



Short-term Effects of Garlic-Based Diets on mRNA Expression of Angiotensinogen, Angiotensin-1 Converting Enzyme, and Atrial Natriuretic Peptide in Cyclosporine-Induced Prehypertensive Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author OOA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author OBA managed the analyses of the study. Authors OOA and OBA managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The short-term effects of garlic, *Allium sativum* L., on the mRNA expression of angiotensin-1 converting enzyme (ACE), angiotensinogen (AGT), and atrial natriuretic peptide (ANP) in cyclosporine-induced prehypertensive rats were investigated in this work. Seven (7) groups of

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animals totaling n=7 were created. Prehypertensive (induced with 25mg/kg cyclosporine) and normal rats were given 10% and 20% diets based on garlic for 7 days. Alteration of Na⁺ and K⁺ levels, increased systolic and diastolic blood pressures, and ACE, AGT & ANP mRNA expressions were all associated with cyclosporin-induced prehypertension. In rats placed on garlic-based diets, these effects were reversed.

Keywords: Hypertension; cyclosporine; garlic; renin-angiotensin system.

1. INTRODUCTION

High blood pressure is a worldwide health problem and the majority of hypertension cases are primary (essential) hypertension, which is high blood pressure caused by general lifestyle choices and hereditary factors [1,2,3]. Heart attack, stroke, and other significant health problems are more likely when hypertension is not under control [4,5].

Prehypertension, also known as borderline hypertension, is a kind of hypertension when a person's blood pressure readings are higher than usual but not high enough to be categorized as hypertension [6]. It is asymptomatic and generally goes unnoticed until more severe stages of hypertension emerge, according to research [6]. Prehypertension may make people more likely to have cardiovascular disease as they mature. As a result, it is connected to the morbidity of high blood pressure. Several steps are taken to reduce the risk of this development, with the primary goal being to decrease blood pressure [7]. These treatments might be pharmaceutical or nutritional. It is strongly advised to first try dietary methods before seeking medication assistance [8].

Additionally, it has been said that herbs and spices have medicinal properties because of their abundance in bioactive compounds, essential oils, and complex organic component mixes [9,10]. Herbs and spices are used in traditional medicine as well as for culinary purposes because of their well-known health benefits. Recent studies on the medicinal and dietary advantages of herbs and spices have given rise to new views in the fields of nutraceuticals and functional foods [11,12].

Garlic is one of these plants that have both culinary and medicinal uses. Considering how pungent and spicy it tastes, it is often used as a spice or condiment all over the world [13]. The bulb of the garlic plant is the part that is used most often. Vitamins, minerals, and a sizable quantity of potassium are all abundant in raw

garlic. Due to its health benefits, which include its use in traditional medicine to treat high blood pressure, it has apparently grown to be one of the most popular spices [14,15,16]. Hypertensive people often use this therapeutic spice as regular dietary additions in an attempt to control blood pressure.

It is commonly thought that a person's diet has a big influence on their health [17]. Changes in gene expression have been studied in response to numerous dietary interventions through the field of nutrigenomics research [18]. Therefore, diet may significantly affect how genes are expressed, particularly when it comes to those factors implicated in the etiology of illnesses like hypertension [19].

Furthermore, earlier research has shown a variety of molecular pathways by which garlic may exert its antihypertensive effects [20]. The renin-angiotensin system (RAS), a hormonal mechanism, regulates blood pressure as well as fluid and electrolyte balance [21]. A few of the RAS's major actors are angiotensinogen (AGT), angiotensin-1 converting enzyme (ACE), and angiotensin II [22]. Increased blood pressure results from aberrant RAS activity [23]. Atrial natriuretic peptide (ANP) is also activated by the blood pressure-raising consequences of aberrant RAS activity [24].

Garlic's long-term or chronic effects on people with chronic hypertension have received a lot of attention from researchers, but concerns affecting borderline hypertension have received less attention, while being just as significant. This study was carried out in order to determine the short-term effects of garlic on borderline blood pressure, genetic expression of AGT, ACE, Na⁺, and K⁺ in cyclosporine-induced rats.

2. METHODOLOGY

2.1 Preparation of Garlic Powder

Garlic powder was prepared using the freeze-drying protocol. Fresh garlic bulbs were selected, thoroughly washed, peeled, and blended using

an electric blender. The slurry obtained was transferred into containers for freeze-drying [25].

2.2 Handling of Animals

The Animal House of the Department of Biochemistry at the University of Ilorin in Kwara State provided the Wistar strain of male albino rats used in this experiment. The rats ranged in weight from 130 to 160 grams. The National Council for Animal Experiments Control's and other international standards were followed while using the animals.

2.3 Cyclosporine Administration

The rats used in the experiment received unrestricted supply of commercial rat pellet diet and clean water. Following acclimation, the rats were split into the seven groups (n=7) shown in Table 1. Prehypertensive rats received 25 mg/kg body weight dosage of freshly produced cyclosporine intraperitoneally daily for seven days [26]. The standard therapy for one of the experimental groups was the ACE inhibitor captopril (10 mg/kg/day). Along with this, the rats were simultaneously given their different experimental diets.

2.4 Formulation of Garlic-based Diets

A modified method was used to formulate garlic-based diets [25] (Table 1).

2.5 Isolation of Total RNA and cDNA Conversion

The rats were euthanized with diethyl ether after receiving experimental meals based on garlic for 7 days. Freshly excised tissues (kidney and heart) were used to isolate total RNA. This was followed by cDNA conversion [27].

2.6 Gene Amplification

Using the Polymerase Chain Reaction, gene amplification was also carried out for the expression of the genes whose primers (SnapGene software) are indicated in Table 2 [27].

2.7 Processing and Quantification of Agarose Gel Bands

Images of in-gel amplicon bands were photographed and processed using the Keynote platform. Using Image-J software, gel density measurement was carried out [28].

2.8 Blood Pressure Assessment

All the rats had their blood pressure measured using the tail cuff technique. Systolic and diastolic blood pressure readings were recorded in millimeters of mercury (mmHg) [29].

Table 1. Garlic-based diet formulation

Ingredients (g/kg)	Groups						
	1	2	3	4	5	6	7
Sucrose	100	100	100	100	100	100	100
Skimmed milk	200	200	200	200	200	200	200
Vitamin-mineral mix	50	50	50	50	50	50	50
Cellulose (Rice bran)	40	40	40	40	40	40	40
Oil	50	50	50	50	50	50	50
Corn Starch	560	560	460	360	460	360	560
Garlic powder	-	-	100	200	100	200	-

Group 1: Normotensive group fed basal diet; Group 2: Prehypertensive rats fed basal diet; Group 3: Prehypertensive rats fed basal diet containing 10% garlic powder; Group 4: Prehypertensive rats fed basal diet containing 20% garlic powder; Group 5: Normotensive group fed basal diet containing 10% garlic powder. Group 6: Normotensive group fed basal diet containing 20% garlic powder. Group 7: Prehypertensive rats fed basal diet + administered Captopril (10 mg/kg/day)

Table 2. Gene sequences for ACE, AGT and ANP primers

Genes	Forward (5' – 3')	Reverse (3' – 5')
ACE	TTTTCCGCGGACGAGGC	CTTCCTCCTGGAGCCGC
AGT	CTGGAGCTAAAGGACA CACAGA	GGTGGATGTATACGC GGTCC
ANP	GCAAACATCAGATCGTGCC	TTGCTCCAATATGGCCTGGG
Beta-actin	CTGGCTCCTAGCACCATGAA	CGCAGCTCAGTAACAG TCCG

2.9 Blood Plasma Preparation

Following seven days of experimental feeding with garlic-based diets, blood samples were drawn into lithium heparin bottles through ocular puncture, where they were centrifuged to prepare the plasma for analysis. Blood plasma was then aspirated into plain bottles and kept in storage until it was needed for analysis.

2.10 Plasma Sodium Ion Assessment

The sample (50 μ l) was also added to the test tube while 50 μ l of distilled water was also added to the blank tube. This was followed by continuous vigorous shaking for 3 minutes. This was also followed by high speed centrifugation (15000g) for 10 minutes and the absorbance of the supernatant fluids was measured at 550nm [30].

2.11 Plasma Potassium Ion Assessment

Potassium ion concentration in plasma was also determined by colorimetric technique. Two test tubes were labeled as blank and sample tubes. 1ml of potassium reagent was pipetted into the test tubes. Accordingly, samples to the respective tubes at 10 μ l. The solutions were mixed and allowed to sit at room temperature for 3 minutes. After 3 minutes, the absorbance of the solutions was read at 500nm [31].

2.12 Statistical Analysis

For gene expression studies, all results for the mRNA expression were analyzed using GraphPad Prism 5.01. The results of all biochemical analysis are expressed as Mean \pm standard deviation (SD). The presence of variability was examined using one-way analysis of variance (ANOVA) combined with Duncan Multiple Range Test (using SPSS 20 software).

3. RESULTS

Table 3 shows the systolic and diastolic blood pressures of the experimental animals before induction, after induction and concurrent feeding with garlic-based diets for 7 days. Significant increase in systolic and diastolic blood pressure was observed due to cyclosporine administration ($p < 0.05$) but garlic-based diets caused a significant decline in systolic and diastolic blood pressure readings.

Fig. 1 shows the results of the effect of garlic-based diets on mRNA expression of Angiotensin-

1 Converting Enzyme (ACE) in the kidneys of cyclosporine-induced rats. The prehypertensive rats showed the highest increase in ACE expression ($p < 0.05$) compared to the normotensive rats fed basal diet. The results also show a significant decrease ($p < 0.05$) in ACE expression in the prehypertensive rats fed 10% and 20% garlic-based diets; normotensive rats fed 10% and 20% garlic-based diets and prehypertensive rats treated with 10mg/kg captopril when compared with the prehypertensive rats. The normotensive rats fed 20% garlic-based diet and prehypertensive rats treated with 10mg/kg captopril showed significantly low ($p < 0.05$) expression of ACE when compared with the normotensive rats fed basal diet and the other groups.

Fig. 2 shows the results of the effect of garlic-based diets on mRNA expression of Angiotensinogen (AGT) in the kidneys of cyclosporine-induced rats. The prehypertensive rats showed a markedly significant increase ($p < 0.05$) in AGT expression compared to the normotensive rats fed basal diet. It is also observed that prehypertensive rats fed 10% and 20% garlic-based diets; normotensive rats fed 10% and 20% garlic-based diets and prehypertensive rats treated with 10mg/kg captopril showed significantly decreased expression ($p < 0.05$) of AGT when compared with the prehypertensive rats.

Fig. 3 shows the results of the effect of garlic-based diets on mRNA expression of Atrial Natriuretic Peptide (ANP) in the heart of cyclosporine-induced rats. The prehypertensive rats showed significant increase ($p < 0.05$) in ANP expression when compared with the normotensive rats fed basal diet. Also, prehypertensive rats fed 20% garlic-based diet and normotensive rats fed 10% garlic-based diet showed significant increase ($p < 0.05$) in ANP expression when compared to normotensive rats fed basal diet. However, ANP expression was found to be significantly decreased ($p < 0.05$) in normotensive rats fed 20% garlic-based diet and prehypertensive rats treated with 10mg/kg captopril when compared with the other groups.

Table 4 shows the effect of garlic-based diets on Na^+ and K^+ levels in the plasma of cyclosporine-induced rats. Plasma Na^+ level was significantly increased ($p < 0.05$) in untreated prehypertensive rats compared with normotensive rats fed basal diet and other experimental groups. Also, a significant reduction ($p < 0.05$) in Na^+ level was observed in

prehypertensive rats fed 20% garlic-based diet when compared with prehypertensive rats fed 10% garlic-based diet. No significant difference ($p < 0.05$) was observed between Na^+ levels of normotensive rats fed basal diet (Group 1) and prehypertensive rats treated with 10mg/kg captopril.

K^+ levels were significantly reduced ($p < 0.05$) in prehypertensive rats when compared with normotensive rats fed basal diet (Group 1) and the other experimental groups. Also, the results show an increase in plasma K^+ level in

prehypertensive rats fed 10% and 20% garlic-based diets when compared with prehypertensive rats although insignificant ($p < 0.05$). A significant increase ($p < 0.05$) in K^+ level was observed in normotensive rats fed 10% garlic-based diet when compared with normotensive rats fed basal diet. However, there is no significant difference between the K^+ levels observed in normotensive rats fed basal diet, prehypertensive rats fed 10% garlic-based diet, normotensive rats fed 20% garlic-based diet and prehypertensive rats treated with 10mg/kg captopril ($p < 0.05$).

Table 3. Systolic and diastolic blood pressures (mm/Hg) of experimental rats before and after induction with cyclosporine

Group B.P	Before induction		After induction	
	Systolic B.P	Diastolic B.P	Systolic B.P	Diastolic
1	98.25 ± 16.50 ^a	73.00 ± 19.49 ^a	101.00 ± 18.57 ^{ab}	62.00 ± 21.97 ^a
2	95.50 ± 4.43 ^a	71.25 ± 10.87 ^a	131.50 ± 13.67 ^c	108.75 ± 9.22 ^b
3	98.25 ± 11.79 ^a	78.50 ± 13.13 ^a	82.50 ± 7.33 ^a	62.75 ± 6.13 ^a
4	97.50 ± 2.38 ^a	69.75 ± 18.57 ^a	80.75 ± 14.97 ^a	60.75 ± 13.72 ^a
5	99.75 ± 9.39 ^a	72.50 ± 13.08 ^a	109.25 ± 21.73 ^{bc}	82.00 ± 26.23 ^a
6	92.25 ± 10.21 ^a	69.25 ± 10.53 ^a	85.5 ± 1.91 ^{ab}	66.25 ± 3.50 ^a
7	95.00 ± 16.09 ^a	67.33 ± 28.92 ^a	94.50 ± 20.73 ^{ab}	73.00 ± 20.59 ^a

Results are expressed as Mean ± Standard deviation of four ($n=4$) determinations for each group using Analysis of Variance (ANOVA) followed by Duncan's (multiple range test) Post Hoc test; values in the same row for each parameter with superscript having the same alphabets, are not significantly different at $p < 0.05$

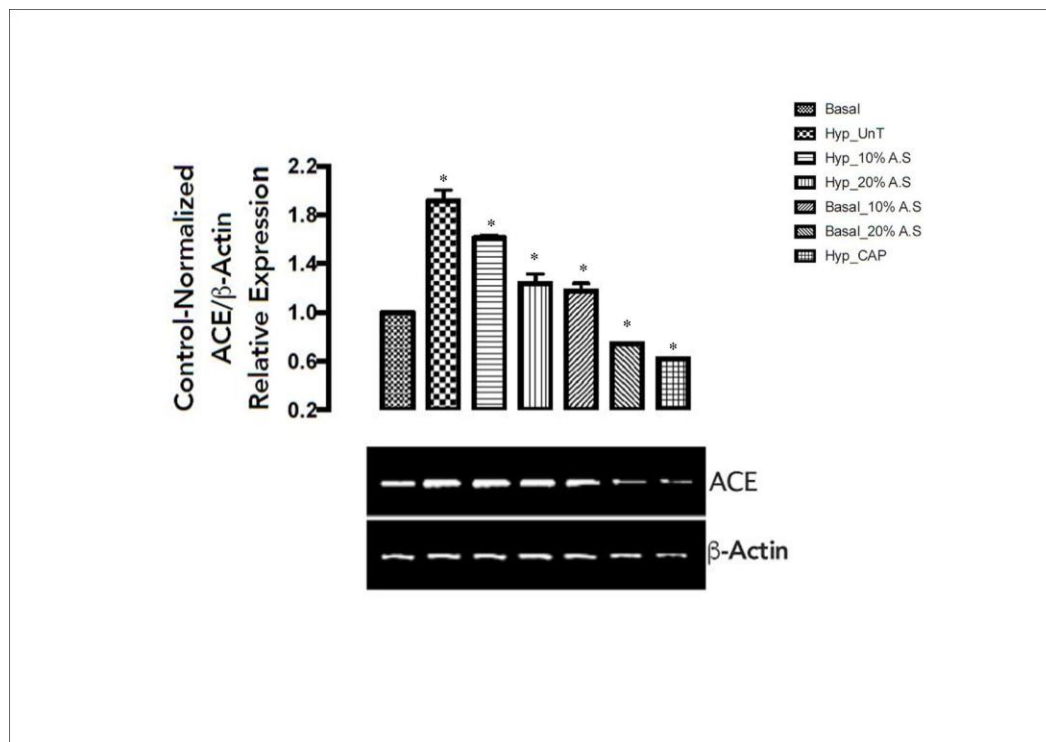


Fig. 1. Short-term effects of garlic-based diets on mRNA expression of Angiotensin 1-converting enzyme (ACE) in the kidneys of cyclosporin-induced prehypertensive rats after feeding for 7 days

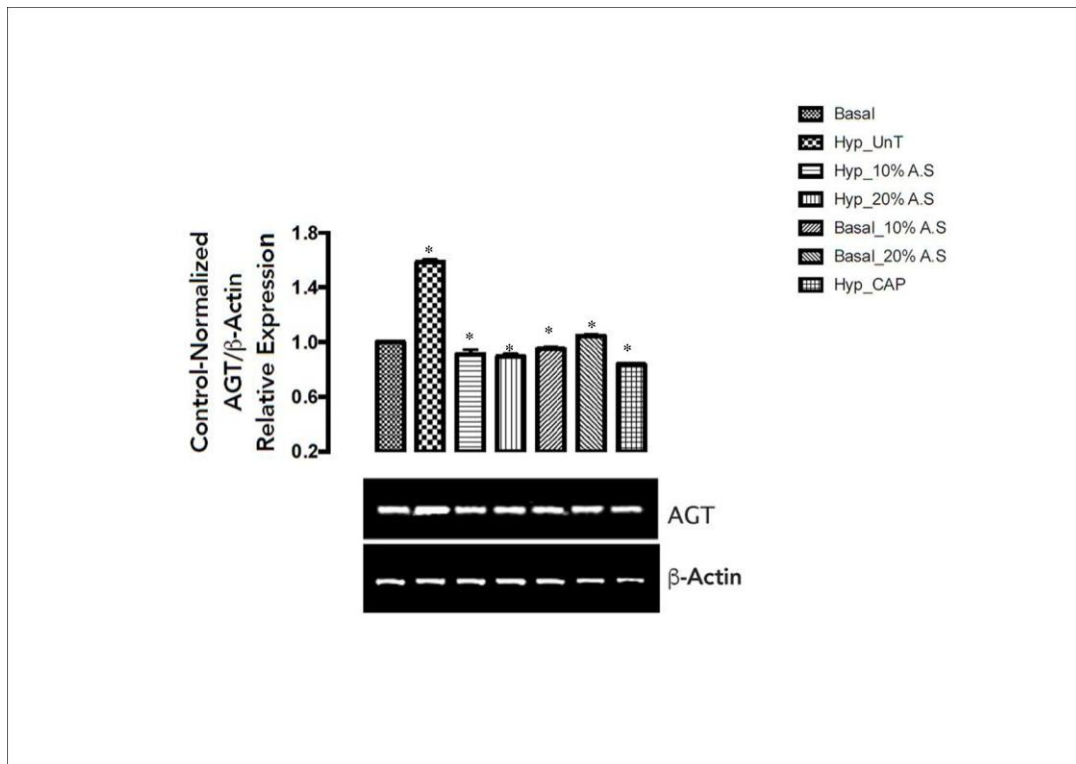


Fig. 2. Short-term effects of garlic-based diets on mRNA expression of angiotensinogen (AGT) in the kidneys of cyclosporin-induced prehypertensive rats after feeding for 7 days

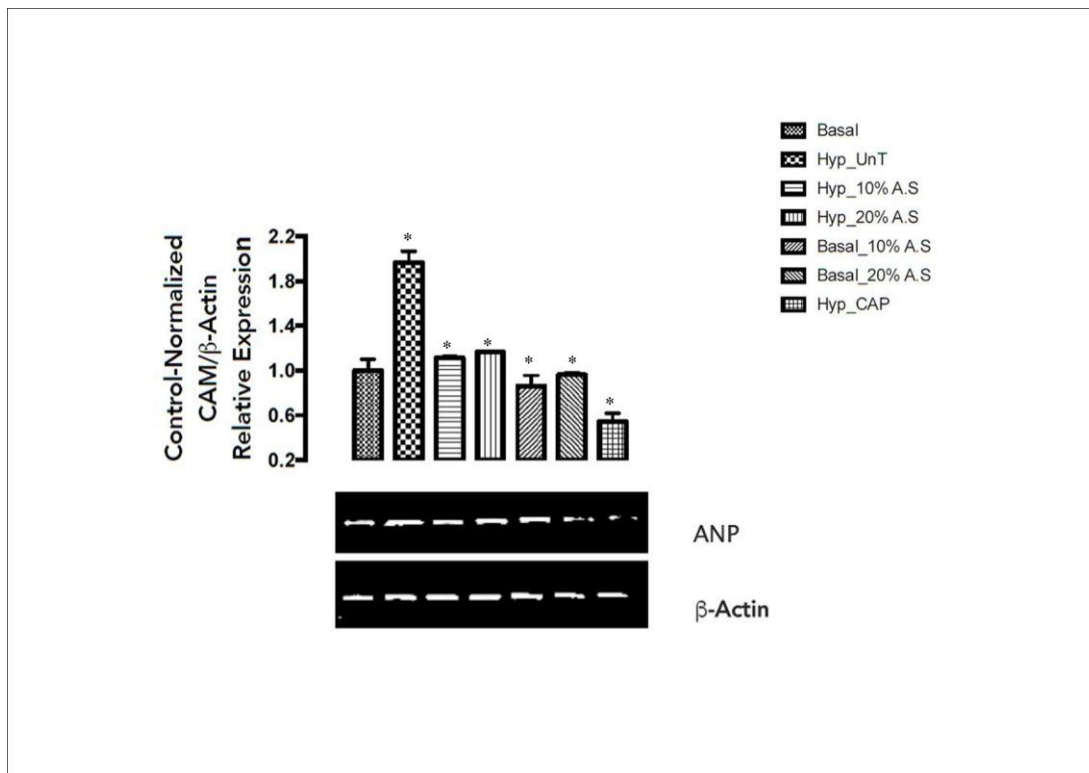


Fig. 3. Short-term effects of garlic-based diets on mRNA expression of ANP in the heart of cyclosporin-induced prehypertensive rats after feeding for 7 days

Table 4. Short-term effects of garlic-based diets on plasma Na⁺ and K⁺ levels in prehypertensive rats after feeding for 7 days

Group	Na ⁺ (mEq/dl)	K ⁺ (mEq/dl)
1	121.00 ± 0.40 ^a	3.97 ± 0.64 ^{ab}
2	153.60 ± 3.21 ^b	3.16 ± 0.39 ^a
3	141.20 ± 0.96 ^{ab}	3.30 ± 0.31 ^{ab}
4	129.20 ± 0.48 ^{ab}	3.58 ± 0.69 ^a
5	124.00 ± 1.76 ^a	4.16 ± 0.35 ^b
6	139.77 ± 0.79 ^{ab}	3.66 ± 0.16 ^{ab}
7	121.70 ± 0.47 ^a	3.80 ± 0.48 ^{ab}

4. DISCUSSION

In this work, cyclosporine-induced prehypertensive rats' responses to dietary inclusion with garlic powder on the mRNA expression of a few important RAS-related genes were evaluated. Similar to what was observed in prehypertensive rats, cyclosporin (25mg/kg body weight) administration for 7 days led to a significant rise in both systolic and diastolic blood pressure readings ($p < 0.05$) (Table 3). Along with this, ACE, AGT, and ANP mRNA expressions were also significantly upregulated ($p < 0.05$) (Fig. 1-3). These findings concur with earlier studies on the impact of cyclosporine on the mRNA expression of ACE, AGT, and ANP [32,33]. Cyclosporine-induced prehypertension also resulted in a significant increase in Na⁺ levels and a significant decrease in K⁺ levels in the plasma of untreated prehypertensive rats ($p < 0.05$). As a result, these findings are comparable to confirmed cases of rat model hypertension caused by cyclosporine [26,34,35]. This study also indicates that mRNA expression of ACE, AGT and ANP are also upregulated in prehypertensive rats.

As shown in Table 3, when garlic-based diets were fed to prehypertensive rats (at 10% and 20%), systolic and diastolic blood pressure significantly decreased ($p < 0.05$). Garlic's ability to lower blood pressure is thought to be mediated by the RAS. Angiotensin I serve as the starting material for the enzyme angiotensin-1 converting enzyme (ACE), which then transforms it into the vasoactive peptide angiotensin II. Angiotensin II works to tighten blood vessels, which raises blood pressure [36]. The ACE and AGT genes, respectively, are responsible for genetically regulating the production of ACE and AGT [33]. The discovery that ACE and AGT mRNA expression was significantly higher in spontaneously hypertensive rats indicates that ACE and AGT gene expression is higher in

prehypertensive rats [33]. As shown in Figs. 1 and 2, this was also confirmed by this study in the prehypertensive rats that were induced with cyclosporin. The study's findings also revealed a significant graded downregulation ($p < 0.05$) of ACE and AGT mRNA expression in prehypertensive rats after feeding with garlic-based diets (10% and 20%) for 7 days. This suggests that dietary inclusion of garlic may be able to reduce the amount of AGT and ACE produced and secreted into the bloodstream in response to high blood pressure within a short period. This may be due to increased stimulation and release of natriuretic peptides from the heart in an effort to lower blood pressure by inhibiting the production of angiotensin II, which is responsible for increased vasoconstriction, salt reabsorption, and the release of aldosterone. Additionally, it might be the cause of the observed decrease in systolic and diastolic blood pressures in prehypertensive rats fed diets containing 10% and 20% garlic.

From our findings, it was discovered that garlic also caused significantly lower mRNA expression of ACE and AGT in normotensive rats that were given diets containing 10% and 20% of garlic. AGT expression did not significantly increase in the rats fed 20% garlic-fed rats compared to the normotensive rats ($p < 0.05$). This may have been a result of the body's corrective reaction to decreasing blood pressure brought on by consuming garlic. When blood pressure drops under normal circumstances, the body detects it and activates the RAS to bring blood pressure back to normal. Therefore, the RAS's response to steadily reducing blood pressure observed in the rats fed 20% garlic can be linked to the increase in AGT expression. These results raise the possibility of garlic to be able to reduce blood pressure within a short period. Additionally, high doses of garlic consumption by normotensive patients may result in RAS activation, which may increase blood pressure.

The balance of salt and water in the body system is maintained by the heart, along with other organs, in order to maintain homeostasis. The heart produces a cardiac peptide hormone called ANP in response to high blood pressure conditions marked by increased retention of sodium and water in the extracellular fluid [37,38]. The ANP gene, which encodes ANP, is in charge of producing ANP from cardiac cells [39]. While trying to study the transition from cardiac hypertrophy to hypertensive heart failure, previous research found that the ANP gene expression was upregulated in hypertensive

patients [32]. This was further supported in the current study, where prehypertensive rats also displayed a significant rise in ANP gene expression in the heart (Fig. 3). However, intervention with diets containing 10% and 20% of garlic resulted in a significant downregulation of ANP mRNA expression in prehypertensive rats ($p < 0.05$). This demonstrates that garlic might have a favorable impact on the cardiac cells' ability to produce and secrete ANP. Since ANP concentration rises in correlation with an increase in blood pressure, downregulated expression of the ANP gene suggests that the cardiac cells' response to high blood pressure, which causes an increase in ANP concentration, was reduced [32]. As a result, the reduction in ANP mRNA expression and the significant reduction ($p < 0.05$) in systolic and blood pressures seen in prehypertensive rats fed garlic-based diets (10% and 20%) further demonstrate that garlic can correct borderline hypertension and have a positive impact on renal function by inhibiting angiotensin II and aldosterone responsible for increased sodium and water retention that causes rise in blood pressure. This is also connected to the earlier observed downregulation of the genes ACE and AGT brought on by the garlic-based diets. ANP mRNA expression in heart cells was found to be markedly upregulated in normotensive rats fed a diet containing 10% garlic as opposed to normotensive rats fed 20% garlic-based diet in this study. The system's counterproductive response in an effort to exert homeostatic control may also be to blame for this.

Under wholesome circumstances, normal blood pressure range is present in normotensive rats. Given that garlic can lower blood pressure [40], rats with normal blood pressure fed 10% garlic-based diet were also able to further lower their blood pressure. The body's control centers then work to combat the decreased blood pressure that was brought on by eating a diet high in garlic in order to bring blood pressure back into a normal range. This might be accomplished by stimulating the renal sympathetic nervous system or by renin-angiotensin system activation. The stimulation is mediated by baroreceptors which are able to sense changes in blood pressure [41]. Garlic-based diets fed to normotensive rats was able to slightly upregulate the expression of AGT gene, suggesting that garlic may mediate its antihypertensive effect by modulating the RAS within a short period, according to the study's current findings. Angiotensinogen concentration released into circulation would rise in response to

an upregulation in AGT gene expression. An increase in angiotensin II levels would then follow. The cardiac cells release a concentration of ANP into the blood that corresponds to how sensitive they are to high levels of angiotensin II. The increased ANP expression seen in normotensive rats fed a diet containing 20% garlic-based diet is supported by this. These results suggest that garlic, which primarily affects the renin-angiotensin system, can elicit a positive effect on blood pressure regulation within a short period after administration.

In the effort to achieve homeostasis, maintaining electrolytes is equally crucial. The RAS controls sodium ions (Na^+) and potassium ions (K^+), which are the major electrolytes in the extracellular and intracellular fluids, respectively [42]. Through the assistance of the renal parasympathetic system, the kidneys respond by secreting renin in response to a decrease in intrarenal blood pressure at the juxtaglomerular cells [43]. Angiotensin II concentration ultimately raises as a result of this. Angiotensin II increases K^+ excretion and induces tubular salt (Na^+ and Cl^-) reabsorption at the kidneys. As a result, the extracellular fluid's Na^+ concentration rises, increasing osmolality, tonicity, and blood pressure in the process. As a result, hypertensive subjects exhibit high Na^+ and low K^+ levels. When compared to the other experimental groups, plasma Na^+ levels in this study's untreated prehypertensive rats were found to be significantly higher while plasma K^+ levels were significantly lower ($p < 0.05$) (Table 4). On the other hand, garlic-based diets were able to enhance natriuresis, as demonstrated by the negligible decrease in plasma Na^+ and enhanced K^+ reabsorption. As demonstrated by the prehypertensive rats fed diets based on garlic, improved natriuresis and K^+ reabsorption would result in a decrease in blood pressure (Table 3). For prehypertensive rats, captopril, a powerful ACE inhibitor, was the standard medication. The plasma Na^+ level in the prehypertensive rats receiving 10 mg/kg captopril significantly decreased ($p < 0.05$), while plasma K^+ reabsorption significantly increased. The same findings were seen in rats fed diets based on garlic, and their plasma Na^+ and K^+ levels were statistically comparable to those seen in prehypertensive rats given 10 mg/kg captopril.

5. CONCLUSION

The findings of this research point to the possibility of garlic to lower high blood pressure

within a short timeframe. For prehypertensive individuals who are susceptible to future consequences from continuous blood pressure increases while avoiding the adverse effects of antihypertensive medicines, this might also be helpful.

ETHICAL APPROVAL

The Ekiti State University research ethical committee gave its approval.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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