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ORIGINAL ARTICLE

INFLUENCE OF ARSENIC ON SELECTED BIOCHEMICAL BLOOD PARAMETERS IN RATS FED DIET WITH DIFFERENT FAT AND PROTEIN CONTENT

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ABSTRACT

Background. Arsenic is widely distributed in the environment. The main routes of absorption of inorganic arsenic compounds are the lungs and the gastrointestinal tract. Arsenates both (III) and (V) are absorbed from the gastrointestinal tract in 55-95%, while the organic arsenic compounds in 75-85%. Arsenic poisoning leads to damage the activities and morphological changes in the stomach and intestines, causing the occurrence of nausea, vomiting and diarrhoea. Arsenic compounds may also be the cause of the development of certain cancers (lung, skin and liver). The first changes caused by arsenic poisoning usually remain unnoticed. Arsenic affects haematological and both lipid and carbohydrate metabolism. It also causes changes in the organs involved in metabolism, so biochemical parameters or enzymes activity are therefore a good indicator of poisoning changes.

Objective. The aim of this study was to examine the influence of protein and fat content in diet on selected biochemical blood parameters in rats.

Materials and Methods. Rats (11 groups n = 88) were fed with 5 types of diet: control, low-protein, high-protein, low- fat and high-fat. Animals received water without arsenic (control group) or water with 10 or 20 µg As/mL.

Results. In animals fed a low protein diets, regardless of the dose of arsenic, it was a decreasing of total cholesterol, triglycerides, and glucose in serum observed, compared to the control group. In the groups fed with low-protein diet revealed a significantly less damage in the liver as compared to the control group. In animals fed high-protein diets and with varying addition of arsenic a significant higher concentration of various biochemical parameters were found, in comparison to the respective control groups. In animals fed the high protein diet and poisoned with 20 µg As/mL of the arsenic significantly higher liver damage were found, compared to control group.

Conclusions. Symptoms of arsenic hepatotoxicity measured with enzyme activity were highest in the groups of animals fed with low-protein diet. The parameters of lipid and carbohydrate metabolism depended mostly on diet than the dose of arsenic.

Key words: *arsenic*, *biochemical blood parameter, protein, rats*

STRESZCZENIE

Wprowadzenie. Arsen jest pierwiastkiem szeroko rozpowszechnionym w środowisku człowieka. Głównymi drogami wchłaniania nieorganicznych związków arsenu są płuca oraz przewód pokarmowy. Arseniany (III) i arseniany (V) wchłaniają się z przewodu pokarmowego z wysoką wydajnością rzędu 55-95%. Organiczne związki arsenu ulegają wchłanianiu z przewodu pokarmowego z wydajnością 75-85%. Zatrucia związkami arsenu prowadzą do uszkodzenia czynności oraz zmian morfologicznych w żołądku i jelitach, powodują wystąpienie nudności, wymiotów i biegunki. Związki arsenu mogą być również przyczyną rozwoju nowotworów (płuc, skóry i wątroby).

Cel. Celem badań była ocena wpływu zawartości białka i tłuszczu w diecie szczurów na wybrane parametry biochemiczne krwi szczurów zatruwanych arsenem.

Materiał i metody. Szczury (11 grup samców n=88) karmiono 5 rodzajami diet: kontrolną, nisko- i wysokobiałkową oraz nisko- i wysokotłuszczową, oraz zatruwano arsenem w ilości 10 i 20 µg/ml.

Wyniki. U szczurów karmionych dietami niskobiałkowymi, niezależnie od dawki arsenu, stwierdzono tendencję do obniżania się stężenia cholesterolu ogółem, triglicerydów i glukozy w surowicy krwi w porównaniu do grupy kontrolnej. W grupach

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zwierząt, które spożywały dietę niskobiałkową wykazano istotnie statystycznie mniejsze uszkodzenie wątroby w porównaniu do grup kontrolnych. U zwierząt karmionych dietami wysokobiałkowymi oraz z różnym dodatkiem arsenu stwierdzono istotne statystycznie wyższe stężenie poszczególnych parametrów biochemicznych w porównaniu do odpowiednich grup kontrolnych. U zwierząt karmionych dietą wysokobiałkową zatruwanych arsenem w ilości 20 μg/ml wykazano istotnie większe uszkodzenie wątroby w porównaniu z grupą kontrolną.
Wnioski. Objawy hepatotoksyczności arsenu mierzone aktywnością enzymów były największe w grupie zwierząt karmionych dietą niskobiałkową. Na parametry gospodarki lipidowej i węglowodanowej większy wpływ miała zastosowana dieta niż dawka arsenu.

Słowa kluczowe: *arsen, parametry biochemiczne krwi, dieta, szczury*

INTRODUCTION

Arsenic is widely distributed in environment and is one of the most important global environmental toxicants. Short exposure results in acute effects characterized by vomiting, abdominal colic, and diarrhea. In rare cases, these symptoms may lead to death. The long term, regular exposure to arsenic compounds both food and inhalation manifests in skin lesion and disorder in functioning of blood, neural and breathing systems. Investigations prove the negative arsenic influence on reproductive abilities at both sexes. It causes decrease the masses of sexual organs and occurrence tissue disease. There are extensive documentations that among women consuming water containing arsenic increase fetal, neonatal and postnatal mortalities, and elevations in low birth weights, spontaneous abortions, still-birth, pre-eclampsia and congenital malformations [1, 2, 3]. Long term exposure to arsenic has a depressant effect on hematopoietic system; sometimes occur anemia, erythrocyte karyorexis, granulocytopaenia, thrombocytopaenia.

Most of people in the world are characterized by not proper nutrition style. In developing countries usually occurs malnutrition. In developed countries diet is usually high energetic, high proteins and fats, especially animal fats, in diet. Anomalous nutrition can lead to higher organism sensitiveness for all toxic trace elements including arsenic [6].

Protein – energetic malnutrition occurred in developing countries can lead to lower organism ability to arsenic detoxification. In developed countries, in spite of arsenic content in foodstuff is lower, higher intake can cause excessive arsenic intake.

Blood and blood components are widely used for biological monitoring. Changes of biochemical parameters measured in blood can reflect abnormalities in organism functioning caused by both: toxins - included arsenic, and improper diet.

The aim of this study was to examine the influence of protein and fat content in diet on selected biochemical blood parameters in rats.

MATERIAL AND METHODS

A total number of 88 male buffalo rats (body weight 200 - 270 g, age - 6 weeks) were randomly divided into 11 groups (8 animals per each group). Rats were housed in plastic cage (4 per cage) in a 12 h light/dark cycle. Animals were acclimated to laboratory conditions for 1 week prior the start of this study. Rats obtained half--synthetic diet AIN-93 recommended for the laboratory rodents with the modifications.

1. control diet: 14 % protein and 4 % fat,
2. high protein diet: 18 % proteins,
3. low protein diet: 10 % proteins,
4. high fat diet: 6 % fat
5. low fat diet: 2 % of fat.

Five groups of rats received throughout the whole period 10 μg/mL and another five groups of rats 20 μg/mL sodium arsenate dissolved in distilled water. 11-th group was a control group. Water consumption and feed intake was measured every day over a 6-week exposure trial. Animal body weights were measured once a week.

The biological experience in the animal model was approved by the Local Ethical Committee (no 36/2008).

Whole blood was collected from the sinus plexus into a Vacutainer serum separation tube for serum analysis. A heparinized blood sample was obtained for automated hematology analysis and differential whole blood cell counts. Blood samples were analyzed on an automated hematology analyzer ADC Vet previously calibrated for rats to measure hematocrit and hemoglobin.

Contents of glucose, total cholesterol and triglycerides were assayed in blood serum with the use of BioSystems kits. Additional assays were performed for activities of hepatic enzymes: alanine aminotransferase (ALAT) and aspartate aminotransferase (ASPAT) with the kinetic method using pyridoxal phosphate and NADH. ALAT catalyzes the transfer of the amino groups from alanine to 2–oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase coupled reaction. ASPAT catalyzes the transfer of the amino groups from aspirate to 2-oxoglutarate, forming oxa-

Table 1. Feed intake and body weight gains in rats

Groups of the rats		Feed intake [g/day]	Water intake [g/day]	Weight gains [g/6 week]
Diet	Arsenic dose [mg/kg]	mean± SD	mean± SD	mean± SD
Control diet (n=8)	0	20.48 ± 2.20	26.35±2.78	151.67±6.09
Control diet (n=8)	10	16.47 ± 2.25	29.50±2.91	134.29±2.81
Low protein diet (n=8)	10	16.90 ± 2.45	34.08±3.70	81.90±3.02
High protein diet (n=8)	10	16.10 ± 2.68	35.74±2.33	87.5±9.86
Low fat diet (n=8)	10	17.67 ± 2.67	26.96±3.84	87.14±7.19
High fat diet (n=8)	10	20.7 ± 2.38	31.96±2.55	131.43±17.48
Control diet (n=8)	20	15.84±2.38	28.48±3.64	136.25±19.14
Low protein diet (n=8)	20	16.90±2.37	33.42±2.38	72.50±8.50
High protein diet (n=8)	20	15.66±2.72	34.66±5.27	95.00±15.26
Low fat diet (n=8)	20	20.13±2.37	25.70±3.61	142.86±7.56
High fat diet (n=8)	20	20.17± 2.90	27.30±2.74	77.14± 9.23

lacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase coupled reaction.

To determine differences between mean contents of the biochemical parameters depending on the protein and fat content and the dose of arsenic the statistical evaluation were done using the *Duncan's* test [17].

RESULTS

Results of mean feed intake and body weight gains were shown in Table 1 whereas biochemical parameters, i.e. hematocrit, hemoglobin, glucose, total cholesterol (TC), and triglycerides (TGC) determined in blood serum were shown in Table 2.

The highest body weight gain were observed in control group (151.67 g). In the other groups body weight gain were 6 to 53 % lower than in control diet. The lowest body weight gain was observed in group of rats fed with low protein diet and poisoned with 20 µg/mL of arsenic. Mean feed consumption ranged from 15.66 g in group of rats fed with high protein diet and poisoned with 20 µg As/mL to 20.70 g in group of rats fed with high fat diet and water with 10 µg As/mL. Mean water consumption ranged from 26.35 g in control group to 35.74 g in rats fed with high protein diet and poisoned with 10 µg As/mL.

There were no significant influence of arsenic or diet on Hematocrit and Hemoglobin observed.

The mean serum glucose concentrations ranged from 101.55 – 151.36 mg/dL. Lowest concentrations of glucose were in group of rats fed high protein diet, irrespective of arsenic level in drinking water. The highest concentration of glucose was observed in group fed with low protein diet and poisoned with 20 µg As/mL (Table 3).

The mean concentration of total cholesterol ranged from 46.71-153.44 mg/dl. Highest amount of cholesterol were found in group of rats fed with high protein diet.

In groups of rats fed with high protein diet, irrespective of contents of the arsenic, the concentration of triglycerides in the blood serum was high and it averaged 95.95 mg/dL. A highest concentration of triglycerides

Table 2**.** Hematocrit, hemoglobin, glucose, total cholesterol (TC) and triglycerides (TGC) in blood serum

Groups of the rats		Hematocrit [%]	Hemoglobin [mmol/L]	Glucose [mg/dL]	Total cholesterol (TC) [mg/dL]	Triglycerides (TGC) [mg/dL]
Diet	Arsenic dose [µg/ml]	mean± SD	mean± SD	mean± SD	mean± SD	mean± SD
Control (n=8)	0	40.5 ± 0.7^{a}	9.1 ± 0.2^{a}	147.40 ± 33.26^{bc}	97.85 ± 22.68^{b}	127.84 ± 24.46^{a}
Control (n=8)	10	39.8 ± 0.8^{a}	9.0 ± 0.2^{a}	143.81 ± 32.69^{bc}	99.41 ± 12.92^{b}	254.48 ± 22.48^{b}
Low protein (n=8)	10	39.3 ± 0.8^{a}	8.8 ± 0.2^{a}	139.01 ± 23.66^{bc}	47.72 ± 14.41^{ab}	59.67 ± 16.93^{a}
High protein (n=8)	10	39.7 ± 1.0^{a}	8.9 ± 0.3^{a}	101.55 ± 2.98^{a}	153.44 ± 14.87^{c}	99.23 ± 31.88^{a}
Low fat (n=8)	10	39.1 ± 0.5^{a}	7.6 ± 3.4^{a}	118..22 ± 46.79^{ab}	46.71 ± 10.12^{ab}	65.27 ± 60.99^{a}
High fat (n=8)	10	42.1 ± 1.8^{a}	9.4 ± 0.3^{a}	103.07 ± 25.00^{a}	57.14 ± 22.13^{ab}	39.07 ± 30.25^{a}
Control (n=8)	20	41.0 ± 1.1^{a}	9.1 ± 0.3^{a}	108.03 ± 3.73^{ab}	67.47 ± 7.79^{ab}	285.38 ± 95.33^{b}
Low protein (n=8)	20	40.3 ± 1.0^{a}	8.8 ± 0.1^{a}	151.36 ± 30.80^{c}	48.10 ± 20.69^{ab}	51.09 ± 30.37^{a}
High protein (n=8)	20	41.7 ± 1.6^{a}	9.2 ± 0.3^{a}	102.55 ± 3.73^{a}	135.36 ± 17.06^{c}	92.67 ± 19.25^{a}
Low fat (n=8)	20	39.6 ± 1.2^{a}	8.9 ± 0.2^{a}	102.63 ± 28.70^{a}	40.59 ± 6.90^{ab}	31.61 ± 19.59^{a}
High fat (n=8)	20	40.9 ± 1.1^{a}	9.2 ± 0.2^{a}	117.06 ± 25.41^{ab}	56.38 ± 7.54^{ab}	41.40 ± 25.33^{a}

$^{a, b, c}$ – homogenous groups, $p<0,05$

Table 3. Activities of ASPAT and ALAT in blood serum of rats

Groups of the rats		Asparagine aminotransferase (ASPAT) [U/l]	Alanine aminotransferase (ALAT) [U/l]
Diet	Arsenic dose [μg/ml]	mean± SD	mean± SD
Control (n=8)	0	14.22 ± 13.13[a]	7.78 ± 7.40[a]
Control (n=8)	10	9.44 ± 7.90[a]	3.81 ± 3.07[a]
Low protein (n=8)	10	8.89 ± 3.97[a]	11.48 ± 4.54[a]
High protein (n=8)	10	15.37 ± 8.71[a]	10.42 ± 8.08[a]
Low fat (n=8)	10	9.55 ± 8.31[a]	7.96 ± 5.27[a]
High fat (n=8)	10	8.89 ± 7.84[a]	4.44 ± 2.57[a]
Control (n=8)	20	12.44 ± 9.13[a]	5.55 ± 3.14[a]
Low protein (n=8)	20	15.37 ± 8.71[a]	10.42 ± 8.08[a]
High protein (n=8)	20	10.69 ± 7.79[a]	4.89 ± 4.60[a]
Low fat (n=8)	20	12.66 ± 12.13[a]	8.41 ± 6.47[a]
High fat (n=8)	20	8.67 ± 6.80[a]	7.94 ± 5.79[a]

[a, b, c] – homogenous groups, $p<0,05$

was found in rats fed with control diet and poisoned with both of arsenic doses.

Results of activities of hepatic enzymes (ALAT and ASPAT) were shown in Table 3. ALAT and ASPAT are found in serum and in various body tissues, but it is most commonly associated with the liver. Elevated levels of ALAT and ASPAT often suggest the liver damages as viral hepatitis, biliary duct problems, infectious mononucleosis, or myopathy. For this reason, ALAT and ASPAT are commonly used as a way of screening for liver dysfunctions. In that study there were no statistically important differences between levels both ALAT and ASPAT in blood observed.

DISCUSSION

This study were taken to examine the influence of protein and fat content in diet and dose of arsenic in drinking water on glucose level, lipid metabolism and ALAT, ASPAT activity.

During experiment the lowest body weight increases in low fat and low protein diet were observed. This might be caused by inadequate amount of those macronutrients and additionally this might be intensified by arsenic poisoning. High protein diet also caused low body weight increase. Probably because of inducing satiety, faster metabolism and lower feed consumption.

Arsenic has a influence on hematopoietic system, hematocrit and hemoglobin values can be a marker of anemia with subsequent result of inhibition of erythropoesis in the hemopoietic system. In present studies there were no changes in those parameters observed. *Savabieasfahani* et al. [15] determined similar value of those parameters in blood of rats exposed to 0, 5, or 10 ppm sodium arsenite in drinking water for 6 weeks. Also *Dwivedi* and *Flora* [4] after 56 days arsenic treatment did not observed changes in blond parameters in rats poisoned with arsenic. *Garcia* et al. [5] observed a decreased number of red blood cells, hemoglobin concentration and hematocrit value but in rats' pups poisoned with arsenic. They also observed that arsenic intoxication produced a significant increase in cholesterol (+34%), triglycerides (+52%), what is consistent with the results obtained in this paper. Also *Wang* et al. [18] showed that different level of arsenic in diet causes decrease of total cholesterol and triglycerides level. The same tendency was observed in current study for both high fat and protein diet. The highest amount of cholesterol in high protein diet groups could be caused by higher homocysteine generation.

That is known that chronic arsenic poisoning increase prevalence of diabetes mellitus [13]. Even at low – dose levels of arsenic damage intracellular hormonal carbohydrate regulatory system. In present study low protein diet intensified that process and high protein diet protected organism from arsenic-induced diabetes mellitus.

Liver is the target organ of arsenic toxicity and the leakage of hepatic enzymes such as ALAT, ASPAT are commonly used as an indirect biochemical index of hepatocellular damage. In this studies the highest activities of ALAT, ASPAT were found in low protein diet. *Nandi* et al. [12] showed that levels of ALAT increased during the experiment but statistically substantial differences were observed after 8 weeks. The level of ASPAT did not change during the experiment. *Messarah* et al. [11] found higher enzymes activities in rats poisoned with arsenic, they had also shown that selenium could reduce arsenic hepatotoxicity.

Based on this study the lowest changes in blood parameters were observed in rats fed with control diet. Among rats fed with high protein diet the highest level of cholesterol were found. Low protein diet caused deterioration of glucose profile. These results also show that higher dose of arsenic among rats which were given control or low fat diet reduced concentration of glucose.

Few literature data shows the influence of the arsenic for biochemical parameters but there are no data on the interaction of anomalous nutrition with arsenic toxicity, and a role of macronutrient in arsenic toxicity.

CONCLUSIONS

1. Symptoms of arsenic hepatotoxicity measured with enzyme activity were highest in the groups of animals fed with low-protein diet.
2. The parameters of lipid and carbohydrate metabolism depended mostly on the kind of diet than the dose of arsenic.

Conflict of interest

The authors declare no conflict of interest.

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