Effects of aqueous extract of garlic *(Allium sativum)* on the left ventricle myocardium of high salt-fed adult Wistar rats

Olusola S. Saka¹, A. Omobola Komolafe¹, Oludare Ogunlade², A. Rotimi Owolabi², Ahmed A. Olayode¹, Babatunde E. Arayombo¹

¹Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun-State, Nigeria
²Department of Physiological Sciences, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun-State, Nigeria

Abstract

**Objectives:** The aim of this study was to evaluate the beneficial effect of aqueous extract of garlic *(Allium sativum)* on the morphology of myocardium of left ventricle in high salt-fed diet Wistar rats.

**Methods:** Twenty-five female Wistar rats weighing 130–180 g were divided into five groups (n=5, each). Group A were fed with standard laboratory pellets, while Groups B, C, D and E were fed a high-salt diet for five weeks. Following this, daily administration of aqueous garlic extract was done orally to Groups C, D and E, respectively for 3 weeks. The left ventricle of the heart was excised, processed in paraffin wax and stained with haematoxylin and eosin, Masson’s trichrome and Verhoeff-Van Gieson stains. One-way ANOVA was used to analyze data, followed by Student-Newman-Keuls (SNK) test for multiple comparisons.

**Results:** We found that the relative change in heart weight in the high salt-fed group was lower, though not statistically significant (p=0.175) than the control group. There was significant increase (p<0.05) in plasma levels of sodium and potassium in Group B when compared with control, but this was dose-dependently reversed by aqueous garlic extract in Groups D and E. Histological and histochemical results revealed morphological alterations in the left ventricle of Group B which were also reversed in Groups D and E.

**Conclusion:** The results of this study indicate that high salt diet-induced histochemical and histomorphological changes in the left ventricle of Wistar rats were significantly reversed by oral administration of garlic extract.

**Keywords:** garlic; heart; high salt diet; ventricle

Introduction

High salt intake has been reported to promote elevation of blood pressure and cardiovascular changes including left ventricular hypertrophy (LVH), cardiac fibrosis and endothelial dysfunction.¹Salt overload increases LVH two-fold, as evaluated by cardiac weight, in 7-week-old spontaneous hypertensive rats (SHR).² Similarly, 12-week-old SHRs develop cardiac hypertrophy when subjected to high salt intake.³ LVH significantly contributes to impairment of cardiac systolic and diastolic function with serious implication on hemodynamics. Left ventricular hypertrophy (LVH) has thus been identified as an important predictor of prognosis in cardiovascular diseases.⁴ Hence, reversal or prevention of LVH is an important component of treatment of cardiovascular diseases, directed at reducing morbidity and mortality.⁵ Increasing evidence also suggests that salt intake may have a direct effect on LVH independent of blood pressure.⁶ A review of nine cross-sectional studies showed a close positive cor-
relation between salt intake and left ventricle mass, with correlation coefficients ranging from 0.22 to 0.61.\[7\]

Presently, the mainstay of treatment modality for LVH is pharmacotherapy for effective blood pressure control and prevention of cardiovascular changes. These drugs have been reported with varying degrees of success, side effects, and are not always affordable.

*Allium sativum* is reported to have many biological activities, including protective roles on the cardiovascular system.\[8,9\] It has been established that garlic has antihypertensive and antioxidant properties.\[10–13\] Allium species such as onions and garlic are used as foodstuff, condiment, flavoring, and traditional medicine.\[14\] It is of the onion genus *“Allium”* and is commonly referred to as garlic. Epidemiologic studies have suggested an inverse relationship between nutritional garlic intake and the occurrence of cardiovascular disorders, and this was attributed to the protective effect of garlic.\[15\]

In many developing countries, a large proportion of the population rely heavily on traditional practitioners and medicinal plants to meet primary health care needs. Many herbs have remained as an alternative to conventional therapy especially in poor areas.\[16\] Garlic has attracted particular attention of modern medicine due to its widespread health use around the world, and the belief that it helps in maintaining good health, wards off illnesses and provides more vigour.\[17\] One of the primary active compounds that give garlic its characteristic odor and many of its healing benefits is called alliin. In the undamaged clove, over 70% of the sulphur compounds exists as (+)-S-allyl-L-cysteine sulphoxide or alliin, (+)-S-trans-1-propenyl-L-cysteine sulphoxide or isoalliin and S–methylcysteine sulphoxide or S–methylcysteine sulphoxide or isoalliin and methin and γ glutamyl peptides such as γ glutamyl-S-allylcysteine and γ-glutamyl-S-trans-1-propenyl cysteine. Sulphur is divided approximately 50% between the cysteine sulfoxides and the γ glutamyl peptides.\[18\] The function of the cysteine sulfoxides is protection, since their breakdown products have antibiotic and fungicidal properties, while the γ glutamyl peptides appear to have a storage function for N and S. The major alkylcysteine sulfoxide in garlic is alliin (85%), with isoalliin (5%) and methin (10%) occupying much more minor roles.\[19\]

Taking into consideration the abundant folkloric evidence in support of antioxidant and cardioprotective potentials of garlic, this study explored the possible protective potential of garlic on high salt diet-induced histomorphological changes in the myocardium of Wistar rats with a view to suggest the application of garlic as an alternative and more affordable therapy relative to the currently available pharmacotherapy in the treatment of cardiovascular disorders associated with cardiac hypertrophy.

### Materials and Methods

#### Animal care and management

Twenty-five female Wistar rats weighing 130-180 g obtained from the Animal Holding of Faculty of Basic Medical Sciences, OAU Ile-Ife were used for this study. The rats were randomly assigned into 5 groups of 5 rats each (Groups A, B, C, D and E). They were maintained on standard laboratory rat pellets before the start of the experiment and water was provided ad libitum. The animal handling and care was in line with the rules and guideline of the Health Research and Ethics Committee of the Institute of Public Health, Obafemi Awolowo University Ile-Ife, Nigeria.

#### Plant material and preparation of extract

Cloves of garlic bulb were procured from Sabo market in Ile-Ife and identified by a taxonomist in the Department of Botany, OAU, Ile Ife. The raw garlic cloves were peeled, chopped into small pieces and blended. The juice was filtered, and the filtrate was freeze-dried using a lyophilizer and stored in a desiccator. An aliquot portion of the crude extract residue was dissolved in distilled water for administration on each day of the experiment.

#### Preparation of high salt diet

High salt diet was prepared by replacing 0.3% sodium chloride in standard diet formula with 8% sodium chloride.\[20\]

#### Animal treatment

Group A was the control, Group B was negative control, while Groups C, D and E were test groups. Rats in Group A were fed with standard laboratory pellets, while Groups B, C, D and E were fed on the high-salt diet for five weeks. Thereafter, daily administration of 50 mg/kg, 100 mg/kg and 150 mg/kg of the garlic extract were given orally to Groups C, D and E respectively for 3 weeks, while rats in Group B received normal saline in place of garlic extract. Oral administration was done, using oral cannula at volume of 0.6 ml/kg/day.

#### Sacrifice of animals

At the end of the experiment, the rats were sacrificed under intramuscular ketamine (30 mg/kg) anesthesia. The heart was removed for further anatomical studies.

#### Histological techniques

Sections of 5 μm thickness were cut from the paraffin embedded tissues and stained with haematoxylin and eosin to demonstrate the general histoarchitecture of the heart.\[21\] Masson’s trichrome stain was used to demon-
strate collagen fibers in the myocardium of the left ventricle of the heart, while Verhoeff-Van Gieson stain was used to demonstrate elastic fibers in myocardium of the left ventricle.\(^{[22,23]}\)

**Determination of relative heart weight (%)**

At sacrifice, the heart weight was determined using a top loader sensitive balance (Mettler-Toledo Garvens GmbH, Giesen, Germany). The relative weight of the heart (%) to the body weight at sacrifice was evaluated.

**Measurement of serum electrolytes**

Blood samples from each rats was collected separately into clean capped plain tubes and allowed to stand for 30 minutes for clotting to occur. These were then centrifuged at 2500 revolution per minutes for 15 minutes. The serum was extracted into clean test tubes for sodium and potassium analysis. This was measured using flame photometry method at wave lengths 590nm for sodium and 770nm for potassium.

**Photomicrography**

Stained sections were viewed under a LEICA research microscope (LEICA DM750, Switzerland) with digital camera attached (LEICA ICC50) and digital photomicrographs were taken at various magnifications.

**Measurements of ventricular thickness and lumen diameter**

Photomicrographs of haematoxylin and eosin stained sections were imported on to the Motic Images Plus, Version 2.0 software (Motic China Group Co. Ltd, Shenzhen, China) for histomorphometric analysis, to measure the ventricular thickness and diameter of the cardiomyocytes.

**Statistical Analysis**

One-way ANOVA was used to analyze data, followed by Student Newman-Keuls (SNK) test for multiple comparisons. GraphPad Prism 5, Version 5.03 (GraphPad Software, Inc., La Jolla, CA) was used as the statistical package. Statistically significant difference was set at \(p<0.05\).

**Results**

One-way ANOVA revealed that, the relative heart weight in the high salt-fed group was lower, though not significant \((p=0.175)\) than the control group (Table 1). Post hoc analysis showed that serum sodium ion concentration of Group B was significantly higher than the control group \((p<0.05)\), but was reversed by aqueous garlic extract in Groups C, D and E (Figure 1, Table 2). There was significant difference in serum potassium ion concentration across all experimental groups \((F_4, 20=28.54; p<0.05)\). Serum potassium ion concentration of Group B was significantly lower than the control Group \((p<0.05)\), but was significantly reversed by the aqueous extract in Groups C, D and E (Figure 2, Table 3). A significant difference in thickness of the left ventricular wall was observed across all experimental groups \((F_4, 20=140.5; p<0.05)\).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Absolute heart weight (g)</th>
<th>Relative heart weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Control)</td>
<td>0.66±0.04</td>
<td>0.39±0.02</td>
</tr>
<tr>
<td>B (high salt diet)</td>
<td>0.66±0.1</td>
<td>0.51±0.06</td>
</tr>
<tr>
<td>C (high salt diet+50 mg/kg of AGE)</td>
<td>0.65±0.09</td>
<td>0.39±0.03</td>
</tr>
<tr>
<td>D (high salt diet+100 mg/kg of AGE)</td>
<td>0.78±0.08</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td>E (high salt diet+150 mg/kg of AGE)</td>
<td>0.68±0.03</td>
<td>0.42±0.02</td>
</tr>
</tbody>
</table>

\(*n=5, values are expressed as relative heart mean weight (%) ± SEM. p<0.05\)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum sodium ion concentration in Groups A–D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>137.3±0.42</td>
</tr>
<tr>
<td>B</td>
<td>144.9±0.06*</td>
</tr>
<tr>
<td>C</td>
<td>141.8±0.19**</td>
</tr>
<tr>
<td>D</td>
<td>141.1±0.27**</td>
</tr>
<tr>
<td>E</td>
<td>139.1±0.02*</td>
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</tbody>
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\(*relative to control, †relative to Group B, p<0.05)
Histological findings
Normal cardiac muscle histology was observed in control rats as shown in Figure 4a. Control rats showed regular arrangement with clear striations of myocardial fibers without histological alterations. In high salt-fed group in Figure 4b, the cross-banding pattern of cardiac cells was distorted. Figures 4d and 4e showed that cardiac muscles had cross striation similar to control group in Figure 5a. The increase in collagen fibers was evident on histological sections of left ventricle of the rats on a high salt-fed group (Figure 5b). The amount of elastic fibers in Figures 6b and 6c were reduced when compared with the control group (Figure 6a). In Figures 6d and 6e, there were significant increases in the deposition of elastic fibers as evident in the intense staining intensity.

Discussion
Histological and histochemical studies of the myocardium of left ventricle and selected serum electrolytes of high salt-fed adult Wistar rats administered with aqueous extract of garlic were carried out in this study. The thickness of the left ventricle was significantly higher in the high salt-fed group compared to the control group.
The micrograph of the control group showed normal histology of the heart, with regular arrangement of clear striation of myocardial fibers without histological alterations; however, the cardiac cells became distorted following administration of high salt diet. In this study, the relative heart weight of the high salt-fed group was higher than the control group, though the level of difference was not statistically significant (p=0.175). This may be attributable to the relatively small sample size used in this study. Similarly, the serum sodium was significantly higher in the high salt-fed group than the control group (p<0.05). This suggests that high salt diet resulted in hypernatriemia, myocardial damage and hypertrophy of the ventricular wall. This is in agreement with previous reports showing that high salt intake induced LVH. While some studies have linked the high salt diet-induced hypertrophy to salt-induced hypertension, others have shown that the hypertrophy is independent of high blood pressure. In normotensive rats, a high salt diet did not increase blood pressure even when there was significant cardiac hypertrophy. Increasing evidence from multiple clinical studies

Figure 4. Photomicrograph of transverse section of rat left ventricle. Control (a), high salt-fed only (b), high salt + 50 mg/kg garlic extract (c), high salt + 100 mg/kg garlic extract (d), high salt + 150 mg/kg of garlic extract (e). Black arrows show nucleus of cardiac muscle cell, blue arrows show the interstitial connective tissue and red arrow shows branching of cardiomyocytes (Haematoxylin and eosin stain x400). [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]
showed that excess salt intake was related to cardiovascular organ damage, independent of blood pressure.\textsuperscript{27} The pathophysiological mechanisms responsible for high salt diet-induced hypertrophy remain debatable.

The active ingredient in garlic extract is known as allicin (diallyl thiosulfinate) which is mainly organosulfur and has been shown to inhibit the renin-angiotensin-aldosterone system and prostaglandin synthesis.\textsuperscript{28} Garlic was reported to exhibit potent angiotensin converting enzyme (ACE) inhibitory activity.\textsuperscript{29} The reversal of high salt diet-induced myocardial distortion, ventricular hypertrophy can be explained by the ACE inhibitory activity of the garlic extract. The reduction in ACE activity results in reduction in plasma level of angiotensin II (a known

\begin{figure}[h]
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\includegraphics[width=\textwidth]{image}
\caption{Photomicrograph of transverse section of a rat left ventricle. Control (a), high salt-fed only (b), high salt + 50 mg/kg garlic extract (c), high salt + 100 mg/kg garlic extract (d), high salt + 150 mg/kg of garlic extract (e). Red arrows indicate collagen deposits (Masson’s trichrome stain x400). [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]}
\end{figure}
potent vasoconstrictor). Angiotensin II is also known to cause cardiovascular changes and sodium ion retention through stimulation of release of aldosterone. \(^{30,31}\) Garlic extract-induced inhibition of ACE probably leads to reduction in angiotensin II, leading to decreased adrenal production of aldosterone. \(^{32}\) This reduction in aldosterone production ultimately decreases the reabsorption of sodium and water from distal convoluted renal tubule, resulting in natriuresis and thereby decreasing plasma volume. \(^{33}\) The garlic extract-induced reduction of plasma level of angiotensin II followed by natri- and diuresis may be responsible for the reversal of the high salt-induced ventricular hypertrophy reported in this study. This result is similar to other reports in which high-salt diet induced

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**Figure 6.** Photomicrograph of transverse section of a rat left ventricle. Control (a), high salt-fed only (b), high salt + 50 mg/kg garlic extract (c), high salt + 100 mg/kg garlic extract (d), high salt + 150 mg/kg of garlic extract (e). Structures with black pigmentation are elastic fibers (Verhoeff -Van Gieson stain x400). [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]
cardiac hypertrophy in rats was inhibited by spironolactone. Another evidence is increased distribution of collagen fibers in the left ventricle of the high salt-fed group which was reversed by the garlic extract.

**Conclusion**

The results of this study indicate that high salt diet causes significant histomorphological changes on myocardium of the left ventricle of rats as evidenced by myocardioctye distortion and ventricular hypertrophy. Garlic extract has protective and ameliorative properties on these high salt diet-induced changes in the cardiovascular system probably via inhibition of renin-angiotensin-aldosterone system. Further studies on the effect of garlic extract on natriuresis and markers of activity of renin-angiotensin system probably via inhibition of renin-angiotensin-aldosterone system probably via inhibition of renin-angiotensin-aldosterone system. Therefore, garlic extract has protective and ameliorative properties on cardiac hypertrophy in rats as evidenced by myocardioctye distortion and ventricular hypertrophy. Garlic extract has protective and ameliorative properties on these high salt diet-induced changes in the cardiovascular system probably via inhibition of renin-angiotensin-aldosterone system. Further studies on the effect of garlic extract on natriuresis and markers of activity of renin-angiotensin-aldosterone system will be required to support this suggestion.

**References**