Care Unit (LACU) and Neuroscience lab (NeuRon) for providing a good environment and facilities to conduct my study.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Bisphenol A (BPA) is a synthetic chemical primarily used in the production of plastic and epoxy resin-based products. These materials can be found in a huge array of consumer products, including food and beverage packaging, sports products, toy items, dental materials, thermal paper, and many more. BPA is routinely added to those products to act as a hardening agent and stabilizer (Vom Saal et al., 2020). Due to its remarkable properties, the growing demand for BPA is increasing year by year leading to significant annual production of around 8 million tonnes worldwide (Vasiljevic & Harner, 2021). Asian Pacific region is known as the largest producer and consumer of BPA contributing to approximately 52% of the market share, while 36% is produced by the USA and Western Europe (Manzoor et al., 2022). In Malaysia, it is estimated that a total of 9 billion plastic products are used by consumers each year where Malaysia has the highest annual per capita plastic use, at 16.78 kg per person compared to China, Indonesia, Philippines, Thailand and Vietnam (Abdullah et al., 2023; WWF Malaysia, 2020).

The widespread use of BPA has raised concerns as its toxicity can potentially pose a health hazard to humans. This compound reportedly can be leached from any plastic or resin packaging and migrate into foodstuff and environment (Acconcia et al., 2015; Ellahi & Rashid, 2017). BPA leaching is possible when BPA-based products are repetitively used and exposed to heat, detergents or irradiation (Khalili Sadrabad et al., 2023). For instance, BPA migration into the foodstuff is found at a detectable range in plastic water bottles (9.13 to 257.67 ng/l) and canned food (0.06 to 32 ng/cm^2) in which higher temperatures lead to higher migration of BPA (Takao et al., 2002; Yun et al., 2018). In Malaysia, BPA levels ranging from 5 to 50,100 ng/l were found in various rivers that serve as the primary water sources for the local population. (Duong et al., 2010; Sabri et al., 2022; Santhi et al., 2012; Shehab et al., 2020). However, wastewater treatment in Malaysia is not able to completely remove this compound as water quality analysis revealed that BPA was detected in household taps with concentrations ranging from 3.5 to 59.8 ng/l, highlighting the presence of BPA in the daily life of the consumers