

Effects of Garlic extracts on the myocardium of left ventricle of the heart of high salt fed adult wistar rats

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ABSTRACT

This study was designed to evaluate the effects of garlic extracts on the morphology of myocardium of left ventricle of the heart and also determine the body weight and relative weight of the organs on high salt fed adult Wistar rats. Twenty-five healthy female Wistar rats weighing 130-180 g were randomly assigned into 5 groups of 5 rats each (Groups A, B, C, D and E). Rats in group A was 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⁻¹, 100 mg kg⁻¹ and 150 mg kg⁻¹ of the garlic extract were giving orally to groups C, D and E respectively for 3 weeks. The rats were sacrificed under ketamine anesthesia (5mg/kg i.m). The left ventricle of the heart was excised, processed routinely in paraffin wax and stained with routine special stained. Result showed significant decreased ($p < 0.05$) in weight of all high salt fed groups when ($F = 46.90$, $p < 0.05$) compared with control. Whereas, treatment with garlic extract help in weight management in high salt fed+garlic extract treated groups and also no significant difference ($p < 0.05$) in the relative heart weight when ($F = 1.773$, $p < 0.05$) compared the control with other groups. Histological results revealed morphological alterations in the left ventricle in high salt fed group. In conclusion, Garlic extract has ameliorative property at the level of 100 mg kg⁻¹ or 150 mg kg⁻¹ of the extract on high salt fed induced cardiac injury.

Keywords: High salt fed; *Allium sativum* (Garlic); Histomorphological; Heart; Aorta; Cardiovascular; Lactate Dehydrogenase (LDH).

INTRODUCTION

Allium sativum is reported to have many biological activities, including protective roles on the cardiovascular system [1]. It had been established that garlic has as an antihypertensive [2] and an antioxidant [3] properties. Aged garlic extracts are considered superior to those of raw garlic in terms of their anti-oxidation properties and for ameliorating physiological and psychological stress [2].

Epidemiologic studies have suggested an inverse relationship between nutritional garlic intake and the occurrence of cardiovascular disorders that could be attributed to protective effect of garlic against these disorders [4]. *Allium* species, which is one of such plant, is used as foodstuff, condiment, flavoring, and folk medicine [5]. The biological responses of garlic have been largely attributed to reduction of risk factors for cardiovascular diseases and cancer, stimulation of immune function, enhanced detoxification of foreign compound,

hepatoprotective effects, anti-microbial effects, and antioxidant effects [5].

High salt intake in humans is associated with numerous complications; it can elevate blood pressure in some individuals [6]. In experimental animals it causes endothelial dysfunction [7], increase in plasma brain natriuretic peptide concentration and perivascular inflammation [8] and deactivation of ATP-sensitive potassium channels and Na-K ATPase pumps on the vascular smooth muscle membrane [9].

MATERIALS AND METHOD

Animal Care and Management: Twenty-five healthy female Wistar rats weighing 130-180 g obtained from the Animal Holding of Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife were used for this research. 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 pellet before the start of the experiment and water was provided *ad libitum*.

Ethical clearance for the study was obtained from Health Research and Ethics Committee of the Institute of Public Health, Obafemi Awolowo University, Ile-Ife.

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, Obafemi Awolowo University, Ile-Ife. A voucher specimen was deposited at the herbarium for future reference. The raw garlic cloves were peeled, chopped into small pieces and blended. The juice was filtered, and the filtrate was freeze-dried using lyophilizer and stored in a desiccator. An aliquot portion of the crude extract residue was dissolved in distilled water which was used on each day of the experiment.

Preparation of High Salt Diet: High salt diet containing 8% sodium chloride was prepared specially by replacing a standard diet containing 0.3% sodium chloride with 8% sodium chloride [9].

Animal Treatment: Group A was the normal control, group B was negative control, while groups C, D and E were the 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⁻¹, 100 mg kg⁻¹ and 150 mg kg⁻¹ of the garlic extract were given orally to groups C, D and E respectively for 3 weeks, while rats in group B were left untreated for the same period. The extract solution was administered orally, using oral cannula.

Sacrifice of Animals: At the end of the experiment, the rats were sacrificed under ketamin anesthesia. The left ventricle of the heart was excised and weighed.

Measurement of Body Weight: The body weights of the animals were taken using a top loader weighing balance.

Determination of Heart Weight: At sacrifice, the heart weight was determined using a top loader sensitive balance (Mettler Toledo Germany). The relative weight of the heart (%) was calculated from the body weight at sacrifice.

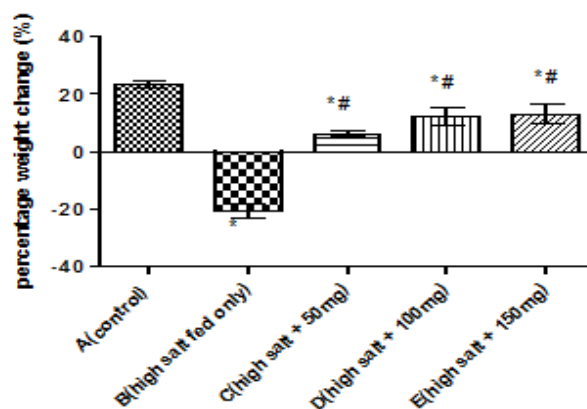
$$\text{Relative weight of the heart} = \frac{\text{weight of the heart (g)}}{\text{body weight at sacrifice (g)}} \times 100$$

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 (×400). Photomicrographs of stained sections will be imported onto the ImageJ software for histomorphometric analysis.

Statistical Analysis: One-way ANOVA was used to analyse data, followed by Student Newman-keuls (SNK) test for multiple comparisons. GraphPad Prism 5 (Version 5.03, Graphpad Inc.) was the statistical package used for data analysis. Significant difference was set at p<0.05.

RESULTS

Body and Organ Weight: Fig. 1 indicates that there was an increase in weight for all treated groups and control, weight was significantly lower (p<0.05) in high salt fed group (-20.88±2.56%) when compared with control group (23.61±0.95%). Also treated groups C, D and E respectively (6.16±0.95, 12.10±3.11 and 13.20±3.39%) were significantly different (p<0.05) from control (23.61±0.95). However, groups C, D and E (6.16±0.95, 12.10±3.11 and 13.20±3.39%) were significantly different from group B (-20.88±2.56%). Table 4.1 shows the relative organ weight of the control and treated groups. There was no significant difference (p<0.05) between the heart weight of the control group (0.39 ± 0.02g) when compared with those that receive high salt diet (0.51 ± 0.06g) and garlic extract groups (0.39 ± 0.03, 0.43 ± 0.04 and 0.42 ± 0.02g).



*= significantly different from control

#= significantly different from toxic group (high salt fed)

Fig. 1: Final Body Weight Change of Control and Treated Rats

Table 1: Relative Organ Weight of the Heart of Control and Treated Rats

Groups	Heart Absolute Weight (g)	Relative Heart Weight (%) ±SEM
A (Control)	0.66 ± 0.04	0.39 ± 0.02
B (High salt diet)	0.66 ± 0.10	0.51 ± 0.06
C (High salt diet + 50 mg kg ⁻¹ of garlic extract)	0.65 ± 0.09	0.39 ± 0.03
D (High salt diet + 100 mg kg ⁻¹ of garlic extract)	0.78 ± 0.08	0.43 ± 0.04
E (High salt diet + 150 mg kg ⁻¹ of garlic extract)	0.68 ± 0.03	0.42 ± 0.02

Values are mean ± SEM of data obtained.

No significant different across the groups (p<0.05)

Histological Findings: The normal histology of the cardiac muscle could be seen in the control rats as shown in plate 1A. Control rats show the regular arrangement with clear striation of myocardial fibers without any histological alterations, showing an outline of the normal heart. In high salt fed (untreated) group in plate 1B, a cross-banding pattern of cardiac cells was ruined and the cardiac muscles had thicker muscular wall. In the plate 1C, the muscle is not as thicker as the control. In plate 1D and plate 1E which are the groups of 100 mg kg⁻¹ of garlic extract and 150 mg kg⁻¹ of garlic extract respectively, show that cardiac muscles had cross striation similar to control group in plate 1A but slightly thicker muscular walls than control group. The increased muscles of collagen fibers were evident on histological section from left ventricle of the rats on a high salt fed group (plate 2B).

Plate 1: Photomicrographs of Transverse Section of Rats' Left Ventricle.

A (Control), B (High salt fed only), C (High salt + 50 mg kg⁻¹ of garlic extract), D (High salt + 100 mg kg⁻¹ of garlic extract), E (High salt + 150 mg kg⁻¹ of garlic extract). H&E × 400

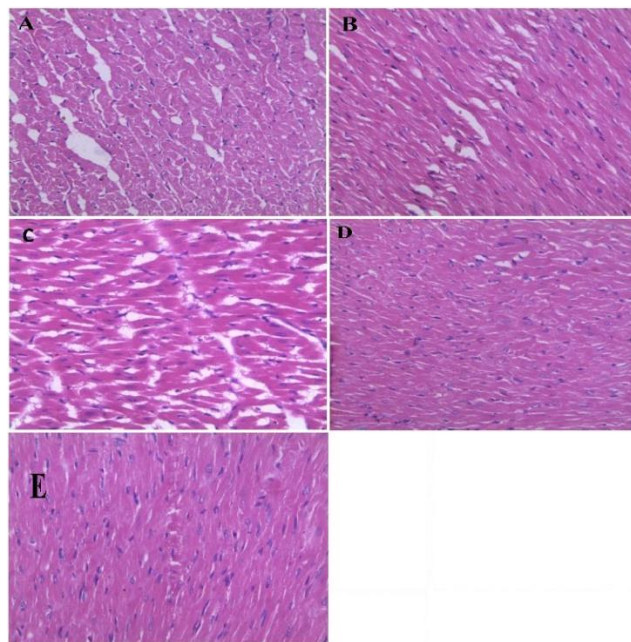
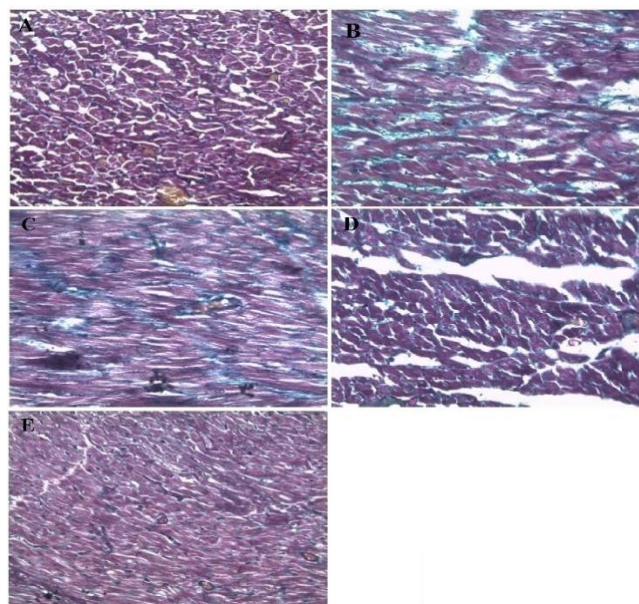


Plate 2: Photomicrographs of Transverse Section of Rats' Left Ventricle.

A (Control), B (High salt fed only), C (High salt + 50 mg kg⁻¹ of garlic extract), D (High salt + 100 mg kg⁻¹ of garlic extract), E (High salt + 150 mg kg⁻¹ of garlic extract). MT × 400



DISCUSSION

Effects of garlic extract on the studies of the myocardium of left ventricle of the heart of high salt fed adult wistar rats were carried out on Wistar rats in this study. In the present study, it was observed that high salt loaded on the experimental animals led to cardiac hypertrophy. This was in agreement with the study of Feng *et al.* ^[10], that a higher salt intake was related to an increased risk of cardiovascular disease. Some reports had shown that high salt intake increased left ventricular hypertrophy ^[11]. Additionally, others have shown that this effect was related to salt-induced hypertension ^[12]. Experimental studies in rats demonstrated that an increase in salt intake for a few weeks not only caused left ventricle hypertrophy, but also led to widespread interstitial fibrosis in the left ventricle and intramyocardial arteries and arterioles ^[13]. Tissue culture experiments showed that increasing bath sodium concentration in an amount similar to the increase in plasma sodium which was seen with an increase in salt intake caused marked cellular hypertrophy in both arterial smooth muscle and cardiac myocytes ^[13]. The hypertrophy was due to an increase in the rate of protein synthesis and a decrease in the rate of protein degradation ^[13].

The hypertrophy caused by high salt intake was ameliorated by the garlic extract. The active ingredient in garlic extract is known as Allicin (diallyl thiosulfinate) which is a mainly organosulfur ^[14] had shown to inhibit the renin angiotensin aldosterone system (RAAS) and prostaglandin synthesis. The change in this balance favors vasodilation. This in part, is thought to be from garlic's inhibition of angiotensin converting enzyme (ACE) activity ^[15]. The reduction in ACE activity results to reduction in plasma level of angiotensin II, which is known to be a direct vasoconstriction. Reduction in angiotensin II would decrease adrenal production of aldosterone ^[16]. This reduction in aldosterone production would ultimately decrease the reabsorption of sodium and water from distal convoluted renal tubule, thereby decreasing plasma volume ^[16]. This result is similar to other reports in which high-salt diet induced cardiac hypertrophy in normotensive rats was inhibited by spironolactone ^[17]. Comparatively, cardiac hypertrophy in spontaneously hypertensive rats subjected to high salt intake was not reversed by an

angiotensin-converting enzyme inhibitor, a beta-blocker or a calcium antagonist ^[18]. This supported the idea that cardiac hypertrophy mediated by salt overload may be induced, at least in part, directly by tissue aldosterone production, independently from the action of angiotensin II. However, blocking the AT1 receptor reduces cardiac hypertrophy without affecting blood pressure in young salt loaded spontaneously hypertensive rats ^[19].

In the present study, the salt loaded rats were observed to have reduced body weight and the least weight gain was compared to other groups although they had the highest body weight at the start of the experiment. This reduction in body weight in the salt loaded rats would have been as a malabsorption due to consequence of intestinal damage caused by the high salt feed to the salt loaded rats. This would result in poor absorption of nutrients necessary for growth resulting in the lower weight recorded in the salt loaded group. Another probable reason for the lower weight of the salt loaded group may be as a consequence of some tissue damage and wasting caused by the high salt concentration. This finding was in agreement with previous reports which showed that salt loading caused derangement of several tissues including the heart ^[20]. Salt loading causes insulin resistance and reduction in glucose uptake by the glucose receptors leading to loss in weight due to poor uptake of glucose by the tissues ^[21]. High salt diet causes decrease in food intake accompanied with slow growth rate and decrease in body weight ^[22]. The weight gain in the groups treated with the extract may be possible that the extract have prevented tissue and intestinal damage to enhance reabsorption of nutrient. It has also been reported that the extract has a blood glucose reducing effect ^[5] which implies that the extract enhances glucose uptake by the tissues which could be probable reason for the better weight observed in extract fed groups mostly, 100 mg kg⁻¹ and 150 mg kg⁻¹ compared to the salt loaded rat ^[23].

No significant changes were observed in the relative weight of heart of experimental groups and control group which due to antioxidant and cardioprotective properties of garlic extract. Histological results of this study clearly support earlier report that high salt fed cause the damage to the tissue of the heart ^[20]. In

the present study, the micrograph of the control group showed normal histology of the heart, with the regular arrangement of clear striation of myocardial fibers without any histological alterations. In this study, following administration of high salt fed rats as observed in plates 1B and 2B. Structural alteration was observed in alignment of the myofibrils striation with the thicker muscular wall. One of the major adverse effects of high salt fed on left ventricle of the heart includes elevation of blood pressure in some individual as reported by Ofem *et al.* [20]. Left ventricular hypertrophy is an increase in myocardial mass that leads to a structural rearrangement of the components of the ventricular wall and may result in ventricular stiffening [24]. It also raises blood pressure in human which is the major risk factors for left ventricular hypertrophy, left ventricular dysfunction and long term treatment of hypertension and finally lead to heart failure [5]. In this present study, plates 1D and 1E (high salt fed + garlic extract in 100 mg kg⁻¹ and 150 mg kg⁻¹ respectively) show slightly thicker muscular wall and cardiac muscle with striation similar to control. This is an indication that aqueous garlic extract was able to ameliorate the myocardial damage done by high salt. It was also supported with the research of Kilikdar *et al.* [25] that aqueous garlic extract may be beneficial in ameliorating effect on lead-induced oxidative damage in the rat heart. Banerjee *et al.* [14] also reported on the antioxidant effect of aqueous

garlic extract on cardiovascular disorders. The accumulation of collagen was also noticed in high salt group than other groups which is associated with the high salt intake. Cardiac fibroblasts are mainly responsible for synthesizing extracellular collagen I and collagen III in the left ventricle myocardium. Cardiac fibrosis is pathophysiologically associated with hypertension and cardiac hypertrophy [26]. This was supported with the finding of Ahn *et al.* [12] that accumulation of ventricular collagen associated with excessive salt intake appears to be an important contributor to the LV filling abnormalities. In young spontaneously hypertensive rats, it has been reported that salt overload leads to interstitial and perivascular fibrosis [26]. In turn, fibrosis is related to enhance left ventricular stiffness, which leads to impairment in left ventricle relaxation [27]. Garlic extract has ameliorative effects on induced cardiac damage as reported by Kilikdar *et al.* [25]. Studies have indicated that active compound in raw garlic is a sulfur compound called Allicin, which is produced when raw garlic is chewed [28].

CONCLUSION

In conclusion, garlic extract has ameliorative properties at the level of 100 mg kg⁻¹ and 150 mg kg⁻¹ of the extract on high salt induced cardiac injury and this was dose dependent and it was observed that low or no ameliorative properties were displayed in the 50 mg kg⁻¹ administered rats.

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