

RESEARCH ARTICLE

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THE EFFECTS OF ISOLATED MYCOTOXINS FROM SOME HERBAL PLANTS ON HISTOLOGY OF LIVER AND KIDNEY OF FEMALE ALBINO RATS

ABSTRACT:

Twenty-two samples of well-known herbs in Riyadh, Saudi Arabia were collected and analyzed for mycotoxins production and its effect on the liver and kidneys of tested rats was studied. One hundred and thirty-five adult female albino rats were divided into three groups where in the first group (n=110) was fed with an aqueous extract from herbal plants. The second group (n=20) was fed on with an aqueous extract of the isolated fungal species. The third group comprised the control group which was given water only and normal feeding (n=5). After 5 weeks, rats were necropsed. Kidney and liver samples were obtained for histological analysis. *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus ochraceus* were the most dominant and frequently isolated (47.3%, 46.5%, and 18.1%, respectively), followed by *Penicillium citrinum* (11.0%). *A. flavus* revealed production of 350 ug/L of aflatoxins B₁, B₂, G₁, and G₂. *A. ochraceus* produced 460 ug/L of aflatoxin B₁, B₂, G₁, and G₂ and 350 ug/L of sterigmatocystin. *A. fumigatus* produced 240 ug/L of ochratoxin A and *P. citrinum* produced 650 ug/L of aflatoxin B₁, B₂, G₁, and G₂. There was no significant histologic abnormality seen in kidney specimens taken from all groups. On the contrary, there were minimal to mild periportal lymphohistiocytic inflammation with rare foci of non-caseating granulomatous inflammation to occasional focus of lobular necrosis seen in liver specimens taken from rats fed with herbal extract and in rats fed with liquid cultures of mycotoxin-producing fungal isolates.

KEY WORDS:

mycotoxins, herbal plants, nephrotoxicity, hepatotoxicity

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INTRODUCTION:

Mycotoxins are toxic fungal metabolites that are produced by a diverse group of fungi. This has been confirmed in several studies (Aziz *et al.*, 1998; Halt, 1998; Abou-Arab *et al.*, 1999; Efuntoye, 1999; Costa and Seussel, 2002; Elshafie *et al.*, 2002). The most common mycotoxins are produced by fungi in the genera; *Aspergillus*, *Penicillium* and *Fusarium* (Robens and Richard, 1992).

Aflatoxins are the most notorious of the fungal toxins. (Shepard 2008) These compounds not only produce acute toxic syndromes but are also carcinogenic. Ochratoxin A and citrinin has been associated with porcine nephropathy (Pfohl-Leszkwicz *et al.*, 2007) In humans, Balkan nephropathy has been suggested as a consequence of ingestion of fungal toxins (Long and Voice, 2007) In rat model experiments, altered renal function with increased urine protein and glucose were observed after repeated injections of mycotoxins (Rached *et al.*, 2008).

Ochratoxin A induced liver injury involves a reduction in the ability to counterbalance oxidative stress leading to altered gap junction intercellular communication and loss of cell adhesion and polarity. The mild oxidative damage in combination with other cytotoxic effects triggers the promotion of liver tumors after exposure to ochratoxin A (Gagliano *et al.*, 2006). The cancer-producing capability of the aflatoxins has been demonstrated in laboratory animals and is suggested from epidemiologic data to occur in man. Aflatoxin B₁ decreases serum proteins, which are sensitive early indicators of hepatotoxicity (Quezada *et al.*, 2000). The most important mycotoxins are aflatoxins which are hepatotoxic and hepatocarcinogenic, and ochratoxins, which are nephrotoxic and nephrocarcinogenic (Rastogi *et al.*, 2001; Orsi *et al.*, 2007). This study was conducted to isolate and evaluate fungal contamination of some herbal plants and to extract the Mycotoxin from these herbal plants, as well as to evaluate the effects of Mycotoxin on the histology of liver and kidney of female albino rats.

MATERIAL AND METHODS:

This experiment was conducted at the laboratory of Biology Department of University of Nora Bent Abdulrahman, Riyadh, Saudi Arabia in February and March 2008.

Herbal plants sampling:

Twenty-two samples of well-known plant herbs from random herb markets in the city of

Riyadh, Saudi Arabia were collected between February and March 2008.

The herbal products were chosen on the basis of their commercial availability and popularity of use (Table 1). Every sample weighed 3 kilograms and was preserved in clean plastic bags at a temperature ranging from 4° to 5°C.

Table 1. Herbal plants analyzed

Common Name	Used part	Scientific name	Known use
1. Aloe	Gum	<i>Aloe vera</i>	For wound and burns
2. Anise	Seeds	<i>Pimpinella anisum</i>	For digestion, anti-bloating
3. Caraway	seeds	<i>Carum carvi</i>	For digestive disorders
4. Chamomile	flowers	<i>Matricaria chamomilia</i>	For digestive ailments
5. Cinnamon	The bark of trees	<i>Cinnamomum zeylanicum</i>	Food flavoring, spice
6. Cumin	seeds	<i>Cuminum cyminum</i>	Stimulant, antimicrobial
7. Dill	seeds	<i>Anethum graveolens</i>	For insomnia
8. Ducrosia	leaves	<i>Ducrosia anethifolia</i> (D C.)Boiss	CNS depressant
9. Dymock	Gum	<i>Astralagus sarcocolla</i>	To boost immune system
10. Fennel	seeds	<i>Foeniculum vulgare</i>	Analgesic, anti-inflammatory
11. Fennel flower plant	seeds	<i>Nigella sativa</i>	For cancer prevention
12. Garden cress	seeds	<i>Lepidium sativum</i>	Prevent post-natal complications
13. Garden sage	leaves	<i>Salvia officinalis</i>	Antiseptic, estrogenic
14. Ginger	rhizom	<i>Zingiber officinale</i>	Arthritis, rheumatism
15. Green mist	seeds	<i>Ammi visnaga</i>	For kidney stones, antispasmodic
16. Hassaniya (in Arabic)	whole plant	<i>Calligonum comosum</i>	Anti-inflammatory, anti-ulcer
17. Hulls	the coffee 'bean	<i>Coffea arabica</i>	Coffee drink
18. White wormwood	whole plant	<i>Artemisia herba alba</i>	Anti-oxidant
19. Myrrh	Gum	<i>Commiphora myrrah</i>	Incense
20. Fenugreek	seeds	<i>Trigonella foenum</i> <i>Trigonella foenum-graecum</i>	Lowers blood pressure, carminative, increases milk production and/or flow, depresses the central nervous system, cardiogenic, aphrodisiac, hypoglycemic, diuretic, hemostatic, antiinflammatory, emollient, and rids the body of impurities and toxins
21. Thyme	leaves	<i>Thymus serpyllum</i>	Aphrodisiac
22. Mixed herbs of chamomile, cumin, cinnamon, ammi, anise, thyme, mung beans, caraway seeds			

*Reference: www.wikipedia.org/wiki/list_of_plants_used_as_medicine
<http://www.impgc.com/index.php> (Indian Medicinal Plants Growers' Consortium)

Isolation and evaluation of fungal contamination:

The fungal flora of each sample was detected by using the dilution plate method according to Van-Walbeek *et al.* (1968). Two types of media were used: 1- Glucose-Czapeck's agar medium in which glucose (10 gm/L) replaced sucrose and 2- potato dextrose

agar medium, chloramphenicol (20 ug/ml) and Rose Bengal (30 ppm) were added to suppress bacterial growth. Every sample was examined for total fungi count (*Aspergillus* and *Penicillium*). Five grams of each sample were mixed with 45 ml of distilled water from which tenfold serial dilution were made. Three ml from each dilution was inoculated in sterile

petri dishes and mixed well. Three culture plates were prepared for each treatment. Plates were incubated upside down at $26 \pm 2^\circ\text{C}$ for 7 days. After incubation, the fungal colonies were counted, recorded and the colony-forming units (cfu) per gram were calculated. The fungal isolates were stored at 25°C . Identification was performed by cultural and morphological characteristics (NMKL, 2005).

1- Extraction of mycotoxins from of fungal isolates:

Aflatoxin was extracted using SMKY (sucrose 20 gm/L, magnesium sulphate 0.59 gm/L, potassium nitrate 3 gm/L, yeast extract 7 gm/L) medium (Bugno *et al.*, 2006). Twenty-five grams of each herbal sample was shaken with 50 ml of chloroform in 250 ml flask for 24 hours. The defatted residue was re-extracted for another 24 hours in a shaker with 50 ml chloroform. Chloroform extracts were combined, washed with an equal volume of distilled water, dried over anhydrous sodium sulfate, filtered, then concentrated and left to dry. The dried materials were transferred to vials with small amount of chloroform which was evaporated to near dryness. The analysis of extract for the detection of different aflatoxins was performed using the HELICA Total Aflatoxin Assay (Hellica Biosystems, Fullerton, California, USA). An aflatoxin specific antibody optimized to cross react with all four subtypes of aflatoxin was coated to a polystyrene microwell. Toxins were extracted from a ground sample with 70% methanol. The extracted sample and HRP-conjugated aflatoxin B₁ were mixed and added to the antibody-coated microwell. Aflatoxin from the extracted sample and HRP-conjugated aflatoxin B₁ compete to bind with the antibody coated to the microwell. Microwell contents were decanted and non-specific reactants are removed by washing. An enzyme substrate (TMB) was added and color (blue) develops. The intensity of the color was directly proportional to the amount of bound conjugate and inversely proportional to the concentration of aflatoxin in the sample or standard. Therefore, as the concentration of aflatoxin in the sample or standard increases, the intensity of the blue color will decrease. An acidic stop solution is added which changes the chromagen color from blue to yellow. The microwells were measured optically by a microplate reader with an absorbance filter of 450nm (OD₄₅₀). The optical densities of the samples were compared to the OD's of the kit standards and an interpretative result is determined. Ochratoxin A was extracted by acetonitrile-4 % KClaq (9:1), separated by migration in toluene- ethyl acetate- formic acid (5:4:1, v/v/v) and quantified by fluorimetry at 33 nm (Boudra, 1995). sterigmatocystin was extracted by The compound exhibits a dull brick red fluorescens under short wave UV light. Fluorescence change to yellow on spraying with aluminum chloride solution (20 g Al Cl₃ 6H₂O in 100 ml ethanol) and the plates heated at 100°C for 5

minutes (Josefsson and Moller, 1977). Mycotoxins were identified by comparison with appropriate standards for aflatoxin and ochratoxin A. (Supelco, USA) according to AOAC (1984).

2- Evaluation of mycotoxins from herbal plants on kidney and liver of test rats:

To evaluate the nephrotoxic and hepatotoxic effects of mycotoxins extracted from plants sample, 135 adult female albino rats weighing between 130-160 grams were procured from the Pharmacy College of King Saud University in Riyadh, Saudi Arabia. The animals were housed in well ventilated aluminum cages with a temperature of 18° to 25°C at the animal lab of King's Khalid University Hospital, Riyadh, Saudi Arabia. All rats were maintained on a standard pellet diet (mouse breeding diet, Pillsbury Ltd., Birmingham, UK) and tap water. Pellet diet's overall nutrient composition was 36.2% carbohydrate, 20.9% protein, 4.4% fat, 38.5% fiber with added vitamins and minerals and with a metabolizable energy content of 1.18 MJ/100 g. Test rats were grouped into 3 as follows:

Group 1: (110 rats) In addition to feeding with standard pellet diet, each herbal plant was assigned 5 rats of which were given water extracts of each herb. The herbal extract was prepared by using 1.5 mg per 1 ml of the herbs as the solute and sterile hot water as the solvent. The solution was mixed well and was placed in sterile glass jars with a firmly closed lid. The herbal extract was left for a couple of hours to cool down and settle before it was filtered. Fifty milliliters were taken from every filtered water extract and was given orally to female albino rats for 5 weeks.

Group 2: (20 rats) In addition to feeding with standard pellet diet, rats were given liquid cultures of fungi that were isolated and found to produce mycotoxins. These liquid cultures were filtered and were placed in sterile glass jars with a firmly closed lid. Aliquots of 50 ml were given to the test rats for 5 weeks.

Group 3: Control group composed of five (5) rats which were given normal drinking water aside from feeding with standard pellet diet. No herbal extract or fungal isolate was given to this group.

After five weeks, rats were necropsed and tissue specimens from the liver and kidneys were collected immediately after slaughter. The tissue samples were fixed in 10% formalin. Tissue samples were embedded in paraffin wax and prepared for cutting at 5 μm , stained with hemtaoxylin and eosin and examined microscopically for histopathological changes and features.

RESULTS:

Fungal contaminants and their frequency of distribution in herbal plants: _

Table 2 presents the frequency distribution of total fungal (*Aspergillus* and *penicillium*) counts in the 25 herbal plants analyzed. The results shown in table 3 revealed that 39.1% of the samples exceeded

the limit determined by the US Pharmacopoeia. *Calligonum comosum* (2×10^5 cfu/g), grained mixed herbs (24×10^3 cfu/g) and *Salvia officinalis* (23×10^3 cfu/g) were the most contaminated samples. The predominant mycoflora obtained was distributed in 4 genera (Table 2). The genus *Aspergillus* was the most dominant (142 isolates) followed by

Penicillium (14 isolates) and these two genera were found in 85.0% and 11.0% of the samples analyzed. *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus ochraceus* were the most dominant and frequently isolated (47.3%, 46.5% and 18.1%, respectively), followed by *Penicillium citrinum* (11.0%).

Table 2. Frequency distribution of total *Aspergillus* and *Penicillium* in 25 herbal plants analyzed

Common Name	Fungal isolates	total Asp spp (cfu/g)
1. Aloe	<i>Aspergillus fumigatus</i>	3×10^1
2. Anise	<i>Aspergillus flavus</i>	9×10^2
3. Caraway	<i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i>	18×10^1
4. Chamomile	<i>Aspergillus flavus</i>	17×10^2
5. Cinnamon	<i>Aspergillus flavus</i>	9×10^1
6. Cumin	<i>Aspergillus fumigatus</i>	4×10^1
7. Dill	<i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i>	64×10^2
8. Ducrosia	<i>Aspergillus flavus</i>	25×10^1
9. Dymock	<i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i>	21×10^1
10. Fennel	<i>Aspergillus fumigatus</i> , <i>Penicillium citrinum</i>	13×10^1
11. Fennel flower plant	<i>Aspergillus fumigatus</i>	32×10^1
12. Garden cress	None isolated (often used as antibiotic)	-
13. Garden sage	<i>Aspergillus fumigatus</i> , <i>Aspergillus ochraceus</i>	23×10^3
14. Ginger	<i>Aspergillus fumigatus</i> , <i>Penicillium citrinum</i>	23×10^1
15. Green mist	<i>Aspergillus fumigatus</i>	5×10^1
16. Hassaniya (in Arabic)	<i>Aspergillus fumigatus</i>	2×10^5
17. Hulls	<i>Aspergillus flavus</i>	50×10^1
18. Myrrh	None isolated (often used as antibiotic)	-
19. Fenugreek	<i>Aspergillus ochraceus</i>	21×10^1
20. Thyme	<i>Aspergillus ochraceus</i>	18×10^2
21. White wormwood	<i>Aspergillus ochraceus</i>	12×10^1
22. Mixed herbs	<i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i>	24×10^3

Cfu: Colony forming units

3- Mycotoxin production by isolated fungal strains:

The three strains of *Aspergillus* and a strain of *Penicillium* isolated were evaluated for their ability to produce aflatoxins B₁ and B₂ and ochratoxin A. The total number of isolates, toxigenic isolates and types of mycotoxins they produce are presented in table 3. *A. flavus* revealed production of 350 ug/L of aflatoxins B₁, B₂, G₁ and G₂. *A. ochraceus* produced 460 ug/L of aflatoxin B₁, B₂, G₁ and G₂ and 350 ug/L of sterigmatocystin. *A. fumigatus* produced 240 ug/L of ochratoxin A and *P. citrinum* produced 650 ug/L of aflatoxin B₁, B₂, G₁ and G₂.

Table 3. Mycotoxins concentration on isolated fungal species

Fungal isolates	No. of isolates	Mycotoxins	Conc (ug/L)
<i>Aspergillus flavus</i>	2	Aflatoxin B ₁ , B ₂ , G ₁ & G ₂	350
<i>Aspergillus ochraceus</i>	4	Aflatoxin B ₁ , B ₂ , G ₁ , G ₂ and sterigmatocystin	460
<i>Aspergillus fumigatus</i>	15	Ochratoxin A	240
<i>Penicillium citrinum</i>	8	Aflatoxin B ₁ , B ₂ , G ₁ & G ₂	650

4- Mycotoxic effects on liver and kidneys: Effects on the Kidneys:

There was no significant histological abnormalities were seen in the kidney

specimens taken from rats fed with herbal extract (Group 1). However, 75 of 110 (68%) kidney specimens from this group showed mild patchy mononuclear inflammation. On the other hand, rats fed with liquid cultures of mycotoxin-producing fungal isolates (Group 2), mild patchy mononuclear inflammation was seen in all 15 kidney samples. There was also presence of yellowish brown pigment deposition seen in the hilar lymph nodes (Fig. 1). Kidneys from control group showed no significant changes except for mild focal chronic interstitial inflammation (Fig. 2).

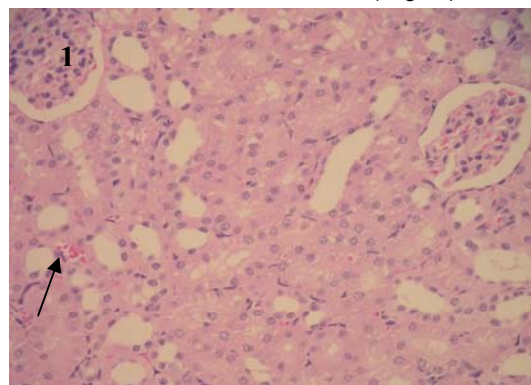


Fig. 1. Photomicrograph of a T. S. of the control Kidneys showing mild focal chronic interstitial inflammation only (H&E x200).

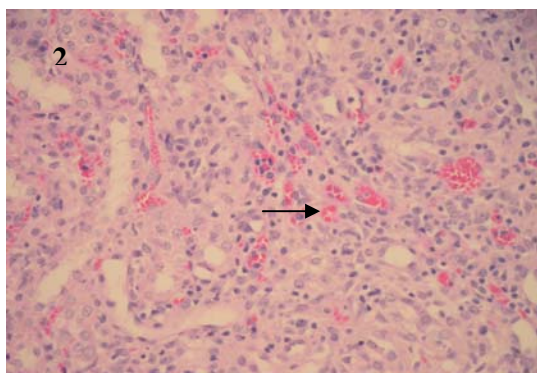


Fig. 2. Photomicrograph of a T. S. of the Kidney showing mild patchy mononuclear inflammation and the presence of yellowish brown pigment deposition seen in the hilar lymph nodes (H&E x200).

Effects on the Liver:

There were minimal to mild periportal lymphohistiocytic inflammation with rare foci of non-caseating granulomatous inflammation (Fig. 3) seen in liver specimens taken from rats fed with herbal extract (Group 1). On the other hand, in rats fed with liquid cultures of mycotoxin-producing fungal isolates (Group 2), mild periportal lymphohistiocytic infiltrates with patchy lobular inflammation were seen in all 15 liver samples (Fig. 4).

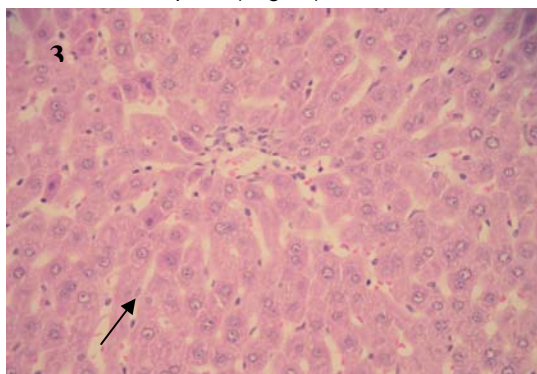


Fig. 3. Photomicrograph of a section of the Liver of control group showing mild portal chronic inflammation composed of lymphohistiocytes and few plasma cells (H&E x400).

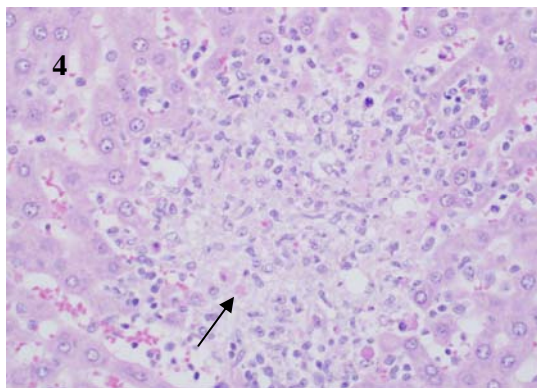


Fig. 4. Photomicrograph of a section of the Liver showing granuloma formation with multinucleated giant cells (H&E x400).

In addition to this, occasional small groups of histiocytes in the lobule with occasional focus of lobular necrosis were seen in group 2 rats. Liver samples from control group showed mild portal chronic inflammation composed of lymphohistiocytes and few plasma cells (Fig. 5).

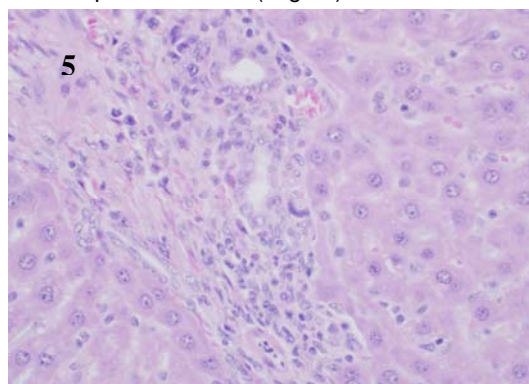


Fig. 5. Photomicrograph of a section of the Liver showing periportal inflammation (H&E x400).

DISCUSSION:

In the present study, *A. fumigatus* was the predominant fungal species in concordance with previous reports. (Halt *et al.*, 1998; Efuntoye, 1999). Aflatoxin production was evident in the present results and ranged from 350 ug/L produced by *A. flavus* to as much as 650 ug/L by *P. citrinum* as previous reports (Mislivec *et al.*, 1968; Bugno *et al.*, 2006). Ochratoxin A was produced in concentration of 240 ug/L by *A. fumigatus*. *A. ochraceus* also produced 350 ug/L of sterigmatocystin. Supported by the evidence of liver histological changes, these mycotoxins proved deleterious to humans. Aflatoxins decrease native resistance to disease and this phenomenon may cause vaccines to fail. Liver dysfunction can cause a decreased in blood clotting factors. Sterigmatocystin as produced by *A. ochraceus* in this study is considered carcinogenic and causes liver damage. (Robens and Richard, 1992). Clinical signs of bloody diarrhea and deaths have been reported. It is a precursor in the synthetic pathway for aflatoxins. On the other hand, aflatoxins are more toxic hepatotoxins. They are also carcinogenic and suppress the natural immunity to infection. Although, the mechanism of cellular damage caused by AFB₁ has not been fully elucidated, lipid peroxidation may be one of the main manifestations of oxidative damage with marked increases in lipid peroxide levels and a concomitant decrease in enzymic antioxidant levels.

The very little effect of these mycotoxins in the kidney has been a subject of debate. There is little doubt that it can produce alterations in renal function. (Peraica *et al.*, 2008; Mally, 2009) In a previous report, the

creatinine and urea levels were consistently high in tested rats which may explain the nephrotoxic potential of the mycotoxins present in these herbs (Berndt *et al.*, 1980). The histologic picture of tubular necrosis indicates classical acute tubular necrosis associated with many nephrotoxins (Marin-Kuan, 2007). Such lesions occur regardless of the chemical type of nephrotoxin involved, a result that has been suggested to be consistent with a disruption of vascular events within the kidney. Unfortunately though, the present study did not reveal specific and more detailed changes in the kidneys as an effect of these mycotoxins. Moreover, preliminary studies suggest the possibility of potentiation of the effect of one of these fungal toxins by

the other. It was suggested that a possible inhibitory effects of combined doses of fungal toxins that were ineffective when present alone (Hussain, 2009).

CONCLUSION:

This study confirms the capability of these fungal species in the production of mycotoxins with induction of hepatotoxicity. Nephrotoxic effects of these mycotoxins may be minimal but alterations in renal function may be evident with long-term administration or feeding experiments to establish the possible potentiation effects of these mycotoxins not only on renal function but on hepatic function as well.

REFERENCES:

- Abou-Arab AAK, Kawther MS, El-Tantawi ME, Badaea RI, Khayria N. 1999. Quantity estimation of some contaminants in commonly used medicinal plants in the Egyptian market. *Food Chem.*, 67: 357-363
- AOAC. 1984. Association of official Analytical Chemist official Methods of Analysis (13rd Ed.) Washington, Dc. pp. 480-494
- Aziz NH, Youssef YA, El-Fouly MZ, Moussa LA. 1998. Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins. *Bot. Bull. Acad. Sinica*, 39: 279-285
- Berndt WO, Hayes AW, Phillips RD. 1980. Effects of mycotoxins on renal function: mycotoxin nephropathy. *Kidney Int.*, 18: 656-664
- Boudra H, Le Bars P, Bars JLe. 1995. Thermostability of ochratoxin A in wheat under two moisture conditions. *Appl. Environ. Microbiol.*, 61(3): 1156-1158.
- Bugno A, Almodovar AB, Pereira TC, Pinto TJA, Sabino M. 2006. Occurrence of toxigenic fungi in herbal drugs. *Braz. J. Microbiol.*, 37: 47-51
- Costa LLF, Seussel VM. 2002. Toxigenic fungi in beans classes black and color cultivated in the state of Santa Catarina, Brazil. *Braz. J. Microbiol.*, 33: 138-144
- Efuntoye MO. 1999. Mycotoxins of fungal strains from stored herbal plants and mycotoxin contents of Nigerian crude herbal drugs. *Mycopathology*, 147: 43-48
- Elshafie AE, Al-Rashdi TA, Al-Bahry SN, Bakheit CS. 2002. Fungi and aflatoxins associated with spices in the Sultanate of Oman. *Mycopathologia*, 155: 155-160.
- Gagliano N, Donne ID, Torri C, Migliori M, Grizzi F, Milzani A, Filippi C, Annoni G, Colombo P, Costa F, Ceva-Grimaldi G, Bertelli AAE, Giovannini L, Gioia M. 2006. Early cytotoxic effects of Ochratoxin A in rat liver: a morphological, biochemical and molecular study. *Toxicology*, 15: 214-224.
- Halt M. 1998. Moulds and mycotoxins in herb tea and medicinal plants. *Eur. J Epidemiol.*, 14: 269-274
- Hussain S, Khan MZ, Khan A, Javed I, Asi MR. 2009. Toxicopathological effects in induced by concurrent exposure to aflatoxin and cypermethrin. *Toxicon*, 53: 33-41.
- Josefsson BGE, Moller TE. 1977. Screening method for the detection of aflatoxins, ochratoxin, patulin, sterigmatocystin, and zearalenone in cereals. *J. Assoc. Anal. Chem.*, 60: 1369-1371.
- Long DT, Voice TC. 2007. Role of exposure analysis in solving the mystery of Balkan endemic nephropathy. *Croat Med. J.*, 48: 300-311
- Mally A, Dekant W. 2009. Mycotoxins and the kidney: modes of action for renal tumor formation by ochratoxin A in rodents. *Mol. Nutr. Food Res.*, 53: 467-478
- Marin-Kuan M, Nestler S, Verguet C, Bezencon C, Piguet D, Delatour T, Mantle P, Cavin C, Schilter B. 2007. MAPK-ERK activation in kidney of male chronically fed ochratoxin A at a dose causing a significant incidence of renal carcinoma. *Toxicol. Appl. Pharmacol.*, 224: 174-181.
- Mislivec PB, Huvter JH, Tuite J. 1968. Assay for Aflatoxin Production By the Genera *Aspergillus* and *Penicillium*. *Appl. Microbiol.*, 7(16): 1053-1055.
- NMKL. 2005. Mould and yeasts determination in food .Nordic Association of Agricultural Scientists. No. 98. 9th Edn. Nordic Committee on Food Analysis, Oslo, Norway
- Orsi RB, Oliveira CA, Dilkin P, Xavier JG, Direito GM, Correa B. 2007. Effects of oral administration of aflatoxin B1 and fumonisin B1 in rabbits. *Chem. Biol. Interact.*, 170: 201-208.
- Peraica M, Domijan AM, Miletic-Medved M, Fuchs R. 2008. The involvement of mycotoxins in the development of endemic nephropathy. *Wien. Klin. Wochenschr.*, 120(13-14): 402-407
- Pfohl-Leskowicz A, Manderville RA. 2007. Ochratoxin A: an overview on toxicity and carcinogenicity in animals and humans. *Mol. Nutr. Food Res.*, 51: 61-99.
- Quezada T, Cuellar H, Jaramillo-Juarez F, Valdivia AG, Reyes JL. 2000. Effects of aflatoxin B1 on the liver and kidney of broiler chickens during development. *Pharm. Toxicol. Endocrinol.*, 125: 265-272.
- Rached E, Hoffmann D, Blumbach K, Weber K, Dekant W, Mally A. 2008. Evaluation of putative biomarkers of nephrotoxicity after

- exposure to ochratoxin A in vivo and in vitro. Toxicol. Sci., 103: 371-381.
- Rastogi R, Srivastava AK, Rastogi AK. 2001. Long term effect of aflatoxin B1 on lipid peroxidation in rat liver and kidney: effect of picroliv and silymarin. Phytoter. Res., 15: 307-310.
- Robens JF, Richard JL. 1992. Richard Aflatoxins in animal and human health. Rev Environ Contam. Toxicol., 127: 69-94.
- Shephard GS. 2008. Risk assessment of aflatoxins in food in Africa. Food Addit. Contam. 25(10): 1248-1258.
- Van-Walbeek W, Scolt P, Totcher F. 1968. Mycotoxins from Food Borne Fungi. J. Microbiol., 14: 131-137.

تأثير السموم الفطرية المعزولة من بعض الاعشاب الطبية على التركيب النسيجي لكبد وكلى اناث الجرذان البيضاء مشاعل المغيريج، سعاد الوكيل

جامعة الاميرة نورة بنت عبدالرحمن، كلية العلوم، قسم الاحياء، الرياض المملكة العربية السعودية

الفطر *A. fumigatus* انتج (240 ug/L) من *Penicillium. citrinum* (650 ug/L) واعطى ochratoxin A من *aflatoxin B₁, B₂, G₁ G₂* وبالفحص النسيجي لعينات الكبد والكلى لم يلاحظ وجود أي مظاهر نسيجية غير طبيعية في عينات الكلى التي أخذت من جميع مجموعات البحث . أما في عينات الكبد فقد وجدت التهابات بسيطة الى متوسطة حول القناة المحمولة بالكبد مع بؤر نادرة من الالتهابات الحبيبية و أحيانا بؤر من الفصيصات الكبدية الميتة وذلك في عينات الكبد التي أخذت من الجرذان التي تمت تغذيتها على مستخلص الأعشاب و المزارع السائلة لمستخلص الفطر المنتجة للسموم .

المحكمون:

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استخدم في هذه الدراسة 22 عينة من اعشاب طبية تتداول في محلات العطارة في مدينة الرياض لتقديرالسموم الفطرية فيها وتأثيرها على التركيب النسيجي لكل من كبد وكلى اناث الجرذان البيضاء. تم في هذا البحث استخدام (135) من اناث الجرذان البيضاء حيث قسمت الى ثلاث مجموعات (المجموعة الاولى وعددها 110) زودت بالمستخلص المائي للأعشاب الطبية، و(المجموعة الثانية وعددها 20 جرد) زودت بالبيئة التي نميت عليها الفطريات والتي عزلت من الاعشاب وكانت ملوثة لها .واما (المجموعة الثالثة وعددها 5) كانت المجموعة الضابطة فقد زودت بالماء , استغرق زمن التجربة خمسة اسابيع تم بعد ذلك ذبح الجرذان وأخذ عينات من الكبد والكلى لعمل الدراسات النسيجية عليها. وقد كانت الاعشاب ملوثة بجنس الاسيرجلس والبنسليوم وقد تم تقدير نسبة السموم الفطرية منها وكانت نسبتها كما يلي من *A. flavus* (350 ug/L) و *aflatoxins B₁, B₂, G₁, G₂* وكانت السموم المعزولة هي *A. ochraceus* ومن *A. ochraceus* كانت الكمية المنتجة من السموم (460 ug/L) لكل من *aflatoxin B₁, B₂, G₁* و *aflatoxin B₁, B₂, G₁* and *G₂* , 350 ug/L₃ , and أعطى *sterigmatocystin*