STUDIES ON THE EFFECTS OF BENZ [a] ANTHRACENE AND BENZO [ghi] PERYLENE ON THE LUNG OF RATS

SANAZ MOVASSAGH

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STUDIES ON THE EFFECTS OF BENZ[a]ANTHRACENE AND BENZO[ghi]PERYLENE ON THE LUNG OF RATS

By

SANAZ MOVASSAGH

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

January 2003
SAY, O' MY LORD! ADVANCE ME IN KNOWLEDGE
Holy Quran (Surah Taha-144)

To my beloved spouse for all what he do and done for me

Thankfulness and Gratefulness
STUDIES ON THE EFFECTS OF BENZ[a]ANTHRACENE AND BENZO[ghi]PERYLENE ON THE LUNG OF RATS

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January 2003

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

Faculty: Veterinary Medicine

Air pollution or haze contains a variety of detrimental agents including benz[a]anthracene (BA) and benzo[ghi]perylene (B[ghi]P). These carcinogenic compounds which are found during the Malaysian haze episodes affected on health and economic status of nation. In view of developing strategies and bringing about a remission of noxious haze effects in humans, acute and chronic exposure to BA, B[ghi]P and their combination was studied in rats.

The acute exposure studies was conducted to evaluate apoptosis in the lung of rats following treatment with BA, B[ghi]P and BA+B[ghi]P. Rats not receiving any treatment served as control while those administered with BA, B[ghi]P and BA+B[ghi]P were instilled intratracheally at the dose of 8 ng (4 μl), 16 ng (8 μl) and 12 ng (6 μl) respectively. Animals in treated groups were euthanised at 1, 4, 8, 16 and 24 hours post-instillation (p.i.) and rats from the control group were only killed 24 hours p.i. Apoptosis assessment was made on haematoxylin-eosin stained histologic sections, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) analysis and DNA laddering of trachea and lung samples.
Different stages of apoptosis were found in the pneumocyte and bronchial epithelium of all treated rats euthanised at the respective time post-instillation and the percentage of apoptotic cells increased with advancement of time, especially in 16 and 24 hours p.i. which was the highest for each group (P<0.05). This was also confirmed by TUNEL analysis and DNA laddering.

The chronic exposure studies was conducted on changes in the lung of BA, B[ghi]P and BA+B[ghi]P-induced rats during a three month period encompassing gross and histopathologic findings, immune response, levels of marker enzyme and the effect of raw garlic as an anti-tumour factor.

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

The results showed growth disturbances in pneumocytes and bronchial epithelium of rats from the BA, B[ghi]P and BA+B[ghi]P groups. Apoptosis was also detected in these groups. In addition, rats treated with BA+B[ghi]P had the lowest levels of IgA, IgG, alveolar macrophages activities and glutathione S-transferase in the lung.

In conclusion, environmental hazard of BA, B[ghi]P and their combination to lungs of rats as target organs causes deleterious changes either by short or long term exposures and garlic has a great potential in alleviating the chronic effects.

Apoptosis pada pelbagai peringkat telah dijumpai pada pneumosit dan epitelium bronkiol tikus yang dibunuh pada p.i. yang berasingan, di mana peratusan sel apoptosis meningkat sekadar dengan masa, terutamanya pada 16 dan 24 jam p.i. yang mencatat paling tinggi dalam setiap kumpulan (P<0.05). Ini turut dikenalpasti positif dengan TUNEL dan tangga DNA.


Kesimpulannya, pencemaran yang merbahaya dari BA, B[ghi]P dan gabungannya terhadap paru-paru tikus sebagai organ sasar boleh menyebabkan
perubahan, mahupun pada jangka masa pendek atau panjang dan bawang putih mentah mempunyai potensi yang besar untuk mengurangkan kesan kronik BA dan B[ghi]P.
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Finally my thanks to any one who has helped in one way or another toward this degree. Thank Allah for giving me the patience and guidance with wisdom and strength for completing my study.
I certify that an Examination Committee met on 31st January 2003 to conduct the final examination of Sanaz Movassagh on her Master of Science thesis entitled "Studies on the Effects of Benz[a]Anthracene and Benzo[ghi]Perylene on the Lung of Rats" 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.

SANAZ MOVASSAGH

Date: 6.2.2003
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<tr>
<td>AGE</td>
<td>Aged garlic extract</td>
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<tr>
<td>AHH</td>
<td>Aryl hydrocarbon hydroxylase</td>
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<td>AhR</td>
<td>Aryl hydrocarbon receptor</td>
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<tr>
<td>AMØ</td>
<td>Alveolar macrophage</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>BA</td>
<td>Benz[a]anthracene</td>
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<td>BALT</td>
<td>Bronchial associated lymphoid tissue</td>
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<td>B[ghi]P</td>
<td>Benzo[g,h,i]perylene</td>
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<td>CV</td>
<td>Crystal violet</td>
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<td>CYPs</td>
<td>Cytochrome P-450 isoforms</td>
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<tr>
<td>CYP1A1</td>
<td>Cytochrome P-450 1A1</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Cytochrome P-450 2E1</td>
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<tr>
<td>DAS</td>
<td>Diallyl sulfide</td>
</tr>
<tr>
<td>DASO</td>
<td>Diallyl sulfoxide</td>
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<tr>
<td>DASO(2)</td>
<td>Diallyl sulfone</td>
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<td>DADS</td>
<td>Diallyl disulfide</td>
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<td>DMBA</td>
<td>7,12-dimethylbenz(a)anthracene</td>
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<tr>
<td>DNMRT</td>
<td>Duncan’s New Multiple Range Test</td>
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<td>G</td>
<td>Garlic</td>
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<td>GGMSC</td>
<td>Gamma-glutamyl-Se-methylselenocysteine</td>
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<tr>
<td>H&amp;E</td>
<td>Haematoxylin-eosin</td>
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<td>hr</td>
<td>Hour</td>
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<td>LC</td>
<td>Langerhans’cells</td>
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<td>MBAs</td>
<td>Monomethylbenz(a)anthracenes</td>
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<tr>
<td>MDR</td>
<td>Multidrug resistance</td>
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<tr>
<td>MSC</td>
<td>Methylselenocysteine</td>
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<td>OD</td>
<td>Optic density</td>
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<td>PAHs</td>
<td>Polycyclic aromatic hydrocarbons</td>
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<td>PBS</td>
<td>Phosphate buffered saline</td>
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<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<td>PDT</td>
<td>Photodynamic therapy</td>
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<td>p.i.</td>
<td>Post instillation</td>
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<td>PM</td>
<td>Particulate matter</td>
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<td>RBC</td>
<td>Red blood cell</td>
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<td>SAC</td>
<td>S-allyl cysteine</td>
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<tr>
<td>SE</td>
<td>Standard Error</td>
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<td>TdT</td>
<td>Terminal deoxynucleotidyl transferase</td>
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<td>Tr.</td>
<td>Tricaprylin</td>
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<td>TUNEL</td>
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CHAPTER I
INTRODUCTION

Air pollution, which is not a recent phenomenon, provides a wide variety of injurious contaminant chemicals for humans, animals and plants. Suspended particles dispersed through a portion of the atmosphere can cause haze. Combustion products, organic compounds, metals, acid aerosols and cigarette smoke as airborne particulate can interfere with the normal defense processes and metabolic systems of the human body to enhance the incidence of cancer (Gilmour et al., 2001). Among the harmful chemicals released to environment is a class of very stable organic molecules made up of only carbon and hydrogen known as polycyclic aromatic hydrocarbons (PAHs), commonly found in the emissions from domestic, agricultural and industrial settings (Vaessen et al., 1988; Holman, 1999). Exposure to different levels of PAHs is connected with deleterious short and long-term effects on human health (Kramers and Van Der Hijden, 1990). One of the most carcinogenic PAHs is benz[a]anthracene (BA), which is a major component of environmental pollutant present in smoke and flue gases, coal tar, petroleum and organic material burning (Sittig, 1985). Benzo[g,h,i]perylene (B[ghi]P) is also among the carcinogenic PAHs found during natural fires and present in edible oils, cigarette smoke and coal tar (IARC, 1983).

It has been reported that a significant increase in particulate matter concentration produced in polluted regions, can lead to variation in metabolism genes (Lan et al., 2000). High levels of (PAH)-DNA adduct is an indicator for occurrence of lung cancer associated with the rate of mortality caused by indoor coal-burning (Xu et al., 1997).
Exposure studies to BA in animals have yielded skin cancer (Rice et al., 1988) besides embryotoxicity (Christou et al., 1990). Also studies in animals have shown B[ghi]P simultaneously increases incidence of skin tumours when administered with Benzo[a]pyrene (Van Duuren et al., 1973). Effects from BA on lung have shown other kinds of tumours (Klein, 1963), as well as from B[ghi]P (Deutsch-Wenzel et al., 1983). Furthermore, it have proved that B[ghi]P and BA to be mutagenic (Hughes and Phillips, 1993; Whong et al., 1994).

Although data relating chronic or sub-chronic toxicity resulting from exposure of humans to BA is identified, no relevant reports of human effects exposure to B[ghi]P is known. Likewise, direct evidence of acute toxicity for humans in these cases is not available (RAIS, 1997a; 1997b). Some PAHs such as BA are also immuno-suppressors (Saas et al., 1996).

The effect of air pollution on health is a developing issue in the whole world (Anderson, 1999). There are many epidemiological and experimental studies about the health effects of air pollution (Ostro et al., 1999; Rosales-Castillo et al., 2001; Hwang and Chan, 2002).

Regarding to the haze episodes in Malaysia, especially dense one occurred in 1997, there has been considerable interest about pollutant effects arising from it. However, the effect of most of air pollutants on lung function in human or animals is lack of study despite their carcinogenic potentials.
The aim of this study was to determine the effect of haze in humans using rats as models. It is also aimed at evaluating environmental pollution caused by haze and the relationship between the BA and B[ghi]P on the respiratory system.

It is hypothesized that BA and B[ghi]P exert deleterious effects on the respiratory system and this can be alleviated by oral supplementation of garlic. Hence, this study is conducted to assess the effect of BA and B[ghi]P administration on rats based on the following objectives:

1. to determine the manner of cell death during acute exposure to BA and B[ghi]P in lung tissue
2. to detect the presence of lung tumours during chronic exposure
3. to determine the sensitive and reliable indicators of tumours induced by BA and B[ghi]P
4. to assess the efficacy of garlic in alleviating BA and B[ghi]P induced injury
CHAPTER II
REVIEW OF LITERATURE

Air Pollution

The impact of air pollution to human health and economic status of nation is one of the important issues all over the world. Air pollution can be defined as any atmospheric condition that consists of a mixture of numerous compounds emitted by many distributed sources and present above normal ambient levels to produce measurable effects on man, animal, vegetation or materials. The impacts of air pollution encompass animal, public and ecosystem health. Air pollution, both indoors and outdoors, is a significant cause of health problems (Painter, 1974).

The pollution source can be referred to biological, chemical or economical by-products, natural or anthropogenic, vary between biomass burning, volcanoes, wildfires, fuel combustion, smoke, dust and radioactive gases (Elsom, 1992).

Air pollution can be transported hundreds of miles downwind from its origin. Since it cannot recognize political boundaries, states and communities cannot independently solve the pollution problems (OAQPS, 2000).

Haze is a phenomenon defined as tiny particulate suspended in air, originating from large-scale forest and land fires, which reduces visibility due to the scattering of light caused by aerosols. Based upon the experiences, the highest levels of haze are in
winter and lowest in summer (Heil, 2000). It is caused in large part by man-made air pollutants (ARB’s, 2000).

Particulate matter (PM) consist of carbon and mineral bodies of different sizes, produced by the burning of wood, fuels and industrial process, floating in the air and exist as gases, liquid drops or solid particles. They are small enough to be inhaled into the deepest parts of the lung and often grow in size as humidity increases (Heil, 2000).

Ambient air particulate matters are classified into two distinct modes in distribution, namely the coarse and fine particles (Hsiao et al., 2000). While breathing, particles are retained according to their size within the respiratory system, from upper respiratory tract to deeper, which impact on the human health especially elderly people and young children. The smaller are the particles, the more surface they offer for other pollutants to adsorb on explaining why smaller particles generally contain a higher amount of harmful compounds (Heil, 2000).

The PM$_{10}$ is a particle with diameter less than or equal to 10µm and PM$_{2.5}$ has diameter less than 2.5µm. These particles are primary particulate of air pollution that threatens both health and environment (Heil, 1998).

Epidemiological studies have demonstrated an association between different levels of air pollution and various health outcomes including mortality, exacerbation of asthma, chronic bronchitis, respiratory tract infections, ischaemic heart disease and stroke. Air pollution increases the risk of chronic diseases (Kunzli et al., 2001). Acute
Effects of air pollution exposure include irritation of the nose and eyes, lung function changes, respiratory changes, headache, fatigue and nausea and chronic exposures are associated with cough, sputum production and lung function decrements (Sydbom et al., 2001).

Polycyclic aromatic hydrocarbons (PAHs) are a group of large organic molecules found as mixtures formed as a result of incomplete combustion. The physical and chemical properties of PAHs are not all alike, nor are their health effects. A common mechanism of transport is by spontaneous desorption and transfer through the aqueous phase. Plant et al. (1983) showed that molecular volume of PAHs is a rate-determining factor for transfer. The PAHs interact with the aryl hydrocarbon receptor (Ahr) to cause reproductive defects (Matzuk, 2001).

Many PAHs have been identified as cancer-inducing chemicals in animals or humans (Karahalil et al., 1999). One pollutant with low carcinogenic effect can increase the effect of another one (Van Duuren et al., 1973; Van Duuren and Goldschmidt, 1976).

The primary source of emitted PAHs is incomplete combustion of wood or fuel. Sources include motor vehicles, non-road vehicles and equipment, residential wood combustion, industrial and commercial combustion, coke ovens, cigarette smoke, primary aluminum production, petroleum catalytic cracking, and charcoal broiled foods. The PAHs accumulate in terrestrial and aquatic life but can be metabolised by many plants and animals (EPA, 2000). Effects of PAHs reported in animal studies include