

## Histological and Immunohistochemical study on the possible protective effects of curcumin and garlic against aflatoxinB1 induced toxicity on the renal cortex of adult male guinea pig

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**Abstract:** **Introduction:** AflatoxinB1 (AFB1) is considered the most potent mycotoxins. When AFB1 is administrated through contaminated food causes severe kidney damage. curcumin and garlic have antioxidants effects and may be effective in ameliorating the toxic effects of AFB1. **Aim:** This work was carried out to study the histological changes in AFB1-induced toxicity in the kidney and the possible protective role of curcumin and garlic in adult male guinea pigs. **Material & Methods:** In this study, sixty adult male guinea pigs were used. They were divided into six groups (10 rats for each): group I (control), group II (curcumin treated group), group III (garlic treated group), group IV (AFB1 treated group), group V (AFB1 and curcumin treated group) and group VI (AFB1 and garlic treated group). Kidney specimens were obtained at the end and processed. **Results:** Light studies showed degenerative changes; most glomeruli showed marked distorsion, some glomeruli were enlarged, other glomeruli were segmented and atrophied. The renal tubules showed marked degenerative changes. There were hyaline material deposition, dilated congested blood vessels and mononuclear cellular infiltration. Curcumin and garlic treatment decreased the toxic effects of AFB1. Garlic administration showed higher protection against AFB1 toxicity. **Conclusion:** It was concluded that, the garlic has a better protective effect than curcumin against toxic effect of AFB1.

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**Key words:** Aflatoxins, curcumin, garlic, kidney, caspase-3,

### 1. Introduction

Aflatoxins are known as mycotoxins which are secreted by *Aspergillus parasiticus* and *Aspergillus flavus* (Mohanamba et al., 2007). Four aflatoxins commonly present in contaminated food: Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1) and Aflatoxin G2 (AFG2). The acute toxicity of these naturally occurring aflatoxins has been evaluated in a limited number of species (Al-Ghasham et al., 2008).

AFB1 is mostly found in contaminated food and people are exposed to it through their diet. Human exposure to AFB1 through their work has been reported in swine and poultry production (Viegas et al., 2013).

AFB1 is considered the most potent mycotoxins due to its carcinogenic, mutagenic and immunosuppressive effects in animals and human (Abdel-Wahhab, 2005) and considered as carcinogenic agent of Group I to human by the international Agency for Research on cancer (IARC, 1993).

Consumption of high dose of AFB1 through the contaminated food causes weight loss, sever kidney, liver and heart damage, reproductive alterations and death (Abdin et al., 2010).

Aflatoxins are DNA damaging agent due to their capacity to bind covalently to DNA leading to their

damage and mutagenesis followed by cellular dysfunction (Cole and Cox, 1981).

AFB1 causes oxidative stress which is a potent mechanism in initiation and progression of aflatoxicosis by raising lipid peroxidation and decreasing enzymatic and non-enzymatic antioxidants leading to cell damage [Gupta and Sharma, 2011].

Therefore, some antioxidants may be useful in ameliorating the harmful effects of chronic AFB1 toxicity.

There is a wide use of medicinal herbs in the last years because these are a rich source of antioxidants (Abdul majeed, 2011).

Curcumin is the active ingredient of turmeric, extracted from *Curcuma longa*, a member of the ginger family, chemically is diarylheptanoid, responsible for turmeric's yellow color, curcumin is commonly used as cosmetic ingredient, food coloring and food flavoring (Anand et al., 2007).

Curcumin is administrated in various diseases as nephrotoxicity, asthma, diabetes, psoriasis, myeloma, pancreatic and colon cancer (Kedia et al., 2014). Curcumin has a potent antioxidant, antibacterial, anti-inflammatory and cancer preventive properties (Smith et al., 2001).

It inhibits the production of reactive-oxygen species, have anti-inflammatory properties as a result

of inhibition of cyclooxygenases and other enzymes and causes disturbance of cell signal transduction by various mechanisms including suppression of protein kinase C. These effects play a role in the inhibition of tumor cells proliferation and progression, it could act as an alternative chemo-preventive agent (Kuhad and Chopra, 2007).

Garlic is a plant which is grown all over the world, it is native to Siberia and then spread to other parts of the world (Lee et al., 2009). Garlic is used in the treatment of many conditions related to cardiovascular system and to prevent stomach, colon, rectal and breast cancer. Also it is used to treat bladder and prostate cancer (Galeone et al., 2006).

Garlic is also used in the treatment of headache, fever, stomachache, gout, hemorrhoids, bronchitis, sinus congestion and bloody diarrhea (Ban et al., 2009).

Chopping of garlic stimulates the enzyme that converts the phytonutrient alliin to a compound allicin to which their health benefits are attributed (Cavagnaro et al., 2007).

The aim of this study is to evaluate the protective effects of curcumin and garlic against the adverse effects of AFB1 on the renal cortex of adult male guinea pigs.

## 2 Material and Methods

### Chemicals and drugs

**Aflatoxin B1** was obtained from Sigma Company (Saint Louis, Missouri, USA). It is available in the form of powder, white to yellow in color. AFB1 was dissolved in dimethylsulfoxide (DMSO), 3mg AFB1 / 30ml DMSO.

**Curcumin** was obtained from sigma company (Saint Louis, Missouri, USA). It is available in the form of powder, insoluble in water and dissolved in corn oil as a vehicle.

**Garlic** cloves are peeled and chopped, then the sliced garlic is heated to a temperature between 150-160 C. The dehydrated form is grounded into fine particles. It is available in most grocery stores.

### Animals

Sixty healthy adult male guinea pigs of average weight 450-500 g, 3-4 months old, were used in this study. The animals were kept in a healthy standard environmental conditions, with 12-h Light\12-h dark cycle, temperature 22 -24°C, and fed with basal diet and tap water. Ethical protocols for animal treatment were followed.

### Experimental procedure

The guinea pigs were randomly divided into six groups included 10 guinea pigs for each, and were administered orally by gastric tube.

**Group I (control group):** The guinea pigs of this group were further subdivided into two equal subgroups.

Subgroup IA: The guinea pigs received corn oil as a vehicle for four weeks.

Subgroup IB: The guinea pigs received DMSO as a vehicle for four weeks.

**Group II (Curcumin treated group):** The guinea pigs received curcumin 200 mg/kg /d for four weeks (Hammouda et al., 2009).

**Group III (garlic treated group):** The guinea pigs received garlic 24.8 mg /day which was equivalent to 800mg/day for human (Kiesewetter et al., 1993). According to Paget and Barnes (Paget and Barnes, 1964). For four weeks.

**Group IV (AFB1 treated group):** The guinea pigs received AFB1 250 µg/kg/d (Tange et al., 2007), for four weeks.

**Group V (AFB1 and curcumin treated group):**

The guinea pigs received AFB1 and curcumin as in the previous groups for four weeks.

**Group VI (AFB1 and garlic treated group):**

The guinea pigs received AFB1 and garlic as in the previous groups for four weeks.

In groups V &VI, AFB1 administered 2h after the curcumin and garlic intake.

24 h after the last dose, the animals were sacrificed by cervical decapitation. Kidney samples were obtained and cleaned by normal saline and then fixed in 10% formol saline and processed in the usual way to obtain the ordinary paraffin blocks which were prepared for **histological** (Hx&E, Masson's trichrome) (Suvarna et al., 2013), **histochemical** (periodic acid Schiff (PAS)(Bancroft and Gamble, 2013) and **immunohistochemical** (caspase-3) study (Sanii et al., 2012).

## 3. Results

### Histological, histochemical and immunohistochemical results.

#### Groups I, II & III:

**Haematoxylin and Eosin stain** findings of group I (control group) showed the renal cortex which consists of convoluted tubules together with the renal corpuscles. Each corpuscle consists of Bowman's capsule enclosing a tuft of capillaries called glomerulus.

The Bowman's capsule is formed of an inner visceral layer and outer parietal layer which lined with simple squamous epithelium and the two layers are separated by Bowman's space. The proximal convoluted tubules have narrow lumen, intensely eosinophilic cytoplasm and lined with pyramidal cells with clear brush borders and basal vesicular rounded nuclei, spaced somewhat farther apart. Distal convoluted tubules have more clear wide lumen, less

eosinophilic cytoplasm, lined by cubical cells which are more numerous and have basal spherical nuclei (Figs 1 & 2). Group II (curcumin treated group) and group III (garlic treated group) showed no detectable changes as compared to that of the control group.

**In Masson's trichrome** section of group I, there were minimal amounts of collagen fibers around Bowman's capsules, glomerular capillaries and basement membrane of renal tubules (Fig. 3). Groups II & III showed no changes when compared to the control group.

**PAS stain** of group I showed strong reaction in the Bowman's capsule, basement membrane and brush border of renal tubules (Fig. 4). Groups II&III showed no changes in the intensity of the reaction as compared to the control group.

**Immunohistochemical result** of group I showed negative cytoplasmic immunoreactivity for caspase-3 of renal tubules (Fig.5). Groups II & III showed the same results.

#### Group IV (aflatoxin B1 treated group)

**Hx&E sections** of this group showed variable histological changes, most renal glomeruli showed distortion. Some glomeruli were enlarged with dilatation of their tufts and some lining cells showed karyolytic nuclei (Fig. 6). Other glomeruli showed degeneration (Figs. 7&12) and segmentation of their tufts with widening of Bowman's space (Fig. 7). Some renal tubules were dilated with presence of vacuoles in their cytoplasm (Figs. 6,7&8) and the lining tubular cells were destructed with sloughing nuclei, some tubules were lined by flat cells with flat nuclei (Fig. 8). There was hyaline material deposition in the lumen of the renal tubule (Fig. 9). In some sections, there were massive degeneration of some glomeruli and renal tubules (Fig. 10). Congested blood vessels, collagen fiber deposition around blood vessels (Fig. 11) and cellular infiltration (Figs. 9& 12) were detected.

**Masson's trichrome stain** showed an intense increase in collagen fibers around blood vessel, Bowman's capsule and basement of renal tubules (Fig. 13). Treatment with aflatoxin B1 caused decrease of glycogen deposition leading to weak **PAS** reaction in Bowman's capsule and brush borders of some renal tubules and other tubules showed moderate reaction at their brush borders (Fig. 14).

**Immunostain with caspase-3** showed strong positive cytoplasmic immunoreactivity of the renal tubules (Fig. 15).

#### Group V (AFB1 and curcumin treated group)

**Hx&E sections** of this group showed appearance more or less like to control group. Some of the lining tubular cells showed vacuolar degeneration and most glomeruli showed regeneration but few of them showed partial atrophy of their tufts. Also congested

blood vessel was detected (Fig. 16). There was moderate increase in the amount of collagen fibers around blood vessel and Bowman's capsule in **masson's trichrome** section (Fig. 17).

**PAS results** of this group showed moderate reaction in the Bowman's capsule, basement membrane and brush border of renal tubules and negative reaction in some of them (Fig. 18).

**Immunostain with caspase-3** showed moderate cytoplasmic immunoreactivity (Fig. 19).

#### Group VI (AFB1 and garlic treated group)

**Hx&E section** of this group showed histological appearance similar to control group (Fig. 20). In **masson's trichrome** section, there was minimal amount of collagen fibers around Bowman's capsule and basement membrane of renal tubules (Fig.21). This group showed strong **PAS** reaction in Bowman's capsule, basement membrane and brush border of the renal tubules (Fig. 22). **Immunostain with caspase-3** showed negative immunoreactivity as the control group (Fig. 23).

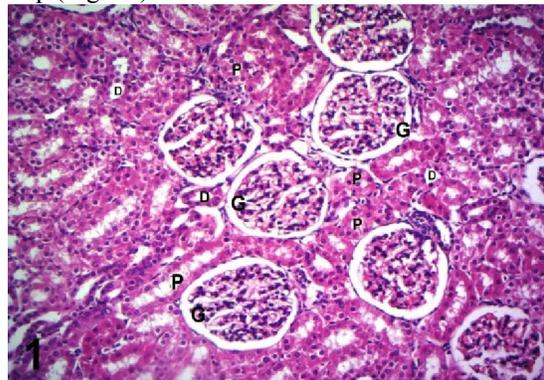


Figure 1: A section of the renal cortex of group I (control group) showing normal appearance of the renal glomeruli (G), proximal convoluted tubules (P) and distal convoluted tubules (D). Hx&E X 200

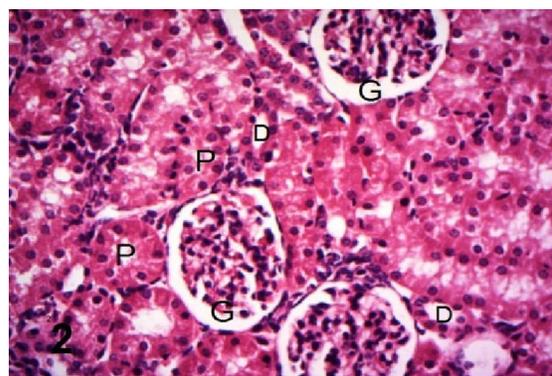


Figure 2: A section of the renal cortex of group I showing normal appearance of the renal glomeruli (G), proximal convoluted tubules (P) with clear brush border and distal convoluted tubules (D). Hx& E X400

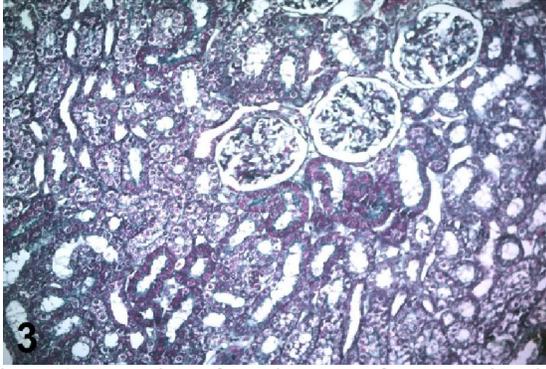


Figure 3: A section of renal cortex of group I showing minimal amounts of collagenous fibers around Bowman's capsule, glomerular capillaries and basement membrane of renal tubules. M.T x 200

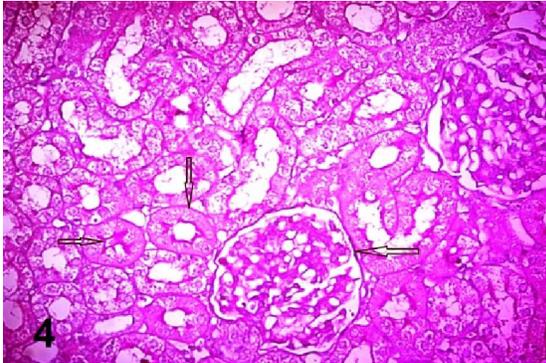


Figure 4: A section of the renal cortex of group I showing strong PAS reaction in Bowman's capsule, basement membrane and brush border of renal tubules (arrows). PAS x 400

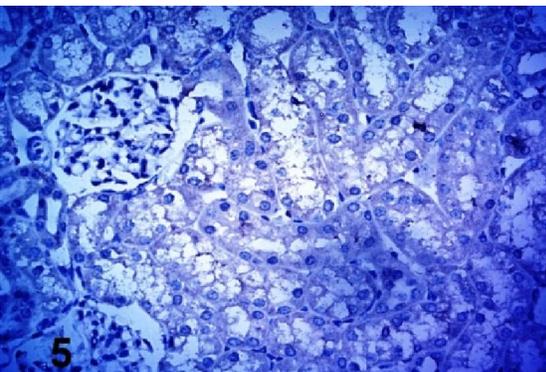


Figure 5: A section of the renal cortex of group I showing negative cytoplasmic immunoreactivity for caspase-3 of the renal tubules. Caspase-3 x400

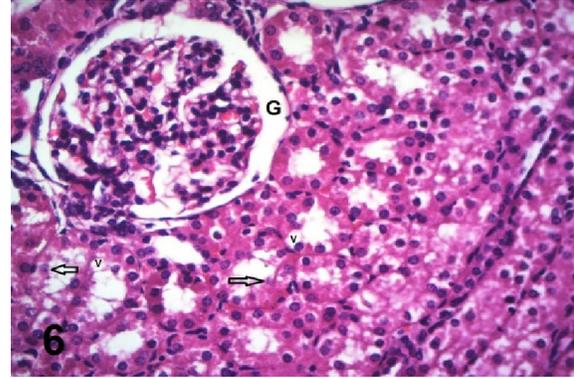


Figure 6: A section of the renal cortex of group IV (AFB1 treated group) showing enlargement of the glomerulus (G). Some tubular cells with karyolytic nuclei (arrows) are observed. Notice, appearance of cytoplasmic vacuoles (V) in some tubular cells. Hx&E X400

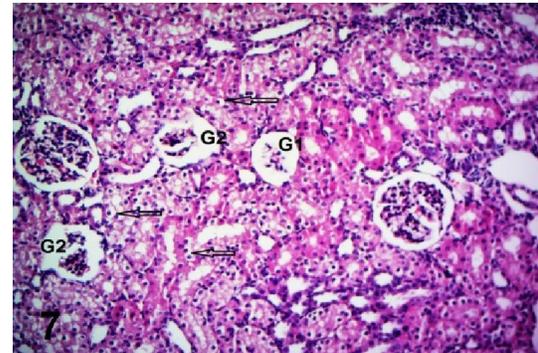


Figure 7: A section of the renal cortex of group IV showing degenerated glomeruli (G1) and others with segmentation of their tufts (G2) and widening of the Bowman's space. Notice, appearance of cytoplasmic vacuoles in the lining cells of the renal tubules (arrows). Hx&E X 200

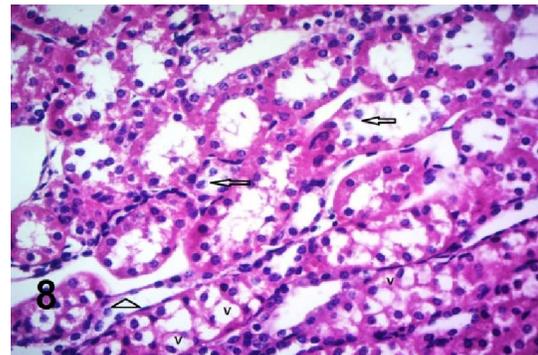


Figure 8: A section of the renal cortex of group IV showing dilated renal tubules with cytoplasmic vacuoles (V) in their lining cells. Other cells are destructed with sloughing nuclei (arrows). Some tubules are lined by flat nuclei (arrow head). Hx&EX 400.

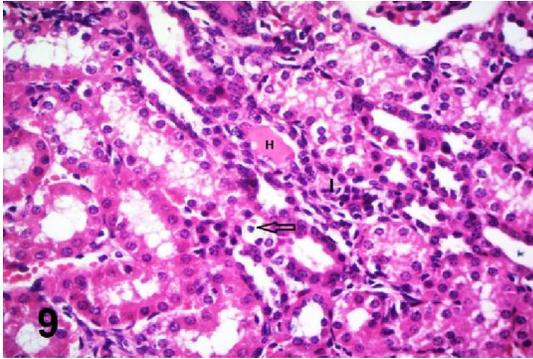


Figure 9: A section of the renal cortex of group IV showing hyaline material deposition (H) in the lumen of the renal tubule. Notice, cellular infiltration (I) and sloughing of the nuclei of some lining cells of renal tubules (arrow). Hx&E X400

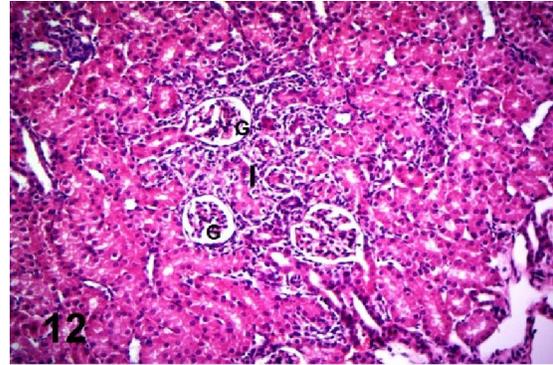


Figure 12: A section of the renal cortex of group IV showing massive cellular infiltration (I) between the glomeruli and renal tubules. Notice, atrophy of some glomeruli (G). Hx&E X200

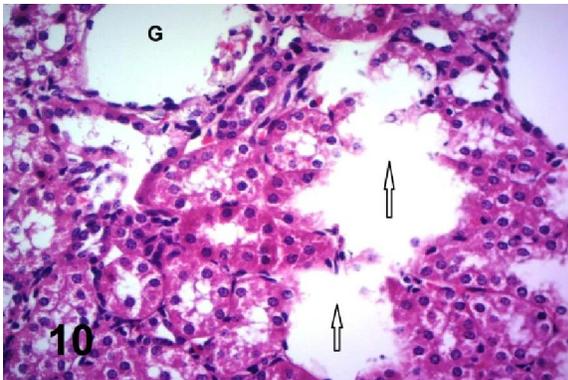


Figure 10: A section of the renal cortex of group IV showing massive degeneration of some glomeruli (G) and some renal tubules (arrows). Hx& E X 400

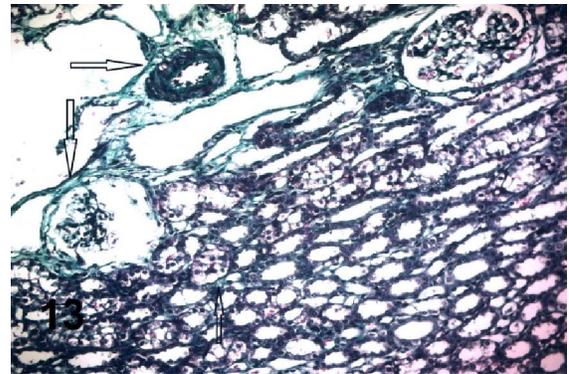


Figure 13: A section of the renal cortex of group IV showing increase of collagen fibers around blood vessel, basement membrane of the renal tubules and Bowman's capsules (arrows). M. T x200

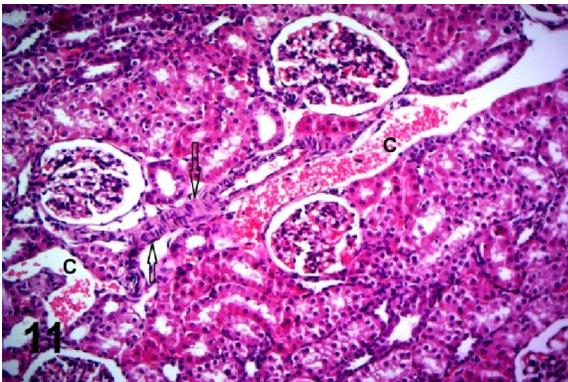


Figure 11: A section of the renal cortex of group IV showing dilated congested blood vessels (C) and collagen fibers deposition around blood vessel (arrows) Hx&E X200

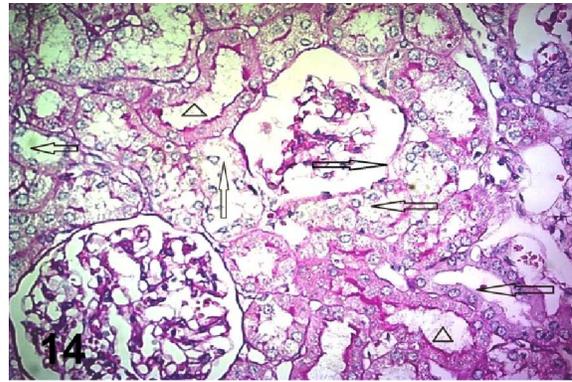


Figure 14: A section of the renal cortex of group IV showing weak PAS reaction in Bowman's capsule and brush border of some renal tubules (arrows) and others tubules showing moderate PAS reaction at their brush borders (arrow heads). PAS X400

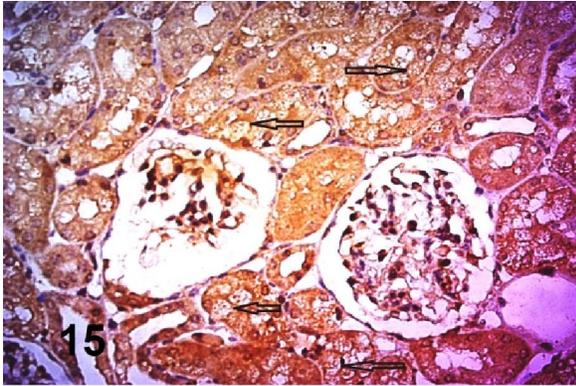


Figure 15: A section of the renal cortex of group IV showing strong positive cytoplasmic immunoreactivity for caspase-3 of the renal tubules (arrows). Caspase-3 x 400

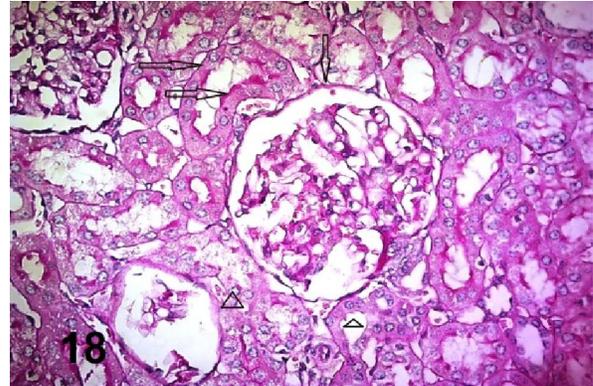


Figure 18: A section of the renal cortex of group V showing moderate PAS reaction in Bowman's capsule, basement membrane and brush border of the renal tubules (arrows) and negative reaction in some of them (arrow heads). PAS x 400

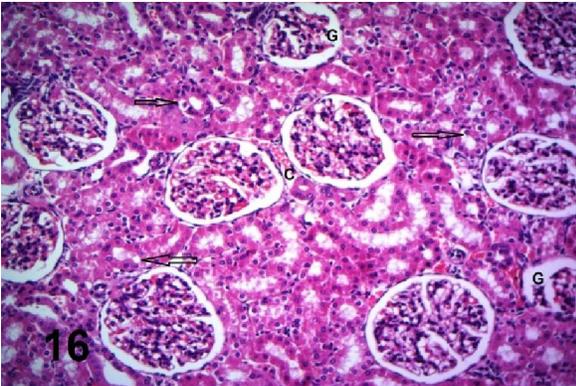


Figure 16: A section of the renal cortex of group V (AFB1 and curcumin treated group) showing cytoplasmic vacuolization of some cells of the renal tubules (arrows) and minimal glomerular degeneration, some have atrophy of their tufts (G). Notice, dilated congested blood vessel(C). Hx& E X 200

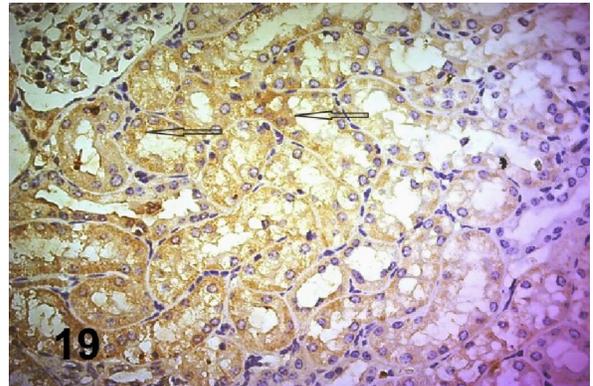


Figure 19: A section of the renal cortex of group V showing moderate cytoplasmic immunoreactivity for caspase-3 of the renal tubules (arrows). Caspase-3 x400

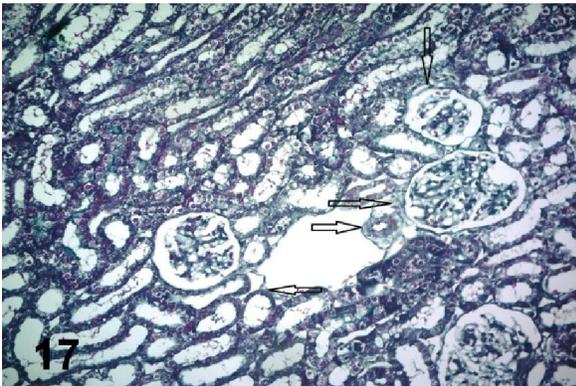


Figure 17: A section of the renal cortex of group V showing moderate amount of collagen fibers around Bowman's capsule and blood vessel (arrows). M.T x 200



Figure 20: A section of the renal cortex of group VI (AFB1 and garlic treated group) showing normal appearance of the glomeruli (G), proximal convoluted tubules (P) and distal convoluted tubules (D). Hx&E x200

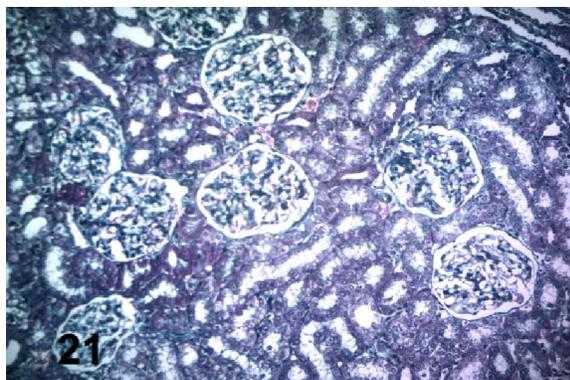


Figure 21: A section of the renal cortex of group VI showing minimal amount of collagen fibers around Bowman's capsule and basement membrane of the renal tubules. M.T x200

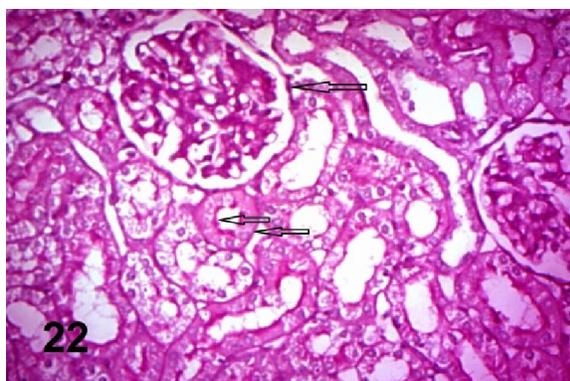


Figure 22: A section of the renal cortex of group VI showing strong PAS reaction in Bowman's capsule, basement membrane and brush border of the renal tubules (arrows). PAS x400

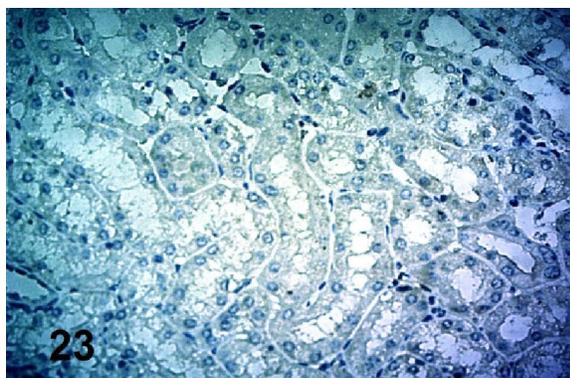


Figure 23: A section of the renal cortex of group VI showing negative cytoplasmic immunoreactivity for caspase -3 of the renal tubules. Caspase-3 x400

#### 4. Discussion

Aflatoxins are a natural contaminants of foods and considered as a metabolites of *Aspergillus parasiticus* and *Aspergillus flavus*. AFB1 is the most toxic aflatoxins, frequently presents as a contaminant

in many products of food and has a potent mutagenic and toxic effects (Gupta and Sharma, 2011).

In the present study, histological changes in AFB1 treated group were observed. The main reason by which AFB1 induces nephrotoxicity is the formation of reactive oxygen species (ROS) thus stimulating a chain reaction producing peroxidation of lipids (Naaz et al., 2007).

AFB1 may cause lipid peroxidation in the cells thus destructing cell membrane integrity (Parveen et al., 2014). Carcinogenicity of AFB1 is mainly attributed to the oxidative damage of DNA and formation of AFB1- DNA adducts, increased level of DNA adducts leads to cell damage and apoptosis (Ellis, 2009).

The histopathological changes of AFB1 treated group were detected. There were enlargement of some glomeruli, while other glomeruli were degenerated with widening of the capsular space, the glomerular tufts were shrunk or segmented. The renal tubular cells showed vacuolar degeneration and sloughing of some epithelial cells, Hyaline casts were also observed.

Bilgic and Yesildere (1992) reported that aflatoxicosis exposure led to degenerative changes in the proximal renal tubules and hyaline casts in the tubular lumen. Some researchers reported that glomerular hypertrophy was explained by function overload which affect the podocytes because their ability of proliferation was limited (Rubin and Strayer, 2008).

The AFB1 and its metabolites causes alterations of renal antioxidants and oxidative stress (Singh et al., 2015).

Glutathion (GSH) is a tripeptide containing cystein and playing an important role in detoxification and interact with ROS by -sh group as a coenzyme. AFB1 produces epoxides that conjugate rapidly with GSH (Janssen et al., 1993), thus decreasing level of GSH in AFB1 treated group suggesting renal tissue oxidation (Chen et al., 2005) as the antioxidant enzymes play an important role against ROS. The present study of AFB1 treated group revealed inflammatory fibrosis, mononuclear cellular infiltration and dilated congested blood vessels between renal tubules and the glomeruli.

Some researchers reported that aflatoxicosis causes hypoproteinaemia by inhibiting protein synthesis thus leading to oedema and cellular infiltration. The appearance of hemorrhage has been reported in aflatoxicosis previously (Saliem et al., 2011).

Aflatoxicosis caused changes in pro-inflammatory cytokines as a step of inflammatory process (Kanaoka and Boyce, 2004). With chronic exposure to AFB1, the bleeding is the result of a lack of clotting factors from extensive liver damage, the

anticoagulant properties of aflatoxin or both (Butter, 1966).

In the present study, caspase-3 reaction showed that the apoptotic cells in AFB1 treated group were higher than the control group. This was in agreement with the previous researches that reported AFB1-induced apoptosis of bone marrow cells, lung (Rai et al., 2001) and kidney cells (Lei et al., 2013).

AFB1 reduces the level of antioxidant enzymes and stimulates the formation of 8-hydroxy deoxyguanosine and ROS (Shen et al., 1995), leading to lipid peroxidation and oxidative damage of DNA (Naaz et al., 2007).

Aflatoxin B1 treated group showed weak PAS reaction in Bowman's capsule and brush borders of some renal tubules. A study on the liver rats given AFB1 7 mg/kg showed focal glycogen loss and cytoplasmic basophilia 16-24 h after treatment (Kanaoka and Boyce., 2004).

Histological examination of group V (AFB1 and curcumin treated group) showed marked improvement of alterations caused by AFB1.

Some of the lining tubular cells showed vacuolar degeneration and most glomeruli showed regeneration but few of them showed partial atrophy of their tufts. Also dilated congested blood vessel was detected. There was moderate increase in the amount of collagen fibers around Bowman's capsule and blood vessel in Masson's trichrome section. This group showed moderate PAS reaction in the Bowman's capsule, basement membrane and brush border of renal tubules and -ve reaction in some of them. Immunostain for caspase-3 showed moderate cytoplasmic immunoreactivity.

This was in agreement with the study that reported that curcumin reduces the activity of microsomal CYO450 in the liver that form AFB1-DNA adducts in rats (El-Agamy, 2010).

Several studies suggest that curcumin might be used as chemotherapeutic agent through several lines of actions (Kaur et al., 2006). Mathuria and Verma, 2007 found that the curcumin reduces the aflatoxin toxicity in the kidney and liver.

While histological examination of group VI (AFB1 and garlic treated group) showed results similar to control group. In Masson's trichrome section, there was minimal amount of collagen fibers around Bowman's capsule and basement membrane of renal tubules. Immunostain section for caspase-3 showed -ve immunore activity as the control group.

Yamasaki and Lau,1997 reported that garlic had direct antioxidant effects and improved serum level of glutathione peroxidase. Also, Yang et al., 1994 found that the chemical contents of garlic have a direct effect on the detoxification enzymes activity.

Curcumin and garlic have protective effect against AFB1- induced toxicity due to their ability to scavenge free radical and to assume antioxidant enzymes (Carmia, 2001).

This study found that garlic is more effective than curcumin, this was coincided with Diab et al., (2002) who reported the effectiveness of curcumin and garlic in reducing toxic effects of AFB1 in the liver, these two drugs improved the values of CAT, TAC, GSH, and However GPx value was improved with garlic only (Diab et al., 2002).

GPx gene expression has an important role in evaluating the protective effect of curcumin and garlic against AFB1 toxicity. GPx is in the first line of the antioxidant defense system. The protein encoded by this gene belongs to glutathione peroxidase family, members of which catalyze the reduction of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and other organic hydroperoxides such as lipid peroxides and uses glutathione as the reductant and thereby protects cells against oxidative damage (Shaymal et al., 2010).

In addition, garlic administration with AFB1 injected fish reflected the highest induction of GPx gene expression. While curcumin in aflatoxicosis caused down- regulations of GPx gene expression (El-Barbary, 2016).

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