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DOCA-Salt Hypertension – Induced Fibro-myocardial damages in Wistar Rat Heart are Reversed by Cumulative Acute Treatment by *Allium Sativum* Extract Compared to Hydrochlorothiazide and Captoten

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ABSTRACT

Hypertension is a major cause of morbidity and mortality associated with coronary heart disease, cerebrovascular and renal disease. Fresh *Allium sativum* bulbs have been alleged to have blood pressure lowering effects. However, exact mechanisms (long-term effects) are not fully understood. This study aimed to investigate functional reversibility effects of blood pressure-induced fibro-myocardial damages in the hearts of male DOCA-salt model wistar laboratory rats by comparing cumulative treatment with *A. sativum* extracts to two known antihypertensive drugs i.e. Hydrochlorothiazide and Captoten. Cardiovascular parameters (Heart rate, Systolic and Diastolic blood pressure) were used as a measure of cardiac functionality over a period of 20 weeks. Sixty four (n = 64) male DOCA-salt wistar rats (250-300g, 36 weeks old) were induced to hypertension using Deoxycorticosterone acetate (DOCA) salt. Doses of 50, 100 and 200 mg/kg body weight of *A. sativum* extracts were prepared as fresh aqueous garlic extract (FAGE-T1), crude garlic extract (CGE-T2) and crude industrial garlic extract (CIGE-T3), with similar doses of Hydrochlorothiazide (HCT-C2), Captoten (CPT-C3) and administered intraperitonially (IP) twice a day. The control group (C1) was treated with normal saline (NS). At week 8 and week 20, eleven (11) selected wistar rats from all the groups were irreversibly anesthetized, sacrificed and their hearts dissected for histological analysis for fibro-myocardial damage and functional reversibility respectively. Histological findings showed that hypertensive experimental groups initially with fibro-myocardial tissue damaged hearts and treated cumulatively by FAGE (200mg/Kg body weight), had reversal effects on their myocardial tissues and function post-hypertension, as compared to hypertensive experimental groups T2, T3, C2 and C3.

Keywords: Hypertension, Cerebrovascular, Fibro-myocardial, Histological, Hydrochlorothiazide and Captoten.
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INTRODUCTION

Garlic (*Alliums Sativum L*.) is a cultivated plant. Its wild progenitor *Allium Longicupis* originated in the high planes of West-Central Asia. In decades, it has been widely used as food and medicine (David, 2005; McMahon, 1993). The plant has had significant foreign influence to Kenya, majorly inhabiting many tribes of the Kenyan Coast. Its effects have been demonstrated in both animals and humans (Brace, 2002), and it has been the subject of intensive scientific research with over 2000 scientific publications on antibacterial, antitumor, antifungal, hypolipidemic, hypogylcemic, antiatherosclerotic, hematinic and hypertensive activities (Alnaqeeb et al., 1996; Al-Qattan et al., 2006; Durak et al., 2004; Gardner et al., 2001; Grazyna, 2008; Zeng et al., 2013). Botanically, *A. sativum* is a member of the Lillaceae family, along with onions, chives, and shallots (Iciek et al., 2009). Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfonate) are the principal bioactive compounds present in the aqueous extract of *A. sativum* or raw *A. sativum* homogenate (Lanzotti, 2006). Hypertension is usually a slowly-developing disorder of middle to old age which

predisposes to cardiovascular disorders (Asdaq and Inamdar, 2011)) that cause most of the morbidity and mortality in the elderly (Lanzotti, 2006). The incidences of hypertension vary markedly by patient subgroup, particularly by gender and race (Frank, 2008). High blood pressure is a condition in which the blood pressure in the arteries is chronically elevated. The heart usually works harder to pump blood to the rest of the body. This could lead to brain and cardiovascular tissue damage resulting in heart attack, stroke, heart failure, aneurysm, or renal failure (Hani, 2012).

The normal human blood pressure (BP) is 100 to 140 mmHg (Systolic) and 60 to 90 mmHg (Diastolic). BP of 140/90 mmHg or above is considered hypertension (HTN). HTN has several sub-classifications including hypertension stage I, hypertension stage II, isolated systolic hypertension, exercise hypertension, pregnancy hypertension, primary hypertension and secondary hypertension (Oparil et al., 2014). Heart Disease: the pressure load placed on the left ventricle results in left ventricular hypertrophy (LVH) (Gurgun et al., 2008). The heart enlarges and dilates, with hypertrophy more marked than dilation, until the left heart begins to fail, particularly when the heart reaches 500 gm in weight due to overstretched fibro-myocardial tissues, endocardial inflammation, degenerative pericardial inflammation and accumulation of acute mast cells. Congestive heart failure and cardiac arrhythmias may result from the failing heart. Diuretics, sometimes called water pills, are medications that act on the kidneys to help the body eliminate sodium and water, reducing blood volume (Guyton, 1990). Thiazide diuretics are often the first, but not the only, choice in high blood pressure medications. Thiazide diuretics include hydrochlorothiazide (HCT), Microzide, chlorthalidone and others. Diuretics or calcium channel blockers may work better for blacks and older people than do angiotensin-converting enzyme (ACE) inhibitors alone (Gao et al., 2014). A common side effect of diuretics is increased urination. ACE inhibitors: These medications such as lisinopril (Zestril), benazepril (Lotensin), captopril (Captoten) (CPT) and others help relax blood vessels by blocking the formation of a natural chemical that narrows blood vessels. People with chronic kidney disease may benefit from having an ACE inhibitor as one of their medications (Asdaq and Inamdar, 2011). In the present study, using cardiovascular parameters (HR, SBP and DBP), the functional reversibility effects of blood pressure-induced fibro-myocardial damages in the hearts of male DOCA-salt model wistar laboratory rats were examined, treated by cumulatively acute concentrations of *A. sativum* extracts, whose effects were compared to two known antihypertensive drugs i.e. Hydrochlorothiazide (HCT) and Captoten (CPT) over a given period of 20 weeks.

MATERIALS AND METHODS

Study Site

This study was mounted at the University of Eldoret (UOE), Department of Biological Sciences animal house. The processing of various *A. sativum* extracts, isolation and determination of concentrations were done at the Department of Chemistry and Biochemistry. The biochemical and Pathophysiological analysis took place at Nairobi Annex Laboratory (Eldoret branch). Electrophysiological procedures were conducted at the Department of Medical Physiology, University of Nairobi (UoN). All laboratory requirements and chemicals were supplied by ScieLab Chemical Suppliers (Nairobi).

Study Design

The study is a Laboratory-based Randomized Controlled Experiment involving control and treatment groups. It constituted a group of Normotensive (C1) and five groups of Hypertension-Induced Wistar laboratory rats (C2, C3, T1, T2 and T3).

Induction of Hypertension and Measurements of Blood Pressure

According to Crofton and Share (1997), Salt retention and fat accumulation is characteristic of human hypertension and can be achieved rapidly in uninephrectomised rats by mineralocorticoid administration (Kandlikar and Fink, 2011). Hypertension was induced to 5 treatment groups out of 6 groups using special diet (Fortified pellets with high lipids (20%) and weekly subcutaneous injections Deoxycorticosterone Acetate (DOCA) (Bell, 1979) salt (10%) and salt loading of 1% Sodium Chloride (NaCl) in drinking water (Mozaffarian et al., 2014). The study was conducted for a period of 20 weeks (6 weeks of HTN induction and 14 weeks of treatment, observation and analysis). Blood pressures were measured using the tail-cuff method via a digital Powerlab recorder against the tail artery. A systolic blood pressure of 150 mmHg and diastolic blood pressure of 100 mmHg were achieved to ascertain the induction of hypertension. Total body weight and rectal body temperatures were recorded daily to keep track of the experiment.

Allium Sativum Preparation and Administration

Sixty-four (64) Wistar laboratory rats were randomly divided into 16 smaller groups depending on the concentrations of the extracts, with four Wistar rats (n = 4) per group as follows; Group C1: Normotensive rats treated with normal physiological saline. Group C2: Treated with (CPT 50, 100, and 200 mg/Kg body weight). Group C3: Treated with Broad-spectrum 1st liner Antihypertensive drug (HCT 50, 100 and 200 mg/Kg body weight). Group T1: treated with Fresh Aqueous Garlic Extract FAGE 50, 100 and 200 mg/Kg body weight. Group T2: treated with Crude Garlic Extract CGE 50, 100 and 200 mg/Kg body weight.

Group T3: treated with Crude Industrial Garlic Extract

CIGE 50, 100 and 200 mg/Kg body weight.

Electrophysiological Studies of The Rat Hearts

Animal Preparation

Eleven (11) selected and carefully marked male DOCA-salt Wistar rats were taken for electrophysiological studies to the University of Nairobi's Department of Medical Physiology animal house. The tests were conducted between 8:00 am and 9:00 am in the morning. Data were collected at the beginning of the study after the animals had acclimatized before induction of hypertension, after 3 weeks following induction of hypertension and an average of 16 tests were conducted.

Protocol for Electrical and Mechanical Studies of The Hearts

Cardiovascular parameters were used to study in-vivo effects of *A. sativum* on the electrical and mechanical activity of the myocardium from 11 of the sampled animal from the 6 groups (1 from the control group and 2 from each of the five treated groups) using Bohr's procedure (Bohr, 1991). The assessments included measurements of the HR, Systolic SBP and DBP and ECG recordings using the PowerLab machine. The left ventricular thickness (LVT), coronary diameter and pulmonary vessel thickness were analyzed histologically as described by Chan et al. (2013). The electrophysiology study was based on clinical protocols used to evaluate cardiac conduction in human patients. For each study, an animal was irreversibly anaesthetized using Ketamine 75 mg/Kg BW + Diazepam 5 mg/Kg BW intraperitoneally (Lasting 20 min with a 2 to 3 h recovery time). A surface Three-limb-lead ECG was then obtained by placement of surface electrodes on the paw of each limb, secured with tape as follows; the white electrode on the right forelimb, the green electrode on the right hind limb and the black electrode on the left hind limb. The ECG channels were amplified (0.1 mV/cm) and filtered between 10 and 100 Hz, and a stable signal was reliably obtained before the electrogram recording. Body temperature, cardiac rhythm, and heart rate were monitored on the transmission screen during the recording. A warming light was used to maintain body temperature within a range of 34°C to 37°C on the warming plate for prevention of hypothermia since the weather was a bit chilly at the time of the experiment. The Powerlab machine was set at a recommended manufacturer's pace necessary for rats. Cardiac rhythm was continuously monitored and recorded (at 100 mm/s), and all ECG frontal axes (P and QRS complex) and time intervals (PR, QRS, QT, RR and P duration) were calculated for each animal in standard fashion (Hiss and Lamb, 1962), using the electrogram tracing and the digital data that were generated by the computer real-time.

Anatomical and Histological Studies of The Heart Tissues

Sacrifice of Animals

At the end of the experiment, the above 11 animals as selected randomly were irreversibly anaesthetized using Ketamine 75 mg/Kg BW + Diazepam 5 mg/Kg BW intraperitoneally (Lasting 20 min with a 2 to 3 h recovery time), the hearts were harvested for further anatomical and histological studies.

Protocol for Determination of Histomorphological Examination

At sacrifice, the weight, length, diameter, ventricular thickness, aortic root dilation and perfusion verse size of cardiomyocytes were measured and recorded (Esler et al., 2010). The heart weights were determined using a top loader sensitive balance (Mettler-Toledo Garvens GmbH, Giesen, Germany). The relative weights of the heart (%) to the bodyweight at sacrifice were evaluated. The hearts were trimmed to remove excess fat, blood vessels or remnants of the lungs. The large vessels were clipped at their roots. The heart dimensions are measured and recorded using anatomical calipers. Two parallel lines were drawn on a polystyrene board 10 cm from each other. The identification case numbers were written along the two lines at 4 to 5 cm intervals. A needle was driven through each heart at the superior end of the ventricular septum to impale the heart to the board such that the upper border of the atria just touches the horizontal line drawn on the board and the apex of the heart points downwards at an angle perpendicular to the horizontal line. Each heart was fixed to its assigned position corresponding to its identification case number. Transverse sections were made across each ventricle at 3 mm and 6 mm from the apex of the heart. The left ventricular thickness is measured and recorded. The tissue sections from the apex and the mid-ventricular region were placed in cassettes and processed histologically, then examined by a pathologist.

Histological Techniques and Photomicrography

The tissues were stored in 10% neutral-buffered formalin for intact preservation. Different cross-sectional planes of the cardiac tissues were sectioned and tissue slides prepared for Histomorphological reading and analysis following prolonged hypertension. Sections of 5 micrometer were cut from the paraffin-embedded tissue and stained with Haemotoxylin and Eosin (H&E Stain) to demonstrate the general histoarchitecture of the heart. Masson's trichrome stain was used to demonstrate collagen fibers in the myocardium of the left ventricles of the heart (Kaye et al., 2000), while Verhoeff-Van Gieson stain was used to demonstrate

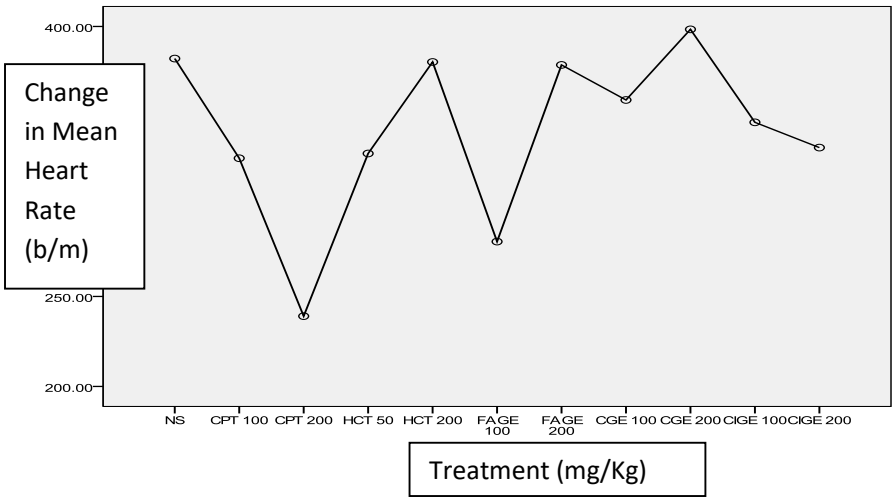

Figure 5. Mean plot effect of *A. sativum* extract treatment on heart rate from a real-time ECG. Each point represents a Mean of four animals per treatment group.

elastic fibers in myocardium of the left ventricles (Wang et al., 2009). H&E Stained sections were viewed under a LEICA research microscope (LEICA DM750, Switzerland) with digital camera attached (LEICA ICC50) and digital photomicrographs were taken at X400 magnification. Photomicrographs of H&E stained sections were imported on to the Motic Images Plus, Version 2.0 software for histomorphometric analysis, to measure the ventricular thickness and diameter of the cardiomyocytes.

Ethical Issues

All procedures and care given to the animals were in accordance to the Laboratory Animal Care Committee Guidelines (LACCG). The institution's Board of Postgraduate Studies was informed of the research project.

Data Collection, Statistical Analysis and Presentation

Three Surface-limb-lead ECG data from 11 DOCA-salt Wistar rats were collected. Using the PowerLab machine, various physiological parameters were analyzed to establish the blood pressure, the hearts structural and functional status. Data was recorded in tables and presented using bar graphs and line graphs. The results were expressed as means ± SEM and were analyzed using One-Way Analysis of Variance (ANOVA), followed by Turkey's HSD Multiple range test as a post-hock test for a significant difference. Paired or unpaired Student's t-test was used for the Statistical analysis. A *p*-value of less than 0.05 was considered significant. All statistical procedures were performed by Statgraphics software version 5.0 (STSC, Inc., Rockville, MD, USA).

RESULTS

Effect of *A. sativum* Extract Treatment on the Heart Rate of DOCA-Salt Hypertension-Induced Laboratory Rat from Real-Time ECG

The heart rate at CPT, HCT, FAGE, CGE, and CIGE were compared with heart rate at NS as shown in Figure 5. The ANOVA Table 1 shows the significance difference between groups. Turkey's HSD multiple comparisons (Table 2) revealed that the observed drops in mean heart rates were significant at all the concentrations except at C3b of HCT 200 mg/kg body weight ($\Delta M=380.27$, $p<0.05$) and T1b of FAGE 200 mg/kg body weight ($\Delta M=378.62$, $p<0.05$. The largest mean significant differences were reported at C2b of CPT 200 mg/kg body weight ($\Delta M=239.01$, $p<0.05$). The increase in mean heart rates at CGE (T2a CGE 100 mg/Kg body weight and T2b CGE 200 mg/Kg body weight) had a mean difference of 16.228 compared to that of NS.

Effect of *A. sativum* Extract Treatment on SBP and DBP of DOCA-Salt Hypertension-Induced Laboratory Rat from Real-Time ECG Recording

The ANOVA output (Table 3) for both SBP and DBP revealed that there were significant differences in mean SBP and DBP at different concentrations of *A. sativum* extracts treatment. *A. sativum* extract treatment was found to have no statistically significant effect on the Mean systolic blood pressure of hypertension-induced laboratory rats. The mean systolic blood pressure values were just at the borderline of the highest systolic blood pressure value. The normal systolic blood pressure is about 90 to 145 mmHg. But upon further assessment, the comparative linear functions of Mean

Table 1. ANOVA tables for electrical and mechanical parameters analysed.

		Sum of Squares	DF	Mean Square	F	Sig.
	Between groups	62305.494	10	6230.549	3449.188	.000
	Within groups	124.640	69	1.806		
RR Interval	Total	62430.134	79			
	Between groups	162207.899	10	16220.790	2887.923	.000
	Within groups	387.557	69	5.617		
Heart Rate	Total	162595.456	79			
	Between groups	22492.378	10	2249.238	28.885	.000
	Within groups	4594.299	59	77.869		
PR Interval	Total	27086.677	69			
	Between groups	7444.909	10	744.491	11.279	.000
	Within groups	3894.517	59	66.009		
P Duration	Total	11339.426	69			
	Between groups	4694.972	10	469.497	22.907	.000
	Within groups	1373.246	67	20.496		
QRS Interval	Total	6068.218	77			
	Between groups	12857.746	10	1285.775	14.979	.000
	Within groups	3776.828	44	85.837		
QT Interval	Total	16634.574	54			

Table 2. Multiple comparison test results of effect of *A. sativum* extract treatment on heart rate from real-time ECG using turkey's HSD.

Multiple Comparisons of Heart Rate (b/m)						
					95% Confidence Interval	
(1) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
	CPT 100	55.37714*	1.26680	.000	51.1600	59.5943
	CPT 200	143.11714*	1.26680	.000	138.9000	147.3343
	HCT 50	52.74000*	1.26680	.000	48.5228	56.9572
	HCT 200	1.89714	1.26680	.916	-2.3200	6.1143
	FAGE 100	101.69429*	1.26680	.000	97.4771	105.9115
	FAGE 200	3.54063	1.19435	.125	-.4354	7.5166
	CGE 100	23.00857*	1.26680	.000	18.7914	27.2257
	CGE 200	-16.22857*	1.26680	.000	-20.4457	-12.0114
	CIGE 100	35.42714*	1.26680	.000	31.2100	39.6443
NS	CIGE 200	49.46411*	1.22658	.000	45.3808	53.5474

*. The mean difference is significant at the 0.05 level.

Table 3. ANOVA table for systolic blood pressure and diastolic blood pressure.

		Sum of Squares	DF	Mean Square	F	Sig.
Systolic	Between groups	8304.176	15	553.612	9.808	.000
	Within groups	9031.636	160	56.448		
	Total	17335.813	175			
Diastolic	Between groups	2983.614	15	198.908	3.936	.000
	Within groups	8085.273	160	50.533		
	Total	11068.886	175			

systolic blood pressures at different concentrations against time with subsequent cumulative treatment of *A. sativum* extracts revealed that Mean systolic blood pressure reduced after induction of hypertension in the

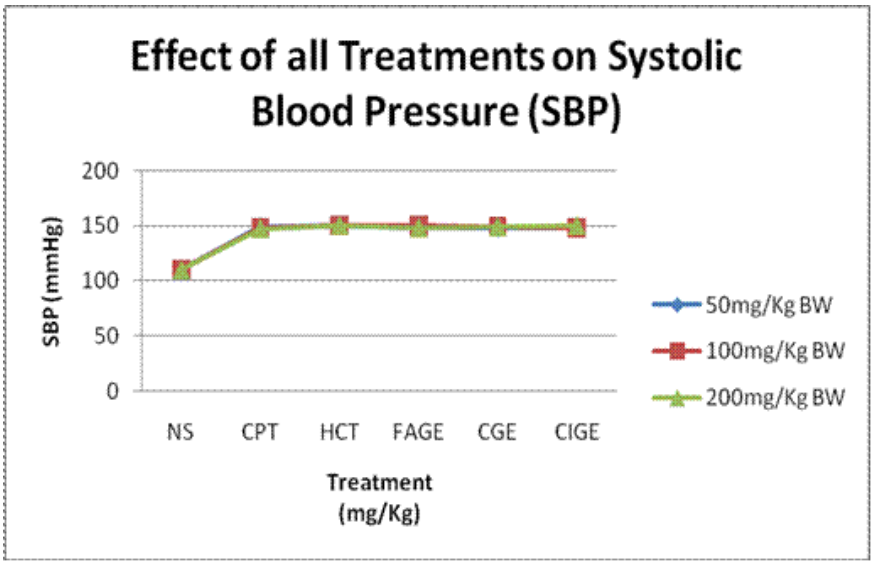

Figure 1. Mean plot effect of *A. sativum* extract treatment on Systolic Blood Pressure (SBP).

Table 4. Multiple comparison test results of effects of *A. sativum* extract treatment on systolic blood pressure in hypertension-induced laboratory rats using Turkeys HSD.

Multiple comparison for systolic blood pressure (mmHg)						
					95% Confidence Interval	
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NS	CPT50	24.00000	3.20363	.000	-35.1534	-12.8466
	CPT 100	23.81818	3.20363	.000	-34.9715	-12.6648
	CPT 200	22.36364	3.20363	.000	-33.5170	-11.2103
	HCT 50	24.36364	3.20363	.000	-35.5170	-13.2103
	HCT 100	25.27273	3.20363	.000	-36.4261	-14.1194
	HCT 200	25.09091	3.20363	.000	-36.2443	-13.9376
	FAGE 50	24.54545	3.20363	.000	-35.6988	-13.3921
	FAGE 100	24.90909	3.20363	.000	-36.0624	-13.7557
	FAGE 200	22.72727	3.20363	.000	-33.8806	-11.5739
	CGE 50	-31.63636*	3.20363	.000	-42.7897	-20.4830
	CGE 100	-27.63636*	3.20363	.000	-38.7897	-16.4830
	CGE 200	-26.81818*	3.20363	.000	-37.9715	-15.6648
	CIGE 50	-32.18182*	3.20363	.000	-43.3352	-21.0285
	CIGE 100	-27.18182*	3.20363	.000	-38.3352	-16.0285
	CIGE 200	-28.09091*	3.20363	.000	-39.2443	-16.9376

*. The mean difference is significant at the 0.05 level.

treatment groups from week 1 to week 20 as depicted in Figure 1. Table 4 shows an examination of the multiple comparisons test results of Mean Systolic blood pressure of treatment group T2 with CGE and T3 with CIGE concentrations with that of the control group at NS revealed highest increments in the Mean systolic blood pressure at the start of the treatments. The largest differences in mean systolic blood pressure were registered at CIGE 50 mg/kg body weight (ΔM=-32.181, $p<0.05$) which is 43.91% increment, CGE 50 mg/kg body weight (ΔM=-31.636, $p<0.05$) which is 41.48% increment and at CIGE 200 mg/kg body weight (ΔM=-28.091, $p<0.05$) which is 25.62% increment, compared with the baseline of the control group at NS (ΔM=-22.36, $p<0.05$). *A. sativum* extract treatment was also found to trigger a decrease and maintained constancy in the normal diastolic blood pressure of hypertension-induced laboratory rats within the reference range for Wistar rat (55 to 66 mmHg). Though within normalcy, the least registered mean diastolic blood pressure was 63.00 mmHg registered at treatment group C2b at a concentration of CPT 200

Table 5. Summary report of mean and SEM of Diastolic Blood Pressure (DBP) in hypertension-induced laboratory rats.

Report		
Diastolic		
Treatment	**Mean**	**Std. Error of Mean**
NS	56.9091	.62457
CPT50	64.8182	2.37341
CPT 100	63.8182	2.19428
CPT 200	63.0000	2.21975
HCT 50	66.0000	2.57258
HCT 100	67.9091*	2.22570
HCT 200	63.1818	2.42644
FAGE 50	67.4545*	2.40592
FAGE 100	66.2727	2.71055
FAGE 200	63.0000	2.29228
CGE 50	73.6364*	1.85508
CGE 100	67.9091*	2.03360
CGE 200	64.1818	2.36573
CIGE 50	74.8182*	.95173
CIGE 100	67.2727*	1.87854
CIGE 200	68.5455*	2.06866
Total	66.1705	.59948

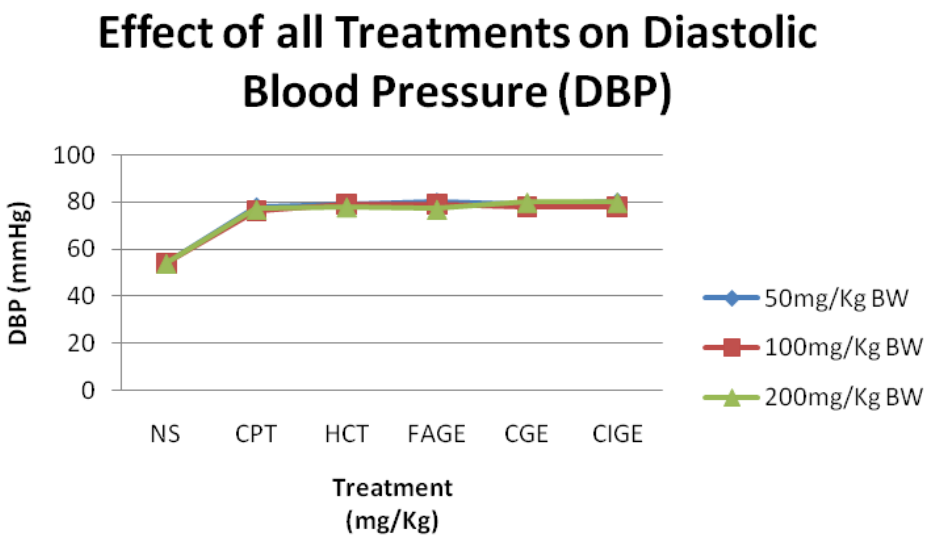

Figure 2. Mean plot effect of *A. sativum* extract treatment on Diastolic Blood Pressure (DSP).

mg/kg body weight, C3b at HCT 200 mg/Kg body weight and treatment group T1b at a concentration of FAGE 200 mg/kg body weight (= 10.7% decrement) compared to the control group with 56.91 mmHg.

The largest Mean diastolic blood pressure was 74.8182 recorded by treatment group T3 at a concentration of CIGE 50 mg/kg body weight (= 31.47% decrement) as shown by Table 5.The linear functions also depicted a decline in the mean diastolic blood pressure at different extract concentrations with subsequent cumulative exposure to the treatments over time for a period of 20 weeks as shown in Figure 2.

Analysis of the multiple comparisons Table 6 revealed that after induction of hypertension, the following treatment groups and concentrations were able to lower or maintain the mean diastolic blood pressure within normal ranges and therefore they were found to be statistically significant compared with the control group that was treated with NS. Treatment groups C3b at HCT 100 mg/kg body weight (ΔM=-11.00, $p<0.05$; = 19.32%); treatment group T2 at CGE 50 mg/kg body weight (ΔM=-16.727, $p<0.05$; = 29.37%); CGE 100 mg/kg body weight (ΔM=-11.000, $p<0.05$; = 19.32%) and treatment group T3 at CIGE 50 mg/kg body weight

Table 6. Multiple comparison test results of effects of *A. sativum* extract treatment on diastolic blood pressure in hypertension-induced laboratory rats using Turkeys HSD.

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	CPT50	-7.90909	3.03114	.400	-18.4619	2.6438
	CPT 100	-6.90909	3.03114	.639	-17.4619	3.6438
	CPT 200	-6.09091	3.03114	.816	-16.6438	4.4619
	HCT 50	-9.09091	3.03114	.181	-19.6438	1.4619
	HCT 100	-11.00000*	3.03114	.032	-21.5528	-.4472
	HCT 200	-6.27273	3.03114	.781	-16.8256	4.2801
	FAGE 50	-10.54545	3.03114	.050	-21.0983	.0074
	FAGE 100	-9.36364	3.03114	.146	-19.9165	1.1892
	FAGE 200	-6.09091	3.03114	.816	-16.6438	4.4619
	CGE 50	-16.72727*	3.03114	.000	-27.2801	-6.1744
	CGE 100	-11.00000*	3.03114	.032	-21.5528	-.4472
	CGE 200	-7.27273	3.03114	.551	-17.8256	3.2801
	CIGE 50	-17.90909*	3.03114	.000	-28.4619	-7.3562
	CIGE 100	-10.36364	3.03114	.060	-20.9165	.1892
NS	CIGE 200	-11.63636*	3.03114	.016	-22.1892	-1.0835

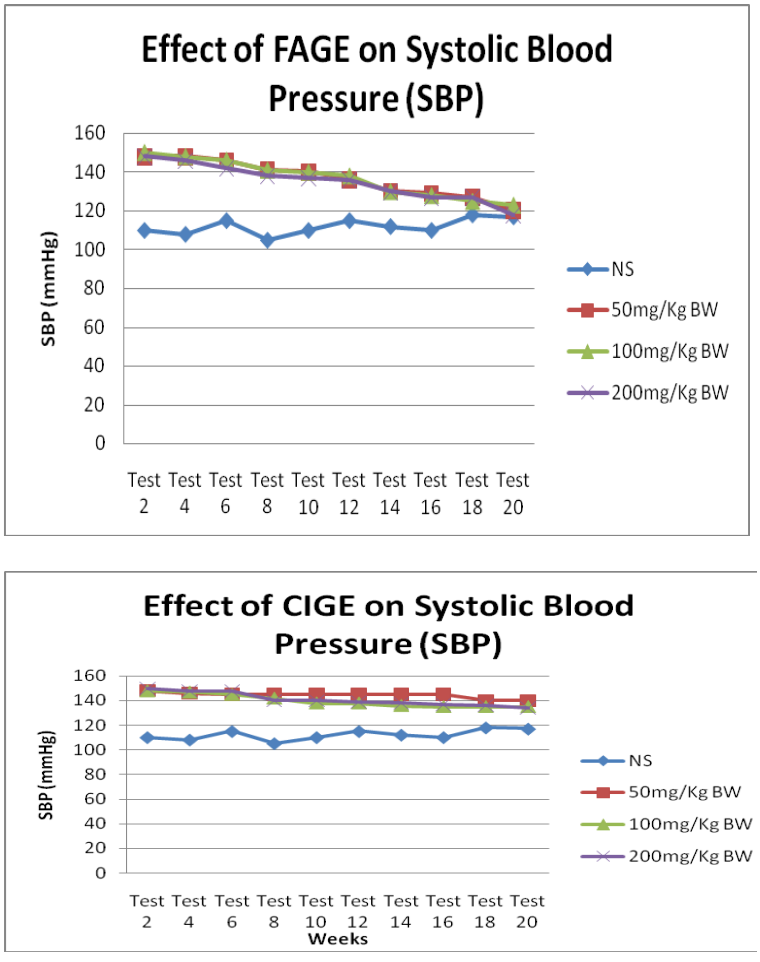

Figure 3. A comparative linear graph of mean plot effect of *A. sativum* extract treatment on systolic blood pressure for 20 weeks.

(ΔM=-17.909, p<0.05; = 31.47) and at CIGE 200 mg/kg body weight (ΔM=-11.636, p<0.05; = 20.45%). The highest mean diastolic blood pressure lowered was recorded in treatment T3 at a concentration of CIGE 50 mg/kg body weight (ΔM=-17.909, p<0.05; = 31.47%), (Figure 4). Figure 3 depicts the comparative effects of

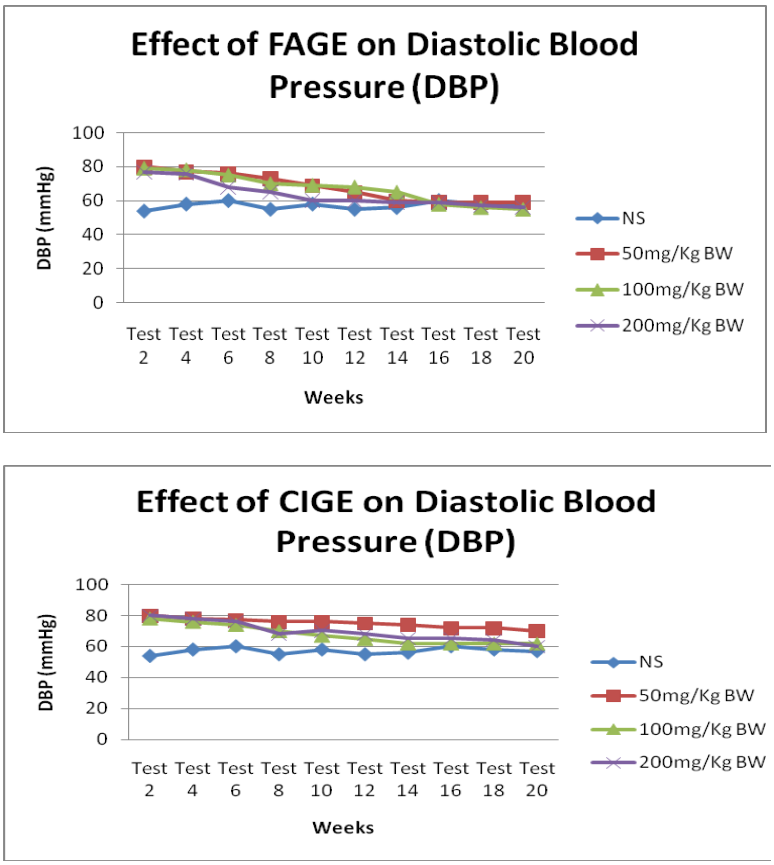

Figure 4. A comparative linear graph of mean plot effect of *A. sativum* extract treatment on Diastolic Blood Pressure (DBP) for 20 weeks.

Table 6. Findings of the effects of *A. sativum* extract treatment on the histology of the heart in hypertension-induced laboratory rats.

Rat	Case Number	Dimensions	Gross Appearance	Left Ventricular Thickness	Microscopic Appearance	Comment
Rat 1	T_1FAGE_{100}	11 x 11 x 9mm	Globular heart	3mm	The heart muscle and vessels appear normal. No degenerative or inflammatory changes were seen.	Normal heart
Rat 2	C_2CPT_{200}	9 x 10 x 8mm	Medium Heart	3mm	There were numerous intramuscular mast cells. Marked pericardial inflammation was noted associated with haemosiderin-laden macrophages	Inflammation
Rat 4	C_3HCT_{200}	10 x 11 x 8mm	Medium Heart	3mm	Features of acute endocardial inflammation seen. Focal endomyocardial fibrosis noted. Mast cells were noted.	Hypertrophy
Rat 5	T_1FAGE_{200}	11 x 12 x 9mm	Globular heart	3mm	The heart muscle and vessels appear normal. No degenerative or inflammatory changes were seen.	Normal
Rat 6	C_2CPT_{100}	10 x 11 x 8mm	Medium Heart	2mm	Mild perivascular inflammation noted	Hypertrophy

FAGE and CIGE on systolic blood pressure for 20 weeks.

Effects of *A. sativum* Extract Treatment on The Histology of The Heart in DOCA-Salt Hypertension-Induced Laboratory Rats

Table 6 shows the experimental tissue results after-treatment of the hearts with different *A. sativum* extract treatments (T1 to T3) in various concentrations (50, 100 and 200 mg/Kg body weight), compared to effects of known antihypertensive drugs (CPT and HCT) as positive controls groups with a normotensive control group pre-treated with normal saline (NS) post hypertension period. The myocardium was sectioned along different planes to establish the effect of

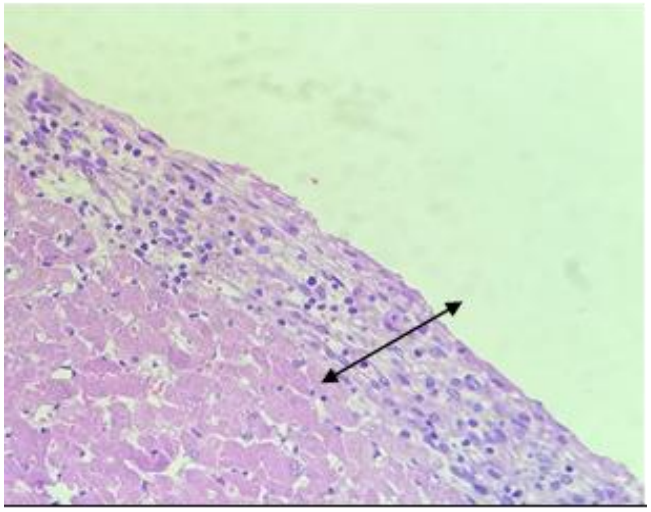

Plate 1: Photomicrograph of a section of the Aortic Root of the Rat heart administered with CPT_{200}. Captoten had no effect Post-Hypertension. H & E stained. X400.

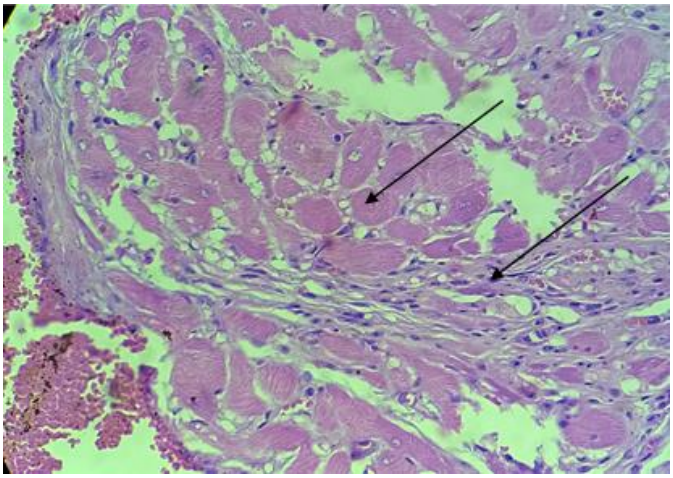

Plate 2: Photomicrograph of a section of the Left Ventricle of the Rat heart administered with $CIGE_{200}$. *Allium sativum* extract had no effect Post-Hypertension. H & E stained. X400.

prolonged hypertension on the heart, followed by establishment of the effect of post-hypertension treatment with the various concentrations of *A. sativum* extracts. The analysis was recorded as seen in Table 6.

DISCUSSION AND CONCLUSION

The heart rate, systolic blood pressure and diastolic blood pressure correlated perfectly with the histological analysis before, during and after induction of hypertension. The normotensive heart (C1NS) showed normal myocardium with normal coronary vessels, no degenerative or inflammatory changes (Plate 6). The rats in the normotensive group were treated with normal saline and the myocardium had normal atrioventricular and semilunar valves, normal myocardial septum, a thicker left ventricle than the right ventricle and normal pericardial layers. The systolic and diastolic blood pressures were normal as depicted by the corresponding tables and figures. The positive control group (C2) treated with an antihypertensive drug CPT 200mg/Kg body weight had numerous intramuscular mast cells, marked pericardial inflammations associated with hemosiderin-laden macrophages as was observed in Plate 1. The observed lesions are positively due to the overload and hypercontractility effects of hypertension and hence could not achieve effective functionality. These was also noted by Gurgun et al in 2008, when they did a study on the effects of short term statin treatment on left ventricular function and inflammatory markers in patients with chronic heart failure. Positive control group C3 were hypertensive wistar rats treated with an antihypertensive drug HCT 200mg/Kg body weight had features of chronic endocardial inflammation, infiltrations of the

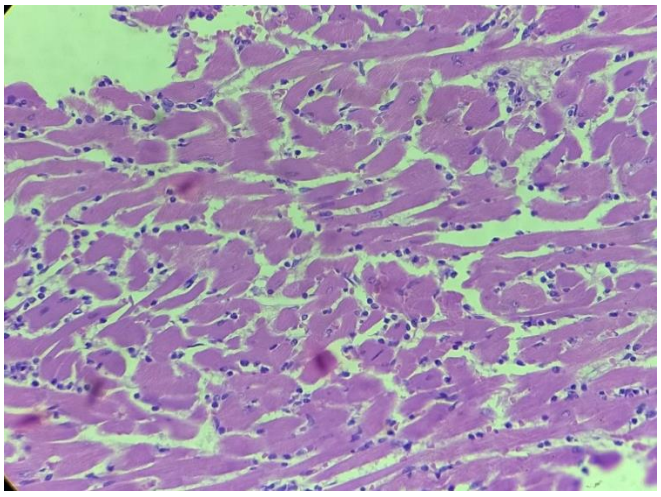

Plate 3: Photomicrograph of a section of the Left Atrial wall of the Rat heart administered with HCT_{200}. Hydrochlorothiazide had no effect on the myocardium Post-Hypertension. H & E stained. X400.

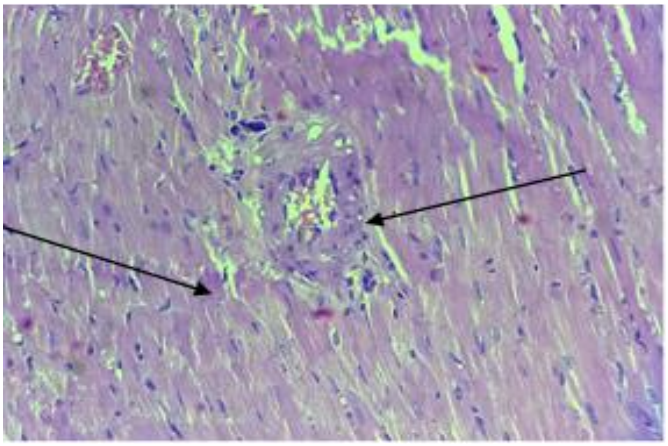

Plate 4: Photomicrograph of a section of the Septal wall of the Rat heart administered with $FAGE_{200}$. Fresh Aqueous Garlic Extract had reversal effect on the myocardiocytes post-hypertension. H & E stained. X400.

endocardium and papillary muscles by lymphocytes and neutrophils with focal endomyocardial fibrosis and presence of mast cells as shown by Plate 3.

This was an indication of left ventricular hypertrophy due to overload and excess demand stretched by prolonged hypertension in order to compensate for the cardiac output required to perfuse the systemic circulation. Analysis of responses of garlic derivatives in the pulmonary vascular bed of the rat by Kaye et al (2000), also yielded similar results on comparative garlic extracts on the heart. Subsequently, there were no signs of tissue repair in the groups treated with the antihypertensives CPT and HCT after prolonged period of hypertension. However, the hypertensive group T1 which initially had myocardial damage and then treated with FAGE 200mg/Kg body weight for 20 weeks, was observed to be normal with no degenerative or inflammatory changes as shown in Plate 4. The inference from this group showed that *A. sativum* extract contain properties that enhanced tissue repair and ability to reinstate of normal myocardial functionality following a period of damage due to hypertension and hence was able to mediate lowering effects of the cardiovascular parameters that were analyzed in this study, compared to the hypertensive groups that were treated with CGE and CIGE despite slight decrease in SBP and DBP observed. In the study of 200mg of garlic powder given three times daily, in addition to hydrochlorothiazide-triamterene (HCT) baseline therapy produced a mean reduction of systolic blood pressure by 10-11 mmHg and of diastolic blood pressure by 68 mmHg versus the control group ($p<0.05$) having no effect. This study is consistent with a study on men with

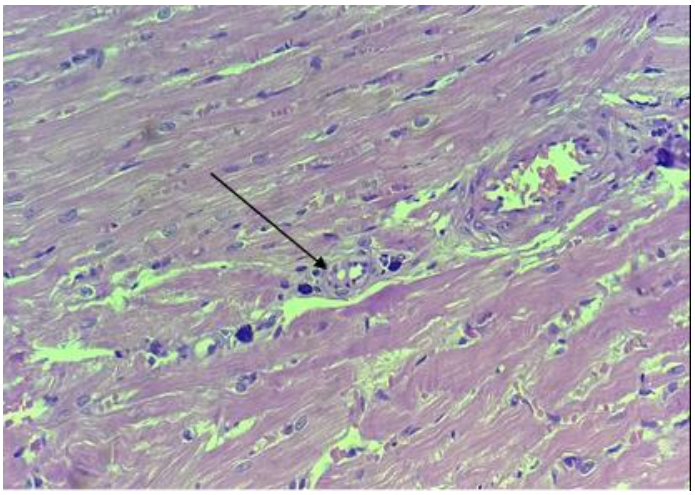

Plate 5: Photomicrograph of a section of the Left Atrial wall of the Rat heart administered with CGE_{100}. Crude Garlic Extract had minimal effect on the myocardiocytes Post-Hypertension. H & E stained. X400.

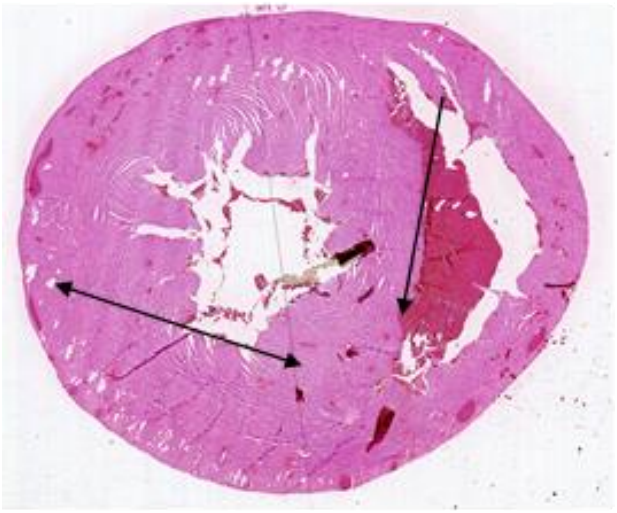

Plate 6: Photomicrograph of a section of the Left Ventricular wall of the Normotensive Rat heart administered with Normal Saline (NS). NS had no effect on the myocardiocytes Pos. H & E stained. X400.

hypercholesterolemia indicated that garlic extract decreased systolic blood pressure compared to the control group ($p<0.05$) by Steiner et al. (1996). *A. sativum* active ingredients are documented for its beneficial effects on major cardiovascular risk factors, including blood pressure. Fresh *A. sativum* extract (FAGE) was found to play a key role in wound healing reversing functionally damaged myocardium to regain effective myocardial contractility post-hypertension. Allicin, the active component of *A. sativum*, has been shown to have anti-inflammatory properties and has been used historically by many cultures to heal wounds (Banerjee and Maulik, 2002).

The observations in reversal of fibro-myocardial damage is supported by a publication on animal studies showed *A. sativum* extract increased wound healing process by reversing damage to myocardiocytes. Alhashim and Lombardo (2018) in their study on mechanism of action on topical garlic on wound healing showed that fibroblasts are activated by allicin, leading to more organized and rapid wound repair. There were more proliferating fibroblasts in previously hypertensive myocardial tissues treated with *A. sativum* extract than in other micrographs. FAGE reversal ability to myocardial damage and restoration of myocardial functionality could possibly be utilizing mechanisms used by HCT more than CPT to mediate its actions on cardiac and renal regulation of blood pressure, especially in the reduction of systolic and diastolic blood pressure. Thiazide diuretics: e.g Hydrochlorothiazide

(HCT - microzide) and chlorthalidone sometimes called water pills are medications that act on the kidneys to help the body eliminate sodium and water, reducing blood volume (Al-Qattan et al, 2006). From this group, CGE was noted to have minor reversal effects on the myocardial damage, hence slight blood pressure lowering effect. Angiotensin-converting enzyme (ACE) inhibitors, are medications — such as captopril (capoten - CPT) — help relax blood vessels by blocking the formation of a natural chemical that narrows blood vessels (7). The heart tissues from wistar rats in group T3, treated with CIGE 200mg/Kg body weight for 20 weeks had features of acute endocardial inflammation, infiltration of the endocardium and papillary muscles by white cells. Focal endomyocardial fibrosis and mast cells were noted. The heart exhibited major cardiac left ventricular hypertrophy as shown in Plate 2. Despite slight reduction in SBP and DBP observed in groups treated with CIGE, conclusive inability to reversal myocardial damage was noted, as shown in one study by Drury, 1985 on cardiovascular disorders in the rat heart.

In summary, the data collected supports the following tentative conclusions: histological findings showed that fresh aqueous garlic extract (FAGE) had reversal effects on the myocardial tissue post-hypertension promoting myocardial healing and tissue repair, reinstating effective functional cardiac contractility as compared to T2, T3, Hydrochlorothiazide and Captoten groups. Though hydrochlorothiazide and Captoten are antihypertensives, they did not reverse fibro-myocardial tissue damage post-hypertension, they could possibly be utilizing different physiological mechanism to lower high blood pressure, hence used as antihypertensives. There is more insight towards current management of hypertension in an affordable manner by use of *A. Sativum* through Complementary/Alternative Medicine..

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