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EFFECTS OF GARLIC (*ALLIUM SATIVUM L.*)-SUPPLEMENTED DIETS ON PLASMA CGMP, NITRIC OXIDE LEVELS AND BLOOD PRESSURE IN NORMOTENSIVE WISTAR RATS

Ajayi Oluwadamilare Oluwaseun[1]*, Ajayi Olubunmi Bolanle[2], Ayodele Oyinlola Roseline[3], Kayode Adekunle Blessing[4], Adedipe Praise Oreoluwa[5], and Akinola Hafsah Olajumoke[6]

Department of Biochemistry. Ekiti State University, Ado-Ekiti. Ekiti State, Nigeria. P. M.B. 5363.

***Corresponding Author: Ajayi Oluwadamilare Oluwaseun**

Department of Biochemistry. Ekiti State University, Ado-Ekiti. Ekiti State, Nigeria. P. M.B. 5363.

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ABSTRACT

This short study assessed the effects of garlic on blood pressure vis-à-vis plasma cGMP and Nitric oxide (NO) levels in normotensive rats fed garlic-based diets for 7 days. The animals used for this study were randomly sorted into three (3) groups (n=4). Group 1: normotensive rats (fed basal diet without garlic powder); Group 2: normotensive rats fed 10% garlic-based diet; Group 3: normotensive rats fed 20% garlic-based diet. After experimental feeding for 7 days, plasma cGMP and NO levels were found to have increased, although insignificantly ($p < 0.05$), in the rats fed 10% and 20% garlic-based diets when compared with rats fed basal diet. Also, systolic and diastolic blood pressure values were found to be significantly increased ($p < 0.05$) in normotensive rats fed 10% garlic-based diet when compared with rats fed basal diet. However, it was observed that systolic blood pressure was significantly decreased ($p < 0.05$) in rats fed 20% garlic-based diets, when compared with normotensive rats fed basal diet and normotensive rats fed 10%. Also, diastolic blood pressure was insignificantly increased ($p < 0.05$) when compared with normotensive rats fed basal diet, but significantly reduced when compared with normotensive rats fed 10% garlic-based diet. These findings may suggest that prolonged consumption of garlic by normotensive subjects especially in high quantities, may affect their blood pressure readings.

KEYZWORDS: Garlic, Hypertension, Nitric oxide, *Allium sativum.*

1.0 INTRODUCTION

Hypertension is rated as one of the most fatal diseases that have contributed to increased mortality and morbidity across the world as stated by the World Health Organization.[1] As a result, several measures have been put in place by scientists to prevent, manage and possibly treat hypertensive cases. Dietary intervention is one of the measures that have been employed over the years in the battle against hypertension.[2] This involved incorporation of medicinal and nutritionally beneficial plant parts into diet.[3] Such plant food sources include herbs and spices which are either used as food additives or consumed directly. Dietary intervention is commonly preferred compared to its pharmacological alternative which involved the use of drugs. This has been attributed to the numerous side effects that antihypertensive drugs may cause to the subjects.[4]

Garlic, *Allium sativum* L., is one of the numerous herbs commonly used by hypertensive patients to lower their blood pressure,[5] and is commonly consumed either as raw garlic or garlic oil. It is a highly nutritious herb containing B-Complex vitamins, vitamin c, potassium, magnesium and zinc amongst other nutrients.[6] Also, garlic is highly rich in sulphur-compounds like allicin, methionine, thiosulfinates, scordinine and so on.[7] Furthermore, its proven effects against cardiovascular diseases have been previously reported.[5,8] Many studies have also confirmed the hypotensive capacity of garlic.[9,10]

Cyclic guanosine monophosphate (CGMP) and Nitric Oxide (NO) are major signaling molecules (second messengers) which have been reported to play important roles in garlic's hypotensive activity.[11,12] Nitric oxide is a potent vasodilator that is synthesized from arginine, catalyzed by nitric oxide synthase. It functions by activating soluble guanylyl cyclase which in turn leads to the synthesis of cGMP, and ultimately works to relax smooth muscles in blood vessels.[13] One of the pathways responsible for the regulation of blood pressure is the Renin-Angiotensin system. Angiotensin II, a potent vasoconstrictor is a key player implicated in increased blood pressure in hypertensive subjects.[14] Nitric oxide and cGMP levels respond to increase in angiotensin II and intracellular Ca^{2+} levels. Thus, NO and cGMP levels are found to be increased in hypertensive subjects.[12,15]

However, it has been discovered that individuals without blood pressure problems also take delight in consuming garlic at will, especially in high quantities. Many individuals consume as many garlic bulbs daily due to its spicy taste and for other possible reasons which have may no medical basis, especially not related with hypertension. Hence, this study assessed the effect of garlic-based diets on blood pressure vis-à-vis plasma cGMP and NO levels in normotensive wistar rats.

2.0 MATERIALS AND METHODS

2.1 Materials

Fresh *Allium sativum* (garlic) cloves were purchased from a local market in Ado-Ekiti, Ekiti State, Nigeria.

2.1.1 Preparation of garlic (*Allium sativum)* powder

The garlic cloves were separated into bulbs, peeled and properly washed. The bulbs were blended and the garlic slurry was freeze-dried at -10°C. The freeze-dried garlic was finally milled into powder and stored in polythene packs at refrigerated temperature for further use.

2.2 Management of experimental animals

Wistar strain of male albino rats weighing between 130g and 160g used for this study was purchased from the Animal house of the Department of Biochemistry, University of Ilorin, Kwara State. All the animals used for this work were maintained according to the international guidelines on Animal care and use for scientific purposes.[16]

2.2.1 Experimental feeding with garlic-based diets

The experimental rats were provided with commercial rat pellets and water *ad libitum*. The rats were randomly allocated into three experimental groups and introduced to their dietary regimen for 7 days (n= 4) as shown in Table 1 below.

2.3 Diet formulation

Allium sativum-based diets were formulated[17] as shown in Table 1.

Table 1: Diet formulation.

	Groups		
Ingredients (g/kg)	**1**	**2**	**3**
Corn Starch	560	560	560
Skimmed milk	200	200	200
Sucrose	100	100	100
Vitamin-mineral mix	50	50	50
Vegetable Oil	50	50	50
Cellulose (Rice bran)	40	40	40
Allium sativum powder	-	100	200

Group 1: Normotensive group; fed basal diet (without *Allium sativum* powder)
Group 2: Normotensive group; fed basal diet containing 10% *Allium sativum* powder.
Group 3: Normotensive group; fed basal diet containing 20% *Allium sativum* powder.

2.4 Measurement of blood pressure

Systolic and diastolic blood pressures were measured in the experimental after 7 days of experimental feeding using tail cuff method. A soft valve extension of the equipment was wrapped round the tail or left leg of the resting experimental rats. The readings were measured in millimeters of mercury (mmHg).[18]

2.5 Preparation of blood plasma

The animals were sacrificed via diethyl ether euthanasia and blood samples were collected after 7 days of experimental feeding with *Allium sativum*-based diets. The blood samples were collected into lithium heparin bottles via ocular puncture and centrifuged for plasma preparation prior to analysis. Thereafter, blood plasma were aspirated into plain bottles and stored at 8^0C until required for assessment of biochemical parameters.

2.6 Determination of plasma CGMP levels

This assay was carried out using ELISA (Enzyme-linked immunosorbent assay) method.[19] 50µl of neutralizing reagent was added into each of the ELISA wells, except the total activity and blank wells. This was followed by the addition of 100µl of 0.1M HCl into the Non-specific binding and standard wells. Thereafter, 100µl of standards and samples was also added to the appropriate wells. 50µl of 0.1M HCl was pipetted into the NSB wells while 50µl of HRP-conjugate was pipetted into each well except the total activity and blank wells. The plates were incubated at room temperature for 2 hours on a plate shaker at 250-500rpm. The contents of the wells were emptied and washed by adding 400µl of wash solution to every well. The washing was repeated two times for a total of three washes. After the final wash, the wells were aspirated and the plate was firmly tapped on a lint free paper towel to remove any wash buffer remaining. This was followed by the addition of 5µl of the HRP-conjugate to the total activity wells. 200µl of the substrate solution was added to every well and incubated at room temperature for 5-30 minutes without shaking. A gradient of blue color became visible during the incubation period. 50µl of stop solution was added to every well. This stops the reaction. The plate reader is blanked against the Blanks wells and the optical density is read at 450nm (for HRP), preferably with correction between 570 and 590nm.

2.7 Determination of plasma NO concentration

This assay employs the quantitative sandwich enzyme immunoassay technique (CUSABIO Nitric oxide assay kit). 100µl of standard or sample was added to each well and incubated for 2 hours at 37°C. The liquid present in each of the wells was removed. 100µl Biotin-antibody was added to each well and also incubated for 1 hour at 37°C. The mixture was aspirated and washed for five times. Thereafter, 90µl TMB substrate was added to the wells and incubated for 30 minutes at the same temperature. 50µl of stop solution was also added to each of the wells and the absorbance was read using a UV-spectrophotometer at 450nm within 5 minutes.

2.8 Statistical analysis

The results of the various biochemical analyses are expressed as Mean ± standard deviation. One-Way Analysis of variance (ANOVA) was used to test for variability using SPSS 20. This was also followed by Duncan Multiple Range Test.

3.0 RESULTS

3.1 Plasma CGMP levels

A significant increase ($p < 0.05$) in plasma cGMP level was observed in rats fed 10% and 20% garlic-based diets when compared with rats fed basal diet. Also, it was observed that there was an insignificant increase ($p < 0.05$) in cGMP level in rats fed 10% garlic-based diet when compared with rats fed 20% garlic-based diets (Table 2).

3.2 Plasma NO levels

An insignificant increase ($p < 0.05$) in plasma cGMP level was observed in rats fed 10% and 20% garlic-based diets when compared with rats fed basal diet. Also, it was observed that there was an insignificant increase ($p < 0.05$) in CGMP level in rats fed 10% garlic-based diet when compared with rats fed 20% garlic-based diets (Table 2).

3.3 Systolic and diastolic blood pressures

It was observed that systolic blood pressure (SBP) was significantly decreased ($p < 0.05$) in rats fed 20% garlic-based diets, when compared with normotensive rats fed basal diet and normotensive rats fed 10%. Also, diastolic blood pressure (DBP) was insignificantly increased ($p < 0.05$) when compared with normotensive rats fed basal diet, but significantly reduced when compared with normotensive rats fed 10% garlic-based diet (Table 3).

Table 2: Plasma cGMP and Nitric oxide levels in normotensive rats fed garlic based diets

Group	cGMP (pg/ml)	Nitric Oxide (mg/dl)
1	1.88 ± 0.09^{a}	140.26 ± 1.81^{a}
2	1.96 ± 0.15^{b}	140.53 ± 0.46^{a}
3	1.97 ± 0.16^{b}	140.83 ± 0.75^{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 tests) Post Hoc test; values in the same row for each parameter with superscript different from control are significantly different at p <0.05.

Group 1: Normotensive group; fed basal diet (without *Allium sativum* powder)
Group 2: Normotensive group; fed basal diet containing 10% *Allium sativum* powder.
Group 3: Normotensive group; fed basal diet containing 20% *Allium sativum* powder.

Table 3: Systolic and diastolic blood pressure values of normotensive rats fed garlic-based diets

Group	SBP (mm/Hg)	DBP (mm/Hg)
1	101.00 ± 18.57^{b}	62.00 ± 21.97^{a}
2	109.25 ± 21.73^{c}	82.00 ± 26.23^{b}
3	85.50 ± 1.91^{a}	66.25 ± 3.50^{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 tests) Post Hoc test; values in the same row for each parameter with superscript different from control are significantly different at p <0.05.

Group 1: Normotensive group; fed basal diet (without *Allium sativum* powder)
Group 2: Normotensive group; fed basal diet containing 10% *Allium sativum* powder.
Group 3: Normotensive group; fed basal diet containing 20% *Allium sativum* powder.

4.0 DISCUSSION

Highly medicinal and nutritious plant parts have been used as supplements with an aim to alleviate symptoms of many diseases[20] including hypertension. Garlic is widely used as a food additive and is also consumed in its various forms by different individuals with hypertension.[5] However, that is not the only stand on which garlic is consumed. Some individuals consume it as a normal food substance without medical reasons attached. This study aimed at assessing the effects of garlic-based diets on blood pressure vis-à-vis plasma cGMP and NO levels in normotensive wistar rats. Cyclic guanosine monophosphate (CGMP) and Nitric oxide (NO) are second messengers linked with a major homeostatic regulator, the Renin-Angiotensin system (RAS), and are therefore important key players in the regulation of blood pressure.[15,21] They are also found at crucial points in signal transduction cascades.[22]

NO is a pluripotent molecule produced by the vascular endothelium and acts as a second messenger. It is able to cause relaxant effects in blood vessels by stimulating the activity of soluble guanylyl cyclase A (GC-A) which in turn produces cGMP.[21] Thus, NO concentration influences the level of intracellular cGMP. The results from this study showed that consumption of garlic-based diets by normotensive rats caused significant increase ($p < 0.05$) in plasma cGMP levels. This was also accompanied by an insignificant increase ($p < 0.05$) in plasma NO levels. Under hypertensive conditions, blood vessels become constricted due to the action of a vasoconstrictive peptide called angiotensin II.[15] This stimulates the release of NO which is a potent vasodilator. NO also stimulates the activity of guanylyl cyclase A (GC-A) which is responsible for the production of cGMP in high levels so as to regulate blood pressure. As observed from this study, consumption of garlic under normotensive conditions may lead to significant decrease in blood pressure, as well as significant increase in cGMP and NO levels. This suggests that excess consumption of garlic may cause abnormalities in major blood pressure regulatory pathways especially the RAS. This is due to the increased levels of plasma CGMP and NO levels observed in normotensive rats fed garlic-based diets. The high SBP and DBP values observed for normotensive rats fed 10% garlic-based diet may be as a result of systemic response to low blood pressure that was possibly induced by the garlic-based diets. This may also explain why CGMP and NO levels were observed to be significantly increased when compared with normotensive rats fed basal diet.

Conclusively, the present observations presented in this study, it may be relevant to presume that consumption of garlic in high quantities by normotensive subjects may have future implications on their blood pressure recordings. Hence, it is relevant to suggest that garlic consumption by normotensive individuals should be done minimally.

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