

Original Research Article

Comparison of the Effects of Different Concentrations of *Allium Sativum* Extracts on Plasma Cholesterol Levels in Salt-Induced Hypertension in Wistar Rats

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Abstract

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Allium sativum (Garlic) has allicin (allyl 2-propenethiosulfinate or diallyl thiosulfonate) as the principal bioactive compounds and has been reported to exhibit blood pressure lowering effects. The activity may be dependent on the concentration and the age status of the bioactive components. The present study was carried out to evaluate the effects of different concentrations of *A. sativum* extracts on plasma cholesterol levels in hypertensive rats. Forty eight (n = 48) male adult wistar rats (250-300g, 36 weeks old) were induced to hypertension using Deoxycorticosterone Acetate salt (DOCA-salt). Doses of 50, 100 and 200 mg/kg body weight of *A. sativum* were each prepared from these three extracts: fresh aqueous garlic extract (FAGE-T1), crude garlic extract (CGE-T2) and crude industrial garlic extract (CIGE-T3). Normal saline was used for the control group (NS). These were administered intraperitoneally (IP) twice a day for 20 weeks. Approximately 1ml of blood was collected from sterilized lateral ear veins of the 48 wistar rats and plasma cholesterol levels were measured, recorded and analyzed. Findings from haematological assays of total plasma cholesterol levels in peripheral blood showed that 200mg/Kg body weight of FAGE (4.25mmol/L hypocholesterolemic activity of 37.54% reduction) was more potent in breakdown of cholesterol in peripheral blood and promoted hypocholesterolemic mechanisms post-hypertension as compared to groups T2 (3.75mmol/L; 21.36%), T3 (3.59mmol/L; 16.18%) and the control was significant at $p \leq 0.05$.

Keywords: Hypocholesterolemic; Hypertension; Homogenate; Cholesterol, Therapeutics, Haematological

INTRODUCTION

Garlic (*Alliums Sativum L.*) is a cultivated plant. Its wild progenitor *A. Longicupis* originated in the high planes of West-Central Asia. In decades, it has been widely used as food and medicine (David, 2005; McMahon and Escott, 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 (Asdaq and Inamdar, 2011;

Brace, 2002), and it has been the subject of intensive scientific research with over 2000 scientific publications on antibacterial, antitumor, antifungal, hypolipidemic, hypoglycemic, antiatherosclerotic, hematinic and hypertensive activities (Alnaqeeb, 1996; Al-Qattan, 2006; Granzyna *et al.*, 2008; Gardner, 2001; Durak *et al.*, 2004; Zeng, 2013).

Hypertension is a slowly-developing disorder of

middle to old age which predisposes to the cardiovascular disorders (Banerjee and Maulik, 2002) that cause most of the morbidity and mortality in the elderly. 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 to heart attack, stroke, heart failure, aneurysm, or renal failure (Hani, 2012). The normal human blood pressure (BP) is 100-120mmHg (Systolic) and 60-90mmHg (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, 2014).

Cholesterol is a biosynthesized organic compound of the sterol type found in most body tissues. Cholesterol and its derivatives are important constituents of cell membranes and precursors of other steroid compounds, but a high concentration in the blood of low-density lipoprotein (which transports cholesterol to the tissues) is associated with an increased risk of coronary heart disease. Its source includes meat, fish, eggs, butter, cheese, and milk. According to some studies, garlic may decrease blood levels of total cholesterol by a few percentage points, but only in the short term due to presence of fiber. It is documented that taking a fiber supplement help meet daily body fiber intake acts on overall cholesterol level and LDL (bad) cholesterol. Therefore, the present study was carried out to evaluate the effects of different concentrations of *A. sativum* extracts on plasma cholesterol levels in hypertensive rats.

MATERIALS AND METHODS

Study Site and Design

This study was carried out in the Department of Biological Sciences laboratory, University of Eldoret (UoE). The study was conducted using a Laboratory-based Randomized Controlled Experimental design involving a control and treatment groups. It constituted a group of Normotensive (C1) and three groups of Hypertension-Induced Wistar laboratory rats (T1, T2 and T3).

Allium Sativum Preparation and Administration

The processing of various concentrations *Allium sativum* extracts, isolation and determination of concentrations were done at the Department of Chemistry and

Biochemistry, University of Eldoret. Forty eight (48) Wistar laboratory rats were randomly divided into 12 smaller groups dependent on the concentrations of the extracts, with each group having the 3 concentrations of four Wistar rats (n = 4) per group as follows; Group C1: Normotensive rats - treated with normal physiological saline. Group T1: treated with Fresh Aqueous Garlic Extract – FAGE 50, 100 and 200mg/Kg body weight. Group T2: treated with Crude Garlic Extract - CGE 50, 100 and 200mg/Kg body weight. Group T3: treated with Crude Industrial Garlic Extract – CIGE 50, 100 and 200mg/Kg body weight. *Groups T1, T2 and T3 were also Hypertensive.* The tests were conducted between 8:00am and 9:00am in the morning.

Induction of Hypertension and Blood Pressure Measurements

Hypertension was induced in the treatment 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 (Drury, 1985; Mozaffarian *et al.*, 2014). The wistar rats were immobilized using restrainers and blood pressures were measured using the tail-cuff method via a digital Powerlab recorder against the tail artery. A systolic blood pressure of 150mmHg and a diastolic blood pressure of 100mmHg were achieved to ascertain the induction of hypertension. Total body weight and rectal body temperatures were recorded daily to keep tract of the experiment. The experimental groups were treated with 50mg/Kg, 100mg/kg and 200mg/Kg body weight concentrations of *Allium sativum* intraperitoneally using insulin syringe. The study was conducted for a period of 20 weeks (6 weeks of HTN induction and 14 weeks of treatment, observation and analysis).

Blood Sample Collection and Analysis of Plasma Cholesterol

Total blood volume of a rat is approximately 6.0 ml per 100 g body weight. Not more than 10% of the total volume per rat was collected every two weeks. The tails were sterilized using 70% alcohol while the animals were conveniently restrained. Using dissecting blades, a small piece of the tail-end was cut and approximately 1ml of blood drawn into Ethylenediamine tetra-acetic acid (EDTA) treated vials. The blood samples were used to analyze and record plasma cholesterol level changes.

Ethical Issues

All procedures, care and treatment to the animals was in

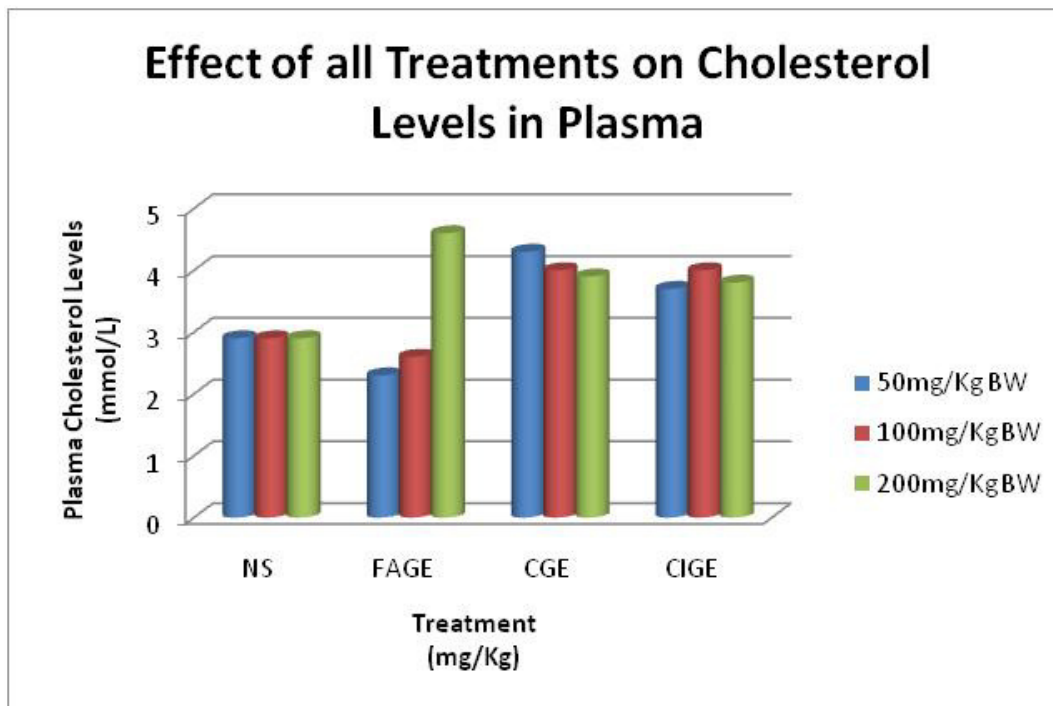


Figure 1. Mean plasma levels of Total Cholesterol following treatments of various concentrations of *Allium sativum* extracts

accordance to the Laboratory Animal Care Committee Guidelines (LACCG), and with the approval of the institution's Board of Postgraduate Studies.

Data collection, Statistical analysis and Presentation

Data was 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. Data was recorded in tables and presented using bar graphs and line graphs. The results were expressed as means \pm SEM and were analyzed using One-Way Analysis of Variance (ANOVA), followed by Turkey's HSD Multiple range test as a post-hoc test for 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

Results in figure 1 on the effect of different concentrations of *Allium sativum* extract treatment on total cholesterol levels in Plasma shows significant difference between groups.

The figure further revealed that there were significant differences in total cholesterol mean levels in Plasma between the control group (NS) and treatment group T2 at CGE 200mg/kg body weight ($\Delta M = -0.809$, $p \leq 0.05$). Mean differences in Plasma total cholesterol levels were however highly significant in treatment group T3 at CIGE 50mg/kg body weight ($\Delta M = -1.209$, $p \leq 0.05$); CIGE 100mg/kg body weight ($\Delta M = -1.209$, $p \leq 0.05$) and CIGE 200mg/kg body weight ($\Delta M = -1.318$, $p \leq 0.05$). The Mean Plasma total cholesterol levels of other treatment groups T1, treatment group T2 at CGE 5mg/Kg body weight and CGE 100mg/Kg body weight were not statistically different from that of the control group.

There is a sharp increase in total Cholesterol from the control group (3.09mmol/L) to the very first treatment concentration of CGE 50mg/Kg body weight (3.75mmol/L), which is 21.36% rise. There is a characteristic steep rise in treatment group T3 at CIGE 100mg/Kg body weight (3.59mmol/L) to 4.25mmol/L at CIGE 50mg/Kg body weight (18.38% rise), with the highest total Cholesterol mean level of 4.50mmol/L at CIGE 200mg/Kg body weight (25.35% rise). Although there were increments, not all were significant compared to the control group.

The comparative bar graph of Mean plot effects of *Allium sativum* extract treatment did not however yield any noticeable trend in Mean Plasma cholesterol levels with respect to subsequent testing as depicted by figures 2.

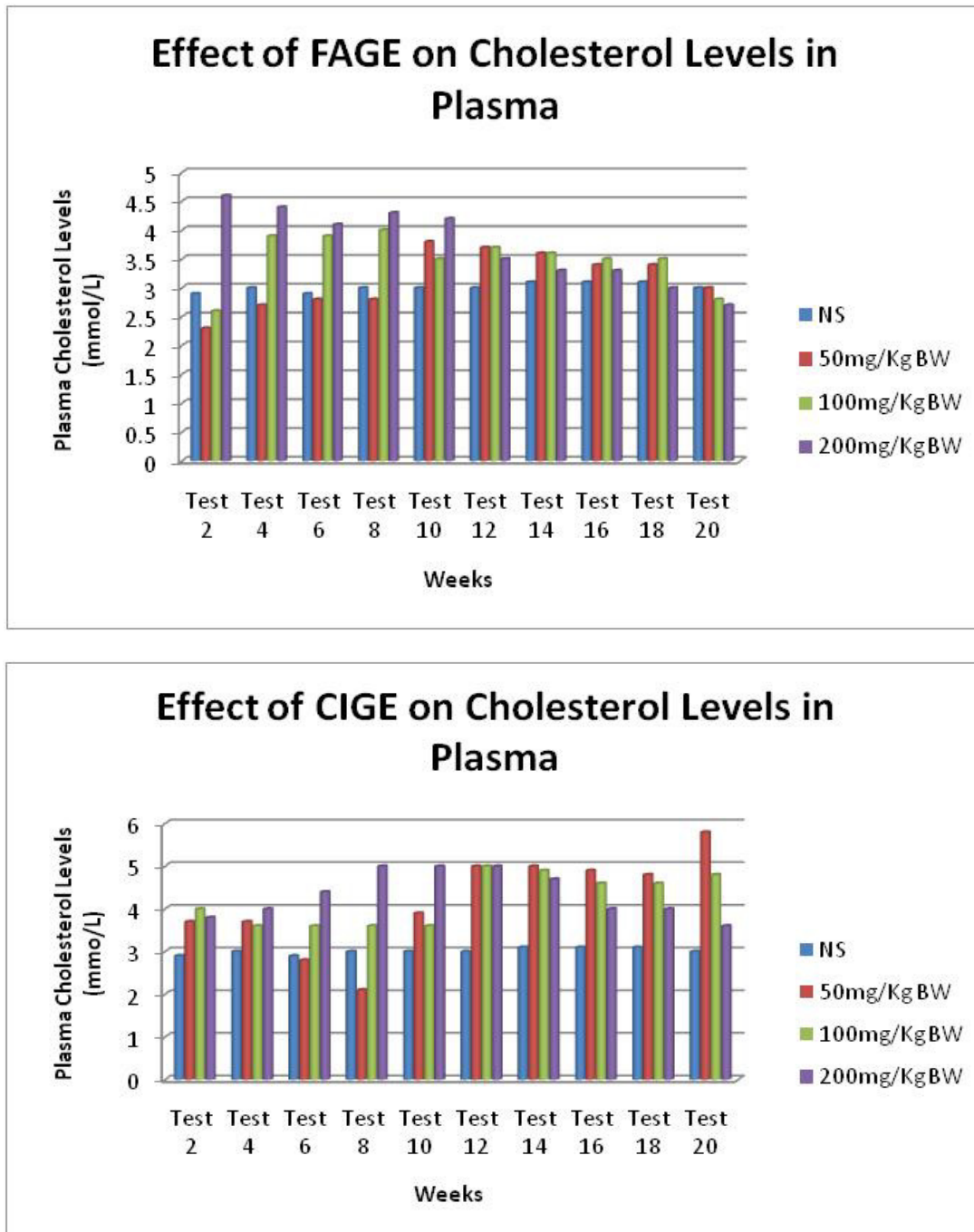


Figure 2. A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of Total Cholesterol for 20 weeks analyzed as 10 tests

DISCUSSION AND CONCLUSION

Figure 1 shows a summary of these findings on the comparison of the effects of different concentrations of *A. Sativum* extracts on total plasma cholesterol levels in peripheral circulation. Out of the three *A. Sativum* extracts, fresh aqueous garlic extracts (FAGE) was found to be more hypocholesterolemic (4.25mmol/L; 37.54%

reduction, $p \leq 0.05$) than crude garlic extracts (CGE) (3.75mmol/L; 21.36% reduction, $p \leq 0.05$) and crude industrial garlic extracts (CIGE) (3.59mmol/L; 16.18% reduction, $p \leq 0.05$). With regards to concentrations, the hypertensive experimental group that was treated with FAGE, particularly at higher concentration of 200mg/Kg body weight proved to be more hypocholesterolemic than experimental groups treated with 50, 100mg/Kg body

weight and compared to the control (NS) that was treated with normal saline post-hypertension.

It was also noted that, as much as CGE and CIGE may contain similar bioactive compounds as FAGE, only after a long period of treatment could they show some hypocholesterolemic activity, in this case from week 10 as shown in figure 2. On the other hand, the hypocholesterolemic activity of FAGE commenced immediately at week 4 and was sustained all the way to the end of the experimental period at week 20. According to Kamanna and Chandrasekhara (1982), garlic administration in rats suffering from hypercholesterolemia, induced by a high-cholesterol diet, significantly reduced serum cholesterol, triglyceride, and LDL, but there was no effect on serum HDL.

This results are in tandem with the data collected in the current study that supports the fact that *A. sativum* is hypocholesterolemic. *A. sativum* extract significantly decreased the mean plasma total cholesterol in the hypertensive group. It is also in agreement with the study on serum levels of cholesterol that suggested garlic is effective in reduction of total serum cholesterol by 17 ± 6 mg/dL and low-density lipoprotein cholesterol by 9 ± 6 mg/dL in subjects with elevated total cholesterol levels (>200 mg/dL). An 8% reduction in total serum cholesterol is of clinical relevance and is associated with a 38% reduction in risk of coronary events at 50 years of age (Ried *et al.*, 2013b). Garlic has been shown to be highly tolerable in all trials and was associated with minimal side effects.

This finding would probably suggest that active constituents of sulphides in *A. sativum* extract oxidatively increased the formation of low-density lipoproteins (LDLs) from the digested fats which do not over-load the heart. LDLs are easily transported within plasma in circulating blood to the rest of the body cells for metabolism and utilization, and hence do not stick and thicken the walls of the myocardium. Several studies have shown that garlic contains active hypocholesterolemic components, known as diallyl disulfide and dipropyl disulfide (Bordia and Bansel, 1973; Bordia *et al.*, 1975; Jain and Vyas, 1975; Jain, 1977). Atherosclerotic features can easily be introduced into the human body by uncontrolled accumulation of fat inside the heart chamber walls and the coronary blood supply system. This study is in agreement with the results of the work conducted by the above research scientists.

It has also been reported that garlic supplements in human subjects lead to the increased resistance of low density lipoprotein to oxidation and may be one of the powerful mechanisms accounting for the antioxidative and anti-atherosclerotic properties of garlic (Munday *et al.*, 1999; Borek, 2001; Lau, 2001). Studies on the effects of short-term supplementation with oily garlic formulation on lipid metabolism (Augusti, 1977; Sodimu *et al.*, 1984) and glucose level (Banerjee *et al.*, 2002) reported hypolipidemic effects. This study is also consistent with a

study by Ou *et al* (2003) on antioxidant status in 70 patients suffering from primary arterial hypertension. The analyzed garlic preparation was found to significantly lower lipid level and the level of lipid peroxidation products in the blood (Ou *et al.*, 2003; Thomson *et al.*, 2006). The results of those studies reported that the garlic preparation may tentatively be used as an adjunct agent in treatment of arterial hypertension because of its hypolipidemic and antioxidant properties (Grazyna *et al.*, 2008). Some reports have indicated that garlic preparations can correct lipid abnormalities and lower blood pressure in patients with hyperlipidemia and arterial hypertension (Durak, *et al.*, 2004; Rahman and Billington, 2000). A study by Warshafsky *et al* (1993) on the effects of garlic on total serum cholesterol – a Meta-Analysis on hypercholesterolemia concluded that garlic showed a significant reduction in total cholesterol levels. The best available evidence suggests that garlic, in an amount approximating one half to one clove per day, decreased total serum cholesterol levels by about 9% in the groups of patients studied.

In conclusion, fresh *A. sativum* extract significantly decreased total cholesterol levels in plasma post-hypertension, as compared to crude garlic extract (CGE) and crude industrial garlic extract (CIGE). The potency of active compounds of garlic is affected by such parameter as shelf-life; hence CIGE (with unknown shelf-life) had minimal hypocholesterolemic effect. Although these are the results, they differ with those of Ried *et al*; which showed that Aged garlic extract was superior to placebo in lowering systolic blood pressure in patients suffering from uncontrolled hypertension. A dosage of 240-960 mg of aged garlic extract containing 0.6-2.4 of S-allylcysteine significantly lowered blood pressure by about 12 mmHg over 12 weeks (Ried *et al.*, 2013a). Antioxidative and anti-atherosclerotic properties could possibly be one of the *A. sativum*'s homeostatic mechanisms employed in the regulation of cholesterol levels in blood, but also by extension regulating blood pressure. Blood pressure medications with addition of *A. sativum*'s active compounds may be used as therapy in the management of hypertension.

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