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**COMPARATIVE EFFECT OF NIFEDIPINE AND GARLIC (*ALLIUM SATIVUM*) ON RENAL
TOXICITY INDUCED BY HYPERTENSION IN WISTAR RATS**

COMPARATIVE EFFECT OF NIFEDIPINE AND GARLIC (*ALLIUM SATIVUM*) ON RENAL TOXICITY INDUCED BY HYPERTENSION IN WISTAR RATS

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ABSTRACT

Introduction

Research has shown that hypertension has lingering effect on several organs and the body in general. One of the most affected organs is a vital organ, the kidney. The aim of this study is to observe the effect of hypertension on the kidney and compare the therapeutic potential of Garlic and Nifedipine on the cyto-architecture and clearance rate of the kidney.

Methods

Twenty (20) male wistar rats weighing between 150-180 g were assigned into four groups (A,B,C,D) with each group comprising of five rats. Group A was designed as a control group which received 0.2 ml of normal saline. Group B received 3 mg/kg body weight of NaCl only, Group C received 3 mg/kg of NaCl and 3mg/kg of Nifedipine while Group D received 3 mg/kg of NaCl and 50mg/kg of Garlic for 28 days.

Results

Administration of Sodium Chloride (NaCl) only in group B treated rats showed significant increase in Concentration of Troponin-T and degeneration of cardiac cells. These effects were lessened in groups C and D treated with NaCl co-administered with Nifedipine and NaCl co-administered with Garlic respectively. Renal-toxicity was observed in group B with significant increase in the concentration of Urea and Creatinine when compared with control and Group D

rats. Histologically, Control and Group D rats showed normal glomeruli with normal mesangial cells, the renal tubules appeared normal with no signs of dilatations and the interstitial spaces appeared normal and cleared.

Conclusion

This study revealed that hypertension induced by NaCl caused reduced efficiency in kidney function (renal dysfunction) in Wistar rats and Garlic was effective in attenuating the effect of the hypertension on the kidney better than the administration of antihypertensive drug (Nifedipine).

Keywords: Renal toxicity, Garlic, Nifedipine, Hypertension, Wistar rats

INTRODUCTION

Chronically elevated blood pressure, or hypertension, raises the risk of morbidity and mortality and damages end organs over time.^{1,2} Compared to younger persons, older persons have far higher rates of it in the population. A similar pattern occurs in both male and female. The product of systemic vascular resistance and cardiac output determines the blood pressure. Consequently, individuals with arterial hypertension may have an increase in cardiac output, an increase in systemic vascular resistance, or both.³ Due to its link to renal illness, arterial hypertension is a major source of morbidity and mortality. Uncontrolled hypertension can

lead to renal artery narrowing, thinning, or hardening. The renal tissue cannot receive adequate blood from these damaged arteries. Damaged renal arteries do not filter blood well.

One of the major organs affected by hypertension is the kidney whose function is mainly to remove nitrogenous waste from the blood stream. An increase in blood pressure (hypertension) will lead to an increase in work load for the kidney which can lead to renal failure.⁴

Cerebrospinal fluid [Na⁺] is increased by a high salt intake. Through the brain's [Na⁺]-sensing circumventricular organs, this causes sympathetic nerve activity (SNA), a key factor in vasoconstriction, to rise. The Na⁺-pump ligand endogenous ouabain (EO) plasma levels similarly rises. Surprisingly, a mechanism that mediates the prolonged rise in SNA is mediated by high cerebrospinal fluid [Na⁺]-evoked, locally released (hypothalamic) EO. Aldosterone, epithelial Na⁺ channels, EO, ouabain-sensitive 2 Na⁺-pumps, and angiotensin II are all part of this hypothalamic signaling pathway (ANG II).⁵

The EO, for example, reduces neuronal nitric oxide synthase protein expression and enhances hypothalamic ANG-II type-1 receptor and NADPH oxidase. The activity of the cerebral cardiovascular control centers that regulate the BP set point and cause long-lasting changes in SNA is modulated via the aldosterone-epithelial Na⁺ channel-EO-2 Na⁺ pump-ANG-II pathway. Through an EO-2 Na⁺ pump-Na⁺/Ca²⁺ exchanger-Ca²⁺ signaling pathway, the EO released by the adrenal cortex directly promotes vasoconstriction in the periphery. Circulating EO also initiates a signaling cascade involving the EO-2 Na⁺ pump and Src kinase. As a result, endothelial vasodilator mechanisms are expressed less in arterial smooth muscle while the Na⁺/Ca²⁺ exchanger-transient receptor potential cation channel Ca²⁺ signaling pathway is expressed more. The arterial structural remodeling and lumen narrowing that are frequently seen in established hypertension are also likely to be directly influenced by EO, a growth factor.⁵

The management of hypertension has changed over the past ten years as it has become clear that there is no safe level of elevated blood pressure. All anti-hypertensive medications must work by reducing peripheral vascular resistance, cardiac output, or both.⁶ Thiazide diuretics, B-blockers, ACE inhibitors, angiotensin II receptor antagonists, calcium channel blockers, α -adrenoceptor blockers, combined α - and β -blockers, direct vasodilators, and some centrally acting drugs like α_2 -adrenoceptor agonists and imidazoline I1 receptor agonists are among the classes of medications that are most frequently used. Nifedipine, Nimodipine, and Amlodipine are examples of dihydropyridine calcium blockers, while verapamil and diltiazem are examples of non-dihydropyridine calcium blockers and both are vital in treating hypertensive patients.⁷ The peripheral vascular resistance is reduced in both groups.^{6,8}

Nifedipine is a calcium channel blocker used to manage angina, high blood pressure, Raynaud's phenomenon, and premature labor. It is one of the treatments of choice for Prinzmetal angina. It may be used to treat severe high blood pressure in pregnancy. Literatures have shown that Nifedipine monotherapy improves renal function in patients with hypertension.⁹ It has been found that the use of Nifedipine aids the passage of kidney stones.¹⁰

Garlic (allicin) has been shown in some studies to be therapeutic to chronic kidney disease (CKD), reducing hypertension and oxidative stress and improving renal dysfunction.¹¹ Garlic may help reduce systolic and diastolic blood pressure levels by up to 11.2 mmHg. In some instances, garlic supplements may work as effectively as standard blood-pressure-lowering medications, with much fewer side effects.¹¹ Thus, this work aimed at investigating the effect of hypertension induced by NaCl on the kidney and compare the therapeutic potential of Garlic and Nifedipine on the cyto-architecture and clearance rate of the kidney.

METHODS

Hypertension was induced in the experiment animals (male wistar rats) by giving oral solution of 3mg/kg of NaCl for 28 days. Synthetic antihypertensive drug (Nifedipine) and naturally used antihypertensive herb (Garlic) were used to treat hypertension.

Ethical Review

These investigations were carried out in conformity with the regulations stipulated by the Health Research Ethics Committee (OS/CHS/2020/0118) College of Health Sciences, Osun State University, Osogbo, Nigeria and in compliance with the guidelines of the National Institute of Health for the care and use of laboratory animals.

Procurement and Preparation of Compounds and Herb

All compounds used (Sodium Chloride and Nifedipine) were pure compounds procured from Hefei TDJ Chemical Co. Ltd China and verified at Pharmacology Department, Osun State University, Osogbo, Nigeria. 50mg of these compounds were dissolved in 200ml of distilled water so that 1ml of the solution contains 0.5mg of the solvent (Stock solution). The solution was allowed to stand for some minutes with constant shaking for proper dissolution.

Garlic root authentication and ethanolic extraction

Garlic roots were purchased from Hefei TDJ Chemical Co. Ltd China and identified in the Department of Plant Biology, Osun State University, Osogbo, Nigeria. The Garlic roots were air dried and grounded using an electric blender (Blender/Miller III, model MS-223, Taiwan, China), following Adeleke et al, 2017.¹²

Animal Grouping

A total number of twenty (20) male wistar rats weighing between 150-180g were used and the animals were randomly split into four groups (A, B, C, and D), each with five rats. Group A was designated as a control group which received 0.2 ml of normal saline, Group B received 3 mg/kg body weight of NaCl only, Group C received 3

mg/kg of NaCl and 3mg/kg of Nifedipine while Group D received 3 mg/kg of NaCl and 50mg/kg of Garlic. Drug administration was done orally using orogastric cannula for a period of 28 days.

Sample collection and preparation

Twenty adult male wistar rats were euthanized by cervical dislocation 12 hours after the last drug administration. Blood was withdrawn from the apex of the heart (left ventricle) for biochemical assay while the kidney and the heart were excised via an abdominal incision, and they were fixed in Neutral buffer saline for histological analysis. The fixed tissues underwent processing into histological slides to check for possible histological changes.

Procedure for Troponin-T concentration

Serum concentration of Troponin-T was calculated after serum sample collection with the aid of commercially available enzyme linked immunosorbent assay (ELISA) one step sandwich assay with streptavidin technology and two specific monoclonal antibodies developed by Katus and colleagues (Enzymun-Test system, Boehringer Mannheim).¹³

Procedure for Urea and Creatinine concentration

Serum urea and creatinine estimation was carried out on the serum sample collected from all the groups using the urease kinetic and Jaffe's reaction methods respectively. The urease kinetic method is an enzymatic method based on two steps. The first step involves the enzyme urease hydrolyzing urea with the production of ammonium. The second step involves the quantitative measurement of ammonium kinetically in the auto analyzer. The concentration of the ammonium is proportional to the urea present in the serum sample. The reaction of creatinine with picric acid in an alkaline solution to produce an orange red complex that is measured spectrophotometrically serves as the basis for the measurement of creatinine.

Histological Processing

Histological processing using Periodic Acid Schiff methods was carried out for the kidney while Masson Trichrome staining was used for the heart. The kidney and heart were fixed in neutral buffer formalin. Before being embedded in molten paraffin wax, the material was dehydrated in progressively stronger alcohols and cleaned in xylene. The rotary microtome was then used to slice the paraffin block containing the tissue into 4µm thick sections. The sections were then transferred to a glass slide, floated in a water bath set at 40 degrees Celsius, and stained with the necessary dyes.

Statistical Analysis

The mean and standard error of mean (S.E.M) of all data were calculated. Comparison of means was made by one way analysis of variance (ANOVA) using Graphpad Prism 5. Tukey's test was used to

adjust for multiple comparisons. P value < 0.05 was considered to be statistically significant.

RESULTS

Serum Troponin-T concentration increases in NaCl treated group

Table 1 showed comparison of concentration of Troponin T among the groups after the administration of Normal saline (Group A), NaCl (Group B), NaCl+Nifedipine (Group C) and NaCl+Garlic (Group D). λ = Comparison with Group A (n=5).

As shown in Table 1 and Figure 1, significant increases in serum Troponin T concentration were observed in Group B ($p<0.01$) and Group C ($p<0.05$) when compared with Group A. Furthermore, no significant difference in serum Troponin T concentration was observed when Group A was compared with Group D.

Table 1: comparison of concentration of Troponin T

	Group A	Group B	Group C	Group D
Mean±SEM	0.33±0.05	1.12±0.10 ^{λ (0.002)}	0.91±0.18 ^{λ (0.04)} _{α(0.367)}	0.84±0.11 ^{λ (0.07)}

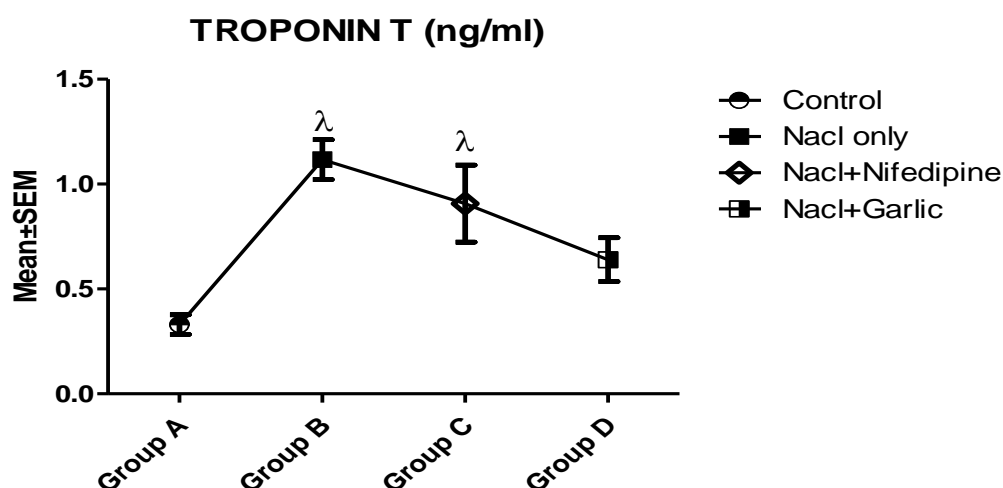


Figure 1: comparison of concentration of Troponin T among the groups after the administration of Normal saline (Group A), NaCl (Group B), NaCl+Nifedipine (Group C) and NaCl+Garlic (Group D). λ = Comparison with Group A (n=5).

Serum Urea concentration increases in NaCl treated group

Urea concentration's mean values after the administration of Normal saline (Group A), NaCl (Group B), NaCl+Nifedipine (Group C) and NaCl+Garlic (Group D). Significant increases in serum urea concentration were observed in NaCl only ($p<0.01$) and NaCl co-administered Nifedipine treated groups ($p<0.05$) when compared with control and

NaCl co-administered Garlic treated groups. Furthermore, there was no significant difference in serum Urea concentration when control was compared with NaCl co-administered with Garlic. Also, Urea concentration was lowered in NaCl co-administered with Garlic when compared with NaCl co-administered with Nifedipine (though not statistically significant) (Table 2 & Figure 2)

Table 2: Comparison in Concentration of Serum Urea among the groups after the administration of Normal saline (Group A), NaCl (Group B), NaCl+Nifedipine (Group C) and NaCl+Garlic (Group D). λ = Comparison with Group A, α = Comparison with NaCl only (n=5).

	Group A	Group B	Group C	Group D
Mean\pmSEM	4.24 \pm 0.40	7.83 \pm 0.15 $\lambda(0.0011)$	6.71 \pm 0.61 $\alpha(0.1482)$ $\lambda(0.0274)$	5.24 \pm 0.13 $\alpha(0.0021)$ $\lambda(0.0754)$

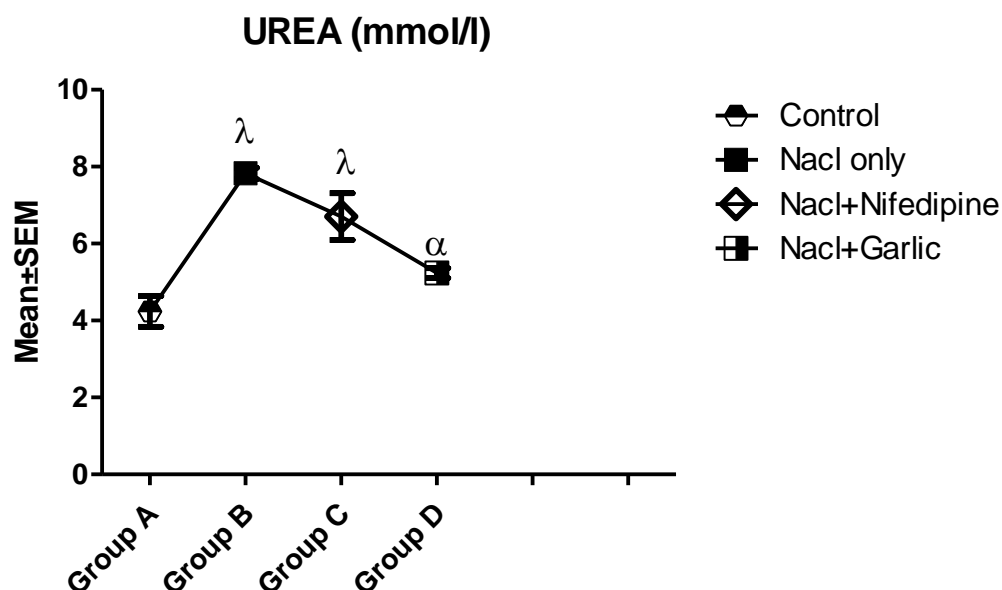


Figure 2: Comparison in Concentration of Urea among the groups after the administration of Normal saline (Group A), NaCl (Group B), NaCl+Nifedipine (Group C) and NaCl+Garlic (Group D). λ = Comparison with control group, α = Comparison with NaCl only group (n=5).

Serum Creatinine concentration increases in NaCl treated group

When compared to the control group, the mean values of serum creatinine concentration in the experimental groups (Table 3, Figure 3) showed a high significant increase ($p < 0.0001$) in NaCl alone treated groups and a slight significant increase in NaCl co-administered with Nifedipine ($p < 0.01$) and NaCl co-administered with Garlic treated groups ($p < 0.05$). Additionally, when compared to the NaCl only treated group, slightly significant decreases in serum Creatinine concentration were seen in the NaCl co-administered with Nifedipine ($p < 0.01$) and NaCl co-administered with Garlic groups ($p < 0.05$). Additionally, compared to NaCl administered alone, the serum urea concentration was lower in NaCl co-administered with garlic and NaCl co-administered with Nifedipine (though not statistically significant).

Histological Observation

Heart Micromorphological Section

Figure 4 shows the heart micromorphological section demonstrated by massontrichrome (MT) staining at high magnification of $\times 400$. The epicardium, myocardium and endocardium as well as the intercalated discs and blood vessels are well demonstrated across the groups. Cardiocytes are seen to be degenerated and distorted in NaCl only treated group but intact profile is seen across other micrographs and is only conspicuous and appreciable at higher magnification. Fenestrations/Striations across the myocardium look intact across groups but not found in NaCl treated group. Areas of observed marked degenerative changes/clotted blood and some mild fibrosis coupled with large fenestrations are represented with black arrows (NaCl only and NaCl co-administered with Nifedipine treated groups). Not very tightly packed involuntary striated appearance was observed across the myocardium of NaCl treated group.

Table 3: Comparison in Concentration of Creatinine among the groups after the administration of Normal saline (Group A), NaCl (Group B), NaCl+Nifedipine (Group C) and NaCl+Garlic (Group D). λ = Comparison with Control group, α = Comparison with NaCl only, β = Comparison with NaCl+Nifedipines group. (n=5).

	Group A	Group B	Group C	Group D
Mean\pmSEM	74.1 \pm 0.66	124.9 \pm 3.85 $\lambda(0.0002)$	104.8 \pm 5.09 $\lambda(0.004) \alpha(0.034)$	93.6 \pm 2.56 $\lambda(0.012) \alpha(0.003)$ $\beta(0.126)$

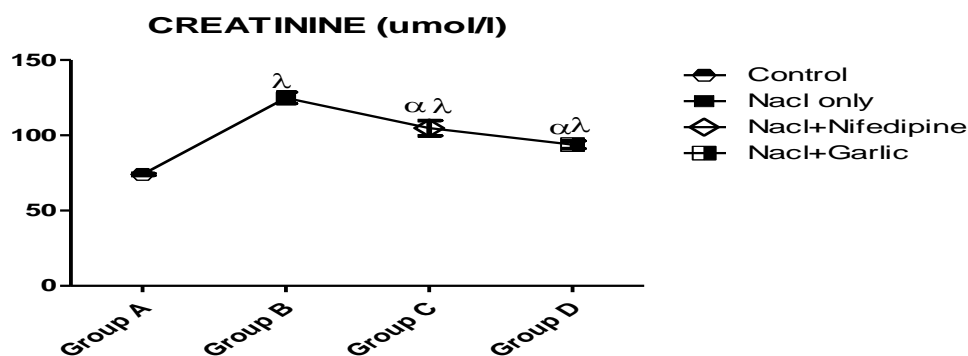


Figure 3: Showed comparison in Concentration of Creatinine among the groups after the administration of Normal saline (Group A), NaCl (Group B), NaCl+Nifedipine (Group C) and NaCl+Garlic (Group D). λ = Comparison with Control group, α = Comparison with NaCl only group. (n=5).

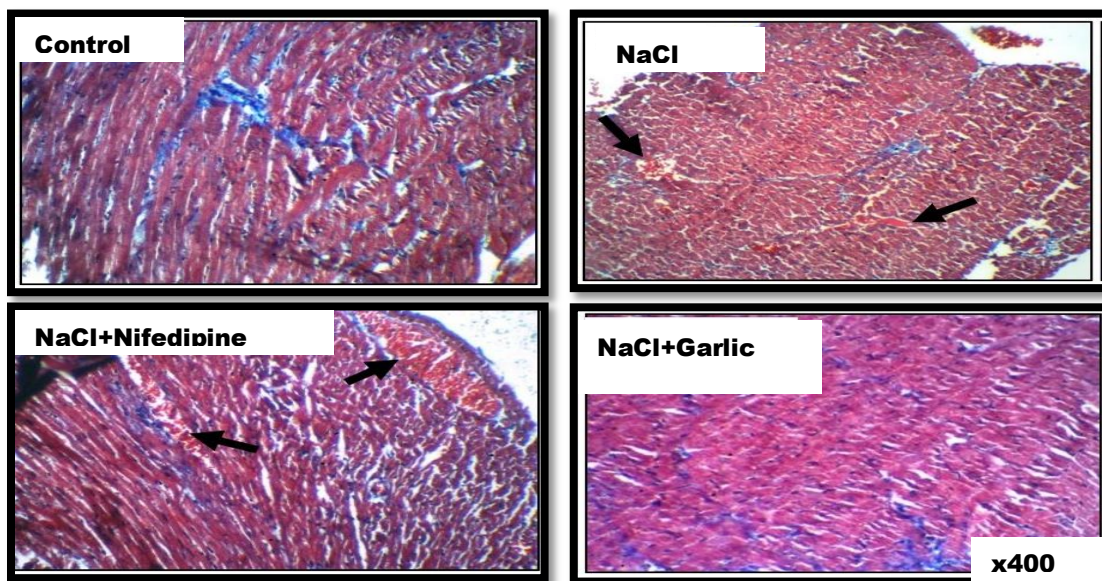


Figure 4: Magnified views of Heart micromorphological section demonstrated by MT staining at high magnification (x400).

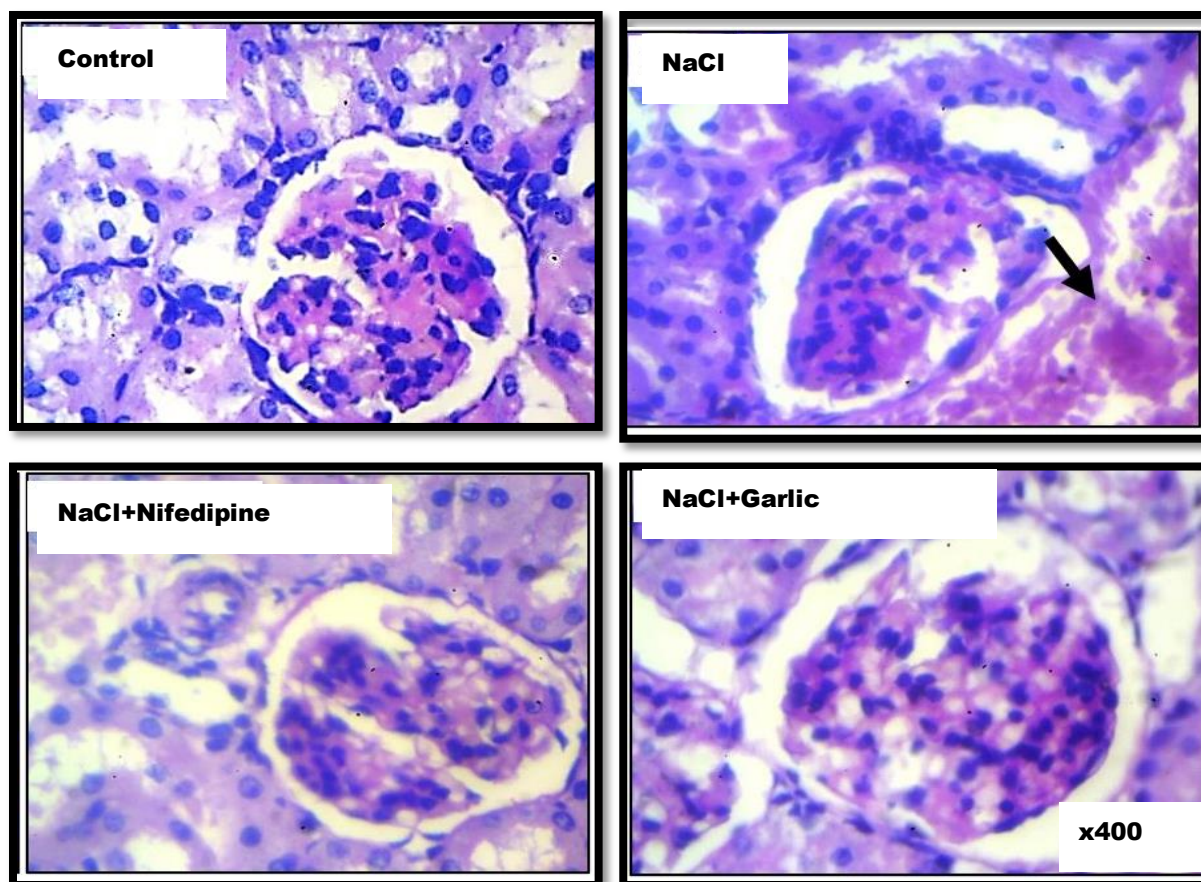


Figure 5: Magnified views of kidney micro-morphological section demonstrated by PAS staining at high magnification (x400)

Kidney Micromorphological Section

Periodic Acid Schiff (PAS) staining for the kidney histo-architecture (Figure 5) showed renal cortex of the Control and NaCl co-administered with Garlic treated group with normal glomeruli and mesangial cells. The renal tubules appear normal with no signs of dilatations while the interstitial spaces appear normal and clear. NaCl co-administered with Nifedipine treated group shows moderate architecture: the renal cortex shows few glomeruli with a mild sclerosis and other normal glomeruli with normal mesangial cells and capsular spaces; the renal tubules appear normal and the interstitial spaces show mild vascular congestion. NaCl only treated group showed poor architecture: the renal cortex shows sclerotic glomeruli with wide capsular spaces, the renal tubules show congestion within the lumen and the interstitial spaces appear infiltrated with presence of red inflammatory cells (black arrow). These may be consistent with features of glomerulonephritis/sclerosis. Diffuse glycogen content was observable in NaCl only treated group relative to other treatment as demonstrated by PAS staining.

DISCUSSION

The ability of the kidney to effectively get rid of waste is vital to the general health of an individual, and a couple of things often reduce the efficacious work of the kidney, one of which is hypertension (high blood pressure). Hypertension is the second most common reason for kidney failure. If uncontrolled, high blood pressure can narrow, weaken, or harden the arteries surrounding the kidneys. The kidney tissue cannot receive enough blood from the damaged arteries.¹⁴ This study compared the

therapeutic roles of synthetic antihypertensive drug (Nifedipine) and naturally used antihypertensive herb (Garlic root) on renal toxicity induced by hypertension.

So as to be able to observe the changes in the heart as a result of hypertension induced by NaCl, serum Troponin-T level was observed. Troponin T is a part of the troponin complex, which are proteins integral to the contraction of skeletal and heart muscles. They are expressed in skeletal and cardiac myocytes. Troponin T binds to tropomyosin and helps position it on actin and together with the rest of the troponin complex, modulates contraction of striated muscle. These proteins are released when the heart muscle has been damaged, such as occurs with a heart attack. The more damage there is to the heart, the greater the amount of troponin T.¹⁵

Garlic has been revealed to have a protective effect on the heart as it decreased the activity and concentration of both Creatinine Kinase and Troponin T.¹⁶ Garlic supplements have shown effectiveness in reducing blood pressure in hypertensive patients, similarly to first-line standard anti-hypertensive medications.¹⁷

All the above findings agreed with our results which showed significant decrease in serum concentration of Troponin T in the NaCl co-administered with Garlic when compared to NaCl only group. Furthermore, slight significant decrease was noted in NaCl co-administered with Garlic when compared to NaCl co-administered with Nifedipine (though not statistically significant).

Furthermore, test for renal-toxicity was assessed by checking the serum urea and creatinine concentration. Our results showed

that there were high significant rises in serum urea and creatinine concentration in NaCl only treated group. This result is in line with Cirillo *et al*, who showed hypertension causes increase in urea concentration.¹⁸ Furthermore, slight increase in serum urea and creatinine concentrations were noted in NaCl co-administered with Nifedipine when compared with the control group. Also, slight decrease was noted in NaCl co-administered with Nifedipine when compared with NaCl only treated group. Literatures have shown that Nifedipine monotherapy improves renal function in patients with hypertension.⁹ Dworkin *et al* showed that calcium blockers are effective in preventing progressive kidney damage.¹⁹ Nifedipine blocks voltage-gated L-type calcium channels in vascular smooth muscle and myocardial cells. This blockage prevents the entry of calcium ions into cells during depolarization, reducing peripheral arterial vascular resistance and dilating coronary arteries. These actions reduce blood pressure and increase the supply of oxygen to the heart, alleviating angina.²⁰

Moreover, Serum urea and creatinine concentration was significantly reduced in NaCl co-administered with Garlic when compared with NaCl only and NaCl co-administered with Nifedipine treated groups. This report is in line with Bagheri *et al* who reported that garlic juice significantly decreased serum urea levels.²¹ Recent evidence has suggested that garlic could be used to promote overall kidney health due to its diuretic properties. Diuretics increase the amount of sodium that is filtered into the urine; salt absorbs water from the blood, decreasing the amount of blood in the circulatory system, which lowers blood pressure.^{22,23,24}

In the histological results, all other treatments aside NaCl group show normal architecture: the renal cortex showed normal

glomeruli with normal mesangial cells and capsular spaces, the renal tubules appeared normal with no signs of dilatations and the interstitial spaces appear normal and clear. This result agrees with Dhar and Farzan who showed unchanged histology after the administration of Nifedipine.²⁵

Histological results observed in NaCl only treated group showed disrupted, degenerated and fenestrated cardiocytes although these effects were lessened in rats administered with NaCl co-administered with Nifedipine. Improved results were observable in rats administered with NaCl co-administered with Garlic. Likewise, kidney histoarchitecture were poor in NaCl only treated group with renal cortex showing sclerotic glomeruli with wide capsular spaces; the renal tubules showed congestion within the lumen and the interstitial spaces appeared infiltrated with presence of red inflammatory cells. These effects were lessened in rats administered with NaCl co-administered with Nifedipine while improved results were noted in NaCl co-administered with Garlic treated group. Our result agreed with Hara *et al.*, who showed improved histopathology after the administration of Garlic.²⁶

CONCLUSION

Our study showed that hypertension induced by NaCl caused reduced efficiency in kidney function (renal dysfunction) in Wistar rats, and garlic was effective in attenuating this renal dysfunction better than with just the administration of a synthetic antihypertensive drug (Nifedipine).

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