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Original scientific paper

DIFFERENTIAL LEUKOCYTE COUNT OF ALLOXAN-TREATED WISTAR RATS

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Abstract. Diabetes is among the most common endocrine diseases with increasing prevalence and represents a constant subject of research. Alloxan induced diabetes in rats corresponds to type 1 diabetes in humans. Intoxication of Wistar rats with alloxan (100 mg / kg) was performed in order to monitor the impact of diabetes on the blood count. Individuals were anesthetized fourteen days after achieving stable hyperglycemia, and blood was collected by cardiac puncture. In all treated groups, there was a significant (p=0.000) increase in the number of leukocytes per liter of blood in relation to the control group of individuals. Much lower percentage of basophils (p=0.002) was observed in the differential blood count of treated animals. Percentage of neutrophils in the blood of treated animals was lower, and those of eosinophils and lymphocytes higher than in control animals, with no statistically significant difference (p> 0.050).

Key words: Wistar rat, alloxan, diabetes, differential blood count.

INTRODUCTION

Diabetes is the most common endocrinological disease in the world, which is also a metabolic disease with multiple etiologies, characterized by chronic hyperglycemia and impaired metabolism of carbohydrates, fats and proteins. It occurs as a result of disordered secretion and action of insulin (Magaš, 2010). Diabetes is one of the most common endocrinological diseases with a steadily increasing prevalence today, especially in the developed world (Rolo and Palmeira, 2006). According to the World Health Organization (WHO, 2011), diabetes is responsible for about 2.2% of illness-related deaths among human. Diabetes takes one life every ten seconds and two people are diagnosed with this disease. It is

a disease of an evolutionary course, of high frequency, which is why it is referred to in the contemporary literature as a pandemic problem of morbidity and mortality. Today's diabetes statistics are devastating because they say that diabetes is among the five most common non- contageous causes of death in the world (Stilinović, 2013). The biochemical changes underlying the pathogenesis of diabetes lead to the appearance of oxidative stress, which is accompanied by an increased presence of free radicals and decreased antioxidant defense of the organism. Oxidative stress, which results from hyperglycemia, is a major cause of the pathological complications typical of diabetes. During hyperglycemia, the production of superoxide radical occurs in the mitochondria, which in subsequent steps can be converted to other forms of reactive oxidative species, which contribute to general oxidative stress (Green *et al.*, 2004).

Alloxan, an organic compound, can induce diabetes in animals by destroying the beta cells of the Langerhans pancreatic islets (Szkudelski, 2001). Alloxan is a well-known and universally used tool for the induction of experimental diabetes. It selectively damages the beta cells, indicating an extremely potent diabetogenic effect. Experimentally induced diabetes in animals shows the same typical symptoms as diabetes mellitus in humans: body mass loss, polydipsia, polyuria, glycosuria, ketonuria, hyperglycemia and ketonemia (Mhammad *et al.*, 2015)

Blood parameters are the best indicator of health. In this regard, a differential blood count of test rats was observed to determine changes resulting from the development of alloxan diabetes.

MATERIAL AND METHODS

For the purpose of the experiment, 40 Wistar rats of both sexes, same approximate age and body weight, were isolated. They were kept in plexiglas cages, on a 12 hour light regime (12 hours in the light and 12 hours in the dark), at a temperature of 22 ± 2 ° C with food and water *ad libitum.* After two weeks of acclimation, the individuals were divided into groups (control K ♂ and K ♀ and treated A ♂ and A♀) and subjected to treatment.

According to the referential accounts, the usual dosage of alloxan to induce hyperglycemia in Wistar rats ranges from 40 mg/kg, i.v. (Mude *et al.*, 2012), 42 mg/kg, i.v. (Lucchesi *et al.*, 2013, 2015), 70 mg/kg, i.v. (Akah *et al.*, 2009), 100 mg/kg, i.p. (Đeri, 2014, Saba *et al.*, 2010), 120 mg/kg, s.c. (Ahmadvand *et al.*, 2012), 125 mg/kg, i.p. (Georgewill i Georgewill, 2009), 140 mg/kg, i.p. (Olurishe *et al.*, 2013), 150 mg/kg, i.p. (Dallatu *et al.*, 2010; Ahmed *et al.*, 2010; Kim and Ha, 2013; Ebong *et al.*, 2014), 160 mg/kg, i.p. (Muhammad *et al.*, 2012), to 200 mg/kg, i.p. (Indradevi *et al.*, 2012) and more. The selected dose of alloxan was 100 mg/kg, i.p. As lower doses of alloxan injected intraperiotoneally can result in autoreversion to normal glycemic status, thanks to the regenerative capacity of Langerhans islet cells (Jain and Arya, 2011), blood glucose is controlled every 48 hours by tail vein blood sample and Accu Check Active digital glucometer (Roche). If necessary, we repeated the treatment with alloxan until stable hyperglycemia (> 11 mmol glucose per liter of blood) was achieved. Twenty individuals of both sexes with stable hyperglycemia were sacrificed after 14 days.

Animals were anesthetized with an intramuscular injection of 50 mg ketamine per kg of body weight (Ketaminol 10 100 ml/mg, Intervet, diluted with 1:10 saline). After anesthesia (15-20 minutes from application), blood was taken by cardiac puncture in vacuteners with K_3EDTA anticoagulant. We determined the leukocyte count by the Thoma chamber counting method. Differential blood count was determined by making smears stained using the Pappenheim method (combined staining by May-Grünwald and Giemsa) (Ivanc and Dekić, 2006).

In accordance with the instructions of the ethical committees and committees for the care of laboratory animals, all animals were sacrificed by decapitation under deep anesthesia (Law on the Protection and Welfare of Animals of the Republika Srpska), in the morning from 08.00 to 10.00 in order to avoid possible variations in the obtained results.

All survey results were analyzed by software in Office Excel 2010, with parameters for mean, minimum, maximum, standard deviation, and coefficient of variation. Data were compared with Student's t-test with a minimal statistically significant difference of r <0.050. All survey results were also processed using the statistical package SPSS 20.0 (ANOVA and LSD test). A 95% confidence level was used in the data processing.

RESULTS AND DISCUSSION

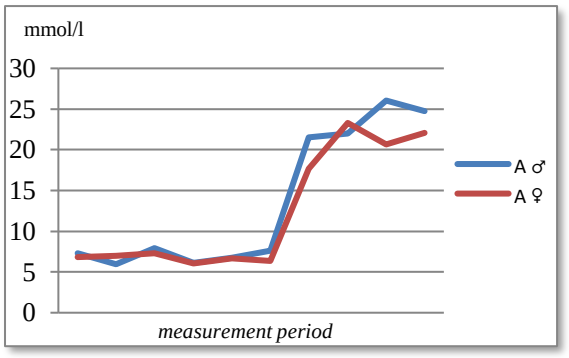

Figure 1. Blood glucose concentrations of test animals

During the experiment, 20 Wistar rats were treated with alloxan, with regular monitoring of blood glucose concentration (Figure 1). Stable diabetes was developed in all experimental subjects, with a mean blood glucose concentration of 24.75 mmol/l in males and 22.1 mmol/l in females at the end of the experiment., There was a significant (p = 0.000) increase in leukocyte counts per liter of blood in all test groups treated with alloxan relative to control individuals (Figure 2).

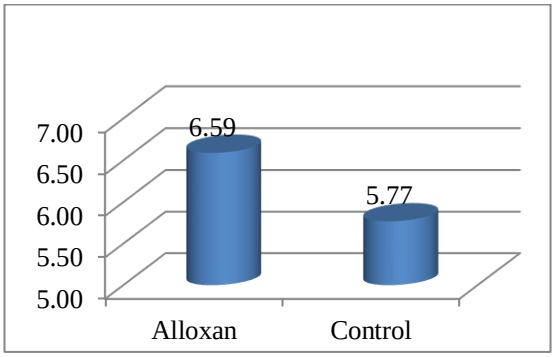

Figure 2. Leukocyte count (10^9 / l) in the blood of test and control individuals

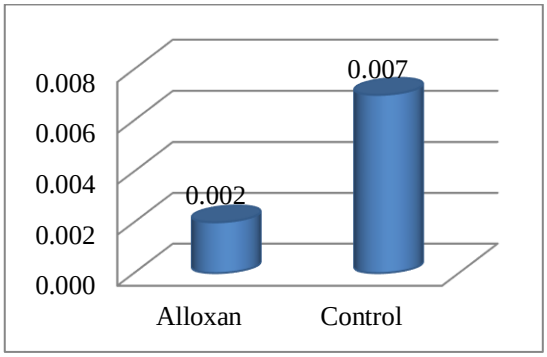

Figure 3. The proportion of basophils in the differential blood count of test and control individuals

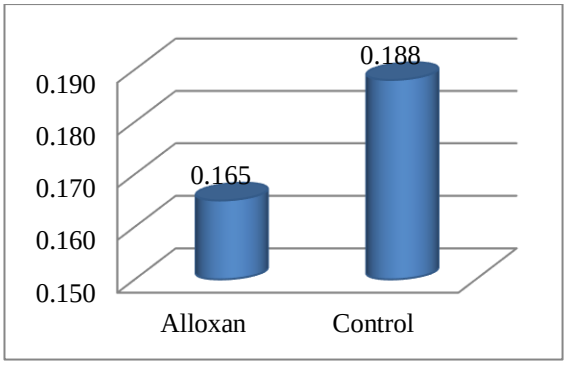

Figure 4. The proportion of neutrophils in the differential blood count of test and control individuals

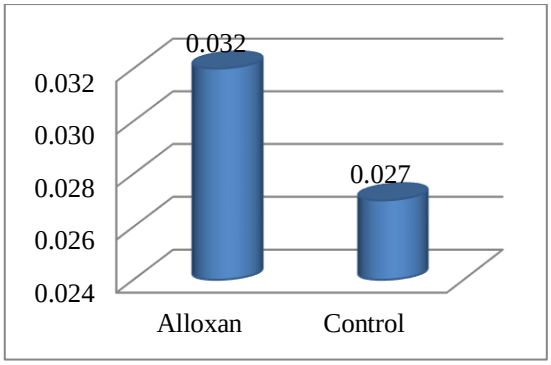

Figure 5. The proportion of eosinophils in the differential blood count of test and control individuals

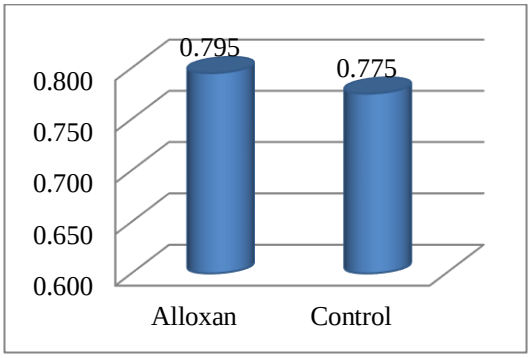

Figure 6. The proportion of lymphocyte in the differential blood count of test and control individuals

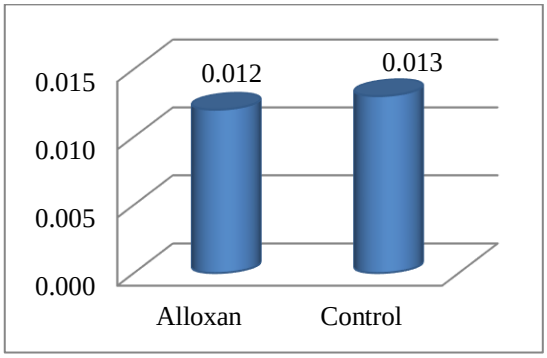

Figure 7. The proportion of monocyte in the differential blood count of test and control individuals

Analyzing the leukocyte formula of the test animals revealed similarity to the control group of individuals, with a slight increase in lymphocyte content and a decrease in neutrophil proportion ($p > 0.050$), while a basophil proportion decreased significantly in diabetic individuals ($p = 0.002$) (Figures 3 , 4 and 6). No significant difference was observed between the eosinophils and monocytes ($p > 0.050$) of the control and test individuals (Figures 5 and 7). There were also minor differences in values referring to gender, with no statistical significance.

By comparing the results with the referential accounts, it can be concluded that the leukocyte formula of control individuals is within the limits of physiological values (Lindstrom *et al.*, 2015; Sharp and Villano, 2013; Thrall *et al.*, 2012; Antai *et al.*, 2009; Moriyama *et al.*, 2008; Rusov, 1984). The results obtained from the analysis of the blood of test animals were in accordance with the study of Muhammad *et al.* (2012), who observed an increase in the total number of leukocytes, as well as changes in the percentage representation of certain forms of leukocytes relative to control individuals. There was also a decrease in the percentage of neutrophilic granulocytes, while percentage of lymphocyte in blood of diabetic rats increased when compared with the healthy individuals. Asanga *et al.* (2013) reported that rats in alloxan diabetes were susceptible to allergic reactions and frequent infections. They also stated that the sensitivity of rats occured as a result of free radicals generated during the metabolism of alloxan, which further affected various tissues in the body. According to

Pankaj and Varma (2013), a variable neutrophil count could be attributed to a change in the total leukocyte count, which negatively affected the body's immune system and phagocytosis activity. Because of neutropenia, there was a reduced ability to defend the body against bacteria, which also entailed frequent infections (Stanković, 2014).

Aloxane tends to be proinflammatory by increasing the number of total leukocytes, as well as altering the ratio of individual types in the leukocyte formula in favor of lymphocytes.

One of the main reasons for the decrease in the number of basophils in the peripheral blood, registered in our study, is the stress, which is evidently present during the experiment. The number of basophils in the blood is affected by the reaction to the chemical substance alloxan, as well as by anemia occurring in the state of artificially induced diabetes.

A slight increase in eosinophilic granulocytes, without statistical significance, was observed in rats with diabetes compared to controls. Eosinophils participate in allergic reactions by performing phagocytosis and antigen-antibody complex removal. The number of monocytes did not change significantly, but there was a slight decrease compared to untreated rats. Because monocytes are produced in the bone marrow, any disease that affects the bone marrow can cause a lower level of monocytes. Increased glucose concentration resulting from the action of alloxan on pancreatic islets of Langerhans leads to oxidative stress. Stress is the result of free radicals, which disrupt the normal functioning of cells and cause many pathological conditions and diseases. A typical primary response to stress is increased secretion of catecholamines (adrenaline) and corticosteroids (cortisol). In rats, the biggest changes due to the action of adrenaline are an increase in glucose concentration and lymphocyte count. It would be expected that an increase in proportion of neutrophil granulocytes would occur after the effect of a stressor on the organism, as it is generally accepted that neutrophilia is the physiological response of the organism to stress. However, the dominant form of leukocytes in rats is lymphocytes, and it is to be expected that the body will act by changing the number of lymphocytes. Lymphocytosis is one of the attendant phenomena of stress, and in proportion to this is the reduction in the percentage of other forms of leukocytes of the treated group (Mahmutović *et al.*, 2013).

CONCLUSIONS

In our study, we examined the effect of alloxan-induced diabetes on the hematological parameters of male and female Wistar rats. Based on the obtained results, the following can be concluded:

- successful induction of diabetes by intraperitoneal administration of alloxan,
- diabetic organisms have significantly more leukocytes per liter of blood than healthy organisms,
- higher proportion of lymphocyte in test animals compared to control,
- the number of neutrophils and basophils showed a tendency to decrease in the treated individuals compared with the control group,
- alloxan-induced diabetes in the Wistar rats had no significant effect on the proportional representation of eosinophils and monocytes in the total blood sample.

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