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INFLUENCE OF THE ZEARALENONE ON THE ACTIVITY OF CHOSEN LIVER ENZYMES IN A RAT

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Abstract: Zearalenone is a mycotoxin compound produced mainly by *Fusarium* species of fungi which is present in cereals cultivated all over the world. The aim of the research was to examine the toxic influence of different doses of zearalenone on liver cells through estimating mycotoxin influence on markers evaluation of biochemical liver damage. The research was carried out on male Wistar rats. The rats were divided into 9 groups of 10 animals each. Group A was orally given 8% ethyl alcohol. Group B, C, D, E was orally given once a day for 10 days a zearalenone alcohol solution properly in dose of 50, 100, 200, 500 µg/kg b.w. Single doses of zearalenone was given to the animals from groups X, Y, Z and W. Control group W – 8% ethyl alcohol, group X dose 1 mg/kg b.w., group Y dose 2 mg/kg b.w., group Z dose 3 mg/kg b.w. For the research, blood was taken from hearts. The blood was centrifuged and the plasma analyzed using spectrophotometric methods: aspartate and alanine aminotransferase, alkaline phosphatase and complete bilirubine. The results of the experiment show that liver cells are exposed to zearalenon activity increased liver aminotransferases (ALT and AST) in blood plasma. Rise of liver aminotransferase level (ALT and AST) in animal's blood plasma after giving zearalenon may confirm the hepatotoxic influence of this mycotoxin. Short-lasting zearalenone influence does not cause changes in the liver aminotransferases.

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INTRODUCTION

Zearalenone (ZEA) is a mycotoxin compound produced mainly by the *Fusarium* species of fungi which is present in cereals cultivated all over the world, such as: wheat, corn, oats, barley, rice and products made from them. Huge amounts of zearalenon are being produced by the *Fusarium* species during cereal storage under high humidity and temperature. The weather conditions at harvesting contribute to an increase in the contents of *Fusarium* fungi and zearalenone mycotoxins produced by them in winter wheat grain [11]. Zearalenone is a stable compound resistant to storage, milling, food processing and cooking. Zearalenone is easily absorbed from the alimentary canal, and metabolised to α-zearalenol and β-zearalenol through 3-hydroxysteroid dehydrogenase in cytosol or hepatocyte organelles. Zearalenon and its metabolites are conjugated through glucuronic acid. Zearalenon attaches to receptors for estrogenes present in uterus, breast gland, adrenal gland, pituitary gland, and affects estrogen-dependent transcription in cell nucleus. Receptor binding zearalenon and by this block estrogen bindings [3, 5, 6, 10, 14].

A serious potential risk for agricultural workers is posed by mycotoxins occurring in grain. Special attention should be paid to toxins produced by *Fusarium* (fusariotoxins), which have been scarcely studied with respect to occupational risk. Most previous studies on *Fusarium* concern their role as cereals' athogens [7]. Zearalenone is found together with other *Fusarium* mycotoxins in "scabby grain toxicosis". This syndrome is associated with nausea, vomiting, abdominal pain, diarrhoea, dizziness and headache. The significance of this finding is not clear [13].

The aim of the research was to examine the toxic influence of different doses of zearalenone on liver cells through

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estimating mycotoxin influence on markers evaluating biochemical liver damage, such us increased aspartate and alanine aminotransferase activity (ALT and AST), alkaline phosphatase (ALP) and bilirubine level.

MATERIALS AND METHODS

The research was carried out on male rats (Wistar breed) of 190–200 g weight. The rats were divided into 9 groups of 10 animals each. Group A was orally given 8% ethyl alcohol. Group B was orally given a zearalenone alcohol solution (daily dose of 50 µg/kg b.w.). Group C (daily dose of 100 µg/kg b.w.), group D (daily dose of 200 µg/kg b.w.), group E (daily dose of 500 µg/kg b.w.). Specified groups were dosed once a day for 10 days. A single dose of zearalenone was orally given to the animals from groups X, Y, Z and W. Control group W – 8% ethyl alcohol, group X dose 1 mg/kg b.w., group Y dose 2 mg/kg b.w., group Z dose 3 mg/kg b.w. For the research, blood was taken from hearts. Cardiac puncture was performed under anesthesia (Vetbutal, dose 25–30 mg/kg b.w.) with a needle inserted between the 4th and 5th rib and into the heart. This technique allowed the collection 5–10 ml of blood from a single animal. After that, the rats were sacrificed by decapitation. The blood was centrifuged and the serum assayed with a spectrophotometer using BIOMAXIMA set: aspartate and alanine aminotransferase, alkaline phosphatase and complete bilirubine [2, 12, 17].

Statistic analysis was conducted by three tests: t-Student test (test t in table), Cochran-Cox test (test Z in table) and Mann-Whitney test (test U in table). The analyses were performed with use of the Statistica for Windows v. 5.0 package (Statsoft, Inc., Tulsa, OH, USA). Results were considered significant for "p" values of <0.05.

RESULTS

The experiment revealed statistical elevation of AST and ALT activity in rat blood serum after 10 days of oral exposure to ZEA (500 µg/kg dose b.w.) in comparison with the control group (Fig. 1). Statistical analysis also showed lack of crucial differences between AST and ALT activities in the control group and groups that were given smaller doses (50, 100, 200 µg/kg b.w.) ZEA during 10 days, and also the groups that were given ZEA in 1 dose (1, 2 or 3 mg/kg b.w.) (Tab. 1). The research showed lack of statistical differences

Table 1. AST and ALT activity in blood serum after ZEA administration.

Group	Mean ± SD	Me	Min. – Max.	Test	Statistically significant
AST					
After 10 days ZEA administration					
A – control	96.6 ± 10.21	96.7	84.4—116.7		
B – 50 µg/kg	101.72 ± 17.44	102.2	76.5–138.5	t = 0.81	p > 0.05
C – 100 µg/kg	111.49 ± 25.46	108.0	85.9–172.3	t = 1.72	p > 0.05
D – 200 µg/kg	109.62 ± 21.92	101.8	86.2–156.2	t = 1.69	p > 0.05
E – 500 µg/kg	120.35 ± 38.55	103.7	80.8–203.6	t = 1.98	p < 0.05*
After 24 hours ZEA administration					
W – control	136.14 ± 32.82	140.4	85.1–183.5		
X – 1 mg/kg	111.35 ± 16.70	110.0	84.1–145.0	Z = 1.1	p > 0.05
Y – 2 mg/kg	120.88 ± 27.35	125.6	78.0–157.4	t = 1.01	p > 0.05
Z – 3 mg/kg	102.14 ± 24.21	103.0	72.6–142.7	t = 1.12	p > 0.05
ALT					
After 10 days ZEA administration					
A – control	45.20 ± 6.33	45.90	35.0–54.7		
B – 50 µg/kg	45.67 ± 9.54	41.20	36.7–65.9	t = 0.13	p > 0.05
C – 100 µg/kg	50.03 ± 6.92	48.65	41.6–62.0	t = 1.63	p > 0.05
D – 200 µg/kg	51.73 ± 14.99	54.10	14.6–70.7	t = 1.27	p > 0.05
E – 500 µg/kg	51.89 ± 7.63	51.80	39.7–65.6	t = 2.17	p < 0.05*
After 24 hours ZEA administration					
W – control	58.98 ± 9.95	60.45	46.1–70.7		
X – 1 mg/kg	55.64 ± 7.44	56.10	43.2–64.5	t = 0.76	p > 0.05
Y – 2 mg/kg	52.11 ± 8.31	49.25	43.0–64.0	t = 1.50	p > 0.05
Z – 3 mg/kg	53.05 ± 6.47	54.40	43.7–61.7	t = 1.41	p > 0.05

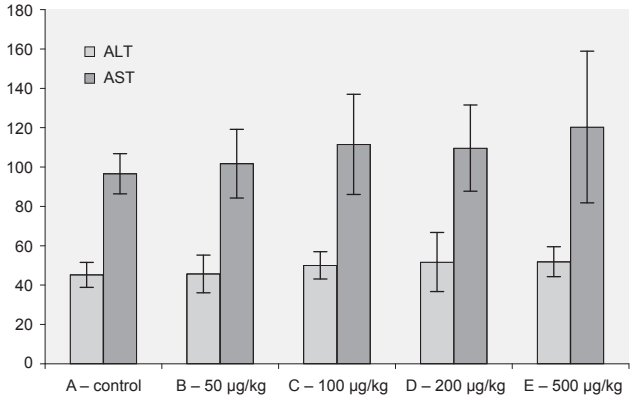

Figure 1. ALT and AST activity in blond serum after 10 days ZEA administration.

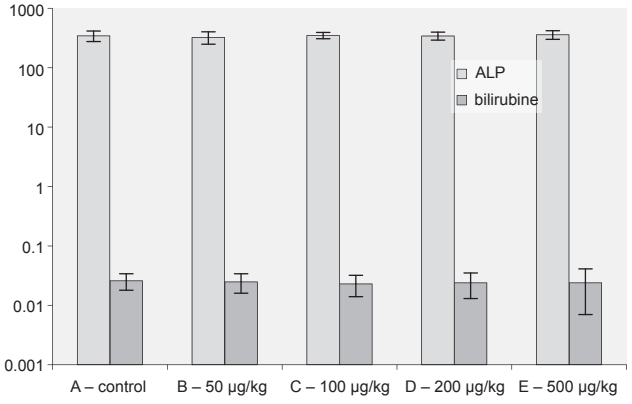

Figure 2. ALP activity and bilirubine concentration in blood serum after 10 days ZEA administration.

in ALP activity or total bilirubine concentration in the control group and groups that were given ZEA (Tab. 2, Fig. 2).

DISCUSSION

On the basis of specific enzimatic tests we can deduce the level of ogran and whole organism damage. Clinically, the elevation of liver transaminases is of great importance. Alanine aminotransferase (ALT) is an exclusively cytoplasmatic enzyme and is more specific for the liver damage than aspartate aminotransferase (AST). AST, apart from the cytoplasmatic form, exists in a mitochondrial form which determines around 80% of its activity. Hepatocyte injury, which results with an increase in aminotransferase level,

Table 2. ALP activity and bilirubine concentration in blood serum after ZEA administration.

Group	Mean ± SD	Me	Min. – Max.	Test	Statistically significant
ALP					
After 10 days ZEA administration					
A – control	345.0 ± 69.27	358.0	193 ± 430.0		
B – 50 µg/kg	324.5 ± 76.34	302.0	257.0–480.0	t = 0.64	p > 0.05
C – 100 µg/kg	349.7 ± 42.64	352.5	274.0–418.0	t = 0.18	p > 0.05
D – 200 µg/kg	343.89 ± 52.64	338.0	282.0–430.0	t = 0.04	p > 0.05
E – 500 µg/kg	359.2 ± 59.28	346.5	320.0–488.0	t = 0.50	p > 0.05
After 24 hours ZEA administration					
W – control	360.73 ± 69.02	346.0	250.0–463.0		
X – 1 mg/kg	346.95 ± 46.36	342.0	274.0–430.0	t = 0.66	p > 0.05
Y – 2 mg/kg	351.95 ± 55.23	339.0	274.0–488.0	t = 0.38	p > 0.05
Z – 3 mg/kg	366.87 ± 100.73	328.0	271.0–556.0	t = 0.16	p > 0.05
Bilirubine					
After 10 days ZEA administration					
A – control	0.026 ± 0.008	0.02	0.02 -0.04		
B – 50 µg/kg	0.025 ± 0.009	0.025	0.01–0.04	U = 54.0	p > 0.05
C – 100 µg/kg	0.023 ± 0.009	0.02	0.01–0.04	U = 43.5	p > 0.05
D – 200 µg/kg	0.024 ± 0.011	0.02	0.01–0.04	U = 50.0	p > 0.05
E – 500 µg/kg	0.024 ± 0.017	0.02	0.01–0.07	U = 44.5	p > 0.05
After 24 hours ZEA administration					
W – control	0.024 ± 0.009	0.02	0.01–0.04		
X – 1 mg/kg	0.025 ± 0.009	0.025	0.01–0.04	U = 29.0	p > 0.05
Y – 2 mg/kg	0.025 ± 0.009	0.025	0.01–0.04	U = 29.0	p > 0.05
Z – 3 mg/kg	0.025 ± 0.008	0.02	0.02–0.04	U = 29.5	p > 0.05

may happen with various different types of liver damage caused by eg. alcohol abuse, chronic viral hepatitis or immunological diseases. Such an increase in aminotransferase can also be observed after nonsteroidal anti-inflammatory drugs, antibiotics or statins [16].

Maaroufi's *et al.* [9] experiment the first time when zearalenone influence on biochemical liver damage markers was researched. During that research, female rats were given zearalenone intraperitoneally, dissolved in sterile olive oil. The animals were divided into groups that were given zearalenone intraperitoneally in doses 1.5, 3.0 and 5.0 mg/kg of body mass, and a group that was given only olive oil. The animals in this research were sacrificed after 48 hours. The animals blood was used to mark standard hematological parameters and also aminotransferase levels (ALT, AST), alkaline phosphatase (ALP) and bilirubine. In every group that was given zearalenone, increases in the biochemical levels of markers of liver damage ware observed. The research results show toxic liver damage which weakens the blood coagulation process. Aminotransferase level in the group of animals that were given 5 mg/kg body mass was 100 times higher than in the control group, which indicates hepatolisis and does not exclude cholestasis [9]. Changes in such biological and hematological parameters were observed in a similar research. Mice were orally given zearalenone in 40 and 500 mg/kg of body mass, and after 48 hours bilirubine in animals blood AST, ALT, ALP was marked. The research results show liver cells damage and moderate cholestasis [1].

The next research during which zearalenone effects on chosen enzymes in blood serum were studied was by Conkova and associates in which zearalenone was orally given to rabbits. The animals were divided in control groups and groups that were given zearalenone in 10 μg/kg and 100 μg/kg body mass during 14 days. The enzyme level in blood serum was assayed for aminotransferase AST and ALT, alkaline phosphatase, gamma glutamyltranspeptidase (GGTP) and lactic acid dehydrogenase (LDH). Zearalenone was given orally during 14 days. Blood samples were collected at 0, 24, 72, 168 and 336 hour of the experiment. In the group that was given zearalenone in 10 μg/kg body mass dose there was a significant ALP increase at 168 and 336 hour of the experiment. In the group that was given zearalenone in 100 μg/kg a dose significant increase of AST, ALT, ALP, GGTP and LDH activity was observed in 168 and 336 hour [4]. Similar results were obtained by Ben Salah-Abbes J. *et al.* [15] when giving zearalenone orally to female Balb/c rats in 40 mg/kg body mass. During that research, a significant increase of such parameters as ALT, AST, ALP, bilirubine and creatinine was shown, together with significant changes in most of the hematological parameters.

In the present research, a statistically significant increase in AST and ALT activity in rats blood serum was shown after 10 days of giving zearalenone into the stomach in 500 μg/kg dose, compared to the control group. Statistical analysis also revealed lack of statistically significant differences between AST and ALT activity in the control group and groups that were given lesser doses of zearaelenone (50, 100, 200 μg/kg) during 10 days. A lack of statistically significant differences was also shown between AST and ALT activity in the control group and groups that were given zearaelenon in one dose (1, 2 or 3 mg/kg), and sacrificed after 24 hours.

Alkaline phosphatase (ALP) in tissues takes part in the transport of phosphate ions through cell membranes. Cholestasis (bile stasis) increases its synthesis. Liver diseases cause a pathological ALP level increase [8].

Bilirubine is a compound created by heme disintegration moved in blood by albumin to hepatocytes. In the endoplasmic reticulum it is joined with glucuronic acid and in this state is secreted to bile. Hyperbilirubinaemia and significant aminotransferase increase may suggest toxic or ischemic liver damage [8].

The present research and statistical analysis of obtained results revealed lack of statistically significant differences for ALP activity and bilirubine concentration between the control group and groups which were given zearaelenone for 10 days in different doses. Moreover, it revealed a lack of a statistically significant change in ALP activity and bilirubine concentration between the control group and groups that were given zearaelenone in one dose (1, 2 and 3 mg/kg), and sacrificed after 24 hours. The outcome is probably connected with the lesser zearaelenone dose applied in the present research than in studies by other authors [4, 9].

REFERENCES

1. Abbes S, Ouanes Z, Ben Salah-Abbes J, Houas Z, Oueslati R, Bacha H, Othman O: The protective effect of hydrated sodium calcium aluminosilicate against haematological, biochemical and pathological changes induced by Zearalenone in mice. *Toxicon* 2006, **47(5)**, 567-574.

2. Bowers GN, McComb RB: A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. *Clin Chem* 1966, **12(2)**, 70-89.

3. Codex Committee on Food Additives and Contaminants. Posting date. Joint FAO/WHO Express comminattee on Food Additives: Position paper on zearalenone. Publication CCFAC 00/19. Codex Alimentarius Commission, Rome, Italy 2000.

4. Conkova E, Laciakova A, Pastorova B, Seidel H, Kovac G: The effect of zearalenone on some enzymatic parameters in rabbits. *Toxicol Lett* 2001, **121(3)**, 145-149.

5. European Commission, Opinion of the scientific panel on contaminants in the food chain in a request from the commission related to zearalenone as undesirable substance in animal feed. *EFSA J* 2004, **89**, 1-35.

6. European Commission. SCOOP, task 3.2.10. Collection of occurrence data of *Fusarium* toxins in food and assessment of dietary intake by the population of EU Member States. European Commission, Directorate-General Health and Consumer Protection, Reports on tasks for scientific co-operation, 2003.

7. Krysińska-Traczyk E, Perkowski J, Dutkiewicz J: Levels of fungi and mycotoxins in the samples of grain and grain dust collected from five various cereal crops in eastern Poland. *Ann Agric Environ Med* 2007, **14**, 159-167.

8. Limdi JK, Hyde GM: Evaluation of abnormal liver function tests. *Postgrad Med J* 2003, **79**, 307-312.

9. Maaroufi K, Chekir L, Creppy EE, Ellouz F, Bacha H: Zearalenone induces modifications of haematological and biochemical parameters in rats. *Toxicon* 1996, **34(5)**, 535-540.

10. Malekinejad H, Maas-Bakker RF, Fink-Gremmels J: Enzyme kinetics of zearalenone biotransformation: pH and cofactor effects. *Arch Toxicol* 2005, **79(10)**, 547-553.

11. Mankeviciene A, Butkute B, Dabkevicius Z, Suproniene S: *Fusarium* mycotoxins in Lithuanian cereals from the 2004–2005 harvests. *Ann Agric Environ Med* 2007, **14**, 103-107.

12. Michaeelsson M: Bilirubin detection in serum and urine. Almqvist & Wiksells, Boktryckeri Aktiebolag, Uppsala 1961.

13. Peraica M, Radić B, Lucić A, Pavlović M: Toxic effects of mycotoxins in humans. *Bull World Health Organ* 1999, **77(9)**, 754-766.

14. Placinta CM, D'Mello JPF, Macdonald AMC: A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Anim Feed Sci Technol* 1999, **78**, 21-37.

15. Ben Salah-Abbes J, Aabbes S, Ouanes Z, Houas Z, Abdel-Wahhab MA, Bacha H, Oueslati R: Tunisian radish extract (*Raphanus sativus*) enhances the antioxidant status and protects against oxidative stress induced by zearalenone in Balb/c mice. *J Appl Toxicol* 2008, **28(1)**, 6-14.

16. Tennant BC: Assessment of hepatic function. **In:** Loeb WF, Quimby FW: *Clinical Biochemistry of Laboratory Animals,* 501–517. Taylor & Francis, London 1999.

17. Thefeld W, Hoffemeister H, Busch EW, Koller PU, Vollamar J: Referenzwerte für die Bestimmung der Transaminasen GOT und GPT sowie der alkalischen Phosphatase im Serum mit optimierten Methoden. *Dtsch Med Wochenschr* 1974, **99**, 343-351.