UNIVERSITI PUTRA MALAYSIA

ANTI-HYPERTENSIVE EFFECT OF PROTEOLYSATE GENERATED FROM STONE FISH (ACTINOPYGA LECANORA JAEGER) IN RATS

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By

MAHDOKHT SADEGH VISHKAEI

Thesis Submitted to the School of Graduate Studies, University Putra Malaysia in Fulfilment of the Requirement for the Degree of Master of science

April 2015

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DEDICATION

To my mom, the bravest woman I have ever seen who beat cancer lonely with her smile when I was doing my master in Malaysia. I give my deepest expression of apologise for not being with you. All that I am I owe to your endless sacrifice, true and unwavering love and sincere support from the moment I was born until now. Hoping my quest to get this degree of higher education is making your dream a reality.

To my father, who earns an honest living for us. I give my deepest expression of love and appreciation for all the sacrifices you have made during my life and study and for your sincere and unconditional supports. I am honored to have you as my father.

To my sister Bita, who always takes care of me from the time I was born until now for sharing laughter and wiping tears and for all the sacrifices that you have made to all our family members. Side by side or miles apart, we are connected by heart.

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

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April 2015

Chairman: Prof Nazamid Saari, PhD
Faculty: Food Science and Technology

Bioactive peptides within the original food-derived proteins are short sequences of amino acids that are inactive in the sequence of the parent protein. However, they can be activated through different ways including enzymatic hydrolysis. Among all the bioactive peptides, antihypertensive bioactive peptides are considered as a vitally important peptides since they are able to function as Angiotensin converting enzyme (ACE) inhibitors and have effective role in curing hypertension which is a common and serious chronic health problem and known as the most important risk factor for development of many diseases such as stroke. The ACE inhibitory effect of *Actinopyga lecanora* proteolysate *in vitro* had been reported. Hence, this study aimed to evaluate the ACE inhibitory potential of *A. lecanora* proteolysate *in vivo* (in normotensive rats). In this regard, the ACE inhibitory capability of the proteolysate to prevent increasing blood pressure, after inducing hypertension by angiotensin I was examined in normal rats. The pre-fed rats with the proteolysates at various doses (200, 400, 800 mg/kg body weight) revealed the significant ($p \leq 0.05$) suppression effect compared with control groups after inducing hypertension. Furthermore, different doses of the proteolysate (200, 400, 800 mg/kg body weight) were examined to decrease the blood pressure of hypertension-induced rats. Results depicted that 800 mg proteolysate/kg body weight significantly reduced blood pressure without a negative effect on normal blood pressure ($p \leq 0.05$). Sub-acute toxicity study based on OECD guideline demonstrated no toxicity effect of the proteolysate *in vivo*. The present study indicated that the proteolysate at a dose of 1000 mg/kg daily did not cause toxicity signs such as death, changes in activity, or piloerection. Since there are no significant differences between treated groups and control groups, hematological and biochemical analysis confirmed the safety of the proteolysate ($p > 0.05$). In addition, there were no significant differences between organs weights of the treated groups and the control groups. Morphologically, neither histopathological changes nor gross abnormalities were observed. However, the proteolysate caused a significant decrease in body weight in relation to the control groups ($p \leq 0.05$) probably due to appetite stimulation by the proteolysate, leading to decreased food consumption in the sub-acute group. It is concluded that the proteolysate generated from *A. lecanora* possess a significant *anti-hypertensive* effect and would be potentially used as a natural alternative of ACE inhibitors.

Abstrak tesis ini dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk Ijazah Sarjana Sains

Kesan anti -hipertensi proteolisat daripada ikan batu (*Actinopyga lecanora*) ke atas tikus

Oleh

Mahdokht Sadegh Vishkaei

April 2015

Pengerusi: Prof. Nazamid Saari, PhD
Fakulti: Sains dan Teknologi Makanan

Peptida bioaktif dalam protein makanan adalah jujukan pendek asid amino yang tidak aktif dalam jujukan protein induk. Walau bagaimanapun, mereka boleh diaktifkan melalui pelbagai cara termasuk hidrolisis enzim. Antara semua peptida bioaktif, peptida bioaktif anti-hipertensi dianggap sebagai peptida yang amat penting kerana mereka dapat berfungsi sebagai perencat kepada enzim penukar angiotensin (ACE) dan seterusnya boleh berperanan secara berkesan mengawal tekanan darah tinggi yang merupakan masalah kesihatan yang meluas dan kronik. Hipertensi turut dikenali sebagai faktor risiko yang paling penting menyebabkan pelbagai penyakit seperti strok. Kesan proteolisat *Actinopyga lecanora* ke atas perencatan ACE dalam kajian *in vitro* telah dilaporkan. Oleh itu, kajian ini bertujuan untuk menilai potensi proteolisat *A. lecanora* sebagai perencat ACE secara in *vivo* menggunakan tikus normotensif. Dalam hal ini, keupayaan perencatan ACE oleh proteolisat untuk mengekang peningkatan tekanan darah telah diperiksa pada tikus normal, selepas hipertensi didorong menggunakan angiotensin 1. Tikus pra -makan dengan proteolisat pada pelbagai dos (200, 400, 800 mg / kg berat badan) menunjukkan kesan pengekangan yang ketara ($p \leq 0.05$) berbanding dengan kumpulan tikus kawalan selepas tekanan darah tinggi didorong. Dos proteolisat pada kepekatan yang berbeza (200, 400, 800 mg/kg berat badan) telah diperiksa untuk mengurangkan tekanan darah tikus hipertensi. Keputusan menunjukkan bahawa penggunaan 800 mg proteolisat/kg berat badan menurunkan tekanan darah tanpa kesan negatif ke atas tekanan darah yang normal ($p \leq 0.05$). Kajian ketoksikan sub-akut berdasarkan garis panduan OECD menunjukkan tiada kesan ketoksikan proteolisat dalam *in vivo*. Kajian ini menunjukkan bahawa proteolisat pada kadar dos 1000 mg/kg berat badan setiap hari tidak menyebabkan tanda-tanda keracunan seperti kematian, perubahan dalam aktiviti, atau piloereksi. Oleh kerana tidak ada perbezaan yang signifikan di antara kumpulan rawatan dan kumpulan kawalan, hematologi dan analisis biokimia mengesahkan keselamatan penggunaan proteolisat ($p > 0.05$). Selain itu, tidak terdapat perbezaan yang ketara di antara berat organ-organ kumpulan yang dirawat dan berat organ-organ kumpulan kawalan. Secara

morfologi, tiada perubahan histopatologi mahupun keabnormalan kasar diperhatikan. Walau bagaimanapun, proteolisat menyebabkan penurunan yang ketara dalam berat badan kumpulan tikus kawalan ($p \leq 0.05$) adalah kemungkinan disebabkan oleh rangsangan selera makan dengan proteolisat yang membawa kepada penurunan pengambilan makanan dalam kumpulan sub-akut. Kajian ini dapat disimpulkan bahawa proteolisat yang dihasilkan dari *A. lecanora* mempunyai kesan anti-hipertensi yang ketara dan berpotensi digunakan sebagai perencat semulajadi alternatif kepada ACE.

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

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LIST OF ABBREVIATIONS

ACE	Angiotensin I-converting enzyme
GI	Gastrointestinal
CVDs	Cardiovascular diseases
SHR	Spontaneously hypertensive rats
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
IC_{50}	The half maximal inhibitory concentration
BP	Blood pressure
HBP	High blood pressure
BPM	Beat per minute
WKS	Weeks
mg	Milligram
kg	Kilogram
bw	Body weight
SBP	Systolic blood pressure
DBP	Diastolic blood pressure

RAS	Renin angiotensin system
Zn	Zinc
TRP	Tryptophan
mmHg	Millimetres of mercury
pH	Hydrogen ion exponent
rpm	Revolution per minute
°C	Degrees celsius
min	Minute
mM	Millimolar
h	Hour
%	Percentage
Sd	Sprague dawley
mL	*Millilitre*
µg	Microgram
mg	Milligram
OECD	The Organisation for economic co-operation and development
WBC	White blood cell
RBC	Red blood cell
Hgb	Hemoglobin
Hct	Hematocrit
MCV	Mean corpuscular volume

MCHC	Mean corpuscular hemoglobin Concentration
MCH	Mean corpuscular hemoglobin
PLT	Platelet count
BUN	Blood urea nitrogen
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
P	Probability
SD	Standard deviation
MW	Molecular weight
MWCO	Molecular weight cut off
et al.	And others
ANOVA	Analysis of variance
Da	Dalton
G	Gram
E/S	Enzyme-substrate ratio

CHAPTER 1

INTRODUCTION

Hypertension is a common and critical chronic health problem, and recognized as a most important risk issue for development of numerous diseases including cardiovascular disease (CVD), stroke, arteriosclerosis, as well as myocardial infarction affecting 15–20% of adults throughout the world and estimates more than 1.56 billion of the population worldwide will suffer from hypertension by 2025 (Reiner, 2009; Rampal, Azhar, & Rahman, 2008). In Asian countries, hypertension is actually popular and has effects on public health. (Ahhmed & Muguruma, 2010). In Malaysia, the prevalence of high blood pressure is high, although levels of consciousness, treatment and control are low. Consequently, there is an urgent need for a comprehensive integrated population-based intervention program to improve the rising issue of hypertension throughout Malaysians (Rampal et al., 2008). Nowadays, the effective synthetic ACE inhibitors stabilize blood pressure. However, they do not remove the root cause, which is as yet unknown. Accordingly, in most cases, hypertension is treated non-specifically because it is of unknown type or is diagnosed at an advanced stage (Ahhmed & Muguruma, 2010). Moreover, synthetic ACE inhibitors can have adverse effects such as skin rashes, taste disturbances and cough (Alashi et al., 2014). Therefore, the search for methods which relate to diet and prevent hypertension is markedly of interest with the probability of functional foods. The most well-established mechanism which is based on the blood-pressure-lowering effect is angiotensin-converting enzyme (ACE) activity inhibition.

ACE inhibitors with the source of protein hydrolysate have been acquired from various food (animal sources and plant sources) such as bovine casein (Miguel, Contreras, Recio, & Aleixandre, 2009), fermented foods (Je, Park, Byun, Jung, & Kim, 2005), red algae (Qu et al., 2010). However, just *in vivo* study can certainly confirm that any specific hydrolysate has antihypertensive effect or not, which usually is based on its destiny to come across gastrointestinal (GI) enzymes and brush-border membrane peptidases after administration orally.

Actinopyga lecanora, a kind of sea cucumber which is known as stone fish with moderately high protein substance, was investigated as raw material for the production of bioactive peptides. Due to its comparatively higher protein substance and base on the results of previous study on its *in vitro* ACE inhibitory effect, *A. lecanora* would be a possible source for the generation of bioactive peptides and bromelain generated proteolysate can exhibit a significant anti-hypertensive effect as well as curative effect *in vivo*. As there is no well-established scientific *in vivo* information reported on the antihypertensive activity of proteolysate derived from *A. lecanora*, accordingly this

study aimed to investigate the antihypertensive activity of *A. lecanora* proteolysate *in vivo*. The main research questions were as follows:

- Whether different doses of proteolysate can significantly affect normal blood pressure before inducing hypertension by Angiotensin I?
- Whether different doses of proteolysate can significantly prevent blood pressure to increase? And whether different doses of proteolysate can markedly decrease blood pressure as an alternative therapy?
- Are there any significant differences between different doses of proteolysate?
- Are there any significant differences between the effective dose and anti-hypertensive synthetic drug?
- Whether the proteolysate cause any toxicity for human in the case of consumption?

The current study aimed to evaluate ACE inhibitory potential of *A. lecanora* proteolysate *in vivo* (in normotensive rats). In this regard, the effect of different doses of proteolysate on normal blood pressure were investigated. In addition, the effect of the proteolysate to prevent blood pressure to increase, and the capability of the proteolysate to decrease blood pressure were evaluated. The toxicity study was determined by assessing body weight gain, organs weight, haematological and biochemical parameters, macroscopic and microscopic findings. The main goal of the present work was to investigate an ACE inhibitor proteolysate derived from a marine source with desirable functional characteristics comparable with a synthetic drug for hypertension.

In the current study, the main objectives were as follows:

- To examine the ACE inhibitory capability of the proteolysate to prevent increasing blood pressure in normal rats after inducing hypertension by angiotensin I.

- To examine the capability of the proteolysate to decrease the blood pressure of hypertension-induced rats.
- To determine toxicity of proteolysate in rats using OECD guideline.

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