

Zearalenone Effect on Uterine Weight of Rats

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Summary

Mycotoxins are today considered one of the main contaminants of food and feed. Widespread zearalenone and its metabolites have potent estrogenic and anabolic activity, proven in numerous studies worldwide. The aim is to investigate influence of zearalenone on the uterine weight of rats depending on the applied dose and duration of the observation period. In a controlled experimental study, 63 adult female Wistar rats were divided into three groups, depending on the oral test dose of zearalenone applied: 0.1, 0.3 and 0.5 mg / kg of body weight. At the end of each of the four observation periods of seven days, animals were sacrificed under general anesthesia with ether, and after an autopsy the mass of the uterus was determined. Zearalenone in the dose of 0.1 mg / kg of body weight has caused a significant increase in uterine weight between the first and fourth observation interval. Doses of 0.3 and 0.5 mg zearalenone / kg caused a decrease in uterine weight, which was at a dose of 0.5 mg / kg highly significant between all observational intervals. After 7 days of applying of the toxin, uterine weights did not differ significantly with respect to applied dose. After 14, 21 and 28 days, differences in uterine weight were highly significant, depending on the dose of zearalenone. The results show that prolonged application of large doses of zearalenone produced a significant decrease in uterine weights in experimental animals.

Key words

zearalenone, rat, uterus

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Introduction

Many chemicals of anthropogenic origin, as well as compounds that occur naturally, such as mycotoxins including zearalenone, can enter the food chain and cause various health disorders in humans and animals. In recent years, the presence of estrogenic substances in food, either as natural constituents, either as contaminants, caused an increase of attention because of the presumed possible etiological role in the development of some diseases.

Zearalenone, a product from the mold *Fusarium*, known as non-steroidal estrogenic mycotoxin, shows strong uterotrophic activity in most animals. This mycotoxin and its metabolites α - and β -zearalenol belong to a rare group of natural products, β -resorcylic acid lactones, which are capacity to bind to estrogen receptors of the uterus due to the chemical identity of the estradiol. The reactions of animals to zearalenone application or taking zearalenone with food depends on a number of factors, primarily on the type of animal. So the pigs are more sensitive to zearalenone (Obremski et al., 2003; Zwierzchowski et al., 2005) compared to rats, poultry and cattle (Fitzpatrick et al., 1989; Weaver et al., 1986).

Studies on various animal species have demonstrated that zearalenone and its metabolites have strong estrogenic and anabolic activity (Kuiper-Goodman et al., 1987). Zearalenone and its metabolites showed competitive binding to estrogen receptors in the uterus during the numerous *in vitro* experiments. A study conducted by Katzenellenbogen et al. (1979) demonstrated the direct interaction of estrogen receptor and the nature of the biological activities of three estrogen Resorcylic acid lactone (P-1492 zearalenone) or its derivatives (P-1496 and P-1560 epimeric zearalenone) in the immature rat uterus. These compounds "compete" with estradiol for cytoplasmic receptors. Similar studies were made by Mitak et al. (2002) by feeding rats over 5 day period during estrus. The animals were fed zearalenone at a dose of 2.5 mg / kg body weight, the number of receptor relationships for H-estradiol to uterine cytosol was observed. The results indicate that the zearalenone competes with β -estradiol for binding to estrogen receptors of the uterus. Fitzpatrick et al. (1989) investigated the binding affinity of α and β -zearalenol, and the estrogenic effect in the uterus and fallopian tubes in various animals (rats, pigs and chickens), as indicators of estrogenic potential. The results showed that the relative binding affinity may be partially explained by differences in the sensitivity of different species of animals to zearalenone food intake. Thus, the relative binding affinity of α -zearalenol was 10-20 times higher than zearalenone, and about 100 times larger than the β -zearalenol, while the binding affinity of α -zearalenol was higher in pigs than in rats and chickens.

During the years, there are more frequent reports about the harmful effects of zearalenone in humans and animals (Ewald et al., 1991). Zearalenone toxicosis of animals usually occurs periodically during the early occurrence of rainy autumns and particularly described in pigs (Ozegovic and Pepenjak, 1995; Ozegovic, 1989). Often it is suspected that reproductive disorders in domestic animals and hyperestrogenic syndromes in humans are caused by intake of zearalenone (Agog, 2004). Reaction of

certain organs in experimental animals after intake of zearalenone is different (Becci et al, 1982). The body of the uterus with endometrium are the most sensitive parts of the female reproductive tract that are exposed to adverse effects of zearalenone. There are many pathological disorders of that organ, often chronic and recurrent nature (Kumar et al., 1994). Zearalenone and its metabolites during numerous *in vitro* experiments showed competitive binding to estrogen receptors in the uterus. It is proved that the toxin causes an increase in the uterus and mammary glands, swelling of the vulva, testicular atrophy, and hatching the birth canal in rats, mice, and guinea pigs (Ruddick et al., 1976). Disorders also include reduction in fertility, damage to the reproductive tract, increased embryonic resorption, changes in weight of adrenal, thyroid, and pituitary glands, changes in serum levels of progesterone and 17 β -estradiol (Zinedine et al., 2007; WHO, 2000).

Toxicological studies of testing the effects of zearalenone in experimental animals are the basis for defining their maximum permitted concentration in food and animal feed in order to protect human and animal health.

The work aims to determine the effect of oral administration of zearalenone on the rat uterine weight, depending on the applied dose and duration of the observation period.

Material and methods

Testing was conducted on 68 female Wistar rats aged cca two months, bred at the Department of Pharmacology and Toxicology of Veterinary Faculty of Sarajevo University. The tested animals were divided into groups: A (22 specimens), B (23) and C (23), depending on the dose of a given toxin. Three levels were determined: 0.1 mg / kg body weight (group A), 0.3 mg / kg body weight (group B) and 0.5 mg / kg body weight (group C). The experiment used a pure toxin zearalenone (Sigma-Z-2125 EECNo241-864-O). Zearalenone was applied perorally (through gastric sonde) at regular intervals (24-hour cycle). Observation period (duration of application) included four different time intervals: I - 7 days, II - 14 days, III - 21 day, and IV - 28 days. The animals were sacrificed every seven days (after the last day of giving the toxins) under a glass bell in the general-ether anesthesia, as follows: the 5 animals from each group after 7 days, 5 animals from group A and 6 from group B and C after 14 days and finally 6 animals from each group after 21 and 28 days. After sacrificing the dissection and inspection of body cavities and organs was approached towards to detect macroscopic changes. After the first cut along the mediosagittal line, ovarian and the third lateral Fallopian tube dissection was performed, as well as uterus on the line of demarcation of the body and cervix by groups.

The measured values of the mass of the uterus are described in the measures of central tendency and variability measures, and with Kruskal-Wallis's test and intergroup comparison, significant differences in uterine weight were compared to applied dose and duration of the observation period was estimated. Differences at $p < 0.05$ were considered statistically significant and highly statistically significant at $p < 0.01$.

Results

Uterine weight depending on the applied dose

After 7 days of application of different doses of zearalenone, uterine weight of rats did not differ significantly: $T=0.24$ and $p>0.05$ (Table 1).

After 14 days of applying different doses of of zearalenone, uterine weight in rats statistically highly significant differed: $T=14.23$ and $p<0.01$ (Table 2). Intergroup differences of uterine weight, depending on the dose (less weight of the uterus associated with a higher dose of the toxins) were also statistically highly significant ($p<0.01$).

After 21 days of applying different doses of of zearalenone, uterine weight in rats statistically highly significant differed: $T=15.17$ and $p<0.01$ (Table 3). Intergroup differences of uterine weight, depending on the dose (less weight of the uterus associated with a higher dose of the toxins) were also statistically highly significant ($p<0.01$).

Table 1. Uterine masses after 7 days of application of zearalenone

Group	Zearalenon dose (mg/kg)	n	Uterine masses (g)			S.D.
			x	min.	max.	
A	0.1	5	0.81	0.63	0.94	0.14
B	0.3	5	0.83	0.63	1.02	0.16
C	0.5	5	0.84	0.62	1.03	0.17

Table 2. Uterine masses after 14 days of application of zearalenone

Group	Zearalenon dose (mg/kg)	n	Uterine masses (g)			S.D.
			x	min.	max.	
A	0.1	5	0.87	0.84	0.94	0.04
B	0.3	6	0.80	0.78	0.84	0.03
C	0.5	6	0.71	0.69	0.76	0.03

Table 3. Uterine masses after 21 days of application of zearalenone

Group	Zearalenon dose (mg/kg)	n	Uterine masses (g)			S.D.
			x	min.	max.	
A	0.1	6	0.87	0.84	0.90	0.02
B	0.3	6	0.78	0.75	0.83	0.04
C	0.5	6	0.63	0.61	0.65	0.02

Table 4. Uterine masses after 28 days of application of zearalenone

Group	Zearalenon dose (mg/kg)	n	Uterine masses (g)			S.D.
			x	min.	max.	
A	0.1	6	0.91	0.87	0.94	0.04
B	0.3	6	0.78	0.74	0.82	0.03
C	0.5	6	0.57	0.54	0.59	0.02

After 28 days of applying different doses of of zearalenone, uterine weight in rats statistically highly significant differed: $T=15.15$ and $p<0.01$ (Table 4). Intergroup differences of uterine weight, depending on the dose (less weight of the uterus associated with a higher dose of the toxins) were also statistically highly significant ($p<0.01$).

Weights of the uterus, depending on the duration of the observation period

Uterine weight of rats in Group A, treated with zearalenone of 0.1 mg / kg, increased during the observational interval, but not statistically significant ($t=5.585$, $p>0.05$). The only statistically significant difference was between 7th and 28th day: $p<0.05$ (Table 5).

Table 5. Uterine masses of rats in group A after the observational interval

Days	n	Uterine masses (g)			S.D.	
		x	min.	max.		
A group (0.1 mg/kg)	7	5	0.81	0.63	0.94	0.14
	14	5	0.87	0.84	0.94	0.04
	21	6	0.87	0.84	0.90	0.02
	28	6	0.91	0.87	0.94	0.04

Table 6. Uterine masses of rats in group B after the observational interval

Days	n	Uterine masses (g)			S.D.	
		x	min.	max.		
B group (0.3 mg/kg)	7	5	0.83	0.63	1.02	0.16
	14	6	0.80	0.78	0.84	0.03
	21	6	0.78	0.75	0.83	0.04
	28	6	0.78	0.74	0.82	0.03

Table 7. Uterine masses of rats in group C after the observational interval

Days	n	Uterine masses (g)			S.D.	
		x	min.	max.		
C group (0.5 mg/kg)	7	5	0.84	0.62	1.03	0.17
	14	6	0.71	0.69	0.76	0.03
	21	6	0.63	0.61	0.65	0.02
	28	6	0.57	0.54	0.59	0.02

Uterine weight of rats in Group B, treated with zearalenone of 0.3 mg / kg, decreased during the observational interval, but not statistically significant: $T=1.78$ and $p>0.05$ (Table 6).

Uterine weight of rats in Group C, treated with zearalenone of 0.5 mg / kg, decreased statistically highly significant during the observational interval $T=17.72$ and $p<0.01$ (Table 7). The only not statistically significant ($p>0.05$) was difference between 7th and 14th day of application.

Discussion

It was determined that *Fusarium* toxins, particularly zearalenone, cause numerous toxic effects, both in laboratory and in domestic animals. Zearalenone originating from food show estrogenic effects *in vitro* and acts via estrogen receptor mechanism. In numerous studies worldwide zearalenone estrogen effects on reproductive organs of animals, especially the uterus has been proven. Rats belong to a group of animals that show sensitivity to estrogenic effects of zearalenone. The research of Beccia et al. (1982) showed significant increase in uterine masses in rats at exposure doses of 3 mg / kg body weight per day. In female rats, average weight 175-200 g, after application of zearalenone subcutaneously, during 3 days at a dose of 0.2 mg / kg, there was an increase of uterine masses (Turcotte et al., 2005). Dose of 250 mg / kg used in the experiment in the diet of rats aged 23 days and 48 hours significantly increases the weight of the uterus (James and Smith, 1982; Smith, 1980) that confirms that the uterus is one of the sensitive organs to the mycotoxin zearalenone. This is in agreement with the results of our study.

Zearalenone estrogenic effect on the uterus depends on the dose and period of application, which we confirmed in our research. In the group of rats that were treated with 0.5 mg zearalenone / kg body weight we found that the value of uterine weight was significantly reduced during all observational periods, and that on the 28th day was the smallest. Larger doses of zearalenone caused a significantly greater decrease in uterine weight. Similar results were showed by Ruzsás et al. (1979). In rats who ate maize contaminated with molds that produce toxin, decreased gonad weight has been shown. However, the study of the effects of dietary exposure of zearalenone on the uterus of rats (doses of 0.03 to 1.0 mg / kg body weight) showed an increase in uterine weight depending on the dose, and significant increase was observed after three days exposure to the highest doses (Heneweer et al., 2007). In our study a significant increase in uterine weight at doses of 0.1 mg zearalenone / kg body weight between 7 and 28 days of application was concluded.

Also in other species of animals uterus activity of zearalenone has been demonstrated. In pigs that were fed corn containing zearalenone for 70 days at a dose of: 0.01, 0.06, 0.15, 0.22 and 0.42 mg / kg, the highest dose had significantly increased mean weight of the uterus immediately after sacrifice (Döll et al., 2004). Contaminated corn (1.2 mg zearalenone / kg maize) was administered to pigs, and it was observed that the mean uterine weight increased (Döll et al., 2003). In young pigs of both sexes who received oral doses of 5, 10 and 15 mg / kg of body weight of purified zearalenone, seven days after application it was established that the reproductive organs of females had four times more mass than the control group (Farnworth and Trenholm, 1983). Lopez et al. (1988) in Argentina were first to prove poisoning of twenty pigs that were fed corn containing 56 ppm of zearalenone. Poisoned animals had signs of zearalenone estrogenic effects such as increased mammary glands, swelling of the vagina and increase of weight of reproductive organs.

Conclusions

- Intergroup differences in uterine weight depending on the applied dose of zearalenone were statistically highly significant different after 14, 21 and 28 days, with smaller uterine masses statistically highly significant associated with a higher dose of zearalenone.
- Zearalenone in the test dose of 0.1 mg / kg caused increased uterine mass that was statistically significant between the 7th and 28th day. The test dose of 0.3 mg / kg caused a decrease in uterine masses that was not statistically significant between observational interval. The test dose of 0.5 mg / kg caused a statistically highly significant decrease in uterine mass during all observational periods.
- Zearalenone is modeling uterine weight in rats depending on the dose and duration of application.

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