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Original scientific paper

SYNERGISTIC EFFECTS OF LEAD-ACETATE AND ALLOXAN ON BODY WEIGHT GAIN AND ORGANOSOMATIC LIVER INDEX OF WISTAR RAT INFECTED WITH ESCHERICHIA

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Abstract. Different agents, both biotic and abiotic, cause health changes in most vulnerable members of the human population. Diabetics, as very sensitive members of the population, are significantly more susceptible to bacterial infections, as well as to the toxic effect of certain pollutants. Alloxan is a common diabetogenic agens often used to induce experimental diabetes in laboratory rats, giving the clinical picture as type 1 diabetes in humans. The aim of this study was to determine common effects of lead-acetate and alloxan on body weight gain and organosomatic liver index of Wistar rat infected with escherichia. Significantly lower weight gain ($p < 0.05$) was achieved by the test subjects exposed to bacterial infection, as well as by lead-acetate toxication. The negative value of this parameter was determined for the synergistic effect of the test substances. Values of organosomatic liver indexes showed a significant increase in diabetic rats exposed to infection in relation to the control group and rats treated with only one agens. In diabetic patients, hyperglycaemia may harm both innate and acquired immunity, thereby reducing resistance to pathogenic and associated illnesses. Environmental pollutants, including lead, can cause different changes in the body of a diabetic, so it is necessary to avoid any contact with this toxicant.

Key words: Wistar rat, aloxan, lead-acetate, bacterial infection, organosomatic liver index, body weight gain.

INTRODUCTION

Increased concentrations of toxicants in the environment result in their accumulation in plants, and consequently in food chains and humans, as end points. Some toxicants are less significant due to their minimal impact on living systems, while others are extremely toxic and create a range of health problems (Obradović and Đekić, 2012). Among toxic chemicals, metals are critical environmental toxicants because of their bioaccumulation ability, inability to biodegrade, and pronounced harmfulness within ecological systems (Sainath *et al.*, 2011). Lead is one of the most prevalent toxic metals due to its widespread use in various industrial products, which is considered a serious risk by many professions around the world. It has a negative effect on all organ systems, primarily the central nervous system, hematopoietic, hepatic and renal systems, where it creates serious disorders (Flora *et al.*, 2012). During pregnancy, lead in particular exerts its negative effect, passing through the placental barrier (Lagerkvist, *et al.*, 1996). Numerous experiments on laboratory animals have shown that lead alters the morphological features of the ovary, causes changes in placental function, fetal development, as well as during lactation, when lead reaches the mammalian organism with milk (Mornjaković *et al.*, 1995; Mornjaković, 1994).

Lead exposure during gestation and lactation leads to numerous neurological disorders such as memory deficits, neurological abnormalities (Soodi *et al.*, 2008), and changes in common behavioral patterns (Seddik *et al.*, 2010). It also has the effect of reducing body length and mass (Mornjaković *et al.*, 2000) and increasing the mass of individual internal organs (Abdel-Moneim *et al.*, 2011). Increasing organ/body mass ratios have been documented for lead-acetate treated rat kidneys and liver (Abdel-Moneim *et al.*, 2011).

Diabetes is a very common disease in people with a steadily increasing prevalence and is a constant subject of research. Aloxan is a well-known and universally used agent for causing experimental diabetes in laboratory animals (rats, mice, rabbits). It selectively damages beta cells, exhibiting an extremely potent diabetogenic effect (Muhammad *et al.*, 2012). Experimentally induced diabetes in animals shows typical symptoms of diabetes mellitus in humans: weight loss, polydipsia, polyuria, glucosuria, ketonuria, hyperglycemia and ketonemia. Rohilla and Ali (2012) observed that injection of alloxan resulted in a typical state of insulin dependent type I diabetes syndrome. For the duration of DM, chronic hyperglycemia impairs the immune system, causing damage and dysfunction to various organs, particularly the eye, kidneys, nerves, heart and blood vessels (Badr, 2012). The discovery that alloxan is also present in the blood of humans, more specifically children with established diabetes, calls into question the assertion that it does not exert a toxic effect on the beta cells of the Langerhans Islands (Mrozikiewicz *et al.*, 1994).

Escherichia coli is a universal resident of the intestinal tract of humans and warm-blooded animals. Together with related bacteria, it accounts for 0.1% of the intestinal flora (Eckburg *et al.*, 2005). Bacterial infection caused by *Escherichia coli* inoculation can lead to serious pathological processes in the body such as sepsis and meningitis in the neonatal period (Korhonen *et al.*, 1985; Martindale *et al.*, 2000), and various systemic infections such as urinary tract infection and colonization gastrointestinal tract at all periods of ontogeny

(Sarff *et al.*, 1975; Orsakov and Orsakov, 1985; Plos *et al.*, 1995; Karch *et al.*, 2005). Extraintestinal Escherichia-induced infection includes septicemia and polyseratosis, neonatal meningitis and urinary tract infection in humans and animals (Ngeleka *et al.*, 1993).

As diabetics are more susceptible to infections and toxins of various pollutants due to their poor health, the aim of the study was to determine the combined effect of lead-acetatand alloxan on morphometric parameters (in this case, body weight gain and organosomatic liver index) of Wistar rats infected with Escherichia.

MATERIAL AND METHODS

The experimental part of the work was carried out on 160 Wistar rats of equal age, approximate body mass, and equal gender representation in the Laboratory for performing animal experiments at the Faculty of Science, University of Banja Luka. The animals were kept in group plexiglass cages, 12-hour light regime (07.00-19.00), air temperature 22 ° C (± 2), with food (product of Veterinary Institute Subotica, Subotica, Serbia) and water *ad libitum*.

The treatment included lead-acetate intoxication (Pb group designation), alloxan (All label) and escheria infection (E for infected individuals and 0 for uninfected individuals). The obtained values of the monitored parameters of the treated individuals were compared with the same in the control individuals (code K).

Escherichia infection was caused by intraperitoneal inoculation of 0.2 ml of bacterial suspension of *Escherichia coli*, strain ATCC 11775, serotype O1: K1: H7, which contained $3x10^7$ CFU / ml, determined spectrophotometrically, by determining the optical density by the standard method (Reynolds, 2011).

Alloxan (Alloxan monohydrate 98%, Sigma, New Jersey, USA) was administered peritoneally at a dose of 100 mg / kg to induce type 1 diabetes mellitus. Blood glucose levels were monitored every 48 hours by tail blood sampling and using the Accu Check Active (Roche) digital glucometer.

Animals were anesthetized with ketamine i.m. in dosage of 50 mg/kg body weight (Ketaminol 10, 100 mg / ml, Intervet). In accordance with the instructions of the ethical committees and committees for the care of laboratory animals, all individuals were sacrificed by decapitation under deep anesthesia (Law on the Protection and Welfare of Animals of the Republika Srpska).

The mass of individuals and separated internal organs in grams was determined by weighing on the technical scales (KERN, PFB 1200-2). Organosomatic indices represent the ratio of total body mass to the mass of a particular organ (Busacker *et al.*, 1990).

OSI =[organ weight (g) / total body weight (g)] x 100

Percentage of body weight gain represents the ratio of body mass after a monitored time compared with the mass recorded at the beginning of the experiment (Mamikutty *et al.*, 2014).

BWG = [end mass t. (g) - initial mass (g)] / initial mass t. (g) x 100.

The results of the survey were analyzed with Office Excel 2010 using parameters for mean, minimum, maximum, standard deviation and standard error and processed using the statistical package SPSS 20.0. (ANOVA and LSD test) for the 95% confidence level.

RESULTS AND DISCUSSION

The obtained values of the monitored morphometric parameters of the individuals undergoing different treatment were compared with the obtained values for the control groups, K-0 and K-E (Table 1, Figures 1 and 2).

Table 1. Values of statistical significance in the comparison of the results of the morphometric parameters of the liver mass index and body weight gain of the control and treated individuals

	Statistical significance of liver mass index	Statistical significance of weight gain	
K-E	0.162	0.099	K-0
Pb-0	0.001	0.000	K-0
	0.035	0.000	K-E
Pb-E	0.001	0.000	K-0
	0.040	0.000	K-E
All-0	0.000	0.000	K-0
	0.018	0.000	K-E
All-E	0.120	0.000	K-0
	0.874	0.000	K-E
All-Pb-0	0.000	0.000	K-0
	0.000	0.000	K-E
All-Pb-E	0.000	0.000	K-0
	0.000	0.000	K-E
All-Pb-0	0.000	0.000	**Pb-0**
	0.000	0.000	**All-0**
All-Pb-E	0.000	0.000	**Pb-0**
	0.000	0.000	**Pb-E**
	0.000	0.000	**All-0**
	0.000	0.000	**All-E**
	0.042	0.369	**All-Pb-0**

The observed values of the monitored parameters of the individuals exposed to the action of single agents were compared with the values obtained for the double and triple treatment units. The statistical significance values are presented in Table 1. As expected, the mean values of the liver index (Figure 1, Table 1) increased in all test groups both infected and uninfected with Escherichia relative to the average of the negative and positive controls (K-0 and KE), although in the case of the All-E group the difference is not statistically

significant (p> 0.050). Infection of control group individuals did not lead to significant deviations in the values of this parameter relative to uninfected individuals. Dual intoxication with lead-acetate and alloxan, as well as additional Escherichia infection, caused greater variations in the proportion of liver weight in total body weight. Significant differences emerged between All-Pb-0 individuals compared to All-0 and Pb-0, as well as analysis of values obtained for All-Pb-E individuals relative to All-0, Pb-0, All- E, Pb-E, and All-Pb-0 (Figure 1, Table 1).

Control group animals as well as lead-acetate intoxicated rats with and without infection achieved a positive weight gain (Figure 2), significantly lower in treated individuals when compared to the untreated ones. All alloxan intoxicated animals suffered significant body weight loss, the highest in group treated with alloxan, lead-acetate, and bacterial infection (All-Pb-E group). Animals subjected to dual intoxication, when compared to individual treatments and control group individuals (Table 1), achieved significant weight loss.

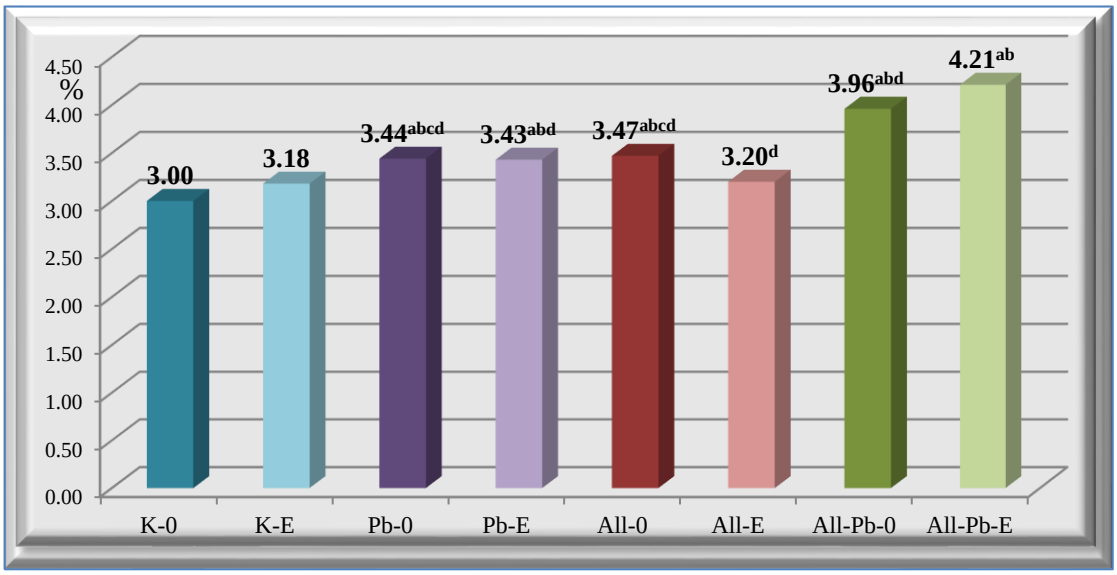

Figure 1. Mean values of the liver indices of controls and individuals under different treatment *

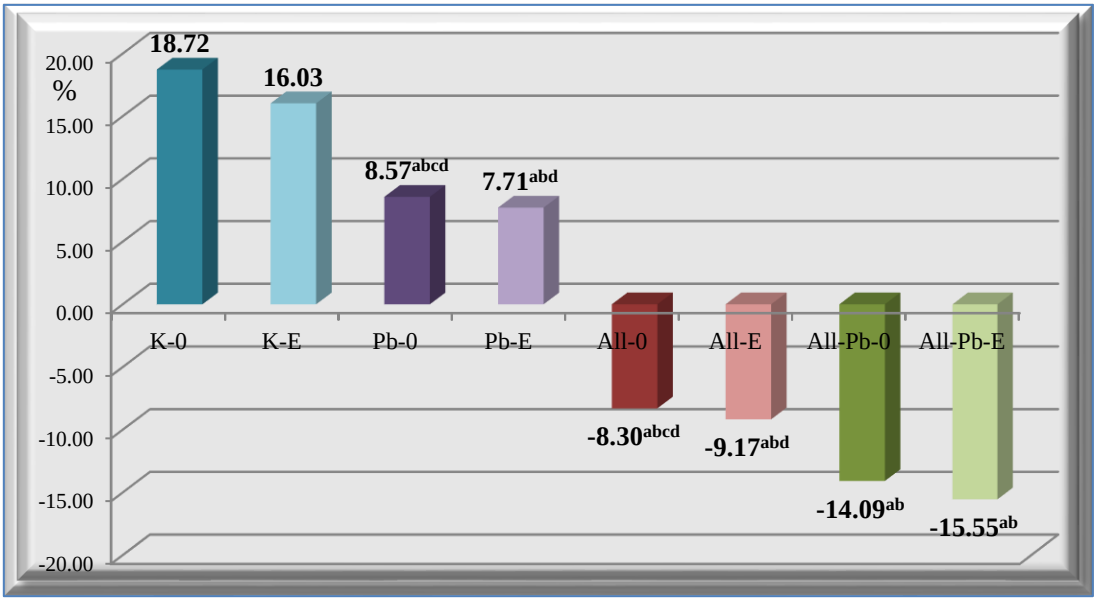

Figure 2. Weight gain means values of controls and test animals*

Male rats have bigger body mass than female rats, especially because of the larger adipose tissue (Pecora and Highman; 1956; Cesta, 2006; Yamano *et al.*, 1998). Male rats have higher increase in the body mass, commonly (Piao *et al.*, 2013). The range of reference values for organosomatic indices of laboratory rats varies from author to author (Addou-Benounan *et al.*, 2009; Onyeanusi *et al.*, 2009; Pecora and Highman, 1956; Gatsing *et al.*, 2005; Mulla *et al.*, 2010; Piao *et al.*, 2013; Blamey and Evans, 1971; Uduak *et al.*, 2013 and others), where the obtained values of all monitored parameters of control animals are within the mentioned ranges. Zou and coworkers (2006) found that infection caused by *Escherichia coli* did not alter organosomatic indices of liver, spleen, small intestine, and lungs of laboratory rats. We reached the same results in our experiment for control animals (C-E group).

The obtained values of the morphometric parameters monitored at control individuals infected with Escherichia are within the reference values for Wistar rats (Addou-Benounan *et al.*, 2009; Onyeanusi *et al.*, 2009; Pecora and Highman, 1956; Gatsing *et al.*, 2005; Mulla *et al.* al., 2010; Piao *et al.*, 2013; Blamey and Evans, 1971; Uduak *et al.*, 2013 and others).

Amjad *et al.* (2013) observed that higher concentrations of lead-acetate led to a significant decrease in the body weight of Wistar rats, which was not observed in our experiment due to the shorter exposure time and lower toxicant concentrations. Significantly less weight gain of lead-acetate-consumed individuals was observed compared to controls ($p<0.005$), but not weight loss, as stated by Amjad *et al.*

Ibrahim *et al.* (2012) noted an increase of renal, hepatic, cardiac and spleen organosomatic indices in Wistar rats exposed to lead-acetate, which was also observed in individuals covered by this experiment, with data presented for organosomatic liver index.

Isaac and coworkers (2013) noted that lead-acetate intoxication (6 mg / kg body weight) during fourteen days resulted in a significant weight loss of Wistar rats. Deveci

*Significant difference in relation to the values of [a] negative control, [b] positive control; [c] group All-Pb-0, [d] group All-Pb-E

(2006), as well as Deveci *et al.* (2011), observed a significant (- 20%) weight loss after two months of lead-acetate intoxication via water *ad libitum* at a concentration of 500 ppm. Similar data, 17% weight loss, were obtained by de Figueiredo *et al.* (2014) after two months of lead-acetate intoxication with a concentration of 30 mg / l deionized water.

Lead-acetate intoxication with a concentration of 15 mg/kg body weight resulted in a weight loss of 5% (relative to the initial weight) over a period of 7-14 days (Olchowik *et al.*, 2014).

Allouche *et al.* (2011) believe that lead may influence weight loss in the first exposure period, in order to adapt the organism to the long-term exposure of this metal and compensate for weight loss. At the same time, the proportion of liver mass increases significantly with the increase of lead-acetate concentration and exposure time.

Ebong *et al.* (2014) observed that treatment with alloxan at a dose of 150mg/kg body weight resulted in a significant weight loss after 14 days, an increase in renal index values and a decrease in liver mass index values. Weight loss has been associated with hyperglycemia, both in experimental models and in humans. Tissue degradation is typical of poor glycemic control in diabetes and is most commonly associated with protein and fat mobilization. Diabetes causes damage to the tissues of the pancreas, liver, kidney and heart, which are directly related to the metabolic changes of lipid peroxidase.

Indradevi *et al.* (2012) caused diabetes in Wistar rats with *i.p*. injection of alloxan at a dose of 200 mg/kg. Fifteen days after treatment, the animals were sacrificed. They observed a significant weight loss in individuals with confirmed hyperglycemia, as well as an increase in liver, spleen, and kidney mass indices compared to controls.

Akah *et al.* (2009) also observed an increase in the index of kidney, liver, heart, and spleen masses in the treatment of Wistar rats with alloxan at a dose of 70 mg / kg, *i.v.*, and a total treatment length of 37 days.

Lucchesi *et al.* (2015) examined the long-term effect of alloxan (42 mg / kg i.v.) on rat liver, noting a significant increase in its proportion to total body weight after 6, 14 and 26 weeks. They observed morphological and ultrastructural lesions in the liver, with changes ranging from fatty degeneration of hepatocytes to steatohepatitis and periportal fibrosis. The changes were reflected in the presence of fat vacuoles in hepatocytes, dilated sinusoids, and progressive loss of general organ structure. Follow-up lesions on the liver of diabetic animals affected all organ structures, from portal areas and sinusoids, to hepatocytes and cytoplasmic organelles, primarily mitochondria, endoplasmic reticulum and nucleus.

During diabetes, due to insulin deficiency or resistance, as well as insensitivity of cells to insulin activity, glucose transport to cells is reduced, and its lack results in gluconeogenesis (lipolysis, proteolysis, etc.), which further leads to the weight loss due to fat and protein burning (Onwuli *et al.*, 2014).

Ananthi and coworkers (2003) observed an average weight loss of approximately 30% after 70 days of hyperglycemia in rats intoxicated with alloxan at a dose of 150mg/kg.

Mude *et al.* (2012) noted a significant weight loss in alloxan intoxicated individuals at 40 mg/kg body weight, 10 and 21 days after administration. Diabetes caused by alloxan (120 mg/kg body weight, i.p.) caused a significant weight loss, polydipsia, as well as an increase in renal and liver mass index values in Wistar rats (Ewenighi *et al.*, 2015). Diabetic rat body

weight loss indicated the degradation of structural proteins due to diabetes (Ananthi *et al.*, 2003).

Saba *et al.* (2010) observed that four weeks of alloxan-induced hyperglycemia (100 mg/kg body weight, i.p.) resulted in a significant decrease in body weight of treated individuals, an increase in liver and kidney mass index, and a decrease in spleen and heart index. On the other hand, after two days of exposure to alloxan hyperglycemia (150 mg/kg body weight, ip), Kim *et al.* (2013) reported a positive weight gain but significantly lower than those obtained for control (untreated) individuals, whereas Sarma and Das (2008) reported a negative increase in the weight of diabetic individuals at the same dose of alloxan and after fifteen days of exposure to increased glucose concentration.

Patients with diabetes are more prone to infections than non-diabetics (Larkin *et al.*, 1985). Uncommon infections such as rhinocerebral and pulmonary mucormycosis, emphysema cholecystitis and pyelonephritis, necrotizing cellulitis or fascitis, and malignant external otitis occurred more frequently (Seidel *et al.*, 2003).

Moderate and severe glucosuria have been shown to increase bacterial growth, which explains the increased susceptibility of diabetics to urinary tract infections, with no difference in the growth of uropathogenic and non-uropathogenic *Escherichia coli* strains (Geerlings *et al.*, 1999).

Studies involving various diabetic animal models support the idea that hosts with diabetes are more susceptible to bacteremia or sepsis. The insulin treatment and appropriate glycemic control can increase resistance to sepsis in diabetic individuals and animals. (Yeh *et al.*, 2014).

According to Huang *et al.* (2013), environmental exposure to lead can progressively accelerate nephropathy in patients with advanced diabetes type 2, regardless of glycemic control and therapy. It is believed that diabetics should avoid any contact with this toxicant and if they have lead concentrations in their body greater than 80 µg, chelation therapy should be administered.

CONCLUSIONS

From the morphometric point of view, the effect of lead-acetate and alloxan intoxication on Wistar rats infected with escheria by intraperitoneal injection was investigated under laboratory conditions.

Significant changes occurred especially with the synergistic effect of lead-acetate and alloxan with the effect of infection on the control groups. The increase in organosomatic liver index was significant, while the weight gain was significantly reduced in individuals exposed to alloxan treatment.

An explanation for these changes is probably to be found in the fact that hyperglycemia causes tissue degradation due to protein and fat mobilization. Likewise, glucose transport into cells is reduced during diabetes and its deficiency results in gluconeogenesis (lipolysis, proteolysis, and the like), which further leads to a decrease in body weight due to fat and protein burning.

Lead-acetate intoxication also leads to significant changes in the body mass of the experimental individuals, as well as the proportion of individual organs. The two-week lead-acetate intoxication of the experimental animals resulted in a significantly lower mass gain than in the untreated animals. Lead induces a wide range of physiological and biochemical disorders, as well as disorders of the function of various organs in animals and humans. The reason for the increase of some organosomatic indices lies in the fact that lead-acetate on one side accumulates in them, and on the other, leads to a reduced weight gain which increases the proportion of individual organs.

Looking at the overall synergistic effect of the treatments used, one can observe their complex effect reflected in the negative values of body weight gain, as well as the significantly increased mass proportions of individual organs. In particular, infections caused by various bacteria are much more common in diabetics and hyperglycemia can impair both innate and acquired immunity, thereby reducing resistance to pathogenic infections and associated diseases. Hyperglycemia increases oxidative stress, which leads to the development of complications in diabetics. Also, lead from the environment can cause numerous changes in the body of diabetics, so diabetics should avoid any contact with this toxicant.

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