SCIENCE LETTERS

eISSN: 2682-8626

Available online at
https://scilett-fsg.uitm.edu.my/

Science Letters 18(2) 2024, 44 – 55.

Science Letters

Antihypertensive Potential of *Lactobacillus rhamnosus* Strain GG on Diastolic, Systolic, and Mean Arterial Pressures in NaCl-Induced Prehypertension Rat Models

Anis Munira Zaharuddin[1], Shafiq Aazmi[1,2*], Mohd Yusri Idorus[3], Faye A Almabhouh[4,5], Chun Wie Chong[6], Khalilah Abdul Khalil[1], Siong Meng Lim[7,8], Kalavathy Ramasamy[7,8], Harbindar Jeet Singh[3,4], Nurunajah Ab Ghani[9]

[1]*School of Biology, Faculty of Applied Science, Universiti Teknologi MARA, Selangor, Malaysia*
[2]*Microbiome Health and Environment (MiHeaRT) Research Interest Group, Faculty of Applied Sciences, Universiti Teknologi MARA, Selangor, Malaysia*
[3]*Institute of Medical Molecular Biotechnology, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Selangor, Malaysia*
[4]*Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia*
[5]*Department of Biology and Biotechnology, Faculty of Science Islamic University of Gaza, Gaza Strip, Palestine*
[6]*School of Pharmacy, Monash University Malaysia, Subang Jaya, Selangor, Malaysia*
[7]*Faculty of Pharmacy, Universiti Teknologi MARA Cawangan Selangor, Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia*
[8]*Collaborative Drug Discovery Research (CDDR) Group, Faculty of Pharmacy, University Teknologi MARA (UiTM) Cawangan Selangor, Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia*
[9]*Atta-ur-Rahman Institute for Natural Product Discovery (AuRIns), Universiti Teknologi MARA, Puncak Alam, Selangor, Malaysia*

ARTICLE INFO

Article history:
Received 10 February 2024
Revised 20 April 2024
Accepted 8 May 2024
Online first
Published 24 June 2024

Keywords:
Lactobacillus rhamnosus
probiotics
high salt diet
NaCl
prehypertension
hypertension

DOI:
10.24191/sl.v18i2.26289

ABSTRACT

Prehypertension is a warning sign that blood pressure is trending towards unhealthy levels, with a three- to six-fold risk of developing hypertension. It is concerning that most incidents are undiagnosed and rarely the subject of clinical research. Substantial efforts are therefore needed to identify functional foods that effectively control blood pressure from progressing to hypertension. Thus, using prehypertensive rats induced with NaCl, this study explores the possibility of *Lactobacillus rhamnosus* (LGG) (ATCC 53103) having antihypertensive effects. Eighteen rats were divided equally into three groups and given three different diets: a normal diet (ND), a high-salt diet (HSD) supplemented with 4% NaCl, and a high-salt diet with LGG at a dosage of 1 x 10^9 CFU daily for eight weeks. The rats were acclimatized to a normal diet for two weeks before being subjected to eight weeks of dietary and probiotic treatments. The three blood pressure metrics, diastolic blood pressure (DBP), systolic blood pressure (SBP), and mean arterial pressure (MAP), were measured once a week using the tail-cuff method. The levels of DBP (93.4 ± 1.27 mmHg), SBP (134.1 ± 1.97 mmHg), and MAP (104.3 ± 1.03 mmHg) in HSD groups were statistically significantly higher after following the 4% NaCl diet, which successfully mimicked the prehypertension state in humans. No reduction trends were observed on the weekly DBP, SBP, and MAP readings during the eight weeks of LGG treatment. Though still adhering to a high-salt diet, the treated LGG group's average levels of DBP (87.7 ± 0.69 mmHg), SBP (124.5 ± 1.23 mmHg), and MAP (99.3 ± 0.71 mmHg) were significantly lower than those of the HSD group. This suggests that probiotic LGG may have antihypertensive effects. This emphasizes LGG's antihypertensive qualities and suggests that it may be used therapeutically to treat prehypertension and delay the onset of hypertension.

* Corresponding author. *E-mail address*: shafiqaazmi@uitm.edu.my

INTRODUCTION

Hypertension is one of the global health concerns, a major cause of premature death, and the most significant modifiable risk factor for the development of cardiovascular disease [1-3]. According to WHO health statistics from 2023, 1.28 billion adults globally between the ages of 30 and 79 are estimated to have hypertension. Most of these individuals are from low- and middle-income nations [3]. Alarmingly, 46% of adult hypertensive individuals are not aware of their blood pressure status. Only 42% of adult patients with hypertension receive a diagnosis and course of treatment. Of the adult population with hypertension, only one in five has their blood pressure under control [3]. One of the global targets for non-communicable diseases is reducing hypertension prevalence by 33% between 2010 and 2030. In Malaysia, the National Health and Morbidity Survey (NHMS) reported that the prevalence rate for hypertension was 30% in 2019 [4].

The abnormal and persistent elevation of arterial blood pressure is the hallmark of hypertension. Prehypertension is a critical blood pressure state that can lead to changes in vascular structure, metabolic function, and myocardial remodeling if it persists [5, 6]. One recent cohort study conducted by Ismail et al. [2] reported that the prevalence rate of prehypertension was 40.7% among the 7585 Malaysian adults. According to clinical studies, people with prehypertension have a three- to six-fold higher risk of developing hypertension within two years compared to people with normal blood pressure. This increased risk also corresponds with a higher risk of developing cardiovascular diseases [6, 7].

Hypertension and prehypertension are multifactorial, non-communicable diseases in which prolonged poor dietary habits with high salt intake are one of the causative factors [8, 9]. One of the strategies is using functional foods such as probiotics for the management of hypertension and prehypertension. Probiotics may be utilized as an adjunctive treatment for high blood pressure management, according to a recent comprehensive meta-analysis report by Zarezadeh et al. [10] that examined the effects of probiotic supplementation on blood pressure from 21 randomized controlled trials and 14 additional meta-analysis studies. According to Zarezadeh et al. [10], well-known bacteria that are beneficial to metabolic health, including *Lactobacillus, Bifidobacterium, Streptococcus, Bacillus, Enterococcus,* and *Saccharomyces,* can confer antihypertensive effects in both human and animal models. *Lactobacillus rhamnosus* strain GG (ATCC 53103) (LGG) is one of the potential probiotics to manage prehypertension. *Lactobacillus rhamnosus* is a strain of beneficial bacteria commonly found in probiotic supplements and some fermented foods. Jiang and colleagues [11] reported the antihypertensive effects of *L. rhamnosus* CP658 extracted from conventional Daqu in spontaneously hypertensive rats (SHR). However, probiotics are known to have strain-specific health effects that may confer different mechanisms of action and target different aspects of health, as reported in cases of gastroenteritis, anxiety, depression, and obesity [12-15]. Therefore, the health-promoting effect of LGG on elevated blood pressure could be investigated to confirm its antihypertensive effect.

Thus, prehypertension needs to be managed early to combat the progression of hypertension, as the majority of cases were those with unknown prehypertension. Currently, clinical research primarily focuses on the health management of diagnosed hypertensive patients, with limited attention given to the health management of prehypertensive patients [10]. The emphasis on health management for individuals in the prehypertensive phase needs to be heightened, as some patients progress to hypertension due to a lack of effective health management, resulting in irreversible consequences. In order to address this gap, this study investigates the antihypertensive effects of *L. rhamnosus* strain GG (ATCC 53103) in managing prehypertension using high-salt diet-induced rats as an animal model.

https://doi.org/10.24191/sl.v18i2.26289

EXPERIMENTAL

Animals and Study Design

For this investigation, twenty-four male, eight-week-old Sprague Dawley rats weighing between 200 and 250 g were used as animal models. The rats were housed in a controlled environment with free food and water throughout the experiment. The temperature was kept at 23 ± 3 °C, the relative humidity was 55 ± 5%, and the light/dark cycle was 12/12 h. The rats were kept separately and allowed to adapt to their new surroundings for two weeks to prevent the effects of the cage. During the acclimatization weeks (AC 1 and AC 2), all the rats were fed with the standard normal diet pellets (ND) (Gold Coin, Selangor, Malaysia). The rats were then randomized into three groups (n = 8 per group) with respective treatments as described in Table 1. The UiTM Research Ethics Committee approved the experimental design (UiTM CARE: 343/2021). The rats were sacrificed for blood collection at the end of the experiment by terminal cardiac puncture.

Table 1. The treatment groups in the present study

Groups	Rats (n)	Descriptions	Treatments (8 weeks)
HSD	8	HSD and prehypertensive control group	fed with HSD and 1 ml of sterilized PBS
ND	8	ND and Normotensive control group	fed with ND and 1 ml of sterilized PBS
LGG	8	HSD and probiotic LGG group	fed with HSD and 1 ml of LGG 10^9 CFU daily

HSD, a high salt diet with 4 % sodium chloride (NaCl); ND, normal diet; LGG, PBS, phosphate-buffered saline; *Lactobacillus rhamnosus* strain GG ATCC 53103 (LACTOGG®, Malaysia)

Preparation and Oral Supplementation of the Probiotic

LGG was obtained commercially in freeze-dried form, with each capsule dose containing a minimum of 20 billion live *L. rhamnosus* GG (ATCC 53103) LACTOGG®(USA). Using a sterile phosphate-buffered saline (PBS), LGG was suspended and diluted to produce a concentration of 10^9 CFU day^{-1} in 1 mL. Every dosage was prepared fresh to maintain the viability of the probiotics. HSD and normal ND groups were only provided with 1 mL of PBS as the vehicles.

Diets

The standard normal diet pellets (ND) (Gold Coin, Selangor, Malaysia) comprise 14% fat, 25% protein, and 61% carbohydrates. The high salt diet (HSD) was formulated by adding 4% sodium chloride (NaCl) to the standard diet. The rats were given water *ad libitum* to avoid hyperkalemia.

Measurement of Diastolic, Systolic, and Mean Arterial Pressures

We used the non-invasive tail-cuff approach (CODA® High Throughput System) to measure blood pressure following the manufacturer's instructions. The rats were acclimated to the non-invasive tail-cuff approach before the actual blood pressure (BP) measurement. Every week throughout the treatment, each rat's blood pressure was taken. To minimize experimental errors, the diastolic blood pressure (DBP),

https://doi.org/10.24191/sl.v18i2.26289

systolic blood pressure (SBP), and mean arterial pressure (MAP) were measured at predefined intervals between 1200 and 1700. Five measurements were made in each rat to determine the mean value.

Statistical Analysis

GraphPad Prism version 8 (GraphPad Software Inc.; San Diego, CA, USA) was used to analyze the experimental data. The results were displayed as the mean and mean standard error of the mean (SEM). A two-way ANOVA was used to compare all the data between the intervention groups, and Tukey's multiple comparisons test was then performed. Significant statistical results in this study were defined as $P < 0.05$.

RESULTS

Effects of Probiotic LGG on DBP

The weekly changes in diastolic blood pressure (DBP), systolic blood pressure (SBP), and mean arterial pressure (MAP) throughout the experiment were presented in Fig. 1, Fig. 2, and Fig. 3, respectively. During the eight weeks of the diet regimen, HSD with 4% NaCl significantly increased the levels of DBP, SBP, and MAP in comparison to the control ND group (Fig. 1, Fig. 2, and Fig. 3). The effects of 4% HSD on the three blood pressure parameters were significantly apparent after one week of diet treatment, starting from week two until week eight (Fig. 1, Fig. 2, and Fig. 3).

The mean levels of DBP for the HSD group steadily increased from 90.5 ± 3.26 mmHg in week 1 to 94.7 ± 4.25 mmHg in week 8 (Fig. 1). Within the treatment week (Fig. 1), the mean levels of DBP in the HSD group were statistically significantly higher than those in the ND group for almost every week except in the first and fourth weeks. There is a trend towards well-controlled DBP levels for the LGG group (between 87.0 ± 1.24 mmHg and 89.0 ± 1.85 mmHg) compared to HSD (between 83.8 ± 2.57 mmHg and 94.7 ± 4.25 mmHg) after eight weeks of oral LGG supplementation (Fig. 1). However, no statistically significant difference was found. The same goes for the ND group, whose DBP levels were kept stable throughout the treatment period of eight weeks (Fig. 1).

https://doi.org/10.24191/sl.v18i2.26289

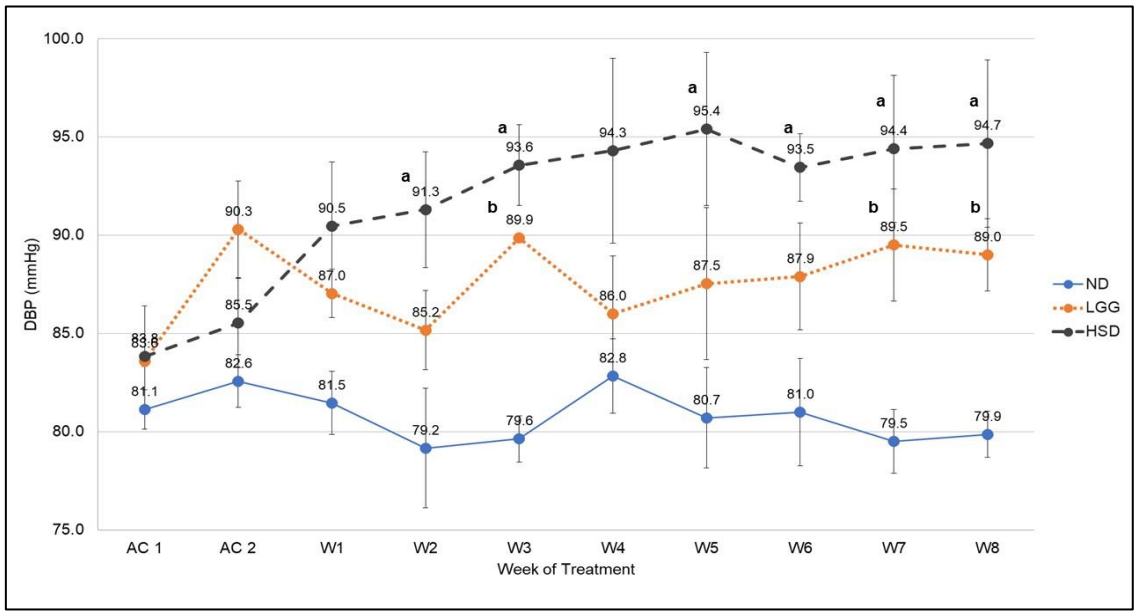

Fig. 1. Changes in DBP of the ND, LC, and HSD animal models throughout the experiment, starting with two weeks of acclimatization period (A1 and AC2) and continuing with eight weeks of dietary and probiotic supplementation (W1 to W8). Each value provided is the mean ± standard error of the mean (SEM). Values with different superscript letters significantly differ ($P < 0.05$) by two-way ANOVA followed by the Tukey *post hoc* test. The letter 'a' indicates significant differences between HSD and ND; 'b' indicates differences between LGG and ND; 'c' indicates differences between HSD and LGG. AC, acclimatization; DBP, diastolic blood pressure; HSD, high salt diet (4% NaCl); ND, normal diet; LGG, HSD plus probiotic *Lactobacillus rhamnosus* strain GG at 10^9 CFU day^{-1} for eight weeks.

Effects of Probiotic LGG on the SBP

The level of SBP increased from 121.5 ± 3.06 mmHg in week 1 to 139.3 ± 5.77 mmHg in week 8. From week 2 onward, the HSD group's weekly SBP level was significantly higher than the ND group (Fig. 2). Compared to the HSD group, the LGG group's SBP level significantly decreased in week 4 (124.1 ± 2.89 mmHg). The SBP level then stayed steady for the remaining weeks until the end of the experiment at week 8 (126.0 ± 3.59 mmHg). In contrast, the ND group had stable SBP levels from the start of the study until the end. The lowest reading was observed in week 3 (112.1 ± 1.40 mmHg), and the highest level was noted in week 8 (116.0 ± 1.66 mmHg) for the ND group (Fig. 2).

https://doi.org/10.24191/sl.v18i2.26289

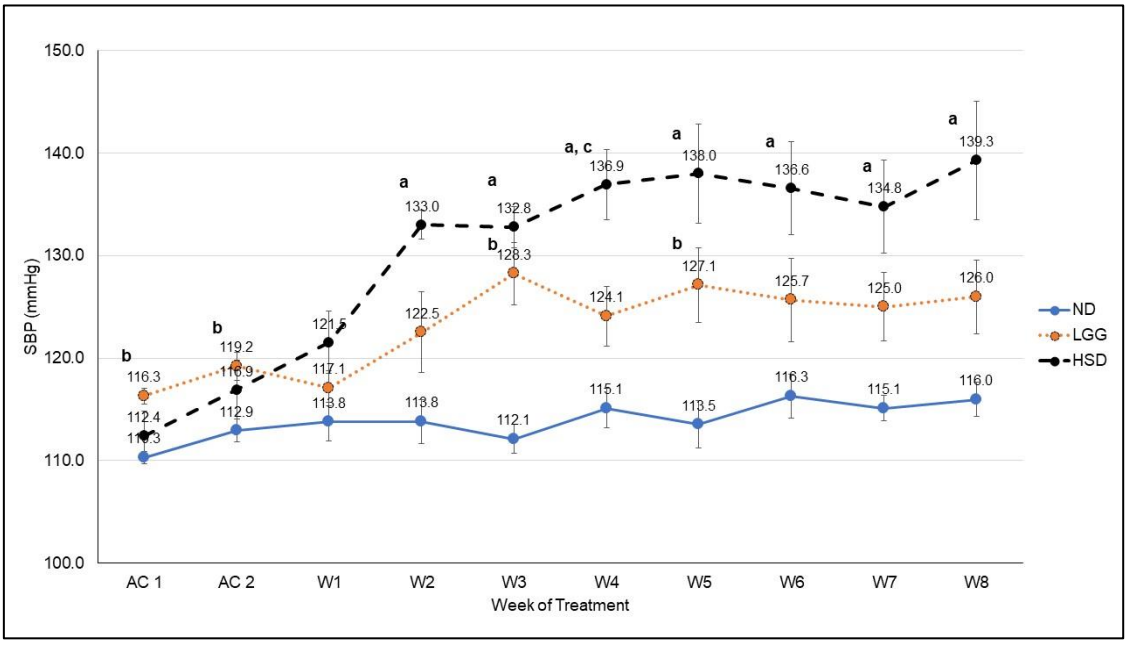

Fig. 2. Changes in SBP of the ND, LC, and HSD animal models throughout the experiment, starting with two weeks of acclimatization period (A1 and AC2) and continuing with eight weeks of dietary and probiotic supplementation (W1 to W8). Each value provided is the mean ± standard error of the mean (SEM). Values with different superscript letters significantly differ ($P < 0.05$) by two-way ANOVA followed by the Tukey *post hoc* test. The letter 'a' indicates significant differences between HSD and ND; 'b' indicates differences between LGG and ND; 'c' indicates differences between HSD and LGG. AC, acclimatization; HSD, high salt diet (4% NaCl); ND, normal diet; SBP, systolic blood pressure; LGG, HSD plus probiotic *Lactobacillus rhamnosus* strain GG at 10^9 CFU day^{-1} for eight weeks.

Effects of LGG on the MAP

The weekly levels of MAP were significantly increased in the HSD group compared to the ND group (Fig. 3). Starting in week 2 (104.9 ± 2.45 mmHg) and continuing until week 8 (109.3 ± 4.71 mmHg), the differences were deemed statistically significant as compared to the ND groups (between 90.4 ± 2.60 mmHg and 91.6 ± 1.19 mmHg). A trend of stable weekly MAP levels was observed in the LGG group after four weeks of oral probiotic supplementation. The recorded reading of MAP on week 4 was 99.1 ± 2.90 mmHg and 101.0 ± 2.37 mmHg at the end of the experiment in week 8 (Fig. 3). Despite a trend towards lower readings in the LGG group, the difference in MAP reading between HSD and LGG was not statistically significant. The ND group also had a stable reading for the weekly MAP throughout the study (Fig. 3).

https://doi.org/10.24191/sl.v18i2.26289

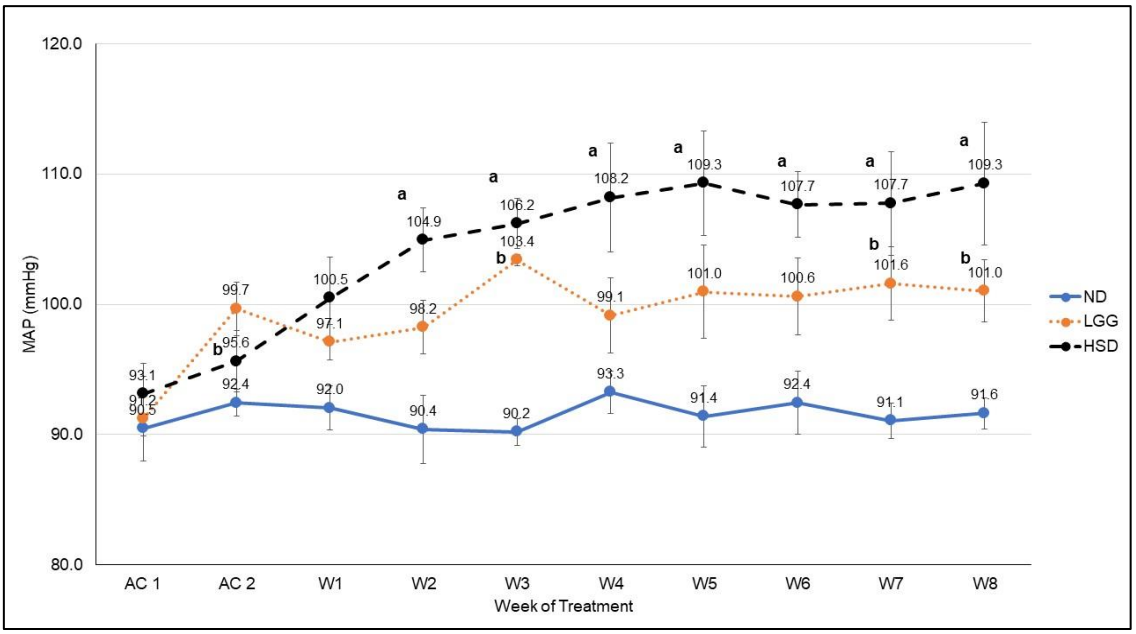

Fig. 3. Changes in MAP of the ND, LC, and HSD animal models throughout the experiment, starting with two weeks of acclimatization period (A1 and AC2) and continuing with eight weeks of dietary and probiotic supplementation (W1 to W8). Each value provided is the mean ± standard error of the mean (SEM). Values with different superscript letters significantly differ ($P < 0.05$) by two-way ANOVA followed by the Tukey *post hoc* test. The letter 'a' indicates significant differences between HSD and ND; 'b' indicates differences between LGG and ND; 'c' indicates differences between HSD and LGG. AC, acclimatization; HSD, high salt diet (4% NaCl); MAP, mean arterial pressure; ND, normal diet; LGG, HSD plus probiotic *Lactobacillus rhamnosus* strain GG at 10^9 CFU day^{-1} for eight weeks.

Therapeutic Potential of LGG on Prehypertension

Overall, there were statistically significant differences in the average blood pressure readings between the groups when comparing the eight weeks of dietary and probiotic treatments (Fig. 4). Figures 4A, 4B, and 4C show that the 4% NaCl diet regimen statistically significantly increased the levels of DBP (93.4 ± 1.27 mmHg), SBP (134.1 ± 1.97 mmHg), and MAP (104.3 ± 1.03 mmHg) in HSD groups. The 4% NaCl diet for eight weeks resulted in diastolic and systolic prehypertension, according to DBP and SBP readings greater than 90 mmHg and 130 mmHg, respectively (Fig. 4A and 4B). When compared to the HSD group, the LGG group exhibited significantly lower average levels of DBP (87.7 ± 0.69 mmHg), SBP (124.5 ± 1.23 mmHg), and MAP (99.3 ± 0.71 mmHg), indicating the therapeutic potential of probiotic LGG. In contrast to the ND group, the LGG group's DBP, SBP, and MAP levels were still noticeably greater. The ND group had the lowest DBP (80.5 ± 0.40 mmHg), SBP (114.5 ± 0.49 mmHg), and MAP (91.5 ± 0.36 mmHg) than the HSD and LGG groups statistically significantly (Fig. 4).

https://doi.org/10.24191/sl.v18i2.26289

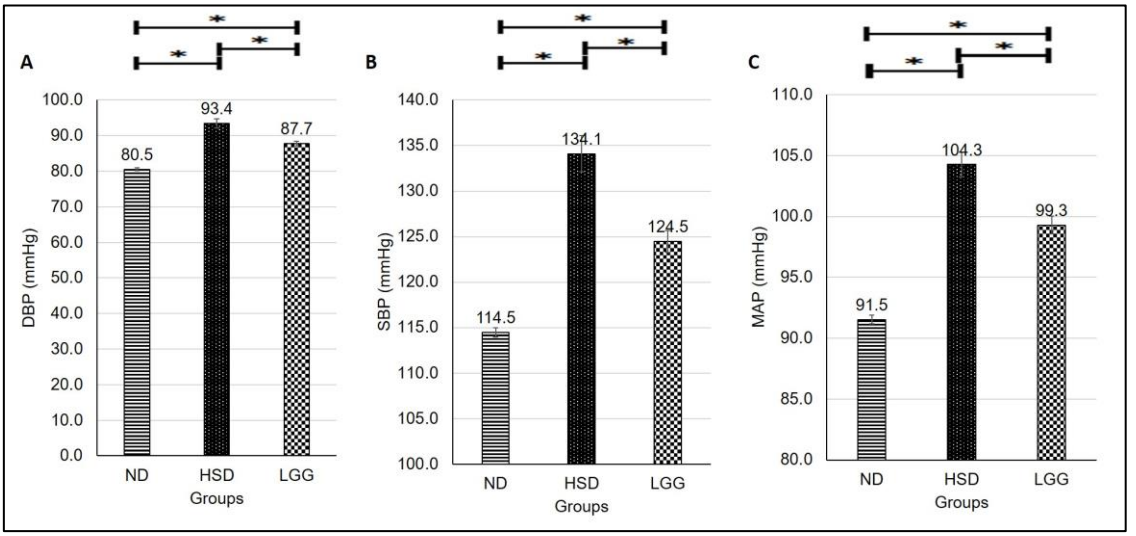

Fig. 4. The effect of LGG on (A) DBP, (B) SBP, and (C) MAP levels after eight weeks of oral supplementation. DBP, diastolic blood pressure; HSD, high salt diet; MAP, mean arterial pressure; ND, normal diet; SBP, systolic blood pressure. Each value provided is the mean ± standard error of the mean (SEM) from eight weeks of treatment. *P < 0.05 shows significant differences between groups tested with two-way ANOVA followed by the Tukey *post hoc* test. HSD, high salt diet (4% NaCl); MAP, mean arterial pressure; ND, normal diet; LGG, HSD + probiotic *Lactobacillus rhamnosus* strain GG at 10^9 CFU day^{-1} for eight weeks.

DISCUSSION

Sprague-Dawley rats fed a high-salt diet containing 4% NaCl were used to successfully create a prehypertensive animal model over eight weeks of a diet regime based on the average DBP (93.4 ± 1.27 mmHg) and SBP (134.1 ± 1.97 mmHg) readings as described in Fig. 4. Blood pressure that is higher than normal but not high enough to be categorized as hypertension is known as prehypertension [2, 16]. High NaCl intake, as high as 4%, is associated with an increased risk of elevated blood pressure in susceptible individuals and animal models, such as rats. The mechanisms underlying this phenomenon are similar between humans and animals [1, 17, 18]. Because of this, when probiotic LGG supplementation is given at 10^9 CFU daily for eight weeks, the current prehypertension animal model may represent a similar metabolic condition in humans. Prehypertension is one of the health conditions that has recently become alarming worldwide, including in Malaysia [2, 16].

The DBP, SBP, and MAP were the three blood pressure measurements used in this study. Monitoring systolic and diastolic blood pressure readings is important for assessing overall cardiovascular health and managing hypertension [6, 19]. The lowest number in a blood pressure reading, the DBP, represents the pressure in the arteries during the period between heartbeats. A normal diastolic blood pressure reading is usually less than 80 mmHg. High diastolic blood pressure, known as diastolic hypertension, is a reading consistently at or above 90 mmHg and can indicate an increased risk of heart disease, stroke, and other health problems. A DBP reading between 80 mmHg and 90 mmHg is considered prehypertension [2, 16].

In contrast, SBP is the top number in a blood pressure reading and represents the pressure in the arteries when the heart beats and pumps blood out. A systolic blood pressure measurement of less than 120 mmHg is typically considered normal. A reading consistently at or above 140 mmHg is referred to as high systolic blood pressure or systolic hypertension, indicating an increased risk of heart disease [6]. A reading of SBP

https://doi.org/10.24191/sl.v18i2.26289

between 120 mmHg and 140 mmHg can be considered prehypertension [2, 16]. Therefore, SBP and DBP readings greater than 120 over 80 and less than 140 over 90 (120/80–140/90) indicate prehypertension. The SBP and DBP readings of 140 over 90 (140/90) or higher indicate hypertension [2, 6, 16]. The present study shows that the 4% high-salt diet during the eight-week study led to prehypertension in the Sprague-Dawley rats in the HSD and LGG groups.

According to Dobrian et al. [18], feeding with the 4% high-salt diet for a longer period, for ten weeks or longer, may cause hypertension. For this reason, the 4% high-salt diet treatment in this study was only administered for eight weeks to raise the prehypertensive state's DBP and SBP levels. Rats were also acclimated over two weeks to verify the direct impact of the oral probiotic LGG supplementation and the 4% high-salt diet on blood pressure during the eight-week treatment period. During this time, they were fed a standard diet to normalize the diet regime before further experiments.

Conversely, the MAP is a computed value representing the average arterial pressure within a single cardiac cycle. It is calculated from systolic and diastolic blood pressure readings, representing the perfusion pressure in the organs, which is essential for sufficient blood flow and oxygen delivery. The MAP readings can serve as an alternative index that effectively captures the individual's total exposure to elevated blood pressure [19]. Normal mean arterial pressure typically falls between 70 mmHg and 110 mmHg. According to preliminary findings reported by Kandil et al. [19], MAP would be more useful and accurate than simply using systolic or diastolic blood pressure measurements (accuracy up to 89.3% and 88.9%, respectively) in determining the cerebrovascular impact of hypertension (accuracy up to 95.2%). This finding highlights the pathophysiological relevance of MAP and validates earlier theories that suggested this straightforward metric might be a better indicator for defining hypertension and conducting hypertension research.

Based on the present study, HSD impacted DBP, SBP, and MAP beginning in week two and increasing throughout the experiment for eight weeks. On the other hand, LGG did not appear to differ significantly in DBP and SBP at week 1 between the ND and HSD groups. For SBP, this pattern continued until the end of the experimental period. However, weeks 7 and 8 saw a noticeable increase in DBP readings. However, distinct patterns were noted in every group, with the blood pressure readings in the LGG group consistently being lower than those in the HD group, suggesting the underlying effects of LGG. The reduction in blood pressure observed with LGG is consistent with the other documented antihypertensive effects of *Lactobacillus rhamnosus* CP658 extracted from conventional Daqu on spontaneously hypertensive rats (SHR) [11]. Although both strains differ, they are from the same genus and species group. Therefore, these results validate *L. rhamnosus*'s antihypertensive properties, necessitating a human clinical trial to verify their beneficial effects on prehypertension and hypertension.

The treated LGG group did not show a significant drop in weekly blood pressure. However, the weekly SBP, DBP, and MAP levels were more stable and under control, even though they were still following a high-salt diet. In addition, the present study shows that the average SBP, DBP, and MAP over the eight weeks of treatment in the treated LGG group are significantly lower than the control HSD group. More statistically significant antihypertensive effects of LGG could be obtained if a higher dosage and longer treatment period are carried out. To significantly reduce the inflammatory response and both DBP and SBP, a higher dosage (2×10^9 CFU daily) and a minimum of 10 weeks of oral supplementation were recommended by a recent umbrella meta-analysis report on the effects of probiotic supplementation on blood pressure from 21 randomized controlled trials and 14 other meta-analysis studies [10].

The modulation and reduction of inflammation is one potential mechanism through which the LGG confers antihypertensive effects on the DBP and SBP [5, 20]. In one study, Ferrian et al. [21] found that single-cell imaging maps inflammatory cell subsets to pulmonary arterial hypertension vasculopathy,

https://doi.org/10.24191/sl.v18i2.26289

supporting the theory that chronic inflammation plays a role in the pathophysiology of pulmonary arterial hypertension. In a different investigation, Nenu et al. [22] demonstrated how a probiotic supplement containing *L. rhamnosus* alters the inflammatory pathways in the liver and gut of a mouse model of hepatocellular carcinoma. This indicates that LGG potentially exerts antihypertensive effects by reducing inflammation in high blood pressure cases. Further research is needed to fully understand this concept to manage prehypertension and prevent the development of hypertension.

CONCLUSION

This study shows that LGG has therapeutic potential in an animal model of prehypertension caused by a high-salt diet. Even with a high-salt diet, the SBP, DBP, and MAP levels could be stabilized with an oral supplement of LGG for eight weeks at a dosage of 1×10^9 CFU daily. To further elucidate the antihypertensive effects of LGG, profiling of the gut microbiome and the metabolic changes associated with the probiotic treatment could be carried out using metagenomic and metabolomics studies. Thus, it highlights the antihypertensive properties of LGG and raises the possibility of using them therapeutically to treat prehypertension and prevent the onset of hypertension, supporting the WHO's 2030 target of a 33% decrease in occurrences.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of the Faculty of Applied Sciences, Laboratory Animal Care Unit (LACU) and Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Collaborative Drug Discovery Research Lab (CDDR), Faculty of Pharmacy, Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA (UiTM), Malaysia, for providing the facilities. This study was supported by the Ministry of Higher Education, Malaysia (RACER/1/2019/STG05/UITM//4).

AUTHOR'S CONTRIBUTION

Anis Munira Zaharuddin wrote and revised the article and performed the research, study, and analysis. The research was planned and overseen by Shafiq Aazmi, who also wrote and revised the article, anchored the revisions made by the review panel, and approved the submission of the work. Reviewing the manuscript and providing the technical and theoretical framework are Mohd Yusri Idorus, Mohd Yusri Idorus, Fayez A. Almabhouh, Chun Wie Chong, Khalilah Abdul Khalil, Siong Meng Lim, Kalavathy Ramasamy and Harbindar Jeet Singh.

CONFLICT OF INTEREST STATEMENT

The authors affirm that they have no financial, commercial, or self-beneficial interests in their research and no conflicts of interest with the funders.

REFERENCES

[1] Abais-Battad, J. M., Saravia, F. L., Lund, H., Dasinger, J. H., Fehrenbach, D. J., Alsheikh, A. J., ... & Mattson, D. L. (2021). Dietary influences on the Dahl SS rat gut microbiota and its effects on salt-sensitive hypertension and renal damage. *Acta Physiologica*, *232*(4), e13662.

https://doi.org/10.24191/sl.v18i2.26289

[2] Ismail, R., Ismail, N. H., Isa, Z. M., Tamil, A. M., Ja'afar, M. H., Nasir, N. M., ... & Yusof, K. H. (2023). Prevalence and factors associated with pre-hypertension and hypertension among adults: Baseline findings of PURE Malaysia cohort study. *American Journal of Medicine Open*, 100049.

[3] WHO. Hypertension. March 1, 2023. https://www.who.int/news-room/fact-sheets/detail/hypertension. Accessed February 24, 2024.

[4] IPH. National Health and Morbidity Survey (NHMS) 2019: Vol. I: NCDs —Non-Communicable Diseases: Risk Factors and Other Health Problems; 2020.

[5] Hall, J. E., Omoto, A. C., Wang, Z., Mouton, A., Li, X., & Hall, M. E. (2024). Pathophysiology of hypertension. In *Hypertension* (pp. 71-86). Elsevier.

[6] Seidu, S., Lawson, C. A., Kunutsor, S. K., Khunti, K., & Rosano, G. M. (2024). Blood pressure levels and adverse cardiovascular outcomes in heart failure: A systematic review and meta-analysis. *European Journal of Heart Failure*.

[7] Huang, J. (2024). Effect of Health Management in Raising Awareness Rate of Disease Prevention and Treatment in Patients with Prehypertension. *Journal of Biosciences and Medicines*, *12*(2), 236-243.

[8] Ding, R., Jiang, Y., Yang, Y., Shi, Y., Ji, Y., Zhen, T., ... & Zhu, S. (2022). Calcitriol ameliorates renal injury with high-salt diet-induced hypertension by upregulating GLIS2 expression and AMPK/mTOR-regulated autophagy. *Gene*, *820*, 146239.

[9] Power, G., & Padilla, J. (2023). (Re) modeling high-salt diet-induced hypertension in mice. *American Journal of Physiology-Heart and Circulatory Physiology*, *324*(4), H470-H472.

[10] Zarezadeh, M., Musazadeh, V., Ghalichi, F., Kavyani, Z., Nasernia, R., Parang, M., ... & Mekary, R. A. (2023). Effects of probiotics supplementation on blood pressure: An umbrella meta-analysis of randomized controlled trials. *Nutrition, Metabolism and Cardiovascular Diseases*, *33*(2), 275-286.

[11] Jiang, Y., Wu, J., Tian, L., Liu, Y., Zhao, F., He, Z., ... & Guan, T. (2024). The therapeutic effects of fermented milk with lactic acid bacteria from traditional Daqu on hypertensive mice. *Journal of Dairy Science*, *107*(2), 742-758.

[12] Ji, Y., Chung, Y. M., Park, S., Jeong, D., Kim, B., & Holzapfel, W. H. (2019). Dose-dependent and strain-dependent anti-obesity effects of Lactobacillus sakei in a diet induced obese murine model. *PeerJ*, *7*, e6651.

[13] Hojsak, I., & Kolaček, S. (2024). Role of Probiotics in the Treatment and Prevention of Common Gastrointestinal Conditions in Children. *Pediatric Gastroenterology, Hepatology & Nutrition*, *27*(1), 1

[14] De Santa, F., Strimpakos, G., Marchetti, N., Gargari, G., Torcinaro, A., Arioli, S., ... & Farioli-Vecchioli, S. (2024). Effect of a multi-strain probiotic mixture consumption on anxiety and depression symptoms induced in adult mice by postnatal maternal separation. *Microbiome*, *12*(1), 29.

[15] Tyagi, A. M. (2024). Mechanism of action of gut microbiota and probiotic Lactobacillus rhamnosus GG on skeletal remodeling in mice. *Endocrinology, Diabetes & Metabolism*, *7*(1), e440.

[16] Zaki, N. A. M., Ambak, R., Othman, F., Wong, N. I., Man, C. S., Morad, M. F. A., ... & Baharudin, A. (2021). The prevalence of hypertension among Malaysian adults and its associated risk factors: data from Malaysian Community Salt Study (MyCoSS). *Journal of Health, Population and Nutrition*, *40*(1), 1-6.

[17] Tobian, L. (1991). Salt and hypertension. Lessons from animal models that relate to human hypertension. *Hypertension*, *17*(1_supplement), I52.

[18] Dobrian, A. D., Schriver, S. D., Lynch, T., & Prewitt, R. L. (2003). Effect of salt on hypertension and oxidative stress in a rat model of diet-induced obesity. *American Journal of Physiology-Renal Physiology*, *285*(4), F619-F628.

https://doi.org/10.24191/sl.v18i2.26289

[19] Kandil, H., Soliman, A., Alghamdi, N. S., Jennings, J. R., & El-Baz, A. (2023). Using Mean Arterial Pressure in Hypertension Diagnosis versus Using Either Systolic or Diastolic Blood Pressure Measurements. *Biomedicines*, *11*(3), 849.

[20] Ren, D., Ding, M., Su, J., Ye, J., He, X., Zhang, Y., & Shang, X. (2024). Stachyose in combination with L. rhamnosus GG ameliorates acute hypobaric hypoxia-induced intestinal barrier dysfunction through alleviating inflammatory response and oxidative stress. *Free Radical Biology and Medicine*, *212*, 505-519.

[21] Ferrian, S., Cao, A., McCaffrey, E. F., Saito, T., Greenwald, N. F., Nicolls, M. R., ... & Angelo, M. (2024). Single-cell imaging maps inflammatory cell subsets to pulmonary arterial hypertension vasculopathy. *American Journal of Respiratory and Critical Care Medicine*, *209*(2), 206-218.

[22] Nenu, I., Baldea, I., Coadă, C. A., Crăciun, R. C., Moldovan, R., Tudor, D., ... & Filip, G. A. (2024). Lactobacillus rhamnosus probiotic treatment modulates gut and liver inflammatory pathways in a hepatocellular carcinoma murine model. A preliminary study. *Food and Chemical Toxicology*, *183*, 114314.

https://doi.org/10.24191/sl.v18i2.26289