Jundishapur J Microbiol. 2012;**5**(3):497-501. DOI: 10.5812/jjm.4251

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Jundishapur Journal of Microbiology

www.jjmicrobiol.com

Survey of T-2 Toxin Present in Cereals Destined for Human Consumption

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ARTICLE INFO

Article type:
Original Article

Article history:
Received: 15 Jun 2011
Revised: 23 Jan 2012
Accepted: 25 Jan 2012

Keywords:
Trichothecenes
T-2 Toxin
Grain
Mycotoxins

ABSTRACT

Background: A variety of agricultural products are exposed to fungal contamination from the early stages of planting, until their final consumption. T-2 mycotoxin is toxic to humans and to all animal species, it is mainly produced by the various *Fusarium* species including; *F. sporotrichioides*, *F. poae*, *F equiseti*, and *F. acuminatum*, and occasionally by other genera species, therefore, measuring T-2 toxin levels is very important in cereals.
Objectives: We examined the occurrence and levels of T-2 mycotoxin in grains for human consumption.
Materials and Methods: Rice, barley and wheat samples, 23, 16 and 7 respectively, were collected from the staple stores of nine food cooking centers in Tehran. After pulverizing the samples, they were extracted using a methanol-water solution (70:30), then analysed with an enzyme linked immunosorbent assay (ELISA), based on the monoclonal antibodies, the amount of T-2 mycotoxin was measured in their extracts.
Results: All of the tested samples were contaminated with T-2 toxin at different levels ranging from 7.9 to 65.9 μg/kg (mean: 17.9 ± 2.1). Wheat samples had the highest level of contamination at approximately 42.4 μg/kg (± 8.4). However, both barley and rice were also affected with contamination levels of 18.3 (±2) and 12.5 (± 0.56) μg/kg respectively.
Conclusions: Although the majority of samples were based on Iranian national standards, a small number of specimens (13.9 %) were contaminated at higher than acceptable limits. The extent of the impurities with T-2 toxin is an indicator of the current normal prevalence of mycotoxins in agricultural products destined for human consumption in this country, and the risk of exposure to the chronic effects of this toxin. Overall, this study showed that the level of mycotoxins in food products should be checked before they are bought or consumed.

Implication for health policy/practice/research/medical education:
Our study suggested that the levels of mycotoxins in products should be detected before buying and be discarded from human consumptive cycle if the grains are contaminated more than allowable limit.

Please cite this paper as:
Riazipour M, Imani Fooladi AA, Bagherpour G. Survey of T-2 Toxin Present in Cereals Destined for Human Consumption. *Jundishapur J Microbiol*. 2012;**5**(3):497-501. DOI: 10.5812/jjm.4251

1. Background

A multitude of agricultural products are exposed to fungal contamination from the early stages of planting until their eventual consumption (1, 2). If the contaminating fungi belong to a toxin producing species, it may produce mycotoxin as a secondary metabolite at some stage of its growth. As mycotoxins are usually classified as stable compounds (2), the different processes which are conducted on the grains before they are consumed, such as cooking at normal temperature ranges (less than 150°C), do not decrease toxicity levels in the majority of cases. (3).

Besides eating, humans confront mycotoxins through inhalation and skin contact. Most of the reported cases

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DOI: 10.5812/jjm.4251

of acute poisoning with mycotoxins have occurred in developing countries. On the contrary, acute and collective cases have rarely been seen in developed countries, and their only concern is its chronic effects, i.e. mutagenic and carcinogenic.

Trichothecenes are a dominant family of mycotoxins, which are commonly found in grains and grain-based products. They may be produced by mold species such as; *Cryptomela, Myrothecium, Stachybotrys, Trichoderma, Hypocrea, Trichothecium,* and *Verticimonosporium genera,* but the *Fusarium* species play the most significant role in secreting these poisons (4). About 150 kinds of trichothecenes have been identified, of which the T-2 toxin is the most important and this is mainly produced by *F. sporotrichioides* (5).The greatest effect of T-2 and other trichothecenes is its ability to inhibit protein synthesis, which leads to secondary disorders in DNA and RNA synthesis. This toxin mainly affects fast dividing cells such as; epithelial, epidermal, lymphoid and erythroid cells and decreases immunoglobulin G (IgG) and other humoral factors like cytokines (5).

Typical clinical symptoms of trichothecenes' contamination include; loss of appetite, vomiting, diarrhea, gastrointestinal bleeding, vertigo and a weakened immune system, alteration of cell membrane functions and lipid peroxidation (6, 7). However, necrotic lesions of the oral cavity, esophagus and stomach and in particular, pronounced leukopenia consisting primarily of bone-marrow hypoplasia and aplasia are the major pathological changes reported among its victims (8).

There are numerous reports about food contamination with T-2 toxin in different countries, this shows a wide range of occurrence from zero (9, 10) to 100 % (11-15). Although, food contamination with mycotoxins in Iran has been investigated in several studies (16-18), measuring the amount of T-2 toxin has rarely been carried out which is a significant oversight (19, 20).

2. Objectives

The aim of this study is to provide a measure of the significance of cereal impurities with T-2 toxin being the most poisonous member of the trichothecenes family. This was achieved by a scrutiny of cereals for human consumption in some of the food cooking centers that serve a multiple number of meals in Tehran.

3. Materials and Methods

This cross-sectional study was carried out both in the field and in laboratory situations in winter (January) 2006.

3.1. Sampling

Samples were collected through standard methods from the grain and legume kitchen stores of 9 food cooking centers in Tehran. (Iran Standard and Industrial Research Institute, Work Guideline No. 2087). In this method, the number of specimens was taken from three parts different of each batch (both sides and middle of the batches). Next, by mixing these three parts, approximately 200 g was taken as a final sample and kept in a closed container in the refrigerator.

3.2. Preparation of Samples and Extracting

100 g of each sample was powdered by mixer and sifted with a soft sieve. Then, they were distributed in closed, non-penetrable containers and kept at -20°C until the next tests; a methanol-water solution (70:30) was used for the extraction. 25 ml of the solvent was added to 5 g of the powdered sample. Then, a mixer (Panasonic Mixer Grinder MX-AC210) mixed it for 10 minutes at a speed of 150 rpm. Next, it was centrifuged for 30 minutes at 10 000 rpm and the supernatant was collected and kept at -20°C.

3.3. ELISA for Measuring T-2 Toxin

A commercially available competitive based ELISA kit (r-Biopharm RIDASCREEN, Art.No.R3801) was used to measure T-2 toxin levels, according to the manufacturer's instructions.

3.4. Standard Graph of Measuring T-2 Toxin Density

A semi-logarithmic standard graph was used for calculating the toxin density in the tested samples. Absorption percentage of each standard was calculated according to the following formula:

$$AbsorbanceStandard\% = \frac{\text{Absorbance of standard}}{AbsorbanceofNegCon} \times 100$$

(Negative control is standard without T-2 toxin)

Then by considering the horizontal axis as a logarithm of the standards concentration and the vertical axis as the amount of their absorption in 450 nm, the standard graph was drawn and toxin density in the unknown samples was calculated by Curve software (Microsoft office Excel 2007) (*Figure 1*).

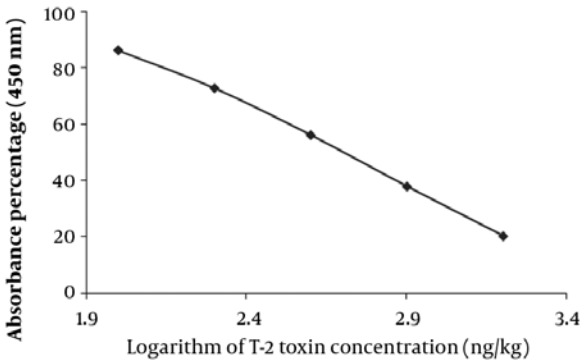

Figure 1. Standard Graph of T-2 Concentration in Grains

3.5. Statistical Analysis

INSTATA statistical software was used for analysis data. ANOVA and a t-test were used for average variance of the different grains and comparing different centers with each other and $P < 0.05$ was considered to be statistically significant.

4. Results

The grains which were used as the primary ingredients for cooking in all of the studied food centers were; rice, wheat and barley. Rice was available in all of the centers, but only 8 and 5 centers had barley and wheat, respectively. Overall, 46 primary samples were collected from the centers; rice, barley and wheat, based on their quantities, were assigned 23, 16 and 7 samples, respectively. The grains were contaminated by T-2 mycotoxin at an average of 17.9 (± 2.1) μg/kg, and the range of contamination varied from 7.9 to 65.4 (mode = 14). The average contamination of rice was 12.5 (±0.56) (mode = 12.06); wheat: 42.4 (±8.4) (mode = 43.7) and barley 18.3 (± 2) (mode = 17.4) μg / kg. *Figure 2* shows the T-2 toxin contamination in three kinds of grains. Variance analysis unraveled the differences among these three grains (P <0.0001) and a t-test revealed that the average level in wheat destined for human consumption is significantly higher than that found in rice (P <0.0001) and barley (P < 0.0005).

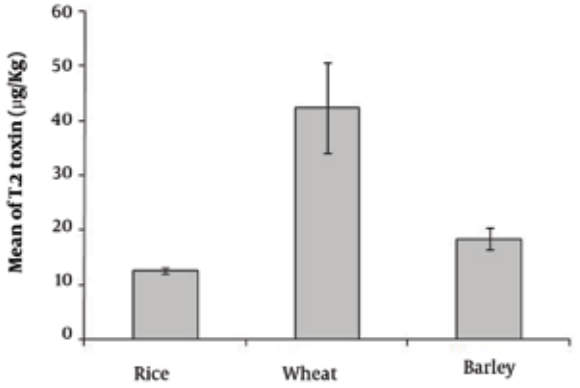

Figure 2. Distribution of Mycotoxin T-2 Contamination in Food Cooking Centers in Winter 2006, Based on Type of Grain

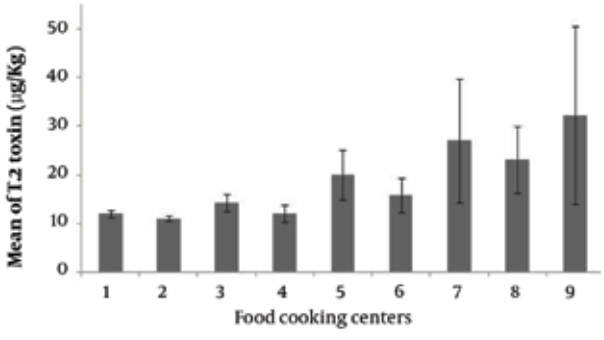

Figure 3. Distribution of Mycotoxin T-2 Contamination in Grains (Rice, Wheat, Barley) at Food Cooking Centers in Winter 2006, Based on Studied Centers

Figure 3 depicts the distribution of grain contamination in the studied centers. The maximum and minimum range of grain contamination pertains to cooking center No. 2 with an average contamination of 10.98 (± 0.48) (n = 5) and cooking center No. 9 with 32.18 (± 18) (n = 3) μg/kg, respectively. Although the graph's shape demonstrates a difference, variance analysis did not show any significant difference among the centers.

5. Discussion

Having carcinogenic potential and poisonous effects, mycotoxins are considered to be one of the most important regulatory issues. In countries with adequate information about mycotoxin occurrence, regular tests to control foodstuffs and detect widespread and serious toxins are currently being performed and this leads to the exclusion of products with higher than allowable limits (20, 21). In Iran, a limited number of mycotoxins including aflatoxins, fumonisins, zearalenon and, ochratoxins are only being measured in export products, but they are not usually checked in foodstuffs for domestic consumption. Trichothecenes are a major family of mycotoxins and T-2 toxin is the most poisonous. Pondering the effects of these toxins for consumers (5, 7), it is crucial that adequate information about how often people are exposed to these kinds of toxins is provided.

It has been reported that fungi, which produce trichothecenes, exist in a number of different foodstuffs (16) and different results have been reported from various studies on measuring *Fusarium* toxins including the T-2 toxin. Yazdanpanah *et al.* by testing 35 immediately harvested wheat samples showed that although some evidence of impurity with other *Fusarium* toxins such as; newelnon, newsolanyol and zearalenon were found, not a single sample was contaminated with T-2 toxin (22). In another study, he showed that *Fusarium* poisons such as T-2 toxin, often contaminate 24 different corn-based human foods, however, the amounts in the majority of cases were low (19). Furthermore, by testing 23 samples of one wheat-based food, Daraei *et al.* reported a high prevalence of *Fusarium* toxins; however, none of them were contaminated with a high dosage level of these toxins.

In this study, we tested the contamination levels of prospective consumptive grains in the stores of some of the food cooking centers for an important mycotoxin from the trichothecenes family. Subsequently, it was demonstrated that all of the provided samples were contaminated more or less by T-2 mycotoxin. In spite of that, different results have been reported from studies in other countries about the occurrence of contamination with T-2 toxin in grains and other crops. Schollenberger indicated that except for two pure samples, all of the other 125 wheat, barley, corn and German corn foodstuff sam-

ples, were contaminated with one or more kind of mycotoxin (23).

Muller proved in various years that 27-61 % of corn (13) and 0-14 % of provender wheat (11) harvested from south German were contaminated with T-2 toxin. Moreover, in The study by Hussein, *et al.* 85 % of New Zealand corn samples had one or several *Fusarium* mycotoxins and T-2 toxin was found in 65 % of them (11). Lepschy *et al.*, also showed that 38 % of wheat, barley, rye, oat and flour were contaminated with T-2 toxin (13). Verabcheva *et al.*, on the contrary, showed that only one specimen of 140 wheat samples which were destined for human consumption, was contaminated with T-2 toxin (0.7 %) (21). And in the Halger study, not a single sample from the central and western parts of the U.S was contaminated with T-2 toxin.

A high incidence of grain contamination with T-2 toxin is an indicator of appropriate conditions for grain contamination with trichothecene mycotoxin producing fungi. It seems that even in a short period of time, with exposure to appropriate conditions for the growth and production of mycotoxins, it is probable that high amounts of this toxin and other mycotoxins will be produced. Yazdanpanah *et al.* demonstrated that keeping samples containing trichothecenes, such as T-2, for 8 days at 5°C in the laboratory, raises their level of toxins from 14-35 to 110-538 ng/g (16).

In spite of the high prevalence of contamination, our outcomes showed that only a few of the grain samples were contaminated beyond the national standard for allowable T-2 limits. Various attitudes toward mycotoxins' health effects lead to a variety of standards for different countries. These differences are mostly based on the economical situation and each country's crop sensitivity to mycotoxins (20). For countries which adopted T-2 toxin standards for human foodstuffs, the permitted amount varies from 20 μg/kg (Slovakia) to 300 μg/kg (Hungary). However, most countries (such as Russia, Bulgaria, Armenia, and Estonia) have accepted 100 μg/kg as their standard (17). In our country the permitted limit for some of the mycotoxins has been identified (Iran Standard and Industrial Research, Regulation No.2925). Upon this, the allowable limit for T-2 toxins in animal food is recommended to be 25μg/kg, but the maximum allowable limit for human consumption is usually lower than those found in animal food, therefore, it is expected that the T-2 toxin permitted level would be lower than 25 μg/kg.

So, none of the samples were contaminated at higher than allowable limits based on most countries standards. However, considering Iranian National Standards, 13.9 % of the tested grains had higher than permitted levels of contamination for animal food and they should not be consumed even by animals, while these foodstuffs have been designated for human use. Wheat and barley were the grains with higher than allowable contamination limits. 80 % of the wheat samples and 12.5 % of the barley samples were contaminated beyond the permitted levels, but not a single sample of rice was contaminated at higher than allowable limits. In recent years, sporadic studies have been carried out on the contamination of consumptive grains with other mycotoxins in our country; these show the differences of grain contamination between each other and with crops from different areas.

Shephard, for example, showed that the occurrence and amount of fumonism in collected corn samples from Mazandaran are higher than those belonging to Esfahan (19, 22). Furthermore Yazdanpanah (15) reported that the amount of aflatoxin B1, aflatoxin B2 and ochratoxin were; 88.8 %, 66.6 % and 2.5 % respectively, in the corn samples of Golestan and Mazandaran provinces. However, all of the barley samples were not contaminated with aflatoxin or ochratoxin (18). Moreover, Hadiani *et al.* found that 7.5 % of corn which was planted in Mazandaran, contained levels of zearalenon at lower levels than the extreme allowable limits of this toxin in Iran (9). But Hedayati found 80.5 % of wheat samples from Mazandaran stores were contaminated with zearalenon, of which 64.4 % was higher than the Iranian allowable standard (200 μg/kg) *et al.* Ghyasian, by collecting 52 corn samples from four corn planting *et al.* areas in Iran; showed that all Mazandaran samples and 53 %, 42 %, and 57 % of the samples from Fars, Kermanshah, and Khuzestan, respectively, were contaminated by fumonisins (7).

Based on our outcomes there were no significant differences between the grains contaminated at the centers studied. Although the maximum and minimum levels of contamination pertained to cooking center No. 9 and No. 2 (*Figure 3*), respectively, their differences were not statistically significant. All of the centers studied were providing their consumptive grains over a short time and they did not store them for long periods of time. All of the grains are purchased from the same source, and they are brought there from diverse parts of Iran. So, it is expectable that the contamination distribution is also similar to each other.

Based on the results of this study, which shows the extent of cereal contamination with T-2 toxin, it is suggested that the levels of contamination should be detected before buying and that they are discarded from the human consumption cycle if the grains are contaminated at higher than allowable limits. As far as discerning differences between the different areas where cereals are sourced, a study using grains with identified sources should be carried out to define the effect of geographical status on levels of T-2 toxin contamination. In order to prevent the growth of fungi in storage, it is important that grains are not stored for a long time and its rapid consumption is recommended.

Acknowledgments

None Declared.

Financial Disclosure

None Declared.

Funding/Support

This work was supported by the grants from the Molecular Biology, Research Center, Baqiyatallah University of Medical Sciences, Tehran , Iran.

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