

Full Length Research Paper

Impact of fungal content of some Arabic nuts to induce kidney toxicity and agonistic action of natural resources

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Human and animals are exposed to a great extent of the danger of mycotoxic infection and their toxins due to wide spread of fungal growth on grains and food. Aflatoxin B1 (AFB1) is one of the most dangerous toxins that are produced by fungi as a secondary metabolic products. This study aims to isolate and identify the fungi from some grains present in Arabian nuts. 30 samples were collected from commercial stores in Jeddah in the period from 2008 to 2009. Histological study was done on female rat kidney that was treated with isolated fungus *Aspergillus flavus* and the carcinogenic substance aflatoxin B1 (AFB1). Results showed the occurrence of several harmful effects of the experimental fungi and AFB1 on the kidney. Effects of "musk" and "sidr" extract as natural treating sources were confined in this study. They proved their effectiveness in treating renal mycotoxicity.

Key words: Arabian nuts, *Aspergillus flavus*, aflatoxin B1, kidney, musk deer, sidr.

INTRODUCTION

Kidney is an important organ because of its role in getting rid of harmful materials and excretions of drugs and body waste products (Seldin and Giebisch, 1992). Urinary tract can be affected by bacteria and fungi, contaminated food. Grains and nuts such as *Corylus avellana*, *Prunus amigdalus* and *Pistacia vera* are labeled to be more contaminated by AFB1, Penicillin and Ocrotoxin (Essa and Ahmad, 2007). Aflatoxins produced by *Aspergillus* as a secondary metabolic product spread all over the world and can be found in all food types. It has phytotoxicity and activity against wheat, mustard which can suppress the peanuts when roots are provided with adose of 0, 1, 5, and 10/ppr. Moreover AFB1 is characterized by its molecular weight 312 and it is formed in colorless soluble crystal forms, 269 to 268 (Abd -El, 2000). Human and animals are exposed to aflatoxins dangerous effects as the food is considered to be the major source (Harbi et al., 1995) that lead workers to concentrate on the benefits from natural sources which can keep human and environment healthy.

Saddiq (2008) pointed the positive efficient effect of

infection (Williams et al., 2004). Using cisplatin in treating cancer of rats in one dose equals to 7.5 mg/kg necrosis in renal tubules and shedding of their epithelia as well as severe histological changes where found (Al - musk and sidr in treatment of pulmonary mycotoxicity, that was confirmed by Saddiq and Elyani (2009) who demonstrated the possibility of using natural musk in treatment of liver toxicity in the teratogenic Aflatoxin B1, they also showed the positive effect of sidr in reducing liver histological changes. The aim of this study to isolate and identify the developing fungi on some of the Arabian nuts such as *P. vera*, *Prunus dulcis*, *Juglans* and *Anacardium occidentale*, more over studying of the histological effect on renal rat tissue that infected with isolated *A. flavus* and that infected with Aflatoxin B1 and the extent of response to sidr and musk extract for treating the renal tissue also comparing that with normal renal tissue of the same age.

MATERIALS AND METHODS

Arabic nut sample

The study was done at the period on 2008 to 2009 of some Arabic nuts. Random sample where chosen:

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Kingdom: Plantae, (unranked): Angiosperms, (unranked): Eudicots, (unranked): Rosids, Order: Sapindales, Family: Anacardiaceae, Genus: *Pistacia*, Species: *P. vera* (Kay et al., 2007).

Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Rosales, Family: Rosaceae, Genus: *Prunus*, Subgenus: *Amygdalus*, Species: *P. dulcis* (Cantor et al., 2006).

Kingdom: Plantae, Division: Magnoliophyta, Order: Fagales, Class: Magnoliopsida, Family: Juglandaceae, Subfamily: Juglandoideae, Tribe: Juglandaeae, Subtribe: Juglandinae, Genus: *Juglans* (Chauhan, 2004).

Kingdom: Plantae, (unranked): Angiosperms, (unranked): Eudicots, (unranked): Rosids, Order: Sapindales, Family: Anacardiaceae, Genus: *Anacardium*, Species: *A. occidentale* (Alexander, 2008). From some commercial stores in Jeddah KSA sold by Kg/wt. Each sample was collected alone in sterile sac, stored in low grade temperature to avoid any pollution produced to be examined.

Aflatoxin B₁, carcinogenic substance

A product of Ubichem- London. UK. chemical name: Tetrahydro-4-methoxy. Molecular formula: C₁₇H₁₂O₆.

Musk

Musk is formed of many compounds, the main compound which causes the odour is musk one, and contain 1.4% volatile oil and was black to brown color, estroil hormones, the most important were musk pyridine in addition to some alkaloids and enzymes, and is used as powder. The natural black animal musk extracted from the umbilicus of deer. Musk was obtained from Korashi stores KSA-Jeddah and was preserved in natural environmental circumstances at temperature 25 to 28 °C.

Sidr (*Zizyphus spina- Christi*)

Rhamnaceae rhamnales

They are huge long living trees with alternate leaves with hermaphrodite flower. The flower is monoecious having sweet taste and fruits are drupe type. The plant is used as blood filter and in treatment of diarrhea and gall bladder. Leaves and cortex are used in treatment of wounds and skin diseases. Sidr leaves were collected from trees in Jeddah. Leaves contain alkaloids, flavonoids, sterols, saponin, and jelly substances (Al- Eready and Al-Farag, 1418; Saad, 2000 and Kandil et al., 2003).

Female rats

Albino mice (*Mus musculus*) strain MFI were used. Their weight varied between 150 to 170 gm. They were obtained from king Fahd research medical center - King Abd Elaziz University- Jeddah. They were injected intra peritoneally with the pathogenic fungus *A. flavus* and the other treated group was injected by aflatoxin B₁.

Media

Sabouraud dextrose agar (S.D.A) media were used as follow: 65 gm of the media were used. The media was previously prepared

Oxid CM41/L distilled water, then sterilized in wet sterilization by autoclave at 15 L/square inch for 20 min.

Isolation of fungi from the tested samples

Fungi on nuts were isolated by direct method (Johnson et al., 1959) and dilution method (Warcup, 1957) using 6 repeated/sample by isolation on glucose chapeks media for fungal isolation, dish was incubated at 28±2°C for 7 days. Colonis were counted and identified as (Gilman, 1957; Raper and Thom, 1949; Raper and Fennell, 1965; Ellis, 1971, 1976) that results were confirmed in agriculture centre. Ain Saim University, Cairo.

Preparation of aflatoxin (B1)

Experimental animals were treated with carcinogenic dose of aflatoxin B₁ by dissolving it into concentration of 20 µl in di-methylsulfoxide (2 mg/ml) equivalent 1.0 ml/100 g of body weight (Ha et al., 1999).

Preparation of (therapeutic) material

Musk

Hydrous extracts was prepared of black musk from deer musk with concentration of 0.02% given to rats with a dose of 1 ml/kg body weight.

Sidr leaves

Sidr plants were washed and left to dry, then after washing and grinding 100 gm/200 ml were taken for distilled and sterilized water (Adzu et al., 2001) and after 24 h filtered and preserved in dark glasses in refrigerator till is used, then it was given to rats in dose of 1 ml/kg body weight.

Histological study by light microscopes: (Bancroft and Gamble, 2002)

Kidneys

Kidneys were dissected from rats and fixed by following standard methods of dehydration and clearing. Then the samples were embedded in paraffin and cut into 3 micron thickness of the control samples treated and infected. Kidney tissues were fixed by neutral buffered neutral formalin. Sections were stained with haematoxylin and eosin stain.

Fixatives used for study

Neutral buffered solutions for histological study by light microscope.

Staining used for the study

Haematoxylin and eosin stain (H and E) stain gives clear cytoplasm differentiation and nuclear and gives good idea about histological structure of the samples of the study and reveals some pathogenic changes.

Statistical study

Effect of different techniques by using Spsspc ++ program to find T,

Table 1. Percentage of fungi isolated from some Arabic nuts were collected from the commercial stores in Jeddah, in the period from (2008 to 2009) on Sabouraud dextrose agar media.

Sample	Isolated fungi		
	Names	Percentage of growth	Total
<i>Pistacia Vera</i>	<i>Fusarium oxysporum</i>	5.80	212
	<i>Aspergillus flavus</i>	8.42	
	<i>Alternaria</i>	1.25	
	<i>Penicillium sp.</i>	3.36	
<i>Prunus dulcis</i>	<i>Ulocladium alternariae</i>	2.80	155
	<i>Alternaria alternata</i>	6.22	
	<i>Mucar sp.</i>	1.33	
	<i>Aspergillus flavus</i>	9.45	
<i>Juglans</i>	<i>Fusarium moniliforme</i>	4.54	96
	<i>Penicillium sp.</i>	3.89	
	<i>Aspergillus flavus</i>	5.12	
	<i>Ulocladium alternariae</i>	1,87	
<i>Anacardium occidentale</i>	<i>Mucar sp.</i>	3.15	50
	<i>Ulocladium atrium</i>	1.28	
	<i>Ulocladium alternariae</i>	0.0	

Test (Abo- Zied., 2003).

RESULTS

Fungal isolation and identification from nut grains

Fungal isolation and identification of some Arabic nuts was held by collecting the grains from four commercial stores in Jeddah KSA (Table 1). The total number of isolated fungi reached *P. dulcis* 155, *Juglans* 96, *A. occidentale* 50. The most isolates were from *P. vera* that reached 212 isolate and that was after 5 days from incubation on the experimental media. It was primary isolated and confirmed in the research collecting microbes center in Egypt- Mircinen that is part of the University of Ain shams Faculty of Agriculture. The most dominant was *A. flavus*. The percentage of fungal isolation reached 8.42, 9.45, 5.12 and 6.77% from samples of *P. vera*, *P. dulcis*, *Juglans* and *A. occidentale*, respectively. It has been selected for the study of the effect of Aflatoxin B1 on rat renal tissue.

Morphological examination of the rat kidney

The urinary system is composed of two kidneys and two ureters. The ureters open in one urinary bladder and then urethra. Each kidney is found in the dorsal upper part of

the abdominal cavity behind the peritoneum and was covered by isolating layer of fat tissue that fix the kidney in position. The right kidney is lower in position than the left because the liver is situated above it. The kidney is similar to one of the bean grains in shape and had one convex and other concave border. The concave one contains the helium in which the renal blood vessels and nerves pass and the ureter developed. The kidney is covered by fibrous capsule increases in thickness at the hilus area, white tissue extend through renal tissues which is formed of stroma. The kidney is responsible of getting rid of body toxins and waste products especially toxic nitrogen which is the protein end products formed in liver. In addition the kidney plays a role in acid –base balance and keeping constant blood pressure by excretion of the waste water.

Histological examination of the control rats kidney

Histological examination of serial sections of control rat kidney showed that the normal architecture of the kidney is formed of outer cortex and inner medulla. The cortex is differentiated into two layers: outer layer containing renal corpuscles and the major part of proximal and the distal tubules, and an inner layer consists of ascending and descending parts of loops of Henle and the collecting tubules. Renal corpuscle is formed of glomerulus which is formed of tuft of capillaries, and enclosed in a Bowman

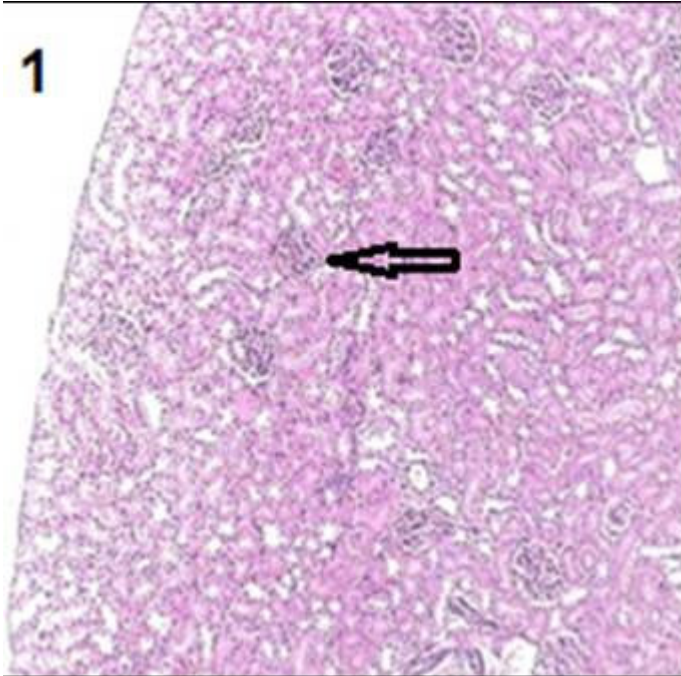


Figure 1. Histological examination of section of control rat kidney showing the normal architecture of both cortex (▶) and renal capsule H and E (x 100).

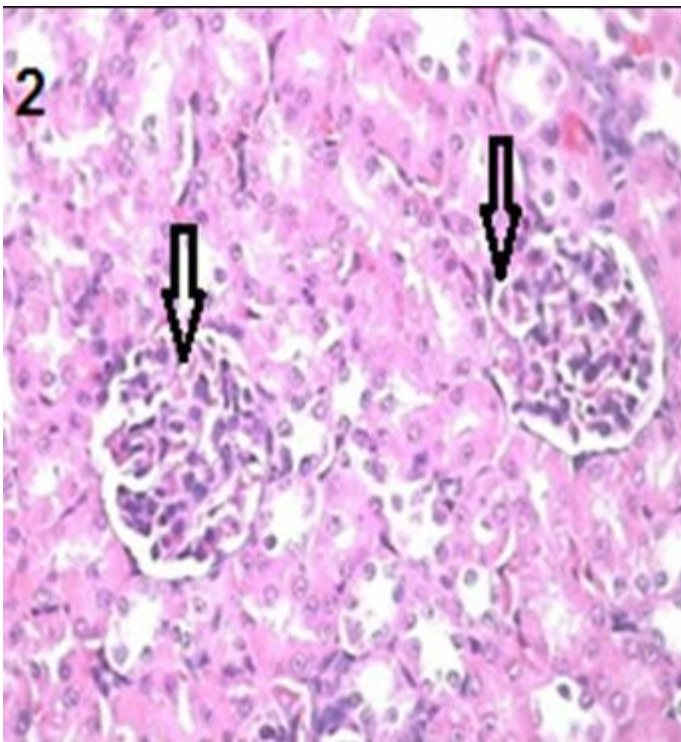


Figure 2. Histological examination of section of control rat kidney showing part of nephron cytology: Malpighian (renal) corpuscles (→) which is formed of the Glomerulus and Bowman's capsule. Proximal (▶) and distal convoluted tubule (▶) are seen H and E (x 400).

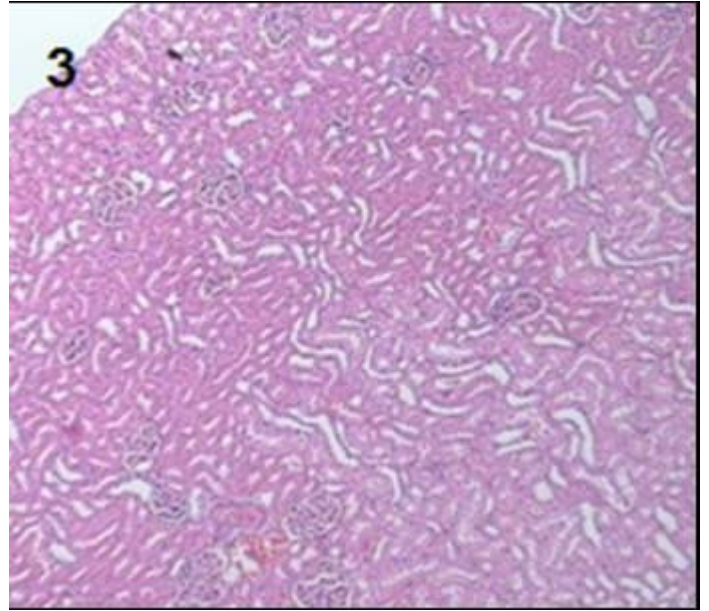


Figure 3. Histological examination of section of rat kidney of animals treated with musk only showing the normal architecture of both cortex and renal capsule H and E (x 100).

capsule. The Bowman's capsule is formed of two layers the inner visceral layer and the outer parietal layer which is formed of squamous layer separated by urinary space. Renal tubules are divided into proximal and distal convoluted tubules; and the collecting tubules, proximal convoluted tubule appeared rounded or oval in cross section lined with simple, acidophilic, cuboidal, epithelial lining cells with: large round nuclei; cell surface facing the lumen is formed of very many microvilli (brush border) that appeared intact in control group. The distal convoluted tubule had wide lumina compared to proximal convoluted tubules, and its cells has little brush border is seen because only a few short microvilli are present. Cell nuclei appeared near to the lamina of the tubules (Figures 1 and 2).

Histological examination of serial sections of control rat renal tissue showed also few changes of the normal architecture of the kidney. The changes were in the form of accumulation of RBC in the interstitial tissues and presence of some clotted substances in the lumina of the renal tubules as well a shortage of some parts of the brush borders.

Histological examination of the kidney of the treated animals with musk or sidr

Histological examination of section of control rat kidney of animals treated with musk only, or sidr only or both musk and sidr, showed that the rat kidney almost kept the normal architecture, no harmful effects occurred from those substances on kidney. Figure 3 shows the

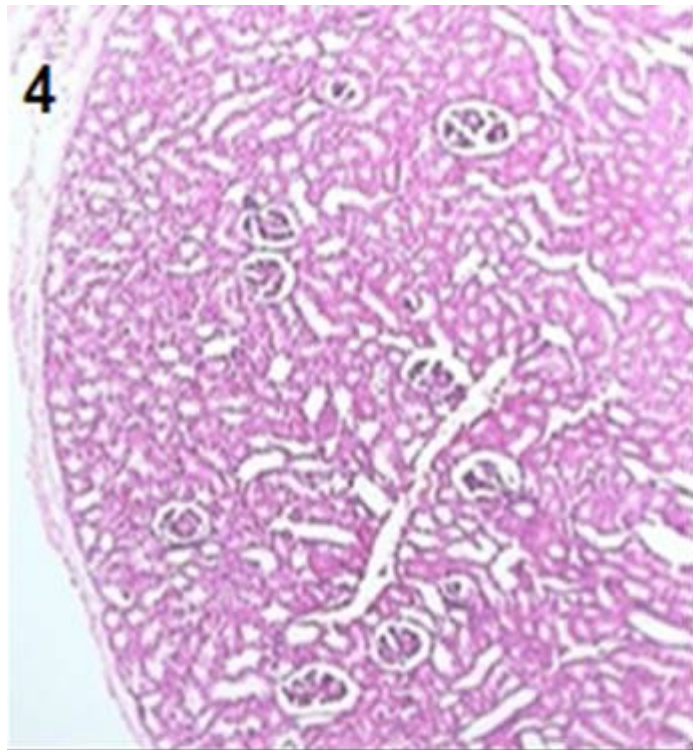


Figure 4. Histological examination of section of rat kidney of animals treated with sidr only showing the normal architecture of both cortex and renal capsule H and E (x 100).

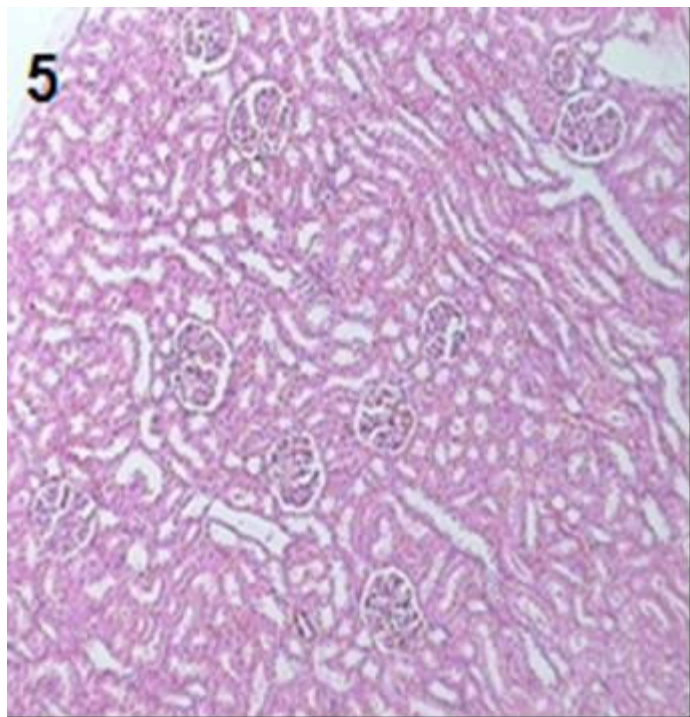


Figure 5. Histological examination of section of rat kidney of animals treated with musk and sidr showing the normal architecture of both cortex and renal capsule H and E (x 100).

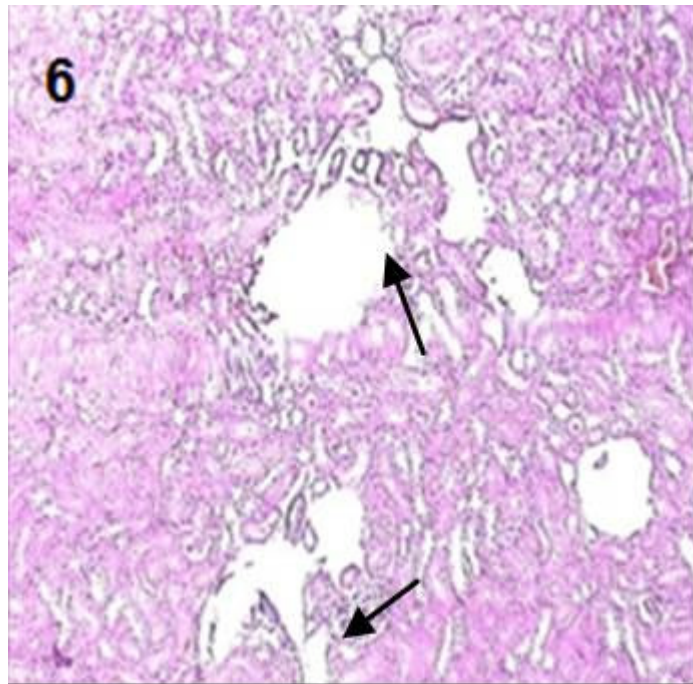


Figure 6. Section in rat kidney treated with the experimental fungus showing degeneration of normal architecture (—>) H and E (x 100).

histological examination of section of rat kidney of animals treated with musk only, Figure 4 shows part of the histological examination of section of rat kidney of animals treated with sidr only and Figure 5 shows the part of the histological examination of section of rat kidney of animals treated with both musk and sidr.

Histological examination of the kidney of the rats treated with the experimental fungus

Histological examination of section of rat kidney treated with the experimental fungus showed many changes of renal architecture compared to control one. The changes were in most component of the renal tissue in the form of degeneration (Figure 6), shrinkage of Malpighian (renal) corpuscles (Figure 7), enlargement of Malpighian (renal) corpuscles (Figure 8), fragmentation of the glomerular tuft enlarged wide spaces in Malpighian (renal) corpuscles (Figure 9) with hemorrhage and congestion between the tubules (Figure 10) and changes occurred in proximal and distal convoluted tubule are also seen as they had wide lumens which degenerated their walls (Figure 11).

Histological examination of the kidney of the rats treated with AFB1

Histological examination of sections of rat kidney treated

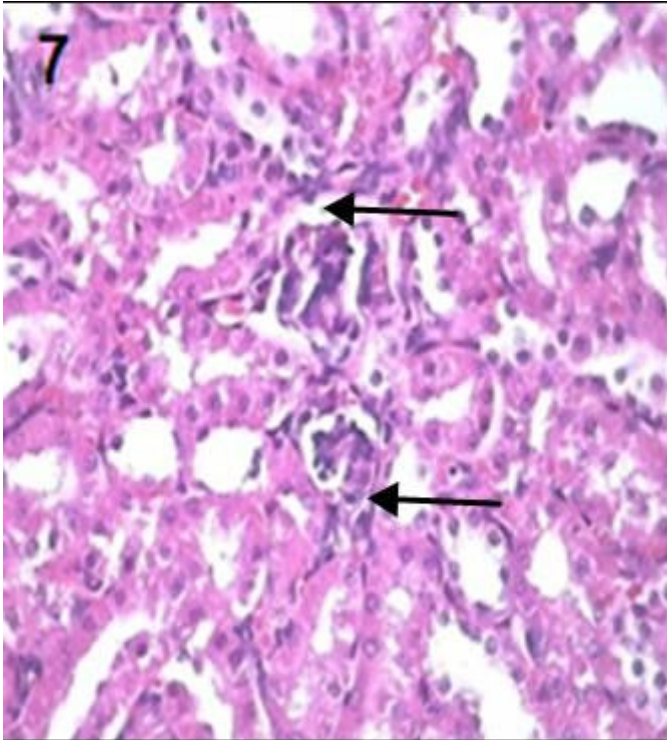


Figure 7. Section in rat kidney treated with the experimental fungus showing shrinkage of Malpighian (renal) corpuscles (—>) H and E (x 400).

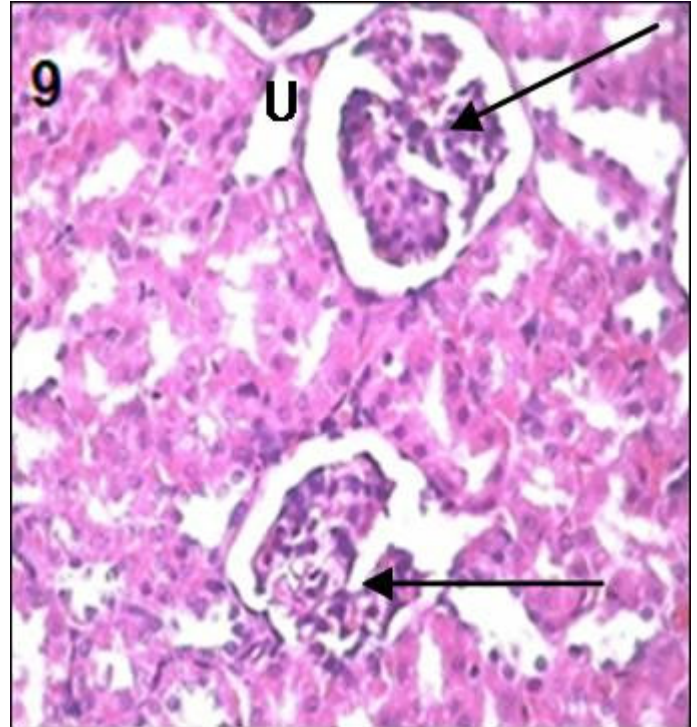


Figure 9. Section in rat kidney treated with the experimental fungus showing fragmentation of the Glomeruli (—>) and the enlarged wide spaces in Malpighian (renal) corpuscles. (U) H and E (x 400).

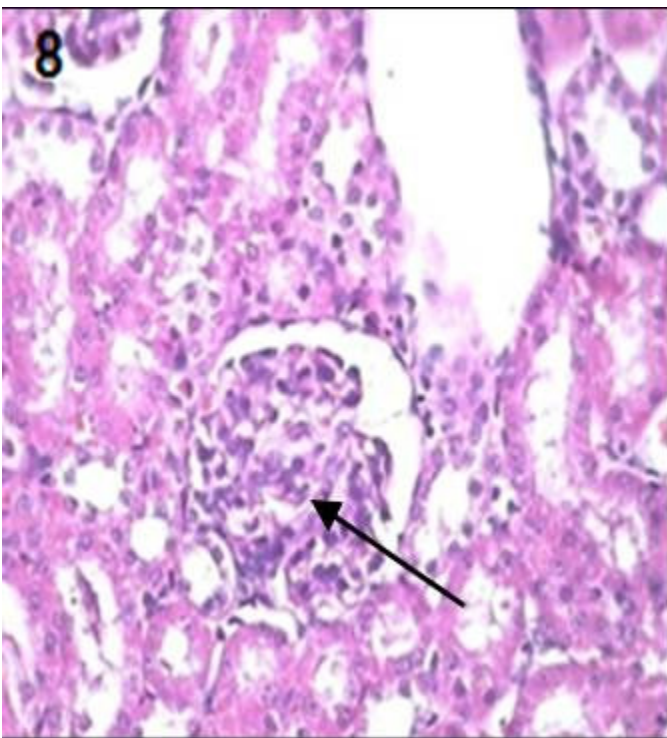


Figure 8. Section in rat kidney treated with the experimental fungus showing enlargement of Malpighian (renal) corpuscles (—>). H and E (x 400).

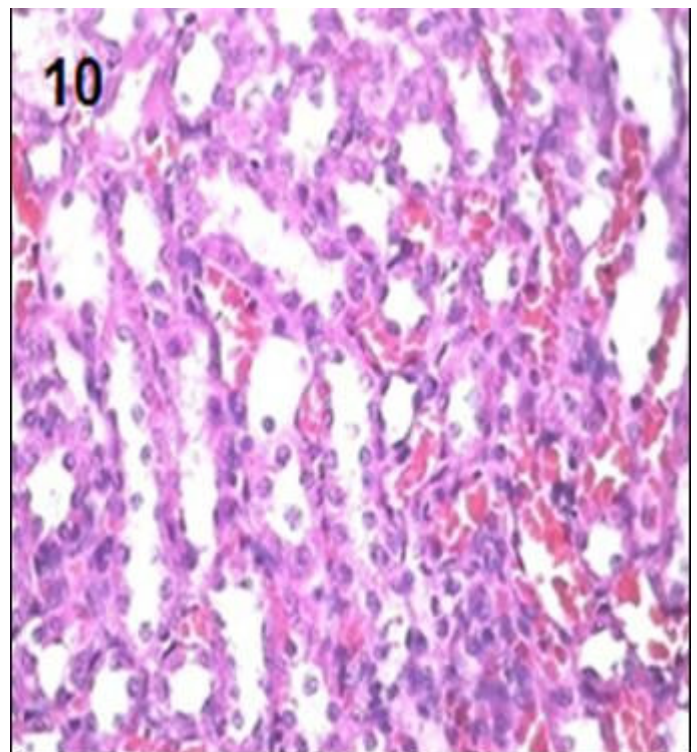


Figure 10. Section in rat kidney treated with the experimental fungus showing hemorrhage between the tubules. H and E (x 400).

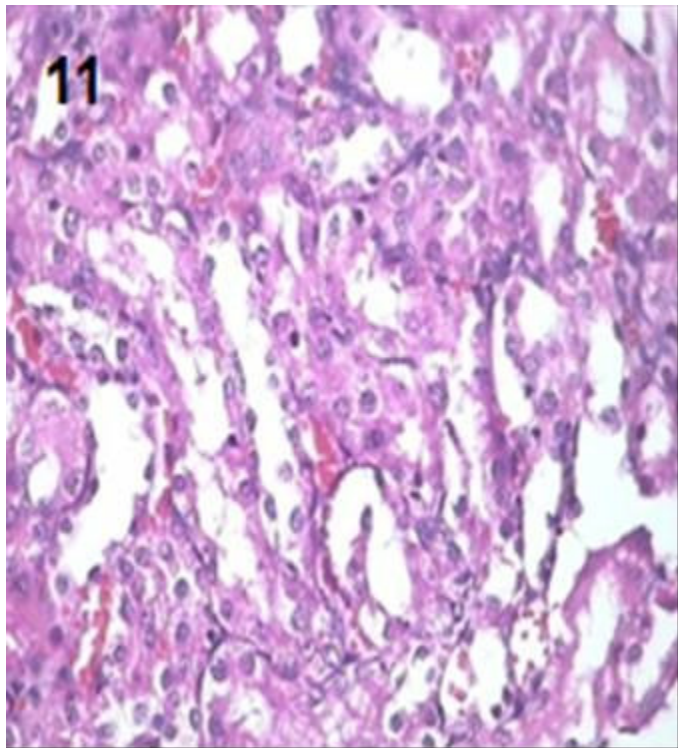


Figure 11. Section in rat kidney treated with the experimental fungus showing the changes occurred in Proximal and distal convoluted tubule H and E (x 400).

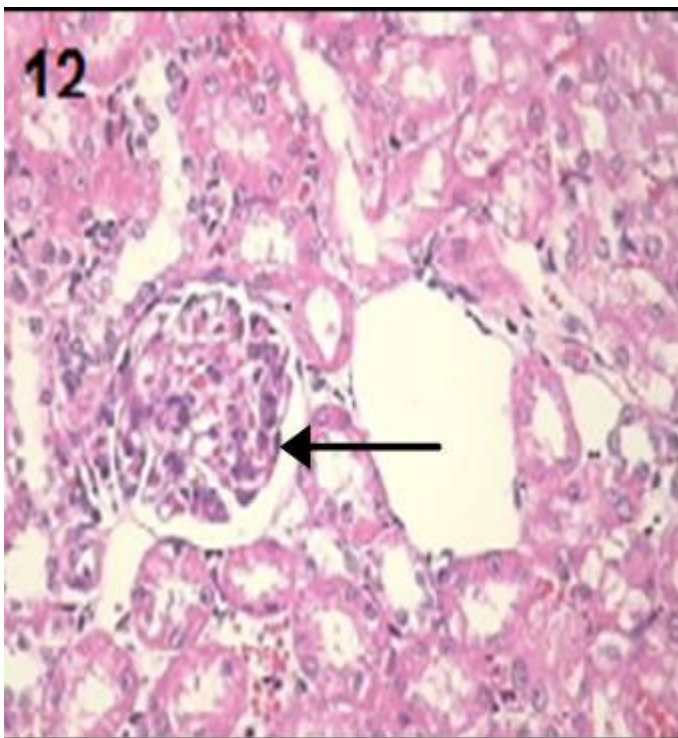


Figure 12. Section in rat kidney treated AFB1 showing degeneration (→) of Malpighian (renal) corpuscles and tubules. H and E (x 400).

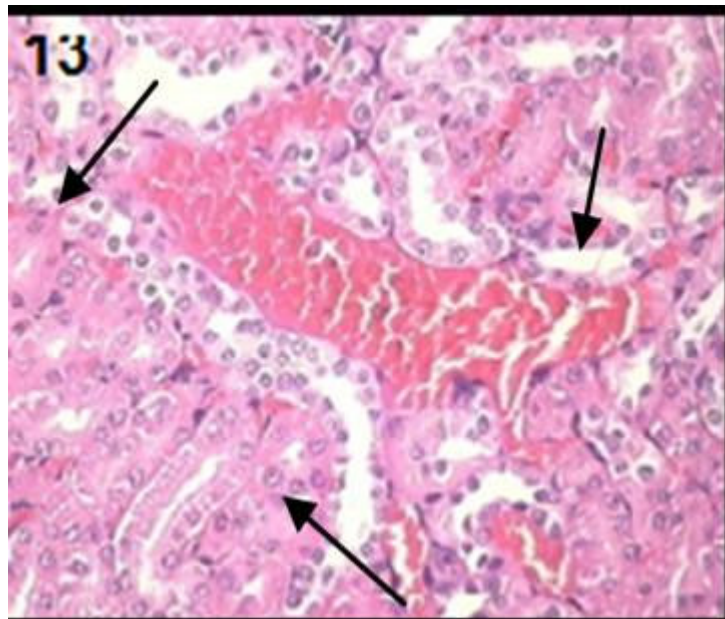


Figure 13. Section in rat kidney treated AFB1 showing areas of bleeding and congestion in renal tissues H and E (x 400).

with the AFB1 showed degeneration of normal kidney including Malpighian (renal) corpuscles and tubule (Figure 12). Histological examination of blood vessels in renal tissues showed areas of bleeding and congestion (Figure 13). Histological examination of section of rat renal tissue treated with the AFB1 showed enlarged deformed Malpighian (renal) corpuscles and degeneration of the cells forming of proximal and distal convoluted tubules (Figure 14).

Histological examination of the renal tissue of the rats treated with the experimental fungus or AFB1 and treated with musk only or sidr or both musk and sidr

Histological examination of the kidney of the rats treated with the experimental fungus or AFB1 and treated with musk only or sidr only, or treated with both musk and sidr showed efficiency of the natural products in treating both the experimental fungus or AFB1 and treated renal tissues. Rat renal tissue appeared similar to control rat tissues and almost kept the normal architecture. Figure 15 shows part of the sections in rat renal tissue of animals treated with the experimental fungus and treated with musk only showing the similar architecture to the normal kidney. Figure 16 shows part of the section in rat renal tissue of animals treated with the experimental fungus and treated with sidr only. In addition Figure 17 shows part of the section in rat renal tissue of animals treated with the experimental fungus and treated with musk and sidr. Histological examination of the renal tissue of the rats treated with the AFB1 and treated with

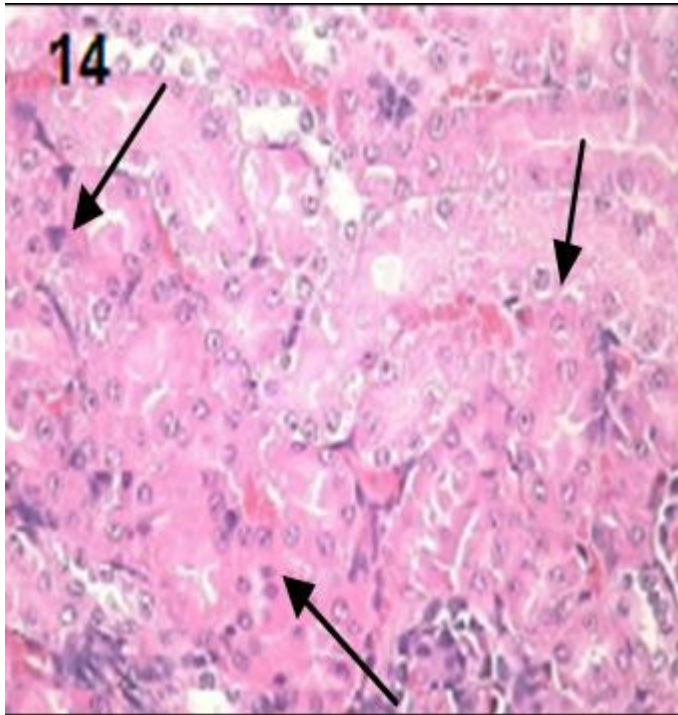


Figure 14. Section in rat kidney treated AFB1 showing degeneration of some cells of Proximal and distal convoluted tubules (→). H and E (x 400).

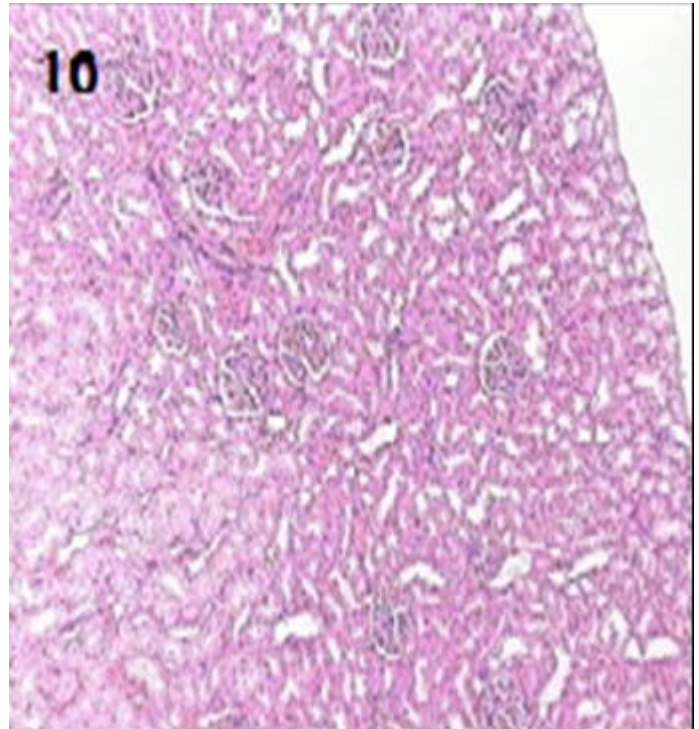


Figure 16. Section in rat kidney of animals treated with the experimental fungus and treated with sidr only showing the architecture which is similar to control. H and E (x 100).

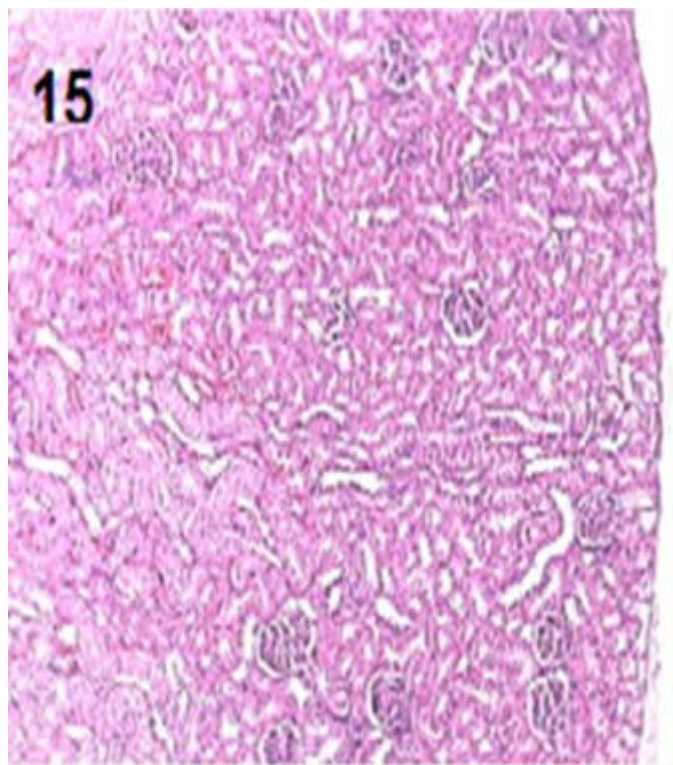


Figure 15. Section in rat kidney of animals treated with the experimental fungus and treated with musk only showing the architecture which is similar to control. H and E (x 100).

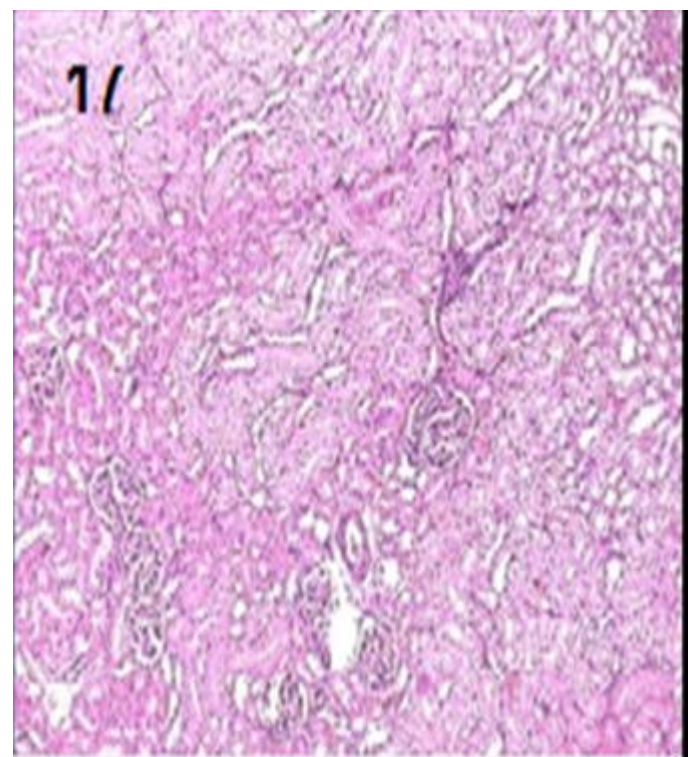


Figure 17. Section in rat kidney of animals treated with the experimental fungus and treated with musk and sidr showing the architecture which is similar to control. H and E (x 100).

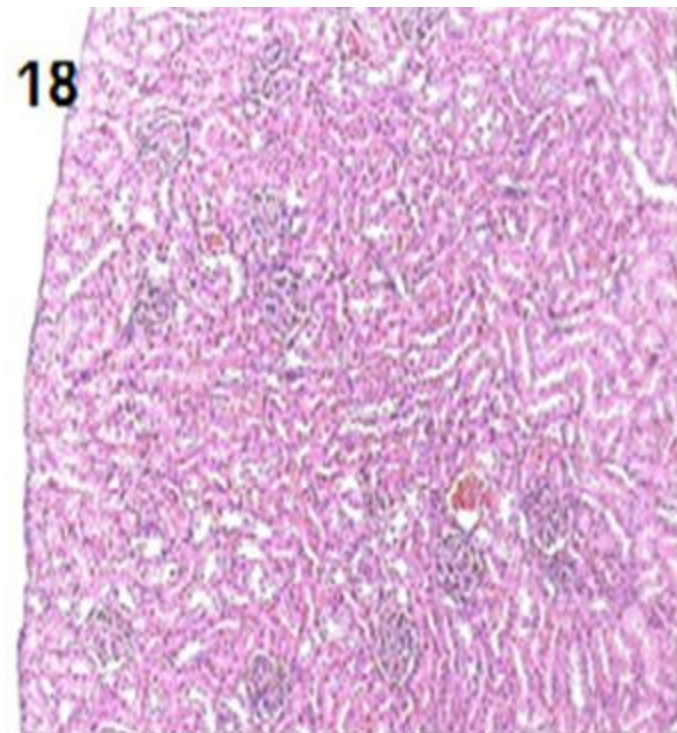


Figure 18. Section in rat kidney of animals treated with the AFBI and treated with musk only showing the architecture which is similar to control. H and E (x 100).

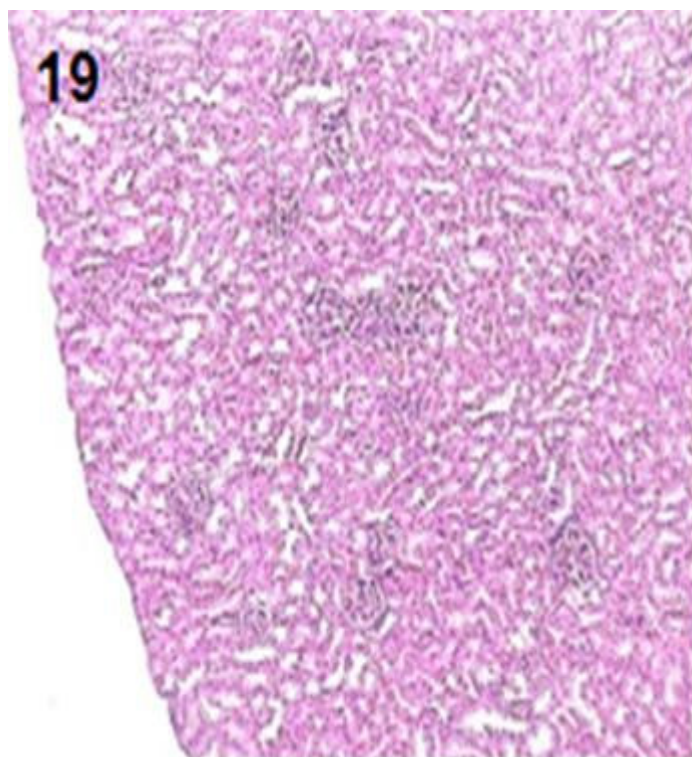


Figure 19. Section in rat kidney of animals treated with the AFBI and treated with sidr only showing the architecture which is similar to control. H and E (x 100).

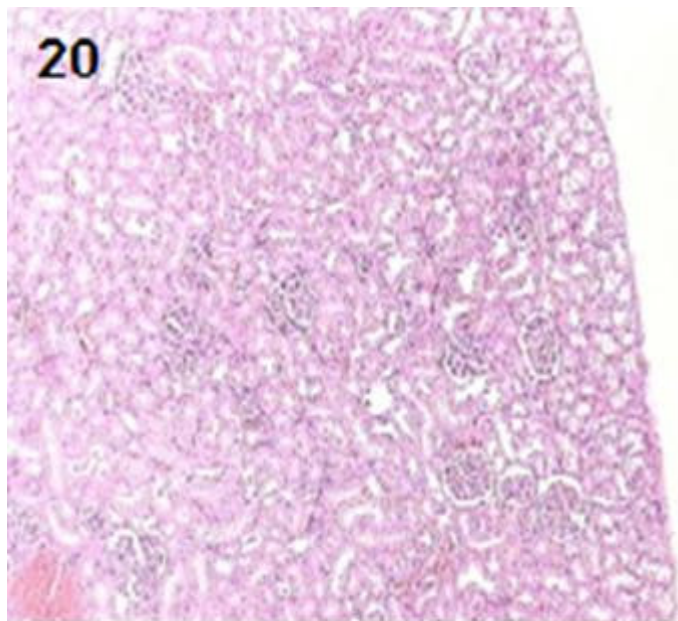


Figure 20. Section in rat kidney of animals treated with the AFBI and treated with musk and sidr showing the architecture which is similar to control. H and E (x 100).

musk only shows similar architecture to control tissues (Figure 18).

Histological examination of part of the section in renal tissue of the rats treated with the AFBI and treated with sidr only (Figure 19) and with both musk and sidr (Figure 20) showed similarity to the normal renal architecture.

DISCUSSION

In this study collection of 30 samples from Arabian nuts were collected from commercial stores and isolated the fungus from them. Plant grains and nuts are usually infected by fungi on their surface or in their subject by direct piercing of plant tissues or through natural openings such as stomatique and lenticulaire (Al-Meleeqy and Zakia, 1998). Grains and nuts such as *Corylus avellana*, *Prunus Amigdalu* and *Pistacia vera* and pepper are contamination by fungi and aflaloxins which cause food to spoil and decrease their technical use, and hence they can cause health hazards for human contaminated food with fungi and toxins (Chelkowskii, 1991; Hassan, 1999 and William et al., 2004). The present study included fungal survey of the external and internal mycoflora of four nut samples: *Prunus dulcis*, *Juglans*, *Pistacia vera* and *Anacardium occidentale*. They were collected in the period of 2008 to 2009 from four commercial different stores in Jeddah City, KSA. Results of isolations were recorded, thus most experimental histological study on rat tissue.

Aflatoxin B1 is one of the main causes of diseases and it is also carcinogenic substances that causes cancer

colon in human. Fungi cause decreases renal function and renal tissue damage, they cause decrease enzymes level that can provide protection of renal tissue damage, free supra oxidant of fatty radicals and suppress free oxygen radicals and prevent conjugation with cell components. That decrease and shortage of internal enzyme level leads to free radical formation that cause important role in impaired renal function and cause destruction of cells in rats (Kanbak et al., 2001), It was noted by Fajardo et al. (1980) that renal infection appeared when fungal toxicity appeared. Solidification of Malpighian (renal) corpuscles and blood precipitates in renal tubules. It was added by Yagmurca et al. (2004) that treatment with bee honey prevents hyper oxidation of fat and oxidation of protein and keep renal tissue damage. Moreover vacuoles and shedding of the tubules occurred. It was explained by Yalmaz et al. (2006) that fungal infection causes congestion and shedding of cells lining proximal and distal convoluted tubules.

In the present study the effect of Aflatoxin B1 and fungi on renal tissues were noted. Histological examination of the renal tissue of the rats treated with the experimental fungus or AFB1 showed degenerated, swelling and deformed cells lining proximal and distal convoluted tubules with vaculation. Degenerated of cell nuclei with protein precipitate were noted inside renal tubules. Reviw of literature showed that aflatoxin B1 lead to great changes in renal tissues and impaired renal function when animal and human were exposed to toxicity. Increased urine volume and high level of cereatenin were noted when a study on rats was performed that (Nower et al., 1983) indicated Malpighian (renal) corpuscles damage and that was also noted in the present study of the histological examination of the renal tissue of the rats. There are many histological studies that confirm the affection of rats renal tissue by aflatoxin B1 in the form of degeneration defome bleeding and congestion of swelling Malpighian (renal) corpuscles (Hegazy, 1988) that was also noted in the present study.

In the present study changes that may occur in the nuclei of the cells forming the renal tissue, also swelling vaculation on some cells and congested of blood vessel this results were confirmed with Hassen et al. (2004), Jakhar and Sadana (2004) and Raju et al. (2005) they found the scontamination with fungal toxin aflatoxin B1 or drugs caused change in fat content and congestion in gall bladder of chickens and lymphatic aggregations. It was demonstrated by Al -Hazmy et al. (2008) that taking by mouth milk contaminated with aflatoxin M1 for 6 months lead to severe damage in liver and kidney rat tissues. The damage was in the form of liver cell programmed death (apoptosis) and appearance of inclusions inside cell nuclei and enlargement of some cells, as well as the appearance of more than one nucleus that may be considered the beginning of tumor formation in liver and damage in endothelial cells lining the kidney tubules more over precipitation of proteins. In the present work

histological examination in this study of affected kidney by fungus and AFB1, treated with musk and sidr tissues showed that the kidney tissue structures were similar to the control. That was similar to the finding of Saddiq and Elyani (2009) who pointed out that the suppressive effect of hydrous extract of musk and sidr in suppression of fungal growth of *A. flavus* in microbiological experimental study on solid enriched media or on the accumulated biomass on the liquid supporad had suppressive effects on liver toxicity.

The present study was in agreement of the early findings that sidr extract containing flavinords, cumarine, mturpines, saponins, alkaloids and organic acids that (Hassanean et al., 1993; Pollman et al., 1997), have an efficient role in suppression of pathogenic *Fusarium Solani* that causes rotten cucumber roots (Sheik, 2008). On the other hand Waggas (2006) proved that sidr could be used as safe sedative that was confirmed after using sidr leaves extract which were used in neural transmission in white rat brain. The present study proved that sidr and musk extracts give an effective potency to overcome kidney toxicity. Musk and sidr extracts can be a natural safe sources of treating kidney toxicity and avoiding tissue metaplasia.

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