UNIVERSITI PUTRA MALAYSIA

TOXICITY EVALUATION OF CURCUMIN ANALOGUE ON ZEBRAFISH

TAN HONG WEI

FBSB 2015 80

TOXICITY EVALUATION OF CURCUMIN ANALOGUE ON ZEBRAFISH

By

TAN HONG WEI

165060

Dissertation submitted in partial fulfilment for the requirement for the course of BCM 4999
Project in the Department of Biochemistry
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

JUNE 2015

PENGESAHAN

Dengan ini adalah disahkan bahawa tesis projek yang bertajuk "Toxicity Evaluation of Curcumin Analogue On Zebrafish" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh TAN HONG WEI (165060) sebagai syarat untuk kursus BCM4999 Projek.

Disahkan oleh,

..... Tarikh : .....
(Dr. Syahida Ahmad)
Penyelia projek
Jabatan Biokimia
Fakulti Bioteknologi dan Sains Biomolekul
Universiti Putra Malaysia

..... Tarikh : .....
(Prof. Dato' Dr. Abu Bakar Salleh)
Ketua Jabatan Biokimia
Fakulti Bioteknologi dan Sains Biomolekul
Universiti Putra Malaysia

ACKNOWLEDGEMENT

I would like to express my immeasurable appreciation and deepest gratitude to several individuals throughout all the processes in completing my final year research project. First and foremost, I would like to thank my final year project supervisor, Dr Syahida Ahmad for her guidances, supervision, constructive comments, and motivation. Her invaluable guidance assisted me all the time in my research and in accomplishing my dissertation. Besides, her immense knowledges and academic suggestions have contributed towards the success of this research and completion of my dissertation.

Special thanks to Dr Muhammad Fuad Matori from the Faculty of Veterinary Medicine, Universiti Putra Malaysia for his precise suggestions, motivations, and professional comments.

Besides, sincere appreciation to my mentor, Nur Amiza Binti Nazarudin, that guided me throughtout the experiments and gave me continuous supports in completing the research. Her helps made a great contribution in completing my thesis.

Special thanks to the staff of the histopathology lab, Faculty of Veterinary Medicine for their helps in the course of this experiments. Meanwhile, thanks to Puan Sharipah, Yakubu, Ibrahim, my labmates, and others who contributed to the success of this project.

Last but not least, my sincere gratitude goes to my beloved parents that give constant supports and motivations to me. Thank you.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Aristolochic acid
AAN	Aristolochic acid nephropathy
COX	Cyclooxygenase
DMSO	Dimethyl Sulfoxide
dpf	Day–post- fertilization
DPX	Distyrene Plasticizer Xylene
E.M	Embryo Medium
GI	Gastro-intestinal
g/mol	Gram per mole
hpf	Hour –post- fertilization
H & E	Hematoxylin and Eosin
LC_{50}	Lethal concentration 50
ml	milli litre
µM	Micromolar
mM	Millimolar
MW	Molecular weight
NSAID	Non- steroidal anti-inflammatory drug

ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most broadly prescribed medications in the world. But, NSAIDs confer side effects towards the gastrointestinal(GI) system.In Malaysia, the usage of one of the NSAIDs,celecoxib, a Cyclooxygenase-2 (COX-2) inhibitors, had increased by two fold from 2006 to 2007. Curcumin analogues are of great interest in pharmacological research due to its advantages compared with pure curcumin. Curcumin analogues are proposed to be used as an alternative curative drugs for the treatment of certain ailments such as inflammatory diseases.Recently, zebrafish has emerge as a toxicology test model organism for the research of vertebrae genetics and developmental biology. The aim of this research is to determine the effect of curcumin analogue (MS65) on zebrafish embryos survivability and heart beat, zebrafish embryos development, and the kidney and intestine of adult zebrafish. The estimated LC_{50} of curcumin analogue were 12.5µM for embryos and larvae. Histological analysis of the MS65 treated kidney of adult zebrafish showed low levels of necrosis while eroded villi was associated with MS65 treated zebrafish intestine. Meanwhile, zebrafish larvae incubated with MS65 developed yolk sac edema. Hence, the low toxicity shown by curcumin analogue (MS65) on zebrafish embryos and larvae led to the proposal of curcumin analogue (MS65) to be developed as an anti-inflammatory drugs. Further pre-clinical and clinical toxicity studies have to be done before MS65 can be developed as a new drug.

ABSTRAK

Ubat anti-keradangan bukan steroid (NSAIDs) adalah antara ubat yang mempunyai preskripsi yang meluas di persada dunia. Walau bagaimanapun, penggunaan NSAIDs memberi kesan sampingan terhadap sistem gastrousus (GI). Di Malaysia, penggunaan salah satu NSAID, iaitu celecoxib yang merupakan perencat COX-2, telah menunjukkan peningkatan dua kali ganda dari tahun 2006 hingga 2007. Analog kurkumin telah mendapat perhatian yang sewajarnya dalam kajian farmakologi ekoran kebaikananya berbanding kurkumin tulen. Analog kurkumin telah dicadangkan sebagai ubat alternatif untuk rawatan sesetengah penyakit seperti penyakit keradangan. Baru-baru ini, ikan zebra telah muncul sebagai organisma model ujian toksikologi untuk penyelidikan dalam bidang genetik vertebra dan biologi pembangunan. Tujuan penyelidikan ini dijalankan adalah untuk menentukan kesan analog kurkumin (MS65) terhadap kemandirian dan denyutan jantung embrio ikan zebra, pertumbuhan embrio ikan zebra, dan buah pinggang dan usus ikan zebra dewasa. Anggaran nilai LC_{50} bagi analog kurkumin (MS65) adalah 12.5µM bagi embrio dan larva. Analisis histologi buah pinggang ikan zebra dewasa yang dirawat dengan MS65 menunjukkan tahap nekrosis yang rendah manakala kehakisan villi kelihatan pada bahagian usus yang dirawat. Sementara itu, larva ikan zebra yang dirawat dengan MS65 mempamerkan edema pada kuning kantung. Oleh itu, tahap ketoksikan yang rendah yang ditunjukkan oleh analog kurkumin (MS65) terhadap embrio dan larva ikan zebra telah mencadangkannya untuk digunakan sebagai ubat alternatif untuk rawatan penyakit tertentu seperti penyakit keradangan. Kajian lanjutan pra-klinikal dan klinikal perlu dijalankan sebelum MS65 boleh dijadikan sebagai ubat yang baharu.

Chapter 1.0

INTRODUCTION

1.1 Research Background

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most broadly prescribed drugs in the world (Miura, 2012). But, NSAIDs confer side effects towards the gastrointestinal (GI) system. The drugs exert inhibitory effects to cyclooxygenase 1 (COX-1) that is expressed continuously in the GI mucosa. The systemic administration of some NSAIDs that trigger GI mucosal injury is the secretion into the bile and routed to enterohepatic transport system (Lichtenberger, 2001). Celecoxib, a specific COX-2 inhibitor, is a novel non-steroidal anti-inflammatory drug (NSAID) and is utilized mainly for the curing of rheumatoid arthritis, osteoarthritis and pain (Hyun et al., 2015).

Many selective COX-2 inhibitors such as celecoxib, rofecoxib, valdecoxib and etoricoxib may cause elevated risk of profound and potentially fatal detrimental cardiovascular thrombotic events, myocardial infarction and stroke (Bansal et al., 2014).In Malaysia, the usage of cyclooxygenase 2 inhibitors (coxibs) has display significant increment. One of the COX-2 inhibitors, celecoxib was chiefly administered in public hospitals while its utilization had increased by two fold from 2006 to 2007 (Hussein et al., 2007). Curcumin is one of the phytochemicals isolated from traditional herb *Curcuma longa*. It is a non-polar bioactive compound (Anand et al., 2007). Two centuries ago, Vogel and Pelletier discovered curcumin in the rhizome of *Curcuma longa* (turmeric) (S. Prasad et al., 2014).

A number of studies pose that curcumin has anti-cancer, anti-viral, anti-amyloid, anti-arthritic, anti-oxidation and anti-inflammation features. The mechanisms involved are complicated and encompass several molecular interactions (Chuah et al., 2014). Likewise, cardiovascular protection is also another pharmacological activity of curcumin. The potentials of curcumin as an agent for prevention and treatment of humankind diseases are chiefly due to its natural safety and pharmacological efficacy (Z. Liu et al., 2014).

Evidences suggest that curcumin has cardio-protective impacts. C- reactive protein, a predictor and independent risk factor of cardiovascular disease, reduced

significantly with curcumin supplementation. This polyphenol is effective against atherosclerosis and myocardial infarction (Prasad et al., 2014). Moreover, the modulation of enzymatic actions and expressions of genes have attributed to the ability of curcumin as therapeutic agent (Wu et al., 2007).The plausible biochemical bioactivity of curcumin comprises the potency to diminish catalytic cyclooxygense, lipoxygenase and phospholipase A2 (Saja et al., 2007). Likewise, curcumin functions as a COX-2 inhibitor by modulating immune-inflammation (Lopresti et al., 2014).

So, numerous integrated methods are implemented to synthesize new analogues so as to bolster bioavailability of curcumin (Katsori et al., 2011). A series of curcumin analogues with more stable structures and sound pharmacokinetics properties were synthesized (Yuan et al., 2014).

Recently, zebrafish has emerged as a toxicology test model organism for the research of vertebrae genetics and developmental biology. Extensive studies demonstrated that zebrafish share high levels of physiological and genetic resemblance to mammals (He et al., 2014). Hence, the evaluation of toxicities of compounds towards humans can be investigated by utilizing zebrafish as a test model.

Curcumin analogues are of great interest due to its advantages compared with pure curcumin. In the meantime, curcumin analogue (MS65) was synthesized in the Chemical Lab of Institute of Bioscience, Universiti Putra Malaysia. Curcumin analogue (MS65) are proposed to be used as an alternative curative drugs for the treatment of certain ailments such as inflammatory-associated diseases.

1.2 OBJECTIVES

1. To determine the effect of curcumin analogue (MS65) on zebrafish embryos survivability and heart beat

2. To determine the effect of curcumin analogue (MS65) on zebrafish embryos development

3. To determine the effect of curcumin analogue (MS65) on the kidney and intestine of adult zebrafish

REFERENCES

Akande, M. G., Orn, S. and Norrgren, L. 2010. Evaluation of the Toxic Effcets of Clozapine in Zebrafish (*Danio rerio*) embryos with the Fish Embryo Toxicity Test. *International Journal On Pharmaceutical and Biomedical Research (IJPBR)* 1(4) : 90-94.

Banaee, M., Davoodi, M. H. and Zoheiri, F. 2013. Histopathological changes induced by paraquat on some tissues of gourami fish (*Trichogastertrichopterus*). *Open Veterinary Journal* 3(1) :36-42.

Bansal, S., Bala, M., Suthar, S. K., Choudhary, S., Bhattacharya, S., Bhardwaj, V., Singla, S. and Joseph, A. 2014. Design and synthesis of novel 2-phenyl-5-(1,3-diphenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazoles as selective COX-2 inhibitors with potent anti-inflammatory activity. *European Journal of Medicinal Chemistry* 80:167-174.

Chede, P. S. 2013. Phytochemical Analysis of *Citrus Sinensis* Peel. *International Journal of Pharma and Bio Sciences* 4(1) :339-343.

Chen, L., Yang, G. and Grosser, T. 2013. Prostanoids and inflammatory pain. *Prostaglandins and other Lipid Mediators* 104-105 :58-66.

Chin, D., Huebbe, P., Frank, J., Rimbach, G. and Pallauf, K. 2014. Curcumin may impair iron status when fed to mice for six months. *Redox Biology* 2: 563-569.

Choudhary, N. and Sekhon, B. S. 2012. Potential therapeutic effect of curcumin-an update. *J Pharm Educ Res* 3(2) : 64-71.

Christiane, N. V. and Ralf, D. 2011.Zebrafish.Oxford University Press Inc .

Chua, S. S. and Paraidathathu, T. 2005. Utilization of Non-steroidal Anti-inflammatory Drugs (NSAIDs) through Community Pharmacies in Malaysia. *Asia Pacific Journal of Public Health* 17(2): 117-123.

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

Du, Z.Y., Wei, X., Huang, M. T., Zheng, X., Liu, Y., Conney, A. H. and Zhang, K. 2013. Anti-proliferative, anti-inflammatory and antioxidant effects of curcumin analogue A_2. *Archives of Pharmacal Research* 36: 1204-1210.

Esatbeyoglu, T., Huebbe, P., Ernst, I. M. A., Chin, D., Wagner, A. E. and Rimbach, G. 2012.Curcumin-From Molecule to Biological Function. *Angewandte Chemie International Edition* 51: 5308-5332.

Fu, X., Liu, Y., Li, W., Bai, Y., Liao,Y., and Liu, H. 2011. Determination of dissociation constants of aristolochic acid I and II by capillary electrophoresis with carboxymethyl – coated capillary. *Talanta* 85: 813-815.

Ganguly, S., Dora, K. C., Sarkar, S., Chowdhury, S. and Ahmed, S. 2012. Zebrafish-General Biology and a Biomedical Research Tool. *International Journal of Science, Environment and Technology* 1(3) : 193-196.

Goel, A., Jhurani, S. and Aggarwal, B. B. 2008. Review: Multi- targeted therapy by curcumin: how spicy is it ? . Mol. Nutr. Food Res 52 : 1-21.

Goldsmith, J. R., Cocchiaro, J. L., Rawls, J. F. and Jobin, C. 2013.Glafenine-induced intestinal injury in zebrafish is ameliorated by μ-opiod signaling via enhancement of Atf6-dependent cellular stress responses. *Disease Models & Mechanisms* 6: 146-159.

Hawkins, P. T. and Stephens, L. R. 2015.P13K signaling in inflammation. *Biochimica et Biophysica Acta* 1851: 882-897.

Henrotin, Y., Clutterbuck, A. L., Allaway, D., Lodwig, E. M., Harris, P., Hartert, M. M., Shakibaei, M., and Mobasheri, A. 2010. Biological actions of curcumin on articular chondrocytes. *Osteoarthritis and Cartillage* 18: 141-149.

He, J. H., Gao, J. M., Huang, C. J., and Li, C. Q. 2014. Zebrafish models for assessing developmental and reproductive toxicity. *Neurotoxicology and Teratology* 42: 35-42 .

Hegazy, R., Alashlab, M., and Amin, M. 2011.Cardiorenal Effects of Newer NSAIDs (Celecoxib) versus Classic NSAIDs (Ibuprofen) in patients with Arthritis. *Journal of Toxicology* 2011:1-8.

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.

Hussein, H., Hussain, H., Salbiah, M. S., Muhaini, O., Low, Y. L., and Beh, P. K. 2007. Chapter 18 : Use of drugs for rheumatological and bone disorders. *Malaysian Statistics On Medicine* 2007 : 109-115.

Iqbal, E., Salim, K, A. and Lim, L. B. L. 2015. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University-Science* : 1-9.

Jalaludeen, M. D., Arunachalam, M., Raja, M., Nandagopal, S., Showket, A. B., Sundar, S. and Palanimuthu, D. 2012. Histopathology of the gill, liver and kidney tissues of the freshwater fish Tilapia Mossambica exposed to cadmium sulphate. *International Journal of Advanced Biological Research* 2(4) : 572-578.

Kais, B., Schneider, K. E., Keiter, S., Henn, K., Ackermann, C. and Braunbeck, T. 2013. DMSO modifies the permeability of the zebrafish (*Danio rerio*) chorion-Implications for the fish embryo test (FET). *Aquatic Toxicology* 140-141 : 229-238 .

Kant, V., Gopal, A., Pathak, N. N., Kumar, P., Tandan, S. K., and Kumar, D. 2014. Antioxidant and anti-inflammatory potential of curcumin accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats. *International Immunopharmacology* 20:322-330 .

Kochel, T. J. and Fulton, A. M. 2015. Multiple drug resistance-associated protein 4 (MRP4), prostaglandin transporter (PGT), and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) as determinants of PGE_2 levels in cancer. *Prostaglandin & other Lipid Mediators* 116-117 : 99-103.

Kwak, D. H., Park, J. H., Lee, H. S., Moon, J. S. and Lee, S.2014. Aristolochic Acid 1 Induces Ovarian Toxicity by inhibition of Akt Phosphorylation. *Chem. Res. Toxicol* 27: 2128-2135.

Lee, K. H., Aziz, F. H., Syahida, A., Abas, F., Sharri, K., Israf, D. A. and Lajis, N. H. 2009.Synthesis and biological evaluation of curcumin-like diarylpentanoid analogues for anti-inflammatory, antioxidant and anti-tyrosinase activities. *European Journal of Medicinal Chemistry* 44 :3195-3200.

Lichtenberger, L. M. 2001. Where is the evidence that cyclooxygenase inhibition is the primary cause of non-steroidal anti-inflammatory drug (NSAID)-induced gastrointestinal injury? Topical injury revisited. *Biochemical Pharmacology* 61: 631-637.

Lopresti, A. L., Maes, M., Maker, G. L., Hood, S. D., and Drummond, P. D.2014. Curcumin for the treatment of major depression : A randomized, double-blind, placebo controlled study. *Journal of Affective Disorders* 167: 368-375 .

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.

Lucas, S. M., Rothwell, N. J. and Gibson, R. M. 2006. The role of inflammation in CNS injury and disease. *British Journal of Pharmacology* 147 : S232-S240.

Brand, M., Granato, M. and Volhard, C. N. 2011. Chapter 1 : Keeping and raising zebrafish. *Zebrafish* :7-37.

Menke, A. L., 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.

Miura, T. 2012. Reactivity of non-steroidal anti-inflammatory drugs with peroxidase: a classification of non-steroidal anti-inflammatory drugs. *Journal of Pharmacy and Pharmacology* 64: 1461-1471.

Merrell, J. G., McLaughlin, S. W., Tie, L., Laurencin, C. T., Chen, A. F., and Nair, L. S. 2009.Curcumin –Loaded Poly(ε-Caprolactone) Nanofibres: Diabetic Wound Dressing With Anti-Oxidant and Anti-Inflammatory Properties. *Clinical and Experimental Pharmacology and Physiology* 36: 1149-1156.

Moallem, S. A., Imenshahidi, M., Shahini, N., Javan, A. R.,Karimi, M., Alibolandi, M., Ghandadi, M., Etemad, L., Motamedshariaty, V., Hosseini, T., and Hadizadeh F. 2013. Synthesis, Anti-inflammatory and Anti- Nociceptive Activities and Cytotoxic Effect of Novel Thiazolidin-4-ones Derivatives as Selective Cyclooxygenase (COX-2) Inhibitors. *Iran Journal of Basic Medical Sciences* 16:1238-1244 .

Nasrallah, R., Robertson, S. J., Karsh, J. and Hebert, R. L. 2013.Celecoxib modifies glomerular basement membrane, mesangium and podocytes in OVE26 mice, but ibuprofen is more detrimental. *Clinical Science* 124: 685-694.

Patricia McGrath. 2012. *Zebrafish : Methods for Assessing Drug Safety and Toxicity*. John Wiley & Sons, Inc.

Potter, P. E. 2013. Curcumin : a natural substance with potential efficacy in Alzheimer's disease. *Journal of Experimental Pharmacology* 5: 23-31.

Prakash, D., Gupta, C., and Sharma, G. 2012. Importance of Phytochemicals in Nutraceuticals. *Journal of Chinese Medicine Research and Development* 1(3) :70-78.

Prasad, S., Gupta, S. C., Tyagi, A. K. and Aggarwal, B. B. 2014.Curcumin, a component of golden spice : From bedside to bench and back. *Biotechnology Advances* 32: 1053-1064.

Rietjens, I. M. C. M., Martena, M. J., Boersma, M. G., Spiegelenber, W. andAlink, G. M. 2005.Review: Molecular mechanisms of toxicity of importance food-borne phytotoxins. *Mol. Nutr. Food Res* 49: 131-158.

Ricciotti, E. and FitzGerald, G. A. 2011. Prostaglandin and Inflammation. *Arterioscler Thromb Vasc Biol* 31(5) : 986-1000.

Roberts, R. J. 2012. Fish Pathology Fourth Edition. *Blackwell Publishing Ltd*.

Saxena, M., Saxena, J., Nema, R., Singh, D., and Gupta, A. 2013.Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry* 1(6) : 168-182.

Schlansky, B., and Hwang, J. H. 2009.Prevention of non-steroidal anti-inflammatory drug-induced gastropathy. *Journal of Gastroenterology* 44: 44-52.

Sevigny, M. B., Graham, K., Ponce, E., Louie, M. C. and Mitchell, K. 2012. Glycosylation of human cyclooxygenase-2 (COX-2) decreases the efficacy of certain COX-2 inhibitors. *Pharmacological Research* 65 : 445-550.

Shaikh, P. Z. 2011. Cytokines & their physiologic and pharmacologic functions in inflammation: A review. *International Journal of Pharmacy & Life Sciences* 2(11) : 1247-1263 .

Sostres, C., Gargallo, C. J., and Lanas, A. 2013.Nonsteroidal anti-inflammatory drugs and upper and lower gastrointestinal mucosal damage. *Arthritis Research & Therapy* 15(3): S3.

Takeuchi, K., Tanaka, A., Kato, S., Amagase, K., and Satoh, H. 2010.Roles of COX Inhibition in pathogenesis of NSAID-induced small intestinal damage. *Clinica Chimica Acta* 411: 459-466.

Tewari, A. K., Singh, V. P., Yadav, P., Gupta, G., Singh, A., Goel, R. K., Shinde, P., and Mohan, C. G. 2014. Synthesis, biological evaluation and molecular modeling study of

pyrazole derivatives as selective COX-2 inhibitors and anti-inflammatory agents. *Bioorganic Chemistry*56: 8-15.

Wang, S., Liu, K., Wang, X., He, Q. and Chen, X. 2010.Toxic effects of celastrol on embryonic development of zebrafish (*Danio rerio*).*Drug and Chemical Toxicology* 34(1): 61-65.

Wu, C. J., Chou, Y, C., Cheng, Y, W., Hsiao, C. J., Wang, C. H., Wang, H. Y., Sheu, J. R., and Hsiao, G. 2011. Aristolochic acid down regulates monocytic matrix metalloproteinase-9 by inhibiting nuclear factor-Kb activation. *Chemico-Biological Interactions* 192: 209-219.

Wu, J. Y., Lin, C.Y., Lin, T. W., Ken, C. F., and Wen, Y. D. 2007. Curcumin Affects Development of Zebrafish Embryo. *Biol. Pharm. Bull*. 30(7): 1336-1339.

Xie, X. W., Pan, L. Y., and Sun, Y. W. 2014. Growing with the world: rapid development of the zebrafish research in China and the China Zebrafish Resource Center. *Science China Life Sciences.* 58(4) : 396-399.

Xue, X., Yu, J. L., Sun, D. Q., Zou, W., Kong, F., Wu, J., Liu, H. P., Qu, X. J., and Wang, R. M. 2013.Curcumin as a multidrug resistance modulator- A quick review. *Biomedicine& Preventive Nutrition* 3: 173-176.

Xu, D. J., Bu, J. W., Gu, S. Y., Xia, Y. M., Du, J. L., and Wang, Y.W. 2011. Celecoxib Impairs Heart Development via Inhibiting Cyclooxygenase-2 Activity in Zebrafish Embryos. *Anesthesiology* 114: 391-400.