ANTI- CATARACT AND ANTI-DIABETIC PROPERTIES of Citrus hystrix DC. LEAF EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

NOR SHAHIRA SOLEHAH BINTI UMRAN

IB 2020 31

ANTI- CATARACT AND ANTI-DIABETIC PROPERTIES of *Citrus hystrix* DC. LEAF EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS.

By

NOR SHAHIRA SOLEHAH BINTI UMRAN

Thesis Submission to the School of Graduated Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

July 2019

DEDICATION

I dedicated this thesis to my parents and husband
who were always right there beside me through thick and thin

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

ANTI- CATARACT AND ANTI-DIABETIC PROPERTIES of *Citrus hystrix* DC. LEAF EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS.

By

NOR SHAHIRA SOLEHAH BINTI UMRAN

July 2019

Chairman: Prof. Suhaila Mohamed, PhD
Faculty: Institute of Bioscience

Citrus hystrix leaf is an important South-East Asian culinary ingredient with anti-oxidant, anti-inflammation, and cardio-protective properties. Inflammation, hyperglycaemia and oxidation are significant contributors to diabetic cataract formation. This study demonstrated the mitigating effects of *Citrus hystrix* leaf extract on diabetes and cataract development in female Sprague Dawley rats. Diabetes was induced by intraperitoneal streptozotocin (75 mg/kg) injection before the rats were orally administrated with the 150 and 300 mg of extract per kg body weight or metformin (250 mg/kg) for 8 weeks after diabetes development. The extract gradually and significantly decreased fasting blood glucose levels ($p<0.05$), reduced serum malondialdehyde (MDA), prostaglandin E2 (PGE2), vascular endothelial growth factor (VEGF), and tumor necrosis factor alpha (TNF-α) levels. Histological evidence showed the cataracts development were significantly suppressed by the 150 mg/kg extract ($p<0.05$), performing better than metformin, by ameliorating systemic inflammation (TNF-α, PGE2, VEGF), oxidative stress (malondialdehyde), hyperglycemia and lens-opacification. Good correlations were found between cataract incidence with fasting blood glucose ($r^2=0.90$), serum PGE2 ($r^2=0.91$), MDA ($r^2=0.99$), VEGF ($r^2=0.71$), and TNF-α levels ($r^2=0.49$) suggesting these biomarkers may probably help predict cataract risk. The citrus compounds suppressed PGE2, VEGF, oxidation (MDA) and inflammation to probably prevent fluid influx, lens-fibers osmotic over-hydration, to mitigate diabetic cataract development. This study showed the limonoids and flavonoid

rich *Citrus hystrix* leaf consumption, is a good anti-hyperglycemic/ anti-diabetic agent, with potent anti-oxidant and anti-inflammation properties that help preventing diabetic cataract development.

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

KESAN ANTI-KATARAK DAN ANTI-DIABETES EKSTRAK DAUN *Citrus hystrix* DC. DALAM TIKUS YANG DIARUH OLEH STREPTOZOTOCIN.

Oleh

NOR SHAHIRA SOLEHAH BINTI UMRAN

Julai 2019

Chair: Prof. Suhaila Mohamed, PhD
Faculty: Institut Biosains

Daun *Citrus hystrix* adalah ramuan penting masakan Asia Tenggara yang mengandungi anti-oksidan, anti-keradangan, dan sifat perlindungan kardio. Keradangan, hiperglisemia dan pengoksidaan adalah penyumbang utama kepada pembentukan katarak diabetik. Kajian ini menunjukkan kesan pengurangan ekstrak daun *Citrus hystrix* pada diabetis dan perkembangan katarak pada tikus. Diabetes diaruh dengan suntikan streptozotocin secara intraperitonum (75 mg/kg) kepada tikus sebelum diberikan 150 dan 300 mg ekstrak per kg berat badan atau metformin (250 mg/kg) secara oral selama 8 minggu selepas pembentukan diabetes. Ekstrak secara beransur-ansur telah menurunkan kadar glukosa darah semasa puasa, mengurangkan kadar serum malondialdehid (MDA), prostaglandin E2 (PGE2), faktor pertumbuhan endothelial vaskular (VEGF), tahap faktor nekrosis alfa dan (TNF-α). Bukti histologi menunjukkan perkembangan katarak yang ditindas dengan ketara ($p<0.05$) terutamanya ekstrak 150 mg/kg, lebih baik daripada metformin, dengan memperbaiki keradangan sistemik (TNF-α, PGE2, VEGF), tekanan oksidatif (malondialdehid), hiperglisemia dan pembekuan lensa. Terdapat korelasi yang baik antara pembentukan katarak dengan kadar glukosa puasa ($r^2=0.90$), serum PGE2 ($r^2=0.91$), MDA ($r^2=0.99$), VEGF ($r^2=0.71$), and TNF-α levels ($r^2=0.49$) dan semua biomarker ini boleh digunakan

untuk meramal risiko katarak. Kajian membuktikan ekstrak daun Limau Purut dapat menurunkan kadar PGE2, VEGF, pengoksidaan (MDA) dan keradangan yang mungkin menghalang kemasukan bendalir, osmotik kanta, serat kanta, untuk mengurangkan perkembangan katarak diabetes. Kajian ini menunjukan pengambilan daun *Citrus hystrix* yang kaya dengan limonoid dan flavonoid adalah ejen anti-hiperglisemia/ anti-diabetes yang baik, anti-oksida dan anti-keradangan yang kuat untuk menghalang pembentukan katarak diabetes.

ACKNOWLEDGEMENTS

Alhamdulillah, all praise to Allah for the successful project and completion of this thesis. Many thanks to wonderful people that made this journey a success.

I would like to express my sincere gratitude to my supervisor, Prof. Dr. Suhaila Mohamed, for her continuous support and motivation, so much wisdom for me to learn in academics as well as in life. To my co-supervisors, Dr. Lau Seng Fong and Dr. Norhaizan Mohd Esa, for their time and expertise throughout this study.

My big thanks for those who assisted me during this project, Puan Latifah Mohd Hanan for preparing histological slides. To my research group member; Iffah Nadhira, Nur Iliyani and Nazmun Nahar, thank you for lending hand whenever I ask from the start until the end. I am also thankful for getting to do my work at UPM-MAKNA Cancer Research Laboratory (IBS, UPM), Animal house of Faculty of Medicine and Health Science,UPM) and Faculty of Veterinary Medicine,UPM.

I would like to acknowledge Universiti Putra Malaysia and Kementerian Pengajian Tinggi Malaysia that provide necessary financial support for this research.

Last but not least, thank you to my parents, Umran Bin Mansor and Ramlah Binti Awang, my family and family-in-law, for the du'a and understanding, my dearest husband, Mohd Afiq bin Mohd Danial, for so much love and strong support.

I certify that a Thesis Examination Committee has met on (9 July 2019) to conduct the final examination of Nor Shahira Solehah Binti Umran on her thesis entitled "Anti-Cataract and Anti-Diabetic Properties of *Citrus Hystrix* DC. Leaf Extract In Streptozotocin-Induced Diabetic Rats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Rozita binti Rosli, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Noordin Mohamed Mustapha, PhD
Professor
Faculty of Veterinary
Medicine Universiti Putra
Malaysia (Internal Examiner)

Kamisah Yusof, PhD
Associate Professor,
Faculty of Medicine
National University of Malaysia
Malaysia
(External Examiner)

ZURIATI AHMAD ZUKARNAIN, PhD
Professor Ts. and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 30 June 2020

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:

Suhaila Mohamed, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Lau Seng Fong, DVM, PhD
Associate Professor,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Norhaizan Mohd Esa, PhD
Associate Professor,
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

_____

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No: _____

Declaration by graduate student

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of
Chairman of
Supervisory
Committee: _____

Signature: _____
Name of
Member of
Supervisory
Committee: _____

Signature: _____
Name of
Member of
Supervisory
Committee: _____

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENT		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xiii
LIST OF FIGURES		xiv
LIST OF ABBREVIATIONS		xvi
CHAPTER		
1.	**INTRODUCTION**	1
1.1.	Research Background	1
1.2.	Hypothesis	2
1.3.	General Objective	2
1.4.	Specific Objectives	2
2.	**LITERATURE REVIEW**	3
2.1.	Prevalence of Cataract	3
2.1.1	Cataract Incidence by Gender, Ethnic Age	4
2.2.	Anatomy and Physiology of Eyes Lens	5
2.3.	Diabetic Cataract	7
2.3.1	Risk Factors for Cataract	7
2.3.2	Signs and Symptoms	7
2.3.3	Treatments Approach	8
2.4.	Pathogenesis of Diabetic Cataract	8
2.4.1	Non-enzymatic glycation	8

2.4.2 Oxidative Stress Pathways 10
2.4.3 Polyol Pathways 11
2.5. Role of Phytochemical and Its Mechanism of Actions in Diabetic Cataract 13
2.6. *Citrus hystrix* 14
2.6.1. Taxonomical Classification 14
2.6.2. Botanical Description 14
2.6.3. Phytochemical Constituents in Citrus 15
2.7. Streptozotocin (STZ) 16
2.7.1. Structure of STZ 17
2.7.2. Solubility and Stability 18
2.7.3. Diabetogenic of STZ 18
2.8. Metformin 20

3. RESEARCH METHODOLOGY

3.1. Chemicals and Drugs 21
3.2. Preparation of solutions 21
3.3. Plant Material and Ethanol Extraction 21
3.4. Animal Study 21
3.4.1 Diabetes Induction 22
3.5. Cataract Formation Evaluation 23
3.6. Enzyme-linked Immunosorbent Assay (ELISA) 23
3.7. Histological Analysis 23
3.8. Biochemical Parameters and Neurological Tests 24
3.8.1 Biochemical Parameters 24
3.8.2 Thermal Hyperalgesia 24
3.8.3 Cold Allodynia 24
3.8.4 Tail Immersion 25
3.8.5 Walking Function Tests 25
3.9. Statistical analysis 25

4. RESULTS 26

4.1. Animal Study 26
4.1.1 Effects on Diabetes 26
4.2. Cataract Formation Evaluation 27
4.3. Oxidative Stress and Inflammation Biomarkers 29
4.4. Biochemical Parameters and Preliminary Neurological Tests 31

5. DISCUSSION 35

5.1. Animal Study (Body Weight and Fasting Blood Glucose) 35
5.2. Cataract Formation Evaluation 35
5.3. Oxidative Stress and Inflammation Biomarkers 36
5.4. Biochemical Parameters and Preliminary Neurological Tests 39

6. SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH 41

6.1. Summary and conclusion 41
6.2. Limitations and recommendations for future research 41

REFERENCES 42
APPENDICES 49
BIODATA OF STUDENT 51
LIST OF PUBLICATIONS 52

LIST OF TABLES

Table		Page
2.1	Potential risk factors for cataract.	7
4.1	Biochemical parameters	32

LIST OF FIGURES

Figure		Page
2.1	Major global cause of blindness in 2010	3
2.2	Gender and ethnic distribution of cataract surgery patient in Malaysia	4
2.3	Age distribution cataract surgery patient in Malaysia	5
2.4	Structure of eyes lens	6
2.5	Comparison between healthy and cataract eye lens	6
2.6	Non-enzymatic reactions	9
2.7	Oxidative stress pathways	11
2.8	Polyol pathways	12
2.9	*Citrus hystrix* plant	15
2.10	Structure of STZ	17
2.11	Mechanism of action of STZ	19
2.12	Structure of metformin	20
3.1	Experimental design	22
4.1	Body weight	26
4.2	Fasting blood glucose level	27
4.3	Cataract formation	28
4.4	Malonylaldehyde (MDA)	29
4.5	Tumor necrosis factor alpha (TNF-α)	30
4.6	Prostaglandins E 2 (PGE2)	30
4.7	Vascular endothelial growth factor (VEGF)	31
4.8	Thermal hyperalgesia	32
4.9	Cold allodynia	33
4.10	Tail immersion	33

4.11	Walking function test – distance covered in 1 minute	34
4.12	Walking function test – time taken to walk 1 meter distance	34
5.1	Anti-diabetic cataract pathways	38

LIST OF ABBREVIATIONS

CLE	*Citrus hystrix* Leaf Extract
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
COX-2	Cyclooxygenase 2
DC	Diabetic Cataract
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
H&E	Hematoxylin and eosin
HPLC	High performance liquid chromatography
IL	Interleukin
MDA	Malondialdehyde
PGE_2	Prostaglandin E_2
ROS	Reactive oxygen species
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

CHAPTER 1

INTRODUCTION

1.1 Research Background

Cataract causes visual impairment associated with diabetes and aging, being the fifth most frequent cause of blindness especially in 8-25% of diabetic patients (Yuan et al., 2017). Cataract (cortical, nuclear, posterior subcapsular, and combined forms) is characterized by cloudy or opaque areas in the normally clear eye lens. Hyperglycemia increases free-radical (oxidative) stress and osmotic damage within the lens via the polyol pathway where glucose is reduced by aldose reductase to sorbitol that accumulates within the lens resulting in secondary osmotic over-hydration of the lens fibers and the subsequent cataract development (Somya et al., 2015). Both diabetes and cataract are health and economic burdens, where diabetes management is insufficient and cataract surgery is often inaccessible or unaffordable (Yuan et al., 2017).

Streptozotocin (STZ) has been used in research for many years to induce diabetes mellitus (DM) in rats and mice because of its toxic effects on pancreatic β-cells. It is a strong alkylating agent that can methylate DNA and its cytotoxicity depends on DNA alkylation. The STZ-induced rat serves as an excellent model to study the molecular, cellular, and morphological changes in the brain induced by stress in DM (Busineni et al., 2015).

Metformin has multifunctional profile such as anti-inflammation actions, anti-cancer and cardiovascular protection (Forez et al., 2014; Malinska et al., 2016). Metformin was selected as the positive control drug because unlike insulin injection, it is orally administered, relatively safe, well tolerated for increasing insulin sensitivity and reducing glucose production by the liver (Hundal & Inzucchi, 2003). Metformin is currently used as the first drug of choice for all types of chronic hyperglycaemia, because of its safety and efficacy, and the dose used were within the range of the experimental doses of CLE.

Kaffir lime (*Citrus hystrix*), is a popular flavor ingredient in Southeast Asia cuisine. It is rich in obacunone (limonoids), and the flavonoids diosmin, hesperidine, lutein, isoquercitrin and didymin (Siti et al., 2015). Limonoids and flavonoids, have good anti-oxidant, anticancer, anti-inflammatory and free-radical scavenging properties, thus are potential agents against diabetic complications (Chetna et al., 2015). *Citrus hystrix* leaf was chosen due to its health-promoting properties and easy to find. There is no report on anti-diabetic cataract on *Citrus hystrix* leaf.

This study demonstrate the anti-cataract and anti-hyperglycemic effect of *Citrus hystrix* leaf extract, by investigating the effects on the eye lens opacities formation, oxidative stress and inflammatory biomarkers in the diabetes rats for 8 weeks, and compared to normal control rats.

1.2 Hypothesis

The *Citrus hystrix* may/may not mitigate diabetes and diabetes cataract by suppressing oxidative stress and inflammation.

1.3 General Objective

To evaluate the effect of *Citrus hystrix* leaf extracts (CLE) on diabetes and diabetic cataract in rat model and investigate the possible mechanisms involved.

1.4 Specific Objectives

1. To examine the effect of CLE on blood sugar level in STZ-induced rat.
2. To study the effect of CLE on cataract development in STZ-induced rat.
3. To study the effect of CLE on serum MDA, VEGF, TNF-α and PGE2 level in STZ-induced rat.
4. To evaluate the effectiveness of CLE on diabetic kidney, liver and neural function.

REFERENCES

Agardh, E., B. Hultberg, and C. Agardh. 2000. Effects of inhibition of glycation and oxidative stress on the development of cataract and retinal vessel abnormalities in diabetic rats. Current Eye Research 21: 543–549.

Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med. 1994; 331:1480–1487.

Ajani, E.O., Sabiu, S., Odufuwa, K.T., Ibrahim, T.B., Salau, B.A., 2017. Evaluation of lens aldose reductase inhibitory and free radical scavenging potential of fractions of lonchocarpus cyanescens: Potential for cataract remediation. Pharmacogn. J. 9, 62–69. doi:10.5530/pj.2017.1.12

Amandeep Kaur; Vikas Gupta; Ajay Francis Christopher; Manzoor Ahmad Malik; Parveen Bansal, Nutraceuticals in prevention of cataract – An evidence based approach, Saudi Journal of Opthalmology, (2017), 31, 30-37.

Anwer, T., M. Sharma, K.K. Pillai, S.E. Haque, M.M. Alam, and M.S. Zaman. 2007. Protective effect of bezafibrate on streptozotocininduced oxidative stress and toxicity in rats. Toxicology 229: 165–172.

Arumugam Abirami, Gunasekaran Nagarani, Perumal Siddhuraju, In vitro antioxidant, anti-diabetic, cholinesterase and tyrosinase inhibitorypotential of fresh juice from *Citrus hystrix* and C. maxima fruits, Food Science and Human Wellness 3 (2014) 16–25.

Aziman N, Abdullah N, Noor ZM, Zulkifli KS, Kamarudin WSSW. Phytochemical Constituents and *In Vitro* Bioactivity of Ethanolic Aromatic Herb Extracts. *Sains Malaysiana*.
2012;41(11):1437–1444

Bai, J., Zheng, Y., Wang, G., Liu, P., 2016. Protective Effect of D-Limonene against Oxidative Stress-Induced Cell Damage in Human Lens Epithelial Cells via the p38 Pathway. Oxid. Med. Cell. Longev. 2016. doi:10.1155/2016/5962832

Baynes, J. W. (1991). Role of oxidative stress in development of complication in diabetes. Diabetes, 40, 405– 412.

Bhadada, S.V., Bhadada, V.J., Goyal, R.K., 2016. Preventive Effect of Tephrosia purpurea on Selenite-Induced Experimental Cataract.Curr. Eye Res. 41, 222–231 doi:10.3109/02713683.2015.1011281

Bickol N. Mukesh, Anhchuong Le, Peter N. Dimitrov, BOrth; Shazia Ahmed, Hugh R. Taylor, AC, MD; Catherine A. McCarty, Development of Cataract and Associated Risk Factors, Arch Ophthalmol. (2006);124:79-85

Busineni Jayasimha Goud et al. Streptozotocin - A Diabetogenic Agent in Animal Models Ijppr.Human, (2015); Vol. 3 (1): 253-269

Calcutt NA, Jorge MC, Yaksh TL, Chaplan SR. Tactile allodynia and formalin hyperalgesia in streptozotocin-diabetic rats: effects of insulin, aldose reductase inhibition and lidocaine. Pain. 1996; 68(2-3): 293-299.

Chang, K. C., Paek, K. S., Kim, H. J., Lee, Y. S., Yabe-Nishimura, C., & Seo, H. G. (2002). Substrate-induced up-regulation of aldose reductase by methylglyoxal, a reactive oxoaldehyde elevated in diabetes. Molecular Pharmacology, 61, 1184–1191.

Chaniphun Butryee, Pongtorn Sungpuag & Chureeporn Chitchumroonchokchai, Effect of processing on the flavonoid content and antioxidant capacity of *Citrus hystrix*

leaf, International Journal of Food Sciences and Nutrition, August 2009; 60(S2): 162-174

Chetna Mishra, Babita Singh, Seema Singh, M.J.A. Siddiqui, Abbas Ali Mahdi, Role Of Phytochemicals In Diabetes Lipotoxicity: An Overview, International Journal Of Research And Development In Pharmacy And Life Sciences, June - July, 2015, Vol. 4, No.4, Pp 1604-1610

Chetna Mishra,, Babita Singh, Seema Singh, M.J.A. Siddiqui, Abbas Ali Mahdi, Role Of Phytochemicals In Diabetes Lipotoxicity: An Overview, International Journal of Research and Development in Pharmacy and Life Sciences, 2015, Vol. 4, No.4, pp 1604-1610

Chew, W. E. Benson, N. A. Remaley, et al., "Results after lens extraction in patients with diabetic retinopathy: early treatment diabetic retinopathy study report number 25," Archives of Ophthalmology, vol. 117, no. 12, pp. 1600– 1606, 1999.

Chiu CJ, Morris MS, Rogers G, et al. Carbohydrate intake and glycemic index in relation to the odds of early cortical and nuclear lens opacities. Am J Clin Nutr 2005; 81(6): 1411–1416.

Chylack Jr, L.T., and J.H. Kinoshita. 1972. The interaction of the lens and the vitreous. I. The high glucose cataract in a lens-vitreous preparation. Experimental Eye Research 14: 58–64.

Claudia M. Garcia, Ying-Bo Shui, Meera Kamath, Justin DeVillar, Randall S. Johnson, Hans-Peter Gerber, Napoleone Ferrara, Michael L. Robinson, David C. Beebe, The function of VEGF-A in lens development: Formation of the hyaloid capillary network and protection against transient nuclear cataracts, Experimental Eye Research 88 (2009) 270–276

Dewanjee S, Gangopadhyay M, Sahu R, Karmakar S. Cadmium induced pathophysiology: prophylactic role of edible jute (Corchorus olitorius) leaves with special emphasis on oxidative stress and mitochondrial involvement. Food Chem Toxicol. 2013;60:188–98.

Dutta M, Ghosh D, Ghosh AK, Bose G, Chattopadhyay A. High fat diet aggravates arsenic induced oxidative stress in rat heart and liver. Food Chem Toxicol. 2014;66:262–77.

Emanuela Ricciotti and Garret A. FitzGerald, Prostaglandins and Inflammation, National Institutes of Health, Arterioscler Thromb Vasc Biol. 2011 May ; 31(5): 986–1000. doi:10.1161/ATVBAHA.110.207449

Eleazu et al:Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. Journal of Diabetes & Metabolic Disorders 2013, 12:60

Firuzi, O., Miri, R., Tavakkoli, M., Saso, L., 2011. Antioxidant therapy: Current status and future, Firuzi, O., Miri, R., Tavakkoli, M., Saso, L., 2011. Antioxidant therapy: Current status and future prospects. Curr. Med. Chem. 18.

Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B. Metformin: from mechanisms of action to therapies. Cell Metab 2014;20:953–966

Girao, H., Mota, C., & Pereira, P. (1999). Cholesterol may act as an antioxidant in lens membranes. Current Eye Research, 18 (6), 448–454

Hegde, K.R., and S.D. Varma. 2005. Prevention of cataract by pyruvate in experimentally diabetic mice. Molecular and Cellular Biochemistry 269: 115–120.

Herna´ ndez, C., Simo´ , R., 2007. Strategies for blocking angiogenesis in diabetic retinopathy: from basic science to clinical practice. Expert Opin. Invest. Drugs 16, 1209–1226.

Irina G. Obrosova, Stephen S. M. Chung, Peter F. Kador, Diabetic cataracts: mechanisms and management, Diabetes Metab Res Rev 2010; 26: 172–180.

Jacob, R. F., Cenedella, R. J., & Mason, R. P. (1999). Direct evidence for immiscible cholesterol domains in human ocular lens fiber cell plasma membranes. Journal of Biological Chemistry, 274 (44), 31613– 31618.

Jain D, Bansal MK, Dalvi R, Upganlawar A, Somani R. Protective effect of diosmin against diabetic neuropathy in experimental rats. J Integr Med. 2014; 12(1): 35-41.

Jamilah B, Gedi, MA, Suhaila M, Zaidul, ISMd. Phenolics in Citrus hystrix leaves obtained using supercritical carbon dioxide extraction. Int Food Res Journal. 2011;18(3):941- 948.

Junod A, Lambert AE, Orci L, Pictet R, Gonet AE, REnold AE: Studies of the diabetogenic action of streptozotocin. ProcSocExpBiol Med 1967, 126:201–205

Kemmochi, Y., K. Miyajima, T. Ohta, T. Sasase, Y. Yasui, K. Toyoda, K. Kakimoto, T. Shoda, and A. Kakehashi. 2014. Ocular inflammation in uveal tract in aged obese type 2 diabetic rats (Spontaneously Diabetic Torii fatty rats). Journal of Diabetes Research 2014: 629016.

Khanra Ritu, Saikat Dewanjee, Tarun K Dua, Ranabir Sahu, Moumita Gangopadhyay, Vincenzo De Feo and Muhammad Zia-Ul-Haq, Abroma augusta L. (Malvaceae) leaf extract attenuates diabetes induced nephropathy and cardiomyopathy via inhibition of oxidative stress and inflammatory response, Journal of Translational Medicine (2015) 13:6

Kim SI, Prevalence and risk factors for cataracts in persons with type 2 diabetes mellitus. Korean J Ophthalmol 2006; 20(4): 201–204

Kim SJ, Flach AJ, Jampol LM. Nonsteroidal anti-inflammatory drugs in ophthalmology. Surv Ophthalmol. 2010;55:108–133.

Kowluru, R.A., and M. Kanwar. 2007. Effects of curcumin on retinal oxidative stress and inflammation in diabetes. Nutrition & metabolism 4: 8.

Kozlowska, A., Szostak-Wegierek, D., 2018. Plant flavonoids in health, prevention, and treatment of chronic diseases, Nutritional Antioxidant Therapies: Treatments and Perspectives. doi:10.1007/978-3-319-67625-8_14

Krishnamoorthy. 2015. Neuroprotective effects of transcription factor Brn3b in an ocular hypertension rat model of glaucoma. Investigative Ophthalmology & Visual Science 56: 893–907.

Kunkel-Bagden E, Bregman BS. Spinal cord transplants enhance the recovery of locomotor function after spinal cord injury at birth. Exp Brain Res. 1990; 81(1): 25-34.

Kyselova*, M. Stefek, V. Bauer, Pharmacological prevention of diabetic cataract, Journal of Diabetes and Its Complications 18 (2004) 129–140

Lamoreaux, W.J., Fitzgerald, M.E., Reiner, A., Hasty, K.A., Charles, S.T., 1998. Vascular endothelial growth factor increases release of gelatinase A and decreases release of tissue inhibitor of metalloproteinases by microvascular endothelial cells in vitro. Microvasc. Res. 55, 29–42.

Lawrence BM, Hogg JW, Terhune SJ. Constituents of the leaf and peel oils of Citrus hystrix DC. *Phytochem.* 1971;10:1404-5.

Laohavechvanich P, Muangnoi C, Butryee C Kriengsinyos W. Protective effect of makrut lime leaf (Citrus hystrix) in HepG2 cells: Implications for oxidative stress. *Science Asia*. 2010;36:112–117.

Ling SL, Mohamed S. Alpha-Tocopherol content in 62 edible tropical plants. *J Agric Food Chem*. 2001;49:3101-3105.

Li, X., Cui, X., Wang, J., Yang, J., Sun, X., Li, X., Zhu, Q., Li, W., 2013. Rhizome of anemarrhena asphodeloides counteracts diabetic ophthalmopathy progression in streptozotocin-induced diabetic rats. Phyther. Res. 27, 1243–1250. doi:10.1002/ptr.4866

Lu M, Taylor A, Chylack LT Jr, et al. Dietary linolenic acid intake is positively associated with five-year change in eye lens nuclear density. J Am Coll Nutr 2007; 26(2): 133–40.

Lu, H., Y. Chen, X. Sun, B. Tong, and X.H. Fan. 2014. Effects of *Citrus hystrix* leaf on retinal oxidative stress and inflammation in diabetes. RSC Advances 7: 4898–4904.

Mario, U. D., & Pugliese, G. (2001). 15th Golgi lecture: from hyperglycaemia to the dysregulation of vascular remodelling in diabetes. Diabetologia, 44, 674– 692.

Melder, R.J., Koenig, G.C., Witwer, B.P., Safabakhsh, N., Munn, L.L., Jain, R.K., 1996. During angiogenesis, vascular endothelial growth factor and basic fibroblast growth factor regulate natural killer cell adhesion to tumor endothelium. Nat. Med. 2, 992–997.

Ming-Yueh Lee, Pik-Pin Goh, Mohamad Aziz Salowi, Tassha Hilda Adnan, BSc,þ and Mariam Ismail, The Malaysian Cataract Surgery Registry: Cataract Surgery Practice Pattern, Asia Pacific Journal of Ophthalmology, Volume 3, Number 6, November/December 2014

Monica, L., P.P. Immaculada, R. Nino, and T. Marirosa. 2004. Structure, conformation, and electronic properties of apigenin, *Citrus hystrix* leaf, and taxifolin antioxidants. A first principle theoretical study. The Journal of Physical Chemistry A 108: 92–96.

Murakami A, Nakamura Y, Koshimizu K, Ohigashi H. Glyceroglycolipids from Citrus hystrix, a traditional herb in Thailand, potently inhibit the tumorpromoting activity of 12-O-tetradecanoylphorbol 13-acetate in mouse skin. *J Agric Food Chem*. 1995;43:2779–2783.

Nishikawa, T., Edelstein, D., & Brownlee, M. (2000). The missing link: a single unifying mechanism for diabetic complications. Kidney International, 58 (77), S26– S30.

Ohta, Y., Yamasaki, T., Niwa, T., & Majima, Y. (2000). Preventive effect of vitamin E-containing liposome instillation on cataract in 12-month-old rats fed a 25% galactose diet. Journal of Ocular Pharmacology and Therapeutics, 16 (4), 323– 335.

Othenin-Girard, P., N. Pittet, and C.P. Herbort. 1993. Anterior segment inflammation in cataract operation: Comparison of intraocular lens implanted in the lens capsule and in the sulcus. Canadian Journal of Ophthalmology 28: 55–57.

Ottonello, S., C. Foroni, A. Carta, S. Petrucco, and G. Maraini. 2000. Oxidative stress and age-related cataract. Ophthalmologica 214: 78–85.

Panzram G. Mortality and survival in type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 1987;30:123–131

Pari L, Srinivasan S. Antihyperglycemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats. Biomed Pharmacother. 2010; 64(7): 477-481

Patel, D., Prasad, S., Kumar, R., Hemalatha, S., 2011. Cataract: A major secondary complication of diabetes, its epidemiology and an overview on major medicinal plants screened for anticataract activity. Asian Pacific J. Trop. Dis. 1, 323–329. doi:10.1016/S2222-1808(11)60075-3

Patel BM, Raghunathan S, Porwal U. Cardioprotective effects of magnesium valproate in type 2 diabetes mellitus. Eur J Pharmacol. 2014;728:128–34.

Paul Robertson, The COX-2/PGE2/EP3/Gi/o/cAMP/GSIS Pathway in the Islet: The Beat Goes On, Diabetes 2017;66:1464–1466

Pornpimol Raksakantong, Sirithon Siriamornpun & Naret Meeso, Effect of drying methods on volatile compounds, fatty acids and antioxidant property of Thai kaffir lime (*Citrus hystrix* D.C.), International Journal of Food Science and Technology 2012, 47, 603–612

Ramı´rez, C., T.L. Russo, G. Delfino, S.M. Peviani, C. Alcaˆntara, and T.F. Salvini. 2013. Effect of tibiotarsal joint inflammation on gene expression and cross-sectional area in rat soleus muscle. Brazilian Journal of Physical Therapy 17: 244–254.

Rania Nasrallah, Ramzi Hassouneh, and Richard L. Hébert, PGE2, Kidney Disease, and Cardiovascular Risk: Beyond Hypertension and Diabetes, J Am Soc Nephrol 27: 666–676, 2016. doi: 10.1681/ASN.2015050528

Rakieten, N., Gordon, B. S., Beaty, A., Cooney, D. A.,Davis, R. D., and Schein, P. S.: Pancreatic islet cell tumors produced by the combined action of streptozotocin and nicotinamide. Proc. Soc. Exp. Biol. Med. 737:280-83, 1971

Reddy R, Kim SJ. Critical appraisal of ophthalmic ketorolac (Acuvail) in the treatment of pain and inflammation following cataract surgery. Clin Ophthalmol. 2011;5:751–758.

Reddy S, Tajunisah I, Low K, Karmila A. Prevalence of eye diseases and visual impairment in urban population-a study from University of Malaya Medical Centre. *Malays Fam Physician* 2008;3(1):25-28.

Rossini AA, Like AA, Chick WL, Appel MC, Cahill GF., Jr 1977. Studies of streptozotocin-induced insulitis and diabetes. ProcNatlAcadSci USA 74:2485–2489

Sato, S., Mori, K., Wyman, M., & Kador, P. F. (1998). Dose-dependent prevention of sugar cataracts in galactose-fed dogs by the aldose reductase inhibitor M79175. Experimental Eye Research, 66, 217– 222.

Satyam, S.M., L.K. Bairy, R. Pirasanthan, and R.L. Vaishnav. 2015. Grape seed extract and Zinc containing nutritional food supplement delays onset and progression of Streptozocin-induced diabetic cataract in Wistar rats. Journal of Food Science and Technology 52: 2824–2832.

Semenza, G.L., et al., 1994. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. J. Biol. Chem. 269 (38), 23757–23763.

Shui, Y.-B., et al., 2003. Vascular endothelial growth factor expression and signaling in the lens. Invest. Ophthalmol. Vis. Sci. 44 (9), 3911–3919

Siti, H.N., Kamisah, Y., Nur Iliyani, M.I., Mohamed, S., Jaarin, K., 2017. Citrus leaf extract reduces blood pressure and vascular damage in repeatedly heated palm oil diet-Induced hypertensive rats. Biomed. Pharmacother. 87. doi:10.1016/j.biopha.2016.12.075

Sowmya, V., Kalekhan, F., Kamath, K., Baliga, M.S., 2015. Fruits in the Prevention of Cataractogenesis by Targeting the Aldose Reductase: Promise from Preclinical Observations. Foods and Dietary Supplements in the Prevention and Treatment of Disease in Older Adults. doi:10.1016/B978-0-12-418680-4.00011-7

Srisukha V, Tribuddharatb C, Nukoolkarnc V, Bunyapraphatsarac N, Chokephaibulkitd, Phoomniyomb KS, Chuanphungb S, Srifuengfung S. Antibacterial activity of essential oils from *Citrus hystrix* (makrut lime) against respiratory tract pathogens. *Science Asia*. 2012;38:212–217.

Stankowska, D.L., A.Z. Minton, M.A. Rutledge, B.H. Mueller, N.R. Phatak, S. He, H.Y. Ma, M.J. Forster, T. Yorio, and R.R.

Suryanarayana, P., K. Krishnaswamy, and G.B. Reddy. 2003. Effect of curcumin on galactose-induced cataractogenesis in rats. Molecular Vision 9: 223–230.

Suryanarayana, P., Saraswat, M., Mrudula, T., Krishna, T.P., Krishnaswamy, K., Reddy, G.B., 2005. Curcumin and turmeric delay streptozotocin-induced diabetic cataract in rats. Invest. Ophthalmol. Vis. Sci. 46, 2092–9. doi:10.1167/iovs.04-1304

Taysi, S., S. Okumus, M. Akyuz, N. Uzun, A. Aksoy, E. Demir, M. Orkmez, M. Tarakcioglu, and M. Adli. 2012. Zinc administration modulates radiation-induced oxidative injury in lens of rat. Pharmacognosy Magazine 8: 245–249.

Thevi T, Basri M, Reddy S. Prevalence of eye diseases and visual impairment among the rural population-a case study of temerloh hospital. *Malays Fam Physician* 2012;7(1):6-10.

Thevi T, Godinho MA. Predictive factors of visual outcome of Malaysian cataract patients: a retrospective study. Int J Ophthalmol 2017;10(9): 1452-1459

Thiraphatthanavong, P., J. Wattanathorn, S. Muchimapura, T.M. Wipawee, P. Wannanon, T.U. Terdthai, B. Suriharn, and K. Lertrat. 2014. Preventive effect of Zea mays L. (purple waxy corn) on experimental diabetic cataract. BioMed Research International 2014: 507435.

Thomas & Ramwell: Streptozotocin: a nitric oxide carrying molecule and its effect on vasodilation. Eur. J. Pharmacol.161, 279 (1989).

Tomar, A., Chandeshwar, I., Pradesh, U., 2013. Pharmacological Importance of Citrus Fruits. Int. J. Pharm. Sci. Res. 4, 156–160.

Tranos, S. S. Wickremasinghe, N. T. Stangos, F, Topouzis, I. Tsinopoulos, and C. E. Pavesio, "Macular edema," Survey of Ophthalmology, vol. 49, no. 5, pp. 470–490, 2004.

Utami, R., Kawiji, Khasanah, L.U. and Nasution, M.I.A, Preservative effects of kaffir lime (*Citrus hystrix* DC) leaves oleoresin incorporation on cassava starch-based edible coatings for refrigerated fresh beef, International Food Research Journal 24(4): 1464- 1472(August 2017).

Valentovic MA, Alejandro N, Betts CA, Brown PI, Ramos K: Streptozotocin (STZ) diabetes enhances benzo(alpha)pyrene induced renal injury in Sprague Dawley rats. ToxicolLett 2006, 164:214–220.

Vanamala, Greg Cobb, Nancy D Turner, Joanne R Lupton, Kil Sun Yoo, Leonard M Pike, Bhimanagouda S Patil, Bioactive Compounds of Grapefruit (Citrus paradisi Cv. Rio Red) Respond Differently to Postharvest Irradiation, Storage, and Freeze Drying, J. Agric. Food Chem., 2005, 53 (10), pp 3980–3985

VanMarle, J., & Vrensen, G. F. (2000). Cholesterol content of focal opacities and multilamellar bodies in the human lens: filipin cytochemistry and freeze fracture. Ophthalmic Research, 32 (6), 285– 291.

Vanner, E.A., and M.W. Stewart. 2014. Meta-analysis comparing same-day versus delayed vitrectomy clinical outcomes for intravitreal retained lens fragments after age-related cataract surgery. Clinical Ophthalmology 8: 2261–2276.

Varma, S. D. (1991). Scientific basis for medical therapy of cataracts by antioxidants. American Journal of Clinical Nutrition, 53 (1), 335– 345.

Weber C, Pernis B, Ting W, Rosenkrantz K, Reemtsma K. 1984. Murine streptozotocin diabetes: influences of the major histocompatibility complex, genetic background, and blood transfusion. Diabetologia27Suppl:160–162 .

World Health Organization. http://www.who.int/blindness/causes. Accessed 7 September 2018.

Xu, Y., J. Zhang, J. Liu, S. Li, C. Li, W. Wang, R. Ma, and Y. Liu. 2015. *Citrus hystrix* leaf attenuate the D-galactose-induced renal damage by attenuation of oxidative stress and inflammation. Natural Product Research 29: 1078–1082.

Yanqing Zhang ,Yuan Zhang ,Robert N. Bone,WanxingCui,Ji-Bin Peng,Gene P. Siegal, Hongjun Wang, Hongju Wu :Regeneration of Pancreatic Non-β Endocrine Cells in Adult Mice following a Single Diabetes-Inducing Dose

Yanni SE, Barnett JM, Clark ML, Penn JS. The role of PGE2 receptor EP4 in pathologic ocular angiogenesis. Invest Ophthalmol Vis Sci. 2009;50:5479–5486

Yoshida S, Nakama T, Ishikawa K. Antiangiogenic shift in vitreous after vitrectomy in patients with proliferative diabetic retinopathy, Invest Ophthalmology 2012;53(11):6997-7003

Yuan Chen, Xiao-Bo Sun, Hong-e Lu, Fang Wang, Xiao-Hui Fan, Effect of luteoin in delaying cataract in STZ-induced diabetic rats, Archives Pharmacal Research (2017) 40:88–95

Zainal M, Ismail SM, Ropilah AR, et al Prevalence of blindness and low vision in Malaysian population: results from the National Eye Survey 1996 British Journal of Ophthalmology 2002;86:951-956

Zhu, X., K. Zhang, W. He, J. Yang, X. Sun, C. Jiang, J. Dai, and Y. Lu. 2015. Proinflammatory status in the aqueous humor of high myopic cataract eyes. Experimental Eye Research. doi:10.1016/j. exer.2015.03.017

Zou, Z., Xi, W., Hu, Y., Nie, C., Zhou, Z., Antioxidant activity of Citrus fruits, Food Chemistry (2015), doi: http://dx.doi.org/10.1016/j.foodchem.2015.09.072