

## Aflatoxin B<sub>1</sub>-Induced Kidney Damage in Rats

May Fadhil Majid Al-Habib (Ph.D.)\*

Akram Abood Jaffar (Ph.D.)\*\*

Hayder Hammadi Abdul-Ameer (FICMS) \*\*\*

### Summary:

**Background:** Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is a widely distributed mycotoxin in nature. Several investigations have shown its biological effects on different organs and in different animal species. However, the effects of AFB<sub>1</sub> on the rat kidney have not been much elucidated histologically.

**Objective:** This study aims to demonstrate the effects of AFB<sub>1</sub> contaminated diet on the rat kidney from the histological and morphometric aspects.

**Method:** Twelve mature albino rats were divided equally into a control group fed with usual diet and a treated group which was daily fed with diet contaminated with 20 mg AFB<sub>1</sub>/kg of body weight for 30 days. Semithin sections from renal cortex were stained with methylene blue and examined by light microscopy. Corpuscular changes were also detected morphometrically in terms of the ratio between the area of Bowman's capsule and the area of its contained glomerulus (B/G ratio).

**Results:** The treated group showed a marked increase in body weight. Histologically, there was evidence of acute tubular necrosis and increase in urinary space. Morphometrically, there was a diffuse significant increase in the B/G ratio compared to the control.

**Conclusion:** Gain in weight can be attributed to fluid retention that accompanies the ensuing renal damage. The dietary dose of AFB<sub>1</sub> (20 mg/kg of body weight) for 30 days was sufficient to produce acute tubular necrosis. The corpuscular changes indicated by the increase in the B/G ratio can be attributed to compensatory hypertrophy.

**Key words:** Aflatoxin, Kidney, Rat, Morphometry

**J Fac Med Baghdad**  
**Vol. 49, No. 1, 2007**

Received: May 2006

Accepted: Oct. 2006

### Introduction:

Aflatoxins are a group of closely related mycotoxins that are widely distributed in nature in different agricultural communities. It has been demonstrated that *Aspergillus flavus* can infect corn and produce Aflatoxins specially type B<sub>1</sub> (AFB<sub>1</sub>)<sup>i</sup>. Aflatoxins, have a very wide range of biological activities particularly B<sub>1</sub>, which causes great economic losses and poses health hazards both to human and farm animals<sup>ii</sup>.

The problem of using contaminated food with toxigenic fungi is still one of the most important stigmas in the field of nourishment of human and animals. These toxigenic fungi are able to produce secondary metabolites that may produce a toxic biological effect<sup>iii</sup>. Aflatoxins attracted attention of researchers especially after the epidemic of (X) disease and Yellow rice disease in Japan<sup>iv</sup>.

Aflatoxins have been considered as potent human carcinogens implicated in the etiology of hepatocellular carcinoma<sup>v</sup>. Low level exposure to AFB<sub>1</sub> may present health risk where it was found to impair specific and non specific immune responses<sup>vi,vii</sup>. Several investigations have shown the serious effects of Aflatoxins on liver, lymphocytes, macrophages, and lung<sup>v,vii,viii</sup>.

AFB<sub>1</sub> was shown to be excreted in human urine<sup>ix</sup>. Kidney damage induced by aflatoxicosis was demonstrated in fish<sup>x</sup> and chicken<sup>xi</sup>; however, the effects of AFB<sub>1</sub> contaminated diet on the rat kidney has not been much elucidated histologically<sup>xii</sup>. This study aims to demonstrate the effects of AFB<sub>1</sub> on the rat kidney from the histological and morphometric aspects.

### Materials and Methods:

#### 1. Isolation of fungi

The Aflatoxins producing fungi were isolated from seed samples (rice, peanut and wheat) according to a standard method<sup>xiii</sup> at the Department of Technical Biology, College of Sciences, Al-Nahrain University. The fungi isolates were identified by direct examination with light microscope using lactophenol stain.

#### 2. Spore suspension preparation

Spore suspensions were prepared by inoculating the isolation of *A. parasiticus* into slants containing Czapek's dox agar medium. The slants were incubated at 30°C for 7 days and kept under 5°C in the refrigerator<sup>xiv</sup>.

#### 3. Laboratory animals

Mature albino rats were isolated in a relatively controlled environment at 37°C with free access of tap water. The rats were divided into 2 groups (6 rats for each group) as following:

a. *Group I (control):* were fed with usual diet for 30 days.

(\*)Lecturer, Section of Histology and Embryology, College of Medicine, Al-Nahrain University.

(\*\*)Assistant Professor, Department of Human Anatomy, College of Medicine, Al-Nahrain University.

(\*\*\*)Lecturer, Department of Human Anatomy, College of Medicine, Al-Nahrain University.

b. *Group II (treated)*: were daily fed with diet contaminated with the spore of isolated *A. parasiticus* 20 mg/kg of body weight for 30 days.

#### 4. Sacrifice, sectioning, and staining

At the end of the treatment period, animals of both groups were sacrificed by spinal dislocation. After weighing the animal, the abdomen was dissected to obtain the kidneys which were cut into small pieces (2×2×2mm).

The kidney specimens were post fixed in 1% osmium tetroxide for 1 hour, dehydrated, cleared in propylene oxide, and embedded in araldite.

Tissue capsules were sectioned at (0.5-1µm) by glass knives which were made by (LKB) knife maker in Riechert Jung ultracut microtome. Cortical sections were stained with few drops of 1% methylene blue, placed on a hot plate at 60°C for 15 seconds then washed with distilled water, and let to dry<sup>xv</sup>.

#### 5. Morphometry and statistical analysis

Section images were obtained by a video camera fitted to a light microscope. The camera was connected to a computer system equipped a measurement software, Global Lab Image/II (GLI software, Data Translation Inc. USA, www.datatranslation.com). The final magnification of the image appearing on the computer monitor screen was found to be x450.

Corpuscular changes in the treated and control group were detected morphometrically by measuring the area of the Bowman's capsule and its contained glomerulus using the "Poly freehand line" tool of the GLI software for tracing. Since it was intended to detect changes in terms of the ratio between the area of Bowman's capsule and the area of its contained glomerulus (B/G ratio), then area measurements were expressed in terms of pixels. No further action was taken to evaluate area measurements in international units.

A total of 100 renal corpuscles were randomly measured in the experimental animals' sections (50 from the subcortical (SC) region and 50 from the juxtamedullary (JM) region). In the control animal sections, a total of 30 renal corpuscles were randomly measured.

Area data were saved into Microsoft® Office Excel 2003 worksheet (Part of Microsoft Office Professional Edition 2003, Copyright© 1985-2003 Microsoft Corporation). The B/G ratio was calculated by dividing the area of the Bowman's capsule by the area of the contained glomerulus. B/G ratios were subjected to statistical analysis. The differences between the means were assessed using the Student's unpaired t-test included within the "data analysis" tool. A (*P*) value of less than 0.01 was considered as statistically significant.

#### Results:

Animals treated with AFB<sub>1</sub> showed a marked increase in body weight from 303.5±126 – 363.5±126g (mean ± SE) in comparison to the control group which did not show a significant increase (from 292±133-313±133g) in body weight at the end of the experiment period.

Kidney sections of experimental animals showed evidence of increased urinary space which was of varying extent in different experimental animals (Fig.1 A&B). In animal sections which showed maximum urinary space (Fig.1A), it was difficult to differentiate between proximal and distal convoluted tubules on general histological basis. Most of the tubules were not showing a lumen, their lining cells showed spaces within, they were very darkly stained so that the staining intensity was not a criterion to differentiate between proximal and distal tubules. Most of the tubules have lost their roundness. Brush borders in the proximal convoluted tubules could hardly be detected.

B/G ratios in the SC and JM regions of the experimental animals were pooled together and compared with the B/G ratio of the control animals. Descriptive statistics of the B/G ratio of the control and experimental animals are shown in Table-1.

There was a significant statistical difference between the B/G ratio of the control and experimental animals (t-test, *P*=9.47E-07). On the other hand, the B/G ratio in the experimental animals was not statistically different between the SC and JM renal corpuscles (t-test, *P*=0.587).

**Table-1: Descriptive statistics of (B/G) ratio of control and experimental animals.**

	<i>Experimental animals</i>	<i>Control animals</i>
<b>Mean</b>	<b>1.217</b>	<b>1.072</b>
<b>Median</b>	<b>1.178</b>	<b>1.075</b>
<b>Standard Deviation</b>	<b>0.1527</b>	<b>0.0282</b>
<b>Minimum</b>	<b>1.078</b>	<b>1.011</b>
<b>Maximum</b>	<b>1.938</b>	<b>1.140</b>
<b>Count</b>	<b>100</b>	<b>30</b>

#### Discussion:

The effect of AFB<sub>1</sub> on many organs in the body and in different species has been studied either following intraperitoneal administration of AFB<sub>1</sub><sup>x</sup> or by the use of contaminated diet<sup>xvi</sup>.

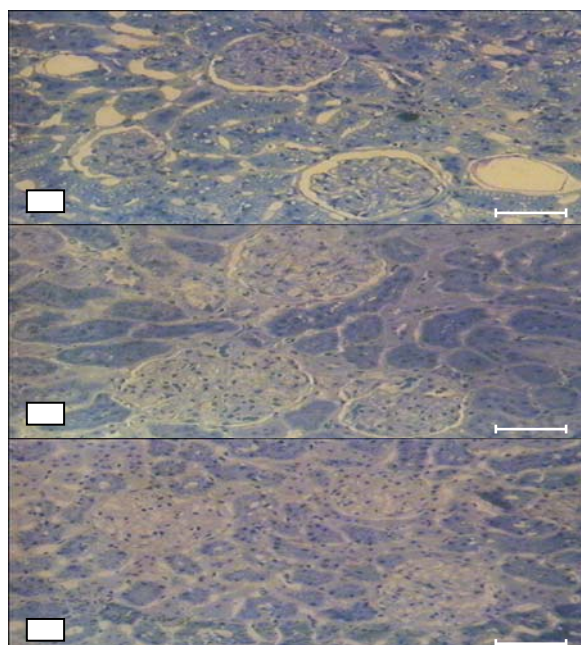
The effects of aflatoxin on the kidney has been shown to be dose related in species other than the rat. In fish, intraperitoneal doses of 7.5-13.75mg/kg body weight revealed toxic changes in the kidney that were not demonstrated with lower doses<sup>x</sup>. In chicken, AFB<sub>1</sub> added to diet (3mg/kg of feed for 21 days) produced thickening of glomerular basement

membrane<sup>xvii</sup>. In mink, dietary aflatoxin (102µg/kg) supplements produced liver lesions in the absence of histopathologic alterations in the kidney<sup>xviii</sup>. In pig, hepatic and renal lesions were directly related to aflatoxins dose. Nephrotubular lipidosis was observed at dietary doses of 650-800 µg/kg<sup>xix</sup>.

The amount of daily intake that produces toxicity differs according to the sensitivity of different animal species and the intake of diet components such as vitamins known to prevent carcinogenesis<sup>xx</sup>. The oral dose of AFB<sub>1</sub> used in this study (20 mg/kg of body weight) for 30 days was sufficient to produce renal damage.

In this study, rats treated with AFB<sub>1</sub> have shown an increase in total body weight. Gain in body weight following aflatoxicosis was also observed in turkey poult<sup>xxi</sup> and in rabbits<sup>xvi</sup>. It has been suggested that weight gain might be due to increase of water intake and swelling of some organs after treatment as a reaction for the effect of AFB<sub>1</sub> which perhaps stimulate the thirst center resulting in an increase in water consumption in an attempt to assist in the excretion<sup>xxii</sup>. However, the ensuing renal damage which was revealed in this study should be considered as a cause of water retention that inevitably results in weight gain.

Oral AFB<sub>1</sub> administration to rats was shown to affect both tubules and glomeruli. In fish, degenerative to necrotic changes were shown in kidney tubules during AFB<sub>1</sub> administration<sup>x</sup>. There has been evidence of renal damage in chicken including glomerular hypertrophy and degeneration of tubular epithelium<sup>xi</sup>. The distortion of tubules, blockage, and absence of clear brush borders of the proximal convoluted tubules (Fig.1) are signs of acute tubular necrosis. Heavy metals, organic solvents, drugs, and poisonous fungi have been incriminated as toxic causes of acute tubular necrosis with similar histopathological picture<sup>xxiii</sup>.



Vacuolation of the cells of the proximal tubules has been demonstrated to be a morphological change consistent with potassium depletion that is a feature of the diuretic stage of acute tubular necrosis<sup>xxiv</sup>.

Renal glomerular morphometry has been used to assess kidney damage from a variety of sources<sup>xxv,xxvi</sup>. Measurements of Bowman's space and glomerular tufts have been amongst the employed parameters<sup>xxvii,xxviii</sup>. The JM and SC renal corpuscles have been separately analyzed in view of the functional differences between glomeruli localized in these two regions of the renal cortex<sup>xxix</sup>. Measurement of SC and JM renal corpuscles were also employed by other researchers<sup>xxx</sup>.

The B/G ratio was designed to provide a sensitive parameter for the variations in the size of the renal space. For a comparatively large structure such as the Bowman's capsule and the contained glomerulus, wide variations are expected at which the section bisects the structure under study. Absolute area measurements, in this case require meticulous serial sectioning of a particular renal corpuscle in order to select the section level of maximum corpuscular diameter<sup>xxvii</sup>. The B/G ratio seems to be a wise parameter for comparison as the ratio will not be affected no matter at which level the section bisects the Bowman's capsule.

The increase in B/G ratio in the experimental animals indicate that renal changes attributed AFB<sub>1</sub> administration are not confined to tubules but also involve renal corpuscles. However, it should be born in mind that the increase in the ratio does not indicate whether it was due to glomerular shrinkage, corpuscular hypertrophy, or both. In view of the absence of histopathological changes of glomerulonephritis and the presence of acute tubular necrosis, corpuscular compensatory hypertrophy would be the most likely cause.

Fig.1: A comparison of kidney sections between experimental animals (A &B) and control (C). Note that corpuscular changes are at their maximum in section (A) where the urinary space is much wider. The changes are to a lesser extent in section (B). Also note that in section (A), there is much distortion of the tubules whose cuboidal cells are "vacuolated" with the absence of a clear brush border of the proximal convoluted tubules. Methylene blue stain, 0.5-1µm, X100, Calibration bar=100µm.

## References:

1. <sup>1</sup> Raper KB & Funnei DI: *The genus Aspergillus*. Baltimore. Williams & Wilkins. 1965; pp:54-57.
2. <sup>1</sup> Cieglaer A: *Dietary Aflatoxins and human cancer*. *Cancer Res.* 1984; 51:102-105.
3. <sup>1</sup> FAO: *Manual of food quality control training in mycotoxins analysis*. 1990; p:17.
4. <sup>1</sup> Chou MW, Chen W, Mikhailova MW, Nichols J, & Chung KT: *Dietary restriction modulated carcinogen DNA adduct formation and the carcinogen induced DNA strand breaks*. *Toxicol. Lett.* 1997; 92:21-30.
5. <sup>1</sup> Stewart RK, Serahjit-Singh CJ, & Massey TE: *Glutathione S-transferase-catalyzed conjugation of bioactivated Aflatoxin B<sub>1</sub> in rabbit lung and liver*. *Toxicol. Appl. Pharmacol.* 1996;140:499-507.
6. <sup>1</sup> Baily WS: *Acute and chronic effects of Aflatoxins on the liver of domestic and laboratory animals*. *Cancer Res.* 1990; 40:236-250.
7. <sup>1</sup> Barta I, Admokova M, & Peter T: *Effects of aflatoxin B<sub>1</sub> on murine lymphocytic function*. *Toxicology.* 1998; 54:234-237.
8. <sup>1</sup> Dugyala RR & Sharma RP: *The effect of aflatoxin B<sub>1</sub> on cytokine mRNA and corresponding protein levels in peritoneal macrophages and splenic lymphocytes*. *Int. J. Immunopharmacol.* 1996; 18:599-608.
9. <sup>1</sup> Verma RJ & Chaudhari SB: *Detection of aflatoxin in human urine*. *Indian J. Env. Toxicol.* 1997; 7:47-48.
10. <sup>1</sup> Sahoo PK, Mukherjee SC, Nayak SK, & Dey S: *Acute and subacute toxicity of aflatoxin B<sub>1</sub> to rohu, Labeo rohita*. *Indian J. Exp. Biol.* 2001; 39:453-458.
11. <sup>1</sup> Ortatli M & Oguz H: *Ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis*. *Res. Vet. Sci.* 2001; 71:59-66.
12. <sup>1</sup> Rati ER, Shantha T, & Ramesh HP: *The effect of long term feeding and withdrawal of aflatoxin B<sub>1</sub> and ochratoxin A on kidney cell transformation in albino rats*. *Indian J. Exp. Biol.* 1991; 29:813-817.
13. <sup>1</sup> Shotwell OL, Goulden ML, & Bennett GA: *Survey for Zearalenone, Aflatoxin, and Ochratoxin in U.S. Asso. Off. Anal. Chem.* 1980; 63:922-926.
14. <sup>1</sup> Faraj MK: *Regulation of mycotoxins formation in Zea mays*. Ph.D. thesis. Department of bioscience and biotechnology, University of Strathclyde, Glasgow, U.K. 1990
15. <sup>1</sup> Robinson G: *Electron microscopy: (B) Sectioning and staining*. In Bancroft JD & Stevens A (Eds.): *Theory and practice of histological techniques*. 2<sup>nd</sup> Ed. Edinburgh. Churchill Livingstone. 1982; pp:499-505.
16. <sup>1</sup> Soliman KM, El-Faramawy AA, Zakaria SM, & Mekkawy SH: *Monitoring the preventive effect of hydrogen peroxide and gamma-radiation of aflatoxicosis in growing rabbits and the effect of cooking on aflatoxin residues*. *J. Agric. Food Chem.* 2001; 49:3291-3295.
17. <sup>1</sup> Valdivia AG, Martinez A, Damian FJ, Quezada T, Ortíz R, Martínez C, Lamas J, Rodríguez ML, Yamamoto L, Jaramillo F, Loarca-Pina MG, & Reyes JL: *Efficacy of N-acetylcysteine to reduce the effects of aflatoxin B<sub>1</sub> intoxication in broiler chickens*. *Polut. Sci.* 2001; 80:727-734.
18. <sup>1</sup> Bonna RJ, Aulerich RJ, Bursian SJ, Poppenga RH, Braselton WE, & Watson GL: *Efficacy of hydrated sodium calcium aluminosilicate and activated charcoal in reducing the toxicity of dietary aflatoxin to mink*. *Arch. Environ. Contam. Toxicol.* 1991; 20:441-447.
19. <sup>1</sup> Bonomi A, Quarantelli A, Mazzali I, Cabassi E, Corradi A, Di Lecce R, Ubaldi R, Fusari A, & Chizzolini R: *Effects of aflatoxins B<sub>1</sub> and G<sub>1</sub> on productive efficiency, meat yield and quality in fattening pigs (experimental contribution)*. *Rev. Soc. Ital. Sci. Alimen.* 1994; 23:251-277.
20. <sup>1</sup> Creppy EE: *Update of survey, regulation and toxic effects of mycotoxicosis in Europe*. *Toxicol. Lett.* 2002; 127:19-28.
21. <sup>1</sup> Kubena LF, Huff WE, Harvey RB, Yersin AG, Etissalde MH, Witzel DA, Giroir LE, Phillips TD, & Petersen HD: *Effects of a hydrated sodium calcium aluminosilicate on growing turkey poults during aflatoxicosis*. *Poult. Sci.* 1991; 70:1823-1830.
22. <sup>1</sup> Nelson TS & Christensen GM: *Effect of Aflatoxin treatment on body and organ weights treatment on Aflatoxin residue in tissue of two different strains of broiler chickens*. *J. Biol. Chem. Vet.* 1995; 24:369-371.
23. <sup>1</sup> Stevens A & Lowe J: *Pathology*. 2<sup>nd</sup> ed. Edinburgh. Mosby. 2000; pp:371-372.
24. <sup>1</sup> Anderson JR (Ed.): *Muir's textbook of pathology*. 10<sup>th</sup> ed. London. Edward Arnold. 1976; p:783.
25. <sup>1</sup> Mayhew TM, SharmaAK, & McCallum KNC: *Effects of continuous subcutaneous insulin infusion on renal morphology in experimental diabetes: I. Blood glucose levels, body size, kidney weight, and glomerulotubular morphometry*. *J. Pathol.* 1987; 151:147-155.
26. <sup>1</sup> Shea SM & Roskova J: *Glomerular morphometry in the Munich-Wister rat*. *Microcirc. Endothelium Lymphatics.* 1985; 2:499-515.
27. <sup>1</sup> Olivetti G, Anversa P, Rigamonti W, Vitali-Mazza L, & Loud AV: *Morphometry of the renal corpuscle during normal postnatal growth and compensatory hypertrophy: A light microscope study*. *J. Cell Biol.* 1977; 75:573-585.
28. <sup>1</sup> Olivetti G, Giacomelli F, & Weiner J: *Morphometry of superficial glomeruli in acute hypertension in the rat*. *Kidney Inter.* 1985; 27:31-38.
29. <sup>1</sup> Jamison RL: *Intrarenal heterogeneity: The case for two functionally dissimilar populations of nephrons in the mammalian kidney*. *Am. J. Med.* 1973; 54:281-289.
30. <sup>1</sup> Shea SM: *Glomerular morphometry in the Munich Wistar rat: effects of sub-total renal ablation*. *Microcirc. Endothelium Lymphatics.* 1988; 4:327-336.

