
UNIVERSITI PUTRA MALAYSIA

# THE EFFICACY OF GARLIC IN ALLEVIATING PHENANTHRENE AND CHRYSENE INDUCED PULMONARY INJURY IN RATS

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**By**

**WAN MAZLINA BT. MD. SAAD**

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

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## DEDICATION

**This thesis is dedicated with appreciation and love to my late mak (Mawanchik Pawan), late bahamad (Md. Saad Abdullah), Bapak, Mamanah, Inda, Ghoi, Mamanoin, Pak E, Ser, Batah, Bedah, Ain ul, Chek Sha and Kak Siti who are always there for me with full support and courage without which I will not finish this course.**

**"Thank You"**

**-Mali-**

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

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**Faculty : Veterinary Medicine**

Air pollution specifically referring to haze consists of numerous particles including polycyclic aromatic hydrocarbons (PAH). These particles consist of various compound including phenanthrene (Phen) and chrysene (Chry). Both of these are among hazardous compounds which are found during the haze periods back in 1997 in Malaysia. This situation had a great impact on health and also on the economic status of the country as well. In finding strategies and bringing about a remission of noxious haze effects in humans, acute and chronic exposure to Phen, Chry and their combination were studied in rats.

The acute exposure studies were conducted to evaluate apoptosis in the lung of rats following with Phen, Chry and Phen+Chry. Rats not receiving any treatment served as control while those administered with Phen, Chry and Phen+Chry were instilled at the dose of 14.87ng (7.4µl), 7.07ng (3.5 µl) and 21.94ng (10.9µl) respectively. Animals in control and treated group were euthanised at 1, 8 and 24 hours post instillation (p.i.).

Apoptosis detection was made on H&E stained paraffin-embedded sections, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling analysis (TUNEL) and DNA laddering of lung samples. Selected proteases were also examined from lung lavage of all rats.

Apoptosis were observed in pneumocytes and bronchial epithelium of rats euthanised at the specific time p.i and the percentage of apoptotic cells increased with time, especially in 24 hours p.i.which were highest for group especially the combination group ($p<0.05$). This was confirmed by TUNEL analysis and DNA laddering. As for proteases, there was also elevation in the content of total protein, number of neutrophil and level of elastase. This activity also showed increased with time as compared to normal.

The chronic exposure study was conducted on changes in the lung of Phen, Chry and Phen+Chry. These rats were kept for three months to assess histopathologic findings, immune response, level of marker enzyme, proteases and the effects of raw garlic as an anti-tumour factor.

Rats from the control group, Phen, Chry and Phen+Chry were daily fed with commercial rat pellet while rats from garlic (G), Phen+G, Chry+G and Phen+Chry+G were fed with rat pellet mixed with garlic at the rate of 80 mg/kg body-weight/rat/day.

From the observation rats from Phen, Chry and Phen+Chry group showed growth disturbances in pneumocytes and bronchial epitheliam including pulmonary mechanisms.

Morphological changes including hyperplasia and metaplasia were among the finding in treatment groups. Necrosis was also detected in these groups. Rats induced with Phen and Chry particles showed a low level of IgG and IgA and alveolar macrophage activity in the lung. Furthermore, rats treated with these particles showed an elevation in glutathione –S-transferase and proteases activity. Rats treated with garlic had no significant lesions and marked elevation in pulmonary defense mechanism.

Lastly, acute and chronic exposure to environmental pollutants such as Phen and Chry incites injury to the lung. Garlic has shown to be a great potential in alleviating chronic injury induced by Phen and Chry in the treated rats.

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

**KEBERKESANAN BAWANG PUTIH DALAM MENGATASI KEROSAKAN PULMONARI AKIBAT INDUS PHENANTHRENE DAN CHRYSENE PADA TIKUS**

**Oleh**

**WAN MAZLINA BT. MD. SAAD**

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Pencemaran udara atau lebih dikenali sebagai jerebu mengandungi pelbagai bahan merbahaya termasuk polisiklik aromatik hidrokarbon (PAH). PAH ini terdiri daripada pelbagai komponen termasuk phenanthrene (Phen) dan chrysene (Chry). Kedua-dua komponen ini adalah antara bahan pencemaran merbahaya yang terdapat dalam jerebu tahun 1997 di Malaysia. Jerebu 1997 bukan sahaja memberi kesan terhadap tahap kesihatan malah juga mempengaruhi keadaan ekonomi negara pada masa itu. Dalam mencari strategi dan kesannya terhadap manusia; dua pendedahan iaitu akut dan kronik dijalankan ke atas tikus untuk Phen, Chry dan gabungan kedua-duanya.

Kesan akut dijalankan untuk menilai bilangan apoptosis didalam peparu tikus yang diberi suntikan Phen, Chry dan Phen+Chry. Tikus yang tidak diberi sebarang suntikan dianggap sebagai kawalan manakala kumpulan-kumpulan tikus lain disuntik Phen sebanyak 14.87 ng (7.4µl), Chry 7.07 ng (3.5µl) dan 21.94 ng (10.9µl) untuk kumpulan Phen+Chry.

Tikus-tikus ini diberi pendedahan selama 1, 8 dan 24 jam. Apoptosis dapat dikesan pada peparu tikus yang diberi rawatan diatas melalui keratan histopatologi yang diwarna dengan H&E, TUNEL dan pengestrakan DNA. Kesan protease juga dikaji melalui basuhan bronkoalveolar dari kumpulan Phen, Chry dan Phen+Chry.

Apoptosis dapat dikesan pada tisu pneumosit dan epitelium bronkiol pada masa pendedahan tertentu. Kesan apoptosis meningkat bersama masa dan kesannya dapat dilihat dengan jelas pada pendedahan selama 24 jam untuk semua kumpulan ($p<0.05$). Keputusan ini dikukuhkan lagi melalui analisis TUNEL dan ekstrak DNA. Peningkatan secara keseluruhan untuk kumpulan Phen, Chry dan Phen+Chry juga dapat diperhatikan bagi kandungan protein keseluruhan, neutrofil dan elastase. Peningkatan ini adalah lebih tinggi berbanding kumpulan tikus kawalan.

Bagi pendedahan kronik (tiga bulan) pula, terdapat pelbagai perubahan pada peparu tikus yang disuntik. Tikus-tikus dipelihara selama tiga bulan untuk kajian histopatologi, gerakbalas sistem keimunan, tahap sasaran enzim, tahap protein dan juga kesan bawang putih sebagai faktor anti-tumor.

Tikus dari kumpulan kawalan, Phen, Chry dan Phen+Chry diberi makanan komersial manakala tikus dari kumpulan bawang putih (G), Phen+G, Chry+G dan Phen+Chry+G diberi makan yang dicampur dengan bawang putih pada kadar 80 mg/kg berat/tikus/hari.

Dari kajian yang dijalankan, dapat disimpulkan bahawa terdapat gangguan pertumbuhan pada sel pneumosit dan epitelium bronkial dimana gangguan mekanisme pulmonari juga turut terlibat. Perubahan dari segi morfologi seperti hiperplasia dan metaplasia adalah antara gangguan yang terdapat pada sel epitelium peparu tikus. Kesan ini juga dapat dilihat pada keratan histologi. Nekrosis juga kelihatan pada kumpulan Phen, Chry dan Phen+Chry.

Tikus-tikus yang disuntik dengan partikel Phen dan Chry menunjukkan aras IgG dan IgA dan aktiviti alveolar makrofaj yang paling rendah pada paru-paru. Walau bagaimanapun, tikus yang disuntik dengan partikel di atas menunjukkan peningkatan pada glutathione-S-transferas (GST) dan aktiviti protease. Kajian ini juga membuktikan bahawa tikus yang menjadikan bawang putih sebagai diet harian, dapat menghalang gangguan pada pertumbuhan sel dan meningkatkan lagi mekanisme pertahanan pulmonari.

Akhir sekali, pendedahan akut dan kronik terhadap bahan merbahaya dari persekitaran dapat memberi kesan kepada peparu. Bawang putih bagaimanapun, telah menunjukkan potensi dapat mengurangkan kesan gangguan kronik Phen, Chry dan Phen+Chry pada tikus-tikus.

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| | |
|---|---|
| AMØ | alveolar macrophage |
| AMT | allylmethyl trisulfide |
| AO | acridine orange |
| $NH_4CL$ | ammonium chloride |
| bp | base pair |
| BAL | bronchoalveolar lavage |
| B(a)P | benzo(a)pyrene |
| C | control |
| CAP | concentrated ambient particles |
| Chry | chrysene |
| $CO_2$ | carbon dioxide |
| COH | coefficient of haze |
| CV | crystal violet |
| DAS | diallyl sulfide |
| DCNB | 1, 2-dichlolo-4-nitrobenzene |
| DNA | deoxyribonucleic acid |
| EDTA | ethylenediaminetetraacetic acid |
| ELISA | enzyme-linked immunosorbent assay |
| EOM | extractable organic material |
| EPA | Environmental Protection Agency |
| G | garlic |

| | |
|---|---|
| GST | glutathione-s-transferase |
| $H_2O_2$ | hydrogen peroxide |
| H&E | haematoxylin and eosin |
| IgA | immunoglobulin A |
| IARC | International Atmosphere Research Centre |
| IgG | immunoglobulin G |
| IL-1 | interleukin -1 |
| IL-2 | interleukin-2 |
| i.p. | intraperitoneal |
| LAK cell | leukocytes activated K cells |
| NaCL | sodium chloride |
| NAS | National Academy of Sciences |
| N-SLAPN | N-methoxyl-succinyl-ala-ala-pro-val-p-nitroanilide |
| OD | optical density |
| PAH | polycyclic aromatic hydrocarbon |
| PBS | phosphate buffered saline |
| Phen | phenanthrene |
| p.i. | post-instillation |
| SD | standard deviation |
| SLAPN | succinyl,alanine,alanine-p-nitroanilide |
| SSC | standard saline citrate |
| TUNEL | terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling analysis |

| | |
|---|---|
| Th-1 | t helper 1 |
| TNF | tumor necrosis factor |
| TNF-α | tumor necrosis factor-alpha |
| UPM | Universiti Putra Malaysia |
| USA | United States of America |
| UV | ultra violet |
| WR | working reagent |

# CHAPTER I

# INTRODUCTION

Air pollution is described as impurities in the air due to various substances that endangers the environment or to health of living things. It usually occur due to massive build up of automobile exhaust, industrial and mine smoke. This pollutant may enter the human blood stream through the nose, mouth, skin and digestive tracts which can cause irritation to the eye, throat and lungs respectively. Epidemiology studies indicate that prolonged exposure to high pollution levels is associated with increased risk of cancer especially that of the lung.

The 1997 Indonesian forest fire was an environment disaster with exceptional proportions. Such a disaster may cause massive transboundary air pollution and discrimate destruction of biodiversity in the world. The immediate consequence of these fires was the production of large amounts of haze in the region, causing visibility and health problems within Southeast Asia including Malaysia.

In Kuala Lumpur, a two to three fold increased in the number of respiratory diseases were recorded during the haze (Brauer, 1997; Benedict, 1998). The haze consists mainly of fine particulate matter in the respirable range with predominantly, carcinogenic polycyclic aromatics hydrocarbon (PAHs).

The PAHs are ubiquitous environmental pollutants and include some of the most carcinogenic materials (Anon, 1983). These compounds are usually present in the environment such as components of complex mixtures in tobacco smoke, polluted air, gasoline and diesel engine exhaust and industrial waste from various manufacturing and chemical processes. The PAHs are the most prominent among the genotoxic and carcinogenic agents present in polluted urban air and have been the focus of attention of most air pollution-related biomarker field studies.

Many of these compounds are potent carcinogen (Santodonato *et al*., 1981) which must be activated metabolically before gaining the ability to attack DNA (Jerina *et al*., 1981). The PAHs exposed experimental animals shows that the level of PAH-DNA adducts correspond with the tumorigenic response in the lung.

Phenanthrene (Phen) and chrysene (Chry) are among the PAH found to exceed the normal level during the 1997 haze episode. Phenanthrene is ubiquitous in the environment as a product of incomplete combustion of biomass and has been identified in ambient air, surface and drinking water and in foods (Anon, 1983; Anon, 1988). In mammals; this PAH is rapidly metabolized by the liver and excreted primarily in the urine. It is also known to be a human skin photosensitiser and mild allergen (Sandmeyer, 1981).

Phenanthrene is absorbed following an oral and dermal exposure (Storer *et al*., 1984) and would be absorbed from the lungs (Anon, 1987). Current theories regarding

the mechanism of metabolic activation of PAHs predicts the carcinogenic potential of Phen.

Chrysene accumulates as a result of anthropogenic activities such as coal combustion and gasification, gasoline exhaust, diesel and air craft exhaust and emission from coke ovens, wood burning stoves and waste incineration (Anon, 1983, Anon, 1990). Human are exposed to Chry by oral, inhalation and dermal routes. Significant exposure to Chry also occurs through the inhalation of mainstream cigarette smoke (Anon, 1983).

Chrysene is preferentially distributed to highly lipophilic regions of the body, most notably adipose and mammary tissue (Bartosek *et al*., 1984). Information on the absorption of Chry in humans is not documented. However, the detection of PAHs, including Chry and its metabolites in the urine of individual who smoke, work in polluted industrial environment with Chry or used therapeutic coal-tar creams (Clonfero *et al*., 1986) provides indirect evidence of inhalation and dermal absorption. Mixture toxicity did appear to increase slightly when Chry, benzo (a) anthracene and anthracene were added to Phen, pyrene and fluoranthrene (Boxall and Maltby, 1997).

Thus, it is the aim of this study to assess the following acute and chronic effects of phenanthrene and chrysene with the following objectives, to determine:

i. the manner of cell death during acute exposure to Phen and Chry
ii. the development and type of chronic changes induced by Phen and Chry
iii. the sensitive and reliable indicator of chronic changes induced by Phen and Chry
iv. the effects of Phen and Chry on proteases and protease inhibitors of rat lung
v. the efficacy of garlic in alleviating Phen and Chry induced injury