

ZEARALENONE-INDUCED LYMPHOPHAGOCYTOSIS (T CELL APOPTOSIS) ON THE RAT'S THYMUS

MIRSAD DORIĆ*, SVJETLANA RADOVIĆ, MIRSAĐ BABIĆ,
SUADA KUSKUNOVIĆ, IVANA TOMIĆ, IVAN SELAK

Institute of Pathology, Faculty of Medicine, University of Sarajevo,
Čekaluša 90, 71000 Sarajevo, Bosnia and Herzegovina

* Corresponding author

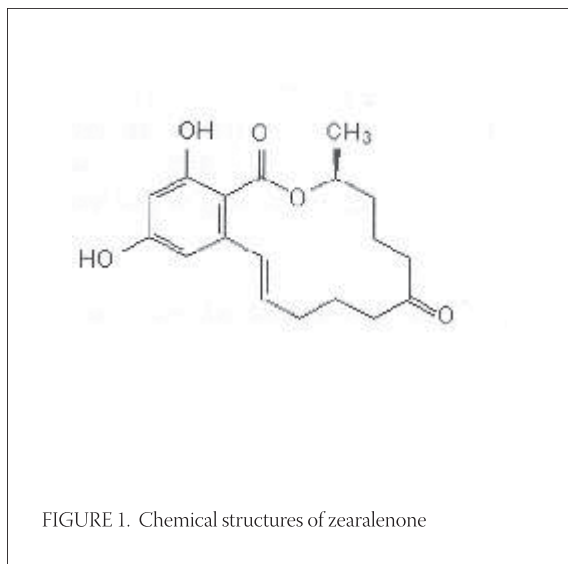
ABSTRACT

The effects of nonsteroidal mycotoxin zearalenone on the lymphoid tissue of thymus in a sense of investigating the subacute toxicity Wistar-albino rats have been examined in the course of the study. We analyzed 42 rats' specimens of both gender, treated with three dosage levels: 0,5; 2 and 4 mg/kg of body weight, after oral submission of the compound, and observed during three different time intervals: 10, 20 and 30 days. Microscopically was semi-quantitatively determined lymphophagocytosis (apoptosis) and cortical thymic cellularity. It was perceived statistically significant growth of lymphophagocytosis compared to a dosage ($p < 0,01$), as well as combination of dosage and interval ($p < 0,001$), while gender had no statistically significant influence on tested parameter ($p > 0,05$). Changes in cortical thyme cellularity were not perceived. Effects of applied doses of zearalenone on the lymphoid tissue of thymus were very mild and in correlation with estrogenicity. They are probably the result of interaction with estrogenic receptors.

KEY WORDS: apoptosis, lymphophagocytosis, thymus, zearalenone

INTRODUCTION

An association of toxic fungal metabolites, so-called mycotoxins, with human and animal health has been made since biblical times when ergotism was suspected to be toxicosis that resulted from such mycotoxins (1). Zearalenone (F₂-toxin) is a naturally occurring estrogenic substance produced by various species of *Fusarium* fungi growing on grains, mainly corn and hay exposed to high moisture on storage, the cause of the numerous mycotoxicosis on the animal farms (2,3). The major effects are on reproduction, including reproductive organs and their func-



tion, leading to hyperestrogenism (4). Hormonal dysregulation and reproductive tract dysfunction were reported to be main effects of zearalenone (5,6). This could be related to its structure (Figure 1.) which shows similarities with estrogenic steroids. The metabolism of zearalenone seems to occur essentially in the liver leading to alpha and beta zearalenol, the latter being not toxic, whereas alpha zearalenol binds 10-20 times more than the parent compound and 100 times more than beta zearalenol to estrogen receptors (7). A health risk to man has been reported, for example precocious pubertal change in children in Puerto-Rico and high incidence of oesophageal cancer in Africa and in China due to zearalenone ingestion (8, 4). Zearalenone has been observed to possess tumor-promoting activity similar to that of estrogen and hypothetically can induce proliferation and carcinogenesis in estrogen-dependent tissues (9). Little is known of the subacute effects of low concentrations of this mycotoxin, or their interaction with the immune system which may be important in pathogenesis of mycotoxicosis. The present study, therefore, was designed to evaluate the effects of zearalenone on the lymphoid tissue of the thymus.

MATERIALS AND METHODS

We have used 42 Wistar albino rats of both sexes, same litter, average weight of 200 g ($\pm 5-10\%$). Treated group was presented with 36 experimental animals (18 males and 18 females), divided into three groups and six subgroups. Each treated group had a control group (six rats), in which sunflower oil was applied. After the euthanasia by ether's anaesthesia, and routine necropsy, samples of thymic tissues, fixed in buffered 10% formalin, then dehydrated and fit into paraffin blocks, sliced microtomi-

cally to a thickness of 5 microns each, fixed on slides and stained standardly with hematoxylin and eosin technique. Toxin zearalenon, produced by IMC, Chemical Group, INC. Terre Haute, in USA, with high level of purity, and which LD 50 is $> 4000\text{mg}/\text{per kg}$ of body weight for rats (10), was used in the experiment. Samples of examined substance were obtained in form of powder, in a manually closed bottle. Before application, the substance was dissolved in sunflower oil (*Oleum heliantii*). Compound was administered orally (by gastric sonda) during correct time intervals, (24-hour cycle) with three dosage levels: 0,5; 2,0 and 4,0 mg/per kg of body weight and observed during 3 time intervals: 10, 20 and 30 days.

QUANTIFICATION

Lymphofagocytes were numbered by using objective fields enlarging 400x (Leitz Diaplan microscope) on 10 randomized successive optic fields. First, measurements on control rats were performed. For the control group, values didn't exceed 20/10 hpf (eng.high-power fields: optic field of great enlargement). Values obtained by this kind of measurements were unanimously classified as:

- 0-absent
- 1-weak (to 20 lymphofagocytes/10 hpf)
- 2-moderate (from 20-70 lymphofagocytes/10 hpf)
- 3-emphasised (above 70 lymphofagocytes/10 hpf)

This investigation was done respecting ethical standards stipulated in Helsinki Declaration.

RESULTS

Our research has shown absence or presence of low degree of lymphofagocytosis (apoptosis) in a group of rats (of both sexes) who obtained the lowest dose of examined substance (0,5 mg/per kg of body weight), unindpendently from time interval of administration (Figure 2), and similar finding has been perceived in control group of examined rats (physiological form). In rats, to which zearalenon was applied in dose of 2 and 4 mg/per kg of body weight, statistically significant growth of lymphofagocytosis was perceived (Figure 3) in comparison to a dosage ($p < 0,01$), and in combination of dosage and interval ($p < 0,001$). The difference in reacting, in comparison to a sex, had not been perceived, although it had been more emphasised in males (Graph 3), but without statistical significance. Measurement of cortical thymic cellularity has not designated to a statistically significant retreat in comparison to a control group.

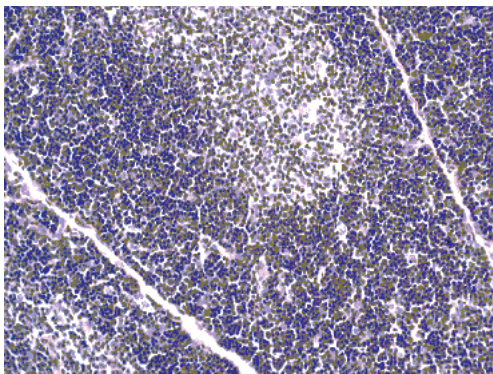


FIGURE 2. The cortex contain numerous lymphocits most, of which have small nuclei with densely packet chromatin. Absence of lymphophagocytosis (control rat). HE, x 200

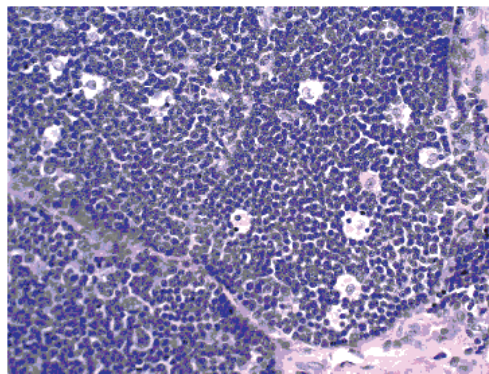
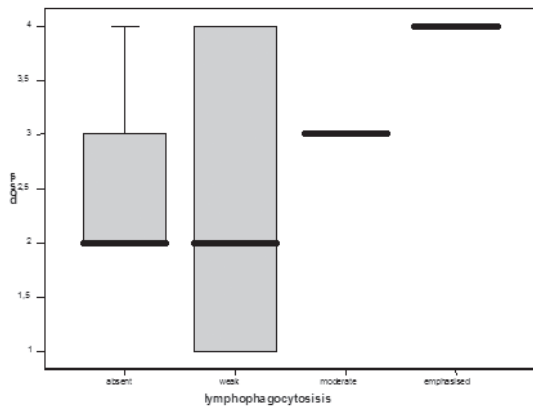
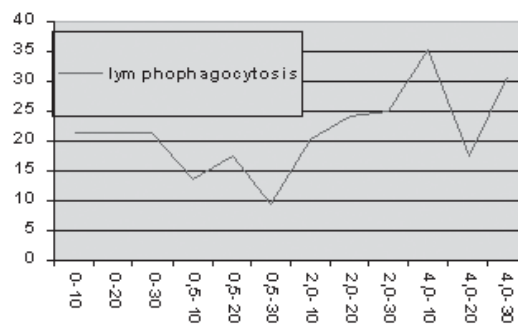


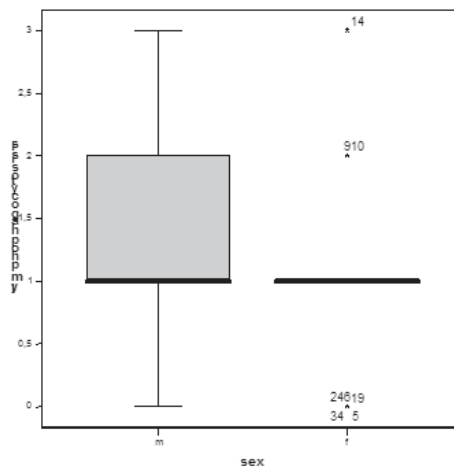
FIGURE 3. Emphasised lymphofagocytosis („starry-sky“ macrophages). Note abundance of tingible-body macrophages. HE, x 400



GRAPH 1. Graphic presentation of semiquantitative analysis of Lymphophagocytosis in relation to the dosis of zearalenone and interval



GRAPH 2. Graphic presentation of semiquantitative analysis of lymphophagocytosis in relation to the dosis of zearalenone and interval



GRAPH 3. Graphic presentation of semiquantitative analysis of lymphophagocytosis in relation to the sex

DISCUSSION

Because of similarity with estrogen, most of former investigation of zearalenone was targeted to reproductive system or so called "estrogen dependent tissues". Studies have shown that phytoestrogens and xenoestrogens bind to estrogenic receptors (ER) but may differ in their ability to induce cell proliferation and trigger ER-regulated products (11). To our knowledge and after a very extensive literature review, this is the first report which describes changes on the thymus of the rats after exposition to zearalenone. Induction of apoptosis *in vivo* is difficult to detect because of rapid clearance of apoptotic cells by phagocytic cells (12). Histologically, cell death was observed as "apoptotic" bodies and lymphocyte phagocytosis by macrophages ("starry-sky" macrophages). Indirectly, we have followed the degree of the apoptosis, by counting phagocytes with apoptotic bodies (lymphophagocytosis), so the values are relative, estimation is partially rough, but satisfying to show capsular region and deep capsular cortex incidents. Our research has shown significant increase of lymphophagocytosis (apoptosis) compared to dose, dose and period in combination, demonstrates satisfying a degree of accordance with recently published studies of estrogen effects to thymic lymphoid tissue. Previous studies have shown that treatment with estrogens triggers thymic atrophy (13,14). However, the precise mechanism remains unclear, and

many hypotheses have been proposed. Studies have suggested that estrogens may induce thymic atrophy by affecting prethymic stem cells in bone marrow or fetal liver (13,14). In contrast, other studies have demonstrated that estrogen alters intrathymic T-cell development (15, 16). It has also been suggested that estrogen might affect lymphocytes indirectly by mediating estrogenic effects on thymic epithelial cells, which have higher expression of estrogen receptors (17). Recent studies suggested that estrogen may trigger the death receptor pathway *in vivo* in T cells, thereby inducing apoptosis (18,19). During the investigation we used low doses of zearalenone, to simulate asymptomatic cases of food and food products consumption. The applied doses were sufficient to start apoptotic process but not to cause the decrease of cellularity in a sense of atrophy because this compound has weaker influence on target tissues (20-160 times) compared to estradiol (9). The current study has significant clinical impact in understanding the effects of the zearalenone on the immune system. The ability of estrogen to induce apoptosis in fetal and adult thymocytes can lead to alterations in the T-cell repertoire in such a way that the immune system may be more skewed to react strongly toward self-antigens and weakly against foreign antigens. Such an immunomodulation can explain the increased susceptibility of estrogen-exposed individuals to autoimmunity and cancer (19). We believe that zearalenone can cause similar effects.

CONCLUSION

1. Zearalenone induces lymphophagocytosis (T cell apoptosis) on the lymphoid tissue of the rat's thymus
2. The degree of induced T cell apoptosis is dose dependent, and of combination of dose/interval
3. Tissue reaction does not show gender differences observed changes, applied doses does not cause a decrease of cellularity in a sense of thymic atrophy
4. Observed changes correlate with estrogenicity

REFERENCES

- (1) Ueno Y. The toxicology of mycotoxins. *CRC Critical Reviews in Toxicology* 1888; 14: 99-133
- (2) Mirocha C.J., Christensen C.M., Nelson G.H. Toxic metabolites produced by fungi implicated in mycotoxicoses. *Biotechnol. Bioengng.* 1968; 10: 469-482
- (3) Mirocha C.J., Christensen C.M. Oestrogenic mycotoxins synthesized by *Fusarium*. In: *Mycotoxins*. 1974; pp 129-148 (Purchase, I. F. H., Ed.) Amsterdam: Esvier
- (4) Kupier-Goodman T., Scott P.M., Watanabe H. Risk assessment of mycotoxin zearalenone. *Regul. Toxicol. Pharmacol.* 1987; 7(3): 253-306
- (5) Etienne M., Jemmali M. Effects of Zearalenone (F₂) on oestrus acivity and reproduction in gilts. *J. Anim.* 1982; 55: 1-10
- (6) Farnworth E.R., Trenholm H.L. The metabolism of the mycotoxin zearalenone and its effects on the reproductive tracts of young male and female pigs. *Can. J. Anim. Sci.* 1993; 63: 967-975
- (7) Fitzpatrick D.W., Picken C.A., Murphy L.C., Buhr M. M. Measurement of the relative binding affinity of zearalenone, α -zearalenol and β -zearalenol for uterine and oviduct oestrogen receptors in swine, rats and chickens: an indicator of oestrogenic potencies. *Comp. Biochem. Physiol.* 1989; 94C: 691-694
- (8) Sanz de Rodriguez C.A., Bougiovanni A.M., Conde de Borrego L. An epidemic of precocious development in Puerto Rican children. *J. Pediatr.* 1985; 107: 393-396
- (9) Tomaszewski J., Miturski R., Semczuk et al.: Tissue zearalenone concentration in normal, hyperplastic and neoplastic human endometrium. *Ginekologia Polska.* 1998; 69 (5) 363-366
- (10) Chi M.S., Mirocha C.J., Weaver G.A., Kurtz H.J. Effect of zearalenone on female white leghorn chickens. *Appl. Environ. Microbiol.* 1980; 39: 1026-1030
- (11) Zava D.T., Blen M., Duve G. Estrogenic activity of natural and synthetic estrogens in human breast cancer cells in culture. *Environ. Health Perspect.* 1997; 105 (Suppl.3): 637-645
- (12) Ashwell J.D., Lu F.W., Vacchio M.S. Glucocorticoids in T cell development and function. *Annu. Rev. Immunol.* 2000; 18: 309-345
- (13) Holladay S.D., Blaylock B.L., Comment C.E., Heindel J.J., Fox W.M., Korach, K.S., Luster M.I. Selective prolymphocyte targeting by prenatal diethylstilbestrol exposure. *Cell. Immunol.* 1993; 152: 131-142
- (14) Staples J.E., Gasiewicz T.A., Fiore N.C., Lubahn D.B., Korach K.S., Silverstone A.E. Estrogen receptor alpha is necessary in thymic development and estradiol-induced thymic alterations. *J. Immunol.* 1999; 163: 4168-4174
- (15) Brunelli R., Frasca D., Baschieri S., Spano M., Fattorossi A., Mosiello L.F., D'Amelio R., Zichella L., Doria G. Changes in thymocyte subsets induced by estradiol administration or pregnancy. *Ann. N.Y. Acad. Sci.* 1992; 650: 109-114
- (16) Rijhsinghani A.G., Thompson K., Bhatia S.K., Waldschmidt T.J. Estrogen blocks early T cell development in the thymus. *Am. J. Reprod. Immunol.* 1996; 36: 269-277
- (17) Luster M.I., Hayes H.T., Korach K., Tucker A.N., Dean J.H., Greenlee W., Boorman G.A. Estrogen immunosuppression is regulated through estrogenic response in the thymus. *J. Immunol.* 1984; 133(1):110-116
- (18) Okasha S.A., Ryu S., Do Y., McKallip R.J., Nagarkatti P.S. Evidence for estradiol-induced apoptosis and dysregulated T cell maturation in the thymus. *Toxicology.* 2001; 163: 49-62
- (19) Yoonkyung D., Seongho R., Mitzi, N., Prakash S.N. Role of death receptor pathway in estradiol-induced T-cell apoptosis in vivo. *Toxicol. Sci.* 2002; 70: 63-72