



UNIVERSITI PUTRA MALAYSIA

**THE EFFICACY OF GARLIC IN ALLEVIATING FLUORANTHENE
AND PYRENE INDUCED PULMONARY INJURY IN RATS**

TEH RASYIDAH BINTI ISMAIL

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By

TEH RASYIDAH 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**

November 2003



DEDICATION

To my beloved family and friends



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

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

Faculty: Veterinary Medicine

Amongst polycyclic aromatic hydrocarbons (PAH) found during the Malaysian 1997 haze episode were fluoranthene (FL) and pyrene (PY). In manifest of evolving strategies and bringing about an attenuation of deleterious effects of these particles in human, both acute symptoms and chronic inflammation of pulmonary system were studied in rats.

The acute exposure studies were performed to determine presence of apoptosis and proteases activity in the lung of rats following treatment with FL, PY and FL+PY. Rats not receiving any treatment served as controls while those administered with FL, PY and FL+PY were instilled intratracheally at the dose of 11ng, 11.5ng and combination of both doses respectively. All rats were euthanised at 1, 8 and 24 hours post- instillation (p.i). Apoptosis estimation was made on haematoxylin-eosin stained histopathologic sections, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) analysis and DNA laddering of lung samples. As for protease analyses, total protein content measured by using BSA kit, elastase-like activity and neutrophil elastase activity were performed in the lung lavage collected from rats.



Different phases of apoptosis were discovered in the pneumocytes and bronchial epithelium of lung samples of all PAH-treated rats. The histopathological finding showed that there was increment of apoptotic cells ($p < 0.05$) with advancement of time, especially in 8 and 24 hours p.i. This was also confirmed by TUNEL analysis and DNA laddering.

The obtained data showed a time-dependent increase in lung lavage total protein content, elastase-like activity and neutrophil elastase activity.

The chronic exposure studies were conducted as described in the acute study but for a three month period. However, an assessment was also made on the efficacy of garlic as an alternative therapeutic agent against FL and PY effects. Rats from the control, FL, PY and FL+PY groups were daily fed on a commercial basal diet while rats from the garlic (G), FL+G, PY+G and FL+PY+G groups were fed the basal ration containing garlic powder incorporated at the rate of 80mg/kg body weight/rat/day.

The outcome demonstrated morphological alteration and growth disorder in pneumocytes and bronchial epithelium of rats from all PAH-treated groups. The PAH-treated groups also showed dreadful effects in proteases activities, levels of IgG, IgA, alveolar macrophages activities and glutathione-S-transferase in the lung.

In contrast, all PAH-treated groups fed with garlic showed significant improvement in pathological changes, proteases activities, immunology, and enzyme activity in the lung.

Concisely, environmental hazard of FL, PY and their combination to lung of rats as target organs triggers deleterious changes either by acute or chronic exposure while garlic has tremendous potential in alleviating the chronic effects of FL and PY.



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sebagai memenuhi keperluan untuk ijazah Master Sains

**KEBERKESANAN BAWANG PUTIH DALAM MENGATASI KEROSAKAN
PULMONARI AKIBAT INDUS FLUORANTHENE DAN PYRENE PADA
TIKUS**

Oleh

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Di antara hidrokarbon polisiklik aromatik (PAH) yang dijumpai semasa episod jerebu Malaysia 1997 adalah fluoranthene (FL) dan pyrene (PY). Kajian mengenai pendedahan simptom akut dan inflamasi kronik sistem pernafasan terhadap FL, PY dan gabungannya telah dijalankan ke atas tikus bagi merancang strategi bagi mengurangkan kesan merbahayanya kepada manusia sejagat.

Kajian pendedahan akut telah dijalankan untuk menentukan kehadiran apoptosis dan aktiviti protease di dalam paru-paru tikus yang menerima rawatan dengan FL, PY dan FL+PY. Tikus yang tidak menerima sebarang rawatan bertindak sebagai kawalan, manakala yang dirawat menerima dos 11ng, 11.5ng dan kombinasi kedua-dua dos yang masing-masing diberi secara intratrakea. Kesemua tikus dibunuh pada 1, 8 dan 24 jam pasca-pemberian (p.i). Penaksiran apoptosis dijalankan secara histopatologi dengan haematoxylin-eosin, analisis pelabelan potongan hujung TUNEL dan tangga DNA pada sampel paru-paru. Bagi analisis protease pula, jumlah kandungan protein yang disukat



dengan menggunakan peralatan BSA, aktiviti elastase dan aktiviti elastase neutrofil telah dijalankan pada cecair paru-paru yang dikumpul dari tikus.

Apoptosis pada pelbagai peringkat telah ditemui pada pneumosit dan epithelium bronkiol sampel paru-paru bagi kesemua kumpulan tikus yang menerima PAH-rawatan. Penemuan histopatologi ini menunjukkan bahawa terdapat peningkatan sel apoptosis ($p < 0.05$) yang berkadar dengan masa, terutamanya pada 16 dan 24 jam p.i. Ini turut ditetapkan oleh analisis TUNEL dan tangga DNA.

Data yang dikumpul menunjukkan peningkatan berkadar langsung dengan masa dalam jumlah kandungan protein, aktiviti elastase dan aktiviti elastase neutrofil bagi sampel cecair paru-paru.

Kajian pendedahan kronik telah dijalankan seperti mana yang diterangkan dalam kajian akut tetapi selama tiga bulan. Walaubagaimanapun, penilaian turut dilakukan pada keberkesanan bawang putih sebagai agen terapeutik alternatif bagi melawan kesan FL dan PY. Tikus dari kumpulan rawatan, FL, PY dan FL+PY diberi makanan komersial manakala tikus dari kumpulan bawang putih (G), FL+G, PY+G dan FL+PY+G diberi makanan yang telah ditambah dengan serbuk bawang putih pada kadar 80mg/kg berat badan/tikus/hari.

Keputusan kajian menunjukkan perubahan morfologi dan gangguan tumbesaran pada pneumosit dan epithelium bronkiol pada kesemua kumpulan tikus yang menerima PAH-

rawatan. Kesemua kumpulan tikus yang menerima PAH-rawatan ini juga menunjukkan kesan teruk dalam aktiviti protease, aras IgG, IgA, aktiviti makrofaj alveolus dan glutathione-S-transferase pada paru-paru.

Sebaliknya, kesemua kumpulan tikus yang menerima PAH-rawatan disamping menerima makanan yang ditambah dengan serbuk bawang putih menunjukkan peningkatan nyata di dalam perubahan patologi, aktiviti protease, imunologi, dan aktiviti enzim pada paru-paru.

Kesimpulannya, pencemaran yang merbahaya oleh FL, PY dan gabungannya terhadap paru-paru tikus sebagai organ sasaran boleh menyebabkan kerosakan samada secara pendedahan akut mahupun kronik manakala bawang putih mempunyai potensi yang hebat untuk mengurangkan kesan kronik FL dan PY.

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I certify that an Examination Committee met on 18th November 2003 to conduct the final examination of Teh Rasyidah Binti Ismail on her Master of Science thesis entitled "The Efficacy of Garlic in Alleviating Fluoranthene and Pyrene Induced Pulmonary Injury in Rats" in accordance with 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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Date: 05/05/2004

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LIST OF ABBREVIATIONS

AGE	Aged garlic extract
AHH	Aryl hydrocarbon hydroxylase
ala	Alanine
AMØ	Alveolar macrophage
AO	Acridine orange
ARDS	Adult respiratory disease symptom
AST	Aspartate amino transferase
BALT	Bronchial alveolar lymphoid tissue
B(a)P	benzo (a) pyrene
BCA	Bicinchoninic acid
BHI	Brain heart infusion
bp	Base pair
BSA	Bovine serum albumin
BW	Body weight
CO ₂	Carbon dioxide
COPD	Chronic obstruction pulmonary disease
Cx	Control
CV	Crystal violet
DAB	3'3'diaminobenzidine-tetrahydrochloride
DAD	Diffuse alveolar damage
DCNB	1,2 dichloro-4nitrobenzene
DGP	Dehydrated garlic powder
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbant assay
EPA	Environmental Protection Agency
FL	fluoranthene
G	garlic
GST	glutathione S-transferase
Hb	Haemoglobin
HDL	High-density lipoprotein
H&E	Hematoxylin and Eosin
HGJ	Heated garlic juice
H ₂ O ₂	Hydrogen peroxide
hr	hour
IgA	immunoglobulin A
IgG	immunoglobulin G
IL	interleukin
KCl	potassium chloride
LD	lethal dose
LDL	Low-density lipoprotein
LOAEL	Lowest-observed-adverse-effect level
M	molar
NaCl	sodium chloride
NH ₄ Cl	Ammonium chloride



NK	Natural killer
N	Nitrogen
NOAEL	No-observed-adverse-effect level
NRS	Normal rat serum
OD	Optical density
p.i.	post-instillation
PAC	polycyclic aromatic compounds
PAH	polycyclic aromatic hydrocarbons
PBS	phosphate buffered solution
pvc	Packed cell volume
PM	particulate matter
pro	Proline
PY	pyrene
RBC	red blood cells
RGJ	Raw garlic juice
ROS	Reactive oxygen species
SD	standard deviation
SLAPN	succinyl, alanine, alanine- <i>p</i> -nitroanilide
SSC	Standard saline citrate
TNF- α	Tumor necrosis factor - alpha
TUNEL	terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling
UV	Ultra violet
val	Valine
WR	Working reagent



CHAPTER I

INTRODUCTION

Transboundary movement of atmospheric pollutants has international policy, economic, human health, and environmental ramifications. Atmospheric pollutants are of particular concern since air masses flow freely across borders, leaving the geographic and political jurisdiction of the originating country and becoming the responsibility of another. Additionally, the transport of air pollutants is a significant contributor to acid rain, poor visibility, climate change, and bioaccumulation of toxics in remote areas. For example, wind blown desert dust and forest fire smoke cross international borders and increase particulate matter concentrations to levels that may exceed regulatory standards and harm human health. Thus, air pollutant monitoring is an important issue for both human and environmental health on a global scale (Pope, 1996).

Particulate matter has impacts on human health by reducing lung function and is of particular concern to those with an existing compromised respiratory function (asthma, children, and the elderly). Generally, particulate matter is classified as less than 10 microns, which is considered inhalable, or fine particulate matter of less than 2.5 microns, which has the most impact on human health (Kim and Kang, 1997).

Health effects do not only depend on the particulate as such, but also on the composition of toxic compounds adsorbed on their surface. Among these compounds is polycyclic aromatic hydrocarbons (PAH) which are formed during combustion processes



of organic material with insufficient oxygen supply (Heil, 1998). The PAH comprise of more than 100 different multi-ringed compounds of which many are known to be carcinogenic (Heil, 1998).

Fluoranthene and pyrene, were among of the PAH collected before, during and after 1997 Malaysian haze episode. Fluoranthene and pyrene are on Environmental Protection Agency (EPA) priority pollutant list (Anon, 1987; Anon, 1990). Several investigators have demonstrated that both fluoranthene and pyrene have acute and chronic adverse effect on human health.

Fluoranthene can be absorbed through the skin following dermal exposure (Storer et al., 1984) and, by analogy to structurally-related PAHs, would be expected to be absorbed from the gastrointestinal tract and lungs (Anon, 1988). Acute toxicity data for animals include an oral LD₅₀ of 2000 mg/kg for rats; a dermal LD₅₀ of 3180 mg/kg for rabbits (Smyth *et al.*, 1962); and an intravenous LD₅₀ of 100 mg/kg for mice (Anon, 1993i). Sub-chronic oral exposure to fluoranthene at doses of greater than or equal to 250 mg/kg produced nephropathy, increased liver weights, and increased liver enzyme levels in rats (Anon, 1988). A single intraperitoneal injection of fluoranthene to pregnant rats caused an increased rate of embryo resorptions (Irvin and Martin, 1987). Furthermore, fluoranthene is a photosensitizing agent that enhances erythema elicited by ultraviolet radiation in guinea pig (Kochevar *et al.*, 1982) and was irritating to the eyes of rabbits (Grant, 1986). Fluoranthene has been shown to be active as a co-carcinogen when applied with benzo[a]pyrene to mice skin (Van Duuren and Goldschmidt, 1976) and was active as



a complete carcinogen in a short-term lung tumor assay with newborn mice (Busby *et al.*, 1984).

Sub-chronic oral exposure to pyrene produced nephropathy, decreased kidney weights, increased liver weights, and slight hematological changes in mice (Anon, 1989) and produced fatty livers in rats (White and White, 1979). A single intraperitoneal injection of pyrene produced swelling and congestion of the liver and increased serum aspartate amino transferase (AST) and bilirubin level in rats (Yoshikawa *et al.*, 1985).

Known scientifically as *Allium sativum*, garlic contains more than 100 biologically useful chemicals, including alliin, alliinase, allicin, S-allylcysteine, diallyl sulfide and allyl methyl trisulfide (Bree, 1994). The healthful properties of garlic are legion and have been identified and validated by hard empirical science in over a thousand scientific reports in the last decade. Areas of beneficial activity include anti-AIDS, anti-cancer and anti-cardiovascular disease and anti-infectious properties (Bree, 1994). Garlic is uniquely the richest dietary source of healthful sulphur compounds, plus organic selenium as well as being one of the best sources of organic germanium besides an impressive array of other essential nutrients and active health-promoting phytochemicals. Thus, this study is aimed at assessing the efficacy of garlic in alleviating fluoranthene and pyrene – induced injury.

The objectives of this study are:

- i. to determine the acute and chronic effects of fluoranthene and pyrene in rats.
- ii. to determine a sensitive and reliable markers of injuries induced by fluoranthene and pyrene.
- iii. to assess the pathological changes in the lungs of rats treated with fluoranthene and pyrene with or without garlic supplementation.