EFFECT OF ALKALINE WATER WITH A pH OF 8.5 ON WEIGHT AND MAIN BLOOD INDICATORS IN RATS

Sorin Marian MÂRZA*, Silvana POPESCU, Mariana TĂTARU, Radu LĂCĂTUŞ, Robert Cristian PURDOIU,Vlad Emil LUCA, Ionel PAPUC

Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

Corresponding author: sorinmarza@yahoo.com

Abstract. Water intended for human consumption called drinking water must have a neutral pH, that is a pH value of 7. Below this pH value, the water is considered acidic; above this value it becomes alkaline. The pH value range tolerated by the human body is between 6.5 and 9. Alkaline water with pH values between 8.0 and 9.5 is considered one of the best types of drinking water due to its hydrating, detoxifying, and antioxidant effect. This study aimed to follow the biological effect of alkaline water consumption on body weight, blood glucose, and some blood biochemical parameters in a population of 20 rats. Rats were divided into two equal groups, a control group, and an experimental group. Both groups of rats were given 300 ml of water 3 times a day for 40 days. The rats in the control group received tap water from the mains and the rats in the experimental group received alkaline water with a pH of 8.5. Analysis of the physicochemical parameters of the two types of water revealed that their values do not exceed the maximum allowed according to the drinking water quality standards, except for the chloride values and the temporary hardness in alkaline water. The values obtained were analyzed and statistically interpreted and showed that consumption of alkaline water with pH 8.5 does not affect usual water intake, body mass values, and biochemical parameters; it does not produce systemic adverse effects, having a hypoglycaemic, antioxidant, and immunostimulatory effect.

Keywords: water, alkaline, rats, weight, blood test

INTRODUCTION

Water is the environment in which most metabolic processes in the body take place. It forms the basis for all body fluids and provides for the transport and absorption of nutrients, as well as the removal of wastes and toxins from the body (Ramsay, 1998). At the same time, water regulates body temperature, ensures the proper functioning of cells, tissues, and organs supports the DNA helix and is the body's main source of energy (Popkin et al., 2010). Water is arguably the most important nutrient and the only one whose absence can be lethal in a few days, and knowing the daily water intake is extremely important. The positive and negative effects of water in the short and long term are well known in the literature. What is lacking is a systematic measurement of total water intake relative to total fluid intake (Jequier and Constant, 2010; Ramsay, 1998). Alkaline water is commercially available under various names and is mainly recommended for supplementing electrolyte loss during excessive sweating. Some animal model studies show that alkaline water supplementation has positive effects on body weight and improves reproductive function in mice (Watanabe and Shirai, 1990; Watanabe, 1995). Consumption of alkaline water can cause an increase in metabolic activity, and due to hyperkalemia, pathological changes in myocardial muscle were observed in rats after 15 weeks (Watanabe et al., 1997).

Alkaline water due to its molecular restructuring and hexagonal structure is better absorbed by the body, which ensures better hydration and also has a suppressive effect on free radical levels in living organisms, leading to disease prevention (Hanaoka et al., 2004). Research in the literature highlights certain benefits of alkaline water in laboratory animals, such as anti-diabetic and antioxidant activity (Jin et al., 2006), DNA-protective (Shirahata et al., 1997), growth-promoting (Watanabe, 1995) and life-extension (Magro et al., 2016). Today there is a multitude of companies on the market offering alkaline water for sale and presenting the health benefits of this type of water. There are a wide variety of waters on the market with a pH above 7 and even some with a pH above 8. Like any water, drinking alkaline water ensures that the body functions properly by hydrating it properly, but it is important to know that everybody has a different pH level, so alkalinization does not start at the same value for everyone. Alkaline water is water that simultaneously produces two positive effects: it strongly alkalinizes water (constant pH above 9) and acts against free radicals through its oxidation-reduction potential (ORP), and its magnesium content ensures the proper functioning of the brain and cardiovascular system (Hanaoka et al., 2004).

WORKING HYPOTHESIS: The consumption of alkaline water with pH 8.5 has beneficial effects on body weight, blood glucose, ionogram, and some blood biochemical parameters in rats. This study aimed to follow the biological effect of drinking alkaline water with pH 8.5 on body weight, blood glucose, ionogram, and some blood biochemical parameters in a group of rats, compared to another group of rats given tap water.

MATERIALS AND METHODS

The biological material used for the experiment was 20 adult female laboratory rats weighing 200-250g, Muridae family, Wistar-Lewis line (Brown, 1985). The animals were purchased from the Experimental Medicine Centre of the Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, and then transferred and housed at the Reproduction Unit and Use of Laboratory Animals of the Faculty of Veterinary Medicine, Cluj-Napoca, Romania, where the experiment was conducted. The rats in the study were divided into two groups: an experimental group and a control group. All rats were provided with standard maintenance, feeding, and watering conditions, with a temperature of 23°C, humidity cycles of 55%, and light/dark cycles of 12 hours according to ISO 10993-2. They were fed standard pelleted rodent food and drinking water was administered 3 times daily. The experimental group received alkaline water with a pH of 8.5, and the control group received tap water. The experiment was approved by the Bioethics Committee of the University of Agricultural Sciences and Veterinary Medicine Cluj Napoca no. 115/26.06.2018 and authorized by the Sanitary-Veterinary and Food Safety Directorate, Cluj-Napoca by Project Authorization no. 130/05.07.2018.

Working materials. The following working materials were used to carry out the experiment: a Berzelius beaker, graduated cylinders, a glucometer, a scale, and a pH meter, alkaline water with pH 8.

Experimental protocol. The experiment was conducted over a period of 40 days under identical experimental conditions for both groups, except for the type of water administered. The rats in both groups received 300 ml of water 3 times/day, which was left in glass dishes. Alkaline water with pH 8.5 was administered to the experimental group immediately after production, and tap water from the public network administered to the control group followed the same protocol. In order to assess the amount of water consumed, after 2 hours of being available, 3 times/day to the rats, the amount of unconsumed water was measured. On the 7th, 14th, 21st, and 40th day of the experiment using the glucometer the blood glucose of each rat was determined, both in the control and experimental groups. On days 1, 5, 15, 21, and 40 of the experiment, each rat was weighed. On the last day of the experiment, on the 40th day, blood was collected from each rat for biochemical examination.

Before starting the experiment, the physicochemical parameters of alkaline water with pH 8.5 and tap water used in the experiment were analyzed. In Romania, tap water is defined and regulated by Law no. 458 of 8 July 2002 - on the quality of tap water, supplemented and amended by Law no. 311 of 28 June 2004. At the EU level, drinking water is regulated by Directive 98/83/EC on the quality of water intended for human consumption.

Working methods. The precision electronic laboratory scale-AM 3000g x 0.1g was used to determine the body weight of rats; the GlucoSure STAR glucometer was used for blood glucose determination, and determination of biochemical parameters ALAT, ASAT, GGT, Protein, Albumin, Urea, Creatinine, Cholesterol, TGL, HDL Cholesterol, Calcium, Magnesium, Phosphorus, Na+, K+, Cl+ UV-VIS screen Master touch spectrophotometer with commercial kits from AMEDA AMP Diagnostics, Austria was used. Normal values are own reference values of the Biochemistry-Hematology Laboratory of FVM Cluj-Napoca. To determine the amount of water consumed and the degree of hydration of the rats, the amount of water not consumed was measured 3 times/day after every 2 hours that water was available and the rats were weighed daily. For statistical analysis and interpretation of the results regarding to changes in body mass, blood glucose, blood biochemical constituents, and haemolucogram in rats, the distribution of values for each variable was performed using the Shapiro-Wilk test ($p>0.05$). The Independent Sample t-test was used to compare the two groups of rats, the control group and the experimental group.

RESULTS AND DISCUSSIONS

Examination of the physicochemical parameters of the two types of water was carried out using the HI83300 photocolorimeter and the results obtained are shown in Table 1.

The analysis of the physicochemical parameters of the two types of water shows that their values do not exceed the maximum allowed, except for chloride values and temporary hardness in alkaline water. The values of ammonia, nitrogen, ammonium hydroxide, sodium nitrate, nitrate, and nitrate in alkaline water are lower compared to those of drinking water, and oxidability, residual chlorine, and electrical conductivity are higher. The results obtained show a significant increase of 1850 mg/l of chlorides in alkaline water whose value was 2100 mg/l compared to drinking water from the network whose value was 20 mg/l. The maximum permitted value for drinking water is 250 mg/l. Also, the temporary hardness of alkaline water was 15.12 German degrees, exceeding the hardness of drinking water by 13.06 German degrees. The maximum permitted value for drinking water is 10 German degrees. The total hardness (TDS) has a very high value for alkaline water, being 1533 ppm compared to tap water from the public network, whose value was 3 ppm, compared to the maximum permitted value of 2000 ppm.

Table 1

Physicochemical examination of water

Parameters studied	Alkaline water pH 8,5	Tap water from the public network	Standard
Ammonia (mg/l)	0,00	0,08	Maximum 0,50 (mg/l)
NH3-N (mg/l)	0,00	0,07	-
NH4 (mg/l)	0,00	0,09	-
Nitrites (mg/l)	0,06	0,06	Maximum 0,50 (mg/l)
NO2-N (mg/l)	0,02	0,02	-
NaNO2 (mg/l)	0,10	0,09	-
Nitrates (mg/l)	0,90	2,20	Maximum 50,0 (mg/l)
NO3-N (mg/l)	0,20	0,50	-
Sulphates (mg/l)	0,00	0,00	Maximum 250 (mg/l)
Iron (mg/l)	0,00	0,00	Maximum 0.2 (mg/l)
pH	8,50	7,11	6,5-9,5
Chlorides (mg/l)	2100*	20,0	Maximum 250 (mg/l)
Temporary hardness (German degrees)	15,12*	1,96	10 German degrees
Oxidability (O2/I)	3,51	1,52	Maximum 5 mg/l
Residual chlorine (mg/l)	0,20	0,10	Maximum 0.5 (mg/l)
Electrical conductivity (µS/cm)	3066	6,00	Maximum 4000 µs
Total hardness (TDS)	1533	3,00	Maximum 2000 ppm

The temporary hardness of water is given by the content of calcium hydro carbonate and magnesium hydro carbonate, and the permanent hardness is given by the content of calcium and magnesium salts (Harvey, 2004). The hardness of water has a positive influence on cardiovascular pathology, hard water being considered a protective factor. Recent studies show that not hardness is beneficial, but Ca, Mg whose compounds are the major determinant of hardness (Gonța et al., 1998; Prodan, 2007).

Chlorines are chemical compounds in which one or more chlorine atoms are covalently bonded. They are very soluble in water, especially sodium chloride.

Chlorides are essential electrolytes and play a key role in maintaining cellular homeostasis and transmitting action potentials in neurons (Jentsch et al., 2002).
The amount of tap water and the amount of alkaline water administered, remained, and consumed/per day in rats in the control and experimental groups are shown in Fig. 1.

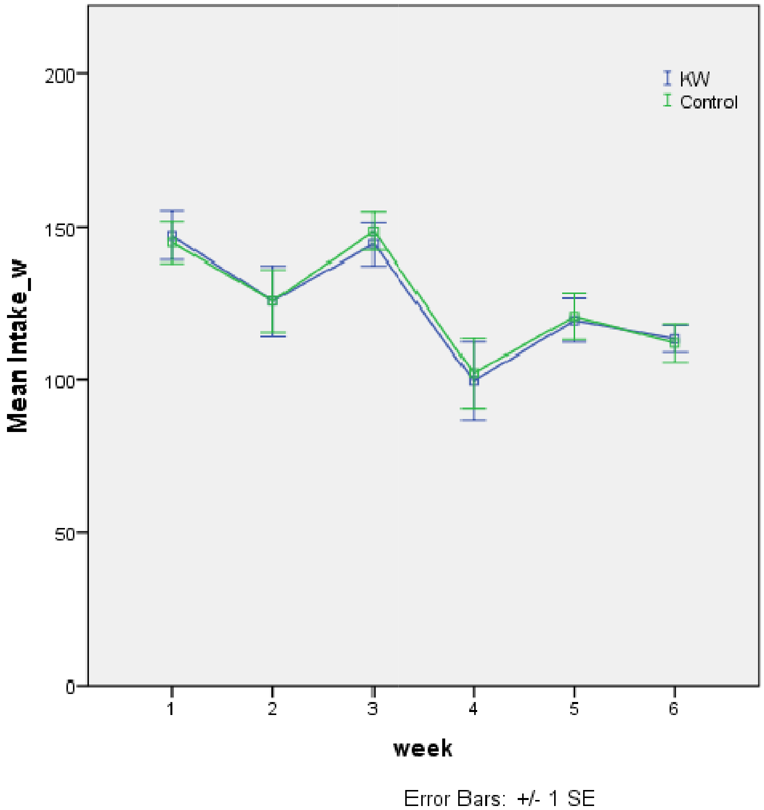

Figure 1. Total alkaline water consumption/day in experimental rats

From the 900 ml of water administered daily to the two groups of rats, it was noted that there was no statistically significant difference in daily water consumption, which averaged around 130±25ml.

Following the application of the Shapiro-Wilk test ($p>0.05$), the distribution of values for body mass and blood glucose values was performed. Body mass and blood glucose variables were normally distributed, both in the control group that consumed tap water and in the experimental group that consumed alkaline water. In the 5th week of the experiment, it was noted that body mass is not a normally distributed variable for the experimental group that consumed alkaline water. Body mass variables at 7 days and 28 days after the start of the experiment, and blood glucose variables at 7 days and 21 days after the start of the experiment are characterized by avarage±standard deviation, and body mass at 35 days is characterized by median and IQR (interquartile range). The characterization is rendered in Figures 2 and 3.

Following the application of the Independent Sample t-test for the study of the effect of alkaline water on the mass of the rats, it was found that in the first 4 weeks, 2 weeks of adaptation, and the first 2 weeks of the experiment there is not enough evidence to support the claim that there are statistically significant differences ($p>0.05$) between the mean body mass values of the control and experimental groups. For the

3rd week of the study, body mass cannot be assimilated with the normal distribution and after running the Independent Sample Mann-Whitney U test, it is found that at the 95% significance level there is not enough evidence for significant differences between the control and experimental groups. The lack of statistically significant differences between the mean body masses of the two groups can be seen in Figure 2 because the error bars overlap during the study period.

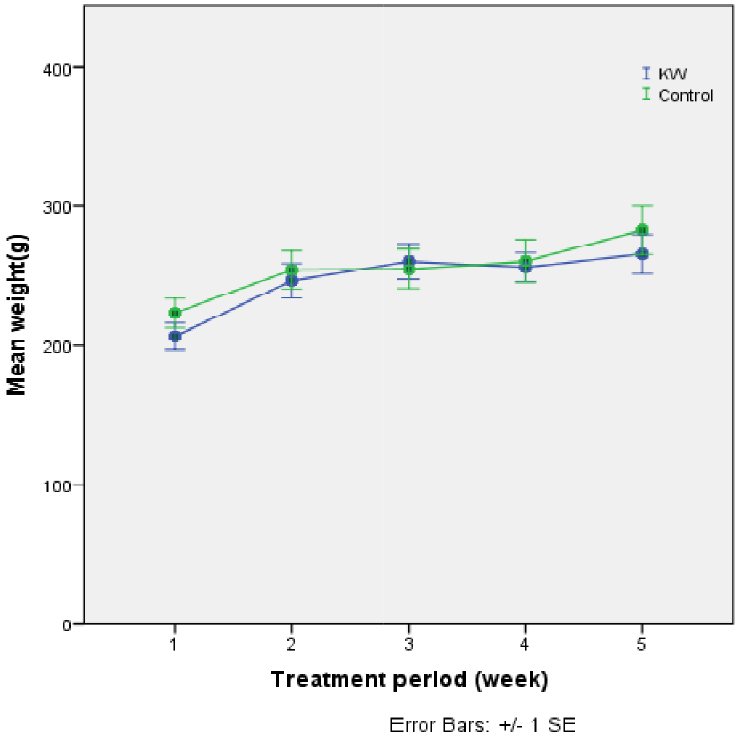

Figure 2. Changes in body mass are represented by mean mass values every 7 days for the control group (n=10) and the experimental group (n=10), together with the standard error (SE) at the 0.05 significance level. The first 2 weeks represent the adaptation period and the following 3 weeks represent the actual experiment period

For the assessment of the hydration index in humans, there are more than 13 techniques commonly used in physiology, in clinics, in certain branches of industry, in the military, in sports, and yet none of these techniques offers an indisputable argument for the superiority of one that could be used in all situations and for all categories of people (Armstrong, 2007). Of the many techniques used to assess hydration, the weighing method was chosen because the change in body weight provides the simplest and most accurate assessment technique when measurements are taken serially and immediately after water consumption (Armstrong, 2007). Similar to our experimental study, human research on the effect of alkaline water and standard purified water following exercise-induced dehydration showed a reduction in blood viscosity following consumption of alkaline water compared to standard purified water, but showed no significant difference in body mass changes between the two types of water (Weidman et al., 2016). Although studies have not identified a „gold standard" regarding hydration patterns, changes in body mass reflect body water losses and gains, secondary to sweating and water intake, respectively, and can serve as a marker of body hydration (Armstrong, 2007). Measurement of total body mass, defined as the fluid occupying the intracellular and extracellular spaces and representing 63.3% of body mass, can be used as a technique to assess body hydration (Dirckx, 2001).

Following the application of the Independent Sample t-test to assess the effect of alkaline water on the blood glucose of rats, it was found that there was insufficient evidence over the 3 weeks to support the claim that the differences were statistically significant ($p>0.05$) between the mean blood glucose values of the control and experimental groups.

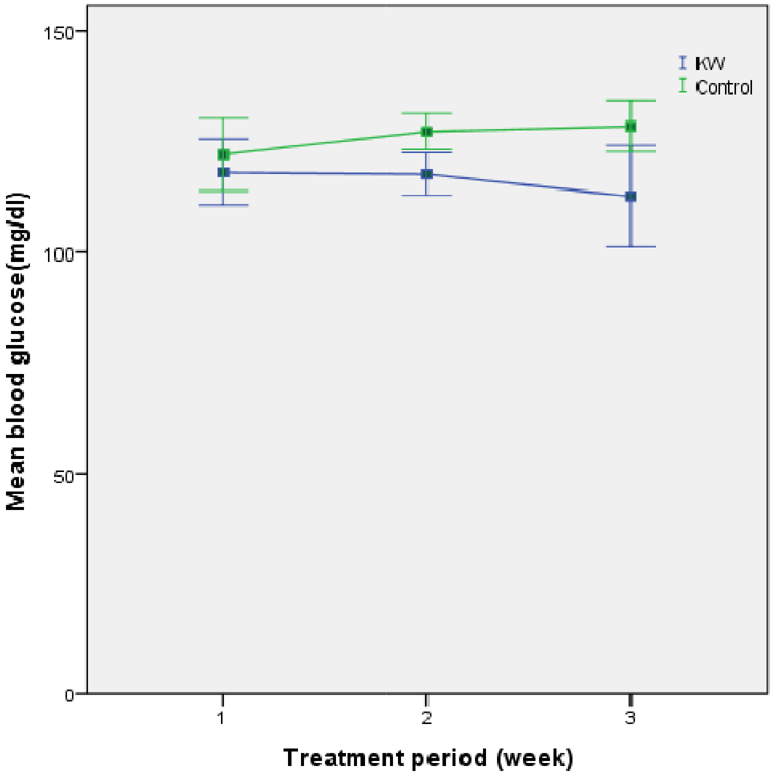

Figure 3. Average blood glucose values from 7 in 7 for the control group (n=10) and the experimental group (n=10), together with the SE standard error at the 0.05 level of significance

Analyzing Figure 3, both individually and in comparison, with Figure 2, it can be seen that the overlap of the error bars occurs only in the first week, and then they become more distant, which shows a certain hypoglycaemic effect that could occur if the experiment is repeated for a longer period.

Rats can be used as model animals for type II diabetes whose symptoms include the main risk factors for atherosclerosis, hyperglycemia, and hyperlipidemia (Kawano et al., 1992; Saito et al., 2000). Blood glucose in the control group ranged from 122±18 at 7 days; 127.2±9 at 14 days; 128.4±13 at 21 days and 129±21 at 40 days, and in the experimental group blood glucose ranged from 118±16 at 7 days; 117.6±10 at 14 days; 112.6±25 at 21 days and 110.3±18 at 40 days. Blood glucose in the experimental group was consistently lower than that of the control group. These results indicate that alkaline water with pH 8.5 administered to the experimental group induced a reduction in blood glucose. This reduction in blood glucose is due to the increased hexokinase activity of alkaline water (Warnholtz et al., 1999; Jin et al., 2006).

In the literature, cholesterol levels, triglycerides, GOT, GPT and body weight have lower values in the experimental group given alkaline water compared to the control group given tap water due to the antioxidant effect of alkaline water which caused changes in total lipid metabolism (Hanaoka, 2001; Hoyos et al., 2000; Jin et al., 2006). Cholesterol values in rats range from 40-130 mg/dl and triglycerides from 26-145 mg/dl (Wilson et al. 2012; Flecknell, 2014). In the groups of rats studied, the distribution of blood biochemical constituent data using the Shapiro-Wilk test (p>0.05) reveals that all variables are normally distributed in both the control and experimental groups. The characterization is given in Table 4.

Table 4

Variables of blood biochemical constituents in the control group and the experimental group

Variable	Control group (n=10)	Experimental group (n=10)
ALAT U/L	30.550±13.849	27.180±6.639
ASAT U/L	47.900±8.517	54.036±5.354
GGT mg/dl	11.460±5.63	5.800±1.825
PROTEINS g/dl	6.740±0.966	6.000±0.218
ALBUMIN g/dl	2.340±0.440	2.898±0.311
UREA mg/dl	46.720±8.111	48.520±9.219
CREATININE mg/dl	0.626±0.134	0.456±0.117
CHOLESTEROL mg/dl	60.540±15.098	49.560±2.496
TRIGLYCERIDE mg/dl	101.260±22.106	103.576±11.688
HDL CHOLESTEROL mg/dl	43.360±3.654	42.220±1.571
CALCIUM mg/dl	10.840±1.053	11.040±0.876
MAGNEZIUM mg/dl	2.920±0.335	2.530±0.293
PHOSPHORUS mg/dl	4.862±0.637	5.564±0.974
Na+ nmol/l	130.160±9.017	130.800±6.556
K + nmol/l	4.644±0.275	4.862±0.437
Cl- nmol/l	105.240±6.053	101.980±5.435

Analyzing the effects of alkaline water on blood biochemical characteristics, following the application of the Independent Sample t-test, there is insufficient evidence to support the claim of statistically significant differences (p>0.05) between the mean values of these characteristics between the control and experimental groups. Only in the case of albumin between the control group (M=2.340, SD=0.440) and the experimental group (M=2.898 SD=0.311) is there a statistically significant (p=0.049) difference in means M=0.558 at the 95% level of significance, CI=[0.003, 1.113].

The study of the distribution of data for each haemolucogram variable in the two groups of rats using the Shapiro-Wilk test (p>0.05) shows that variables that are not normally distributed are found in the total leukocyte volume (WBG µl) of the control group (p=Sig. =0.001), total lymphocyte count in percentage value (LYM %) in the control group (Sig.=0.005) and mean erythrocyte volume (MCVfl) in the experimental group (p=0.006), and all other variables in both control and experimental groups are normally distributed. Physiological values of haemolucogram in rats were: WBC: 4-12x10^9/l, LYM: 2-14.1x10^9/l, MID: 0-0.98x10^9/l, GRA: 0.1-5.4x10^9/l, RBC: 9-15x10^{12}/l, HGB 90-150 mg/dl, HCT: 24-45%, PLT: 250-750x10^9/l;

The characterization of haemolucogram variables in the two groups is shown in Table 5.

Table 5

Haemolucogram variables in the control group and the experimental group

Variable	Control group (n=10)	Experimental group (n=10)
WBC (10^9/l)	9.790 (9.475, 14.080)	13.474±1.550*
LYM (10^9/l)	6.830 (6.360,10.090)	9.136±1.167*
MID (10^9/l)	0.922±0.272	1.040±0.252
GRA (10^9/l)	2.512±1.009	3.524±0.262
RBC (10^9/l)	8.374±0.472	8.284±0.307
HGB (g/dl)	135.000±7.176	132.400±6.804
HCT (%)	41.332±2.564	39.930±1.866
MCV (fl)	49.200±1.483	48.000 (48.000,49.000)
MCH (pg)	16.120±0.370	16.040±0.378
MCHC (g/dl)	326.400±4.669	330.600±6.542

Analyzing the effects of alkaline water on haemolucogram characteristics, following the application of the Independent Sample t-test for variables that can be assimilated to a Gaussian distribution, it is found that there is not enough evidence to support the claim of a statistically significant difference (p>0.05) between the mean values of the characteristics of the control and experimental groups. For the variables that cannot be assimilated to the normal distribution, after running the Independent Sample Mann-Whitney U test, it is found that at the 95% significance level there is not enough evidence for significant differences between the control and experimental groups, for WBC p=0.151, LYM p=0.151, and MCV p=0.310. However, a slight leukocytosis given by lymphocytosis is observed in the alkaline water-treated group, with lymphocyte values not exceeding the physiological limit of the species, suggesting a possible increase in non-specific immunity, a possible immunostimulatory effect given by the antioxidant effects of alkaline water consumption.

A complete blood count may reveal general pathological conditions of the body evidenced by anemia, systemic infections, or blood neoplasia. It is also performed to monitor disease or drug treatment (Smith C, 2011). At the end of the experiment, all values recorded in the erythrogram analysis are within the physiological limits of the species. This indicates the absence of chronic systemic anemia (Giknis, 2008). After 40 days, no inflammatory reaction due to alkaline water consumption can be observed. The lack of adverse systemic hematological reactions also correlates with the clinical symptoms of the rats being well maintained.

CONCLUSIONS

The data obtained in this study revealed that the consumption of alkaline water with pH 8.5 does not produce adverse systemic effects, but has a hypoglycaemic, antioxidant, and immunostimulatory effect. To establish a greater number of systemic biological effects, specific recommendations for alkaline water consumption require studies over a longer period and in larger numbers of rats and a standardized experimental protocol.

REFERENCES

1. Armstrong L. E., 2007., Assessing Hydration Status: The Elusive Gold Standard, Journal of the American College of Nutrition, Vol. 26, No. 5, 575S–584S.
2. Brown R.E., 1985. The rodents Il: suborder Myomorpha. In social Odours in Mammals, Oxford University Press, Oxford Volume 1, 345-457.
3. Dirckx J.H., ed. 2001. Stedman's Concise Medical Dictionary for the Health Professions, 4th ed. Philadelphia: Lippincott Williams & Wilkins.
4. Gonţa M., Şalaru I., Sireţanu D. 1998. Impactul mediului ambiant asupra sănătăţii. - Chişinău, p.22.
5. Hanaoka, K., 2001. Antioxidant effects of reduced waterproduced by electrolysis of sodium chloride solutions.J. Appl. Electrochem.,31, 1307–1313.
6. Hanaoka K., Sun D., Lawrence, R. Kamitani Y., Fernandes G., 2004. The mechanism of the enhanced antioxidant effects against superoxide anion radicals of reduced water produced by electrolysis, Biophysical Chemistry, vol. 107, no. 1, pp. 71–82.
7. Harvey P.A. 2004. Borehole sustainability in rural Africa: Analysis of routine field data. In Proceedings of 30th WEDC International Conference, Vientiane, Lao PDR.
8. Hoyos, M., Guerrero, J. M., Perez-Cano, R., Olivan, J.,Fabiani, F., Garcia-Perganeda, A., and Osuna, C., 2000. Serumcholesterol and lipid peroxidation are decreased bymelatonin in diet-induced hypercholesterolemic rats.J. Pineal. Res.,28, 150–155.
9. Jequier E, Constant F. 2010. Water as an essential nutrient: the physiological basis of hydration. Eur J Clin Nutr. 64:115–123.
10. Jentsch T.J., Stein V., Weinreich F., Zdebik A.A,. 2002. Molecular Structure and Physiological Function of Chloride Channels. Physiological Reviews. 82 (2): 503–568.
11. Jin D., Ryu S. H., Kim H. W., Lim S.J., Ryang Y.S., Chung C.H., Park S.K., Lee K.J., 2006. Anti-diabetic effect of alkaline-reduced water on OLETF rats, Bioscience, Biotechnology and Biochemistry, vol. 70, no. 1, pp. 31–37.
12. Kawano, K., Hirashima, T., Mori, S., Saitoh, Y.,Kurosumi, M., and Natori, T., 1992. Spontaneous long-termhyperglycemic rat with diabetic complications. OtsukaLong-Evans Tokushima Fatty (OLETF) strain.Diabetes,41, 1422–1428.
13. Magro M., Corain L., Ferro S., Baratella D., Bonaiuto E., Terzo M., Corraducci V., Salmaso L., Vianello F., 2016. Alkaline Water and Longevity: A Murine Study. Evidence-Based Complementary and Alternative Medicine. Hindawi Publishing Corporation.
14. Popkin B, M., D'Anci K., E., Rosenberg I.H., 2010. Water, Hydration and Health. Nutr Rev., 68 (8): 439–458.
15. Prodan M.L., 2007. Impactul apei potabile asupra sănătăţii populaţiei, Revista Științifică a Universității de Stat din Moldova, nr.7.
16. Ramsay D.J. 1998. Homeostatic control of water balance. In: Arnaud MJ, editor. Hydration Throughout Life. Montrouge: John Libbey Eurotext; pp. 9–18.

17. Saito, Y., Nakamura, T., Ohyama, Y., Suzuki, T., Iida,A., Shiraki-Iida, T., Kuro-o, M., Nabeshima, Y.,Kurabayashi, M., Nagai, R., 2000. In vivoklotho genedelivery protects against endothelial dysfunction inmultiple risk factor syndrome.Biochem. Biophys. Res.Commun.,276, 767–772.

18. Shirahata S., Kabayama S., Nakano M., Miura T., Kusumoto K., Gotoh M., Hayashi H., Otsubo K., Morisawa S., Katakura Y., 1997. Electrolyzedreduced water scavenges active oxygen species and protects DNA from oxidative damage, Biochemical and Biophysical Research Communications, vol. 234, no. 1, pp. 269–274.

19. Warnholtz, A., Nickenig, G., Schulz, E., Macharzina, R.,Brasen, J. H., Skatchkov, M., Heitzer, T., Stasch, J. P.,Griendling, K. K., Harrison, D. G., Bohm, M., Meinertz,T., Munzel, T., 1999. Increased NADH-oxidase-mediatedsuperoxide production in the early stages of atheroscle-rosis: evidence for involvement of the renin–angiotensinsystem.Circulation,99, 2027–2033.

20. Watanabe T., Shirai W., 1990. Influence of alkaline ionized water on reproductive functions in the rat, International Journal of Fertility and Sterility, vol. 35, pp. 748–751.

21. Watanabe T., 1995. Effect of alkaline ionized water on reproduction in gestational and lactational rats. Journal of Toxicological Sciences, vol. 20, no. 2, pp. 135–142.

22. Watanabe T., Kishikawa Y., Shirai W., 1997 . Influence of alkaline ionized water on rat erythrocyte hexokinase activity and myocardium, Journal of Toxicological Sciences, vol. 22,no. 2, pp. 141–152.

23. Weidman J., Holsworth Jr. R.E., Brossman B., Cho D.J., Cyr J. St., 2016. Effect of electrolyzed high-pH alkaline water on blood viscosity in healthy adults. Journal of the International Society of Sports Nutrition.

24. Tukey J. W., 1977. Exploratory Data Analysis. Addison-Wesley.

25. ***Legea nr. 458 din 8 iulie 2002 - privind calitatea apei potabile.

26. ***Legea nr. 311 din 28 iunie 2004.

27. ***Directiva 98/83/CE privind calitatea apei destinate consumului uman.

28. ***STAS 1342-91 - Apă potabilă.

29. ***https://www.aparatapa.ro/?c=produse&lc=ro&gclid

30. Giknis M.L.A, Clifford C.B, 2008, Clinical laboratory parameters for CRL:WI(HAN) Rats, Charles River

31. Smith C., Jarecki A., 2011, Atlas of Comparative Diagnostic and Experimental Hematology, 2nd Edition, Wiley Blackwell