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The Effect of Drinking Water Quality on the Morphological and Biochemical Characteristics of Blood and Organs of Female White Rats

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Abstract: Numerous experimental studies have proven by now the action of chemical composition of water, especially in the vicinity of threshold and subthreshold levels. However, the effect of waters of the Mordovia Republic on the blood parameters and morphology of animal and human internal organs has not been studied. We performed a comparative study of the effects of tap water of the city of Saransk coming from artesian wells and water with balanced ion composition on the blood parameters and internal organ morphology of non-pregnant and pregnant female white rats. This was done using an Axio Imager.M2 digital microscope with AxioVision SE64 Rel. 4.8.3 and ZEN 2011 software for image analysis. Here it was shown that tap water of Saransk, which contains increased quantities of iron, calcium, magnesium and fluoride ions, has no adverse influence on the organs non-pregnant female animals but causes considerable changes at the biochemical, histological and cytological levels in pregnant animals.

Key words: Water · Ionic composition of water · Balanced ion contents · Water with increased salinity · Blood · Intestine · Kidneys

INTRODUCTION

The composition of the external environment is undoubtedly important for the normal vital activity. A deficiency or surplus of particular elements in the environment may have a pronounced effect on the vital activity of an organism, which depends on the chemical composition of water, air and food because all chemical elements enter the body mainly with vegetable and animal food and drinking water [1-3].

Providing people with high-quality drinking water is currently a global problem because water quality and quantity affect the health level of people and the sanitary and epidemiological welfare of population as a whole [4].

The quality of water becomes especially important during pregnancy. The more pure the water, the more substances it can dissolve and deliver to the fetus, thus creating normal conditions for the formation of a new organism [6].

Pathologies in pregnant women and the progenies were found to be highly correlated with chemical water pollutants, especially in the case of central water supply [7].

Kidneys, which are the key excretory organs maintaining the water and salt balance of the body [7, 8] and the small intestine, which is directly contacting with water, are the first to respond to a change in the composition of consumed water [9].

The water supply of the population of the Republic of Mordovia relies on subsurface water-bearing beds, which are well protected from external pollution. The artesian drinking water is epidemiologically safe and has a good taste; however, as regards the chemical composition, it has increased salinity. Increased contents of fluorine and iron and relatively high total hardness were found for water from artesian wells of Saransk [10].

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The effect of drinking water produced from the artesian wells of the city of Saransk, Republic of Mordovia, on the blood parameters or morphology of organs has not yet been studied. Therefore, it appeared pertinent to study the responsiveness of tissues and organs of laboratory animals to the action of an important environmental factor, i.e., drinking water.

MATERIALS AND METHODS

White outbred mature female rats weighing 180–200 g were used as biological test subjects. Altogether 240 animals were used. According to the set tasks, the animals were divided into groups.

The first control group comprised non-pregnant female rats and the second control group comprised pregnant female rats. Both control groups were given bottled non-sparkling drinking water with balanced contents of micro and macro elements according to the requirements of Sanitary Regulations and Standards (SanPiNs). The first test group was composed of non-pregnant rats and the second test group included pregnant rats. The test groups of animals were given drinking water with increased contents of iron, calcium, magnesium and fluoride ions from the central water supply system of Saransk.

The experiment was performed in summer and autumn, indoors, at a temperature of 22–25°C and a relative humidity of 67–70%. The animals were maintained under usual vivarium conditions and had free access to food and water. The pregnancy was ascertained by detecting mating of a female rat that was in estrus. The animals were considered to be pregnant from the morning of the day in which spermatozoa were detected in the vaginal contents. On the 21st day of the experiment, the animals of each group were decapitated under ether/chloroform narcosis with observance of the humane principles outlined in the European Union Directives (86/609/EEC) and the Declaration of Helsinki and in accordance with the Guidelines for Research Involving Experimental Animals.

The subjects examined included blood, kidneys and empty intestine (jejunum) of white rats.

The red and white blood cell counts, the hemoglobin level and the erythrocyte sedimentation rate (ESR) were determined in the blood of animals by morphological methods. The total levels of protein, creatinine and urea were determined by biochemical methods in the blood serum.

The morphology of internal organs of animals was studied on 10×10 mm pieces of a kidney and jejunum. For the histological investigation, the material was fixed in a 10% solution of neutral formalin. The fixed samples were washed with running water, dehydrated by placing into alcohols with increasing concentration and embedded into paraffin by the common procedure. Histological section with thickness of 7–10 µm were prepared and stained by hematoxylin-eosin and by the van Gieson's method.

The jejunum as a part of the small intestine was examined to determine the thicknesses of the whole intestinal wall, mucous membrane, muscular layer and serous coat; the height and the width of villi; the crypt depth and width; the area of enterocytes and the nuclear area of enterocytes in the villi and crypts; the number and the area of goblet cells; and the area of Paneth cells.

The kidney was examined to determine the area of renal corpuscle and glomerulus, epithelial cells and their nuclei, the diameter of the proximal convoluted tubule (PCT) and the diameter of the tubular lumen. The cyto- and morphometry of the kidneys and intestines of white rats were performed using an Axio Imager.M2 digital microscope with AxioVision SE64 Rel. 4.8.3 and ZEN 2011 software for image analysis.

The statistical treatment of the obtained data was performed using FStat and Excel software. The statistical tests were performed according to the Student t-test with the significance value $p \leq 0.05$.

RESULTS

According to the studies, the blood of pregnant animals (C2) that consumed ion-balanced water contained 12.8% higher red blood cell count, 29.6% higher white blood cell count ($P \leq 0.05$) and 13.3% higher ESR as compared with non-pregnant female rats (C1). In all probability, this was the physiological response of the body to stimulation of metabolism during pregnancy. The decrease in the total protein level by 13.3% and the decrease in the blood serum creatinine level by 37.5% ($P \leq 0.05$) in pregnant animals is due to an increase in the blood volume, enhancement of the renal plasma flow and filtration (Table 1).

The blood of pregnant white rats (T2) affected by tap water with increased contents of iron, calcium and magnesium ions had 20.4% higher red blood cell count, 46.0 % higher white blood cell count, 11.7% higher hemoglobin level and much higher (by 275%) ESR

Table 1: Blood characteristics of white rats upon consumption of ion-balanced water and high-iron, -magnesium and -fluoride tap water

Characteristics	Control 1	Test 1	Control 2	Test 2
Total red blood cell count ($\times 10^{12}$/L)	4.52±0.01	4.90±0.08	5.10±1.06	5.90±0.51*
Total white blood cell count ($\times 10^{9}$/L)	5.90±0.30	6.10±0.05	7.65±0.55$^{\Delta}$	8.91±0.94*
Hemoglobin level (g/L)	101.00±9.16	110.02±9.28	110.00±10.80	123.00±9.90*
ESR (mm/h)	1.50±0.08	2.00±0.01*	1.70±0.50	5.50±0.94**
Total protein level (g/L)	39.00±3.28	36.80±3.29	34.50±3.05	32.20±2.71
Urea level (mmol/L)	5.10±0.76	5.60±0.15	5.05±0.50	6.00±0.09
Creatinine level (mol/L)	0.08±0.008	0.09±0.002	0.05±0.001$^{\Delta}$	0.11±0.043*

Note: the asterisk * denotes statistically significant differences between the control and test groups (*– $P \le 0.05$, ** – $P \le 0.01$); $^{\Delta}$ indicates the statistically significant differences between the control groups ($^{\Delta}$– $P \le 0.05$, $^{\Delta\Delta}$ – $P \le 0.01$).

compared with the non-pregnant animals (T1). Simultaneously, the total protein level decreased by 12.5% and creatinine level increased by 22.2%. The observed changes of the blood in test animals indicate that water with higher hardness affects the body responsiveness of pregnant female rats.

The decrease in the total blood protein level may be related to both kidney dysfunction upon exposure to water with increased hardness and partial dilution caused by water retention in the body.

The increase in the biochemical characteristics such as the blood serum creatinine level (by 12.5%) and urea level in pregnant animals that were given tap water also attests to kidney dysfunction and may be regarded as an early symptom of renal disease.

Comparative study of the blood of non-pregnant rats (C1) consuming ion-balanced water and non-pregnant rats (T1) that were given tap water demonstrated a statistically significant increase only for the ESR. Thus, tap water does not has a marked influence on the blood parameters of non-pregnant rats and is harmless for them.

Meanwhile, the blood parameters of pregnant rats (T2) that consumed tap water differ from the parameters found for non-pregnant animals (T1) that were given ion-balanced water: statistically significant increase was observed for the red blood cell count (by 15.6%), white blood cell count (by 16.4%), hemoglobin level (by 11.8%), ESR (by 323.5%), urea level (by 18.8%) and creatinine level (by 220.0%). Thus, on long use, tap water has a non-specific adverse effect on the blood parameters of pregnant rats.

The morphological and cytometric investigations demonstrated that the most pronounced changes in the intestine and kidneys develop when animals consume the tap water of the city of Saransk characterized with increased contents of iron, calcium, magnesium and fluoride ions.

Upon exposure to tap water, in the intestine of non-pregnant white rats (T1), the height of the intestinal villi increased by 6.85%, the thickness of the villi increased by 12.21%, the crypt depth became greater by 8.57% and the crypt width increased by 29.72% with respect to the control group (C1) ($P \le 0.05$) (Table 2).

The cyto- and morphometric analysis of the intestinal wall for this group of animals (T1) demonstrated that the area of villus enterocytes increased with respect to the control (C1) by 16.87% ($P \le 0.05$). A statistically significant increase was observed for the nuclear area of villus enterocytes (by 18.43%) ($P \le 0.05$) and the nuclear area of crypt enterocytes (by 27.84%) ($P \le 0.05$) (Table 2). Also it was found that the action of the Saransk tap water (T1) with increased contents of iron, calcium, magnesium and fluoride ions resulted in 50.14% greater amount of goblet cells in the intestine of this group of animals with respect to the control group (C1) ($P \le 0.05$).

The study showed that in pregnant rats that consumed tap water of the city of Saransk (T2), the intestinal wall thickness increased by 40.6% compared with that of the pregnant rats that consumed drinking water (C2) ($P \le 0.05$) mainly at the expense of the mucous membrane; simultaneously, the crypt depth and width increased by 22.14% ($P \le 0.05$) and 22.51% ($P \le 0.05$), respectively.

Comparative analysis of the morphological and morphometric changes showed that the most pronounced changes in kidneys were observed for pregnant rats consuming tap water (T2) versus pregnant rats consuming drinking water (C2). These changes include decrease in the number and increase in the inhomogeneity of renal corpuscles; increase in the average area of a renal corpuscle by 19.54% ($P \le 0.05$) and in the area of a glomerulus by 21.16% ($P \le 0.05$); increase in the diameter of the proximal convoluted tubule by 7.28% and narrowing down of the tubular lumen by 12.69%; the area of epithelial cells increased simultaneously by 13.71%.

Table 2: Morpho- and cytometric characteristics of the jejunum wall of pregnant and non-pregnant white rats upon consumption of ion-balanced water and high-iron, -calcium, -magnesium and -fluoride tap water

Characteristics	Control 1	Test 1	Control 2	Test 2
Villus height, µm	288.98±20.20	308.78±26.28	264.15±25.18	263.72±21.24*
Villus width, µm	77.68±6.78	87.17±5.43	81.34±7.05	93.75±8.22*
Crypt depth, µm	261.57±23.47	284.00±24.09	248.19±20.18$^{\Delta}$	303.10±23.18*
Crypt width, µm	38.25±3.09	49.62±4.84*	39.98±3.06	48.98±3.76*
Intestinal wall thickness, µm	684.45±60.53	887.43±80.71*	681.19±60.45	957.91±76.57*
Mucous membrane thickness, µm	542.28±53.43	780.25±70.77*	546.87±54.38	798.28±75.09*
Muscular layer thickness, µm	150.64±16.66	124.45±10.11*	152.36±17.61	113.45±9.51*
Serous coat thickness, µm	15.17±4.60	16.15±1.08*	15.56±2.19	17.05±0.61*
Area of villus enterocytes, µm^2	164.15±12.02	178.48±13.13	185.44±11.01	187.00±10.08
Area of crypt enterocytes, µm^2	166.37±10.2	184.62±12.43*	188.87±10.06	190.49±10.03
Area of goblet cells, µm^2	73.15±2.56	72.38±5.30	72.19±5.44	69.38±5.34
Number of goblet cells in a villus	18.25±3.11	27.40±2.63*	19.76±3.98	28.10±2.03*
Nuclear area of the villus enterocytes, µm^2	39.48±1.15	46.76±2.03*	41.06±4.34	49.16±2.89*
N/C ratio of villus enterocytes	3.15	2.9	3.59	2.8
Nuclear area of the crypt enterocytes, µm^2	24.68±1.91	31.55±2.57*	25.72±1.41	27.18±0.43*
N/C ratio of crypt enterocytes	5.73	4.85	6.34	6.02
Area of Paneth cells, µm^2	158.07±12.6	163.99±9.10	162.4±12.07	162.19±11.00

Note: * $P \leq 0.05$ compared with the control group animals; the asterisk * denotes statistically significant differences between the control and test groups (*– $P \leq 0.05$, ** – $P \leq 0.01$); $^{\Delta}$ indicates the statistically significant differences between the control groups ($^{\Delta}$– $P \leq 0.05$, $^{\Delta\Delta}$ – $P \leq 0.01$).

Table 3: Morphometry of kidneys of white rats that consumed ion-balanced water and high-iron, -calcium, -magnesium and -fluoride tap water

Characteristics	Control 1	Test 1	Control 2	Test 2
The number of corpuscles per field of view, items	10.25±1.18	9.74±1.06	9.80±1.17	8.90±1.01*
Renal corpuscle area, µm^2	5849.03±193.65	6623.66±180.20	6689.48±321.00*	7996.85±469.68*
Kidney glomerulus area, µm^2	4079.71±159.16	4494.26±154.52	4375.05±149.50	5300.44±148.25 *
PCT diameter, µm	32.28±2.60	33.07±1.81	33.09±2.65	35.50±2.87*
Diameter of the tubular lumen, µm.	11.93±1.52	12.01±0.30	12.45±0.57	10.87±0.49*
Epithelial cell area, µm^2.	109.46±2.26	113.82±3.30	117.47±3.27	133.58±4.90
Nuclear area of the epithelial cell, µm^2.	31.15±2.89	31.97±2.08$^{\Delta}$	30.18±2.70	27.32±1.99
N/C ratio of the epithelial cell	2.51	2.56	2.89	3.88

Note: N/C ratio is the nuclear-cytoplasmic ratio; the asterisk * denotes statistically significant differences between the control and test groups (*– $P \leq 0,05$, ** – $P \leq 0,01$); $^{\Delta}$ indicates the statistically significant differences between the control groups ($^{\Delta}$– $P \leq 0.05$, $^{\Delta\Delta}$ – $P \leq 0.01$).

CONCLUSION

The whole body of data obtained during the experimental study attests to active participation of blood, small intestine and kidneys in the homeostasing of the internal environment of the body upon consumption of water with increased contents of iron, calcium, magnesium and fluoride ions, this participation involving an intricate rearrangement of morphofunctional parameters and their components at the tissue and cellular levels of organization.

The water of the city of Saransk has no statistically significant influence on the blood parameters of non-pregnant rats and can be recommended for test animals.

The consumption of this water by non-pregnant rats induced increase in the crypt width and the nuclear areas of crypt and villus epithelial cells. In the pregnant rats that were given the Saransk tap water, the intestinal wall width and the crypt depth and width increased with respect to the pregnant rats that consumed ion-balanced water.

The observed histological and morphometric changes in the small intestine of white rats upon consumption of the Saransk tap water with increased contents of iron, calcium, magnesium and fluoride ions can be classified as adaptive response at the tissue and cellular levels.

When both pregnant and non-pregnant rats consume ion-balanced water, no statistically significant differences are detected in histological, cytochemical, or morphometric state of kidneys. The most pronounced changes in the kidneys are formed when pregnant rats consume tap water of Saransk, in particular, the number of renal corpuscles decreases and they become inhomogeneous; the average size of renal corpuscle and the area of the glomerulus increase.

Resume: Thus, the study showed that the tap water of the city of Saransk characterized by increased contents of iron, calcium, magnesium and fluoride ions is not a strong stress factor for non-pregnant rats. It can rather be classified as a "low-intensity factor", long-term exposure to which does not induce any qualitative changes at the cellular or tissue level provided that the subject organism is in the optimal condition. However, the female animals that are subjected to additional load caused by pregnancy suffer from more significant adverse effect of the high-iron, calcium, magnesium and fluoride water.

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