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Vitamin E influence on selected parameters in rats after ochratoxin A intoxication

Mycotoxins are a group of toxic, biologically active metabolites produced by mould fungi. They are present in food and feeding stuff. One of the most frequently appearing mycotoxins in the natural environment is ochratoxin A (OTA), which is produced by numerous mould fungi, mostly of the families *Penicilium* and *Aspergillus* (6,13,17,18). OTA toxicity is being confirmed by many authors. It has been proved that OTA has the greatest affinity to the kidneys, it can destroy also other organs (2,3,9). Additionally the experiments conducted on laboratory animals indicated genotoxic, mutagenic and cancerogenic properties of OTA. It has also been proved that OTA demonstrates certain immunosuppressive features (8,12,15,16).

This research aimed to determine vitamin E influence on the degree of OTA toxic activity basing on selected red and white blood cell systems parameters analysis in rats.

MATERIAL AND METHODS

The experiments were conducted on 45 eight-week- old albino Wistar female rats with the initial body weight of 170-190g, maintained on a 12-hr light/dark cycle and fed a standard granulated rodent laboratory chow (Fodder Manufactures, Motycz, Poland). The animals were randomly divided into 3 groups of 15:

- group I – control, receiving distilled water orally once a day,
- group II treated with OTA 250 μg/kg of body mass p. o. once a day,
- group III treated with vitamin E 100 mg/kg and after 20 min. OTA 250 μg/kg of body mass p. o. once a day.

After 4 weeks of vitamin E and OTA oral administration the samples of blood were taken from rats' left heart ventricles under ether anaesthesia. Red blood cells and white blood cells were taken in Thom's chamber, hematocrit was determined with the use of micromethod and hemoglobin was counted

with the cyanomethaemoglobin method (1). White blood cell system percentage composition was counted with the Pappenheim's method (10).

Statistical analysis was carried out with the use of Student's t-tests.

RESULTS

The results are given in Table 1 and Table 2.

Table 1. Selected hematological parameters of rat blood after orally given doses of ochratoxin A and vitamin E

Group	Hematocrit	Hemoglobin Mmol/l	Erythrocytes x 10^{12}/l	Leucocytes x 10^{9}
Group I (control)	0.45 ± 0.04 (0.35-0.48)	8.98 ± 0.70 (7.56-9.49)	4.84 ± 0.38 (3.72-5.09)	5.52 ± 1.04 (4.40-7.80)
Group II (ochratoxin 250 µg/kg b.m.)	0.40 ± 0.04 (0.32-0.46)	8.06 ± 0.76 (6.82-9.24)	4.49 ± 0.39 (3.50-4.99)	4.40 ± 0.73 (3.20-5.60)
Group III (ochratoxin 250 µg/kg m.c. and Vit.E 100 mg/kg b.m.)	0.42 ± 0.05 (0.32-0.50)	8.33 ± 1.11 (6.39-9.92)	4.69 ± 0.61 (3.50-5.41)	5.50 ± 1.47 (4.40-8.00)
Differences significance	I : II p<0.05 I : III p<0.05 II : III p<0.05	I : II p<0.01 I : III p<0.01 II : III p<0.05	I : II p<0.05 I : III p<0.05 II : III p<0.05	I : II p<0.01 I : III p<0.01 II : III p<0.01

Table 2. White blood cells smear after orally given doses of ochratoxin A and vitamin E

Group	Lymphocytes	Eosinophils	Monocytes	Band neutrophils	Segmented neutrophils
Group I (control)	79.00 ± 6.81 (67.00-89.00)	0.20 ± 0.41 (0-1.00)	0.53 ± 0.63 (0-2.00)	4.07 ± 1.62 (2.00-8.00)	16.20 ± 6.47 (8.00-27.00)
Grupa II (ochratoxin 250 µg/kg b.m.)	75.73 ± 7.49 (65.00-86.00)	0.13 ± 0.35 (0-1.00)	0.80 ± 0.56 (0-2.00)	4.07 ± 1.03 (3.00-6.00)	19.27 ± 6.73 (9.00-29.00)
Group III (ochratoxin 250 µg/kg m.c. and Vit.E 100 mg/kg b.m.)	64.33 ± 5.95 (49.00-81.00)	0.12 ± 0.05 (0-1.00)	0.84 ± 0.51 (0-2.00)	4.37 ± 1.08 (3.00-6.00)	30.34 ± 4.91 (16.00-43.00)
Differences significance	I : II ns I : III p<0.001 II : III p<0.01	I : II ns I : III ns II : III ns	I : II ns I : III ns II : III ns	I : II ns I : III ns II : III ns	I : II ns I : III p<0.001 II : III p<0.01

In group II there was found a statistically significant decrease of all the examined parameters of red blood cell system compared to the control group. There was also found a decrease in total white blood cells compared to control with no statistically significant differences in its proportions.

Although an average hematocrit, hemoglobin concentration and red blood cells in group III were still significantly lower than in control group, they were higher than in group II. There was no significant white blood cells decrease in group III compared to control group. Concurrently the neutrophilic leucocytes percentage increased and was accompanied by loss of total lymphocytes count compared to both: control and group II.

DISCUSSION

OTA is a widespread mycotoxin. However its toxic, mutagenic or teratogenic activity mechanisms are not fully understood. The papers issued about OTA metabolism suggest that its primary activity mechanism is blocking t-RNA phenyloalanine synthetase (3) as well as mitochondrial breathing failure (11). F i n k - G r e m m e s et al. proved that OTA's cytotoxic activity in their experiments on hepatic cells cultures depends on its concentration and time of exposition (4). However, S t o r m e r et al. discovered that OTA can block DNA synthetase in lymphocytes T. It has also been found that OTA leads to reactions causing lipid metabolism failure (11). The influence of OTA on the above metabolic changes may result in enormous abnormalities in intensively multiplying cells as the erythropoietic system and cells growing in the bone marrow. In G u p t a ' s et al. works it was found that OTA administered in doses of 5mg/kg of body mass significantly reduces red blood cells and Ht in the peripherial blood in rats. It also causes decrease in the amount of proliferating cells in the red bone marrow including precursors of erythrocytes, leucocytes and megacariocytes. In this work after p. o. administration of OTA 250 mg/kg of body mass it was found that all the parameters of the red blood cell system were decreased as well as white blood cells compared to the control group. There was no change in the percentage proportion of the white blood cell system in this group. It is very likely that not only as high doses as administered in this research, but also low and medium ones can result in similar effects if present in long period of time. It is connected with the fact that OTA has a long half-life and the substance is very slowly eliminated from the organism (9).

H o e h l e r et al. indicated that OTA's toxic activity in animals can also result from its influence on increased oxygen free radicals release that aggravate cell metabolism failures. Vitamin E is a well known antioxidant. It easily dissolves in fats and concentrates in cell membranes next to unsaturated fatty acids as it stops their oxidation chain. The aim of the research was to evaluate the influence of vit. E on OTA's toxic activity against red and white blood cell systems in rats.

After OTA administration 250 mg/kg of body mass p. o. plus vit. E 100mg/kg of body mass (in group III) in rats it was found that the average hematocrit, hemoglobin and red blood cells were still significantly lower than in control group but kept to be higher than in group II where animals were treated with OTA without vit. E. There was no white blood cells loss in group III. Neutrophilic leucocytes proportion was higher and lymphocyte count was lower than in control and group II. It is assumed that vit. E minimizes OTA's toxic activity due to its antioxidative properties.

The above results confirm the negative OTA's influence on both red and white blood cell system parameters and indicate that vit. E can diminish it if administered concurrently. However, further investigation of this matter is needed.

CONCLUSIONS

1. The results obtained in this research suggest negative influence of OTA on red blood cell parameters.
2. OTA acting via white blood cell system may lead to the failure of organisms' resistance mechanisms.
3. Vit. E administration may decrease OTA's toxicity.

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SUMMARY

The aim of the research was to evaluate vitamin E influence on ochratoxin A (OTA) toxicity based on analysis of selected parameters of white and red cell system in rats. The animals were divided into groups: I – control, II – treated with OTA, III – treated with OTA and vitamin E. In group II a significant decrease in the values of hematocrit, hemoglobin level, red and white cell counts was observed. In group III an average hematocrit, hemoglobin level were significantly lower than in control group, nevertheless they were higher than in group II.

Wpływ witaminy E na wybrane parametry układu biało- i czerwonokrwinkowego szczura po intoksykacji ochratoksyną A

Celem pracy była ocena wpływu witaminy E (vit. E) na wielkość toksycznego działania ochratoksyny A (OTA) na podstawowe analizy wybranych parametrów układu biało- i czerwonokrwinkowego szczurów. Badaniami objęto 45 szczurów szczepu Wistar. Zwierzęta podzielono losowo na grupy : I grupa – kontrolna, II grupa otrzymywała doustnie OTA w dawce 250 mg/kg mc., III grupa otrzymywała doustnie 250 mg/kg mc. OTA oraz vit. E w dawce 100mg/kg mc.

W II grupie stwierdzono istotne obniżenie wartości parametrów układu czerwonokrwinkowego oraz całkowitej ilości krwinek białych w stosunku do grupy kontrolnej. W grupie III średnie wartości Ht, Hb oraz ilość erytrocytów były nadal istotnie niższe niż w grupie kontrolnej, pozostawały jednak na poziomie istotnie wyższym w stosunku do wartości obserwowanych w grupie II. Ilość krwinek białych w tej grupie nie różniła się od wartości kontrolnych. Wzrósł jednak odsetek granulocytów obojętnochłonnych z jednoczesnym spadkiem ilości limfocytów w stosunku do kontroli oraz do II grupy badanych zwierząt.