COMPARATIVE LH RECEPTOR RESPONSES TO AFLATOXIN B1 USING RAT INTERSTITIAL CELL TESTOSTERONE ASSAY

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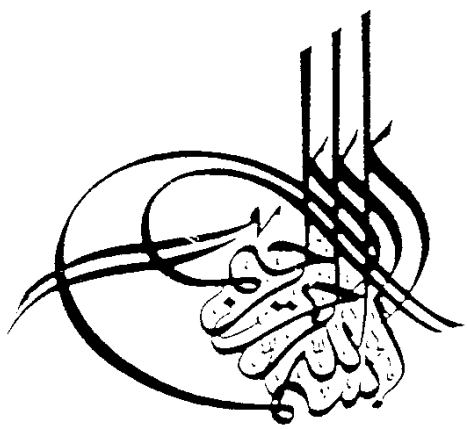

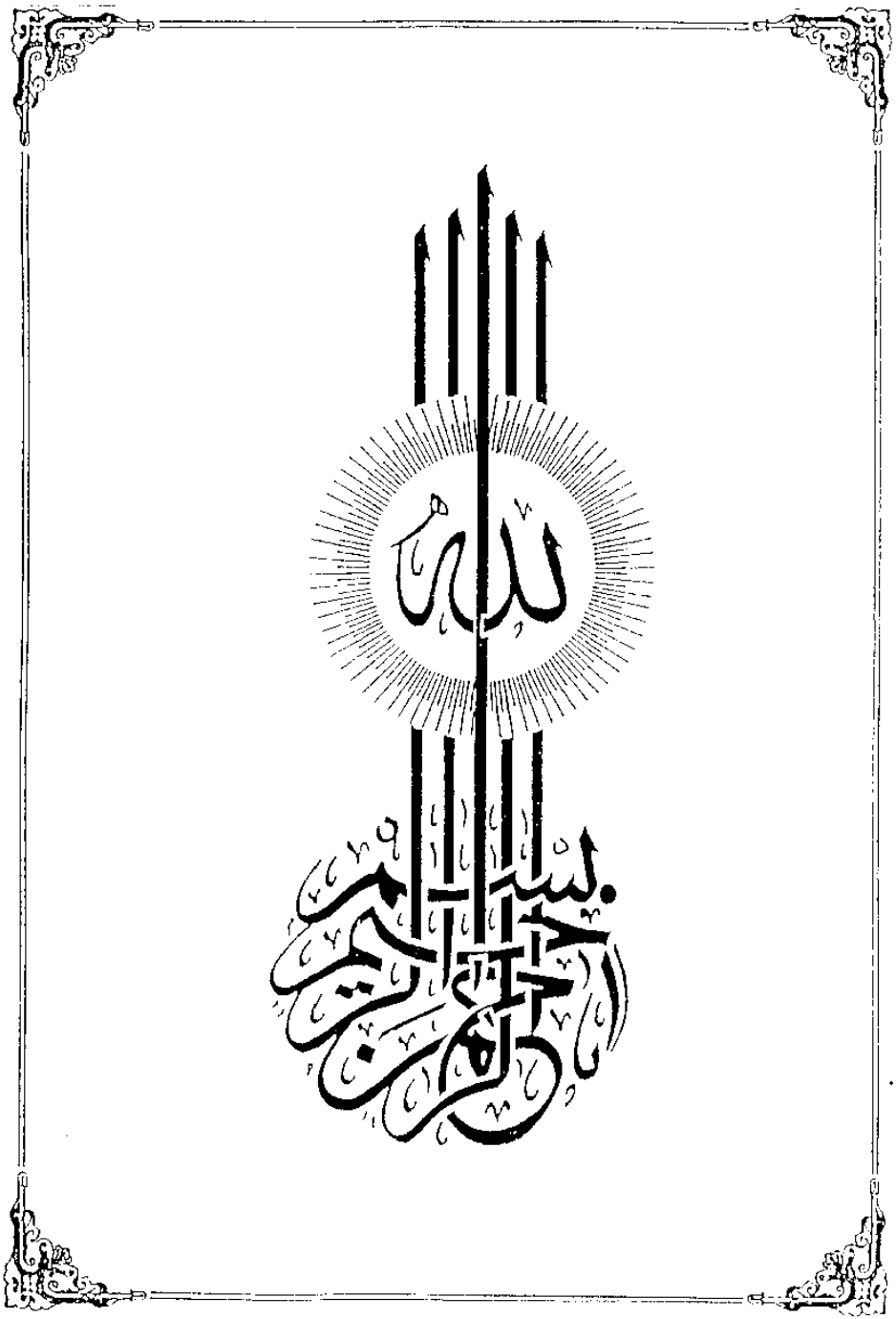

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INTRODUCTION

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Public concern about food contamination in general and especially human foodstuff, have been strongly revived during 1994 and early 1995 in Egypt. Moreover, premonitory symptom was called by Egyptian medical corps, denoting the widespread of hepatic diseases all over the country. The campaign was criticized as being exaggerated. Nevertheless, increasing numbers of hepatocellular carcinoma cases, suggests the seriousness of this health problem. Meanwhile, it is documented that hepatocellularcarcinoma is endemic in tropical areas in Africa and Asia (WHO, 1983).

Field survey of normal individuals in Egypt revealed a positive serum aflatoxicosis (Nasser *et al.*, 1990). Aflatoxin B1 serum levels were higher in patients with hepatocellular carcinoma and liver cirrhosis in the same study. On the other hand some data gathered during the past decades assures the existence of aflatoxin in almost all food and agricultural products being used as human and /or animal nutrients in Egypt.

Food products of wide range were found contaminated with aflatoxin B1 including corn (Magdi and Abou-Salem, 1988a), meat products (Magdi and Abou-Salem, 1988b ; Magdi 1993), chickpea (Premlata and Khalid, 1989), smoked herring (Hammad and El-Baz, 1988), grains of Egyptian maize cultivation (Amra and Ahmed, 1991), spices for human consumption (Baghdadi and Eltawila, 1990). Levels of aflatoxin B1 contamination ranged between 140 to 2400 µg/kg (Magdi and Abou-Salem, 1988a). The LD_{50} for Aflatoxin B1 in rabbits

is 300 µg/kg body weight (Newberne and Butler, 1969). Therefore, it seems certain that under Egyptian environment heavy aflatoxin B1 contamination represents a constant hazard inevitably introduced into human and animal body systems, *via* foodstuff.

Most of the published work on the effect of aflatoxin B1, centered on carcinogenic effects of acute doses. Establishment of lowest permissible aflatoxin B1 contamination levels, entailed investigating its carcinogenic and mutagenic effects.

Aflatoxin B1 pollution

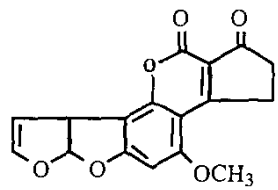
Aflatoxin B1 pollution was detected in England firstly (Wogan, 1966). The aflatoxin B1 was found in a wide variety of products for human and animal consumption. It has been concluded that infestation with aflatoxin B1 producing fungi of field crops is apt to occur during the post harvest period (UNEP and WHO , 1979). Maise, wheat, sorghum, oats and rice represent major field crops for human and animal consumption which were found to be contaminated with Aflatoxin B1 producing fungi (Shotwell *et al.*, 1969a, 1969b and 1970; Shank *et al.*, 1972). It was also found in peanuts, tree nuts, copra (and copra meal) and cotton seeds (Stoloff, 1976). Animals being raised for meat, milk and egg production were found to acquire aflatoxin B1 metabolites after feeding on aflatoxin B1 contaminated diets (Strzelecki and Gasiorowska, 1974; Rodricks and stoloff, 1977; Kiermeier, 1977).

The aflatoxin B1 contamination is known to be intensified in regions of the world, where high environmental temperature and humidity prevails most of the year. Eventually, temperate zones of the world are less developed. Some African and South East Asian countries (Busby and Wogan, 1984), represent a traditionally recognized sites of heavy fungal contamination of agricultural grain crops. In these areas, aflatoxin B1, constitutes a regular contaminant of human and animal food supply. Human liver cancer incidence in these countries was directly linked to pollution with aflatoxin B1 in food products (Van Rensburg *et al.*, 1985 ; Yeh *et al.*, 1989), also aflatoxicosis was observed in other parts of the world such as New Zeland (Becroft and Webster, 1972) Czechoslovakia (Dvorackova *et al.*, 1974) and United states (Chaves-Carballo *et al.*, 1976).

Two fungal strains were found to be of much importance due to their extensive occurrence in agricultural products, generally used in human and animal nutrition. These strains are *Aspergillus flavus* (Link) and *Aspergillus parasiticus* (Speare) (Goldblatt, 1969). Both fungi are capable of producing aflatoxins. The optimum temperature for aflatoxins production is 27°C with a 18.3% moisture content (Davis and Diener, 1970).

There have been 17 compounds designated as aflatoxins which were isolated and characterized. The term aflatoxins, however was commonly coherent with only 4 compounds (B1, B2, G1 and G2). The nomenclature was given on the basis of fluorescent colour, where B stand for blue and G for green, while the subscripts to relative migration on chromatographic separation. Structure of aflatoxin B1

was disclosed (Asoa *et al*, 1963 and 1965), with a molecular weight 321, as follows,



Aflatoxin B1

Biodistribution of aflatoxin B1 in Rats

The main route of aflatoxin B1 intoxication in mammals is *via* contaminated food. The wide variety of foodstuff liable for growth of AFB1 producing fungi makes it almost for every individual of mammalian species to encounter more or less degree of aflatoxin B1 intoxication.

On the other hand, several experimental designs were carried out to investigate the effect of aflatoxin B1 on mammalian physiological function. Intoxication have to be made *via* intubation or intraperitoneal injection in such experiments.

Extensive studies during the past decades have shown that aflatoxin B1 is the most toxic compound and represents the dominating mycotoxin produced by respective fungi. Therefore most investigations related to biodistribution and metabolism has centered on aflatoxin B1.

In white rats; intraperitoneal injection of a single ^{14}C-AFB1 dose of 0.07 mg/kg. body weight was performed to trace biodistribution of the labelled toxin. Retention of 20% of the radioactivity was noticed 24 hours after injection. The highest AFB1 concentration was found in liver which contained 5 to 8% radioactivity, which is equivalent to the remainder of the whole carcass (Wogan *et al.*, 1967).

In human studies, diagnosis of AFB1 intoxication was confirmed by occurrence of 520 µg/kg body weight in liver biopsy. Subjects with AFB1 intoxication were found to have carcinoma in liver and rectum (Phillips *et al.*, 1976).

In humans the main organ subject to AFB1 retention is unequivocally the liver. Moreover the exposure of various parts of the intestinal tract to the mycotoxins was attributed to excretion in bile. Occurrence of AFB1 metabolites in mother's breast milk was confirmed with resultant Juvenile cirrhosis (Robinson, 1967; Yadgri *et al.*, 1970).

In other animals, used as source of meat and milk for human nutrition, distribution in other regions of the animal body is an important issue. In poultry fed with rations contaminated with AFB1, was found in its liver and muscle tissues (Mintzlaff *et al.*, 1974). When feed contaminated with AFB1 was offered to cattle, their milk contained aflatoxin M1 (Allcroft *et al.*, 1968), which is less toxic than AFB1, which bears the same mycotoxic effects.

Metabolic biotransformation of AFB1

Metabolism of AFB1 occurs mainly in liver cells. The endoplasmic reticulum is the site of metabolic action (Fig. I), where upon various compounds produced (UNEP and WHO, 1979). AFB1 was found to be the most potent of aflatoxins followed by G1, B2, G2 and P1 in order of effectiveness. The extra oxygen in G group apparently, decreased its activity relative to the B group. On the other hand saturation of the double bond results in decreasing toxic potency by around 1/4 (Wogan, 1966). Demethylation represents another route of biotransformation, leading to AFP1 which is less toxic than AFB1 (Hsieh and Wong 1981). Comparative studies of AFB1 and AFP1 lethal potency in newborn mice were undertaken (Büchi *et al.*, 1973). The LD_{50} for AFB1 is 9.5 mg/kg while the value for AFP1 is 95-190 mg/kg. body weight. The LD_{50} for rats is 6 mg/Kg body weight (Butler, 1964) .

Elimination of AFB1 in bile as polar metabolites was described as early as 1978 by Degen and Neumann. In similar studies over 50% of administrated AFB1 dose was eliminated as glutathione conjugates in rats (Holeski *et al.*, 1987; Monroe *et al.*, 1986). Conjugation with glucuronic and taurocholic acids (Bassir and Osiyemi, 1967) was described as major routes of AFB1 elimination. Further studies on carcinogenic effects of AFB1 in male white Swiss mice showed that when animals were given 1 ppm mycotoxin in their diet; only 15% developed liver tumor (Newberne and Butler, 1969). Conversely, the same dietary AFB1 intoxication was ineffective in two other Swiss strains of mice (C3HFB1/Hen and C57B1/5NB). The difference