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THE EFFECTS OF EXPERIMENTAL ADMINISTRATION OF LOW DOSES OF ZEARALENONE ON BLOOD CELL COUNT AND NEUTROPHIL TO LYMPHOCYTE RATIO IN RATS

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ABSTRACT

The effects of different level of zearalenone administration on the red and white blood cells in a sense of investigating the toxicity in Wistar rats have been examined in the course of the study. The blood samples of 20 rats perionetally injectedfour dosage levels of zearalenone(0, 0.5,1 and 1.5 mg/kg of body weight)were analyzed for numbers of red blood cell, white blood cells, neutrophils, monocytes and platelets.There were differences among treatments for red blood cells count. Red blood cells decreased as zearalenone dose increased (linear effect, $P<0.025$). White blood cells decreased in a linear manner as zearalenone dose increased ($P<0.001$). The highest count of lymphocytes was related to control group and the lowest one was for rats received 1.5 g/kg BW zearalenone. In a linear manner, neutrophils count increased as zearalenone dose increased ($P<0.015$). The ratio of neutrophil to lymphocyte increased linearly ($P<0.001$) as zearalenone dose increased. The lowest monocyte counts were related to control group and the lowest one was for rats received 1.5 g/kg BW zearalenone. The orthogonal contrast showed the linear effect ($P<0.001$) and quadratic effect ($P<0.043$) of zearalenone on monocytes counts. There was no significant difference among treatments for platelets, but orthogonal contrast showed

linear decrease of platelets as irradiation dose increased. It was concluded that zearalenone under low dosages could affect blood cell count and the ratio of neutrophil to lymphocyte ratio.

Keywords: Red blood cells, White blood cells, Zearalenone, Rat

INTRODUCTION

Zearalenone is a potent estrogenic substance produced by various species of Fusarium fungi growing on grains or feeds exposed to high moisture on farm and duringstorage[1]. Fusarium toxins especially zearalenone has been shown to cause diverse toxic effects in laboratory animals, livestock and humans [2-5]. Studies in various species (mice, rats, pigs and monkeys) have shown that zearalenone and its metabolites have potent estrogenic activities [6] with a high binding affinity for estrogen receptors. The immune system is a potential target for estrogenic endocrine disruptors, because various cells of the immune system have estrogen receptors [7]. Zearalenone has also been shown to be immunotoxic [8], and genotoxic [9] in different species. This mycotoxin has been classified as a genotoxic, because after binding to estradiol receptors in the uterus, mammary gland, hypothalamus and pituitary gland [2], induces lipid peroxidation[10], cell death, inhibits protein and DNA synthesisand disturbs enzymatic and hematological parameter in mice. Abbès et al. [8] reported that dietary zearalenone resulted in decreaseof blood IgA and IgG concentrations and expression of some genes on blood lymphocyte in mice. Also, Swamy*etal*. [11] demonstrated that immunosuppressive effect of zearalenone was mediated by depressed T and B cell activity in chickens. ZEA and its derivatives showed divergent effects on important parameters of innate immunity in swine, such as cell proliferation (Marin et al., 2010). Based on study of Ben-Salah Abbès et al., [12] high doses of zearalenone significantly decreased in total white blood cell counts, immunoglobulin profiles (IgG and IgM), B cells, T-cell sub-types and natural killer cells and pro-inflammatory cytokines.Zearalenone research has mainly focused on the reproductive system [13], with little information of the effect of zearalenone on immune cells counts [8,14] especially lymphocytes and neutrophils, cell populations with an important function in the achievement of the innate immune

response. In addition, most toxicological data on animals has been obtained using medium to high doses of zearalenone (2 to 90 mg/kg of body weight). Such high dosages are not commonly consumed or found in animal feeds; therefore, the objective of the present study was to investigate the effects of zearalenone in rats under low dosage.

MATERIAL AND METHODS

Chemicals

Zearalenone (CAS Registry No: 17924-92-4) used in this assays was provided by Cayman Chemical Company (USA). The toxins were dissolved in DMSO (Merck, Darmstadt, Germany) to a concentration of 10 mg/ml and then further dilutions were made in phosphate buffer saline.

Animals and treatment

Twenty female Wistar-albino rats (150-155 g body weight) were obtained from the Pasteur Institute (Tehran, Iran). Prior to dosing, they were acclimatized for 7 days to light from 06:00 to 18:00 h alternating with 12 h darkness. The animals were housed in polycarbonate cages with sterilized softwood bedding in an air-conditioned room with temperature maintained at 25± 2 °C and 40-60% humidity. All rats were provided ultra-pure water and rodent diet ad libitum. After 1 week of acclimation, rats were divided into four groups (two or three per each cage) by randomization of body weight (BW). Rats in the control group received intraperitoneally sterile saline solution, and treatment groups were administered with 0.5, 1 and 1.5 mg zearalenone per kg BWintraperitoneally three times per weekfor4 week, respectively.

Blood sampling and analysis

At the end of the experiment, rats were fasted overnight with free access to water. Rats were anesthetized with diethyl etherand blood was collected into heparinized tubes from heart. These tubes transferred to laboratory for counting red blood cell, white blood cell, heterophil, lymphocyte, monocyteand platelets with automated counters.

Statistical analysis

Collected data were analyzed using completely randomized design using GLM procedure of SAS (SAS Institute, Cary, NC). Basic statistics and variance analysis were performed to test the significance between the treatments. To evaluate the differences between the control and treatments, significant means were analyzed using Duncan's multiple range tests. In all cases, p-values ≤ 0.05 were considered significant.Differences among

treatments were separated using polynomial orthogonal contrasts to determine linear, quadratic, and cubic responses.

RESULTS AND DISCUSSION

The main aim of this study was to evaluate the effect of different level of zearalenone on blood cells counts and the ratio of neutrophil to lymphocyte. There are some studies concerning the effects of zearalenone on some immune parameters [9,11,12,15], but in the literature, there was no report on the effects of this mycotoxin on blood cells counts and especially neutrophil to lymphocyte ratio.

The effect of zearalenone on total and differential blood cells counts is presented in Table 1. There were differences among treatments for red blood cells. Red blood cells decreased as zearalenone dose increased (linear effect, $P<0.025$). In contrast to our finding, Allen et al. [16]reported no significant effect of dietary zearalenone on red blood cells in the broiler chicks. The differences between red blood cells in mammals and poultry maybe a reason of this discrepancy. The adverse effects of zearalenone are partly determined by the processes of elimination, because the biliary excretion and enterohepatic cycling are important processes affecting the fate of zearalenone and explaining a different sensitivity between animals [17]. In agreement with our results, Salah-Abbès et al. [18] reported that ingestion of zearalenone had a significant effect on total red blood cell count in mice.Zearalenone may be induced the lipid peroxidation [10] and disturbed hematological parameter [8], and finally cell death, thus red blood cells and other blood cells counts decreased.

There was difference among treatments for total white blood cellcount**(Table 1)**. White blood cells decreased in a linear manner as zearalenone dose increased ($P<0.001$). In contrast to our finding, Allen *etal*. [16]reported no significant effect of dietary zearalenone on white blood cells of broiler chicks. Mice treated with zearalenone (40 mg/kg) for two weeks showed a decrease in the lymphocyte count, the total white blood cell count, immunoglobulins (IgG and IgM), B cells, T cell subtypes and natural killer cells, and pro-inflammatory cytokines [18]. Zearalenone metabolites (α and β) that produced in liver cause cytotoxicity by inhibiting cell viability, protein and DNA syntheses and inducing oxidative damage and over-expression of stress proteins [19]. In addition, oxidative damage is likely to

be evoked as one of the main pathways of zearalenone toxicity which may initiate event at least in part contribute to the mechanism of zearalenone induced genotoxic and cytotoxic effects [20].

The highest count of lymphocytes was related to the control group and the lowest one was for rats received 1.5 g/kg BW zearalenone. In a linear manner, neutrophils count increased as zearalenone dose increased (P<0.015).The ratio of neutrophil to lymphocyte (N:L ratio) increased linearly (P<0.001) as zearalenone dose increased.Changes in differential white blood cells observed in this study may be related to the effects of zearalenone on oxidative stress. It was demonstrated that diets contaminated with zearalenone at medium levels are already able to induce oxidative stress and compromise the blood phagocytic activity in chickens[21].Meanwhile, production of corticosterone increases from the adrenal gland during oxidative stress. Corticosterone has been found to be immunosuppressive [22], inhibiting the production and actions of antibodies, lymphocyte function, and leukocyte population [22,23]. Based on the results in **Table1**, rats received zearalenone at higher doses maybe experienced physiological stress. The heterophil to lymphocyte ratio has been accepted as a reliable index for determining stress [24]. The increases in heterophil to lymphocyte ratio in ratsreceived zearalenone may be attributed to increased corticosterone secretion.

The lowest monocyte counts were related to control group and the lowest one was for rats received 1.5 g/kg BW zearalenone. The orthogonal contrast showed the linear effect (P<0.001) and quadratic effect (P<0.043) of zearalenone on monocytes counts. There was no significant difference among treatments for platelets, but orthogonal contrast showed linear decrease of platelets as zearalenone dose increased.

In contrast to our finding, Forsell *etal*. [25] reported no differences from the control group found in differential counts especially monocytes of mice fed a diet supplemented with 10.0 mg/kg zearalenonefor 6 weeks. Cytotoxicity and reactive oxygen species (ROS) generation are mechanisms of mycotoxins mediated toxicity. ROS are chemically reactive molecules containing oxygen. They are highly reactive due to the presence of unpaired electrons. ROS formed as a natural byproduct of the normal metabolism of oxygen have important roles in cell signaling and homeostasis.

However, during times of environmental stress, ROS levels can increase dramatically as a result of oxidative stress [26]. Oxidative stress occurs when the concentration of ROS generated exceeds the antioxidant capability of the cell. In other words, oxidative stress describes various deleterious processes resulting from an imbalance between the excessive formation of ROS and limited antioxidant defenses [27]. Zearalenone generate ROS and at higher doses may be induced the lipid peroxidation [10] and disturbed blood cells and platelets.

Table 1: The effects of zearalenone on total and differential blood cells counts

Parameters	Zearalenone dose (mg/kg BW)				SEM	Contrast		
	Control	0.5	1.0	1.5		L	Q	C
Red blood cells × 1000/ml	8.70^{a}	7.78^{ab}	7.76^{ab}	7.50^{b}	0.513	0.025	0.297	0.414
White blood cells × 1000/ml	10.20^{a}	9.89^{a}	9.32^{a}	7.91^{b}	0.589	0.001	0.145	0.720
Lymphocyte × 1000/ml	7.89^{a}	7.35^{ab}	6.83^{b}	5.45^{b}	0.487	0.001	0.177	0.504
Neutrophil × 1000/ml	1.67^{b}	1.95^{a}	1.94^{a}	2.02^{a}	0.132	0.015	0.235	0.296
N:L Ratio	0.21^{c}	0.26^{b}	0.28^{b}	0.37^{a}	0.022	0.001	0.257	0.109
Monocyte Per ml	28.3^{b}	20^{b}	18.7^{b}	17.8^{a}	2.64	0.001	0.043	0.357
Platelets × 1000/ml	275	157	248	205	38.9	0.062	0.588	0.691

^{abc} **Means in the same row with different letter are significantly different (P≤0.05)**

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