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RESEARCH

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Changes in the gut microbiota induced by an oral formulation of multiple peptides and plants contribute to its antihypertensive effects

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Abstract

The homeostasis of the gut microbiota is a crucial factor in regulating peripheral and central blood pressure. Formulating a food-derived mixture of antioxidants, anti-inflammatory compounds and prebiotics to maintain the homeostasis of the gut microbiota will be essential for preventing and alleviating hypertension. Although bioactive peptides and plant extracts have been demonstrated not only to be excellent antioxidants and anti-inflammatory agents but also to promote the homeostasis of the microbiota, few studies have investigated the influence of multiple peptides mixed with plant extracts on the gut microbiota of hypertensive rats. This is the first study to characterize the fecal microbiome of spontaneously hypertensive rats supplemented with a mixture of multiple peptides and plant extracts by integrating 16 S rRNA gene sequencing. During the six-week experiment, the animals were healthy and without mortality. The results revealed that oral supplementation with the formulation reduced both systolic and diastolic blood pressure, improved the gut microbiota by increasing the ratio of Bacteroidetes to Firmicutes, and increased the relative abundance of specific genera, such as Lactobacillus. This study suggested that a synergistic formulation of multiple peptides mixed with plant extracts could be a novel strategy for preventing and alleviating hypertensive diseases.

Keywords Gut microbiota, Multiple peptides, Plant extracts, Antihypertension, 16S rRNA gene sequencing

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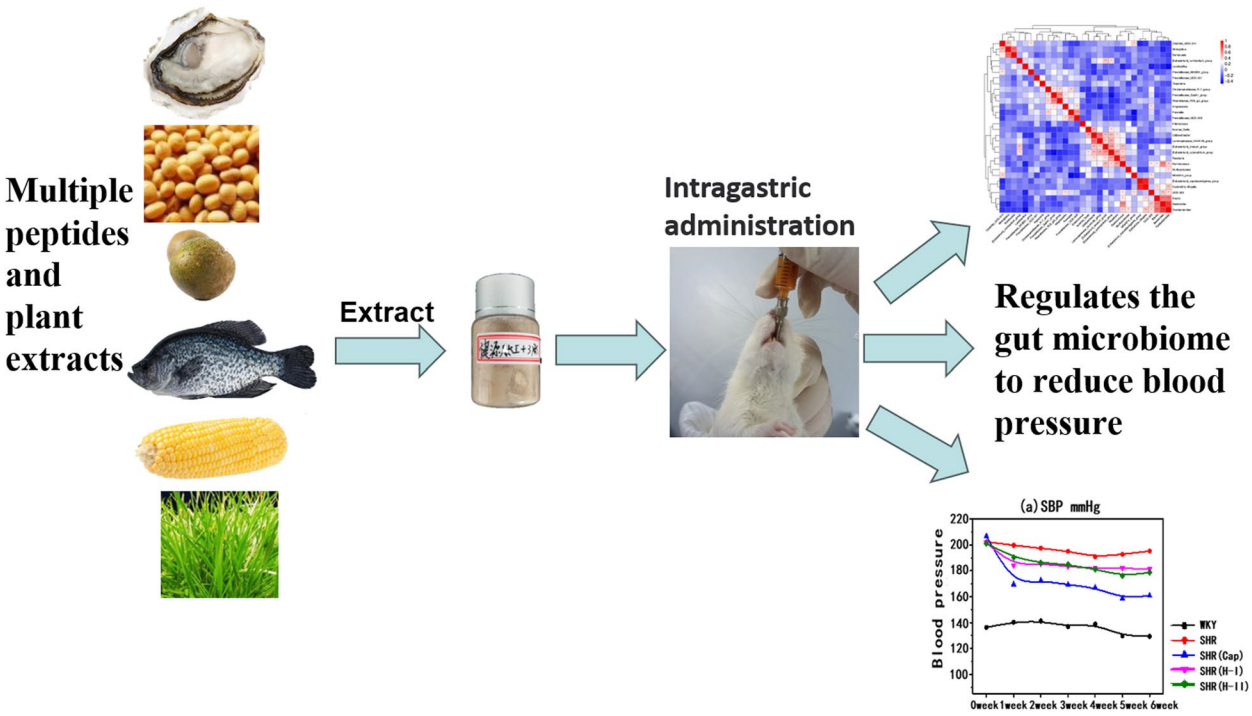
Graphical Abstract



Introduction

Hypertension is one of the leading risk factors for death worldwide, affecting 30% of adults worldwide (WHO 2019). Despite the availability of some antihypertensive drugs, some patients are resistant to these drugs (Drummond et al. 2019). Hypertension is a complex disease resulting from complicated pathological processes and multiple mechanisms and involves many target organs rather than a single factor (Hallow et al. 2014; Oparil et al. 2018; Kutumova et al. 2022). Therefore, taking one or two hypertension drugs fails to adequately control hypertension. Antihypertensive clinical medicines are synthetic and show specific toxicity and numerous adverse effects, such as neutropenia, renal toxicity, angioedema, asthma, and hepatotoxicity (Messerli et al. 2018). Patients with hypertension must take medication for a long time or throughout their life. Moreover, these drugs affect metabolism and gut microbial metabolic pathways, which may result in complications and a greater risk of disease progression (Tokarek et al. 2023; Williams et al. 2018). Creating potential antihypertensive therapies with no or few adverse effects and without disturbing the gut microbiota is highly important (Chiu et al. 2021).

In view of pathological mechanisms, hypertension results from the sophisticated regulatory interplay of many factors, such as oxidative stress, inflammation and the gut microbiota, with alterations in renal, neural and vascular function (Griendling et al. 2021). Oxidative stress develops when there is an imbalance between the generation of oxidant compounds and antioxidant defense mechanisms, and oxidative stress is a crucial factor in initiating irreversible cellular damage and inducing organ inflammation by triggering maladaptive immune responses associated with the development of hypertension (Murray et al. 2021; Tain & Hsu 2022). Oxidative stress accelerates the progression of hypertension, leading to inflammation, fibrosis, vascular dysfunction and associated target organ damage (Harrison et al. 2021). Additionally, the gut microbiome has been highlighted as an emerging modulator of blood pressure. The gut microbiota influences the occurrence and development of hypertension in various ways, such as through the production of short-chain fatty acids and dysfunction of the brain-gut axis. The gut microbiota also changes the content of serotonin, which can cause an imbalance in vagus and sympathetic nerve output associated with hypertension. There is rapidly increasing evidence about the involvement of the gut microbiome (dysbiosis) in

hypertension (Onal et al. 2018) and the significant alterations associated with inflammation in hypertension (Suganya et al. 2020; Santisteban et al. 2017). The homeostasis of the gut microbiota is also critical for cardiovascular health (Tokarek et al. 2023; Tang et al. 2017). Therefore, formulating a food-derived mixture of antioxidants, anti-inflammatory agents and prebiotics that maintains the homeostasis of the gut microbiota with fewer side effects will be a novel strategy for alleviating hypertensive diseases.

Antioxidative and anti-inflammatory compounds and prebiotics can be processed into food products. Natural antioxidants, anti-inflammatory compounds and prebiotics can be found in bioactive peptides and plant extracts in favor of beneficial bacteria that have been intensively studied to prevent and reduce the harm of hypertension in recent years (Tain & Hsu 2022). Bioactive peptides derived from natural plants and animal sources have attracted the interest of scientists to explore their antihypertensive effects due to their great antioxidative, anti-inflammatory and gut microbial modulatory effects (Karami & Akbari-adergani 2019; Márquez et al. 2022; Daliri et al. 2020), with few or no adverse side effects (Karami & Akbari-adergani 2019; Márquez et al. 2022; Daliri et al. 2020). They reduce blood pressure not only by working on the RAAS as ACE inhibitors but also by working on other blood pressure regulation pathways. Additionally, Song et al. (2021) reported that fermented clam peptides significantly reduce blood pressure; alleviate damage to the kidney, thoracic aorta and heart; and improve the gut microbiota by inhibiting the RAS system, maintaining the ET-1/NO balance, and improving intestinal microbial disorders in SHRs. More importantly, peptides generated from legumes have been studied as sources of agents with anti-inflammatory activity. To balance the nutritive and functional properties of foods, the combined use of animal and plant sources has more effective biological activity (Liu et al. 2019).

In contrast to bioactive peptides, plant extracts are rich in polyphenols (including flavonoids and nonflavonoid polyphenols), vitamins and oligosaccharides (Oliveira et al. 2022) and display different mechanisms, which are involved in the improvement of endothelial function, oxidative stress and inflammatory effects (López-Fernández-Sobrino et al. 2021). Oligosaccharides in plant extracts act as prebiotics and are nondigestible food ingredients but promote the growth of probiotics and inhibit the growth of pernicious bacteria. Prebiotics, which are rich in fruit extracts and can alter the microbiota, are significant modulators of the gut microbiota (Tokarek et al. 2023). Gut microbiota dysbiosis is an important factor in the etiology of hypertension. The homeostasis of the gut microbiota impacts peripheral and central blood pressure control mechanisms. The supplementation of prebiotics has been proven to be adequate for restoring the homeostasis of the gut microbiota (Li et al. 2017). Prebiotics can also act directly on host cells to regulate inflammation and enhance immunity, in addition to their indirect effects through the intestinal microflora (Natividad et al. 2020). Together, different phenolic compounds and prebiotics have other functions in regulating inflammation and immunity in the human body to stabilize blood pressure and reduce target organ damage (Yan et al. 2022).

There are significant differences in the degree of response to drug therapy among hypertensive patients. While no single food counteracts hypertension, adopting a plant-based dietary pattern including various polyphenol-rich foods is an advisable practice to improve blood pressure. Given that hypertension is the result of multiple mechanisms, a formulated food-derived mixture, composed of a variety of compounds that present more effective biological activity, should be used to adequately control blood pressure to modulate different pathways and mechanisms, leading to synergistic effects on health. In this study, an oral formulation of multiple peptides and fruit extracts was used for the first time to evaluate the effects of numerous functions for maintaining healthy microbiota and controlling blood pressure by taking advantage of the biological activities of plant extracts and multiple peptides from animals and plants.

Materials and methods

Chemicals and reagents

Chemicals were of analytical grade and were purchased from Shanghai Hengchuang Biotechnology Co., Ltd. (Shanghai, China). The reagents used in the experiment were as follows: captopril (Aladdin, Lot#J2114473), DNA Extraction Kit (BASY-DO, Cat. No. DR 0301050), QIAamp 96 PowerFecal QIAcube HT kit (QIAGEN, Cat. No. 51,531), Qubit dsDNA Assay Kit (Life Technologies, Cat. No. Q32854), Tks Gflex DNA Polymerase (Takara, Cat. No. R060B), Health-originated Peptides (HOP) TM, a food-derived mixture formulation, (Suzhou Health-originated Biotechnology Co., Ltd., Cat. No. SHOB2022-02HPI and SHOB2022-02HPII). The ingredients of the Formula two (HOP-II) are similar in composition to Formula one (HOP-I), except that it contains stachyose (20%), a popular prebiotic. The percentage of each component in Formula one (HOP-I) and Formula two (HOP-II) were as follows: collagen peptides (25%/20%) from American shad, multiple peptides from soybean (12.5%/10%), amaranth (12.5%/10%), corn (12.5%/10%), quinoa (6.25%/5%), oyster (6.25%/5%) and plant extracts from sea-buckthorn (5%/4%), mogroside (5%/4%),

pueraria (2.5%/2%), black chokeberry (5%/4%), mulberry (5%/4%), lemon (1.25%/1%) and licorice (1.25%/1%).

Animal culture

Wistar Kyoto (WKY) (eleven-week-old, $n=8$) male rats and spontaneously hypertensive male rats (SHR) ($n=32$) rats with body weights of 220–250 g were purchased from the Beijing Weitong Lihua Animal Co., Ltd., with ID Number (SCXK 2021-0006, Beijing, China). Rats were housed in a sterile area with room temperature (20 ~ 26°C), humidity 40 ~ 70% and 12 h of darkness/12 hours of light. The WKY and SHR groups were fed with standard laboratory chow and water. After one week of adaptation, the rats were divided into five groups before drug intervention (8 per group): WKY group, SHR group, SHR + Captopril (Cap) group (15 mg/kg rat, per day), SHR + HOP I (H-I) group (800 mg/kg rat, per day), and SHR + HOP-II (H-II) group (800 mg/kg rat, per day). The experimental concentration of the drug was selected according to the effect of the health of rat and lowering blood pressure in the pre-experiment. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the rats were measured by a non-invasive blood pressure measurement system BP2010AUL (Softron Beijing Biotechnology Co., Ltd.) at 0, 12, 24 h after the first administration of medicament. During long-term intervention, rats were given daily gavage. Blood pressure was measured once a week at a fixed time before intragastric administration. An average blood pressure was obtained for each rat after at least seven successful measurements. After six weeks of continuous gavage, the feces of rats collected in a sterile environment after the last blood pressure were measured. Each group collected four tubes of feces samples for DNA extraction (> 2 mg per tube). Keep all samples at -80 °C before the following analysis. All animal experiment was operated by Jiangxi Zhonghong Boyuan Biotechnology Co., LTD (Jiangxi, China). Animal protocols were supervised by the Laboratory Animal Ethics Committee of Jiangxi Zhonghong Boyuan Biotechnology Co., LTD with ID Number (202303150002) was strictly in accordance with the International Animal Protection Directive.

DNA extraction, library construction and 16 S rRNA gene bioinformatics analysis

The methods of DNA extraction, library construction and 16 S rRNA gene bioinformatics analysis are shown in Supplementary Material 1.

Results

Blood pressure

As shown in Fig. 1, the mean systolic blood pressure (SBP)/diastolic blood pressure (DBP) were 136.21 ± 10.08/100 ± 10.15 mmHg, 202.36 ± 6.43/155.65 ± 7.86 mmHg, 206.7 ± 29.36/165.7 ± 22.81 mmHg, 201.52 ± 35.3/161.37 ± 28.51 mmHg, and 201.13 ± 35.84/162 ± 27.77 mmHg (daytime mean) in the WKY, SHR (Cap), SHR (H-I) and SHR (H-II) groups, respectively, before treatment. After six weeks of treatment with captopril, Formula one or Formula two, the mean SBP/DBP values were 129.35 ± 9.03/100.93 ± 8.18 mmHg, 195.21 ± 7.17/161.88 ± 7.82 mmHg, 160.73 ± 31.33/130.33 ± 26.49 mmHg, 181.3 ± 37.41/148.6 ± 36.12 mmHg, and 178.66 ± 37.79/148.28 ± 35.08 mmHg (daytime mean) in the WKY, SHR, SHR (Cap), SHR (H-I) and SHR (H-II) groups, respectively, before treatment. After six weeks of treatment with captopril, Formula one and Formula two were administered. After six weeks of drug exposure, the SBP/DBP decreased by 22.23%/21.4%, 10.03%/9.77% and 11.17%/8.46% in the SHR (Cap), SHR (H-I) and SHR (H-II) groups, respectively. Blood pressure did not significantly change between the WKY and SHR groups.

Microbiome structure analysis

The microbiota of these samples was the most abundant of the 15 most abundant prokaryotic phyla and was dominated by *Firmicutes, Bacteroidota, Proteobacteria, Desulfobacterota, Spirochaetota, Actinobacteriota, Campilobacterota, Deferribacterota, Acidobacteriota, Fusobacteriota, Verrucomicrobiota, Patescibacteria, Gemmatimonadota, Nitrospirota* and *Cyanobacteria* (Fig. 2a). The microbiome structures showed a significant difference at the phylum level between the groups before treatment and after treatment. The relative abundances at the phylum level in the samples from WKY, SHR and all treatment groups (SHR (Cap), SHR (H-I) and SHR (H-II)) before treatment were *Firmicutes* (53.6%, 60.14%, 62.54%), *Bacteroidota* (44.06%, 38.08%, 35.08%), *Proteobacteria* (1%, 0.45%, 0.54%), *Desulfobacterota* (0.77%, 0.53%, 0.66%), *Spirochaetota* (0, 0.097%, 0.21%), *Actinobacteriota* (0.18%, 0.29%, 0.37%), *Campilobacterota* (0.1%, 0.36%, 0.43%), *Deferribacterota* (0, 0.004%, 0.08%) and *Acidobacteriota* (0.03%, 0.009%, 0.029%). However, after six treatments, the relative abundances of the phyla *Firmicutes* (59.27%, 58.21%, 58.83%), *Bacteroidota* (36.97%, 39.55%, 39.37%), *Proteobacteria* (0.90%, 0.54%, 0.57%), *Desulfobacterota* (0.88%, 0.43%, 0.50%), *Spirochaetota* (1.7%, 1.0%, 0.15%), *Actinobacteriota* (0.08%, 0.12%, 0.35%), *Campilobacterota* (0.06%, 0.07%, 0.13%), *Deferribacterota* (0.03%, 0.002%, 0.005%) and *Acidobacteriota* (0.02%, 0.01%, 0.02%, 0.02%, 0.01%, 0.02%) were detected in the WKY, SHR and all treatment groups. The relative abundances of *Bacteroidota* and *Proteobacteria* were greater after captopril and antihypertensive peptide exposure than before treatment. The abundances of other phyla decreased compared with those before treatment. The

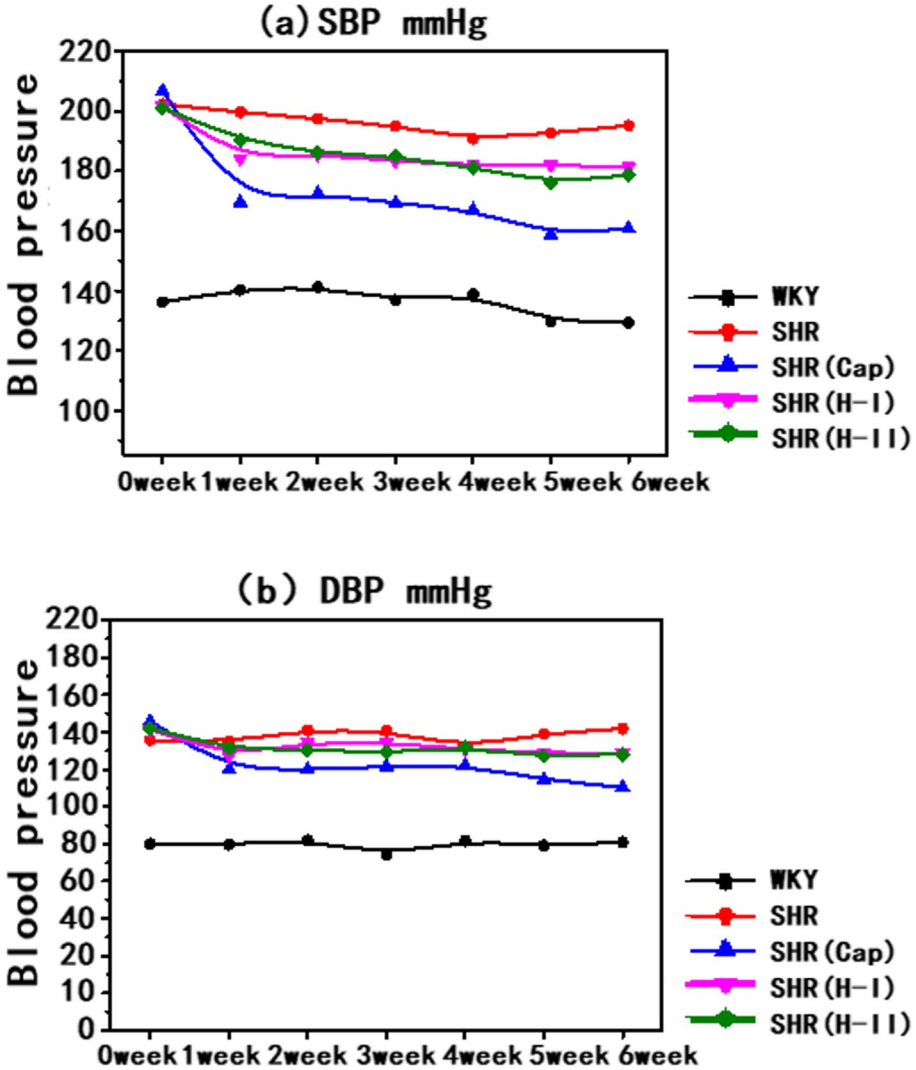


Fig. 1 The blood pressure on different treatment groups. **a** The changes of systolic blood pressure (SBP) during the 6-week experiment; **b** The changes of diastolic blood pressure (DBP) during the 6-week experiment. Note: Wistar Kyoto (WKY) group; spontaneously hypertensive male rats (SHR) group; SHR + Captopril (Cap) group (15 mg/kg rat, per day); SHR + HOP I (H-I) group (800 mg/kg rat, per day); SHR + HOP-II (H-II) group (800 mg/kg rat, per day)

microbiota of these samples was abundant, ranking among the 15 most abundant prokaryotic genera, dominated by *Muribaculaceae, Lactobacillus, Clostridia, Lachnospiraceae, Bacteroides, Alloprevotella, Eubacterium, Prevotella, Christensenellaceae, Ruminococcus, Prevotellaceae, Enterococcus, Monoglobus* and *Prevotellaceae* (Fig. 2b). The relative abundances of *Muribaculaceae, Lactobacillus, Clostridia, Lachnospiraceae, Prevotellaceae* and *Enterococcus* were lower after captopril and antihypertensive peptide exposure than before treatment. Other prokaryotic genera were reduced compared with those before treatment. A heatmap showing the levels of relative abundance is also provided in Fig. 2c.

The richness and diversity indices of the microbiome

The composition of the bacterial communities was analyzed by three major ecological parameters, including Chao richness, Good's coverage and observed species. The index of Good's coverage reflects the sequencing depth. Figure 3a shows that the index of each group was closer to 1, which suggested that the sequencing depth basically covered all species in the samples. Figure 3b and c show that the chao and bserved species numbers significantly changed in the fecal microbiota of the SHR, SHR (Cap), SHR (H-I) and SHR (H-II) groups compared to those of the WKY group after six weeks of treatment.

The distance between the fecal samples of each group was evaluated by beta diversity analysis, and a

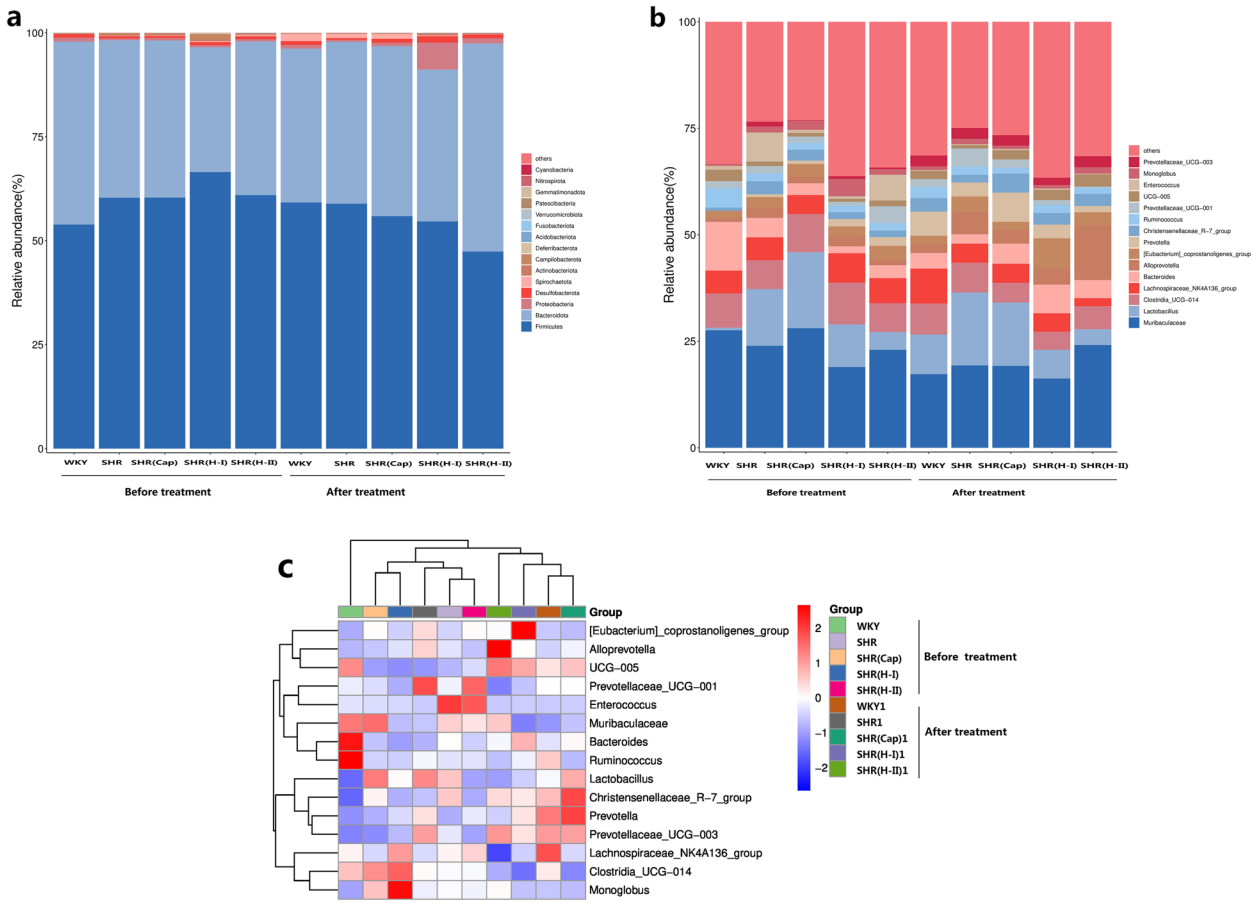


Fig. 2 Sample community structure map. **a** Histogram of the community structure at the phylum level. Note: The top 15 phyla are reported for each sample, and all other phyla are grouped into 'Other'. **b** Histogram of community structure at the genus level. Note: The top 15 families are reported for each sample, and all other families are grouped into 'Other'. Columns represent samples, different colors represent different annotation information, and others represent all species except Top. **c** Species abundance heatmap. The above figure shows the species abundance of TOP15 in each sample under different classification levels of Phyla family species. Group represents different groups; the left clustering tree represents the clustering of species; and the upper clustering tree represents the clustering of samples. The above clustering branch Group represents samples from different groups. Orange indicates a high relative abundance of species, while blue indicates a low relative abundance. Note: Wistar Kyoto (WKY) group; spontaneously hypertensive male rats (SHR) group; SHR + Captopril (Cap) group (15 mg/kg rat, per day); SHR + HOP I (H-I) group (800 mg/kg rat, per day); SHR + HOP-II (H-II) group (800 mg/kg rat, per day)

two-dimensional scatter map was generated by principal coordinate analysis (PCoA). In the end, the similarities or differences in community composition among different groups of samples were discussed. Figure 4a shows good clusters of samples in all the groups after treatment, with bad clusters in all the SHR groups before treatment. A clear separation was observed in the PCoA between the two clusters representing the microbial compositions of WKY rats and all SHR groups before treatment, revealing differences between intestinal environments. However, after six weeks of captopril and antihypertensive treatments, the WKY, SHR, SHR (Cap), SHR (H-I) and SHR (H-II) did not significantly differ, which indicated that the composition of the intestinal flora tended to be similar. Figure 4b reveals the similar relative abundance of the microbiome in the fecal samples. The relatively close proximity between WKY, SHR, SHR (Cap), SHR (H-I) and SHR (H-II) revealed that the multiple peptides mixed with plant extracts and captopril had similar effects on improving the intestinal flora.

Figure 5a, b shows the combination of phylogenetic trees and species abundance data. The phylogenetic trees were built based on the top 100 ASVs with the most tags and the abundance of each amplicon sequence variant (ASV) (Fig. 5a). Figure 5b shows the abundance of the ASVs in different samples. The species correlations at various taxonomic levels at different times are shown by the correlation heatmap and correlation network map (Fig. 5c, $P < 0.01$).

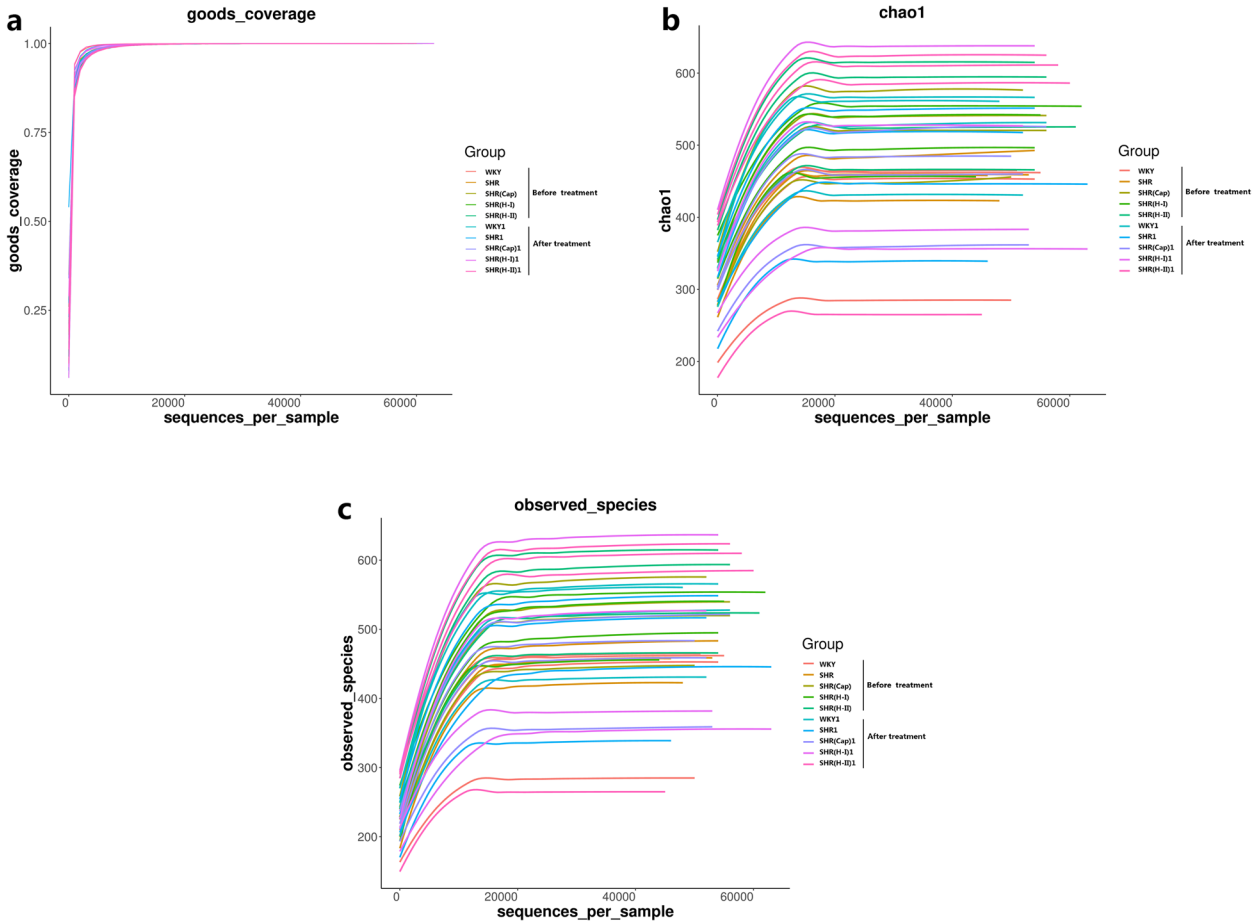


Fig. 3 Amplicon sequence variant (ASV)/diversity index dilution curve. **a** goods coverage; **b** Chao1; **c** observed species; **d** Shannon diversity; **e** Simpson diversity. In the diagram, a curve represents a sample, the horizontal coordinate is the depth of random sampling (that is, the number of sequences sampled), the vertical coordinate is the exponential value, and the legend is the group name. When the curve flattens out with the increase of the number of extracted sequences, it indicates that the amount of sequencing data in the sample is reasonable, and more data will only generate a small number of new ASVs; otherwise, it indicates that continued sequencing may generate more new ASVs. Note: Wistar Kyoto (WKY) group; spontaneously hypertensive male rats (SHR) group; SHR + Captopril (Cap) group (15 mg/kg rat, per day); SHR + HOP I (H-I) group (800 mg/kg rat, per day); SHR + HOP-II (H-II) group (800 mg/kg rat, per day)

Discussion

The gut microbiota plays a vital role in maintaining the intestinal immune system (McDermott & Huffnagle 2004; Chow et al. 2010). Dysbiosis of the gut microbiota induces abnormal immune responses. A close relationship between the gut microbiota and blood pressure has been proven in biological models (Li et al. 2017; Adnan et al. 2017). Some researchers have demonstrated that dysbiosis of the microbiota induces hypertension after transplantation to nonhypertensive populations (Toral et al. 2019a, b). This is the first report of specific changes in fecal microbiota composition and function in a SHR model after exposure to multiple peptides mixed with plant extracts and captopril. The composition of the fecal microbiota was altered in the SHR groups. The microbial abundance of the fecal microbiome was calculated to investigate the changes in taxonomy at the phylum and genus levels. 16 S rRNA gene sequencing revealed changes in the fecal microbiota composition in the treatment groups (the SHR (Cap), SHR (H-I) and SHR (H-II) groups) compared to the WKY and SHR groups. The abundances of *Bacteroidota, Proteobacteria* and *Actinobacteria* increased, and the *Firmicutes/Bacteroidota* ratio decreased in the SHR (Cap), SHR (H-I) and SHR (H-II) groups compared to those in the WKY and SHR groups at the phylum level. Actinobacteria and Proteobacteria can maintain intestinal homoeostasis (Blandford et al. 2018). Bacteroidetes and Firmicutes play essential roles in fatty acid absorption and lipid metabolism in the gut (Semova et al. 2012; Binda et al. 2018). The absorption of

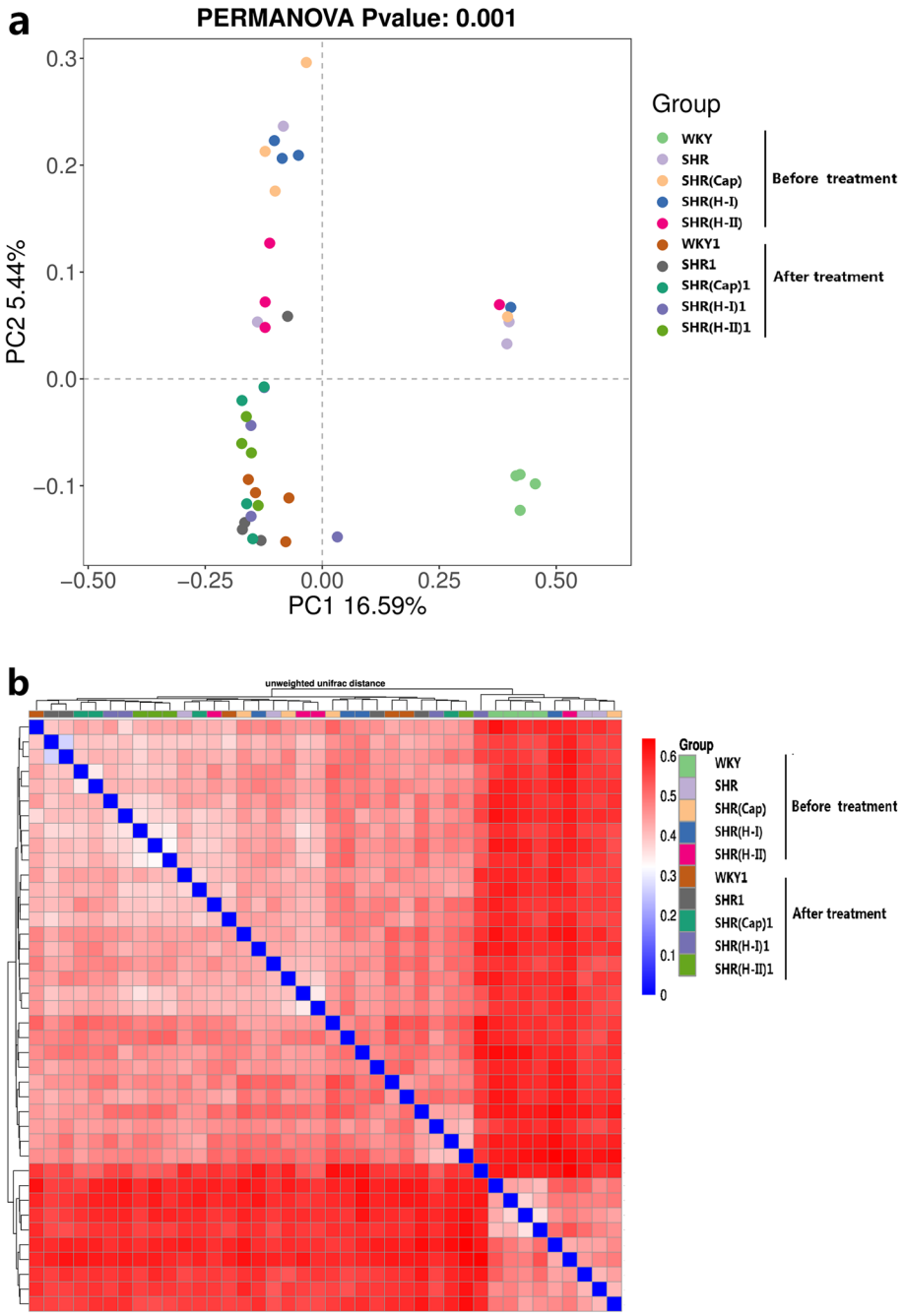


Fig. 4 **a** Principal coordinates analysis (PCoA) (2D), the horizontal coordinate (PC1) and the vertical coordinate (PC2) are the two main coordinates with the largest difference in interpretation between samples. The same color means the same grouping. A point is a sample, and similar samples will gather together. **b** Sample distance heatmap: the bluer the colour is, the closer the distance between the samples, and the higher the similarity. Note: Wistar Kyoto (WKY) group; spontaneously hypertensive male rats (SHR) group; SHR + Captopril (Cap) group (15 mg/kg rat, per day); SHR + HOP I (H-I) group (800 mg/kg rat, per day); SHR + HOP-II (H-II) group (800 mg/kg rat, per day)

intestinal fatty acids was significantly correlated with the ratio of *Bacteroidetes* to *Firmicutes* (Semova et al. 2012). Yang et al. (2015) reported that minocycline rebalanced hypertension-related dysbiotic gut microbiota by reducing the *Firmicutes/Bacteroidota* ratio in angiotensin II-induced hypertension. The blood pressure data were consistent with this result. SHR (Cap), SHR (H-I) and SHR (H-II) treatment reduced blood pressure by regulating the Firmicutes/Bacteroidetes ratio. The abundances of *Desulfobacterota, Spirochaetota, Actinobacteriota,*

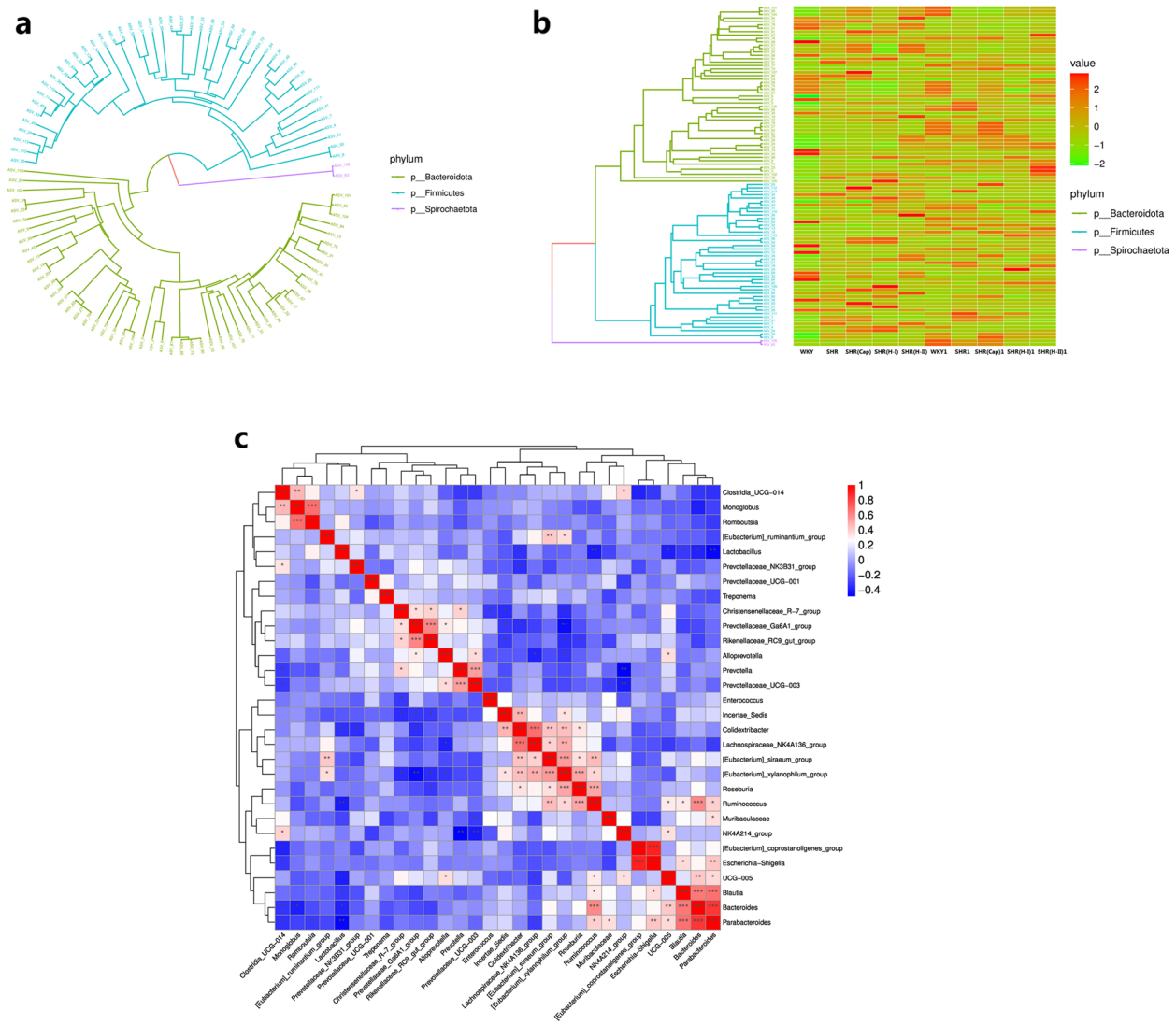


Fig. 5 **a** Top 100 evolutionary tree; **b** Amplicon sequence variant (ASV) abundance map. Note: the evolutionary tree and the phylum information are on the left; the abundance map on the right corresponds to the abundance of ASVs on the left in each sample; **c** Correlation heatmap: red indicates a positively correlation, and blue indicates a negative correlation. The species on the map all had $P < 0.01$. Note: Wistar Kyoto (WKY) group; spontaneously hypertensive male rats (SHR) group; SHR + Captopril (Cap) group (15 mg/kg rat, per day); SHR + HOP I (H-I) group (800 mg/kg rat, per day); SHR + HOP-II (H-II) group (800 mg/kg rat, per day)

Campilobacterota and *Deferribacterota* were significantly different between the pretreatment groups and after-treatment groups. There were significant differences in taxa among the WKY, SHR and treatment groups at the genus level, including *Muribaculaceae, Lactobacillus, Clostridia, Lachnospiraceae, Prevotellaceae and Enterococcus*. O'Callaghan & O'Toole (2013) suggested that *Lactobacillus* was vital for maintaining intestinal stability and integrity (Lai et al. 2016). A decrease in *Lactobacillus* has the potential to exacerbate heart failure (Adnan et al. 2017). Research has shown a positive correlation between systolic blood pressure and *Lactobacillus* in the SHR model. In our study, the WKY group had a greater abundance of *Lactobacillus* than the SHR group. Therefore, a decrease in the abundance of *Lactobacillus* was associated with hypertension. Captopril and antihypertensive peptides have antihypertensive effects by modifying the gut microbiota. The alpha diversity analysis showed significant differences in fecal microbiota diversity between WKY, SHR and treatment (SHR (Cap), SHR (H-I) and SHR (H-II) groups based on the Chao 1, Simpson and Shannon indices. The richness and diversity of the fecal

microbiota were reversed and enhanced, respectively, in the treatment groups. Beta diversity indices indicate dissimilarities among different groups. The SHR groups deviated from the WKY group before treatment. In contrast, the WKY group and SHR groups were closely clustered and showed more similarities after six weeks of treatment. These results indicated that captopril and antihypertensive treatment improved the composition of the gut microbiota, including *Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria* and *Lactobacillus*, and consequently exerted functional effects on the prevention and treatment of hypertension.

Currently, some bioactive peptides made by enzymolysis from different aquatic organisms and plants, including tuna, sea bream, sea cucumber, wakame and soya bean, exhibit good antihypertensive effects in the SHR model (Kim & Wijesekara 2010; Lee et al. 2010; Fahmi et al. 2004; Suetsuna & Nakano 2000; Zhao et al. 2009). In this study, the formulation of multiple peptides and plant extracts exhibited good antihypertensive activity. The initial blood pressure was 202/155 mmHg in the SHR model group, which was much greater than that in the WKY group (136/100 mmHg). After six weeks of treatment with two kinds of formulated products acquired from multiple peptides mixed with plant extracts, the rats exhibited good hypertension. The SBPs decreased by 20 mmHg after oral exposure (800 mg/kg/day) to the two formulated products for 24 h, and the antihypertensive effect lasted for 24 h. Similar to our study, some researchers found that bioactive peptides extracted from clams (*Ruditapes philippinarum*) and Ruditapes philippinarum fermented with Bacillus natto had antihypertensive effects (Song et al. 2021; Suetsuna 2010). In addition, this is the first study to assess the long-term antihypertensive effect of multiple peptides mixed with plant extracts. Blood pressure decreased significantly after six weeks of continuous administration of antihypertensive formulated products, similar to captopril. The hypotensive function of formulated products is related to their ability to regulate the intestinal flora and increase the proportion of beneficial bacteria. However, sex differences were not investigated in this study. The relationship between sex-independent effects of antihypertensive formulated products needs further investigation in the SHR model. Modifying the gut microbiota with functional peptides is an excellent method for supporting the growth of beneficial bacteria in the gut. Therefore, improving our understanding of antihypertensive formulated products from plants and aquatic organisms and their effects on the gut microbiota can improve the development of health care products.

Conclusions

In this study, two kinds of formulated products reduced hypertension in the SHR model. The formulation of multiple peptides and plant extracts also improved dysbiosis by increasing the proportion of beneficial bacteria at various taxonomic levels. The ratio of *Bacteroidetes* to *Firmicutes* and the relative abundance of *Lactobacillus* increased in the food-derived mixture with antioxidants, anti-inflammatory agents and prebiotic treatment groups. Multiple peptides and plant extracts can be processed into healthcare products with a modulatory influence on the ecology of the intestinal flora as a possible health-promoting regulator of the gut microbiota.

Abbreviations

WKY	Wistar Kyoto
HOP	Health-originated peptides
HOP-I	Health-originated peptides formula one
HOP-II	Health-originated peptides formula two
SHR	Spontaneously hypertensive male rats
PCoA	Principal coordinates analysis
ASVs	Amplicon sequence variants
RAS	Renin-angiotensin system
ACE	Angiotensin converting enzyme
ET-1	Endothelin-1
NO	Nitric oxide
RAAS	Renin-angiotensin-aldosterone system
SBP	the mean systolic blood pressure
DBP	Diastolic blood pressure

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-024-00252-6.

Supplementary Material 1.

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Authors' contributions

Jia Du wrote the main manuscript; Miao Xiao provided resources; Naomi Sudo coordinated the project; Qinghua Liu designed the project, and revised the manuscript. All contributing authors have read and approved the final version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that there is no competing interest associated with this work.

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