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Effects of carbonated natural mineral water baths and mofettes on peripheral arterial flow in rats

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Abstract: (1) Introduction. Disorders of peripheral blood circulation affect people all around the world. Balneotherapy, in the form of carbonated mineral water baths and mofettes, could be considered one of the treatment options. The current study aims to investigate, in an experimental study, the effects of carbonated natural mineral water, with a known composition, and natural mofettes from Băile Tușnad (Romania) on peripheral arterial blood flow. (2) Material and method. A total of 16 rat subjects were studied, divided equally into 4 groups, all evaluated at 2, 4 and 6 weeks after the intervention. Group 1 was considered the control-group, and no treatment was applied. Group 2 was taken to the mofettes daily, for 2 weeks, for exposure to carbon dioxide, dry gas, for 20 minutes/day. Group 3 performed daily mofettes and baths with carbonated mineral water, for two weeks, 20 minutes/day, each procedure. Group 4 took a daily bath with carbonated mineral water, for 2 weeks, 20 min/day. At the end of the two-week treatment, they were evaluated by doppler ultrasound at the femoral artery, respectively at 2 and 4 weeks, to evaluate the effect over time of these treatments. Femoral artery diameter (mm), peak systolic velocity (PVS, cm/s), heart rate (HR, bpm) were quantitatively determined in all 4 groups, at 2, 4 and 6 weeks respectively, and ultrasound images were captured. (3) Results and discussions. In the control group, there were no differences at 2 weeks vs. 4 weeks vs. 6 weeks regardless of the studied parameter. The diameter of the femoral artery increased significantly in group 3 (mofetta and carbonated mineral water, p=0.0183), respectively in group 4 (mineral water p=0.007). Heart rate changed significantly p=0.0183 in the mofette group, as well as in the one treated with carbonated mineral water p= 0.024. The results of the study suggest that carbonated mineral water and mofettes were responsible for the changes in femoral artery flow, compared to the data from the control group. (4) Conclusions. Carbonated mineral water baths and mofettes could increase the peripheral arterial blood flow, during repeated immersion, and the results were beneficial 2 weeks after the end of the treatment, and were maintained after treatment at 4 and 6 weeks, respectively.

Keywords: carbonated mineral waters, mofette, doppler ultrasound, arterial flow.

1. Introduction

The natural mineral waters from the Băile Tușnad spa resort in Romania, belong to the category of mixed carbonated, sodium chloride, calcium, magnesium, ferruginous, bicarbonate, hypotonic mineral waters with a total mineralization of 0.68-17.86 g/liter. They are recommended in the form of external bathing, in certain pathologies, especially

for cardiovascular diseases, arterial hypertension, ischemic heart disease, chronic obliterating arteriopathy [1,2,3]. The natural mofettes from Băile Tușnad contains carbon dioxide (CO_2), and has a known effect of increasing blood flow through the penetration of CO_2 through the skin and at the muscle level [1,2,3]. Clinical studies were conducted on their effectiveness in the recovery of stroke patients [4,5]. Recent studies have investigated the specific effects of sequentially applied carbon dioxide (CO_2) baths and mofettes on oxidative stress parameters and antioxidant parameters in experimentally induced myocardial ischemia in rats [1,2]. Positive results were obtained by applying baths with carbonated mineral waters in patients with arterial occlusive disease [6]. In a full bath, between 10 and over 80ml/min/m² of CO_2 are absorbed through the skin surface, on average 30ml/min/m² of CO_2, but it depends on the measurement method, water temperature, and concentration. A molecular mechanism study showed that CO_2-induced vasodilation in peripheral blood vessels is related to the activation of the nitric oxide guanosine monophosphate signaling cascade and angiogenesis by inducing the synthesis of vascular endothelial growth factor (VEGF) [7]. VEGF causes an increase in vascular permeability and recruits progenitor endothelial cells from the bone marrow, increases the mitosis rate of endothelial cells, being a strong vasodilatory agent [8,9]. Carbon dioxide can affect skin microcirculation and arterial macro-circulation, as controlled experiments on animals have shown that both skin and muscle blood flow and oxygen pressure increased during immersion [10]. CO_2 causes changes in hemodynamics, in microcirculation, in thermoregulation in human skin, being confirmed by spectroscopy, magnetic resonance and thermography. An acute vasodilator action upon CO_2 application and an increase in peripheral blood flow in rats has been demonstrated using in vivo imaging with intravital microscopy [11-15]. Research by Nishimura et al. showed an increase in forearm cutaneous blood flow reaching 200–250% during immersion in carbon dioxide-rich water, from the pre-immersion control value [16]. Similarly, research by Ogoh et al. showed an increase in popliteal artery blood flow during 20 minutes of foot immersion in warm carbonated water [17]. Peripheral blood circulation disorders are worldwide health problems. Balneotherapy using carbonated mineral water baths and mofetotherapy can be one of the treatment options. The aim of this study is to investigate the effects of natural carbonated mineral water, with a known composition, and the natural mofette from Băile Tușnad on peripheral arterial blood flow, in an experimental study.

2. Material and methods

The current experimental study was carried out according to the following stages:

2.1. Preparation of rat subjects

The experimental study was approved by the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca and the Veterinary Sanitary Commission (approval no. 332/17.09.2022). The animals included in the study belong to the Rattus Norvegicus species, Wistar rats, male, 16 weeks old, clinically healthy, weighing between 250-300 g. The animals come from the authorized sanitary-veterinary biobase, Biobase-Center for Experimental Medicine and Practical Skills, from the University of Medicine and Pharmacy "Iuliu-Haţieganu" Cluj-Napoca. All procedures performed on animals were in accordance with the legislation in force. The animals were kept in cages, according to European standards. The animal space was equipped with an adequate ventilation system that meets the requirements of the housed species. The air in the room was constantly refreshed, at a rate of 10-20 air changes/hour. The optimal temperature inside the accommodation spaces was maintained between 20-24° C. The relative humidity in the accommodation spaces was maintained at the level of 55%+- 10%. The lighting was also controlled, to satisfy the biological needs of the animals and to ensure an optimal working environment, with the light-dark cycle present. The food

administered to the animals was in the form of Granulated Combined Feed (NCG), administered ad libidum, through the bunkers located at the level of the stainless steel covers, on the surface of the cages. The animals benefited from biologically uncontaminated drinking water, ad libidum. The bedding used in the cages was dry, absorbent, non-toxic and free of infectious agents or other forms of contamination.

2.2. Experimental design

A total of 16 rat subjects were studied, divided equally into 4 groups and were evaluated at 2 weeks, 4 weeks and 6 weeks post-treatment. Group 1 was considered the control-group, and no treatment was applied. Group 2 was taken to the mofettes daily, for 2 weeks, for exposure to carbon dioxide, dry gas, for 20 minutes/day.Group 3 performed daily mofettes and baths with carbonated mineral water, for two weeks, 20 minutes/day, each procedure. Group 4 took a daily bath with carbonated mineral water, for 2 weeks, 20 min/day. The composition of carbonated mineral water was as follows: chlorine 49.999 mmol/L, bromines 0.020 mmol/L, sulphates 0.0666 mmol/L, HCO3-13,000 mmol/L, sodium 44,161 mmol/L, potassium 2,307 mmol/L, calcium 4,698 mmol/L, magnesium 3.088 mmol/L, iron 0.376 mmol/L, carbon dioxide 27,000 mmol/L, pH 5.8. Total mineralization of the water is 122,036 mmol/L. The general characteristics of this water refers as an carbonated, ferruginous, chlorinated, bicarbonate, sodium, hypotonic natural mineral water.At the end of the two-week treatment, they were evaluated by doppler ultrasound at the femoral artery, respectively at 2 and 4 weeks, to evaluate the effect of these treatments.The animals were anesthetized by intramuscular administration of a cocktail of products (Ketamine 10% and Xylazine Bio 2%) according to the medical prescription. The animals were groomed by trimming, at the level of the inguinal area of the left hind limb. After the installation of anesthesia, the animals were positioned in supine position, the forelimbs and the right hind limb being fixed on the examination table, with the help of surgical tape. The left hind limb of the animal was fixed in a raised position (~ 0.5 – 1 cm) above the examination table, with the help of a cotton roll (butts). By palpating the inner face of the upper thigh area, the pulse of the femoral artery is identified, being easy to identify (Figure 1).

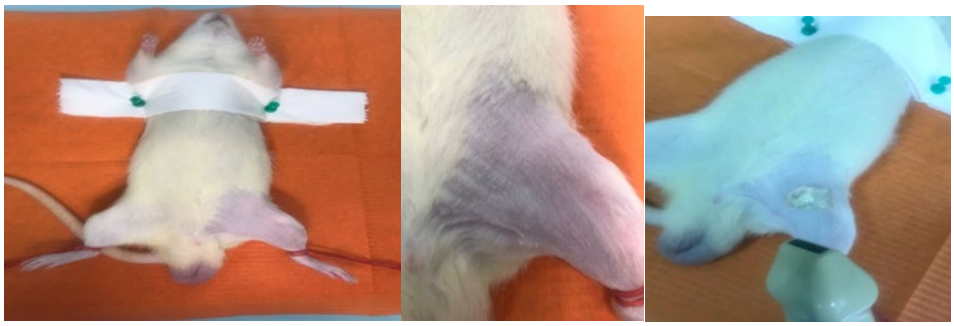

Figure 1. Rat preparation and work area identification for Doppler ultrasound

2.3.Doppler ultrasound – rat femoral artery

A small amount of ultrasound gel was applied to the left hind limb, at the left inguinal area. The parameters of the ultrasound device (ULTRASONIX) are set to mode B. The transducer is set to a depth of 1 mm, so that the superficial femoral artery is easily visible. To confirm the localization of the superficial femoral artery, the PW mode of the ultrasound is selected, so that we can observe the pulsatile blood flow of the artery, compared to the continuous blood flow of the adjacent femoral vein. The determination of the diameter of the femoral artery in the rats included in the study was carried out with the help of the transducer, which was positioned in the transverse section, the imaging

plane being orthogonal to the plane of the vessel. Spectral Doppler was enabled for Doppler measurements. By adjusting the angle as well as the orientation of the transducer, obtaining an angle <700, the spectral Doppler waveforms were highlighted and measured. These waves were well highlighted and presented a white color of maximum intensity, so that the errors resulting from the incorrect settings of the device as well as from the incorrect placement of the transducer were minimized. Femoral artery diameter (mm), peak systolic volume (PVS, cm/s), heart rate (HR, bpm) were quantitatively determined in all 4 groups, at 2, 4 and 6 weeks respectively, and ultrasound images were captured.

2.4. Data analysis

The statistical analysis of the data was performed using the statistical package MedCalc 10.3.0.0 (MedCalc Software, Ostend, Belgium). Considering the small number of cases in each group, non-parametric tests were applied. The numerical data were presented as mean ± standard deviation, respectively the median, and the qualitative data as numbers, respectively percentages. The Friedman test was used to evaluate the differences within the same group at 2 weeks vs 4 weeks vs 6 weeks. Using the Kruskal-Wallis test, the differences between the four groups were evaluated. A p-value less than 0.05 was considered statistically significant.

3. Results

An equal number of 4 subjects were included in each group. Overall, there were significant differences at 2 weeks vs. 4 weeks vs. 6 weeks regarding: (1) femoral artery diameter (mm) 0.73 ±0.05 (0.73) vs 0.82 ± 0.09 (0.85) vs 0.87 ± 0.1 (0.92), p<0.001; (2) PVS (cm/s) – statistical significance was at the limit of p=0.071 - 9.67 ± 3.16 (10.7) vs 11.02 ± 4.04 (11.07) vs 11.37 ± 3.71 (12.17); HR (bpm) p <0.001 - 16.75 ± 1.06 (17) vs 17.62 ± 1.4 (18) vs 19.5 ± 1.86 (20).

In group 1 (control) there were no differences at 2 weeks vs 4 weeks vs 6 weeks regardless of the studied parameter. Complete data regarding the other three lots are presented in Tables 1 and 2. The diameter of the femoral artery increased significantly in group 3 (mofette and carbonated mineral water, p=0.0183), and in group 4 (mineral water, p=0.007). There were no changes regarding the PSV in any of the groups The heart rate changed significantly (p=0.0183) in the mofette group, as well as in the one treated with carbonated mineral water (p=0.024).

Firthermore, it was evaluated the possible differences between the 4 groups at 2 weeks, 4 weeks and 6 weeks. Regarding the diameter of the femoral artery, there was a significant difference at 4 weeks (p=0.0209) and at 6 weeks (p=0.0097). Regarding PSV values, there were differences starting with 2 weeks. For HR, significant differences were recorded only at 6 weeks (p=0.0139). The complete data and resulta can be seen in Tables 1 and 2, and Figures 2-8, respectively.

Table 1. Individual group analysis at 2, 4 and 6 weeks

	Group 1 (control)				Group 2 (mofette)				Group 3 (mofette and carbonated mineral water)				Group 4 (carbonated mineral water)			
Weeks	2	4	6	P	2	4	6	p	2	4	6	p	2	4	6	p
D(mm)	0.69± 0.05 (0.69)	0.69±0.05 (0.69)	0.69±0.05 (0.69)	**NS**	0.73±0.06 (0.74)	0.84 ±0.05 (0.84)	0.90±0.01 (0.90)	**0.084**	0.75 ± 0.03 (0.75)	0.91±0.02 (0.9)	0.94±0.02 (0.94)	**0.0183**	0.74±0.06 (0.72)	0.83±0.08 (0.85)	0.94±.02 (0.94)	**0.007**
PSV (cm/s)	5.71 ± 0.92 (5.97)	5.71±0.92 (5.97)	5.71±0.92 (5.97)	**NS**	10.62±3.92 (10.08)	10.85±2.7 (11.10)	11.38±.81 (11.47)	**NS 0.536**	11.18±.38 (11.22)	13.32±3.52 (13.31)	13.86±1.46 (13.98)	**NS 0.422**	11.16±2.32 (11.41)	14.21±1.75 (14.61)	14.53± 1.1 (14.75)	**NS 0.422**
HR (bpm(	16.75 ±0.95 (16.5)	16.75±0.95 (16.5)	16.75±0.95 (16.5)	**Ns**	15.75±0.95 (15.5)	18 ± 0 (18)	20.75 ± 0.5 (21)	**0.0183**	17.75±0.5 (18)	18.25±1.25 (18)	19.75±1.25 (20)	**NS 0.178**	16.75±0.95 (16.5)	17.50±2.38 (16.5)	20.75± 0.5 (21)	**0.024**

Table 2. Group analysis at 2, 4 and 6 weeks

	p	Significant differences between groups
Diameter - 2 weeks	NS p = 0.6126	
ameter - 4 weeks	p = 0.0209	mineral water vs control; mofette+mineral water vs control; mofette vs control
Diameter - 6 weeks	p = 0.0097	mineral water vs control; mofette+mineral water vs control; mofette vs control mineral water vs mofette; mofette+mineral water vs mofette
PSV- 2 weeks	p = 0.0358	mineral water vs control; mofette+mineral water vs control; mofette vs control
PSV- 4 weeks	p = 0.0174	mineral water vs control; mofette+mineral water vs control; mofette vs control
PSV - 6 weeks	p = 0.0057	mineral water vs control; mofette+mineral water vs control; mofette vs control mineral water vs mofette; mofette+mineral water vs mofette
HR - 2 weeks	p = 0.0750	-
HR - 4 weeks	p = 0.2037	-
HR - 6 weeks	p = 0.0139	mineral water vs control; mofette+mineral water vs control; mofette vs control

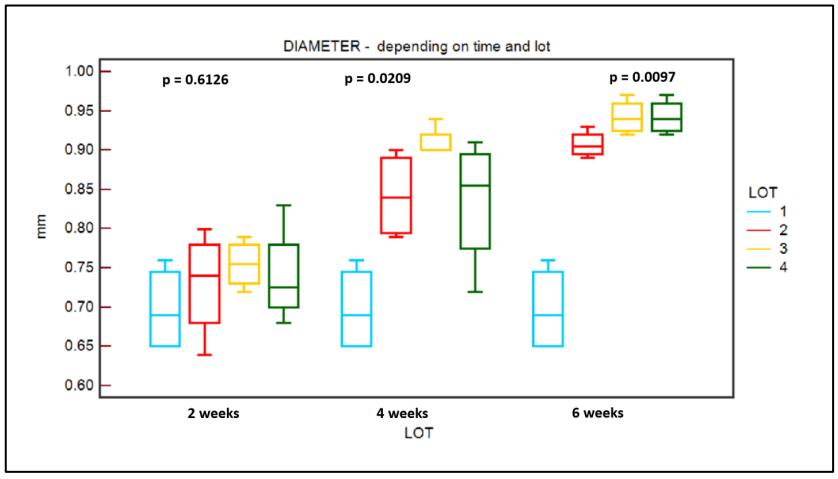

Figure 2. Evolution of diameter(mm) depending on time and lot

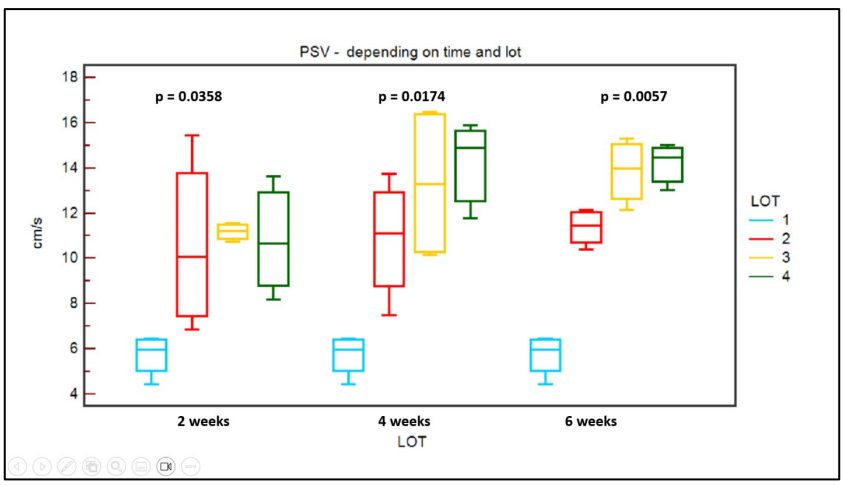

Figure 3. Evolution of PSV(cm/s) depending on time and lot

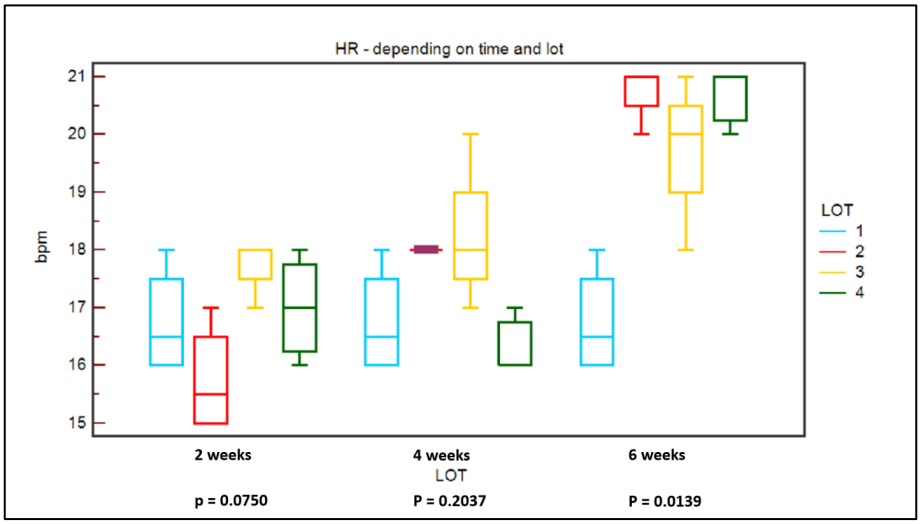

Figure 4. Evolution of HR depending on time and lot

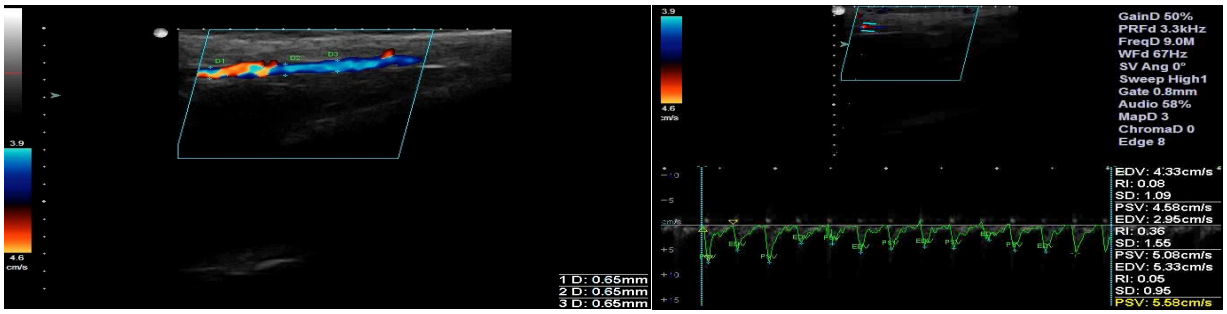

Figure 5. Control group 1 – diameter of the femoral artery remained constant at 0.69 cm at 2, 4, and 6 weeks, as well as PVS and HR

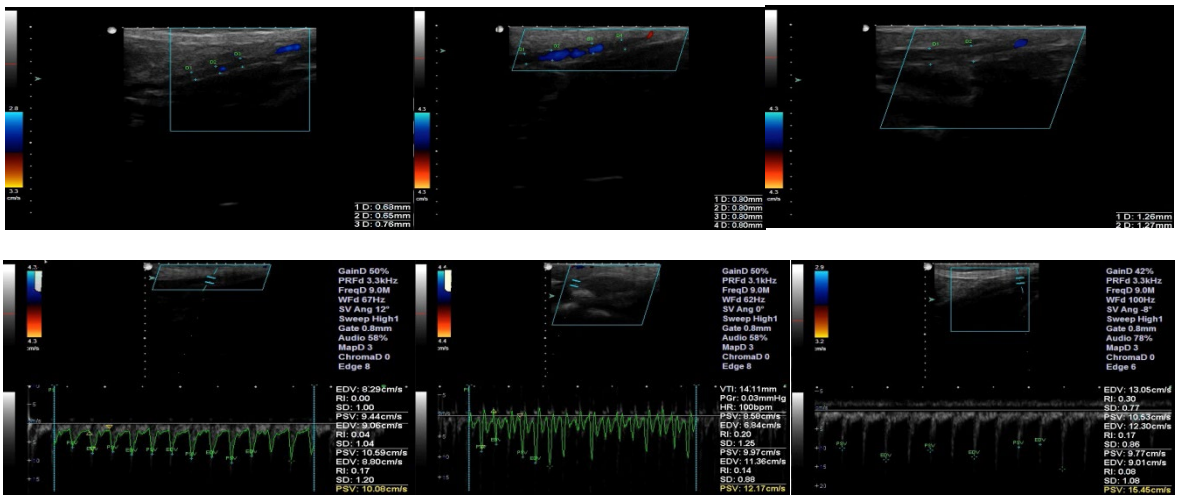

Figure 6. Group 2 (mofette) - femoral artery diameter (cm) and PVS (cm/s) at 2, 4, 6 weeks, the average diameter at 2 weeks was 0.74 cm, at 4 weeks 0.84 cm, and the effect was maintained at the evaluation ultrasound at 6 weeks at 0.90 cm

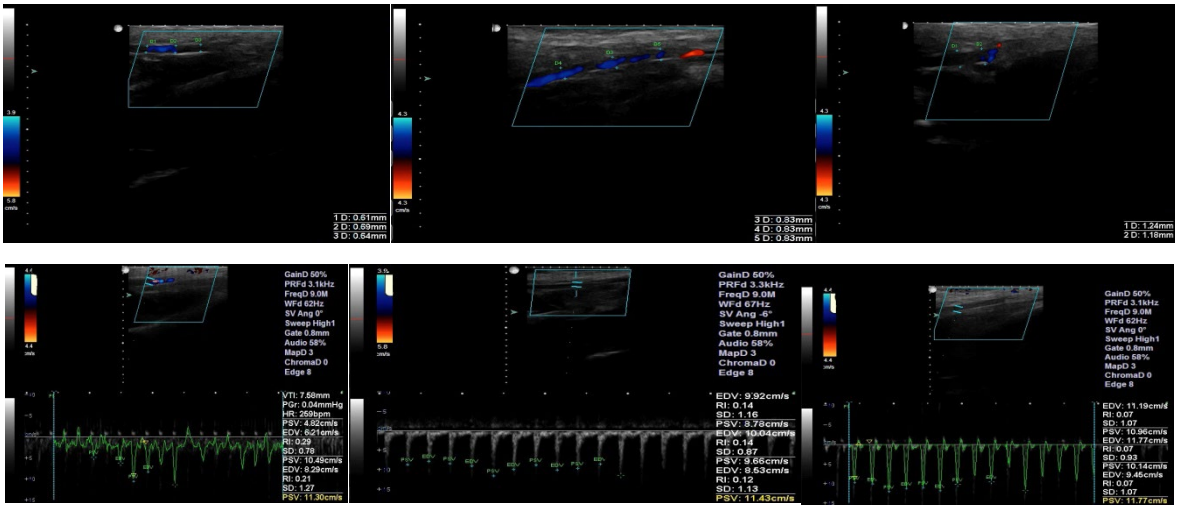

Figure 7. Group 3 (carbonated mineral water and mofette) - femoral artery diameter and PVS (cm/s) at 2, 4 and 6 weeks, the average diameter at 2 weeks was 0.75 cm, at 4 weeks 0.9 cm, at 6 weeks 0.94 cm

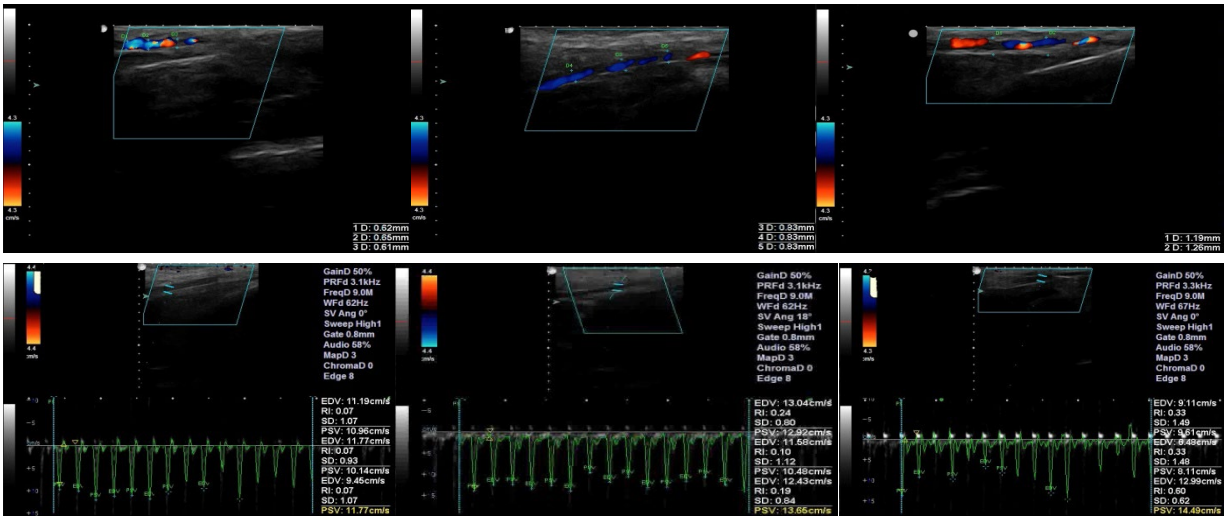

Figure 8. Group 4 (carbonated mineral water) - femoral artery diameter and PVS (cm/s) at 2, 4, 6 weeks, the average value of the diameter at 2 weeks was 0.72cm, at 4 weeks 0.85cm, and at 6 weeks 0.94 cm

4. Discussion

In this experimental study, an increase in blood flow at the femoral artery was found in groups 2, 3 and 4, compared to the control group, at 2 weeks after the end of the treatment, an effect that was maintained including at 4 and 6 weeks after the intervention. In group 1, control, the value of the diameter of the femoral artery remained constant at 0.69 cm, at 2, 4, and 6 weeks, respectively, as did PVS and HR. In group 2, the group that followed the mofette treatment for two weeks, 20 minutes/day, the average diameter at 2 weeks was 0.74 cm, at 4 weeks 0.84 cm, and the effect was also maintained at the ultrasound evaluation from 6 weeks 0.90 cm, with a p-value of 0.084. In group 3, treated with carbonated mineral water by immersing the whole body and exposure to the mofette, 20 minutes/day each, for 2 weeks, the average diameter at 2 weeks was 0.75 cm, at 4 weeks 0.9 cm, at 6 weeks 0.94, with a statistically significant p-value of 0.0183. In group 4, the group that for 2 weeks took a bath with carbonated mineral water, for 20 minutes/day, the average value of the diameter at 2 weeks was 0.72cm, at 4 weeks 0.85cm, and at 6 weeks 0.94cm, with a p-value of 0.007, statistically significant. PVS values (cm/s) in all groups increased at 2,4 and 6 weeks, but they were statistically insignificant, but they were statistically significant with the control group (Table 1,2).

There are published studies that associate the improvement of blood flow with bathing in inorganic salts and carbon dioxide [18,19]. Animal studies have also shown that immersion in carbonated water improves angiogenesis and muscular arterial flow in the lower limbs, improving peripheral circulation, especially when administered repeatedly [20]. Both carbon dioxide and heat are recognized as factors that trigger vasodilation [21,22], but in our study the carbonated mineral water was natural, directly from the spring, at a temperature of 28-30°C. From the statistical analysis of among the 4 groups, significant statistical differences are observed regarding the diameter of the femoral artery, at 2, 4 and 6 weeks. It increased at 4 weeks (Figure 2), but also at 6 weeks after the end of the treatment, which means that the effect was maintained over time. There were also significant statistical differences in terms of PVS (cm/s) and HR (bmp) between the 4 groups, at 2, 4 and 6 weeks respectively (Figures 3 and 4).

The results of the study suggest that carbonated mineral water and mofettes were responsible for the changes in femoral artery flow, compared to the data from the control group.

5. Conclusions

Baths with carbonated natural mineral waters and mofettes could increase the peripheral arterial blood flow, during repeated immersion, and the results are beneficial 2 weeks after the end of the treatment, and were maintained at 4 and 6 weeks, respectively, after the end of the treatment. It is important, however, for natural mineral waters, to know the concentration of anions and cations, the concentration of carbon dioxide, the degree of mineralization, with periodic analyses.

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Informed Consent Statement: "Not applicable."

Data Availability Statement: The data can be provided on reasonable request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Dogaru Gabriela, Bulboaca AE, Stanescu I, Rus V, Bodizs G, Ciumarnean L, Muntean C, Boarescu PM, Neagos A, Festila Dana.The Effect of Carbonated Natural Mineral Water on Oxidative Stress in Experimental Myocardial Ischemia. Revista de chimie . 2019;70(7): 2677-2680
2. Gabriela Dogaru, Adriana Bulboaca, Lorena Ciumărnean, Vasile Rus, Adela-Viviana Sitar-Tăut, Constantin Munteanu, Gyorgy Bodisz, Ioana Stănescu.The effect of mofettes on oxidative stress /antioxidant balance in experimental myocardial ischemic. In Vivo, 2019,33
3. Munteanu Constantin, Dogaru Gabriela, Rotariu Mariana, Onose Gelu. Therapeutic gases used in balneotherapy and rehabilitation medicine - scientific relevance in the last ten years (2011 – 2020) - Synthetic literature review. Balneo and PRM Research Journal DOI: http://dx.doi.org/10.12680/balneo.2021.430 Vol.12, No.2 June 2021 p: 111–122
4. Gabriela Dogaru, Alexandra Ispas, Ioana Stănescu, Marieta Motricala, Molnár Ákos. A clinical study on the efficacy of natural therapeutic factors in Băile Tuşnad for the rehabilitation of post-stroke patients. Balneo Research Journal. 2017;8(1):5-10
5. Gabriela Dogaru, Andreea-Sabina Scripcă, Adina-Eliza Croitoru, Marieta Motricală, Adriana Bulboacă, Ioana Stănescu. A clinical study regarding the improvement of symptoms and the time efficacy of treatments performed in Băile Tuşnad balneoclimatic resort. Balneo Research Journal. 2018;9(2):76-81

6. Gabriela Dogaru, Ioana Stănescu, Daniela Pop, Marieta Motricală, Molnár Ákos. Effects of carbonated mineral water treatment in Băile Tuşnad on chronic arterial occlusive disease – a case report. Balneo Research Journal. 2017;8(3):121-124
7. Fabry R, Monnet P, Schmidt J., et al. Clinical and microcirculatory effects of transcutaneous CO2 therapy in intermittent claudication, Randomized double-blind clinical trial with a parallel design, Vasa, 3, no. 3, 2009, p. 213.
8. Matsumoto T , Bando H . Toi M .Vascular endothelial growth factor: its prognostic, predictive, and therapeutic implications. Lancet Oncol. 2001 Nov;2(11):667-73
9. Elbaz M .VEGF in therapeutic coronary and peripheral artery angiogenesis.Pathol Biol (Paris). 1999 Apr;47(4):380-4
10. Komoto Y, Nakao T, Sunakawa M, Yorozu H. Elevation of tissue PO2 with improvement of tissue perfusion by topically applied CO2. Adv Exp Med Biol .1988;222:637-645.
11. Xu YJ, Elimban V, Dhalla NS. Carbon dioxide water-bath treatment augments peripheral blood flow through the development of angiogenesis. Can J Physiol Pharmacol.2017;95(8):938-44.
12. Irie H, Tatsumi T, Takamiya M, Zen K, Takahashi T, Azuma A, Tateishi K,Nomura T, Hayashi H, Nakajima N, Okigaki M, Matsubara H. Carbon dioxide-rich water bathing enhances collateral blood flow in ischemic hindlimb via mobilization of endothelial progenitor cells and activation of NO-cGMP system.Circulation.2005; 111:1523-1529
13. Dogliotti G, Galliera E, Iorio E, De Bernardi Di Valserra M, Solimene U, Corsi MM. Effect of immersion in CO2-enriched water on free radical release and total antioxidant status in peripheral arterial occlusive disease. Int Angiol. 2011 Feb;30(1):12-7.
14. Andi Rizky Arbaim Hasyar, Haerani Rasyid , Irfan Idris , Irawan Yusuf. Effect of Artificial Carbon Dioxide-Rich Water Immersion on Peripheral Blood Flow in Healthy Volunteers: Preliminary Study about Artificial Carbon Dioxide-Rich Water. Open Access Maced J Med Sci. 2021 Aug 03; 9(A):527-531.
15. S, Imanishi N, Ishizawa T, Yano S, Takeda S,Miyamoto K, et al. The effects of bathing with inorganic salts and carbon dioxide on body temperature, systemic circulation, and food ingestion and absorption. J Japanese Soc Balneol Climatol Phys Med. 2006;69(3):167-78.
16. Nishimura N, Sugenoya J, Matsumoto T, Kato M, Sakakibara H,Nishiyama T, et al. Effects of repeated carbon dioxide-rich water bathing on core temperature, cutaneous blood flow and thermal sensation. Eur J Appl Physiol. 2002;87(4-5):337-42.
17. Ogoh S, Washio T, Suzuki K, Ikeda K, Hori T, Olesen ND, et al.Effect of leg immersion in mild warm carbonated water on skin and muscle blood flow. Physiol Rep. 2018;6(18):e13859.
18. Ogoh S, Nagaoka R, Mizuno T, Kimura S, Shidahara Y, Ishii T, et al. Acute vascular effects of carbonated warm water lower leg immersion in healthy young adults. Physiol Rep. 2016;4(23):e13046.
19. Watanabe S, Imanishi N, Ishizawa T, Yano S, Takeda S, Miyamoto K, et al. The effects of bathing with inorganic salts and carbon dioxide on body temperature, systemic circulation, and food ingestion and absorption. J Japanese Soc Balneol Climatol Phys Med. 2006;69(3):167-78.
20. Irie H, Tatsumi T, Takamiya M, Zen K, Takahashi T, Azuma A, *et al*. Carbon dioxide-rich water bathing enhances collateral blood flow in ischemic hindlimb via mobilization of endothelial progenitor cells and activation of NO-cGMP system. Circulation. 2005;111(12):1523-9.
21. Inoue S, Takemoto M, Chishaki A, Ide T, Nishizaka M, Miyazono M, *et al*. Leg heating using far infra-red radiation in patients with chronic heart failure acutely improves the hemodynamics, vascular endothelial function, and oxidative stress. Intern Med. 2012;51(17):2263-70.
22. Pagourelias E, Zorou PG, Tsaligopoulos M, Athyros VG, Karagiannis A, Efthimiadis GK. Carbon dioxide balneotherapy and cardiovascular disease. Int J Biometeorol. 2010;55(5):657-63.