UNIVERSITI PUTRA MALAYSIA

STUDIES ON THE EFFECT OF BENZO(a)PYRENE IN RATS

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By

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DEDICATION

To my wonderful family

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

STUDIES ON THE EFFECT OF BENZO(a)PYRENE IN RATS

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Chairman: Dr. Noordin Mohamed Mustapha

Faculty: Veterinary Medicine

Benzo(a)pyrene [B(a)P] is a carcinogen found in high concentrations during the Malaysian haze episodes. In view of developing strategies in alleviating deleterious effects of haze in humans, the effect of acute and chronic exposure to B(a)P was studied in rats.

In the acute exposure studies, an evaluation of apoptosis following treatment with B(a)P was assessed. The B(a)P treated rats received 24 ng (13µL) of B(a)P instilled intratracheally, while similar volumes of tricaprylin (Tr) was administered to rats from the Tr group. Rats not receiving any treatment served as controls. An assessment of apoptosis was made on haematoxylin and eosin

(H&E) stained histologic sections, terminal deoxynucleotidal transferase-mediated dUTP-biotin nick end labeling (TUNEL) analysis and DNA laddering of lung samples. Rats in the B(a)P group were killed at 1, 2, 4, 8, 16, and 24 hours post-instillation (p.i.) and rats from the control and Tr groups were only killed 24 hours p.i.

Apoptosis at different stages was found in the pneumocyte and bronchial epithelium of B(a)P-treated rats killed 8, 16 and 24 hours p.i. This was also confirmed positive by TUNEL analysis and DNA laddering.

In the chronic exposure studies, changes in the lung of B(a)P-induced rats during a three month period encompassing p53 expression, proliferating cell nuclear antigen (PCNA) expression, immune response (IgA, IgG levels and alveolar macrophage activity), levels of glutathion s-transferase (GST) marker enzyme and the effect of raw garlic as an anti-tumour agent were studied.

Rats from the control, B(a)P and Tr group were daily fed on a commercial basal diet while rats from the garlic (G) and [B(a)P+G] group were fed the basal ration containing garlic incorporated at the rate equivalent to an intake of 80mg/kg bodyweight/rat/day.

The results showed growth disturbances in pneumocytes and bronchial epithelium of rats from the B(a)P group. Apoptosis was detected in four rats from the B(a)P+G group. The PCNA positive areas were only found in hyperplastic

areas in the lungs of rats from the B(a)P-treated group. In addition, rats treated with B(a)P and B(a)P+G had lower levels of IgA, IgG, alveolar macrophages activities and glutathione S-transferase in the lung.

In conclusion, either short or long term exposures to B(a)P produce detrimental changes to lungs of rats and garlic has great potential in alleviating the chronic effects.

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

KAJIAN KEATAS KESAN BENZO(a) PIREN PADA TIKUS

Oleh

THORIA ALI ABDUL HAMEED DRAGH

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Pengerusi: Dr. Noordin Mohamed Mustapha

Fakulti: Perubatan Veterinar

Kepekatan bahan karsinogen, benzo(a)piren [B(a)P] yang tinggi telah dikesan pada episod jerebu di Malaysia. Kajian mengenai kesan akut dan kronik pendedahan kepada B(a)P telah dijalankan ke atas tikus bagi merancang strategi mengurang kesan merbahaya terhadap manusia.

Dalam kajian pendedahan akut, penilian terhadap apoptosis secara histologi, analisis pelabelan potongan hujung dUTP-biotin deoksinukleotid perantaraan transferase (TUNEL) dan tangga DNA pada lavaj bronkiol-alveolus. Tikus dari kumpulan B(a)P menerima sebanyak 24 ng (13µl) B(a)P yang diberi secara

intratrakea, manakala isipadu tricaprylin (Tr) yang sama telah diberi kepada tikus dari kumpulan Tr. Tikus yang tidak menerima apa-apa rawatan bertindak sebagai kawalan. Tikus dari kumpulan B(a)P telah dibunuh pada 1, 2, 4, 8, 16 dan 24 jam pasca-pemberian (p.i) manakala tikus daripada kumpulan lain hanya dibunuh selepas 24 p.i.

Apoptosis pada pelbagai peringkat telah dilihat pada pneumosit dan epitelium bronkiol tikus dari kumpulan B(a)P yang dibunuh selepas 8, 16, dan 24 jam p.i. Ujian TUNEL dan tangga DNA memberikan keputusan yang sama.

Pada kajian pendedahan kronik, perubahan pada paru-paru tikus dikaji 3 bulan selepas menerima rawatan dengan B(a)P merangkumi p53, antigen pemproliferatan nuklias sel (PCNA), gerakbalas imun, aras enzim petunjuk dan kesan bawang putih mentah sebagai anti-tumor.

Tikus dari kumpulan kawalan, B(a)P dan Tr diberi makan makanan komersial manakala tikus daripada kumpulan bawang (G) dan B(a)P+G menerima makanan dengan penambahan bawang putih mentah pada kadar 80 mg/kg berat/tikus/hari.

Keputusan kajian menunjukan ganguan tumbesaran pada pneumosit dan epitelium bronkiol pada tikus dari kumpulan B(a)P. Apoptosis telah dikesan pada empat ekor tikus dari kumpulan B(a)P+G. Tindakbalas positif PCNA hanya didapati

pada kawasan hiperplasia di paru-paru tikus dari kumpulan B(a)P sahaja. Tambahan lagi, paru-paru tikus yang diberi B(a)P dan B(a)P+G mempunyai aras IgA, IgG, aktiviti makrofaj alveolus dan glutation S-tranferase yang rendah.

Sebagai rumusan, pendedahan akut atau kronik B(a)P menjana perubahan membahayakan pada paru-paru tikus dan bawang putih mempunyai potensi besar untuk mengurangi kesan kronik B(a)P.

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This thesis submitted to the senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Veterinary Sciences.

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AFC	Antibody-forming cells
AmØ	Alveolar macrophage
ANF	α-naphthoflavn
B(a)P	Benzo(a)pyrene
BALT	Bronchial associated lymphoid tissue
bp	Base pair
B-PBS	Bovine serum-phosphate buffered solution
BPDE	Benzo(a)pyrene-7,8-diol-9,10-epoxide
Cx	Control
DAB	3'3'diaminobenzidine-tetrahydrochloride
DAS	Diallyl sulfide
DCNB	1,2 dichloro-4nitrobenzene
DNA	Deoxyribonuclic acid
DNT	Dermonecrotic toxin
ELISA	Enzyme linked immunosorbant assay
g	Grams
G	Garlic
GST	Glutathion S-transferase
H&E	Hematoxylin and eosin
hr	Hour
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
KCl	Potassium chloride
M	Molar
MNU	Methylnitrosurea
ng	Nano-gram
p.i	Post instillation
PAHs	Polycyclic aromatic hydrocarbons
PBS	Phosphate buffered solution
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PM	Particulate matter
RBC	Red blood cells
TNF-α	Tumor necrosis factor-alpha
TUNEL	Terminal deoxynucleotidal transferase-mediated DUTP-biotin nick end labeling
µg	Microgram
µl	Microliter

CHAPTER I

INTRODUCTION

Airborne pollutants produce a wide variety of harmful effects on humans, animals and plants. Chemicals are released due to coal combustion, gasification, emissions, biomass burning, coal and tobacco smoke that may interact with different metabolic systems in human tissues and cell and eventually cause cancer (Tokiwa *et al.*, 1998). Among these chemicals is a group of multi-ring hydrogen and carbon compounds known as polycyclic aromatic hydrocarbons (PAHs), which are commonly found in the emissions from burned plant and petroleum products. Deteriorating air quality due to an increase in PAH concentration will cause increasing adverse effects on human health. (Omland *et al.*, 1994; Hock *et al.*, 1997; Scarlett *et al.*, 1996) One of the most studied of the PAHs is benzo(a)pyrene [B(a)P], which is an ubiquitous environmental pollutant present in coal tar, cigarette smoke and biomass burning (Yang *et al.*, 1997).

In heavily polluted regions, it has been found that air pollution can cause congenital anomalies especially those who are exposed to high levels of PM_{10}, pollutant particles of less than 10 micrometers in diameters. The DNA damage by PAHs has been reported (Leadon *et al.*, 1995) to be due to adduction of that can lead to mutations and increase the probability of developing cancer (Nakanishi *et al.*, 1997).

Studies in animals have shown that dermal exposure to B(a)P and PAH can cause skin cancer. Effects from breathing or ingestion were also reported to draw a connection with other kinds of cancer. Animal studies have shown B(a)P to be teratogenic, embryotoxic, and mutagenic (Lu *et al*., 1986; Nesnow *et al*., 1998a). Other animal tests have shown that exposure to B(a)P may cause reproduction difficulty (Zenzes *et al*., 1999).

Although acute toxicity appears to be low for humans, sub-chronic and chronic toxic effects exist. Some PAHs such as B(a)P are known potent immunosuppressors (White and Holsapple, 1984; Thomas *et al*., 1987).

There is a growing concern about the health effects of air pollution in the world (Scarlett *et al*., 1996). Many epidemiological and experimental studies have been conducted to examine the effect of air pollution on health (Dockery and Pope, 1994; Pope *et al*., 1995; Scarlett *et al*., 1996).

In Malaysia there has been considerable concern about the effect of pollution arising from haze. In 1997, Malaysia and other Southeast Asia countries have been exposed to a severe haze episode. However, no studies have examined the effect of air pollution on lung function in human or animals.

The aim of this study was to determine the effect of haze on health by using rats as models for humans. Likewise, it is also aimed at evaluating

environmental pollution caused by haze and the relationship between the concentration of B(a)P during the haze episode and the outcome of lung injury.

The objectives of this study were:

i. to determine the manner of cell death during acute exposure to B(a)P

ii. to determine the development and type of tumour induced by B(a)P

iii. to determine the sensitive and reliable indicators of tumours induced by B(a)P

iv. to assess the efficacy of garlic in alleviating B(a)P induced injury

CHAPTER II

LITERATURE REVIEW

Air Pollution

Undoubtedly, over decades air pollution is a problem of growing national and international interest. Air pollution can be defined as any atmospheric condition when excessive substances are present above normal ambient levels to produce measurable effects on man, animals, vegetation or materials. Substances refer to any natural or man-made chemical elements or compounds capable of being airborne. These substances may exist in the gaseous, liquid or solid states in the atmosphere (Painter, 1974).

The earliest pollutants noted in the atmosphere were probably of natural origin. Man, plants, animals, and the act of nature would contribute to pollution (Ottar, 1987).

The origin of air pollution varies between smoke, fumes, ashes and gases from volcanoes and forest fires, sand and dust from wind storms in arid region, fog in humid, low-lying areas and natural trepan hazes from pine trees in mountainous regions (Ross, 1972).

Haze is an atmospheric phenomenon caused by the presence of tiny particulate suspended in air. These particulate, which are molecular in size, scatter and absorb sunlight resulting in diminished visibility giving the atmosphere a characteristic opalescent appearance (Hassan *et al*., 1998).

Particulate matter (PM) which is produced by the burning of wood, diesel and other fuels by agriculture activities and industrial processes is made up of ash, smoke, soot, dust, fibers and liquid droplets. Apart from impairing ambient visibility, the smaller PM can be inhaled and over an extended period of time can injure the respiratory system.

Particulate matter can be defined as any material that can remain in the atmosphere or gas stream at standard conditions in the solid or liquid states. The term PM_{10} and $PM_{2.5}$ are primary particulate referred to during the occurrence of an air pollution. The PM_{10} is a particle with an aerodynamic diameter less than or equal to 10μm and $PM_{2.5}$ has an aerodynamic diameter less than 2.5μm. These particles represent health hazard, enhance atmospheric chemical reactions, and environmental response as well as negatively affecting aesthetic appearances (Heil, 1998).

Despite a poorly understood mechanism, epidemiological studies have documented the association between fine particulate air pollution especially particles of the size of PM_{10} or less with the development of pulmonary diseases. Inhalable particles with a diameter over 10μm are predominantly deposited in the