UNIVERSITI PUTRA MALAYSIA

TOXICITY EFFECTS OF CURCUMIN ANALOGUE, 2, 6-BIS(2-FLUOROBENZYLIDENE)CYCLOHEXANONE ON ZEBRAFISH (Danio rerio F. Hamilton, 1822)

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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April 2017

Chairman : Syahida Ahmad, PhD
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Non-steroidal anti-inflammatory drugs (NSAIDs) have been used to reduce pain and treat inflammatory diseases such as Rheumatoid Arthritis and Alzhemeir's Disease. However, treatment with NSAIDs has been associated with significance side effects towards heart diseases in humans and experimental animals in long terms. Our previous study has shown that curcumin analogue, 2,6-bis(2-fluorobenzylidene)cyclohexanone (MS65) demonstrated strong anti-inflammatory activity in cellular model. Thus, in the present study the toxicity effects of newly synthesized curcumin analogue (MS65) was determined on zebrafish (*Danio rerio*) as an animal model. The toxicity effects of MS65 compound were evaluated by measuring survival rate and recording heartbeat of zebrafish embryos from day 0 to day 5, observing the morphological defects in developing zebrafish embryos, investigating the cardiac defects on 72 hours postfertilization of zebrafish larvae via fluorescence immunostaining and observing the toxicity effects on adult zebrafish kidney and intestine via histopathology. The toxicity effects of curcumin, celecoxib and aristolochic acid (AA) were also carried out for comparison. The LC_{50} value of survival rate for MS65 compound on zebrafish embryos were 12.5 µM. The heart rate of zebrafish larvae recorded at 5 day of exposure to 6.25 µM of MS65 was 131 ± 0.15 min^{-1}. The morphological defect result showed pericardial edema toward zebrafish embryos after treated with 6.25 µM of MS65. MF20 Monoclonal antibody stained uniformly in the heart of MS65-treated zebrafish larvae and showed normal size of the heart. In contrast, MF20 staining in AA-treated zebrafish larvae showed a more intense pattern and highlighted the small size of the heart. Histological analysis reveals that MS65-treated adult zebrafish displayed less necrosis in kidney and low level of erosion in intestine. The results demonstrated that MS65 compound showed low toxicity effects toward zebrafish model compared to celecoxib, an NSAID and AA, a toxic compound that caused heart failure in zebrafish embryos. As a conclusion, the

present study indicated that the newly synthesized curcumin analogue, MS65 has low toxicity effects towards zebrafish model and could be pharmacologic potential drug in the treatment of diseases with low toxicity effects.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN-KESAN KETOKSIKAN ANALOG KURKUMIN, 2,6-BIS(2-FLOROBENZILIDIN)SIKLOHEKSANON DALAM IKAN ZEBRA (*Danio rerio* F. Hamilton, 1822)

Oleh

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Ubat anti-radang bukan steroid (NSAID) telah biasa digunakan dalam populasi umum untuk merawat kesakitan dan penyakit radang kronik seperti Artritis Reumatoid dan penyakit Alzhemeir. Walau bagaimanapun, rawatan dengan NSAID telah dikaitkan dengan kesan sampingan penyakit jantung kepada manusia dan haiwan penyelidikan dalam jangka masa yang panjang. Penyelidikan kami sebelum ini telah menunjukkan bahawa analog kurkumin, 2,6-bis(2-florobenzilidin)sikloheksanon (MS65) menghasilkan aktiviti anti-radang yang kukuh dalam model selular. Oleh itu, kesan-kesan ketoksikan analog kurkumin (MS65) yang baru disintesis telah dijalankan dalam penyelidikan ini terhadap ikan zebra (Danio rerio) sebagai model haiwan. Kesan-kesan ketoksikan MS65 telah dinilai dengan mengukur kadar kelangsungan hidup dan denyutan jantung embrio ikan zebra yang telah dirakamkan dari hari 0 sehingga hari ke-5, memerhatikan kecacatan morfologi dalam tumbesaran embrio ikan zebra, mengkaji kecacatan jantung larva ikan zebra pada jam ke-72 selepas persenyawaan melalui pewarnaan immuno dan memerhatikan kesan ketoksikan dalam ikan zebra dewasa melalui histopatologi. Nilai LC_{50} kadar kelangsungan hidup untuk MS65 kompoun terhadap embrio ikan zebra ialah 6.25 µM. Kadar denyutan jantung larva ikan zebra yang telah direkodkan pada 5 hari pendedahan kepada 6.25 µM MS65 ialah 131 ± 0.15 min^{-1}. Hasil penyelidikan kecacatan morfologi MS65 menunjukkan edema perikardium terhadap embrio ikan zebra. MF20 monoklonal antibodi mewarna jantung larva ikan zebra yang dirawat dengan MS65 secara sekata dan menunjukkan saiz jantung yang normal. Sebaliknya, pewarnaan MF20 dalam larva ikan zebra yang dirawat dengan AA menunjukkan corak yang lebih sengit dan saiz jantung yang kecil. Analisis histologi menunjukkan bahawa ikan zebra dewasa yang dirawat dengan MS65 mengalami nekrosis yang rendah dalam buah pinggang dan hakisan yang rendah dalam usus. Hasil kajian menunjukkan bahawa MS65 menunjukkan kesan ketoksikan yang rendah terhadap model ikan zebra berbanding selekosib, salah satu

daripada NSAID dan asid aristolokik (AA), sebatian toksik yang menyebabkan kegagalan jantung pada embrio ikan zebra. Kesimpulannya, hasil kajian ini menunjukkan bahawa analog kurkumin yang baru disintesis, MS65 mempunyai kesan ketoksikan yang rendah terhadap model ikan zebra dan berpotensi menjadi ubat farmakologi untuk merawat penyakit-penyakit dengan kesan-kesan ketoksikan yang rendah.

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

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LIST OF ABBREVIATIONS

AA	Aristolochic acid
ANOVA	Analysis of variance
COX	Cyclooxygenase
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
Coxibs	Cylooxygenase-2 inhibitors
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dph	Day of post fertilization
EM	Embryo medium
g	Gram
h	hour/s
hpf	Hour of post fertilization
IACUC	Institutional Animal Care and Use Committee
iNOS	Inducible nitric oxide synthase
kg	Kilogram
L	Litre
LOX	Lipoxygenase
LC_{50}	Lethal concentration 50%
LD_{50}	Lethal dose 50%
M	Molar
mg	Milligram
mg/L	Milligram/litre

mL	Millilitre
mM	Millimolar
MMP-9	Matrix metallopeptidase
µ	Micron
µg	Microgram
µL	Microlitre
µM	Micromolar
µg/L	Microgram/litre
min	Minute
MS65	2,6-bis(2,5-dimethoxy-benzylidene)cyclohexanone
MW	Molecular weight
NaOH	Sodium hydroxide
NF-κB	Nuclear factor- κB
NSAIDs	Non-steroidal anti-inflammatory drugs
PBS	Phosphate buffer saline
PKA	Protein kinase A
rpm	Rotation per minute
s	second/s
SD	Standard deviation
SEM	Standard error of mean
α	Alpha
ºC	Degree celcius

CHAPTER 1

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) have been commonly used in the general population for treating pain and inflammatory conditions (Rodriguez *et al.*, 2008). NSAIDs are also one of drugs used by patients for the treatment of cardiovascular diseases (Howard and Delafontaine, 2004). Heart problem is one of the common cardiac diseases that result from infection, injury, genetic disorders or toxin-induced damage on the myocardium (Huang *et al.,* 2007). In order to prevent progression and development of heart problems, pharmacotherapy and mechanical interventions are currently used (Singla and Sobel, 2005).

NSAIDs are one of the classes that commonly used as pharmacologic agents to inhibit cyclooxygenase enzyme activity (Ishikawa *et al.*, 2007). Cyclooxygenase (COX) is a rate-limiting enzyme which present in at least two isoforms, COX-1 and COX-2. The first step in the synthesis of prostanoids (prostaglandin and thromboxane) was catalysed by this enzyme (Zidar *et al.,* 2007). Study has shown that NSAIDs act mostly through the inhibition of COX-2 (Rodriguez *et al.,* 2008). Selective COX-2 inhibitors (coxibs) represent a new generation of NSAIDs and have been found to be effective in a wide range of treatments which include acute pain, osteoarthritis and rheumatoid arthritis (Ushiyama *et al.,* 2008). However, study has been reported that long term consumption of these drugs can cause side effects such as mucosal erosion and intestinal bleeding (Petruzzelli *et al.,* 2007). For example, previous study has shown that cyclooxygenase-2 inhibitors (coxibs) are associated with cardiovascular adverse events (Rodriguez *et al.,* 2010).

Therefore, natural plant products have been a recent focused as the current therapies due to great ability for scavenge free radicals. Besides that, plants offer an important source of active natural products because they are widely different in terms of biological properties, structures and mechanisms of actions (Amari *et al.,* 2013). Treatment with curcumin which is derived from turmeric (*Curcuma longa*) has been shown to possess medicinal benefits in treating of cardiovascular, diabetes and inflammatory diseases (Lee *et al.,* 2009). Recently, study has found that synthesized new curcumin analogues have been developed in order to enhance curcumin's bioavailability as anti-inflammatory and anti-cancer agents (Katsori *et al.,* 2011). Study has also reported that curcumin analogue has achieved the potential therapeutic interest to cure immune related due to a vast number of biological targets and no side effects (Srivastava *et al.,* 2011).

Zebrafish has become popular in the field of toxicological studies, genetics, environmental science and drug screening (Hung, 2012). Zebrafish has emerged as a simple model system to study human diseases because of its ex-utero development, optical transparency of the embryo, short generation time and small size (David and Pancharatna, 2009). Moreover, there is high similarity at the physiological,

anatomical and molecular levels between zebrafish cardiovascular, metabolic pathways and nervous systems with those of mammals (Hung *et. al.*, 2012). All these features have made zebrafish as an ideal model for studying the biological activity of natural products with complex chemical components (Hung, 2012). Previous study has shown that curcumin analogue, 2,6-bis(2-fluorobenzylidene)cyclohexanone (MS65) demonstrated strong anti-inflammatory activity in cellular model. Thus in the present study, the effects of curcumin analogue, MS65 were studied and determined using zebrafish embryo, larvae and adult (*Danio rerio*) as the model organism for research.

1.1 Objectives

The general objective of this study is to determine the toxicity effects of curcumin analogue, MS65 on zebrafish (*Danio rerio*) model.

The specific objectives are:

1) To determine the acute toxicity effects of curcumin analogue, MS65 on zebrafish embryos and larvae.
2) To determine the cardiac defect caused by curcumin analogue, MS65 on zebrafish larvae.
3) To histologically evaluate curcumin analogue, MS65 treated adult zebrafish.

REFERENCES

Agrawal, P. and Laddha, K. (2017). Development of validated high-performance thin layer chromatography for quantification of aristolochic acid in different species of the Aristolochiaceae family. *Journal of Food and Drug Analysis*, 25; 425-429.

Amari, N. O., Bouzouina, M., Berkani, A. and Lotmani, B. (2013). Phytochemical screening and antioxidant capacity of the aerial parts of *Thymelaea hirsute L*. *Asian Pacific Journal of Tropical Disease*, 4(2); 104-109.

Awika, J. M. and Rooney, L. W. (2004). Sorghum phytochemicals and their potential impact on human health. *Phytochemistry*, 65; 1199-1221.

Barros, L., Cabrita, L., Boas, M. V., Carvalho, A. M. and Ferreira, I. C. F. R. (2011).Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. *Food Chemistry*, 127; 1600-1608.

Barthelemy, O., Limbourg, T., Collet, J. P., Beygui, F., Silvain, J., Bellemain-A. A., Cayla, G., Chastre, T., Baumgartner, I., Rother, J., Zeymer, U., Bhatt, D. L. and Steg, G., Montalescot, G. (2013). Impact of non-steroidal anti-inflammatory drugs (NSAIDs) on cardiovascular outcomes in patients with stable atherothrombosis or multiple risk factors. *International Journal of Cardiology*, 163; 266-271.

Basharat, Z., Zaib, S. and Yasmin, A. (2017). Computational study of some amoebicidal phytochemicals against heat shock protein of *Naegleria fowleri*. *Gene Reports*, 6; 158-162.

Berghmans, S., Butler, P., Goldsmith, P., Waldron, G., Gardner, I., Golder, Z., Richards, F. M., Kimber, G., Roach, A., Alderton, W. and Fleming, A. (2008). Zebrafish based assays for the assessment of cardiac, visual and gut function – potential safety screens for early drug discovery. *Journal of Pharmacological and Toxicological Methods*, 58; 59-68.

Braunbeck, T. and Lammer, E. (2006). Fish embryo toxicity assays. *Aquatic Ecology & Toxicology*, UBA Contract No.20385422.

Canada, B., Thomas, G., Cheng, K. and Wang, J. Z. (2007). Automated segmentation and classification of zebrafish histology images for high-throughput phenotyping. *Life Science Systems and Applications Workshop*.

Chakraborty, C., Hsu, C. H., Wen, Z. H., Lin, C. S. and Agoramoorthy, G. (2011). Effect of caffeine, norfloxacin and nimesulide on heartbeat and VEGF expression of zebrafish larvae. *Journal of Environmental Biology*, 32; 179-183.

Chigayo, K., Mojapelo, P. E. L., Moleele, S. M. and Misihairabgwi, J. M. (2016). Phytochemical and antioxidant properties of different solvent extracts of *Kirkia wilmsii* tubers. *Asian Pacific Journal of Tropical Biomedicine*, Vol 6 (12); 1037-1043.

David, A. and Pancharatna, K. (2009). Developmental anomalies induced by a non-selective COX inhibitor (ibuprofen) in zebrafish (Danio rerio). *Environmental Toxicology and Pharmacology*, 27; 390-395.

Denvir, M. A., Tucker, C. S., and Mullins, J.J. (2008). Systolic and diastolic ventricular function in zebrafish embryos: Infuence of norepinephrine, MS-222 and temperature. *BMC Biotechnology.* 8:21.

Ding, Y-J and Chen, Y-H. (2012). Developmental nephrotoxicity of aristolochic acid in a zebrafish model. *Toxicology and Applied Pharmacology*, 261:59-65.

Fernandez, J. and Fuentes, R. (2013). Fixation/Permeabilization: New alternative procedure for immunofluorescence and mRNA *In Situ Hybridization* of vertebrate and invertebrate Embryos. *Developmental Dynamics*, 245:503-517.

Gaggin, H. K. and Januzzi, Jr. J. L. (2013). Biomarkers and diagnostics in heart failure. *Biochimica et Biophysica Acta*, 1832; 2242-2450.

Ghosh, M., Singh, A. T. K., Xu, W., Sulchek, T., Gordon, L. I. and Ryan, R. O. (2011). Curcuminnanodisk: formulation and characterization. *Nanomedicine: Nanotechnology, Biology, and Medicine*, 7; 162-167.

Harjo, B., Wibowo, C. and Ng, K. M. (2004). Development of natural product manufacturing processes phytochemicals. *Chemical Engineering Research and Design*, 82(A8); 1010-1028.

Henrotin, Y., Clutterbuckt, A. L., Allaway, D., Lodwig, E. M., Harris, P. and Mathy-Hartert M. (2010). Biological actions of curcumin on articular chondrocytes. *Osteoarthritis and Cartilage*, 18; 141-149.

Hoage, T., Ding, Y. and Xu, X. (2012). Quantifying Cardiac Functions in Embryonic and Adult Zebrafish. *Cardiovascular Development: Methods and Protocols, Methods in Molecular Biology*, Vol. 843.

Howard, P. A. and Delafontaine, P. (2004). Nonsteroidal anti-inflammatory drugs and cardiovascular risk. *Journal of the American College of Cardiology*, Vol. 43, No.4.

Huang, C-C., Chen, P-C., Huang, C-W. and Yu, J. (2007). Aristolochic acid induces heart failure in zebrafish embryos that is mediated by inflammation. *Toxicological Sciences*, 100(2); 486-494.

Hung, M. W., Zhang, Z. J., Li, S., Lei, B., Yuan, S., Cui, G. Z., Hoi, P. M., Chan, K. and Lee, S. M. Y. (2012). From omics to drug metabolism and high content screen of natural product in zebrafish: a new model for discovery of neuroactive compound. *Evidence-Based Complementary and Alternative medicine*, Vol. 2012: 605303.

Chitrangi, S., Nair, P. and Khanna, A. (2017). 3D engineered In vitro hepatospheroids for studying drug toxicity and metabolism. *Toxicology in Vitro*, 38; 8-18.

Haque, M. and Haque, Md. E. (2011). Sub-acute toxicity study of a novel compound E-Octadec-7-en-5-ynoic acid from *Capparis zeylanica* Linn roots. *Agriculture and Biology Journal of North America*, 2(4); 708-712.

Inoue, D. and Wittbrodt, J. (2011). One for All – A Highly Efficient and Versatile Method for Fluorescent Immunostaining in Fish Embryos. *Plos One*, Volume 6: Issue 5.

Ishikawa, T-O., Griffin, K. J. P., Benerjee, U. and Herschman, H. R. (2007). The zebrafish genome contains two inducible, functional cyclooxygenase-2 genes. *Biochemical and Biophysical Research Communications*, 352; 181-187.

Katsori, A.-M., Chatzopoulou, M., Dimas, K., Kontogiorgis, C., Patsilinakos, A., Trangas, T., Litina-A. (2011). Curcumin analogues as possible anti-proliferative & anti-inflammatory agents. *European Journal of Medicinal Chemistry*, 46; 2722-2735.

Kim, K.-H., Antkiewicz, D. S., Yan, L., Eliceiri, K. W., Heideman, W., Peterson, R. E. and Lee, Y. (2007). Lrrc10 is required for early heart development and function in zebrafish. *Developmental Biology*, 308; 494-506.

Kundu, J. K. and Surh, Y-J. (2008). Inflammation: Gearing the journey to cancer. *Mutation Research*, 659; 15-30.

Leal, A. A. D. F., Palmeira, A. C., Castro, G. M. A. D., Simoes, M. O. D. S., Ramos, A. T. and Medeiros, C. C. M. (2013). Homocysteine: Cardiovascular risk factor in children and adolescents? *Revista da Associacao Medica Brasileira*, 92; 7.

Lee, K-H., Aziz, F. H. A., Syahida, A., Abas, F., Shaari, K., Israf, D. A. and Lajis, N. H. (2009). Syhthesis and biological evaluation of curcumin-like diarylpentanoid analogues for anti-inflammatory, antioxidant and anti-tyrosinse activities. *European Journal of Medicinal Chemistry*, 44; 3195-3200.

Li, F., Liu, S., Ouyang, Y., Fan, C., Wang, T., Zhang, C., Zeng, B., Chai, Y. and Wang, X. (2012). Effect of celecoxib on proliferation, collagen expression, ERK1/2 and SMAD2/3 phosphorylation in NH/3T3 fibroblasts. *European Journal of Pharmacology*, 678; 1-5.

Li, Q., Chen, J., Luo, S., Xu, J., Huang, Q and Liu, T. (2015). Synthesis and assessment of the antioxidant and antitumor properties of asymmetric curcumin analogues. *European Journal of Medicinal Chemistry*, 93; 461-469.

Liang, B., Liu, Z., Cao, Y., Zhu, C., Zuo, Y., Huang, L., Wen, G., Shang, N., Chen, Y., Yue, X., Du, J., Li, B., Zhou, B. and Bu, X. (2017). MC37, a new mono-carbonyl curcumin analog, induces G2/M cell cycle arrest and mitochondria-mediated apoptosis in human colorectal cancer cells. *European Journal of Pharmacology*, 796; 139-148.

Liang, G., Yang, S., Zhou, H., Shao, L., Huang, K., Xiao, J., Huang, Z., and Li, X. (2009). Synthesis, crystal structure and anti-inflammatory properties of curcumin analogues. *European Journal of Medicinal Chemistry*, 44; 915-919.

Lill, A., Scholich, K. and Stark, H. (2013). Synthesis of novel dansyl-labeled Celecoxib derivatives. *Tetrahedron Letters*, 54; 6682-6686.

Lincova, E., Hampi, A., Pernicova, Z., Starsichova, A., Krcmar, P., Machala, M., Konzubik, A. and Soucek, K. (2009). Multiple defects in negative regulation of the PKB/Akt pathway sensitise human cancer cells to the antiproliferative effect of non-steroidal anti-inflammatory drugs. *Biochemical Pharmacology*, 78; 561-572.

Liu, R., Gong, C., Tao, L., Yang, W., Zheng, X., Ma, P. and Ding, L. (2015). Influence of genetic polymorphisms on the pharmacokinetics of celecoxib and its two main metabolites in healthy Chinese subjects. *European Journal of Pharmaceutical Sciences*, 79; 13-19.

Luca, D. E., Zaccaria, G. M., Hadhoud, M., Rizzo, G., Ponzini, R., Morbiducci, U. and Santoro, M. M. (2014). ZebraBeat: a flexible platform for the analysis of the cardiac rate in zebrafish embryos. *Scientific Reports*, 4; 4898.

Ma, L., Qin, Y., Shen, Z., Bi, H., Hu, H., Huang, M., Zhou, H., Yu, L., Jiang, H. and Zeng, S. (2015). Aristolochic acid I is a substrate of BCRP but not P-glycoprotein of MRP2. *Journal of Ethnopharmacology*, 172; 430-435.

Maheshwari, R. K., Singh, A. K., Gaddipati, J. and Srimal, R. C. (2006). Multiple biological activities of curcumin: A short review. *Life Sciences*, 78; 2081-2087.

Manikandan, P., Sumitra, M., Aishwarya, S., Manohar, B. M., Lokanadam, B. and Puvakrishnan, R. (2004). Curcumin modulates free radical quenching in myocardial ischaemia in rats. *The International Journal of Biochemistry & Cell Biology*, 36; 1967-1980.

Menke, L. A., Spitsbergen, J. M., Wolterbeek, A. P. M. and Woutersen, R. A. (2011). Normal anatomy and histology of the adult zebrafish. *Toxicologic Pathology*, 39; 759-775.

Nagano, A., Arioka, M., Yanaga, F. T., Matsuzaki, E. and Sasaguri, T. (2017). Celecoxib inhibits osteoblast maturation by suppressing the expression of Wnt target genes. *European Journal of Pharmaceutical Sciences*, 133; 18-24.

Nezhadali, A. and Sadeghzadeh S. (2017). Experimental design-artificial neural network-genetic algorithm optimization and computer-assisted design of celecoxib molecularly imprinted polymer/carbon nanotube sensor. *Journal of Electroanalytical Chemistry*, 795; 32-40.

Nitzsche, D., Melzig, M. F. and Arlt, V. M. (2013). Evaluation of the cytotoxicity and genotoxicity of aristolochic acid I – A component of Aristolochiaceae plant extracts used in homeopathy. *Environmental Toxicology and Pharmacology*, 35; 325-334.

Notoya, M., Nishimura, H., Woo, J. T., Nagai, K., Ishihara, Y. and Hagiwara, H. (2006). Curcumin inhibits the proliferation and mineralization of cultured osteoblasts. *European Journal of Pharmacology*, 534; 55-62.

Okarter, N., Liu, C-S., Sorrells, M. E. and Liu, R. H. (2010). Phytochemical content and antioxidant activity of six diverse varieties of whole wheat. *Food Chemistry*, 119; 249-257.

Oliveira, P. R. de, Bechara, G. H., Denardi, S. E., Pizano, M. A. and Mathias, M. I. C. (2011). Toxicity effect of the acaricide fipronil in semi-engorged females of the tick *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): Preliminary determination of the minimum lethal concentration and LC_{50}. *Experimental Parasitology*, 127; 418-422.

Pares, U. V., Castanaga, L., Lacave, J. M., Oron, M., Reip, P., Berhanu, D., Jones, E. V., Cajaraville, M. P. and Orbea, A. (2014). Comparative toxicity of metal oxide nanoparticles (CuO, ZnO and TiO2) to developing zebrafish embryos. *Journal of Nanoparticles Research*, 16; 2550.

Petruzzelli, M., Vacca, M., Moschetta, A., Sasso, R. C., Palasciano, G., Erpecum, K. J. V. and Portincasa. (2007). Intestinal mucosal damage caused by non-steroidal anti-inflammatory drugs: Role of bile salts. *Clinical Biochemistry*, 40; 503-510.

Pieperhoff, S., Wilson, K. S., Baily, J., Mora, K. D., Maqsood, S., Vass, S., Taylor, J., Pozo, J. D., MacRae, C. A., Mullins, J. J., Denvir, M.A. (2014). Heart on a Plate: Histological and funtional assessment of isolated adult zebrafish hearts maintained in culture. *Isolated Adult Zebrafish Hearts in Culture*, Volume 9: Issue 5.

Pini, B., Grosser, T., Lawson, J. A., Price, T. S., Pack, M. A. and FitzGerald, G. A. (2004). Prostaglandin E synthases in zebrafish. *Arteriosclerosis Thrombosis and Vascular Biology*, 25; 315-320.

Ponnudurai, R. P., Basak, T., Ahmad, S., Bhardwaj, G., Chauhan, R. K., Singh, R. A., Lalwani, M. K., Sivasubbu, S. and Sengupta, S. (2012). Proteomic analysis of zebrafish (Danio rerio) embryos exposed to cyclosporine A. *Journal of Proteomics*, 1004-1017.

Ren, G., Huang, Q., Wu, J., Yuan, J., Yang, G., Yan, Z. and Yao, S. (2014). Cloud point extraction-HPLC method for the determination and pharmacokinetic study of aristolochic acids in rat plasma after oral administration of *Aristolochiae Fructus*. *Journal of Chromatography B*, 953-954; 73-79.

Rodriguez, L. A. G., Gonzalez-Perez, A., Bueno, H. and Hwa, J. (2011). NSAIDs use selectively increases the risk of non-fatal myocardial infarction: a systemic review of randomised trials and observational studies. *Plos One*: Vol. 6 - Issue 2.

Rodriguez, L. A. G., Tacconelli, S. and Patrignani, P. (2008). Role of dose potency in the prediction of risk of myocardial infarction associated with nonsteroidal anti-inflammatory drugs in the general population. *Journal of the American College of Cardiology*. Vol. 52:20.

Rodriguez-Vita J. and Lawrence, T. (2010). The resolution of inflammation and cancer. *Cytokine & Growth Factor Reviews*, 21; 61-65.

Roma, G. C., Oliveira, P. R. de, Pizano, M. A. and Mathias, M. I. C. (2009). Determination of LC_{50} of permethrin acaricide in semi-engorged females of the tick *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae). *Experimental Parasitology*, 123; 269-272.

Sahu, P. K. (2016). Design, structure activity relationship, cytotoxicity and evaluation of antioxidant activity of curcumin derivatives/analogues. *European Journal of Medicinal Chemistry*, 121; 510-516.

Saito, T., Rodger, I. W., Hu, F., Robinson, R., Huynh, T. and Giaid, A. (2004). Inhibition of COX pathway in experimental myocardial infarction. *Journal of Molecular and Cellular Cardiology*, 37; 71-77.

Schoonheim, P. J., Chatzopoulou, A. and Schaaf, M. J. M. (2010). The zebrafish as an in vivo model system for glucocorticoid resistance. *Steroids*, 75; 918-925.

Serluca, F. C. (2008). Development of the proepicardial organ in the zebrafish. *Developmental Biology*, 315; 18-27.

Simon, A., Allais, D. P., Duroux, J. L., Basly, J. P., Durand-Fontanier, S. and Delage, C. (1998). Inhibitory effect of curcuminoids on MCF-7 cell proliferation and structure-activity relationships. *Cancer Letters*, 129; 111-116.

Singla, D. K. and Sobel, B. E. (2005). Enhancement by growth factors of cardiac myocyte differentiation from embryonic stem cells: a promising foundation for cardiac regeneration. *Biochemical and Biophysical Research Communications,* 335; 637-642.

Sostres, C., Gargallo, C. J. and Lanas, A. (2010). Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. *Best Practice & Research Clinical Gastroenterology,* 24; 121-132.

Sreedevi, B., Suvarchala, G. and Philip, G. H. (2014). Morphological and Physiological Abnormalities During Development in Zebrafish Due to Chlorpyrifos. *Indian Journal of Scientific Research,* 5(2); 1-8.

Srivastava, R. M., Singh, S., Dubey, S. K., Misra, K. and Khar, A. (2011). Immunomodulatory and therapeutic activity of curcumin. *International Immunopharmacology,* 11; 331-341.

Struck, S., Schmidt, U., Gruening, B., Jaeger, I. S., Hossbach, J. and Preissner, R. (2008). Toxicity versus potency: elucidation of toxicity properties discriminating between toxins, drugs, and natural compounds. *Toxicity versus Potency*, 231-242.

Teraoka, H., Kubota, A., Dong, W., Kawai, Y., Yamazaki, K., Mori, C., Harada, Y., Peterson, R. E. and Hiraga, T. (2009). Role of the cyclooxygenase 2-thromboxane pathway in 2, 3, 7, 8-tetrachlorodibenzo-ρ-dioxin-induced decrease in mesencephalic vein blood flow in the zebrafish embryo. *Toxicology and Applied Pharmacology,* 234; 33-40.

Tiziani, S., Schwartz, S. J. and Vodovotz, Y. (2008). Intermolecular interactions in phytochemical model systems studied by NMR diffusion measurements. *Food Chemistry,* 107; 962-969.

Tsao, R. and Deng, Z. (2004). Separation procedures for naturally occurring antioxidant phytochemicals. *Journal of Chromatography B,* 812; 85-99.

Ushiyama, S., Yamada, T., Murakami, Y., Kumakura, S., Inoue, S., Suzuki, K., Nakao, A., Kawara, A. and Kimura, T. (2008). Preclinical pharmacology profile of CS-706, a novel cyclooxygenase-2 selective inhibitor, with potent antinociceptive and anti-inflammatory effects. *European Journal of Pharmacology*, 578; 76-86.

Vodovotz, Y., Constantine, G., Rubin, J., Csete, M., Voit, E. O. and An, G. (2009). Mechanistic simulations of inflammation: Current state and future prospects. *Mathematical Biosciences,* 217; 1-10.

Voelker, D., Vess, C., Tillmann, M., Nagel, R., Otto, G.W., Geisler, R., Schirmer, K., Scholz, S. (2007). Differential gene expression as a toxicant sensitive endpoint in zebrafish embryos and larvae. *Aquatic Toxicology,* 81; 355–364.

Wang, H., Guo, X., Hu, X., Li, T., Fu, X. and Liu, R. H. (2017). Comparison of phytochemical profiles, antioxidant and cellular antioxidant activities of different varieties of blueberry (*Vaccinium* spp.). *Food Chemistry*, 217; 773-781.

Wang, W.-D., Huang, C.-J., Lu, Y.-F., Hsin, J.-P., Prabhakar, V. R., Cheng, C.-F. and Hwang, S.-P. L. (2006). Heart-targeted overexpression of *Nip3α* in zebrafish embryos causes abnormal heart development and cardiac dysfunction. *Biochemical and Biophysical Research Communications*, 347; 979-987.

Watanabe, K., Nishimura, Y., Oka, T., Nomoto, T., Kon, T., Shintou, T., Hirano, M., Shimada, Y., Umemoto, N., Kuroyanagi, J., Wang, Z., Zhang, Z., Nishimura, N., Miyazaki, T., Imamura, T. and Tanaka, T. (2010). *In vivo* imaging of zebrafish retinal cells using fluorescent coumarin derivatives. *BioMed Central Neuroscience*, 11:116.

Westbrook, A. M., Szakmary, A. and Schiestl, R. H. (2010). Mechanisms of intestinal inflammation and development of associated cancers: Lessons learned from mouse models. *Mutation Research*, 705; 40-59.

Wilson, I. D. and Nicholson, J. K. (2016). Gut microbiome interactions with drug metabolism, efficacy, and toxicity. *Translational Research*, Vol 179; 204-222.

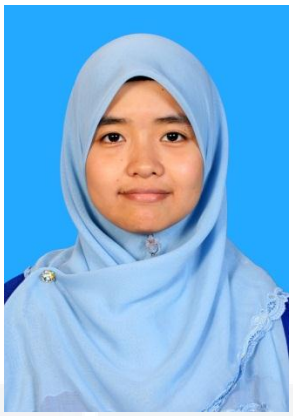
Wu, J.-Y., Lin, C.-Y., Lin, T.-W., Ken, C.-F. and WenY.-D. (2007). Curcumin affects development of zebrafish embryo. *Biological and Pharmaceutical Bulletin*, 30(7); 1336-1339.

Xu, D.-J., Bu, J.-W., Gu, S.-Y., Xia, Y.-M., Du, J.-L. and W, Y.-W. (2011). Celecoxib impairs heart development via inhibiting cyclooxygenase-2 activity in zebrafish embryos. *Anesthesiology*, Vol. 114 (2).

Zidar, N., Strazar, Z. D., Jeruc, J., Jerse, M., Balazic, J., Gartner, U., Jermol, U., Zupanc, T. and Stajer, D. (2007). Expression of cyclooxygenase-1 and cycloogygenase-2 in the normal human heart and in myocardial infarction. *Cardiovascular Pathology*, 16; 300-304.

Zodrow, J. M., Stegeman, J. J. and Tanguay, R. L. (2004). Histological analysis of acute toxicity of 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD) in zebrafish. *Aquatic Toxicology*, 66:25-38.

BIODATA OF STUDENT



The student, Nur Amiza binti Nazarudin was born on 21th of June, 1990 in Kuala Lumpur, Malaysia. She received her early education at Sekolah Kebangsaan Marian Convent, Kuala Lumpur and Sekolah Kebangsaan Danau Kota, Kuala Lumpur for her primary education. Then, she attended Sekolah Menengah Kebangsaan Setapak Indah, Kuala Lumpur for her secondary education. After completed her SPM examination, she completed her matriculation level at Kolej Matrikulasi Negeri Sembilan (KMNS) before furthering her study at tertiary level in Universiti Putra Malaysia (UPM). Amiza graduated with Bachelor of Science in Biochemistry (Hon.) in year 2012. In 2012, she was awarded with two scholarships in the same year which were Graduate Research Fellowship (GRF) by UPM as well as MyBrain15 by Ministry of Education to pursue her Master's degree in Animal Cell Biotechnology at Faculty of Biotechnology and Biomolecular Sciences (FBSB), UPM under supervision of Dr. Syahida Ahmad.

LIST OF PUBLICATIONS

Conference Attended

Poster Presentation

Nur Amiza N., Noor Azmi S., Abas F., Syahida A. (2015). Cardiotoxicity Effects Of Curcumin Analogue 2,6-Bis(2-fluorobenzylidene)cyclohexanone On Zebrafish (*Danio rerio*) Embryo. Biotechnology Joint Symposium 2015, Universiti Putra Malaysia-Sejong University at Faculty of Biotechnology and Biomolecular Sciences. Universiti Putra Malaysia, Serdang (Poster presentation).

Nur Amiza N., Song LK., Abas F., Syahida A. (2016). Toxicity Effects Of Curcumin Analogue 2,6-Bis(2-fluorobenzylidene) Cyclohexanone On Zebrafish (*Danio rerio*). 41st Annual Conference of The Malaysian Society for Biochemistry and Molecular Biology 2016, 17-18 August 2016. Pullman Bangsar Hotel, Kuala Lumpur (Poster presentation).