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The effects of low mineral content water on microbiota, metabolic, and oxidative stress parameters in patients with type 2 diabetes mellitus

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ABSTRACT

Although scientific evidence has shown that natural mineral waters have potential beneficial metabolic effects, there is still very scarce data on their influence on type 2 diabetes mellitus (T2DM). The study was designed to investigate the effects of low mineral water from the "Sneznik-1/79″ source in Serbia on microbiota, metabolic, and oxidative stress parameters in patients with T2DM. In total, 60 patients with confirmed T2DM were included in the study, and they consumed "Sneznik-1/79″ water for 28 days. To examine the positive effects of "Sneznik-1/79″ water, we compared the results before and after the four weeks of "Sneznik-1/79″ water intake. Standard biochemical analyses were carried out, such as glucose level, lipid profile, and stool tests. The blood samples were collected to evaluate the effects of "Sneznik-1/79″ water on the redox status. At the end of the monitoring period, the total cholesterol concentration significantly dropped compared to the initial value. A significant improvement in intestinal peristalsis was observed, which was reflected in the fact that after four weeks, all patients established regular, daily bowel movements. Moreover, consumption of "Sneznik-1/79″ water eliminated the appearance of dysbiosis in 50% of patients. Additionally, the antioxidant capacity was improved by increasing the concentration of superoxide dismutase and reduced glutathione. The result of our study pointed out that the intake of "Sneznik-1/79″ water could be a promising adjuvant therapy for improving intestinal peristalsis as well as reducing the appearance of dysbiosis in T2DM patients.

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1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by fasting or postprandial hyperglycemia due to an absolute or relative deficit in the production or action of insulin [1]. According to the International Diabetes Federation, DM affects more than 390 million patients worldwide, which indicates that DM has reached the proportions of a global epidemic. The most common form of DM is type T2DM which accounts for 90% of all diabetic patients [2]. The exact cause of T2DM is unknown, but hereditary factors, lifestyle, and numerous associated diseases and conditions are believed to play an important role in its development [2,3]. The goal of T2DM treatment is to achieve and maintain optimal levels of glucose, lipids, and blood pressure, as well as to prevent or delay the development of chronic complications [3].

The first step in treating T2DM is non-pharmacological treatment, which involves lifestyle changes, i.e., changes in dietary habits and increasing physical activity alone or in combination with the current medical approach [4]. However, the increasing incidence of T2DM associated with side effects and high costs of available drugs indicates the need to use herbal medicines [5,6] or discover new, less harmful approaches to blood glucose and lipid control.

In addition to recommending numerous diets, recent scientific research has focused on mineral and medicinal waters that can potentially be used to prevent and treat this complex metabolic disorder. It has been documented that adequate water intake is associated with a lower risk of hyperglycemia and developing diabetes [7,8]. Consumption of mineral waters originating from natural sources or underground reservoirs might have even better therapeutic benefits than tap water in patients with T2DM [8,9]. In addition, minerals from natural mineral water have potential pharmacological, physiological, and clinical effects on the body [9].

Literature data indicate that bicarbonate mineral water has numerous health benefits, reflected in reduced cholesterol and glucose levels. Consequently, bicarbonates from mineral water play a significant role in preventing cardiovascular diseases [10–12]. Moreover, low-mineral water intake might improve the redox state in rats with metabolic syndrome, but there is still insufficient evidence for patients with T2DM [13]. Additionally, patients with T2DM have associated complications, such as intestinal motility disorders [14]. Although intestinal motility disorders are classified as a mild complication of the disease, it often directly affects patients' quality of life.

Considering that the mineral water from the "Sneznik-1/79″ source has been traditionally used since the early 20th century for treating gastrointestinal and diabetic problems, we seek to provide scientific justification for its usage. Therefore, we hypothesized that this water could contribute to eliminating the problem of constipation and have other beneficial effects in patients with T2DM.

Considering all the above-presented data, this study aimed to estimate the effects of the low-mineral, weakly acidic water from the "Sneznik-1/79″ source on the microbiota, metabolic, biochemical, and anthropometric parameters in patients with T2DM.

2. Material and methods

2.1. Ethical approval

The study was performed in accordance with the Declaration of Helsinki and principles of Good Clinical Practice and approved by the Ethics Committee of the Merkur Special Hospital, Vrnjacka Banja (number: 01–716/1). Written informed consent to participate was obtained from all participants before enrollment.

2.2. Patients

The study enrolled 60 patients with diagnosed T2DM referred for treatment to the Merkur Special Hospital, Vrnjacka Banja, Serbia. The patients consumed "Sneznik-1/79″ low mineral water for 28 days, and all analyses of interest were measured at two points of interest: day 0, before water consumption, and day 28, after 4-week water consumption. The inclusion criteria were as follows:

1. age (30–60 years);
2. confirmed T2DM according to the criteria of the National Good Clinical Practice Guideline for DM of July 2012
3. absence of diabetes complications (diabetic foot, retinopathy, hemodialysis)

2.3. Consumption of natural mineral water from "sneznik-1/79″

According to the disease stage and the general condition of the patients, water was dosed individually (1.5 to 3 L) by the doctor of the "Merkur" Special Hospital. The time frame for water consumption was four weeks and was chosen according to literature data [12].

2.4. Anthropometric and body composition assessment

Anthropometric and body composition measures were obtained with the subjects dressed in light clothing without shoes. Body weight was measured with a scale to the nearest 0.1 kg, while the height was measured to the nearest 0.5 cm with a stadiometer with an incorporated scale according to standardized procedures [15]. These measures were used to calculate body mass index - BMI (weight (kg)/height (m^2)).

Body composition was measured using a bioelectrical impedance analysis (BIA) by InBody according to the manufacturer's guidelines [16]. The participants removed all objects that could interfere with the bioelectrical impedance assessment. The variables collected were total body water (TBW) and fat mass (FM).

The anthropometric and body composition measurements were carried out in the morning in a room with an ambient temperature of 22–23 °C and relative humidity of 50–60%, after a minimum of 8 h of fasting and after emptying the bladder [17].

2.5. Bowel movement diary

Patients had completed a standardized Bowel movement diary for three weeks prior to the start of the experimental protocol to determine whether constipation was present. Constipation was defined as fewer than three bowel movements per week with dry, hard, small stools. The same diary was then kept in the following four weeks, which was the period of consumption of "Sneznik-1/79″ mineral water. The doctor instructed each patient on how to complete the required diary items. After having a bowel movement, each patient was instructed to fill in the letter corresponding to what happened in the appropriate day and time box: N-normal bowel movement, P-pad or pants change, F-fingers needed to push stool out (splinting), I-incontinence/bowel accident, S-straining to pass stool.

2.6. Stool analysis

Fresh stool samples were collected using a stool kit immediately after spontaneous defecation. The individual samples were collected and delivered to the laboratory within 24 h at the beginning and after four weeks at the end of the study. Samples were immediately stored at −80 °C until analysis.

2.7. Glucose levels

Glucose blood level was determined before and after four weeks of "Sneznik-1/79″ consumption using a glucometer (Accu-Chek, Roche Diagnostics, Indianapolis, IN, USA) [18].

2.8. Lipid profile

The following serum biochemical parameters: total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined spectrophotometrically on a programmed biochemical analyzer (Dimension Xpand, Siemens, IL, USA) using commercial kits (Siemens Healthcare Diagnostics, Frimley, Camberley, Surrey, UK) [18].

2.9. Redox state

2.9.1. Blood sampling

Blood samples were obtained in the same manner for all the patients at two points of interest: day 0, before the start of "Sneznik-1/79″ water consumption, and day 28, after four weeks of "Sneznik-1/79″ water intake. Blood was collected in Vacutainer tubes containing sodium citrate as an anticoagulant and centrifugated to separate the red blood cells (RBCs) from the plasma. After centrifugated, the plasma was separated and stored at −20 °C, while the erythrocyte lysates were prepared by washing the RBC three times in ice-cold saline, following lysis in three volumes of ice-cold distilled water.

In the plasma samples, the following pro-oxidative markers were determined: level of hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-), nitrites (NO_2^-), and index of lipid peroxidation (expressed as thiobarbituric acid reactive substances—TBARS).

In the erythrocyte lysate, the following antioxidative defense system parameters were determined: the activity of superoxide dismutase (SOD) and catalase (CAT), as well as the level of reduced glutathione (GSH) [19].

2.9.2. Pro-oxidative markers

2.9.2.1. Hydrogen peroxide (H_2O_2). The determination of the H_2O_2 level was based on the oxidation of phenol red by H_2O_2 in a reaction catalyzed by horseradish peroxidase. The protocol included adding 800 μl of phenol red solution (PRS) and 10 μl POD (horseradish peroxidase (1: 20)) to 200 μl of plasma sample. After a 10-min incubation at room temperature, the absorbance was measured at a wavelength λ = 610 nm. An equivalent volume of distilled water was used for the blind test [19].

2.9.2.2. Superoxide anion radical (O_2^-). The reaction of nitro blue tetrazolium (NBT) with O_2^- is the reaction for determining O_2^- concentration. The procedure included mixing 50 μl of plasma samples and 950 μl of *ex-tempore*-prepared assay mixture. The absorbance was measured three times with stirring. The measurement was carried out every 60 s at λ = 550 nm. An equivalent volume of distilled water was used for the blind test [19].

2.9.2.3. Nitrites (NO_2^-). The nitric oxide (NO) level was evaluated indirectly by measuring the NO_2^- level, as NO decomposes rapidly, forming an equal amount of nitrite products. First, 200 μL of plasma sample was precipitated with 100 μl of 3 N perchloric acid (PCA) and 400 μL 20 mM ethylenediaminetetraacetic acid (EDTA), put on ice for 15 min, and centrifuged at 6000 rpm for 15 min. After 15 min, the supernatant was separated, and 220 μL of potassium carbonate (K_2CO_3) was added to the sludge. A total of 200 μL of the sample was mixed with 250 μL Griess's reagent and 125 μL of buffer for NO. The samples were incubated at room temperature for 15 min, and the measurement was performed at λ = 550 nm [19].

2.9.2.4. Index of lipid peroxidation measured as TBARS. The index of lipid peroxidation in the plasma samples was determined indirectly by measuring the level of TBARS using 1% thiobarbituric acid (TBA). First, 800 μL of plasma sample was mixed with 400 μl of trichloroacetic acid (TCA), put on ice for 10 min, and centrifuged at 6000 rpm for 15 min. Next, 400 μl of supernatant was incubated with 100 μl of 1% TBA in 0.05 NaOH at 100 °C. After a 15-min incubation, the samples were left for 10 min at room temperature, and the measurement was performed at λ = 530 nm [19].

2.9.3. Antioxidative parameters

2.9.3.1. Superoxide dismutase. SOD activity was evaluated by the epinephrine method. Erythrocyte lysate in the amount of 100 μl was mixed with 1000 μl carbonate buffer, and after a few seconds in a vortex mixer, 100 μl adrenaline was added. The absorbance measurement was performed in duplicate at a wavelength λ = 470 nm. The carbonate buffer and adrenaline mixture was used as a blind test [19].

2.9.3.2. Catalase (CAT). The procedure for CAT determination involved dissolving lysate RBCs with distilled water (1:7 v/v), followed by the addition of ethanol (0.1:1). Then, we added 50 μL CAT buffer, 100 μL prepared lysate sample, and 1000 μL 10 mM H_2O_2 into the test tube. The measurement was performed six consecutive times at a wavelength λ = 360 nm. An equivalent volume of distilled water was used for the blind test [19].

2.9.3.3. Reduced glutathione (GSH). The GSH level was determined by GSH oxidation with 5,5-dithiobis-6,2-nitrobenzoic acid. A total of 50 μL of lysate was mixed with 200 μl of 0.1% EDTA and 385 μL precipitated buffer, put on ice for 15 min, and centrifuged at 4000 rpm for 15 min. After 15 min, the extract was obtained. Next, we added 300 μL extract, 750 μL sodium phosphate dibasic, and 100 μL 5.5-dithiobis-6.2-nitrobenzoic acid (DTNB) into the test tube. After a 10-min incubation, the measurement was performed at λ = 420 nm. Distilled water was used for the blind test [19].

2.10. Statistical analysis

All data were analyzed using SPSS 20.0. The results are expressed as means ± standard deviation (SD). Data normality was checked using the Kolmogorov-Smirnov and Shapiro–Wilk tests. Independent Student's t-test (parametric) and Mann–Whitney *U* test (nonparametric) were used to assess the difference in estimated variables between groups. A p-value <0.05 was regarded as statistically significant.

3. Results

3.1. Anthropometric and body composition assessment

Anthropometric and body composition parameters of the T2DM patients before and after four weeks of "Sneznik-1/79″ mineral water consumption are shown in Table 1. The results showed that four weeks' administration of "Sneznik-1/79″ mineral water did not significantly change any values of measured parameters.

3.2. Bowel movement diary

The data collected from the patient's bowel movement diaries indicate that 62.5% of patients had constipation on admission, which was expected due to their primary disease. After starting the consumption of "Sneznik-1/79″ water, intestinal peristalsis was improved. After four weeks, improvement was noticed in all patients (100%), i.e., all patients established regular, daily bowel movements.

Table 1
Anthropometric and body composition parameters of the patients before and after "Sneznik-1/79″ mineral water consumption (mean ± SD).

Parameters	Before	After
Weight (kg)	88.7 ± 15.0	88.5 ± 14.6
Body mass index (kg/m^2)	30.16 ± 4.61	30.0 ± 4.56
Total body water (kg)	45.31 ± 9.66	43.3 ± 7.51
Fat mass (%)	28.69 ± 10.44	29.1 ± 9.8

3.3. Stool analysis

The results of the stool examination before and after four weeks of "Sneznik-1/79″ mineral water consumption are shown in Table 2. The results showed that the four-week consumption of "Sneznik-1/79″ water had the effect of eliminating the appearance of muscle fibers in the stool in 10% of patients. Additionally, consumption of "Sneznik-1/79″ water eliminated the appearance of dysbiosis in 50% of patients, which can be indirectly seen by the results of the isolation of *Enterococcus* sp. Namely, before consumption of "Sneznik-1/79″ water, 100% *Enterococcus* sp. was present in 50% of patients, while at the end of the study, the presence was noticed in only 10% of patients.

3.4. Blood glucose level

The study results showed that the glucose level was approximately the same at the beginning and after four weeks of consuming "Sneznik-1/79″ mineral water without any significant difference (Fig. 1).

3.5. Lipid profile

TC, TG, HDL, and LDL concentrations, expressed in standard units, are presented in Fig. 4. TG, LDL- and HDL-cholesterol concentrations were lower after the four-week consumption of "Sneznik-1/79″ water, but without statistical verification. On the other hand, total cholesterol concentration was significantly lower at the end of the monitoring period (Fig. 2).

3.6. Systemic redox state

3.6.1. Pro-oxidative markers

Four weeks of "Sneznik-1/79″ water usage have led to a decrease in the level of pro-oxidative markers such as O_2^- and H_2O_2. The level of H_2O_2 was significantly lower in T2DM patients after mineral water consumption. In addition, the O_2^- level was lower, but the difference was not statistically significant. On the other hand, the level of NO_2^- was significantly higher at the end of the monitoring period, while the release of TBARS measured in plasma samples was not significantly different between the two points of interest (Fig. 3).

3.6.2. Antioxidative parameters

Considering the antioxidant defense system parameters, "Sneznik-1/79″ water intake improved antioxidant protection by increasing all three parameters. The activity of SOD was statistically significantly increased after four weeks of "Snežnik" water intake. The same trend was observed in the case of GSH, while CAT activity was also improved, but without statistical verification (Fig. 4).

4. Discussion

Water is an ideal calorie- and sugar-free beverage, and numerous studies have demonstrated the critical role of water intake for maintaining normal metabolism and life functions [20,21]. Since drinking water significantly reduces the intake of calories and sugar, the chances of developing T2DM are consequently reduced by 6% [7]. Likewise, the positive effects of water in improving glycemic parameters in patients with confirmed diabetes have been reported [7,22]. It is also important to note that mineral nutrients affect glucose metabolism and that consuming mineral water prevents or has a beneficial effect on T2DM [23–25].

Taking into account the growing prevalence of T2DM and its related complications, our study aimed to estimate the potential benefits of alkaline "Sneznik-1/79″ water on the body composition, anthropometric, microbiota, metabolic parameters as well as oxidative stress in patients with T2DM. To examine the potential effects of "Sneznik-1/79″ water, we compared the results before and after four weeks of "Sneznik-1/79″ water intake.

The results of our study clearly show that consumption of "Sneznik-1/79″ water was not associated with changes in body

Table 2
The parameters of stool examination.

Parameters	Before	After
Muscle fibers	80% patients	70% patients
Fat drops	15% patients	15% patients
Starch	10% patients	10% patients
Bacteria	Not isolated	Not isolated
Fungi	Not isolated	Not isolated
Enterococcus sp.	100% in 50% patients	100% in 10% patients
	90% in 10% patients	80% in 10% patients
	80% in 20% patients	70% in 20% patients
	70% in 10% patients	
	Reduced intestinal flora in 10% patients	
Dysbiosis	100% patients	50% patients

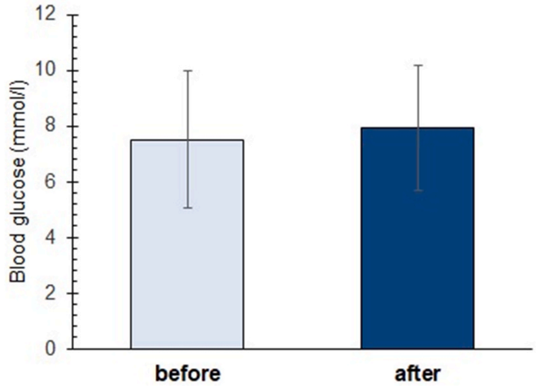

Fig. 1. The blood glucose level before and after "Sneznik-1/79″ mineral water consumption. Values are expressed as mean ± standard deviation. *statistically significant difference $p < 0.05$; #statistically significant difference $p < 0.01$.

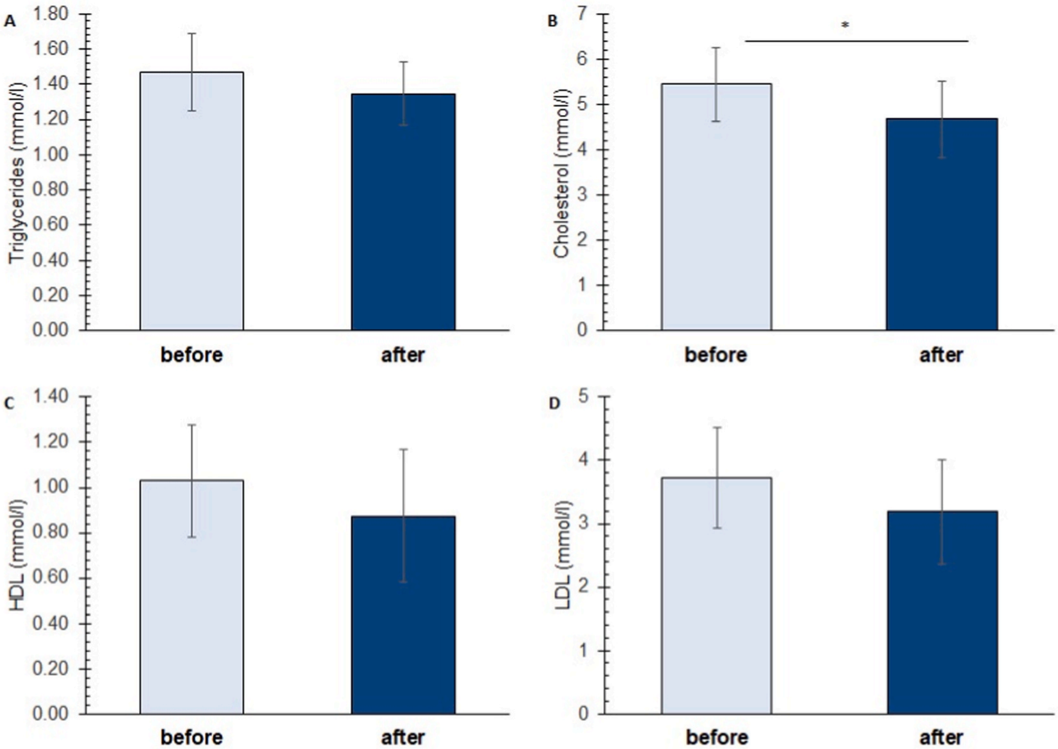

Fig. 2. The lipid profile before and after "Sneznik-1/79″ mineral water consumption: (A) TC; (B) TG; (C) HDL; (D) LDL. Values are expressed as mean ± standard deviation. *statistically significant difference $p < 0.05$; #statistically significant difference $p < 0.01$.

composition parameters, which indicates that there were no metabolic deviations of the subjects during the monitoring period. Additionally, body weight and body mass index were unchanged after four weeks of drinking "Sneznik-1/79″ water. Although electrolytes from mineral waters accomplish various physiological functions, it seems that their concentration in this water and duration of water consumption were insufficient to change adipose tissue activity. Moreover, one research shows that even when rich mineral water was applied, these ions could not affect the secretion of adiponectin from adipocytes and thus do not change the metabolic activity of adipose tissue [26]. Our results are in line with the results of the previous studies, which indicated no significant variations in basic anthropometric characteristics such as body mass and body mass index after consumption of low mineral water [26–28].

On the other hand, it is well known that gastrointestinal symptoms are often observed in T2DM patients, with constipation being one of the most common [29]. Therefore, in the second part of the investigation, we estimated the effects of "Sneznik-1/79″ water on diabetes-induced constipation. As recorded in the Bowel movement diary, the consumption of this water was associated with improved intestinal peristalsis and regular, daily bowel movements in all patients. These findings may be a consequence of the high

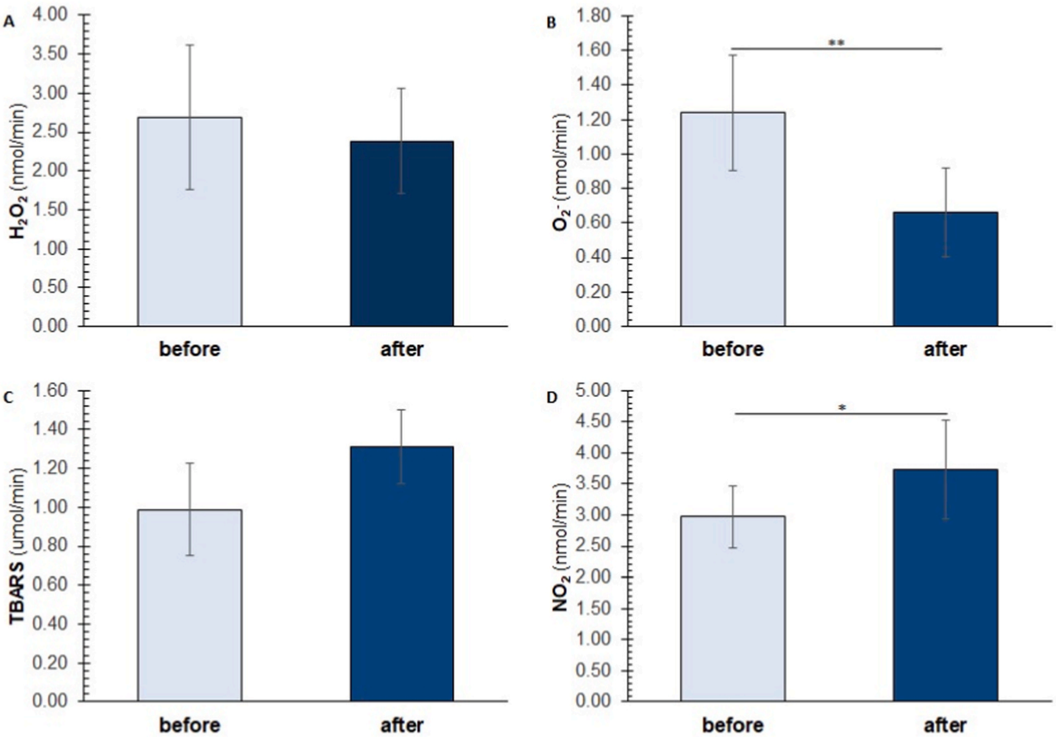

Fig. 3. The level of pro-oxidative markers before and after "Sneznik-1/79″ mineral water consumption: (A) $H_2O_2^-$ (B) O_2^-; (C) NO_2^-; (D) TBARS. Values are expressed as mean ± standard deviation. *statistically significant difference $p < 0.05$ between examined groups; #statistically significant difference $p < 0.01$.

concentration of bicarbonate ions (1098,0 mg/L) in "Sneznik-1/79″ water, considering that bicarbonate-rich waters are proven to stimulate gastric motor and secretory functions as well as the activity of pancreatic enzymes. Furthermore, it was noted that bicarbonates are responsible for improved fat emulsification by bile and enhanced secretion of pancreatic fluids and bile flow [30]. All these actions lead to the re-establishment of normal bowel movements. A similar effect was also recognized in a study showing a clear result of bicarbonate Uliveto water in fecal excretion. Fecal excretion and fecal water content in rats treated with bicarbonate water were significantly higher in comparison to those found in rats treated with control water. A positive effect was observed both under normal conditions and in the presence of bowel motor alterations [31]. Given that Uliveto water increases the rate of colonic transit in animals with constipation, its intake can benefit evacuation disturbances induced by constipation. Likewise, the results of a study conducted by Anti et al. have shown that the intake of 2 L bicarbonate water with 25g fiber intake per day significantly increased stool frequency [32]. Considering that the selective 5-HT3 receptor antagonist, alosetron, blunted the effects of Uliveto water, bicarbonate-alkaline water can also possibly have a beneficial effect by acting through the serotoninergic system [31].

In addition to establishing regular, daily bowel movements, stool analysis results might be one of the most important findings of the current study. We can notice that after four weeks of drinking water from the "Sneznik-1/79″ spring, the stool culture test result indicated dysbiosis in only 50% of patients compared to the initial 100%. In addition, the percent of *Enterococcus* sp. was also lower after intake of this water. The potential explanation for this positive influence can be attributed again to the high concentration of bicarbonate ions in "Sneznik-1/79″ water. Namely, as previously shown in both preclinical and clinical studies, bicarbonates from mineral waters contribute to a rise in gastrointestinal pH levels [30–32]. Movement from acid to alkaline pH, in turn, balances the typical intestinal colonies and favors the restoration of the qualitative and quantitative characteristics of the normal intestinal flora [30–32]. Accordingly, our findings imply that using "Sneznik 1/79″ water is clearly associated with improved intestinal dysbiosis, followed by relieved constipation in these patients.

Other studies have also shown the beneficial effect of mineral waters against constipation, especially sulfate mineral waters [33, 34]. These investigations highlighted that thanks to high levels of sulfate, magnesium, and sodium, sulfate mineral waters reduce functional constipation by increasing intestinal motility and changing colon osmotic pressure [33,34].

Considering the effect of water on the glucose level, a previous study conducted by our research team has shown that the treatment with "Sneznik 1/79″ water normalized glycemic values in diabetic rats during the fourth-week treatment [35]. These results are inconsistent with present findings where the 4-week consumption of "Sneznik 1/79″ water was not associated with changes in glycemic values in T2DM patients. The absence of interaction between this water and glucose metabolism is difficult to explain. Given the lower concentration of calcium (80,1 mg/L) and magnesium (34,9 mg/L) ions, which can help in cell glucose uptake, it can be assumed that the mineral properties of this water are not appropriate for the hypoglycemic effect. Therefore, we can hypothesize that longer

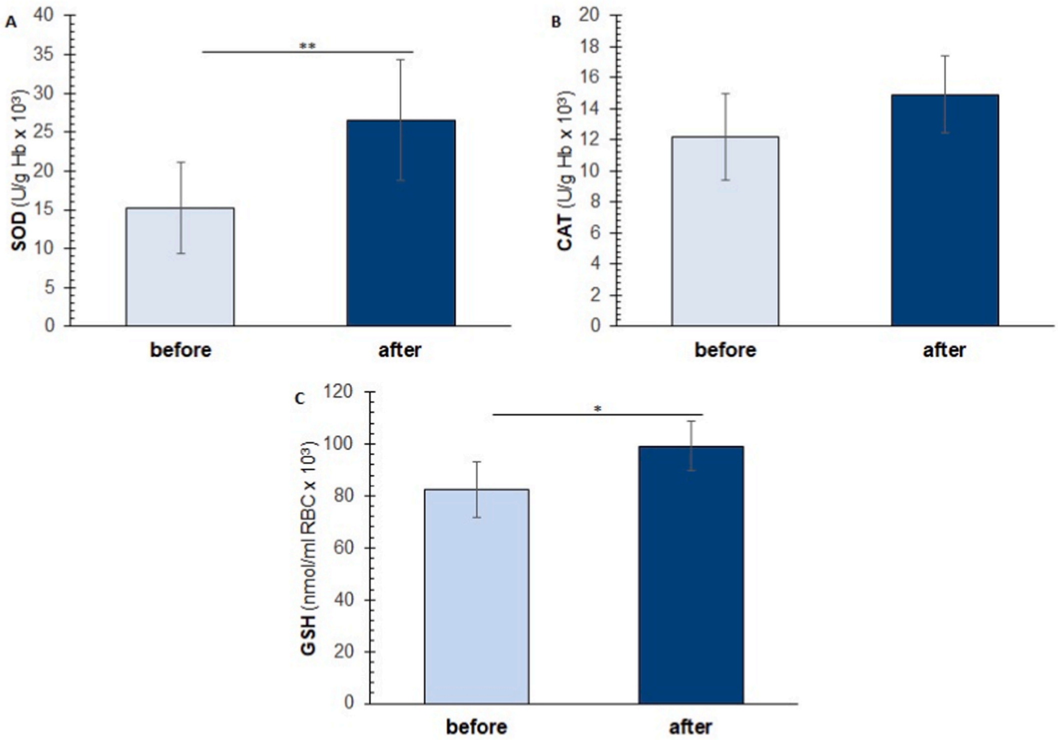

Fig. 4. The antioxidative defense system parameters before and after “Sneznik-1/79″ mineral water consumption: (A) SOD; (B) CAT; (C) GSH. Values are expressed as mean ± standard deviation. *statistically significant difference $p < 0.05$; #statistically significant difference $p < 0.01$.

exposure to this water would then ensure more pronounced glucose changes. On the other hand, our results are in correlation with the research conducted by Pérez-Granados, which indicates that 8-week carbonic sodium bicarbonate mineral water did not significantly affect serum glucose levels in moderately hypercholesterolaemic and normoglycemic adults [26]. Additionally, bicarbonate-rich mineral water did not decrease blood glucose levels even in healthy subjects after ten days of consumption [36]. This might be attributed to external factors such as food intake and exercise influencing blood glucose levels [37].

In addition to high glucose levels, perturbed lipid metabolism also contributes to the development of atherosclerosis in T2DM patients [38,39]. The current investigation has shown that the concentration of lipid parameters was lower at the end of the monitoring period, but statistical verification was observed only for the cholesterol value. However, the dynamics of the lipid status clearly indicate the positive impact of consuming “Sneznik 1/79″ water, which should be investigated in more detail in future studies with data on eating habits and physical activity. The mechanism responsible for the hypocholesterolemic effect of “Sneznik 1/79″ water is based on its alkaline nature or high bicarbonate concentration. As proven earlier, bicarbonate ions have strong choleretic (stimulation of bile production) and cholagogic (stimulation of gallbladder contraction) properties [40,41]. All of these cause enhanced conversion of cholesterol into bile acid and lowers total cholesterol levels. Furthermore, it was also shown that high amounts of bicarbonates have a powerful osmotic effect that can decrease the absorption of fat and cholesterol [12,40,41].

Likewise, other authors have shown that consuming mineral bicarbonate water from Montecatini has a decreasing effect on total and LDL cholesterol in a fasting state [42]. Furthermore, the lipid-lowering potential was confirmed for the bicarbonate water from another spring in Spain, where Schoppen observed that drinking mineral water rich in bicarbonate reduced postprandial lipemia in healthy postmenopausal women [41].

Since we noticed in a previously published paper that “Sneznik 1/79″ water could improve antioxidative parameters in rats with T2DM [14], we wanted to confirm the results in the human population. We obtained a significant increment in the activities of measured antioxidative parameters, such as SOD and GSH. Additionally, water consumption was effective in the reduction of O_2^- production, which can be a possible consequence of the increased concentration of SOD, responsible for converting O_2^- to H_2O_2. The potential antioxidant property of this water may be due to the abundance of many oligoelements (copper, manganese, and zinc) necessary for antioxidant enzyme activity, in particular SOD [43].

In line with our results is the finding that two months of Zamzam water consumption showed a significant increase in total antioxidant capacity and concentration of catalase, superoxide dismutase, and glutathione [43]. It has been previously proved that drinking sulfurous mineral water from Terme of Telese SpA for two weeks may be useful for improving the redox state in T2DM patients, especially in combination with antidiabetic drug treatment [44].

Finally, it is important to emphasize that none of the present findings can be attributed to mineral water intake only since many other factors, such as food intake, physical activity, and medication used, could contribute to the results. Unfortunately, we were not able to receive all of these data in a reliable form, and therefore we omitted them. In addition, our study had other limitations that must be considered when discussing the obtained results. One is the number of participants, and the other is the time frame of water consumption. Both of them should be higher.

5. Conclusion

Our research was the first to note that low mineral water from the “Sneznik-1/79″ spring improved intestinal peristalsis by establishing regular bowel movements. Additionally, it eliminated the appearance of dysbiosis in 50% of patients. This water also improved the antioxidant capacity by increasing the action of superoxide dismutase and reduced glutathione. From a clinical point of view, the result of our study pointed out that the intake of “Sneznik-1/79″ water could be a promising adjuvant therapy in T2DM patients, especially when pharmacological treatment is unsatisfactory. Nevertheless, further basic and applied investigations are necessary to confirm these beneficial effects. Therefore, future research on this mineral water should include a much greater number of respondents and a longer water consumption period.

Author contribution statement

Nikola Jovicic, Marijana Andjic: Performed the experiments; Wrote the paper.
Jovana Novakovic, Nevena Jeremic: Performed the experiments; Analyzed and interpreted the data.
Vladimir I. Živković: Conceived and designed the experiments; Wrote the paper.
Ivan Srejovic, Petar Ristic: Contributed reagents, materials, analysis tools or data.
Dejan Stanojevic: Performed the experiments.
Sergey Bolevich, Vladimir Jakovljevic: Conceived and designed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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