

OCCURRENCE OF FUMONISINS B₁ AND B₂ IN HOMEMADE MEDICINAL PLANTS: EXPOSURE ASSESSMENT IN NORTHERN TURKEY

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(Received: 7 December 2014; accepted: 8 January 2015)

This study was conducted to determine the recent level of contamination with Fumonisin B₁ (FB₁) and Fumonisin B₂ (FB₂) in major medicinal plants and to assess consumer exposure in northern Turkey. FB₁ and FB₂ were investigated by using high performance liquid chromatography (HPLC) with fluorescence detection after derivatization with *o*-phthalaldehyde (OPA). A total of 78 homemade medicinal plant samples from 14 species were analysed. The recovery in thyme was 67.2±5.2% for FB₁ and 80.8±14.3% for FB₂ spiked with 1 µg g⁻¹ of each analyte. The minimum detectable amount for the OPA derivatives of FB₁ and FB₂ were 1 ng per injection and 2.5 ng per injection, respectively. The limit of quantitation (LOQ) S/N=10 was 0.078 and 0.313 µg g⁻¹, and the limit of detection (LOD) S/N=3 was 0.023 and 0.093 µg g⁻¹ for FB₁ and FB₂, respectively. FB₁ was detected in thyme (0.125) and mint (0.125 and 0.256 µg g⁻¹) samples; however, FB₂ toxin was below the detection limit in all samples. These results indicate that toxins might be present in homemade medicinal plants; however, the risk of exposure to fumonisins by the consumption of those plants was lower than the estimated TDI limits (<2 µg kg⁻¹ bw).

Keywords: dietary intake, dried plants, *Fusarium* toxins, HPLC

Fumonisin B₁ (FB₁) and Fumonisin B₂ (FB₂) are secondary metabolites produced by several species of the *Fusarium*, which include *Fusarium verticillioides* (Sacc.), Nirenberg (ex *F. moniliforme* Sheldon) and *Fusarium proliferatum*. They are associated with maize and are frequently present in naturally contaminated corn, feeds, medicinal and other plants throughout the world; particularly where a high humidity exists (CASTELO et al., 1998; MARTINS et al., 2001a; SOLFRIZZO et al., 2001; OMURTAG, 2001; OMURTAG & YAZICIOĞLU, 2004).

Fumonisin was first isolated from maize that caused poisoning in the Transkei in South Africa in 1988 (BEZUIDENHOUT et al., 1988), and thereafter *Fusarium* sp. was also detected in corn silk (MARTINS et al. 2001b) and herbal teas with highest quantity in sage (BOKHARI & ALY, 2013). Moisture content during harvesting and postharvest process of corn are critical to control fungal growth and further fumonisin production (TRUCKSESS et al., 2000; ONO et al., 2002). Studies on the presence of FB₁ conducted in 18 countries revealed a high frequency (93%) in maize samples (DE NIJS et al., 1998). Except 'extrusion cooking' in cereal industry, FB₁ cannot be destroyed by cooking and could therefore enter the human food chain (ALBERTS et al., 1990; CASTELLS et al., 2005).

Despite the difficulty of determining the effects of fumonisins on humans, most of the studies agreed on that consumption of corn and cereal products contaminated with FB₁ may

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contribute to esophageal cancer (CHU & LI, 1994; PIÑEIRO et al., 1997). However, medicinal plants and several food supplements might also bear the risk of contamination (WANG et al., 2000; DI MAVUNGU et al., 2009; ALIZADEH et al., 2012; BRYLA et al., 2013). High levels of fumonisins were also recorded in animal feeds associated with confirmed field outbreaks, i.e. pulmonary oedema (PE) in pig (COLVIN et al., 1993) and induced leukoencephalomalacia (LEM) in horses (KELLERMAN et al., 1990). Hence FB₁ is mentioned as potentially carcinogenic to humans (class 2B carcinogens) (IARC, 2002) and together with other mycotoxins is considered as a cancer inducer in humans (CHU & LI, 1994).

Despite the increased consumption of medicinal plants, researches on their level of fumonisin are rare compared to that in corn. In our consideration, among the instrumental methods developed for the analytical determination of the fumonisins, high-performance liquid chromatography (HPLC) is one of the most widely used with reliable results. Therefore, the purpose of this study was to investigate the level of natural contamination of FB₁ and FB₂ by HPLC in several home-grown medicinal plants, which are most frequently consumed for medicinal and also dietary purposes in Turkey. All of the collected samples (n=78) originated from the northern Anatolia, where mean relative humidity is 77% (SAHIN & CIGIZOGLU, 2012).

1. Materials and methods

Seventy-eight medicinal plants were collected from Ordu, which is a city in the middle region of the Black Sea shore of Turkey, in-between May and July. Leaves of mint (*Mentha piperita*), laurel (*Laurus nobilis*), stinging nettle (*Urtica* sp.), thyme (*Oryganum* sp.), and basil (*Ocimum basilicum*) leaves; flowers of lime (*Tilia* sp.), chamomile (*Anthemis* sp.), corn-poppy (*Papaver rhoeas*), and marshmallow (*Althea officinalis*); herba of horsetail (*Equisetum arvense*) and St. John's wort (*Hypericum perforatum*); fruit of rose hip (*Rosa* sp.), raspberry (*Rubus sanctus*), and coriander (*Coriandrum sativum*) were dried under shade and studied to evaluate the presence of FB₁ and FB₂. Analyses were carried out with regard to the reported HPLC method after OMURTAG and YAZICIOĞLU (2004) as given below.

1.1. Chemicals

The FB₁ and FB₂ standards (F 1147) and (F 3771), respectively, were supplied from Sigma. All chemicals were the products of Sigma, Merck, and JT Baker and all of the solvents were of HPLC grade. Standards, solutions, and mobile phase were prepared in HPLC-grade water obtained from Millipore-Milli Q-RG. Isocratic conditions were applied in the HPLC analytical method.

1.2. Preparation of stock solutions

The standard stock solutions of FB₁ and FB₂ were separately dissolved in the mixture of acetonitrile and water (1:1, v/v) as 250 µg ml⁻¹. From this solution 50 µg ml⁻¹ were prepared for each FB₁ and FB₂ standard stock solutions. All stock solutions were sealed and stored at -20 °C until use.

1.3. Preparation of sample

A sample of 25 g was placed in a Waring blender and extracted with 125 ml of a mixture of methanol and water (3:1, v/v) for 5 min and then filtered through Whatman No. 4 filter paper.

The pH of the extract was adjusted when necessary to pH 5.8–6.5 with 1 M NaOH. A 1 ml portion containing 0.2 g sample was applied to a 1 ml SAX (strong anion exchange, Supelco, Part number SP1984A, sorbent quantity is 100 mg) solid phase extraction column (SPE) previously conditioned with 2 ml methanol followed by 1 ml water at a flow rate ≤ 2 ml min^{-1} with a manifold (Alltech- 210224, 21212). The column was washed successively with 500 μl water and 500 μl methanol. All washes were discarded. FB_1 and FB_2 were eluted with 1 ml methanol/acetic acid (99:1, v/v) at a flow rate ≤ 1 ml min^{-1} . The eluate equivalent to 0.2 g sample was evaporated to dryness under a stream of nitrogen in a water bath at 60 °C.

1.4. Apparatus

The HPLC (Agilent 1100 Series, USA) was a combination of a Model G1311A Quaternary Pump with a Model G1321A Fluorescence Detector. The fluorescence was recorded at excitation (λ_{Ex}) and emission (λ_{Em}) wavelengths of 335 and 455 nm, respectively. The sample loop was 20 μl (Rheodyne 7725i, USA). The column heater was thermostatted column compartment with a Model G1316A. A reverse phase Symmetry C_{18} column was used (4.6 \times 250 mm, 5 μm particle size, part no: WAT054275) (Waters) with a guard column (Hicrom KR100-5C18-10C, 10 mm length). Data was processed through the AgilentChemStation (USA).

1.5. Operating conditions

The mobile phase was methanol/0.1 M sodium dihydrogen phosphate (8:2, v/v), adjusted to apparent pH: 3.33 with *o*-phosphoric acid. The mobile phase filtered through a Millex HV Millipore (0.45 μm) filter, degassed by Agilent 1100 Series (Model G1379A). The flow rate was 0.7 ml min^{-1} . The column temperature was 25 °C. The standard curves of FB_1 ($t_{\text{R}}=10.5$ min) and FB_2 ($t_{\text{R}}=22.5$ min) were linear between the ranges of 1–40 ng and 2.5–40 ng, respectively. FB_1 plot linear regression line was $y=3.2027x+0.1552$ ($r=0.9998$) and FB_2 plot linear regression line was $y=1.3057x-0.7791$ ($r=0.9995$).

1.6. Procedure for HPLC

The residue was first dissolved in 200 μl of methanol and 50 μl of this solution was derivatized by addition of 200 μl *o*-phthalaldehyde (OPA) reagent (40 mg of OPA was dissolved in 1 ml methanol and diluted with 5 ml 0.1 M disodium tetraborate solution, then 50 μl of 2-mercaptoethanol was added to this solution and mixed). OPA solution was prepared daily and stored at room temperature in capped amber vial. The final extracts were 0.2 g ml^{-1} equivalent sample concentrations, and 20 μl (4 mg sample equivalent) of the extracts were injected into HPLC within 1 min of derivatization. For the HPLC quantitation, the samples were injected into the HPLC system under the same conditions as used for preparing calibration graphs and were analysed in triplicate.

2. Results and discussion

In this study, 78 homemade medicinal plants were collected from northern Turkey. FB_1 was detected in three samples (0.125, 0.125, and 0.256 $\mu\text{g g}^{-1}$), however, FB_2 was detected in none.

Table 1. Results of HPLC method for FB₁ and FB₂ in 78 medicinal plant samples that are especially consumed in north Anatolia (Black Sea region in Turkey)

Medicinal plants	(Positive/Total)		Conc. ($\mu\text{g g}^{-1}$)	
	FB ₁	FB ₂	FB ₁	FB ₂
Laurel (<i>Laurus nobilis</i>)	0/1	0/1	nd	nd
Horsetail (<i>Equisetum</i> sp.)	0/1	0/1	nd	nd
Basil (<i>Ocimum basilicum</i>)	0/5	0/5	nd	nd
Mint (<i>Mentha</i> sp.)	2/15	0/15	0.125 0.256	nd
Thyme (<i>Thymus</i> sp.)	1/14	0/14	0.125	nd
Lime (<i>Tilia</i> sp.)	0/8	0/8	nd	nd
Coriander (<i>Coriandrum sativum</i>)	0/8	0/8	nd	nd
Nettle (<i>Urtica dioica</i>)	0/7	0/7	nd	nd
Rosehip (<i>Rosa canina</i>)	0/9	0/9	nd	nd
Poppy (<i>Papaver rhoeas</i>)	0/3	0/3	nd	nd
Marshmallow (<i>Althea officinalis</i>)	0/3	0/3	nd	nd
Raspberry (<i>Rubus</i> sp.)	0/2	0/2	nd	nd
St. John's Wort (<i>Hypericum perforatum</i>)	0/1	0/1	nd	nd
Walnut leaves (<i>Juglandis folium</i>)	0/1	0/1	nd	nd

nd: not detected

The mean recovery results of the analysed five replicates with $1 \mu\text{g g}^{-1}$ FB₁ and FB₂ for thyme, mint, and stinging nettle are given in Table 2.

Table 2. Recovery results of fumonisins from thyme, mint, and stinging nettle

Sample (n=5)	Concentration added ($\mu\text{g g}^{-1}$)		Recovery (%)	
	FB ₁	FB ₂	FB ₁	FB ₂
Thyme	1	1	67.2 \pm 5.2	80.8 \pm 14.3
Mint	1	1	62.1 \pm 7.9	80.4 \pm 7.9
Stinging nettle	1	1	85.9 \pm 3	89.2 \pm 10.8

The minimum detectable amount for the OPA derivatives of FB₁ and FB₂ were 1 ng per injection and 2.5 ng per injection, respectively. The limit of quantitation (LOQ) S/N=10 was 0.078 and 0.313 $\mu\text{g g}^{-1}$ for FB₁ and FB₂, respectively. The limit of detection (LOD) S/N=3 was 0.023 and 0.093 $\mu\text{g g}^{-1}$ for FB₁ and FB₂, respectively.

The levels of fumonisin contamination in feed, corn, and corn-based products range between 0.25 $\mu\text{g g}^{-1}$ and 188 \pm 25.38 $\mu\text{g g}^{-1}$ generally in the country (SONAL & ORUC, 2000; OMURTAG, 2001; OCAK & BOSTAN, 2010). Analyses in corn flour conducted in the northern region of Turkey pointed out that processing of corn is critical in the decontamination from *Fusarium* toxin (NUMANOGLU et al., 2010). However, out of the large number of researches conducted to detect the level of fumonisin in corn and cereals, a few are studied in plants that are used with medicinal and dietary purpose. In Turkey, OMURTAG and YAZICIOGLU (2004) detected FB₁ in mint and stinging nettle (0.160 and 1.487 $\mu\text{g g}^{-1}$) from herbal tea and medicinal plant samples. Besides, the first report in Portugal on the natural and frequent occurrence of FB₁ in medicinal plants prevised the need of risk management strategies on the natural

contamination with this mycotoxin (MARTINS et al., 2001a). In Eastern Cape Province of South Africa, levels of FB₁ was in the range of 34–524 µg kg⁻¹ and 8–1553 µg kg⁻¹ in several dietary and medicinal plants, respectively (SEWRAM et al., 2006).

The medicinal and dietary plants consumed all over the world have an important role in human health. The current incidence and the data available on the toxicity and carcinogenicity of FB₁ and FB₂ indicate that the mycotoxins are a potential risk to human health and their occurrence is also harmful to economy. Surveys conducted in many countries have shown that contamination of food by *Fusarium* species of fungi is a major problem, because growth, depending on the climate conditions, can develop in the field and during storage. Therefore, they need to be controlled and analysed all throughout the processing stage and not only for FB₁ and FB₂ but also for other *Fusarium* mycotoxins. In this context, a guideline on FB₁ and FB₂ in maize and maize products is set in Turkish Food Codex (TGK, 2011), however a specified limit for fumonisins in plants used as food supplement is currently not available.

The Swiss Federal Office of Public Health has established regulations for FB₁ and FB₂ in maize products at 1 µg g⁻¹ (FAO, 1997; SWISS FEDERAL OFFICE OF PUBLIC HEALTH, 1997) and a Tolerable Daily Intake (TDI) value is estimated by the Joint Food and Agriculture Organization-World Health Organization Expert Committee as 2 µg kg⁻¹ bw (WHO, 2000). The European Commission also highlighted this mentioned TDI limit for *Fusarium* toxins in the recent Commission Regulation (EC, 2007). There is evidence that epidemiological studies evaluate the intake of FB₁ in diet as a risk factor to increase the rate of human oesophageal cancer, liver damage, and levels of certain classes of lipids, especially sphingolipids (NJOBEB et al., 2010). Additionally, the intake of high levels of fumonisin during early pregnancy was associated with the risk to neural tube defects of the brain and spinal cord (GELINEAU-VAN WAES et al., 2005; MISSMER et al., 2006).

For this purpose, to estimate the dietary exposure to fumonisin via consumption of contaminated medicinal plants in northern region of Turkey, a simple risk assessment scenario was realized on mint, which was the highest contaminated plant in our study with a mean of 0.191 µg g⁻¹ of the two positive samples. TDI of 2 µg kg⁻¹ bw per day is considered for an adult person of 70 kg (=140 µg per day), inspired by the similar assessment of DI MAVUNGU and co-workers (2009) conducted for ochratoxin A. We supposed that dried mint is widely used in most of the traditional dishes in Turkey and its approximately ratio is one dessert spoon per portion (~2 g mint). Thus, an adult person of 70 kg would consume 0.382 µg, which might be accepted as a tolerable dose (<140 µg per day).

Consequently, increase or decrease of these toxins in foods depend on how the production line and method operates. Accordingly, we assume that drying plant without using standardized methods might increase the risk of unwanted mycotoxins entering the food chain.

3. Conclusions

In conclusion, on the presence of fumonisin little is known about homemade medicinal plants. In our study, regular consumption of low doses was not considered, but detected level of contamination did not bear a relevant risk. Hence, for monitoring the occurrence of FB₁ and FB₂ in those plants, more field studies on the preparation steps are needed.

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This study was supported by the Marmara University Research Council (BAPKO, SAG-D-300409-0113).

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