BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902020000419177

Article

Chronic and acute effects of kefir: the role of angiotensin converting enzyme inhibition instead of nitric oxide balance

Tadeu Uggere de Andrade[1], Karolina Silva Schumacker[1], Karina Silva Schumacker[1], Gabriela Coutinho[1], Mahira Sabino Rezende[1], Silas Nascimento Ronchi[2], Ieda Carneiro Kalil[1], Mirian de Almeida Silva-Cutini[1], Ewelyne Miranda de Lima[1], June Ferreira Maia[1], Girlandia Alexandre Brasil[1*]

[1]Pharmaceutical Sciences Graduate Program, University Vila Velha, Vila Velha, ES, Brazil, [2]Physiological Sciences Department, Health Sciences Center Federal University of Espírito Santo, Vitória, ES, Brazil

Probiotic consumption promotes numerous health benefits. The aim of this study is 1) to evaluate the antihypertensive effect of kefir in a hypertension rat model caused by the administration of the nitric oxide synthesis inhibitor, L-NAME, and 2) to evaluate the acute angiotensin converting enzyme (ACE) inhibitory activity of the soluble nonbacterial fraction (SNBF) of kefir. To develop the first aim, male rats were separated into three groups: control group (C) treated with 0.3 mL/100 g of milk; L-NAME group (LN) received 10 mg/kg of said inhibitor; and Kefir group (K) treated with 0.3 mL/100 g of kefir plus L-NAME (10 mg/kg of said inhibitor). The treatments were given by oral gavage twice a day for four weeks. For the second aim"instead additionally, male rats received angiotensin I (*in bolus*) in three doses (Ang I: 0.03, 3 and 300 µg/kg) and were separated into two groups: a) received captopril (30 mg/kg i.v.) and b)received SNBF of kefir (5 mL/kg i.v.). Blood pressure were evaluated before and after Ang I. After treatment, hemodynamic parameters were evaluated, heart weight was recorded, and body weight gain was calculated. SNBF of kefir did not decrease the blood pressure for L-NAME-treated animals, and no changes were observed in the cardiac parameters. However, the SNBF of kefir demonstrated acute inhibition of ACE *in vivo* similar to that of captopril. Thus, our results suggest that kefir may improve human cardiovascular systems by using mechanisms independent of nitric oxide syntheses. Additionally, the renin angiotensin system is probably the most important system involved in kefir effect regarding hypertension.

Keywords: Nitric oxide. Blood pressure. Probiotic. ACE inhibition. Soluble nonbacterial fraction.

INTRODUCTION

Probiotics are live microorganisms that, when consumed, promote valuable health benefits, and they are also found in medicines as well as naturally in some foods (Sanders 2015). Kefir is a fermented milk that supports gut regulation, reduces inflammation and regulates baroreflex, as demonstrated in previous works (Klippel *et al*. 2016; Rosa *et al*. 2016; Kim *et al*. 2017). Improving the gut microbiome offers many health advantages (Sirisinha 2016). Further, it is well established that dysbiosis is involved in the development of many diseases, including hypertension (Richards *et al*., 2016; Yang, Zubcevic, 2017).

In addition to modulating the gut microbiota, kefir also uses its bioactive peptides to benefit physiological systems, including the cardiovascular system, and to produce immunomodulatory, antioxidant and antithrombotic effects as well as angiotensin converting enzyme (ACE) inhibitory activities (Ebner *et al*. 2015).

In recent studies, our group demonstrated the importance and influence of kefir treatment for hypertension (Friques *et al*. 2015; Klippel *et al*. 2016;

[*]**Correspondence:** G. A. Brasil. Pharmaceutical Sciences Graduate Program (UVV). Phone: 55-27-3421-2091Fax: 55-27-3421-2087. Av. Comissário José Dantas de Melo 21. 29102-920. Boa Vista, Vila Velha, ES, Brazil. Email: gi.faraway@gmail.com

Brasil *et al*. 2018) in an animal model of essential hypertension using Spontaneously Hypertensive Rats (SHR) which indicated that kefir caused a decrease in blood pressure using both its probiotic action and its bioactive peptides. However, the effects of kefir in other animal models for hypertension with shorter treatment time and other mechanisms independent of the ACE inhibitory action have not yet been evaluated.

Furthermore, secondary hypertension is caused by endothelial dysfunction, kidney dysfunction, a decrease in nitric oxide production and other factors (Puar *et al*., 2016; Rimoldi, Scherrer, Messerli, 2018). The synthesis of nitric oxide (NO), a potent vasodilator agent, has an important effect on blood pressure control and impacts the development of hypertension (Kružliak, Novák, Novák, 2014; Tain, Huang, 2014).

Decreased NO synthesis or bioavailability advances an increase in vascular resistance and the development of hypertension. This is supported by the chronic intake of NO synthase from N(ω)-nitro-L-arginine methyl ester (L-NAME) (Dornas, Silva, 2011), which provides an important model for studying the mechanisms of action of new drugs (Yang *et al*. 2012; Zhu *et al*. 2018), ultimately indicating that the kefir treatment acts as donor of NO and promotes acute vasorelaxation.

Therefore, the aim of the present study was to evaluate the effects of a four-week kefir treatment on the blood pressure and cardiac alterations induced in rats by the chronic inhibition of NO synthesis with L-NAME. Additionally, we analyzed the acute ACE inhibitory activity of the soluble, nonbacterial fraction of kefir. Thus, our hypothesis was that treatment with kefir would increase vasorelaxation and subsequently reduce high blood pressure, describing a new mechanism of kefir regarding hypertension.

MATERIAL AND METHODS

Kefir preparation

Kefir was prepared from grains kindly given by Dr. Célia L.L.F. Ferreira of Federal University of Viçosa (UFV). The grains were added to pasteurized milk at a concentration of 4% (m/v) and were kept at room temperature for 24 hours (Santanna *et al*. 2016). The grains were then separated by filtration and the fermented milk obtained was stored in a refrigerator (8 ºC) for an additional 24 hours. Lastly, the fermented milk was maintained at -80 ºC until its usage.

Separation of the soluble, nonbacterial fraction of kefir

After the production of milk the soluble, nonbacterial fraction of kefir (SNBF) was separated according to Tsai *et al*. (2008). Briefly, the milk was heated to 98 ºC for four minutes, followed by centrifugation at 10000 rpm (4 ºC) for 30 minutes and it is noteworthy that the supernatant was filtered. The supernatant was collected and frozen (-80 ºC) until the analysis. The control was prepared with milk constituting a pH adjusted to 4 using acetic acid.

Experimental animals

For the experiments, 2-3-month-old male *Wistar* rats weighing between 200-300 g were used. The animals were grouped in cages with controlled temperature and humidity, a dark-light cycle of 12 hours and food and water *ad libitum*. The animals were provided by the animal care facility from the University of Vila Velha (UVV), Vila Velha, Brazil. All procedures were approved by the Institutional Committee on Animal Care (CEUA, Protocol# 040/2014)and were performed in agreement with the guidelines for the care and use of laboratory animals as recommended by the National Institutes of Health (NIH) (Garber *et al*. 2010).

Chronic evaluations

To determine whether kefir treatment plays a beneficial role in the hypertension that develops due to insufficient nitric oxide formation, the animals received a chronic administration of *N*(ω)-nitro-L-arginine methyl ester (L-NAME; 10 mg/kg) and were separated into three groups (n=7 each) as follows: the control group (C) received milk (0.3 mL/100 g) by gavage as the vehicle for kefir; the L-NAME group (LN) received milk as the vehicle for kefir and L-NAME at 10 mg/kg; and the kefir group (K) received kefir (0.3 mL/kg; Friques *et al*., 2015) and the same dosage of L-NAME. All treatments were given twice a day for four weeks. After that period, the animals were subjected to hemodynamic evaluations. It is important to note that L-NAME was administered through the drinking water.

Acute evaluations

Indeed, kefir is a source of bacteria and yeast, and thus the acute evaluations were performed with the SNBF

of kefir. This process was accomplished by anesthetizing the animals with a mixture of ketamine and xylazine (100/10 mg/kg), and their femoral artery and vein were catheterized. The animals were then separated into two groups (n=6 each) for evaluation as follows: the captopril group received captopril (30 mg/kg; i.v.); and the kefir group received the soluble fraction of kefir (5 mL/kg; i.v.). None of the animals in these groups received pre-treatment with L-NAME. Lastly, the infusion was performed using an infusion pump (Insight®, Ribeirão Preto, São Paulo, Brazil) at a rate of 0.5 mL/min (Mangiapane *et al.* 1994).

To determine the acute capacity of ACE inhibition, three doses of Angiotensin I (Ang I: 0.03; 3 and 300 μg/kg, i.v.) were injected to the animals before and after the administration of either SNBF or captopril. The difference in blood pressure increased after the inhibition (ΔMAP mmHg) and this change was used to determine the ACE inhibition.

Blood pressure measurements

The animals with the chronic treatment were anesthetized with a mixture of ketamine and xylazine (100/10 mg/kg, i.p.). After the anesthesia was complete, the femoral artery and vein were separated and catheterized (Brasil *et al*. 2014). At least 24 hours after the surgical procedure, the blood pressure (Mean Arterial Pressure (MAP), Diastolic Blood Pressure (DBP), Systolic Blood Pressure (SBP)) and heart rate (HR) were determined as previously described (Andrade *et al*. 2011).

Ponderal data

The animals with the chronic treatment were weighed to determine the difference between the initial and final body weights (ΔBW). After treatment, the animals were euthanized with an anesthetic overdose, and their hearts were excised, washed, cleaned and weighed to determine cardiac hypertrophy. The ratio of the heart weight (HW) to the body weight (BW) was used as an index of cardiac hypertrophy.

Statistical analysis

The data are presented as the mean ± standard error mean (S.E.M.). All the results were subjected to a one-way variance analysis (ANOVA) followed by Tukey's post hoc test. The differences were significant when $p<0.05$.

RESULTS

The principal result from the present study was that kefir was unable to prevent the hypertension caused by L-NAME. Nonetheless, kefir promoted acute ACE inhibition, which was probably the main mechanism of action for decreasing the blood pressure in the hypertensive animals.

Kefir did not promote a decrease in the MAP and cardiac hypertrophy by using a model of hypertension caused by L-NAME

As expected, the ingestion of L-NAME at a dose of 10 mg/kg by the *Wistar* rats, promoted an increased mean MAP and, consequently, cardiac hypertrophy (Figure 1). In addition, chronic treatment with kefir did not cause a decrease in this parameter for the kefir-treated group.

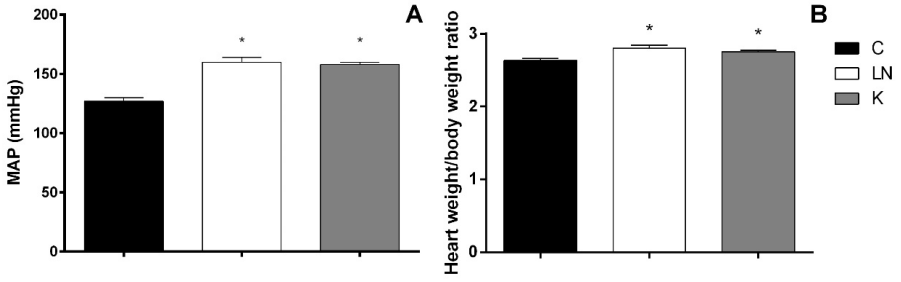

FIGURE 1 – Effect of chronic treatment with kefir and L-NAME on the Mean Arterial Pressure (MAP). * $p<0.05$ compared to the C group. C: control animals that received milk (0.3 mL/100 g twice a day) as a vehicle for kefir; LN: L-NAME-treated animals that received milk (0.3 mL/100 g twice a day) and L-NAME (10 mg/kg); K: kefir-treated animals that received kefir 0.3 mL/100 g twice a day and L-NAME. L-NAME was provided in the drinking water, and the milk and kefir were administered by gavage.

Hemodynamic parameters were not changed after chronic treatment with kefir in the L-NAME model of hypertension

All the other parameters were evaluated after the chronic treatment of L-NAME in hypertensive animals and neither changed after the kefir treatment (Table I).

Hemodynamic parameters were not changed after chronic treatment with kefir in the L-NAME model of hypertension.

Kefir prevented the increase in blood pressure promoted by Ang I

With the aim of determining the potential of the kefir treatment to decrease the blood pressure by other mechanisms, we evaluated acute ACE inhibition *in vivo*. Kefir prevented an increase in blood pressure assisted by Ang I ($p<0.01$) in which the first dose (0.03 μg/kg) exhibited a similar prevention to that of captopril (Figure 2).

Kefir prevented the increase in blood pressure promoted by Ang I.

TABLE I – Values of the parameters evaluated after a chronic treatment with kefir in the L-NAME model of hypertension

Groups	Parameters					
	MAP	SBP	DBP	HR	HW/BW	ΔBW
C	127±3	154±3	100±4	395±15	2.63±0.03	34±5
LN	160±4*	192±3*	121±3*	381±9	2.80±0.04*	39±4
K	158±2*	187±3*	123±4*	391±4	2.75±0.03*	27±6

The values are presented as the mean± standard error of mean (SEM). * $p<0.05$ compared to the C group.

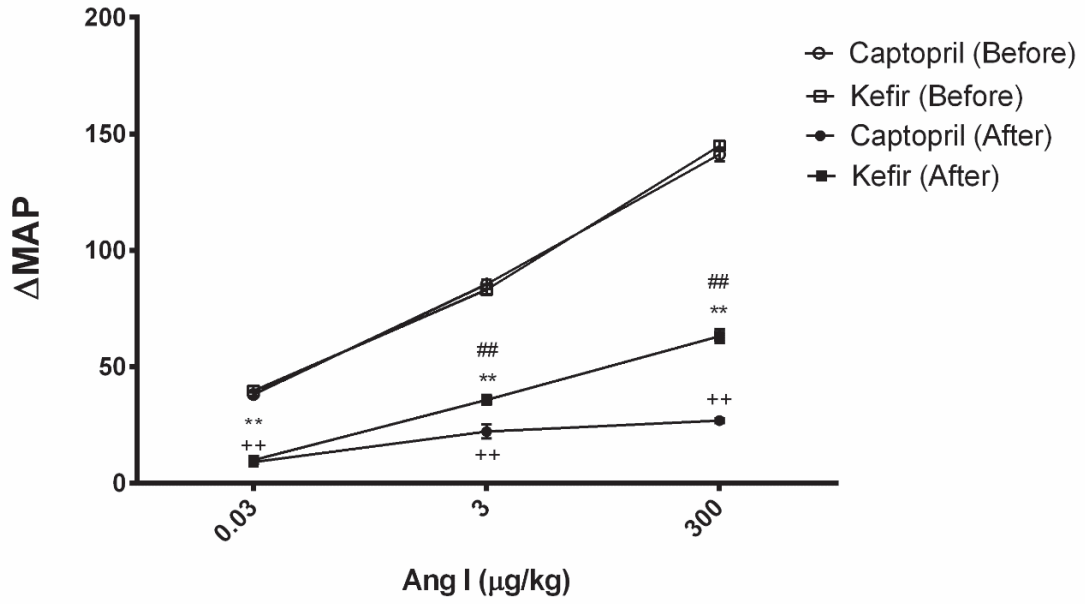

FIGURE 2 – Acute effect of Captopril or kefir on ACE. Both promoted a decrease in the Ang I effect on blood pressure. For the first dose, the effect of kefir was the same as captopril. Captopril and kefir (Before), effect promoted by Ang I before a *bolus* application of the substances. Captopril and kefir (After), effect promoted by Ang I after a *bolus* application of the substances. ** $p<0.01$ compared to the kefir before group; ++ $p<0.01$ compared to the captopril before group; ## $p<0.01$ compared to the captopril after group.

DISCUSSION

Our results demonstrated that, for the first time, kefir was incapable of decreasing blood pressure in L-NAME-induced hypertension after four weeks of treatment. The results suggested that the period of treatment may not have been sufficient to demonstrate the effect of kefir on vascular relaxation nor on NO bioavailability in this animal model. Furthermore, the chief mechanism of action of kefir in hypertension may be the inhibition of the angiotensin converting enzyme.

Hypertension is classified as either essential or secondary, according to the main pathophysiology mechanisms (PK *et al*. 2017), however the former is multifactorial and more prevalent. In 2010, approximately 34 million people were diagnosed with essential hypertension in the United States (Mozaffarian *et al*. 2016), which significantly impacted health system costs (Elliott 2003). On the other hand, secondary hypertension is less prevalent and yet it is responsible for 10% of hypertension in adults (PK *et al*. 2017). Secondary hypertension differs from essential hypertension because it is caused by a known event or pathophysiological condition such as primary aldosteronism, renal stenosis and others (Rimoldi, Scherrer, Messerli, 2018).

Additionally, the hypertension induced by the intake of L-NAME mimics a situation where the endothelium is not capable of synthetizing NO, a potent vasodilator agent (Dornas, Silva, 2011). This kind of model evaluates the capacity of the treatment to donate NO to further treat hypertension promoted by the decreased NO bioavailability.

In this present study, the short treatment with kefir did not cause a decrease in blood pressure in the animals treated with L-NAME. Also, none of the parameters measured (SBP; DBP; HR; HW/BW; ΔBW;) were changed with the kefir treatment. Previously, our group showed that kefir decreased blood pressure after a long treatment period using a spontaneously hypertensive rats model of hypertension (Friques *et al*. 2015; Klippel *et al*. 2016; Brasil *et al*. 2018), which is an animal model that mimics essential hypertension in humans and that includes multiple mechanisms involved in the rise of blood pressure (Dornas, Silva, 2011).

Additionally, there are different systems involved regarding the positive effects of kefir. For example, Klippel *et al*., (2016) demonstrated that treatment with kefir for eight weeks lowered blood pressure and heart rate in SHR animals. This decrease was followed by an ameliorated autonomic control of pressure, indicating the possibility of kefir to act on the neural control of pressure. Friques *et al*., (2015) evaluated the effects of kefir treatment on endothelial function during eight weeks and observed an improvement in said function associated with a restoration of the ROS/NO imbalance. Here, the difference between the results demonstrated the effect of time on the treatment with kefir and the models used.

Recently, studies have focused on showing the potential role of probiotics against hypertension. However, it is also necessary to evaluate the different parameters and mechanisms that influence this activity (Chen *et al*. 2014; Yap *et al*. 2016; Robles-Vera *et al*. 2017). Some other authors demonstrated that, for probiotic action, time is an important factor for reaching beneficial effects (Ahrén *et al*., 2015; Yap *et al*., 2016). Previous study demonstrated that the probiotic effect for *Lactobacillus paracasei,* was time-dependent and the resulting positive effects on the immune stimulation started after nine weeks of administration (Tsai *et al*. 2008). In animal models, Chen *et al*., (2014) evaluated the effect of *Lactobacillus helveticus* strains on hypertension and found that seven weeks of treatment was sufficient to reveal a significant antihypertensive effect for both systolic and diastolic blood pressure, which was accompanied by a reduction in the extent of left ventricular hypertrophy. Yap *et al*., (2016) discovered that administrating *Lactobacillus casei* strain promotes a decrease on systolic and diastolic blood pressure, reverted aorta remodeling and promoted NO production, which improved vasodilation after eight weeks of treatment in hypertensive animals. All the treatments were experimented for longer periods in the SHR models of hypertension.

In contrast, the effect of probiotics on the L-NAME model of hypertension, in a shorter period of time, is also reported in literature (Xu *et al*. 2013; Ahrén *et al*. 2015; Yang *et al*. 2015; Robles-Vera *et al*. 2018). Our results are consistent with data from other authors, showing no effect on systolic and diastolic blood pressure after a four week treatment with a dietary supplement of *Lactobacillus plantarum* together with fermented blueberry (Xu *et al*. 2013; Ahrén *et al*. 2015). Robles-Vera *et al*., (2018) proved that *Lactobacillus fermentum* CECT5716 (LC40) treatment did not prevent the hypertension induced by L-NAME, despite being capable of preventing gut dysbiosis and reducing vascular oxidative stress and pro-inflammatory status.

The health benefits of the ingestion of probiotics is extensively explored (Leite *et al*., 2013; Nielsen, Gürakan, Ünlü, 2014; Sanders, 2015; Beilharz *et al*., 2018) of which the probiotic kefir contributes to the reported positive effects (Quirós *et al*. 2005; Kim *et al*. 2017). These effects are attributed to kefir's ability to modulate the gut microbiome, and it is also observed that dysbiosis negatively affects other systems including the cardiovascular system (Yang *et al*. 2015). Additionally, studies indicate that gut dysbiosis in hypertension varies, according to the model of hypertension, through the modulation of bacteria, which is considered important in the regulation of the immune system, thus influencing both the gut microbiota and blood pressure (Yang, Zubcevic, 2017; Robles-Vera *et al*., 2018).

A microbiological analysis of the grains used in our study was previously conducted and demonstrated the presence of a great source of bacteria (*Acetobacter aceti*, *Acetobacter* sp., *Lactobacillus delbrueckii delbrueckii*, *Lactobacillus fermentum*, *Lactobacillus fructivorans, Enterococcus faecium*, *Leuconostoc* spp.), as well as *Lactobacillus kefiranofaciens* and yeasts (*Candida famata, Candida krusei*) (Friques *et al*. 2015). In addition to its effect on the gut microbiome, the fermentation of milk induces the release of many peptides, which are absorbed by the intestinal mucosa and offers health benefits (Quirós *et al*. 2005; Ebner *et al*. 2015; Santanna *et al*. 2016; Brasil *et al*. 2018). Ebner *et al*., (2015) demonstrated that the fermentation of milk kefir lead to the release of 236 different peptides from which sixteen had previously indicated beneficial properties such as antimicrobial, antioxidant, ACE inhibitor and others. This finding indicates that these peptides, rather than microbiome activity, had positive effects on the organism.

In this study, the acute effect of SNBF of kefir on ACE was evaluated and demonstrated the advancement of an acute ACE inhibition, which was observed by a reduction in the increase in blood pressure promoted by Ang I. This result corroborated with others from our group (Brasil *et al*. 2018), which showed that kefir established ACE inhibition and that other mechanisms of action attributed to the cardiovascular effects of kefir.

With respect to the effects of kefir on other parameters, no effect from the kefir treatment on cardiac hypertrophy and on body weight gain was observed. Additionally, it was expected that the animals treated with L-NAME presented cardiac hypertrophy (Adamcova, Ruzickova, Simko, 2013), as demonstrated by an increased HW/BW ratio, and no changes on body weight gain. (Buckley, Johns, 2011). However, as previously observed, kefir has the capacity to decrease cardiac hypertrophy in an SHR hypertension model (Klippel *et al*. 2016), and the differences in the results observed when compared to our study can be attributed, at least in part, to the divergence in the time of treatment and to the animal model used.

This study was limited by the lack of evaluation on analyzing the bacterial population present in the gut and fecal samples from the animals, bioactive compounds from the soluble and nonbacterial fraction of kefir. Although literature indicates that changes in the gut microbiota after kefir treatment promote a reduction in blood pressure, the presence of peptides and others active compounds in this fraction could bring more reliability to our findings. Therefore, further studies are necessary to identify the bacterial population and the bioactive compounds in the soluble fractions of kefir that explain the unchanged pressure in this model of hypertension.

CONCLUSION

In conclusion our results demonstrated that kefir did not diminish blood pressure in the L-NAME model of hypertension after four weeks of treatment. Additionally, none of the other parameters evaluated were ameliorated from the treatment. Lastly, kefir promoted acute angiotensin-converting enzyme inhibition, proving that the effect on the renin angiotensin system was probably the major mechanism of action for the probiotic.

ACKNOLEDGMENTS

We would like to give acknowledgement and gratitude to FAPES for their financial support. TUA receives fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq: grant number 311925/2018-9).

DECLARETION OF INTEREST

None

REFERENCES

Adamcova M, Ruzickova S, Simko F. Multiplexed immunoassays for simultaneous quantification of cardiovascular biomarkers in the model of hg-nitro-l-arginine

methylester (l-name) hypertensive rat. J Physiol Pharmacol. 2013;64(2):211–7.

Ahrén IL, Xu J, Önning G, Olsson C, Ahrné S, Molin G. Antihypertensive activity of blueberries fermented by Lactobacillus plantarum DSM 15313 and effects on the gut microbiota in healthy rats. Clin Nutr. 2015;34(4):719–26.

Andrade TU De, Loiola LZ, Merces S, Alcure N, Raquel A, Medeiros S, et al. Role of the renin – angiotensin system in the nandrolone-decanoate-induced attenuation of the Bezold – Jarisch reflex. Can J Physiol Pharmacol. 2011;897:891–7.

Beilharz JE, Kaakoush NO, Maniam J, Morris MJ. Cafeteria diet and probiotic therapy: cross talk among memory, neuroplasticity, serotonin receptors and gut microbiota in the rat. Mol Psychiatry. 2017;00(January):1–11.

Brasil GA, Ronchi SN, Nascimento AM do, de Lima EM, Romão W, da Costa HB, et al. Antihypertensive Effect of Carica papaya Via a Reduction in ACE Activity and Improved Baroreflex. Planta Med. 2014;80(17):1580–7.

Brasil GA, Silva-Cutini M de A, Moraes F de SA, Pereira T de MC, Vasquez ECEC, Lenz D, et al. The benefits of soluble non-bacterial fraction of kefir on blood pressure and cardiac hypertrophy in hypertensive rats are mediated by an increase in baroreflex sensitivity and decrease in angiotensin-converting enzyme activity. Nutrition. 2018;51–52(2018):66–72.

Buckley MM, Johns EJ. Impact of L-NAME on the cardiopulmonary reflex in cardiac hypertrophy. Am J Physiol Regul Integr Comp Physiol. 2011;301(5):R1549-56.

Chen Y, Liu W, Xue J, Yang J, Chen X, Shao Y, et al. Angiotensin-converting enzyme inhibitory activity of Lactobacillus helveticus strains from traditional fermented dairy foods and antihypertensive effect of fermented milk of strain H9. J Dairy Sci. 2014;97(11):6680–92.

Dornas WC, Silva ME. Animal models for the study of arterial hypertension. J Biosci. 2011;36(4):731–7.

Ebner J, Arslan AA, Fedorova M, Hoffmann R, Kuçukçetin A, Pischetsrieder M. Peptide profiling of bovine kefir reveals 236 unique peptides released from caseins during its production by starter culture or kefir grains. J Proteomics. 2015;17:41–57.

Elliott WJ. The economic impact of hypertension. J Clin Hypertens. 2003;5(3 Suppl 2):3–13.

Friques AGF, Arpini CM, Kalil IC, Gava AL, Leal MA, Porto ML, et al. Chronic administration of the probiotic kefir improves the endothelial function in spontaneously hypertensive rats. J Transl Med. 2015;13:1–16.

Garber JC, Barbee RW, Bielitzki JT, Clayton LA, Donovan JC, Hendriksen CFM, et al. National Institute of Health: Guide for The Care and Use of Laboratory Animals. 8th ed. Wasington D.C.: The National Academies Press; 2010.

Kim DH, Kim H, Jeong D, Kang IB, Chon JW, Kim HS, et al. Kefir alleviates obesity and hepatic steatosis in high-fat diet-fed mice by modulation of gut microbiota and mycobiota: targeted and untargeted community analysis with correlation of biomarkers. J Nutr Biochem. 2017;44:35–43.

Klippel BF, Duemke LB, Leal MA, Friques AGF, Dantas EM, Dalvi RF, et al. Effects of Kefir on the Cardiac Autonomic Tones and Baroreflex Sensitivity in Spontaneously Hypertensive Rats. Front Physiol. 2016;7:1–12.

Kružliak P, Novák J, Novák M. Vascular Endothelial Growth Factor Inhibitor – Induced Hypertension: From Pathophysiology to Prevention and Treatment Based on Long-Acting Nitric Oxide Donors. Am J Hypertens. 2014;27:3–13.

Leite AM de O, Miguel MAL, Peixoto RS, Rosado AS, Silva JT, Paschoalin VMF. Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage. Brazilian J Microbiol. 2013;44(2):341–9.

Mangiapane ML, Rauch AL, Macandrew JT, Ellery SS, Hoover KW, Knight DR, et al. Vasoconstrictor action of angiotensin I-convertase and the synthetic substrate (Pro11,D-Ala12)-angiotensin I. Hypertension. 1994;23(6):857–60.

Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. AHA Statistical Update Heart Disease and Stroke Statistics — 2016 Update A Report From the American Heart Association. Circulation. 2016;133:e38–360.

Nielsen B, Gürakan GC, Ünlü G. Kefir: A Multifaceted Fermented Dairy Product. Probiotics Antimicrob Proteins. 2014;6(3–4):123–35.

Puar THK, Mok Y, Debajyoti R, Khoo J, How CH, Ng AKH. Secondary hypertension in adults. Singapore Med J. 2016;57(5):228–32.

Quirós A, Hernández-Ledesma B, Ramossss M, Amigo L, Recio I. Angiotensin-Converting Enzyme Inhibitory Activity of Peptides Derived from Caprine Kefir. J Dairy Sci. 2005;88:3480–7.

Richards EM, Pepine CJ, Raizada MK, Kim S. The Gut, Its microbiome, and hypertension. Curr Hypertens Rep. 2016;137(32):10160–3.

Rimoldi SF, Scherrer U, Messerli FH. Clinical update Secondary arterial hypertension : when, who, and how to screen ? Eur Heart J. 2018;(35):1245–54.

Robles-Vera I, Toral M, de la Visitación N, Sánchez M, Romero M, Olivares M, et al. The probiotic Lactobacillus fermentum prevents dysbiosis and vascular oxidative stress in rats with hypertension induced by chronic nitric oxide blockade. Mol Nutr Food Res. 2018;62(19):1800298.

Robles-Vera I, Toral M, Romero M, Jiménez R, Sánchez M, Pérez-Vizcaíno F, et al. Antihypertensive Effects of Probiotics. Curr Hypertens Rep. 2017;19(4).

Rosa DD, Grzeskowiak LM, Ferreira CLLF, Fonseca ACM, Reis SA, Dias MM, et al. Kefir reduces insulin resistance and inflammatory cytokine expression in animal model of metabolic syndrome. Food Funct. 2016;1–34.

Sanders ME. Probiotics in 2015: Their Scope and Use. J Clin Gastroenterol. 2015;49:S2–6.

Santanna AF, Filete PF, Lima EM, Porto ML, Meyrelles SS, Vasquez EC, et al. Chronic administration of the soluble non-bacterial fraction of kefir attenuates lipid deposition in LDLr-/- mice. Nutrition. 2016;35:100–5.

Sirisinha S. The potential impact of gut microbiota on your health: Current status and future challenges. Asian Pacific J Allergy Immunol. 2016;34(4):249–64.

Tain Y, Huang L. Restoration of Asymmetric Dimethylarginine – Nitric Oxide Balance to Prevent the Development of Hypertension. Int J Mol Sci. 2014;15:11773–82.

Tsai J-S, Chen T-J, Pan BS, Gong S-D, Chung M-Y. Antihypertensive effect of bioactive peptides produced by protease-facilitated lactic acid fermentation of milk. Food Chem. 2008;106(2):552–8.

Whelton PK, Carey RM, Aronow WS, Ovbiagele B, Casey DE, et al. Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task force on Clinical Practice Guidelines. J Am Coll Cardiol. 2017;283.

Xu J, Ahrén IL, Prykhodko O, Olsson C, Ahrné S, Molin G. Intake of blueberry fermented by lactobacillus plantarum affects the gut microbiota of L-NAME treated rats. Evidence-based Complement Altern Med. 2013;2013.

Yang H, Hwang I, Koo T, Ahn H, Kim S, Park M, et al. Beneficial effects of Acer okamotoanum sap on L-NAME-induced hypertension-like symptoms in a rat model. Mol Med Rep. 2012;427–31.

Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, et al. Gut Dysbiosis is Linked to Hypertension. Hypertension. 2015;65(6):1331–40.

Yang T, Zubcevic J. Gut-brain axis in regulation of blood pressure. Front Physiol. 2017;8(OCT):1–12.

Yap WB, Ahmad FM, Lim YC, Zainalabidin S. Lactobacillus casei strain C1 attenuates vascular changes in spontaneously hypertensive rats. Korean J Physiol Pharmacol. 2016;20(6):621–8.

Zhu F, Hu J, Lv F, Cheng P, Gao S. Effects of oligomeric grape seed proanthocyanidins on L- NAME-induced hypertension in pregnant mice: Role of oxidative stress and endothelial dysfunction. Phyther Res. 2018;(November 2017):1–12.

Received for publication on 19th February 2019
Accepted for publication on 12nd July 2019