SEARCH FOR AFLATOXINS IN BRONCHIAL BIOPSIES FROM BRONCHIAL TUMOURS USING "ELISA" TECHNIQUES

THESIS

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BY
MAHA ALI MAHER
M.B., B.Ch.

Supervised By

Prof. Dr. **HASSAN HOSNY YOUSSEF**
Professor of Chest Diseases
Ain Shams University

52200

Prof. Dr. **TAREK SAFWAT**
Professor of Chest Diseases
Ain Shams University

Prof. Dr. **MAGDY MOHEB EL-DIN SAAD**
Prof. of Mycotoxins
National Research Centre

FACULTY OF MEDICINE
AIN SHAMS UNIVERSITY

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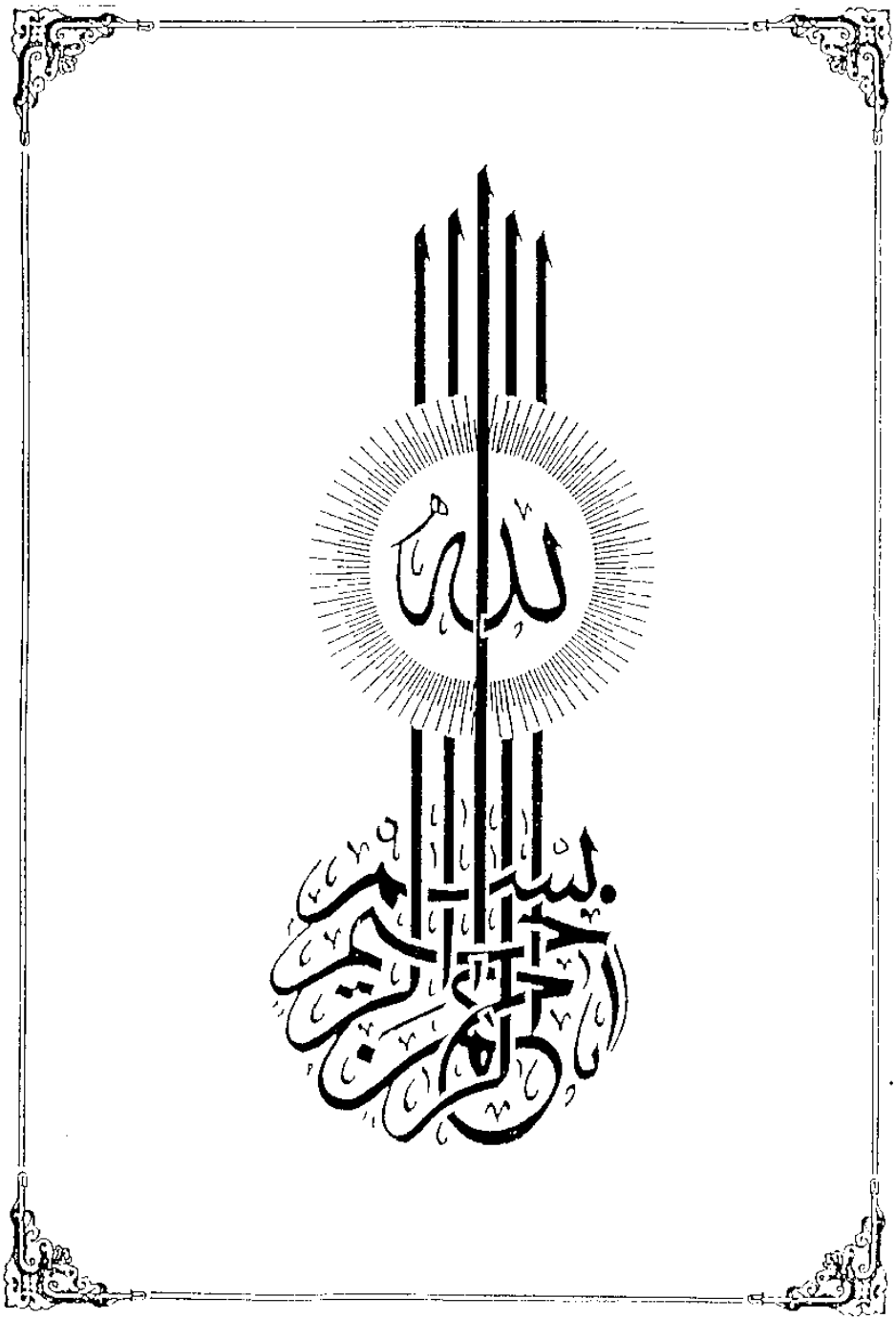

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C O N T E N T S

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LIST OF ABBREVIATIONS

A. Flavus or Asp. Flavus: Aspergillus Flavus.

A. Parasiticus : Aspergillus parasiticus.

A. Oryzae : Aspergillus oryzae.

A. Tamaraii : Aspergillus tamaraii.

AOAC : Association of official Analytical Chemists.

CaO : Calcium oxide.

DEN : Diethylnitrosamine.

ELISA : Enzyme linked immunoassay.

HCC : Hepatocellular carcinoma.

HPLC : High performance liquid chromatography.

IARC : International Agency for Research on Cancer.

ppb : part per billion.

ppm : part per million.

TAT : Tyrosine aminotransferase.

TLC : Thin Layer Chromatography.

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Introduction and AIM OF THE WORK

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INTRODUCTION AND AIM OF WORK

Aflatoxins are defined as a group of mycotoxins produced by some strains of the mould Aspergillus flavus, a fungus that grows on many foodstuffs kept under hot, humid conditions. These are compounds of related structures. Aflatoxin B_1 is the most potent form of them. Humans are exposed to aflatoxins either from consumption of commodities that are contaminated with mycotoxins or by consumption of products of animals that have ingested mycotoxin contaminated feed.

Aflatoxins are shown to be potent animal carcinogens and, therefore, suspect as a cause of cancer in man. Epidemiological studies have shown a positive correlation between the average dietary concentrations of aflatoxins in populations and the incidence of primary liver cancer. (Linsell,1980) ,Lungs have received little,if any attention as regards the effects of aflatoxins on them.

The **aim** of this work is to search for aflatoxin B_1, in bronchial biopsies from patients suspected to have lung cancer, to find the relationship between lung cancer and aflatoxins. The method used for detection and quantitation of aflatoxin B_1 in this work is the ELISA techniques. Regarding our best of knowledge, it is the first time to apply such technique in biological research.

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Review of Literature

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AFLATOXINS

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HISTORICAL ASPECTS OF MYCOTOXINS

The historical record of human and animal mycotoxins is a long one. The best known example is that of ergotism which occurred in the 11th and 18th century in France and Middle Europe (Food and Agriculture Organisation FAO, Food Control Series, No. 4).

Sargeant et al. (1961) was the first who observed that large numbers of turkey poults and ducklings in British farms had died as a result of consuming contaminated groundnut meals imported from Brazil. The lethal agents was initially isolated from groundnut meals and suspected to be produced by the common mould Aspergillus flavus and that toxic metabolite caused hepatic changes among poultry and other farm animals. **Allcroft and Carnaghan (1963)** revealed that Aspergillus flavus produced a toxic factor "aflatoxin" not only responsible for hepatotoxicity in animals but also was carcinogenic to poultry continuously fed on contaminated meals. The production of aflatoxin varied according to the strain of A. flavus and the environmental conditions affecting the mould growth.

The first report of carcinogenicty of aflatoxin

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came from England which concluded: "After six months feeding of 20 percent (toxic) Brazilian groundnut meal in a purified diet, nine out of eleven rats developed multiple liver tumours, and two of these had lung metastases **(Lancaster et al., 1961).**

The chemical structure of the aetiological factor causing "turkey X disease" was determined in 1963 by **Asao et al.**

Isolation and characterization of four closely related toxins were first reported by **Hartely et al. (1963).** They separated the four compounds on silica gel chromatoplates using chloroform-methanol (98:2 v/v) as developing solvent. These compounds designated aflatoxins B_1, B_2, G_1 and G_2 because of their blue and green fluorescent under ultraviolet waves. These four compounds were found to be toxic in varying degrees to ducklings **(De Long et al., 1964).**

Aflatoxins, Production:

Many investigators reported the occurrence and formation of mycotoxins on natural substrate and production of carcinogenesis from the fungal metabolites of Aspergillus flavus **(Wogan, 1965; Baker et al., 1966 and Habish et al., 1972).** The data collected from 6 countries showed

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that of a total of 1390 isolates of the Aspergillus flavus group, 803 or approximately 60% produced some aflatoxins **(Diener and Davis, 1968).**

Isolates generally classed as Aspergillus parasiticus are among the most active aflatoxins producing fungi. Certainly the members of the A. flavus group have the greatest capacity for aflatoxins production **(Conder et al., 1963; McDonald and Harkness, 1964 and Rao et al., 1965).**

Factors influencing aflatoxins production:

Extensive researches were conducted to investigate the factors which affect the growth of fungi producing aflatoxins and production of aflatoxins. It was found that these factors are as follow:

1. The fungus:

Aspergillus flavus is a group of strains **(Raper and Fennell, 1965)** includes A. flavus, A. parasiticus, A. oryzae and A. tamarii. The first two fungi produced aflatoxins. While the last two did not **(Austwick and Ayerst, 1963; Hesseltine et al., 1966 and Parrish et al., 1966).** Where as nearly all strains of Aspergillus parasiticus are toxigenic, the production of aflatoxins by A. flavus

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vary considerably from one strain to another. In both species it is also a function of the environmental condition **(Jarvis, 1971).**

2. The substrate:

Numerous natural substrates have been used to produce aflatoxins in large quantities in the laboratory. The quantity of aflatoxins produced by A. flavus under similar environmental conditions differed according to substrate **(Diener et al., 1965).** It was suggested that substrates rich in carbohydrates supported large yields of aflatoxins than those of oil seeds **(Clinton, 1960 and Diener and Davis, 1968).**

Wildman et al. (1967) also reported that apple, apricot, grape, grapefruits, mixed vegetable juice drinks supported production of aflatoxins.

Generally, Aspergillus flavus had produced aflatoxins on numerous foods including eggs, cheese, condensed and powdered milk, vegetables and fruits **(Stubblefield and Shannon, 1974).**

Martinez et al. (1988) studied the presence of toxigenic Asp. flavus in 144 samples of 33 varieties of

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different spices on sale in the Spanish market. They found that 35 strains of Asp. flavus group were isolated of which 8, turned out to be aflatoxigenic. The aflatoxins B_1 and B_2 were synthesized by toxigenic strains whilst G_1 and G_2 were only produced in the strains isolated in peppers.

Montani et al. (1988) studied the production of aflatoxins B_1, B_2, G_1 and G_2 in corn by Asp. flavus at 30°C and at three water activities. Aflatoxins accumulation was determined at selected times by thin layer chromatography.

The first report of the natural occurrence of aflatoxins in lentils was done by **El-Maraghy & Mohamed(1988)**who studied the fungal flora of 20 samples of lentil seeds collected from Assiut governorate, Egypt. Thin layer chromatographic analysis and a biological test indicated the presence and the toxicity of aflatoxin in the extract of one sample (Aflatoxin B_1, B_2, G_1 and G_2 at 20 mg/Kg, total).

Roy and Chourasia (1989) studied the aflatoxin production in samples of the medicinal plant, Mucuna pruriens seeds. Under the most favourable conditions under test they obtained 1.75 µg/gm of aflatoxin B_1 in the samples.

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