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Full Length Research Paper

Unexpected hazard due to Fumonisins contaminating herbal teas used traditionally by Saudi people

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Fumonisins are mycotoxins synthesized by various species of the genus *Fusarium* and are hazardous for human and animal health. The purpose of this study was to investigate fumonisin B1 (FB1) in herbal tea consumed especially by Saudi population. FB1 was detected using high performance liquid chromatography (HPLC) with fluorescence detection. Forty-seven commercially available samples for infusions preparations were collected and analyzed for FB1. The detectable amount for FB1 ranged from 0 - 266 µg/kg. All the herbal tea samples were evaluated for the fungal contamination and the presence of mycotoxigenic fungi. Results indicate that predominant mycoflora were distributed in 13 genera representing 25 species. From these, the genera *Aspergillus*, *Penicillium* and *Fusarium* which considered extremely important from the mycotoxicological standpoint were the most abundant fungi. The presence of toxigenic moulds represents a potential risk of mycotoxin contamination. Considering the worldwide increased use of herbal products as alternative medicines, it is necessary setting standards for moulds in crude herbal tea in order to reduce the risks for consumers' health.

Key words: Fumonisins, herbal tea, mycotoxins, fungi, Fusarium, toxigenic fungi.

INTRODUCTION

The ubiquitous nature of fungal contamination has resulted in high levels of various mycotoxins in many food crops throughout the world (Aziz et al., 1998; Tutelyan, 2004). Such high levels of mycotoxins, especially aflatoxins, fumonisins B or ochratoxin A, in food and feed commodities may have adverse effects on human and animal health, provoking different kinds of mycotoxicoses including carcinogenic effects (Tutelyan, 2004). According to Yang (1980) and Marasas et al. (1988), outbreaks of human oesophageal cancer were linked to the consumption of fumonisin-contaminated maize.

Fumonisins are mainly produced by *Fusarium verticalioides* and *F. proliferatum,* which are both field pathogens. Although at least 15 different fumonisins have been reported, structurally seven different well known fumonisins, FA1, FA2, FB1, FB2, FB3, FB4 and FC1, have been described (WHO, 2000). FB1 and FB2 are the most abundant in naturally contaminated food such as corn, medicinal plants and herbal tea and they are toxic to Turkey poultry, broiler chickens and may produce nephrotoxicity in rats (Omurtag et al., 2005). International Agency for Research on Cancer has declared *F. moniliforme* toxins as potentially carcinogenic to humans (class 2B carcinogens) and FB1 is a cancer promoter and play an important role in carcinogenesis in humans (Chu and Li, 1994; Yoshizawa et al., 1994).

Field fungal pathogens infect plants while they are growing in the field while post harvest pathogens grow and transit especially under inappropriate conditions of temperature and humidity (Hasan and Abdel-Sater, 1993; Bokhari and Aly, 2009). Indeed, warmer temperatures and higher humidity is most conducive to mold development during this post harvest period. Herbal tea including black, green or contain one or two ingredients like chamomile, lemon or ginger may be stored in adverse climatic conditions and are subjected to increased levels of fungal and mycotoxin contamination.

Authors detected fumonisin B1 and B2 in cereal, cereal

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products, medicinal plants and herbal tea (Atak and Omurtag, 2003; Omurtag et al., 2005). Moreover, fungal contaminated herbal tea samples were analyzed for a range of mycotoxins including ochratoxin A and zearalenone as well as aflatoxins and Ochratoxin A was found in 15% of the analyzed samples. Medicinal plants and probably herbal teas especially if stored improperly, were susceptible to fungal growth and should therefore be routinely tested for the presence of fungi and mycotoxins before entering the market. FB1 was detected in 55 (65.5%) of the 87 samples of herbal tea collected from Portugal and the highest number of positive samples was found in black tea (88.8%) with levels ranging from 80 to 280 µg/kg (Omurtag and Yazıcıoglu, 2004).

With regard to human health, epidemiological studies established a positive correlation between the level of FB1 consummation in a diet and the rate of human esophageal cancer, liver damage and levels of certain classes of lipids, especially sphingolipids. In addition, maternal ingestion of high levels of FB1 during early pregnancy may increase the risk of neural tube defects of the brain and spinal cord (Gelineau-van Waes et al., 2005; Missmer et al., 2006). Studies on risk assessment of mycotoxins in herbs continue to rise throughout the world with respect to mycotoxins contamination of herbal tea. The main objective of this study was to determine the incidence and levels of mycotoxins in herbal tea in Jeddah, Saudi Arabia in addition to different contaminated fungi.

MATERIALS AND METHODS

Collection of herbal tea samples

A total of 47 samples belonging to 15 kinds of herbal tea were collected from supermarkets and street bazaars in Jeddah, Saudi Arabia. Each sample was collected in a sterile polyethylene bag, sealed, transferred immediately to the laboratory and kept in a cool place for fungal determination and mycotoxins analysis.

Moisture content of the herbal tea samples

The moisture content of the samples was directly determined by dry weight method (Aziz, 1987). About 10 g of each sample was transferred to an oven at 60°C under vacuum for 12- 24 h until a constant weight and percentage of water content was calculated.

Mycological analysis

Ten grams of each sample were added to a 90 ml of sterile saline solution in 250 ml Erlenmeyer flasks and homogenized thoroughly on an electrical shaker at constant speed for 15 min. Ten fold serial dilutions was then prepared (Bokhari and Aly, 2009) and one ml of the suitable dilution of the resulting medicinal plant suspension was used to inoculate Petri dishes, each containing 15 ml of agar medium containing 50 µg/ ml chloramphenicol to suppress bacterial growth. Plates were then incubated for 7 days at 28°C and examined visually for fungal growth. Five replicates were performed for each sample and the developing fungi were counted and identified according to the method of Raper and Fennell (1965), Moubasher (1993), Samson et al. (1995) and Pitt and Hocking (1997).

Determination of FB1

Preparation of plant extracts

A ground sample of each plant (kg) with sodium chloride (40 g) was homogenized in a mixture of methanol/water (80:20 v/v) for 5 min and the extract was then filtered through Whatman no. 4 filter paper. The filtrate was collected in a clean vessel, concentrated and taken for fumonisin B1 cleanup.

Cleanup for Fumonisin B1

The analytical method for the determination of FB1 in medicinal plant was carried out according to the method of Sewram et al. (2006). The filtered extract (10 ml) was diluted with 40 ml a solution of phosphate-buffer (pH 7.0), the extract was then filtered and the filtrate was transferred into a polypropylene syringe barrel, which was attached to the FumoniTest immunoaffinity (IA) column (Vicam). The extract was passed through the IA column at a rate of about 1-2 drops/s until air passed through the column. Thereafter, 15 ml of phosphate buffer was passed through the column at a rate of 1-2 drops/s. The fumonisin B1 was eluted from the IA column under gravity by passing HPLC grade methanol (3 ml) through the column at a rate of 1 drop/s and the elute was collected into a glass vial, dried under a stream of nitrogen at 60°C and concentrated at the base of a small vial (4 ml capacity).

Derivatization and HPLC analysis

Fifty (50) µl of FB1 working standard solution was mixed with 225 µl o-phthaldialdehyde (OPA) reagent (40 mg of OPA in1 ml methanol, diluted with 4 ml of 0.1 M disodium tetraborate and 50 µl 2-mercaptoethanol) and 10 µl of reaction mixture was injected to HPLC within 1 min.

Preparation of herbal tea extract derivatives

The purified dry film residue of sample extract was dissolved in 200 µl methanol and 50 µl of this extract was mixed with 225 µl OPA reagent. About 10 µl of the previous mixture was injected to HPLC within 1 min of adding OPA reagent.

HPLC chromatography conditions and determination of FB1

HPLC method was used for the determination of FB1 in positive samples according to the method of Shaphard et al. (1996). The HPLC instrument used for FB1 determination was waters delivery system 600 controller, equipped with fluorescence detector set system at 335 nm excitation and 440 nm emission wavelengths. The chromatography column was Nova-Pak C18 (150 x 3.9 mm). The mobile phase system (water: methanol: acetonitriel, 6:3:1 v/v/v) was at flow rate of 1 ml /min.

The derivative solutions of the standard or herbal tea extract were filtered through a 0.45 µm membrane filter and 10 µl was injected. The quantity of FB1 was determined from chromatographic peak areas using standard solutions containing 10-100 µg/ml FB1 (Sigma F 1147).

Table 1. % of humidity, total fungal counts, number of fungal and toxigenic isolates and quantity of FB1 (µg/kg) in herbal tea samples.

Herbal tea type	Used part	No. of collected Samples	% Moisture content	No. of fungal contaminated samples/No. of examined samples (% of fungal contamination)	Total fungal count x10^4 CFU/g	No. of fungal isolates	No. of toxigenic isolates	% of toxigenic isolates	Quantity of FB1 (µg/kg)
Anise	Seeds	3	7.1	2/3 (66.6%)	3.3	11	1	9.0	11*
Basil	Flowers	3	7	1/3 (33.3%)	1.1	7	1	14.2	7
Black tea +Rosemary	Leave	3	7	1/3 (33.3%)	1.2	16	3	18.7	11*
Cardamom	Seeds	3	7	3/3 (100%)	2.3	12	1	8.3	9*
Chamomile	Flowers	3	6	1/3 (33.3%)	1.9	8	0	0	<1
Green tea+ Thyme	Leave	3	7.1	1/3 (33.3%)	0.33	9	1	11.1	<1
Chrysanthemum	Flowers	3	7.0	3/3 (100%)	3.4	14	1	7.1	82*
Cinnamon	Bark	3	6.3	1/3 (33.3%)	0.22	12	2	16.6	31*
Cloves	Fruit	3	7.1	1/3 (33.3%)	0.9	11	1	9.0	5
Cactus	RootS	3	6.6	3/3 (100%)	1.3	14	1	7.1	111*
Different flowers	Flowers	3	7.5	3/3 (100%)	1.9	9	4	44.4	29*
Green tea+Cumin	Leave	3	6.9	1/3 (33.3%)	0.2	12	1	8.3	>1
Lemon grass	Leave	3	7.1	1/3 (33.3%)	0.11	10	1	10.0	10*
Sage	Leave	5	7.1	5/5 (100%)	4.44	19	4	21.0	266*
Green tea +Mint	Leave	3	7.0	1/3 (33.3%)	0.23	6	1	16.6	33*

*: significant results at $p \leq 0.05$

Statistical analysis

Each experiment has three replicates and mean value was recorded. Student t- test was used to detect any significant differences between samples.

RESULTS

Various unpacked herbal teas including Anise, Basil, Rosemary, Cardamom, Chamomile, Thyme, Chrysanthemum, Cinnamon, Cloves, Cactus, Cumin, Lemon grass, Sage and Mint tea samples as well Black and Green tea were collected from different supermarkets and bazaars in Jeddah, Saudi Arabia. The most contaminated samples (100% contamination) were Sage, different flowers, Chrysanthemum and Cardamom. The percentage of humidity, total fungal counts, % of toxigenic fungi and quantity of FB1 for each sample were determined (Table 1). The percentages of humidity in the collected herbal tea samples were ranged from 6.3-7.5%. The results of this investigation showed that fungal counts reached levels as high as 4.4×10^4 cfu/g. Sage harbored the highest fungal contamination and the lowest counts of fungi were associated with Lemon grass ($0.11x10^4$ cfu/g), Green tea with Cumin ($0.2\ x10^4$ cfu/g), Cinnamon ($0.22\ x10^4$ cfu/g) and Green tea + Thyme ($0.33\ x10^4$ cfu/g). The number of fungal isolates was as the follow: 16 fungal isolates were associated with Black tea +Rosemary, 14 fungal isolates were detected on Cactus herbal tea and Chrysanthemum, 12 fungal isolates were detected on either Green tea + Cumin, Cardamom or Cinnamon. Green tea + Mint (6 isolates) and Basil (7 isolates). The highest number of toxigenic isolates was found on Sage and different flowers herbal tea (4 toxigenic isolates). The lowest number of toxigenic isolates on herbal tea were isolated from Green tea + Mint, Lemon grass, Green tea + Cumin, Cactus, Cloves, Chrysanthemum, Cardamom, Basil and Anise. Samples were analyzed for presence and level of fumonisin B1 (FB1). The quantity of FB1 was ranged from 0-266 µg/kg with mean value of 39.14 µg/kg. The most contaminated herbal tea sample was sage (266 µg/kg) and the less contaminated was both Green tea + Cumin and

Green tea+Thyme.

The most common fungi found (high occurrence) in herbal teas were *Aspergillus niger*, *A. parasiticus, Fusarium sp.* and *Trichoderma* (Table 2). Moreover, *Alternaria alternate, A. humicola, Geotrichum sp, Penicillium citrinum* and *P. corylophylum* were of moderate occurrence. However, *A. ochraceus, A. fumigates, A. sydowi, A. terreus, Chaetomium sp., Cladosporium sp, Eurotium rubrum, Gliocladium sp, Paellomyces sp*. and *Penicillium* sp. were of rare occurrence. The highest count was recorded for *A. niger* (16.4 %) of the total fungal count.

DISCUSSION

Herbal tea samples including Sage, different flowers, Chrysanthemum and Cardamom were highly contaminated with fungi (100%) whereas samples contained Black tea, Green tea, Lemon grass, Cloves, Cinnamon and Chamomile were less contaminated (33.3%). This may be attributed to the caffeine and/or antifungal content of these commodities. There is evidence that medicinal plants or herbal tea may be contaminated with toxigenic fungi including *Aspergillus* and *Fusarium* (Elshafie et al., 1999; Abou Donia, 2008). Certain plant constituents are susceptible to chemical transformation by contaminating microorganisms. Withering leads to enhanced enzymes activity, transforming some of the constituents to other metabolites not initially found in the herb. These newly formed constituent (s) along with the molds such as *Penicillium nigricans* and *P. jensi* may then have adverse effects (De Smet et al., 1992).

The percentages of humidity in the collected herbal tea samples ranged from 6.3-7.5%. Hasan and Abdel-Sater (1993) recorded lower level of moisture content of the tea samples where it was fluctuated from 5.2-6.8%. Increasing water content may enhance fungal contamination and mycotoxin production (Bokhari and Aly, 2009). Moreover, contamination of tea by mycoflora and mycotoxin is favored by high humidity and high water activity (Hasan and Abdel-Sater, 1993). Microbial counts are thus a reflection of the original bioload of microbes as well as of die off that are probably enhanced by oxidation and the presence of active compounds in herbs and spices (Farkas, 2000).

The collected herbal tea samples were contaminated with fungi (33.3-100%) and the total fungal counts were up to 4.4x104. The differences in colony forming units (cfu/g) in different herbal tea were non-significant. Thus, all the tested herbal teas met all the hygienic conditions concerning them according to District Court Action No. 294/1997. Similar results were obtained by Rezacova and Kubatova (2005) who found the most contaminated sample of tea contained only 1.2×10^3 cfu/g, which was lower than the cited notice Act no. 294/1997 ($<10^5$ cfu/g). Lutomski and Kedzia (1980) reported that 10% of the analyzed samples from 95 different crude herbal drugs contained $<10^2$ cfu/g molds, 38% contained 10^2-10^3 cfu/g, 28% had 103-104 and 24% had $>10^4$ cfu/g. Schilcher (1982) analyzed 548 samples of seeds and 221 samples of crude herbal materials (barks, flowers, leaves, fruits, herbs, roots and rhizomas) and found that the total count of aerobic bacteria in samples was 10^2-10^7. Halt (1998) suggested that medicinal plant material and possibly herbal teas, if stored improperly allowing for mould growth should be analyzed for mould and mycotoxin prior to use.

In this study, the mycoflora of herbal tea was distributed in 13 genera comprising 25 species in majority of the tested herbal tea samples; *A. niger* was found and represented 16.4% of the total fungal counts. Many authors carried out mycological examination of teas and recorded the dominance of *A. niger* (Ostry et al., 2000; Ostry et al., 2002; Rezacova and Kukatova, 2005). Rezacova and Kukatova (2005) indicated no relationship between the particular microfungal species and a type of tea studied and high occurrence of *A. niger* in herbal tea samples could be due to low water content of tea which is preferred by *A. niger*. The most important toxigenic fungus *A. flavus* was found in only 9 out of 47 samples (18.8%) and it represented 4.9% of total fungal counts. *A. flavus* is a known producer of aflatoxin and the percentage of occurrence calculated by Rezacova and Kukatova (2005) was 13%, which was lower than the previous percentage. Elshafie et al. (1999) recorded *A. niger* as the most dominant species in all examined tea brands and the percentage of tea contamination with *A. niger* ranged between 0.66 and 30.34%. Abdel-Hafez and El-Maghraby (1992) indicated that *A. flavus, A. fumigatus* and *A. niger* were the most prevalent in some drinks including tea and the total count of fungi was regularly increased with the rise of moisture content and storage periods. They added that out of 20 isolates of *A. flavus*, 15 isolates produced B1, B2, G1 and G2, while five isolates produced B1 and B2 and *A. flavus* had the ability to produce aflatoxin in all kinds of tea after 20 days of incubation at 45% moisture content. Furthermore, the results of Bugno et al. (2006) indicated that the predominant mycoflora from 91 herbal plant was distributed in 10 fungal genera, 89.9% of the isolates corresponded to genera *Aspergillus* and *Penicillium*, which are extremely important from the mycotoxicological standpoint. Moreover, they added that 21.97% of the *Aspergillus* and *Penicillium* isolates proved to have the ability for producing aflatoxins (42.9%), ochratoxin A (22.4%) and citrinine (34.7%). The mycofloral analysis of twenty different kinds of black tea powder (commonly used in Egypt) indicated that about 7 genera, 23 species and 2 varieties were recorded and the most prevalent mould were *Aspergillus, Penicillum, Cladosporium* and *Eurotium* (Hasam and Abdel-Sater, 1993). Moreover, *Aspergillus, Penicillium, Mucor, Rhizopus, Absidia, Alternatia, Cladosporium* and *Trichoderma* were the common genera isolated from 62 samples of medicinal plant

Table 2. Distribution of the detected fungi in samples of herbal tea.

Fungal genera and specie found	Occurrence remark	Number of Appearance/47 samples	Mean value of cfu/g X 10^3	Percentage of occurrence of the total fungal count
Alternaria alternata	M	13	2.5	4.9
Aspergillus ochraceus	R	5	1.0	1.9
Aspergillus flavus	H	9	2.5	4.9
Aspergillus fumigatus	R	5	1.0	1.9
Aspergillus humicola	M	20	2.9	5.6
Aspergillus niger	H	44	8 .4	16.4
Aspergillus parasiticus	H	23	1.0	1.9
Aspergillus sydowi	R	5	1.8	3.5
Aspergillus terreus	R	5	1.34	2.5
Chaetomium sp.	R	5	1.81	3.5
Cladosporium sp	R	5	1.11	2.1
Eurotium rubrum	R	4	2.34	4.5
Fusarium moniliforme	R	4	1.34	2.5
Fusarium spp.	H	34	2.30	4.5
Geotrichum sp	M	23	0.11	0.21
Gliocladium sp	R	4	0.17	0.33
Mucor spp	M	23	2.32	4.1
Paellomyces sp.	R	5	0.99	1.9
Penicillium citrinum	M	15	2.30	4.5
Penicillium corylophylum	M	24	1.41	2.7
Penicillium corylophylum	M	20	0.8 8	1.7
Penicillium sp.	R	5	0.8 8	1.7
Phoma	R	5	0.178	0.33
Rhizopus sp.	R	4	1.94	3.8
Trichoderma sp.	H	36	3.88	7.6

H, High occurrence (more than 25); M, moderate (less than 25 and more than 5); R, rare (equal or less than 5).

material and 11 herbal tea samples (Halt, 1998).

Fusarium sp. and *F. moniliforme* were found in 34 and 4 of the examined herbal tea samples, respectively representing 4.5% and 2.5 % of the total fungal count. Fumonisin B1 is produced mainly by *F. moniliforme* that are prevalent in cereals and other agricultural products (Bezuidenhout et al., 1998). In Egypt, *Aspergillus*, *Fusarium* and *Penicillium* genera were more frequently detected than other genera (*Alternaria, Absidia* spp., *Mucor* spp., *Rhizoctonia* and *Cladosporium* spp.) in herbal samples (Abou Donia, 2008). *Fusarium* spp., *Penicillium* spp., *A. flavus* and *A. niger* were predominant in all tested herbal tea samples with the exception of garden sage samples (Martins et al., 2001a). In Sultanate of Oman, five fungal species were isolated from 48 samples of black tea with *A. niger* as the most dominant isolate followed by *Aspergillus flavus*, *Penicillium* spp. and *Paecelomyces* spp. (Elshafie et al., 1999). Rezacova and Kukatova (2005) did not find any effect of fermentation or origin of plant on the fungal species composition of black, green or herbal tea and most microfungi colonize the tea product later during processing. This hypothesis supports not only the fungal spectrum recorded (generally saprophytic fungi preferring dried food) but also the great difference in species composition between our results and fungal species isolated from soil of tea plants (Agnihothrudu, 1962; Farr et al., 1994). In Prague, Rezacova and Kubatova (2005) isolated 81 species of fungi from 40 samples of teas green, black and herbal teas but presence of *Aspergillus* species with known capacity for ochratoxin A and aflatoxins production led the authors to issue warnings, which sparked more interest and investigation is required.

The results show that fungi that might constitute health hazards for humans might contaminate herbal tea post harvest and during processing which must be conducted under conditions that are more hygienic.

In this study, the detectable amount for FB1 in herbal tea ranged from 0 - 266 µg/kg using HPLC. No FB1 was detected on samples of Green tea + Cumin, Green tea + Thyme and Chamomile (Table 1). Contamination with Fumonisins generally caused acute toxic effects, pulmonary oedema (IARC, 1993) and esophageal cancer (Yang, 1980; Marasas et al., 1981, 1988; Sydenham et al., 1990). Other mycotoxin was detected by Abdel-Hafez and El-Maghraby (1992) who proved that tea powder was

contaminated by aflatoxins (26-81 µg/kg). In Portugal, FB1 was detected in 55 (65.5%) of the 87 samples (18 black tea samples and 69 herbal tea) for infusions preparations. The highest number of positive samples was found in black tea (88.8%) with levels ranging from 80 to 280 µg/kg but chamomile had less contamination of FB1, with concentrations ranging from 20 to 70 µg/kg, and none of the tested samples had contamination of FB2 (Martins et al., 2001b). Using HPLC with fluorescence detection, Omurtag and Yazıcıoglu (2004) detected FB1 in two samples (0.160 and 1.487 µg/g) out of 115 commercially available herbal teas but FB2 was not detected in any sample.

In conclusion, fungi, which could conceivably constitute health hazards for humans, contaminate herbal tea and post-harvest contamination of tea can be reduced or even eliminated if tea processing is carried under the most hygienic conditions.

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