UNIVERSITI PUTRA MALAYSIA

COMPARISON OF JAVANESE MEDAKA (*Oryzias javanicus,* BLEEKER, 1854) AND ZEBRAFISH (*Danio rerio,* HAMILTON, 1822) AS A MODEL ORGANISM FOR NEUROTOXICOLOGICAL STUDY

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By

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Thesis Submitted to the School of Graduated Studies, Universiti Putra Malaysia, in Fullfillment of the Requirement for the Degree of Master of Science

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Abstract of Thesis Presented to the Senate of Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Master of Science

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Zebrafish is increasingly popular in neurotoxicology research due to their small size, low maintenance and efficient for high throughput testing. However, using non-native species may raise concern on ecological aspect as accidental introduction of foreign species to our local aquatic environment could cause ecosystem instability. Therefore, the applicability of using Javanese medaka (JM) in the neurotoxicology and developmental neurotoxicology (DNT) research will be assessed by using zebrafish as a reference model. Assessment of the exploratory, anxiety and social behaviour were conducted by using open field test and aquatic light/dark plus maze. Biochemical assessment was conducted using Fourier Transform Infrared (FTIR) analysis. Without exposure to neurotoxicants, JM displayed less exploratory behaviour and showed no difference for anxiety and social behaviour as compared to the zebrafish. JM exhibited light preference while zebrafish showed dark preference. Exposure to 1% ethanol resulted a reduction in exploratory behaviour, induction in anxiety like behaviour and no alteration in the social behaviour of JM. Meanwhile, zebrafish showed a reduction in anxiety like behaviour and no alteration in exploratory and social behaviour. Treatment with 1.6 µg/L endosulfan resulted increment in exploratory behaviour, induction in anxiety like behaviour and decrement in social behaviour for both fishes. Exposure to 100 mg/L caffeine resulted anxiogenic response and decrement in the exploratory behaviour for both fishes. Alteration in social behaviour was only observed in the zebrafish. Both fishes showed alteration in biochemical profile after exposure to ethanol or endosulfan with regard to different macromolecules in the brain tissues. This study also found that, JM is inefficient for DNT research as they are laborious, time-consuming and not amenable for high throughput screening. More studies need to be conducted to further develop JM as an ideal model organism for neurotoxicology research.

Keywords: Javanese medaka (JM), zebrafish, neurotoxicology, Developmental Neurotoxicology (DNT), ethanol, endosulfan

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk master sains

PERBANDINGAN JAVANESE MEDAKA (*Oryzias javanicus*, BLEEKER, 1854) DAN ZEBRAFISH (*Danio rerio*, HAMILTON, 1822) SEBAGAI ORGANISMA MODEL UNTUK KAJIAN NEUROTOKSIKOLOGI.

Oleh

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Pada masa kini, jumlah bahan kimia yang berada di pasaran semakin meningkat, namun begitu, data berkaitan ketoksikan neuro adalah terhad. Saiz yang kecil, kos penjagaan yang rendah dan boleh digunakan dalam kadar yang banyak untuk tujuan eksperimen telah menjadikan zebrafish sering digunakan dalam kajian neurotoksikologi. Walau bagaimanapun, penggunaan spesies luar menimbulkan isu berkenaan ketidakseimbangan ekosistem sekiranya berlaku pelepasan ikan zebrafish ke persekitaran akuatik tempatan secara tidak sengaja. Oleh itu, kebolehgunaan Javanese medaka (JM) dalam bidang neurotoksikologi dan neurotoksikologi perkembangan (DNT) akan dinilai dan zebrafish akan digunakan sebagai rujukan. Penilaian terhadap kelakuan penerokaan, kebimbangan dan sosial telah dijalankan terhadap kedua-dua jenis ikan dengan menggunakan "open field test" dan "aquatic light/dark plus maze". Analisa biokimia telah dijalankan menggunakan Fourier Transform Infrared (FTIR). Tanpa sebarang rawatan, JM menunjukkan pengurangan kelakuan penerokaan dan tiada perbezaan kelakuan kebimbangan dan sosial apabila dibandingkan dengan zebrafish. Selain itu, JM juga mempamerkan kecenderungan terhadap persekitaran yang lebih terang sementara zebrafish menunjukkan kecenderungan terhadap persekitaran yang lebih gelap. Rawatan dengan 1% etanol, menyebabkan pengurangan kelakuan penerokaan, peningkatan kelakuan kebimbangan dan tiada perubahan kelakuan sosial pada JM. Manakala, zebrafish menunjukkan pengurangan kelakuan kebimbangan dan tiada perubahan kelakuan penerokaan dan sosial. Kedua-dua jenis ikan menunjukkan peningkatan terhadap kelakuan penerokaan dan kebimbangan, serta pengurangan terhadap kelakuan sosial selepas menerima rawatan endosulfan sebanyak 1.6 µg/L. Rawatan terhadap 100 mg/L kafein, telah menyebabkan tindak balas "anxiogenic" dan pengurangan kelakuan penerokaan dalam kedua-dua jenis ikan. Pengurangan terhadap kelakuan sosial hanya dapat dilihat pada zebrafish. Kedua-dua jenis ikan menunjukkan perubahan yang berbeza dalam profil biokimia. Kajian ini juga mendapati bahawa, JM adalah tidak bersesuaian untuk kajian DNT kerana ia mengambil masa yang lama

dan tidak sesuai untuk ujian yang memerlukan hasil yang tinggi. Lebih banyak kajian pada masa akan datang perlu dilakukan untuk menjadikan JM sebagai organisma model untuk kajian neurotoksikologi.

Katakunci: Javanese medaka (JM), zebrafish, neurotoksikologi, neurotoksikologi perkembangan (DNT), etanol, endosulfan

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADHD	Attention deficit hyperactivity disorder
ANOVA	Analysis of variance
ARBD	Alcohol related birth defects
ASD	Autism spectrum disorder
CNS	Central nervous system
cm	Centimetre
DNA	Deoxyribonucleic acid
DNT	Developmental neurotoxicity
FASD	Fetal alcohol spectrum disorder
FTIR	Fourier Transform Infrared
g	Gram
h	Hour
ITCN	Image-based Tool for Counting Nuclei
JM	Javanese medaka
L	Litre
mg/L	Milligram per litre
mm	Milimeter
PBS	Phosphate buffered saline
PFA	Paraformaldehyde
PFOS	Perfluorooctane sulfonate
S	Second
SEM	Standard error of the mean
µM	Micromolar
ºC	Degree celcius
%	Percentage

CHAPTER 1

INTRODUCTION

Neurotoxicity can be defined as any adverse effect on the chemistry, structure or function of the nervous system, during development or at maturity, which is induced by chemical or physical influences (Giordano and Costa, 2012). Meanwhile, developmental neurotoxicity (DNT) can be defined as any negating effect on the chemistry, structure or function of the normal development of nervous system caused by exposure to a toxic substance (Crofton et al., 2011). Ensuing to this matter, there are more than 80 000 industrial chemicals which were produced in large quantities and currently available in the market but their neurotoxicity effects have never fully tested (Grandjean and Landrigan, 2006). A survey done by National health and morbidity study in 2015 state that, the ratio of Malaysians who struggling with mental health problem is 1:3 which covers almost 4.2 million Malaysians people having mental health problem such as depression, anxiety, bipolar disorder and etc.. Environmental factors and interaction between environment and genetic inheritance may contribute to the development of mental health problem. In the year of 2000, National Research Council stated that 3% of developmental disabilities are the direct result of environmental exposure and 25% arised through interactions between environmental factors and individual genetic susceptibility. However, 14 years later, due to the increment cases of neurodevelopmental disorder worldwide, Grandjean & Landrigan, (2014) suggested that genetic factors only contribute to neurodevelopmental disorders at about 30-40% as for the rest which is about 60-70% caused from the environmental exposures and in some cases are caused by the interaction genetically inherited susceptibility. Therefore, neurotoxicology and developmental neurotoxicology (DNT) testing has become one of the requirements in toxicity testing of chemicals (OECD, 2013).

Animal models play a fundamental role in drug discovery, biomedical and ecotoxicology research. The main goal of developing animal models is to understand biological phenomena in the human or a species other than the one investigated, depending on the questions asked by the scientist. Traditional method utilizing mammal as a model organism in neurotoxicology research was practically time consuming, laborious, expensive and inefficient for hight throughput screening. Thus, it is fail to test thousands of chemicals in a short period of time. Therefore, using fish as a model organism has recently become increasingly popular among toxicologist as an alternative model organism to the mammals. The practicality of different type of fishes, either marine or freshwater for scientific research can be justified on different basis. Fish has been used as models for various research disciplines such as engineering (Romano et al., 2017), environmental research (Cossins and Crawford, 2005), genetic research (Gerlai, Lahav, Guo and Rosenthal, 2000), toxicology (Peterson, Mok and Au, 2015) and pharmacology (Maximino, da Silva, Gouveia and Herculano, 2011). Aquatic animal models are favourable for biomedical research community as they are relatively simple (Schartl., 2014), easy to culture and maintained in the laboratory as well as offer a distinct cost benefit as compared to the rodents, especially when ones dealing with high throughput testing.

Zebrafish (*Danio rerio*), originated from South and Southeast Asia, north-eastern India, Bangladesh and Myanmar are gaining popularity in neurobehavioral research and toxicology research as they have been proven to share approximately 70% of the human genes with common set of genes that are highly conserved and similarly regulated in human (Howe et al., 2013). A comprehensive behavioural catalogue for both adult and larvae zebrafish has been published recently (Kalueff et al., 2013), leading to an extensive research related to neurobehavioral research in zebrafish such as pharmacology (Maximino et al., 2011), toxicology (Beaver et al., 2017) and developmental biology (Stewart et al., 2012). Nonetheless, zebrafish are non native species in Malaysia aquatic ecosystem and using them for experiment may raised concern on the ecological aspects as accidental introduction of foreign species into our local aquatic environment could cause instability to our ecosystem. This can be an invasive threat to the native environment and lead to the loss of biological diversity (species, genetic and ecosystem diversity) (Schlaepfer, Sax and Olden, 2011).

Other than zebrafish, Japanese medaka (*Oryzias latipes*), which originated from Asian countries such as Japan, Korea and China are small, resilient vertebrates with mapped and malleable genomes are used by a small, but gradually growing community of researchers (Wittbrodt et al., 2002). Apparently, in Malaysia, a related species to Japanese medaka, namely Javanese medaka (*Oryzias javanicus*), can be found abundantly in the brackish waters of Peninsular Malaysia, Singapore, Indonesia, Thailand, and Western Borneo (Yusof, Ismail, Koito, Kinoshita and Inoue, 2011). This fish has been highlighted as a new experimental model for environmental research in marine and freshwater environment (Ismail and Yusof, 2011; Yusof, Ismail and Alias, 2014; Aziz, Zulkifli, Mohamat-Yusuff, Azmai and Ismail, 2017). Given that Javanese Medaka and zebrafish can be raised in the same laboratory setting, thus, this study aims to assess the suitability of Malaysian local species, Javanese Medaka as a model organism for neurotoxicity research in Malaysia. Of particular interest, this study also intends to assess whether Javanese Medaka is suitable model organism for DNT research or not. A well-established model organism, zebrafish was used as a reference model organism.

Although Malaysia is known as a muslim country, however, it was ranked as 10th largest consumer of alcohol in western pacific region countries and Malaysians spent over $500 million on alcohol each year with a per capita consumption of 7 litres (WHO, 2014). Meanwhile, the usage of endosulfan was banned in many countries including Malaysia. However, a survey done by Customer Association of Penang in 2010 found that our farmers used this ban pesticide in their paddy field. In addition, a study done by Santhi et al., (2011) discovered that endosulfan level in the stingray sample which was collected from coastal water of Malaysia is 2880 ng/g which has exceeded default Maximum Residue Limit (MRL) for fish and fishery products set by the US Food and Drug Administration (FDA). Therefore, ethanol and endosulfan was chosen as testing chemicals in this study. By using these testing chemicals, this study investigated the neurotoxic effects in both fishes at the adult stage. In addition, the suitability of Javanese medaka and zebrafish for DNT research were evaluated. The endpoints for neurotoxicity research involved behavioural assessment and alteration in biochemical profile.

The objectives of this study are:

1. To compare the behavioural activity between the adult of Javanese medaka and zebrafish without any neurotoxicants exposure
2. To compare the behavioural alterations and biochemical profile in the adult of Javanese medaka and zebrafish after exposure to ethanol and endosulfan
3. To assess the suitability of Javanese medaka for neurotoxicology and DNT research.

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Nurul Farhana Ramlan, Nurul Syafida Asma Mohd Sata, Siti Norhidayah Hassan, Noraini Abu Bakar, Syahida Ahmad, Syaizwan Zahmir Zulkifli, Che Azurahanim Che Abdullah and Wan Norhamidah Wan Ibrahim (2017). Time dependent effect of chronic embryonic exposure to ethanol on zebrafish: morphology, biochemical and anxiety alterations. *Behavioral Brain Research*. https://doi.org/10.1016/j.bbr.2017.05.048. (Full length article) Accepted Q2

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