Section A-Research paper
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Mycotoxins of *Triticum aestivum: In silico* toxicity prediction

Shashank Awasthi[1] and Neeraj Wadhwa[2]*
[1,2] Department of Biotechnology, Jaypee Institute of Information technology,
A-10, Sector-62, Noida-201307, U.P. India
*Corresponding author
Email: neeraj.wadhwa@mail.jiit.ac.in

Abstract:

Mycotoxins are natural toxins mainly produced by pathogenic fungi and these are found in plants and consumed crops such as wheat, rice & maize etc and their products. Mycotoxins causes various serious health risk to humans; they can enter human body by contaminated food products.

In the present study we analysed using in silico studies the harmful effect of mycotoxins of wheat (*Triticum aestivum*) on humans utilizing computational tool ProTox-II. In silico toxicity and other toxicity parameters of mycotoxins were analysed using this tool. International authorities EU & USFDA set permissible range of mycotoxins in food based on LD_{50} values, authorities have categorized mycotoxins into five classes (1-5). Class 1 is fatal and class 5 is categorized harmful for human consumptions. Results obtained were compared to permissible LD_{50} value, all the predicted mycotoxins of wheat exceeded from permissible range. Aflatoxin B1, Deoxynivalenol (DON) and HT-2 were predicted in class 1 which is fatal to humans. Other mycotoxins, Ophiobolin, Aflatoxin G1 & M1 were found under class 3, and these are also very harmful to humans. Almost all the toxin are neurotoxic and carcinogens. This study clearly states that some mycotoxins are very harmful and can cause severe damage to liver and other organs of humans and they are carcinogen and immunotoxic also. Therefore, a safe approach should be developed to mitigate the contamination of Mycotoxins by inhibiting the infection of plant pathogenic fungi using biological methods.

Key Words Mycotoxins, ProTox-II, wheat, in silico prediction, LD_{50} value, organ toxicity.

1. Introduction:

Cereals and their products are one of the most consumed and staple crops worldwide, wheat, is the staple foods in the world [1,2]. Mainly, wheat is used to produce bread, pasta, biscuits, and other food products. Durum wheat *(Triticum durum)* is the most common wheat variety

in India, it is also known as bread wheat. Thus, about 60-70 % of the food products are made up of *durum* wheat [3].

Fusarium head blight disease in wheat is caused by *F. graminearum*, in this wheat grain head is initially infected and then infection was spread whole part of the grain [4].

On storage wheat grain gets contaminated by moulds and their toxins commonly termed as mycotoxins [5,6]. Mycotoxins are toxic metabolites produced by various fungal species like *Fusarium, Dreschelra* etc, which grow on grain, this mycotoxin belong to the most toxic contaminants present in various food supplies [7]. It can cause acute & chronic effects in human as well as in animal also.

In grains like wheat, rice and their products may comprise of mycotoxins like deoxynivalenol, zearalenone ochratoxin A and aflatoxins [8,9,10]. In barley, wheat and maize, deoxynivalenol mycotoxins are abundantly present after infection with *Fusarium graminearum* [11,12].

From last 30 years, mycotoxins have been the reason of epidemics in humans and animals all over the world. In Europe thousands of people died because of ergotism caused by mycotoxins. In Russia during 1942-1948, around one lac people lost their livesdue to alimentary toxic leukopenia caused by Mycotoxins [13].

Fusarium toxins such as deoxynivalenol (DON), zearalenone (ZEA), nivalenol (NIV), fumonisins (FUM), T- 2, and HT- 2 toxins are the most common toxins present in wheat [14-21]. It also causes various health diseases for example gastrointestinal upset, abdominal pain, diarrhoea, mental illness [22,23]. It is well reported by several research groups that mycotoxins are the causative agents causing carcinogenicity, genotoxicity, nephrotoxicity, hepatotoxicity, immunotoxicity and gastrointestinal upset, dermatological problems and neurological disorder [24]. Most of the mycotoxins are highly reactive and inhibit the protein synthesis as well. They have immunotoxin effects on humans [25].

In humans, Aflatoxins showed mutagenic and carcinogenic effect leading to development of hepatocellular carcinoma [26]. It also shows toxicity against ROS and responsible for DNA damage & cytolysis, can attack on p53 gene [27,28,29].

Fuminosin mycotoxins present in wheat can cause oesophageal cancer and it also shows embryotoxic and harmful for renal system [30,31,32]

Ochratoxin (OTA) is nephrotoxic causes renal failure in humans [33,34]. While T2 toxins causes defects in DNA stand leads to cervical cancer and it also enhance ROS and lipid peroxidation [35].

Zearalenones present in wheat affects oestrogen hormone leading to various reproductive impairment such as reproductive disorder and low fertility count etc [36]. ZEA also have immunosuppressive property [37].

However toxic properties of chemicals present in various food, drugs and other products needs an early predication of toxicity level as this will be significant for the discovery of various drugs and also important for regulatory bodies like US FDA, US Environmental Protection Agency [38] and European Environment Agency & European Medicines Agency [39]. (Table 1)

Table 1. Mycotoxins permissible limit present in food and their products (40,41,42).

Mycotoxins	**Food products**	**Permissible limit (µg/kg)**	
		European Union	**US FDA**
Zearalenone (ZEA)	Wheat & Other cereals and its products, maize	20-400	NA
Ochratoxin A(OTA)	Wheat & Other cereals and its products, vine, coffee, spices	2-80	NA
Deoxynivalenol (DON)	Cereal and cereal products	200-1750	1000
Patulin	Fruits & Cereals	10-50	50
Aflatoxins B1,B2,M1,M2,G1,G2	Wheat, rice, fruits, nuts	4-15	20
Fumonisins B1,B2,B3	Maize and other cereals	4000	NA
T2 & HT-2	Cereals like wheat, maize, barley, oat and their products	50-1000	NA

In our daily life we are exposed to many harmful chemicals which includes our food cosmetics we use, Over the counter (OTC) and other prescribed medicines, even air we inhale has been polluted by various industrial gases. However, effect of these chemical depends upon the quantity and exposure time, may be useful or harmful [38,39].

Thus, it is necessary to check experimentally, the noxious effects of these chemical compound and their products (38,39). But due to several limitations like fund, time, and ethical concern for animal trials, it is very difficult task to perform experimental for each chemical.

Thus, in silico toxicity predication using pro tox tool is highly beneficial platform for the toxicity assessment of chemicals which can be harmful to all living being [39].

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The aim of the in-silico toxicity prediction was to check the effect of various mycotoxins and their combinations present in wheat and studied their effect on various organs and immune system etc. "In silico toxicity model incorporates the knowledge from various fields such as toxicology, biostatistics, systems biology, computer science and many other relevant discipline" [39,43]. In this analysis, quantitative as well qualitative of mycotoxins present in wheat has been performed like lethal dose (LD_{50} values), cytotoxicity, carcinogenicity, and hepatotoxicity predication respectively. On the basis toxicity predication result, we can provide a substitute approach for mycotoxin control or mycotoxin degradation using biological strategies [44].

2. Materials & methods:

2.1. *In silico* Prediction analysis

ProTox-II tool was used for the in-silico prediction of mycotoxins [38]. In this, PubChem name or chemical structure of mycotoxins were used. Prediction analysis was done based on five classifications: acute toxicity, organ toxicity, toxicological endpoints, toxicological pathways, and toxicity targets, in these five classes, total 33 models were available for analysis [45]. This tool is based on molecular similarity pathways and machine-learning approach [44].

2.2. Prediction of mycotoxins for Oral Toxicity

All selected mycotoxins of wheat name were analysed in ProTox-II, depending on the LD_{50} (mg/kg bodyweight) value range, mycotoxins were categorized under 5 different toxicity class [46].

These Classes are as follows:

Class 1: Fatal if swallowed (LD_{50} value range $\leq$ 5 mg/kg)

Class 2: Fatal if swallowed (LD_{50} value range 5-50 mg/kg)

Class 3: Toxic if swallowed (LD_{50} value range 50-300 mg/kg)

Class 4: Harmful if swallowed (LD_{50} value range 300-2000 mg/kg);

Class 5: may be harmful if swallowed (LD_{50} value range 2000- 5000 mg/kg).

2.3. Prediction of mycotoxins for Toxicity Endpoint and Organ Toxicity

Mycotoxins which were selected for oral toxicity were also predicted toxicity against various organs and endpoint toxicity evaluation. In this, predictive models were based on data from

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both *in vitro* (Tox21 assays, Ames bacterial mutation assays, hepatocytotoxicity assays and immunotoxicity assays) and *in vivo* assays (carcinogenicity, hepatotoxicity etc.) [38,47].

2.4. Prediction of mycotoxins for Toxicological Pathways

Mycotoxins present in wheat and other food products can activate & alter the specific enzymes or receptor involved in various biological pathways, resulting disruption in metabolic process leads to various health issues.

In ProTox-II, mainly two broad Toxicological Pathways were analysed: (1). Nuclear receptor signalling pathways. (2). Stress response pathways.

In nuclear receptor signalling pathways seven pathways were analysed such as hydrocarbon Receptor, androgen Receptor, Androgen ligand binding domain, Aromatase estrogen receptor, Estrogen receptor ligand binding domain, Peroxisome proliferator receptor etc. While in Stress response pathways, total five pathways like nuclear factor /antioxidant responsive pathways, heat shock element pathways, prediction of mitochondrial membrane potential, Tumor suppressor pathway, ATPs domain pathways were predicted.

Aim of this prediction analysis was to evaluate in-depth toxicological study of mycotoxins to identify mechanism related to disease associated pathways. ProTox-II tool was used to check the identification of the harmful health effect of mycotoxins in the human body very proficiently.

3. Result and Discussion:

3.1. *In silico* Toxicity Prediction

Most of the mycotoxins present in wheat are not reported in literatures and *in-vitro* studies is not possible in short duration of time. So, prediction *in-silico* was performed using ProTox-II computational tool to identify the effect of mycotoxins of wheat on humans.

3.2. Prediction of mycotoxins for oral toxicity analysis

Data obtained after oral toxicity prediction by ProTox-II are based on similarity and identification of toxic compounds. Result obtained as a lethal dose (LD_{50} values) of respective mycotoxins against humans. Predicted LD_{50} value and toxicity class of identified mycotoxins of wheat are shown in Table 2.

Table 2. Prediction of Toxicity class and LD_{50} Value of various mycotoxins of wheat

Mycotoxins present in wheat	**LD_{50} Values (mg/kg)**	**Toxicity Class**	**Average Similarity (%)**	**Average accuracy (%)**
Aflatoxin B1	3	1	100	100
Aflatoxin B2	600	4	62.66	68.07
Aflatoxin G1	120	3	68.06	68.07
Aflatoxin G2	832	4	57.56	67.38
Aflatoxin M1	120	3	74.57	69.28
Ochratoxin A (OTA)	20	2	100	100
Fumonisin B1	4280	5	68	68.07
Fumonisin B2	4280	5	68	68.07
Fumonisin B3	4280	5	70.38	69.26
Ophiobolin	238	3	100	100
Zearalenone (ZEA)	500	4	100	100
Deoxynivalenol (DON)	5	1	100	100
Patulin	17	2	100	100
T-2 Toxin	615	4	100	100
HT-2	4	1	100	100

According to the obtained predictions, three mycotoxins Aflatoxin B1, Deoxynivalenol (DON) & HT-2 toxin of wheat showed that they are lethal to humans if consumed, these were present in Class 1 toxicity level having LD_{50} value of 3, 5 and 4 mg/kg respectively (Table 2). LD_{50} value of Ochratoxin A (OTA) and Patulin are 20 & 17 mg/kg respectively showing toxicity level of Class 2. Aflatoxin G1, M1 and Ophiobolin showed Class 3 level toxicity with LD_{50} values of 120, 120 & 238 mg/kg respectively. All predicted values of mycotoxins of wheat when compared with standard permissible range of mycotoxins as per EU & USFDA, showed exceeding from the range and showed that they are lethal & harmful to humans even if present within permissible range.

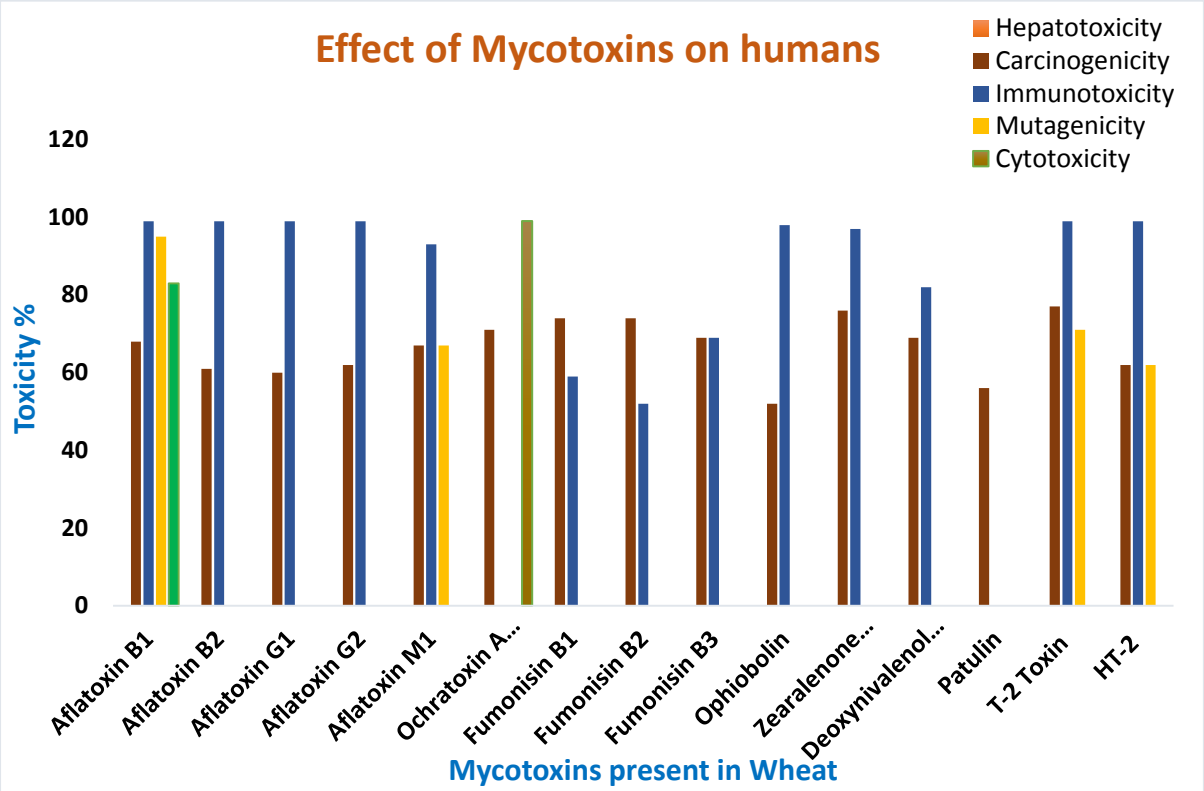
3.3. Prediction of mycotoxins for Organ toxicity and toxicity end points

Organ toxicity and other toxicity parameters were analysed to check the effect of mycotoxins of wheat against liver and other. Mycotoxins are metabolized in the liver, so it was necessary

to check the effect of various mycotoxins of wheat on liver. In Table 3, the results obtained of organ toxicity and varied toxicity endpoints and their predicted values are reported.

Mycotoxins present in wheat	**Effect against Liver**	**Effect against various toxicity end points**			
	Hepatotoxic	**Carcinogenic**	**Immunotoxic**	**Mutagenic**	**Cytotoxic**
Aflatoxin B1	No	Yes	Yes	Yes	Yes
Aflatoxin B2	No	Yes	Yes	No	No
Aflatoxin G1	No	Yes	Yes	No	No
Aflatoxin G2	No	Yes	Yes	No	No
Aflatoxin M1	No	Yes	Yes	Yes	No
Ochratoxin A (OTA)	No	Yes	No	No	Yes
Fumonisin B1	No	Yes	Yes	No	No
Fumonisin B2	No	Yes	Yes	No	No
Fumonisin B3	No	Yes	Yes	No	No
Ophiobolin	No	Yes	Yes	No	No
Zearalenone (ZEA)	No	Yes	Yes	No	No
Deoxynivalenol (DON)	No	No	Yes	No	No
Patulin	No	Yes	No	No	No
T-2 Toxin	No	Yes	Yes	Yes	No
HT-2	No	Yes	Yes	Yes	No

Regarding the organ toxicity (Hepatotoxicity), results showed that all the mycotoxins of wheat were not hepatotoxic for humans. While in toxicity endpoints prediction result, all mycotoxins were carcinogenic except Deoxynivalenol (DON), Wheat mycotoxins except Patulin and Ochratoxin (OTA) are immunotoxic while Aflatoxin B1, M1, T2 and HT-2 toxin showing mutagenic activity and Aflatoxin B1 & Ochratoxin A (OTA) were predicted as cytotoxic (Graph 1).



Graph 1. Effect of mycotoxins of wheat against Organ toxicity and toxicological endpoints

3.4. Prediction of Mycotoxins for toxicological pathways

3.4.1 Nuclear Receptor Signalling pathways

Mycotoxins are the potential threats to human health [45]. Seven different cellular signalling pathways prediction result revealed that Zearalenone (ZEA) mycotoxins of wheat showed that it can be harmful affect to Estrogen Receptor and Estrogen ligand binding receptor. ZEA mycotoxins could affect these cellular pathways in humans (Table 4).

Table 4. Prediction of mycotoxins of wheat against various nuclear receptor signalling pathways

Mycotoxins present in wheat	Nuclear receptor signaling pathways						
	Aryl hydrocarbon Receptor	Androgen Receptor	Androgen Ligand Binding receptor	Aromatase receptor	Estrogen Receptor	Estrogen Ligand Binding receptor	Peroxisome proliferator Receptor
Aflatoxin B1	No	No	No	No	No	No	No
Aflatoxin B2	No	No	No	No	No	No	No
Aflatoxin G1	No	No	No	No	No	No	No
Aflatoxin G2	No	No	No	No	No	No	No

Aflatoxin M1	No	No	No	No	No	No	No
Ochratoxin A (OTA)	No	No	No	No	No	No	No
Fumonisin B1	No	No	No	No	No	No	No
Fumonisin B2	No	No	No	No	No	No	No
Fumonisin B3	No	No	No	No	No	No	No
Ophiobolin	No	No	No	No	No	No	No
Zearalenone (ZEA)	No	No	No	No	**Yes**	**Yes**	No
Deoxynivalenol (DON)	No	No	No	No	No	No	No
Patulin	No	No	No	No	No	No	No
T-2 Toxin	No	No	No	No	No	No	No
HT-2	No	No	No	No	No	No	No

3.4.2. Stress response pathways

In this prediction five stress related pathways were analysed and computational predictions were presented in table. Mycotoxin Zearalenone (ZEA), which showed to be active against 4 pathways such as nuclear factor pathway, heat shock response pathway, mitochondrial membrane potential and tumor suppressor (p53) pathways (Table 5).

Table 5. Stress response pathways predicted using the ProTox-II tool for different mycotoxins of wheat

Mycotoxins present in wheat	**Stress response pathways (% Probability)**				
	Nuclear factor (erythroid)/ antioxidant responsive element	**Heat shock factor response element**	**Mitochondrial Membrane Potential (MMP)**	**Phospho protein (Tumor Suppressor) p53**	**ATPase family AAA domain-containing protein 5**
Aflatoxin B1	No	No	No	No	No
Aflatoxin B2	No	No	No	No	No
Aflatoxin G1	No	No	No	No	No
Aflatoxin G2	No	No	No	No	No
Aflatoxin M1	No	No	No	No	No
Ochratoxin A (OTA)	No	No	No	No	No
Fumonisin B1	No	No	No	No	No
Fumonisin B2	No	No	No	No	No
Fumonisin B3	No	No	No	No	No
Ophiobolin	No	No	No	No	No
Zearalenone	Yes (100)	Yes (100)	Yes (95)	Yes (100)	No

Deoxynivalenol (DON)	No	No	No	No	No
Patulin	No	No	No	No	No
T-2 Toxin	No	No	No	No	No
HT-2	No	No	No	No	No

4. Conclusion

Our findings suggest that mycotoxins produced by phytopathogens in wheat and other food products can lead to health problems are significant. *In-silico* prediction of Mycotoxins were studied using ProTox-II tool, we have predicted toxicity level based on their LD_{50} value and data were compared with standard range controlled by USFDA & EU (Table 1).

Based on oral toxicity result, LD_{50} value and toxicity class of predicted mycotoxins has been presented in Table 2, as per the result Aflatoxin B1, Deoxynivalenol (DON) and HT-2 were presented in Class-1 toxicity which are fatal to human for consumption. While Ochratoxin A (OTA) & patulin were predicted to be belonging to Class 2 and Ophiobolin, Aflatoxin G1 & M1 belong to Class 3 level, both the classes are fatal & toxic to humans. Aflatoxin B2 & G2, Zearalenone (ZEA) and T2 Mycotoxins which were predicted to be of Class 4 toxicity level a causing adverse health effect in humans. In the finding of toxicity end point prediction almost all the mycotoxins are carcinogenic and immunotoxic

To address this issue, it is essential to develop strategies aimed at mitigating mycotoxin development and preventing fungal infections throughout the entire grain production and processing cycle. Mycotoxin of wheat and other crops showed substantial adverse health effect in human and it also affects food supply chain. Mycotoxin contamination in crops and food products has been serious issue for human beings.

. In summary, by implementing a comprehensive approach that includes seed treatment, improved storage practices, advanced processing technologies, and innovative formulations, it will be possible to effectively mitigate mycotoxin development and prevent fungal infections in grains. These strategies can help safeguard food safety and protect human health.

Section A-Research paper
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