Functional and morphological alteration in deoxinivalenol (DON) induced liver and kidney injuries

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Abstract

The aim of the study was to assess the influence of known concentration of deoxinivalenol (DON), naturally produced by fungi, on the blood component and histological structure in liver and kidney. Acting in small amounts they can be toxic to humans and animals. Forty male rats of the Sprague-Dowley breed were divided in 4 groups who received through feed doses of 3 mg DON/kg BW (B, n=10), 5 mg DON/kg BW (C, n=10), 9 mg DON/kg BW (D, n=10) and none DON (A-control group, n=10). The main components of blood as a alanine aminotransferase (ALT), glucose (GLU), serum creatinine (C), serum urea (U) and blood urea nitrogen (BUN) were significantly higher (P ≤ 0.05) and serum albumin (ALB) respectively total serum protein (TP) were significantly lower (P ≤ 0.05) for in experimental groups compared to control group. Complexity of pathological changes in liver and kidney increased with increasing dose DON administered in feed. In the liver tissues were highlighted with the following pathological changes: hyperemia and swelling of hepatic sinusoids, hepatocyte large globular with rich intense eosinophilic cytoplasm, enlarged nuclei with evident nucleolus and hyperchromasia variable light space portal fibrosis with rare inflammatory cells and swelling of blood vessels and accumulation of apoptotic bodies. In renal tissue the pathological changes were represented by degenerative lesions of the renal tubular epithelium and glomerulus level.

Keywords: deoxynivalenol, rat, liver, kidney, histological

Introduction

Numerous species of the Aspergillus, Fusarium and Penicillium genera are known to produce mycotoxins. Mycotoxins, natural secondary metabolites produced by fungi, are agents with various chemical structures which are toxic to humans and animals. Present in feed and food, even in very small amounts, mycotoxins can have a negative effect on animals and humans health. [1, 2].

Deoxynivalenol (DON) ((3 α, 7 α, and 15 α trihydroxy 12, 13-epoxytrichothe-9-en-8-one), produced by fungi of Fusarium genus, in particular F. graminearum, also known as vomitoxina, is the most common mycotoxin from the trichotheccenes group. The other
*Fusarium* species from which DON is isolated are *F. culmorum*, *F. roseum*, and *F. sporotrichioides* [3].

DON is a toxic by-product of *Fusarium* head blight and a contaminant of oats, corn, wheat, barley, rice, and other cereal grains [4]. In 2001, the JECFA published a monograph summarizing safety data of certain mycotoxins found in food, including DON. In general, acute exposure to DON resulted in decreased feed consumption (anorexia) and vomiting (emesis) [5]. The DON dose, present in food or feed, which can determine the morphological and physiological changes in animals and humans, can differ. The Scientific Committee on Food [6] derived a tolerable daily intake of DON for humans of 1 μg/kg body weight after multiplying a no observed adverse effect level of 0.1 mg DON/kg resulting from one long-term study on mice, with a 100 safety factor.

Studies regarding the DON effect were performed on different groups of organisms such as: rats [7, 8], mice [9], pigs [10], broiler chickens [11], and poultry [12]. Analysis of blood parameters evolution in animals, treated with feed contaminated with mycotoxins, reveals changes of hematological parameters (erythrocytes, leukocytes, platelets, hemoglobin concentration, hematocrit), as most of the values fall. This fact suggests that mycotoxins exert a toxic effect directly on the blood system and on the formation of blood elements [9, 14, 15, 16].

Determination of biochemical parameters (alanine aminotransferase (ALT), albumin (ALB), glucose (GLU), total protein (TP), serum creatinine (C), serum urea (U), and blood urea nitrogen (BUN)) is very important and useful in diagnosing disorders in liver and kidney functions. The analysed blood parameters present decreases or increases the normal values, depending on the type of mycotoxin, animal and dosage [17, 18, 19, 15]. Other studies [13, 20] report histological changes in the intestinal, gastric, hepatic and renal tissue. Haematological and biochemical parameters of blood are often confirmed and supported by histological analysis results of liver and kidney tissues [21, 22, 23, 24, 25].

Laboratory studies on animals revealed their overall weight loss [8, 13] on one hand and the weight loss of some organs such as: heart, brain, liver [7,8] on the other hand.

The present study aims to determine the effects of deoxynivalenol (DON, vomitoxina) on rats’ based on biochemical parameters and histological changes in liver and kidney tissues. The obtained results offer new insights into the research field of mycotoxins.

**Materials and methods**

**Animals study**

The study was conducted on a total of 40 male Sprague-Dowley rats weighing 410 ± 10.3 grams, in accordance with the conditions imposed by the Ethical Commission approval based on European Union’s Directive (2010)/63/EU for animal experimentation [26].

The animals, obtained from the biobase of the Faculty of Medicine and Pharmacy “V. Babes” from Timisoara were acclimatised in the laboratory of molecular and cellular biology at standard conditions: i.e., humidity (45-55%), temperature (25° C) and light control (12h light /12h dark). The animals were kept in individual cages and all animals received normal diet and water ad-libitum, which were supplemented with deoxynivalenol (DON, vomitoxina).

DON was daily administered for 8 weeks in increasing doses [g/kg]. The amount of contaminated feed was administrated based on the amount of DON in feed. The animals in question were divided into 4 groups:
Group A (the control group) - 10 rats without DON administration,
Group B - 10 rats administrated with a dose of 3 mg/kg BW DON,
Group C - 10 rats administrated with a dose of 5 mg/kg BW DON,
Group D - 10 rats administrated with a dose of 9 mg/kg BW DON.

Figure 1. Deoxynivalenol structure (DON) [51481-10-8]

The animals were weighed at the beginning of the study and then weekly until the 8th week of administration of DON contaminated feed. The last weighing was before the rat’s slaughter.

In order to collect biological samples, at the end of the study rats were anesthetized by administration of 100 mg ketamine /kg BW and 20 mg xylazine /kg BW. Abdominal incision was performed and the animals were slaughtered by exsanguination. After centrifuging the collected blood, the obtained serum was used to determine the biochemical parameters. After the slaughter, blood and organ samples were taken.

The investigated material of this study is represented by fragments of liver and kidney tissue for histological achievement.

Biochemical analysis
Blood was extracted from the inferior cava vena and collected in heparinized tubes. The samples were centrifuged at 3,000 rpm for 10 min and serum samples were separated and preserved at -20 °C until analysis. The analyzed parameters of serum included alanine aminotransferase (ALT, E.C. 2.6.1.2), albumin (ALB), glucose (GLU), total protein (TP), serum creatinin (C), serum urea (U) and blood urea nitrogen (BUN), which were determined using an automatic analyser (Vitros Fusion 5,1).

Histological analysis
The collected tissue samples from liver and kidneys were processed by usual histological technique that involves fixing in 10% neutral buffered formalin. Subsequently, histological pieces were washed with tap water and then distilled water and processed by paraffin inclusion classic technique comprising the steps of: dehydration in ethanol, clearing in xylene, embedding in paraffin and cut into 4 µm thick sections (microtom MLHL3500), and placed on glass slides.

For overall diagnosis of each case the Hematoxylin-Eosin staining was performed (H-E) [27]. Histological sections were examined under a microscope Nikon Eclipse TE2000-U and digital camera with Nikon BM-6.
Statistical analysis
The data were analyzed using variance analysis. The significance between samples were determined using Mann-Whitney test. The difference was considered significant when \( P < 0.05 \), highly significant when \( P < 0.01 \) and non-significant when \( P > 0.05 \).

Results and discussions

Weight variations
Analyzing the effect of deoxynivalenol on the body weight of male rats, the variations observed were between the experimental groups (B, C, D) and control group (A). The control group presented a 8.63% increase in body weight from the start to the end of experimental period. The animals of experimental groups registered a weight loss in direct correlation with the rise in administrated deoxynivalenol dosage; group B showed a weight loss of 8.83%, group C of 10.22% and group D showed the largest decrease of 11.64% weight (Fig. 2.). The obtained values are in agreement with those presented in the first study on Sprague-Dawley rats, which received deoxynivalenol in their diet in 20 mg/kg BW concentration. At this dose, rats did not refuse feed but showed a weight loss of 10% [28]. Subsequent studies confirm significant weight loss observed at doses of 2.5 g/kg BW [8].

![Figure 2. Weight variation at experimental animals during the study](image)

Blood biochemical parameters
DON influence on the main blood indices is being presented in table 1 and table 2.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Biochemical parameters</th>
<th>ALT U/I</th>
<th>Serum glucose (Glu) mg/dl</th>
<th>Serum albumin (ALB) g/dl</th>
<th>Total serum protein (TP) g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>74.2±4.467</td>
<td>132.6±0.257</td>
<td>5.378±1.893</td>
<td>7.246±0.43</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>84.35±2.582**</td>
<td>161.4±0.351**</td>
<td>4.568±2.015**</td>
<td>7.211±0.28*</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>147.12±3.678**</td>
<td>218.9±0.064**</td>
<td>3.735±1.9**</td>
<td>6.803±0.12**</td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>359±5.617**</td>
<td>301.1±0.027**</td>
<td>3.155±1.726**</td>
<td>6.531±0.06**</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. Number of sample in each group is 10. Group A = Control, Group B, Group C, Group D = Treated group with DON. Significant change in comparison between groups:** Highly significant \( (P \leq 0.01) \) * Significant \( (P \leq 0.05) \) N.S Non significant \( (P > 0.05) \)
Serum transaminase (ALT) had significant higher values ($P \leq 0.01$) in the experimental groups with 13.68 % (group B), 98.27 % (group C) and 383.32 % (group D) compared with controls (group A). ALT is a cytosolic enzyme present in the liver. Pathological changes found in ALT activity can be observed in different diseases (cardiac, skin, muscle, liver diseases) [29]. Some studies indicate that serum aspartate aminotransferase can provide information about: the presence and extent of liver disease, treatment monitoring response, and prognosis. Tests on serum transaminases (ALT and AST) are commonly used as a marker of hepatic cytolysis process, although liver biopsies from patients with normal transaminase have shown some degree of liver injury [30, 18]. Some cells contain specific enzymes that get into the blood only when cells containing them are damaged or destroyed. The presence of significant quantities of blood enzymes indicates the likely tissue damage locations [31].

Glucose concentration increases highly significant ($P \leq 0.01$) with 28.8 mg/dl in group B, 86.3 mg/dl in group C and 168.5 mg/dl in group D compared with the group A.

Serum albumin, in the experimental groups (B, C, D), decreases highly significant ($P \leq 0.01$) with 0.81 g/dl, 1.65 g/dl and 2.22 g/dl compared with the reference group (A).

Total serum protein concentrations in rats treated with DON (B, C, D group) compared to the values recorded in the control group (A) showed highly significant decreases ($P \leq 0.01$) with 0.053 g/dl, 0.46 g/dl, and 0.73 g/dl. Decreases in total serum protein concentration were expected results based on the fact that DON belongs to trichothecenes, a class of mycotoxins which are potent inhibitors of protein synthesis. Decrease in total protein and albumin is due to effects of DON that causes reduced food uptake, and altering protein synthesis. Similar results have been reported in poultry at doses of 3 ppm DON [23, 32].

### Table 2 Values serum parameters

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Biochemical parameters</th>
<th>Serum creatinine (C) mg/dl</th>
<th>Serum urea (U) mg/dl</th>
<th>Serum urea nitrogen (BUN) mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>0.377±0.029</td>
<td>33.3±1.418</td>
<td>14.7±1.229</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>0.400±0.012*</td>
<td>34.63±1.479**</td>
<td>16.3±0.752**</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>0.446±0.009**</td>
<td>38.67±0.849**</td>
<td>17.35±1.119**</td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>0.477±0.020**</td>
<td>46.75±2.917**</td>
<td>19.7±2.83**</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. Number of sample in each group is 10. Group A = Control, Group B, Group C, Group D = Treated group with DON. Significant change in comparison between groups: ** Highly significant ($P \leq 0.01$) * Significant ($P \leq 0.05$) N.S Non significant ($P > 0.05$)

Based on the serum creatinine concentration values were found highly significant ($P \leq 0.01$) increased levels in group B (with 6.1 %), in group C (with 18.3 %) and in group D (with 26.5 %) compared to the control group A.

Serum urea concentration had significant higher values ($P \leq 0.01$) in the groups B (with 1.33 mg/dl), C (5.37 mg/dl), D (13.45 mg/dl) compared to A group (Table 2).

The increased concentrations of serum urea nitrogen in experimental groups (B - with 1.6 mg/dl, C – with 2.65 mg/dl and D – with 5 mg/dl) are highly significant ($P \leq 0.01$), compared to the reference group (A). Increased levels of BUN in laboratory animals which received through feed DON, were also reported in other studies [15, 17].
Histopathological study of the liver

The rats in the control group did not show pathological changes of liver tissue by analysis of liver sections stained with H-E. Analyzing histological changes using coloring technique H-E we found cell nuclei colored in blue-violet and cytoplasm colored pink.

After analyzing histological section stained with HE, were found large globular hepatocytes with eosinophilic cytoplasm, round relative uniform nucleus with granular nuclear chromatin and rare binucleated issues, and in some areas easy pale colagenisation with eosinophilic collagen.

Microscopic analysis revealed uni or polinucleated polygonal hepatocytes arranged in cords form oriented radial towards end-stage liver venula. Analyzing histological liver tissue derived from rats in group B (feed with a dose of 3 mg DON /kg BW) we found the presence of the following: redness and swelling of the liver sinusoids, mild portal fibrosis with rare space inflammatory cells and vessel ectasia (Fig 3.a, Fig 3.b).

On liver tissue sections from rats belonging to group C feed with a dose of 5 mg DON/kg BW, we reported: large hepatocytes, globular with rich intense eosinophilic cytoplasm, enlarged nucleus with evident nucleolus and varied hyperchromasia, slightly altered pericentrilobular vacuolated hepatocytes (Fig 4.a, Figure 4b).

The analysis of liver sections stained with H-E, from the D group animals treated with 9 mg DON / kg BW, presented the most serious alterations of liver parenchyma: vacuolar alteration of periportal hepatocytes and accumulation of apoptotic bodies (Fig 5. a, Fig 5.b). Increasing the concentration of mycotoxins in feed causes the pathological changes in kidney tissue, whose severity increases with the increase in DON concentration.

Studies regarding the identification of histopathological changes in the liver tissue collected from animals which received feed contaminated with DON presented histological changes similar to those identified by us in the liver tissue derived from experimental rats.

The identified pathological changes were represented by hepatocytes vacuolation and cellular membrane damage (necrosis), nuclei grown in size; toxic injury and focal tissue necrosis in target organ, severe vacuolar degeneration of hepatocytes, biliary hyperplasia and mild vacuolar degeneration, and apoptotic cells [15, 25].
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Figure 4. Liver Group C: a – Large hepatocytes, globular with rich intense eosinophilic cytoplasm, larged nuclei slowly ununiform with evident nucleolus and variable hyperchromasia; mild dilation of the vessels in the port area (bottom left) (400x; haematoxylin-eosin stain); b – Centrilobular vein ectasia, mild vacuolar alteration of hepatocytes with mild hypercromatie pericentrilobular vacualizarizari nuclear and perinuclear (400x; haematoxylin-eosin stain).

Figure 5. Liver Group D: a – Acinar zone III hepatocytes (periportal hepatocytes) with cytoplasmic vacuolar alteration issues (400x; haematoxylin-eosin stain); b – Acinar zone III hepatocytes (periportal hepatocytes) with cytoplasmic vacuolar alteration issues, massive vacuolar alteration of hepatocytes, with apoptotic bodies presence (400x; haematoxylin-eosin stain).

Histopathological study of the kidney

The analysis of histological sections taken from rat kidney tissue derived from the control group and the experimental groups B (feed with a dose of 3 mg DON /kg BW) or C (feed with a dose of 5 mg DON /kg BW) did not show existence of obvious pathological changes. The rats which received a lower amount of DON in the feed did not show any pathological changes of renal parenchyma compared with controls.

Compared with the control group, the group D animals administrated with 9 mg DON /kg BW), shown changes in the nephron, in renal tubules, as well as at glomerulus. Thus the histological lesions revealed degenerative renal epithelium with pathological changes in both renal tubules and the glomerulus, and glomerular compression and atrophy (Fig. 6.a). Other pathological changes in the renal capsule was thickened glomerular mesangial cell proliferation (tubular damage) (Fig. 6.b).

Similar results (glomerules of hypertrophic, thickened capsule glomerular mesangial cell proliferation, tubular epithelial cell necrosis) on renal histological sections from rats receiving feed contaminated with DON were reported by Macri et al. [33].
Figure 6. Kidney Group D: a - Intumescent moddy epithelial renal tubules, glomeruli with glomerular atrophy capillaries ball (right) zonal slight thickening of the glomerular capsule (top left); glomerulus compression and atrophy (200x; haematoxylin-eosin stain); b - Thickened capsule glomerular mesangial cell proliferation; parieto-capillary symphysis; intumescent muddy epithelial kidney tubules (200x; haematoxylin-eosin stain)

Conclusions

High values of serum parameters ALT and glucose concentration in DON-exposed rats compared with controls indicates the presence of pathological changes in the liver. Decrease in total serum protein and albumin concentration is due DON's effect of protein synthesis inhibition and feed consumption. Serum biochemical parameters: serum creatinine (C), serum urea (U), and serum urea nitrogen (BUN) displayed a significant increase in response to DON exposure and showed the existence of renal pathological changes. Pathological changes in the liver revealed large and globular hepatocytes with rich intense eosinophilic cytoplasm, enlarged nuclei with evident nucleolus and variable hyperchromasia, redness and swelling of the liver sinusoids, mild portal fibrosis space with rare inflammatory cells and vessel ectasia, vacuolar alteration of pericentrilobular hepatocytes and accumulation of apoptotic bodies. Pathological changes in the kidney shown degenerative lesions of the renal epithelium (renal tubules and the glomerulus), and capsule thickening of glomerulus mesangial cell proliferation.

References

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Ethical Commission approval according to the European Union’s Directive (2010)/63/EU