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# The Effect of Mycotoxins found in some Herbal Plants on Biochemical Parameters in Blood of Female Albino Mice

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**Abstract:** In this study, twenty five samples of well-known herbs in Riyadh, Saudi Arabia were collected and analyzed for Total Fungi Count (TFC). Mycotoxins were extracted and screened using SMKY liquid medium. One hundred and thirty adult female albino mice were grouped into three wherein one group (n =110) was fed with an aqueous extract from herbal plants. The second group (n =15) was fed with an aqueous extract of the isolated fungal species. The third group comprised the control group which was given water only (n =5). All mice were fed with mice breeding diet by Pillsbury, UK. After 5 weeks, mice were fasted and blood was withdrawn for biochemical analysis including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), serum creatinine and urea. *Calligonum comosum* with $2\times10^5$ cfu $g^{-1}$ fungus spore, grained mixed herbs ($24\times10^3$ cfu $g^{-1}$) and *Salvia officinalis* ($23\times10^3$ cfu $g^{-1}$) were the most contaminated samples. The genus *Aspergillus* was the most dominant genus recovered (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 *Aspergillus citrinum* (11.0%). *Aspergillus ochraceus* had 21.7 μg $kg^{-1}$ of Aflatoxin $B_2$ and 7.25 μg $kg^{-1}$ of ochratoxin A, whereas *Aspergillus flavus* had 7.45 μg $kg^{-1}$ of Aflatoxin $B_1$ and *Aspergillus fumigatus* had 3.5 μg $kg^{-1}$ of Aflatoxin $B_2$ and 3.8 μg $kg^{-1}$ of ochratoxin A. Mean creatinine, urea, ALT, AST and GGT were higher in mice fed or treated with herbal and fungal extracts group than the control group. This study confirms previous studies demonstrating the predominance of *Aspergillus* species in herbal and medicinal plants and its capability in the production of aflatoxin with induction of nephrotoxicity and hepatoxicity in animals and even in humans.

**Key words:** Mycotoxins, herbal plants, fungal extracts, medicinal plants, aflatoxin, biochemical parameters of blood

## INTRODUCTION

The occurrence of toxigenic mycoflora and mycotoxins in medicinal and herbal plants has been confirmed in several studies (Aziz, 1998; Halt, 1998; Abou-Arab, 1999; Efuntoye, 1999; Costa and Scussel, 2002; Elshafie *et al*., 2002). Potential contamination of these plants with mycotoxins poses concerns over their use. *Aspergillus* and *Penicillium* are two major reported genera that produce mycotoxins (Rodriguez-Amaya and Sabino, 2002). Several environmental factors are reported to influence mycotoxin production, but temperature and relative humidity are considered to be the most critical (Simsek *et al*., 2002). Since, the mycotoxins are secreted and can contaminate products of vegetal origin consumed by livestock such as cattle, they therefore contaminate human secondarily.

The effects of these mycotoxins especially aflatoxins and ochratoxin on various organs has been reported in some studies. 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 trigger 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 $B_1$ decreases serum proteins, which are sensitive early indicators of hepatotoxicity (Quezada *et al*., 2000). The most important mycotoxins are aflatoxins which are hepatotoxic, hepatocarcinogenic and ochratoxins, which are nephrotoxic and nephrocarcinogenic (Rastogi *et al*., 2001; Orsi *et al*., 2007). This study was conducted to evaluate the predominant mycoflora, the extent of fungal contamination in herbal plants, the ability of fungi isolated to produce mycotoxins and the effects of these

mycotoxins on the liver and kidneys of test mice. Female albino mice were used since most herbs are used by women after delivery in Saudi Arabia.

## MATERIALS AND METHODS

**Sampling:** Twenty five samples of well-known plant herbs from random herb markets in the city of Riyadh, Saudi Arabia were collected between February and March 2008 to assess the predominant mycoflora and the extent of fungal contamination. The herbal products were chosen on the basis of their commercial availability and popularity of use (Table 1). Every sample weighed 3 kg and was preserved in clean plastic bags at a temperature ranging from 4 to 5°C.

**Isolation and evaluation of fungal contamination:** The fungal flora of the samples was detected by using the dilution plate method. Two types of media were used, (1) Glucose-Czapeck's agar medium in which glucose (10 gm $L^{-1}$) replaced sucrose and potato dextrose agar medium, chloramphenicol (20 μg $mL^{-1}$) and Rose Bengal (30 ppm) were added to suppress bacterial growth, (2) Sabouraud's agar and (3) Rose Bengal. Every sample was examined for Total Fungi Count (TFC). Five grams of each sample were mixed with 45 mL of distilled water from which tenfold serial dilution were made. Three milliliter from each dilution was inoculated in sterile petri dishes and mixed well. Plates were incubated upside down at 26±2°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 and followed taxonomic schemes of Raper and Fennel for the genus *Aspergillus* and Pitt for the genus *Penicillium*.

**Extraction and evaluation of toxigenic potential of fungal isolates:** Aflatoxin was extracted using SMKY (Sucrose: Magnesium sulphate: Potassium nitrate: Yeast extract) medium (Bugno *et al*., 2006). Twenty five grams of each raw sample was shaken with 50 mL of chloroform in 250 mL flask for 24 h. The defatted residue was re-extracted for another 24 h 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 is coated to a

Table 1: Herbal plants analyzed

| Common name | Scientific name | Known usege |
|---|---|---|
| Aloe | *Aloe vera* | For wound and burns |
| Anise | *Pimpinella anisum* | For digestion, anti-bloating |
| Caraway | *Carum carvi* | For digestive disorders |
| Chamomile | *Matricaria chamomilia* | For digestive ailments |
| Ciunamon | *Cinnamomum zeylanicum* | Food flavoring, spice |
| Cumin | *Cuminum cyminum* | Stimulant, antimicrobial |
| Dill | *Anethum graveolens* | For insomnia |
| Ducrosia | *Ducrosia ismaelis* | CNS depressant |
| Dymock | *Astralagus sarcocolla* | To boost immune system |
| Fennel | *Foeniculum vulgare* | Analgesic, anti-inflammatory |
| Fennel flower plant | *Nigella sativa* | For cancer prevention |
| Garden cress | *Lepidium sativum* | Prevent post-natal complications |
| Garden sage | *Salvia officinalis* | Antiseptic, estogenic |
| Ginger | *Zingiber officinale* | Arthritis, rheumatism |
| Green mist | *Ammi visnaga* | For kidney stones, antispasmodic |
| Hassaniya (in Arabic) | *Calligonum comosum* | Anti-inflammatory, anti-ulcer |
| Hulls | *Coffea arabica* | Coffee drink |
| Lavender cotton | *Achillea fragrantissima* | For diabetes, kidney stones, UTI |
| Mung beans | *Vigna radiata* | Cuisine |
| Myrrh | *Commiphora myrrah* | Incense |
| Fenugreek | *Trigonella foenum, Trigonella foenum-graecum* | Lowers blood pressure, carminative, increases milk production and/or flow, depresses the central nervous system, cardiotonic, aphrodisiac, hypoglycemic, diuretic, hemostatic, antiinflammatory, emollient and rids the body of impurities and toxins |
| Thyme | *Thymns serpyllum* | Aphrodisiac |
| White wormwood | *Artemisia herba alba* | Anti-oxidant |
| Mixed herbs of chamomile, cumin, cinnamon, ammi, anise, thyme, mung beans, caraway seeds | | |
| Grained herbs (as marketed) | | |

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

from microwell. Toxins are extracted from a ground sample with 70% methanol. The extracted sample and HRP-conjugated aflatoxin $B_1$ are mixed and added to the antibody-coated microwell. Aflatoxin from the extracted sample and HRP-conjugated aflatoxin $B_1$ compete to bind with the antibody coated to the microwell. Microwell contents are decanted and non-specific reactants are removed by washing. An enzyme substrate (TMB) is added and color (blue) develops. The intensity of the color is 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 chromogen color from blue to yellow. The microwells are measured optically by a microplate reader with an absorbance filter of 450 nm ($OD_{450}$). The optical densities of the samples are 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 333 nm (Boudra *et al*., 1995). Mycotoxins were identified by comparison with appropriate standards for aflatoxin and ochratoxin A. (Supelco, USA).

**Evaluation of mycotoxins on test mice:** One hundred and thirty adult female albino mice weighing between 130-160 g 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 mice were maintained on a standard pellet diet (mouse breeding diet, Pillsbury Ltd., Birmingham, UK) and tap water. Overall nutrient composition of the diet 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. A water extract was made using 1.5 per 1 mL of the herbs as the solute and sterile water as the solvent. The solution was mixed well and was placed in sterile glass jars with a firmly closed lid. The 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 mice instead of water (Bokhari *et al*., 2007).

The liquid cultures of fungi that were isolated and found to produce mycotoxins were filtered and were placed in sterile glass jars with a firmly closed lid. Portions of 50 mL were given to the test mice instead of water (Waggas, 2007).

Test mice were segregated into three groups, the herbal extract group, the fungal extract group and the control group. The herbal extract group was given aqueous herb extracts for five weeks through drink bottles. After five weeks, mice were fasted for 12 h then placed under anesthesia using diethyl ether before blood was extracted. Blood was with drawn from the heart by direct cardiac heart puncture and collected in sterile blood tubes (Terumo Europe N.V.3001 Leuven, Belgium) for biochemical analysis including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), serum creatinine and urea. Biochemical analysis of blood was performed by Dimension ® Clinical Chemistry System Flex ®reagent Cartridge (Dade Behring Limited, United Kingdom).

The second group, fungal extract group was given a liquid medium in which fungi has been cultured and filtered. The same protocol used for the herbal extract group was administered for the test mice and the control group. The control group of mice was given normal drinking water for the entire 5 weeks and was also maintained on standard pellet diet.

**Statistical analysis:** Laboratory values for creatinine, urea, ALT, AST and GGT were expressed as Mean±SD. Student t-test was used to compare differences between treated and control group and in between the two variables. A p-value<0.05 was considered statistically significant.

## RESULTS

**Fungal contaminants and their frequency of distribution in herbal plants:** Table 2 presents the frequency distribution of total fungal 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* ($2\times10^5$ cfu $g^{-1}$), grained mixed herbs ($24\times10^3$ cfu $g^{-1}$) and *Salvia officinalis* ($23\times10^3$ cfu $g^{-1}$) were the most contaminated samples. The predominant mycoflora obtained was distributed in 4 genera. 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 *Aspergillus citrinum* (11.0%).

**Effects of the fungal contaminants in herbal plants on test mice:** In the biochemical assessment on test mice fed with specific herbal plant, the level of urea

Table 2: Frequency distribution of total fungal counts in 25 herbal plants analyzed

| Common name | Fungal isolate | Total fungi count (cfu $g^{-1}$) |
|---|---|---|
| Aloe | *Aspergillus fumigatus* | $3\times10^{1}$ |
| Anise | *Aspergillus flavus* | $9\times10^{2}$ |
| Caraway | *Aspergillus fumigatus, Aspergillus flavus* | $18\times10^{1}$ |
| Chamomile | *Aspergillus flavus* | $17\times10^{2}$ |
| Ciunamon | *Aspergillus flavus* | $9\times10^{1}$ |
| Cumin | *Aspergillus fumigatus* | $4\times10^{1}$ |
| Dill | *Aspergillus fumigatus, Aspergillus flavus* | $64\times10^{2}$ |
| Ducrosia | *Aspergillus flavus* | $25\times10^{1}$ |
| Dymock | *Aspergillus fumigatus, Aspergillus flavus* | $21\times10^{1}$ |
| Fennel | *Aspergillus fumigatus, Penicillium citrinum* | $13\times10^{1}$ |
| Fennel flower plant | *Aspergillus fumigatus* | $32\times10^{1}$ |
| Garden cress | None isolated (often used as antibiotic) | - |
| Garden sage | *Aspergillus fumigatus, Aspergillus ochraceus* | $23\times10^{3}$ |
| Ginger | *Aspergillus fumigatus, Penicillium citrinum* | $23\times10^{1}$ |
| Green mist | *Aspergillus fumigatus* | $5\times10^{1}$ |
| Hassaniya (in Arabic) | *Aspergillus fumigatus* | $2\times10^{5}$ |
| Hulls | *Aspergillus flavus* | $50\times10^{1}$ |
| Lavender cotton | *Aspergillus fumigatus, Aspergillus flavus* | $12\times10^{2}$ |
| Mung beans | *Aspergillus flavus, Aspergillus ochraceus* | $13\times10^{1}$ |
| Myrrh | None isolated (often used as antibiotic) | - |
| Sickle fruit | *Aspergillus ochraceus* | $21\times10^{1}$ |
| Thyme | *Aspergillus ochraceus* | $18\times10^{2}$ |
| White wormwood | *Aspergillus ochraceus* | $12\times10^{1}$ |
| Mixed herbs | *Aspergillus fumigatus, Aspergillus flavus* | $57\times10^{2}$ |
| Grained herbs (fennel, ginger, anise, dill and cumin) | *Penicillium citrinum* | $24\times10^{3}$ |

Table 3: Distribution of herbal plant samples according to the counts of fungi (n = 23)*

| Enumeration limits (cfu $g^{-1}$) | No. of samples |
|---|---|
| $2\times10^{1} = 2\times10^{2}$ | 14(60.9%) |
| $2\times10^{2} = 2\times10^{3}$ | 6(26.1%) |
| $2\times10^{3} = 2\times10^{4}$ | 2(8.7%) |
| $2\times 10^{4} = 2\times10^{5}$ | 1(4.3%) |

*No fungi were isolated from 2 herbal plants.

was significantly high with *Ducrosia ismaelis* (9.6±1.05 mmol $L^{-1}$ versus the control group, 6.7±1.03 mmol $L^{-1}$, p = 0.0041). Several other herbs increased the urea levels on test mice including *Carum carvi* (8.4±1.27 mmol $L^{-1}$), *Pimpinella anisum* (8.02±1.6 mmol $L^{-1}$), *Cuminum cyminum* (7.62±.0 mmol $L^{-1}$), *Thymus serpyllum* (7.53±1.2 mmol $L^{-1}$), *Foeniculum vulgare* (7.5±1.2 mmol $L^{-1}$), *Astralagus sarcocolla* (7.44±1.8 mmol $L^{-1}$), *Aloe vera* (7.4±0.9 mmol $L^{-1}$), *Lepidium sativum* (7.36±1.5 mmol $L^{-1}$), *Ammi visnaga* (7.34±1.0 mmol $L^{-1}$), *Zingiber officinale* (7.2±1.5 mmol $L^{-1}$), *Salvia officinalis* (7.13±1.2 mmol $L^{-1}$), *Nigella sativa* (6.98±1.1 mmol $L^{-1}$) and *Achilea fragrantissima* (6.94±1.3 mmol $L^{-1}$). Mice fed with the other test herbs had urea levels below the control group with significantly lower levels in *Trigonella foenum* (5.26±0.5 mmol $L^{-1}$, p = 0.0270) and *Anethum graveolens* (4.7±0.9 mmol $L^{-1}$, p = 0.0163).

The mean creatinine level in the control group was 30±11.7 μmol $L^{-1}$. Twenty one of the tested herbs caused the elevation of the creatinine levels, the extract of *D. ismaelis* posted the highest significant mean creatinine level of 51.6±4.5 μmol $L^{-1}$, p = 0.0072, followed by *A. vera* extract (47.2±10.8 μmol $L^{-1}$, p = 0.0452) and *C. cyminum* (46.4±7.0 μmol $L^{-1}$, p = 0.0307). All extracts from other tested herbs induced higher creatinine levels compared to the control group except extracts of *Z. officinale* (29.4±11.4 μmol $L^{-1}$, p = 0.9366), *N. sativa* (26.2±3.3 mmol $L^{-1}$, p = 0.5051) and *Cinnamum zeylanicum* (25.2±10.5 mmol $L^{-1}$) which resulted in creatinine levels lower than those of the control group.

The mean AST level of the control group was 536±263 U $L^{-1}$. Test mice fed with grained mixed herbs posted the highest AST level with 1238.8±835 U $L^{-1}$, followed by mixed herbs (889.6±357 U $L^{-1}$, p = 0.1152), *A. sarcocolla* (834.6±525 $L^{-1}$, p = 0.2895), Arabica coffea (756.6±573 U $L^{-1}$, p = 0.4575), *Vigna adiata* (654.4±406 U $L^{-1}$, p = 0.5997), *T. serpyllum* (634.5±424 U $L^{-1}$, p = 0.6711), *Z. officinale* (643±227 U $L^{-1}$, p = 0.5243), *C. zeylanicum* (567.8±244 U $L^{-1}$, p = 0.8481), *Commiphora momol* (538.6±225 U $L^{-1}$, p = 0.9870) and *Artemisia herba alba* (462.4±202 U $L^{-1}$, p = 0.6340). All other test herbs resulted in insignificantly lower levels of AST as compared to the control group.

The mean ALT level of the control group was 306.2±345 U $L^{-1}$. Test mice fed with grained mixed herbs posted the highest ALT level with 740.8±987 U $L^{-1}$, followed by *L. sativum* (505.6±542 U $L^{-1}$, p = 0.5078), *T. serpyllum* (399.8±309 U $L^{-1}$, p = 0.7532), *A. sarcocolla* (380.4±577 U $L^{-1}$, p = 0.8116) and *C. arabica* (338.6±535 U $L^{-1}$, p = 0.9123). All other test herbs resulted in ALT levels lower than those of the control group with *C. comosum*, the lowest value recorded was 70.2±15 U $L^{-1}$ (p = 0.1668).

Table 4: The four isolated fungal species and their mycotoxin content (μg kg$^{-1}$)

| Fungal species | Aflatoxin $B_1$ ($AFB_1$) | Aflatoxin $B_2$ ($AFB_2$) | Ochratoxin A (OA) |
|---|---|---|---|
| *Aspergillus fumigatus* | - | 3.50 | 3.80 |
| *Aspergillus flavus* | 7.45 | - | - |
| *Aspergillus ochraceus* | - | 21.70 | 7.25 |
| *Penicillium citrinum* | - | - | - |

Table 5: Biochemical analysis of mycotoxin effect in 130 albino mice*

| Groups | Urea (mmol L$^{-1}$) | Creatinine (μmol L$^{-1}$) | AST (U L$^{-1}$) | ALT (U L$^{-1}$) | GGT (mmol L$^{-1}$) |
|---|---|---|---|---|---|
| Herbal extract group | 7.9±5.3 | 38.2±7.8 | 531.9±396.3 | 212.9±314.4 | 12.9±3.1 |
| (n = 110) | (3.8-11.2) | (14-73) | (62-2413) | (44-2187) | (6-24) |
| Fungal extract group | 6.2±1.3 | 31.5±9.8 | 561.5±287.3 | 213.7±182.5 | 12.2±2.3 |
| (n = 15) | (4.4-8.6) | (15-45) | (246-1336) | (39-610) | (8-15) |
| Control | 6.7±1.0 | 30.0±11.7 | 524.6±264.8 | 206.2±245.4 | 11.1±1.7 |
| (n = 5) | (5-8) | (20-40) | (156-872) | (54-487) | (9-17) |
| **p - values** | | | | | |
| Herbal vs. control | 0.6154 | 0.0264 | 0.9676 | 0.9627 | 0.2011 |
| Fungal vs. control | 0.4450 | 0.7802 | 0.8032 | 0.9424 | 0.3418 |

Values are expressed as Mean±SD (range)

The mean GGT level of the control group was 13.6±2.4 mmol L$^{-1}$. Test mice fed with *Z. officinale* posted the highest GGT level with 15.8±6.2 mmol L$^{-1}$ (p = 0.4821) followed by mixed herbs with 13.8±3.6 mmol L$^{-1}$ (p = 0.9203). All other test herbs posted GGT levels insignificantly lower than that of the control group.

**Mycotoxin production by isolated fungal strains and their effects on the liver and kidneys of test mice:** The four strains of *Aspergillus* and *Penicillium* isolated were evaluated for their ability to produce aflatoxins $B_1$ and $B_2$ and ochratoxin A. *A. flavus* revealed production of 3.5 μg kg$^{-1}$ of aflatoxin $B_2$ and 3.8 μg kg$^{-1}$ of ochratoxin A. *A. flavus* produced 7.45 μg kg$^{-1}$ of aflatoxin $B_1$ whereas *A. ochraceus* produced 21.7 μg kg$^{-1}$ of aflatoxin $B_2$ and 7.25 μg kg$^{-1}$ of ochratoxin A (Table 4).

Biochemical analysis of mycotoxin effects in 130 albino mice showed that mean creatinine was significantly higher in the mice group that was fed with herbal extract than the control group (38.2±7.8 μmol L$^{-1}$ vs. 30.0±11.7 μmol L$^{-1}$, p = 0.0264). Urea was also higher in the herbal extract fed group than the control group (7.8±5.3 mmol L$^{-1}$ vs. 6.7±1.0 mmol L$^{-1}$, p = 0.6154). Similarly, the AST, ALT and the GGT values in the group fed with herbal extracts was higher than that of the control group (p = 0.9676, p = 0.9627 and p = 0.2011, respectively). In the group of mice fed with fungal extract, creatinine, AST, ALT and GGT values were also higher than the control group of mice fed with water only (p = 0.7802, p = 0.8032, p = 0.9424 and p = 0.3418, respectively). Among the three groups, the group of mice fed with fungal extract had the highest values for AST and ALT whereas, the group that was fed with herbal extract posted the highest urea, creatinine and GGT values (Table 5).

## DISCUSSION

In the study, *Aspergillus sp.* were the predominant fungi recovered and as such the major toxigenic species. Present findings support the findings of Bugno *et al.* (2006). Furthermore, in this attempt to determine the mycotoxin content of fungal isolates, *A. fumigatus, A. flavus* and *A. ochraceus* were aflatoxigenic. Interestingly, present study showed the presence of fungi in 23 of 25 herbs analyzed, of which 31.9% of samples were above the enumeration limits set by the US Pharmacopoeia. *A. fumigatus* predominated in present study. Approximately, 39.1% of the samples showed enumeration limits of more than $2\times10^2$, the enumeration limit for total fungal count as set by the US Pharmacopoeia. Considerably though, the high percentage of mycotoxin production as revealed by present results indicated the inherent capacity of these moulds to instigate deleterious effects on humans when consumed. Aflatoxin $B_1$ was high at 7.45 μg kg$^{-1}$ extracted from *A. flavus*. Moreover, Aflatoxin $B_2$ was even higher at 21.7 μg kg$^{-1}$ from *A. ochraceus* and 3.5 μg kg$^{-1}$ from *A. fumigatus*. Ochratoxin A was also extracted from *A. fumigatus* (3.8 μg kg$^{-1}$) and *A. ochraceus* (7.25 μg kg$^{-1}$). These values are higher than levels reported by Elshafie *et al.* (2002) and Halt (1998).

When the extracts of these herbs and even the mycotoxin extracts were tested on test mice, their hepatotoxicity and nephrotoxicity was evident in the results. Fourteen of this tested herbs showed nephrotoxicity with elevations of urea beyond the control group. Furthermore, the creatinine levels were consistently high in almost all of our tested herbs except for *Z. officinalis* (ginger), *C. ceylanicum* (cinnamon) and *V. radiata* (Mung beans), which are understandably

being used in most of oriental and Middle Eastern cuisine. These high urea and creatinine levels explain the nephrotoxicity of the mycotoxins present in these herbs which can be potentially dangerous since they alter kidney function and/or liver function and are teratogenic (Berndt *et al*., 1980). The differences in the biochemical test results between test mice and control groups showed that aflatoxin caused hepatic and renal tissue degeneration, although present results did not reach significant levels. This may indicate that there is an interference with normal development and arrest of differentiation in the various internal organs. Furthermore, the high percentage of aflatoxins ($B_1$ and $B_2$) and ochratoxin A in this study indicate the degree of contamination of these herbs. Use and consumption of these potentially harmful herbs especially in Saudi Arabia where most of these herbs are being used by women after delivery, can be deleterious and hazardous to health. Information campaigns on the potential harm of the herbs and their safe use can benefit the majority.

## CONCLUSION

This study confirms earlier studies demonstrating the predominance of *Aspergillus* species as contaminants in herbal and medicinal plants. Furthermore, the capability of this fungal species in the production of aflatoxin, promoted by appropriate relative humidity and temperature induces nephrotoxicity and hepatoxicity in animals and even in humans.

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