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THE EFFICACY OF SELECTED HERBS IN DELAYING THE AGEING PROCESS AS INDICATED BY THE REDUCTION IN PLASMA MALONDIALDEHYDE LEVELS

By

IMILIA BINTI ISMAIL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

December 2004

To my beloved mum and my sister

…for many years of patience and support

-THANK YOU-

-IMI-

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

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Chairman : Associate Professor Noordin Bin Mohamed Mustapha, Ph.D.

Faculty : Veterinary Medicine

Effects of selected herbs in delaying ageing in rats were studied by measuring the activity of plasma malondialdehyde (MDA) as an index of lipid peroxidation status. Likewise, the activities of anti-oxidative enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were also assessed as indices of anti-oxidant. Descriptive histological changes in the brain, liver, kidney and heart were also performed, while, quantitative histology was determined by counting the necrotic cells and lipofuscin pigments in the liver, the glomeruli in the kidney, and the neuron cells in the brain.

Fifty male Sprague-Dawley rats each of the 4-week and 10-month old respectively were used. All rats were divided equally into 10 groups. While the control group was given the basal diet, other groups were fed the basal diet containing 5% of Sireh (*Piper betle;* S), Bunga Kantan (*Pheaomeria speciosa;* BK), Dukong Anak (*Phyllanthus niruri;* DA) or Pucuk Gajus (*Anacardium occidentale* L.; PG). Blood

samples for biochemical analysis were taken every three weeks by intracardiac puncture.

The results showed that at almost all instances, the concentration of MDA in the controls were significantly ($p<0.05$) higher than any other groups. The concentration of MDA was been markedly reduced by herbal supplementation. Likewise, supplementation had also exerted its protective effect against ageing by increasing the activity of anti-oxidant enzymes. Such effective scavenging mechanism in the herbal supplemented group had led to less ageing lesion development in these rats.

In conclusion, the selected herbs especially those of DA and PG were able to alleviate ageing-induced injuries in rats via boosting the scavenging system or generating anti-oxidant-like compounds.

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

KEBERKESANAN HERBA-HERBA TERPILIH DALAM MELAMBATKAN PROSES PENUAAN DENGAN PENURUNAN ARAS PLASMA MALONDIALDEHID SEBAGAI PENUNJUK

Oleh

IMILIA BINTI ISMAIL

Disember 2004

Pengerusi : Profesor Madya Noordin Bin Mohamed Mustapha, Ph.D.

Fakulti : Perubatan Veterinar

Kesan herba-herba terpilih di dalam melambatkan proses penuaan pada tikus telah dikaji melalui pengukuran aras plasma malondialdehid (MDA), sebagai penunjuk kepada proses peroksidasi lipid. Sehubungan dengan itu, aktiviti enzim anti-oksidan seperti katalase (CAT), superoksid dismutase (SOD) dan glutation peroksidase (GSH-Px) turut dikaji sebagai penunjuk kepada status anti-oksidan. Perubahan histologi dikaji pada tisu otak, hati, buah pinggang dan jantung. Kuantitatif histologi ditentukan dengan pengiraan sel nekrosis dan pigmen lipofusin di dalam hati, glomerulus pada buah pinggang dan neuron pada otak.

Sebanyak 50 ekor tikus Sprague Dawley jantan untuk setiap peringkat umur, iaitu 4 minggu dan 10 bulan telah digunakan sebagai subjek kajian. Tikus-tikus ini dibahagikan sama rata kepada 10 kumpulan. Kumpulan kawalan diberi diet normal, manakala kumpulan rawatan di beri diet yang mengandungi 5% herba sama ada Sireh (*Piper betle; S*), Bunga Kantan (*Pheaomeria speciosa; BK*), Dukong Anak

(*Phyllanthus niruri; DA*) atau Pucuk Gajus (*Anacardium occidentale* L.; PG). Sampel darah untuk analisis biokimia diambil setiap 3 minggu melalui intrakardiak.

Hasil kajian menunjukkan secara kesuluruhannya, aras kepekatan MDA dalam kumpulan kawalan adalah lebih tinggi ($p<0.05$) berbanding dengan kumpulan-kumpulan lain. Kepekatan aras MDA berjaya diturunkan dengan pemberian diet yang mengandungi herba yang dikaji. Di samping itu, pemberian diet herba ini telah menunjukkan kesan yang positif dalam memperlambatkan proses penuaan melalui peningkatan aktivit enzim anti-oksidan. Kesan mekanisma ini turut mengurangkan pembentukan lesi yang wujud disebabkan oleh penuaan di dalam tikus yang dikaji.

Kesimpulannya, herba-herba yang dikaji terutama DA dan PG telah membuktikan kemampuannya dalam memperlambatkan proses penuaan dengan meningkatkan ketahanan sistem badan terhadap radikal bebas atau menghasilkan unsur-unsur seperti anti-oksidan.

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I certify that an Examination Committee met on 31st December 2004 to conduct the final examination of Imilia binti Ismail on her Master of Science thesis entitled "The Efficacy of Selected Herbs in Delaying the Ageing Process as Indicated by the Reduction in Plasma Malondialdehyde Levels " in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

_____

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LIST OF ABBREVIATIONS

BK	Bunga Kantan
CAT	Catalase
CCl_3^-	Trichlorometyl radical
$CCl_3O_2^-$	Peroxy radical
CHP	Cumene hydroperoxide
Cu/Zn-SOD	Copper/zinc-superoxide dismutase
CO_2	Carbon dioxide
Cx	Control
DA	Dukong Anak
DNA	Deoxyribonucleic acid
DTNB	5,5'-Dithio-bis(2-nitrobenzoic acid)
EC-SOD	Extra-cellular superoxide dismutase
GLM	General linear model
GSH	Glutathione
GSH-Px	Glutathione peroxidase
Hb	Haemoglobin
H&E	Haematoxylin and Eosin
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
H_2O	Water
HO_2^-	Peroxy radical
H_2O_2	Hydrogen peroxide
LOOH	Lipid peroxide

MDA	Malondialdehyde
Mn-SOD	Mangenese superoxide dismutase
MT	Metallothionein
NaCl	Sodium chloride
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
$NaWO_4$	Sodium tungsten
$NO^{\cdot}$	Nitric oxide
$^1O_2$	Singlet oxygen
O_2	Oxygen
O_2^{-}	Superoxide radical
$OH^{\cdot}$	Hydroxyl radical
$ONOO^{-}$	Peroxynitrite
PAF	Platelet activating factor
PAS	Periodic Acid Schiff
PG	Pucuk Gajus
PUFA	Polyunsaturated fatty acid
RBC	Red blood cell
$RO^{\cdot}$	Alcocxyl radical
ROO	Peroxyl radical
ROS	Reactive oxygen species
$RS^{\cdot}$	Thyl radical
S	Sireh
Se	Selenium
SDAT	Senile dementia of Alzheimer type

SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TEP	Tetraethoxypropane
WHV	Woodchuck hepatitis virus

CHAPTER I

INTRODUCTION

Ageing in humans, especially those surpassing the age 28 years, has become a major risk factor for disease and death among people nowadays especially in the developed countries (Matsuo *et al.,* 1992). This phenomenon happens because of the rapid changes in lifestyles. Humans do not have or do not allocate time for physical activities and good rest. Furthermore, as time advances, their exposure to potent activator of ageing such as ultra violet (UV) radiation increases.

Despite studies, the actual mechanism of ageing remains to be explained. However, current knowledge on ageing has led to several postulations. Ageing is described as a phenomenon, which results from randomly accumulated damaging effects hampering the ability of an organism to maintain homeostasis (Nohl, 1991). Likewise, ageing can also be defined as a multi-step, time-dependent phenomenon. It is characterized by decreased capability of the cells, tissues, organs and the whole organism to respond to exogenous and endogenous insults from physical, chemical or biological agents (Henning *et al.,* 1991).

The process of ageing occurs due to the presence of free radical or species of reactive oxygen in the body. Free radical theory supports the process of ageing (Newsholme and Leech, 1983). Vervaart and Knight (1996) defined free radical as an atom or a molecule having at least one unpaired electron in its outer orbit. It formation is stimulated by the body's metabolic process and environmental factors. Reformation of free radicals and lipid peroxidation process increase with advancement of age.

Ageing progressively damages cells due to the decreasing antibody system (Henning *et al.,* 1991).

It is well established that free radicals or reactive oxygen species (ROS) are generated *in vivo* and that they can lead to cell and tissue damage. In respons, organisms have developed complex anti-oxidant defense and repair mechanisms to prevent the accumulation of oxidatively damaged molecules. Any perturbation to anti-oxidant balance that favors oxidation leads to cellular damage termed "oxidative stress". Previously it was thought that oxidative stress was damaging to cells in all cases. It is becoming apparent however, that the presence of a degree of oxidative stress within cells is essential for the normal functioning of some intracellular signaling pathways and as a mediator of stress-induced apoptosis (Vervaart & Knight, 1996). Therefore, anti-oxidant is a very important scavengers of free radicals that might delay ageing.

Currently, worldwide traditional medicine is being revalued by extensive research on different plant species and their therapeutic principles. As plants produce several anti-oxidants to control oxidative stress, they can represent a source of new compounds with anti-oxidant activity. Hence, the purpose of our research is to determine the effect of selected plant herbs in delaying the ageing process. Meanwhile, the hypothesis of this research is that the selected herbs posses anti-oxidant properties in alleviating ageing-induced injuries.

In this research, Sprague Dawley rats were used as models of ageing. Blood haemolysate samples were used to measure the activity of anti-oxidant enzymes

while the plasma was used to assess the peroxidation status. Histology of organs was performed to investigate changes during ageing.

Research objective

The objectives of this study are:

i) to assess the peroxidation status of rats during ageing with or without herbal supplementation

ii) to assess the anti-oxidant defense status of rats during ageing with or without herbal supplementation

iii) to study the histologic patterns of tissues undergoing ageing with or without herbal supplementation.

CHAPTER II

LITERATURE REVIEW

Ageing

Ageing is the final phase of human development and may be defined as the aggregate of structural changes that occur with the passage of time; it is characterized by progressive inability to sustain vital function, with death the eventual result. Life expectancy varies according to localities and gender. For example, in the United States, the average male life expectancy at birth is between 70 and 75 years; for female, between 75 and 80 years (Chandrasoma and Taylor, 1998).

It has also been documented that there is a steady loss of function in various critical organs with age. Extrapolation from such observation would indicate that humans have a finite biologic life span of 90-110 years, so that even if cardiovascular disease and cancer were eradicated, the current average life expectancy would increase by only a few years (Vervaart and Knight, 1996).

Senescence might therefore be defined as the sum of the time dependent phenomenon associated with modifications and impairment of cellular function and ageing is the sum of the phenomenon related to the time, dependent on the physiological functions of an organ or organism.

There are several theories and hypothesis proposed on ageing, which includes:

a) free radical theory
b) cross-link theory