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Original Research Article

Comparison of Effects of Various *Allium Sativum* Extracts on Plasma Cholesterol Levels in Hypertensive Rats

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Abstract

An agricultural plant *Allium sativum* (Garlic) has been shown to have medicinal properties among them blood pressure lowering effects. It is a cultivated member of the Lillaceae family, along with onions, chives, and shallots. Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfonate) are the principal bioactive compounds present in the aqueous extract of *Allium sativum* or raw *Allium sativum* homogenate.The activity may be dependent on the concentration and the age status of the bioactive components. To analyze the comparative effects of *A. sativum* extracts on plasma cholesterol levels on hypertensive rats. Forty eight (n = 48) male wistar rats (250-300g) and 36 weeks old were induced to hypertension. Doses of 50, 100 and 200 mg/kg body weight of *A. sativum* were prepared as (Fresh aqueous garlic extract (FAGE-T1), crude garlic extract (CGE-T2) and crude industrial garlic extract (CIGE-T3). These were administered intraperitonially (IP) twice a day for 20 weeks. Blood was collected from the lateral ear vein and plasma cholesterol levels were analyzed. Findings from haematological assays of plasma cholesterol levels in peripheral blood showed that FAGE was more potent in breakdown of cholesterol in peripheral blood and promoted lipolytic mechanisms post-hypertension as compared to groups T2 and T3 with the control was significant at p≤0.05. Fresh *Allium Sativum* provides more insight towards alternative therapeutics and management of hypertension.

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

INTRODUCTION

Garlic (*Alliums Sativum L.*) is a cultivated plant. Its wild progenor *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, hypogylcemic, antiatherosclerotic, hematinic and hypertensive activities (Alnaqeeb, 1996; Al-Qattan, 2006; Granzyna *et al.*, 2008; Gardner, 2001; Durak *et al.,* 2004; Zeng, 2013).

Hypertension is usually 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 proportion 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.

MATERIALS AND METHODS

Study Site and Design

This study was carried out in the Department of Biological Sciences, Universiy 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 *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 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). 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 treatment groups were treated with various preparations and concentrations of *Allium sativum* intraperitonially 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 and care was given to the animals in accordance to the University of Eldoret Laboratory Animal Care Committee Guidelines (LACCG), and with the approval by the institution’s Board of Postgraduate Research and Ethics Committee (BPREC).

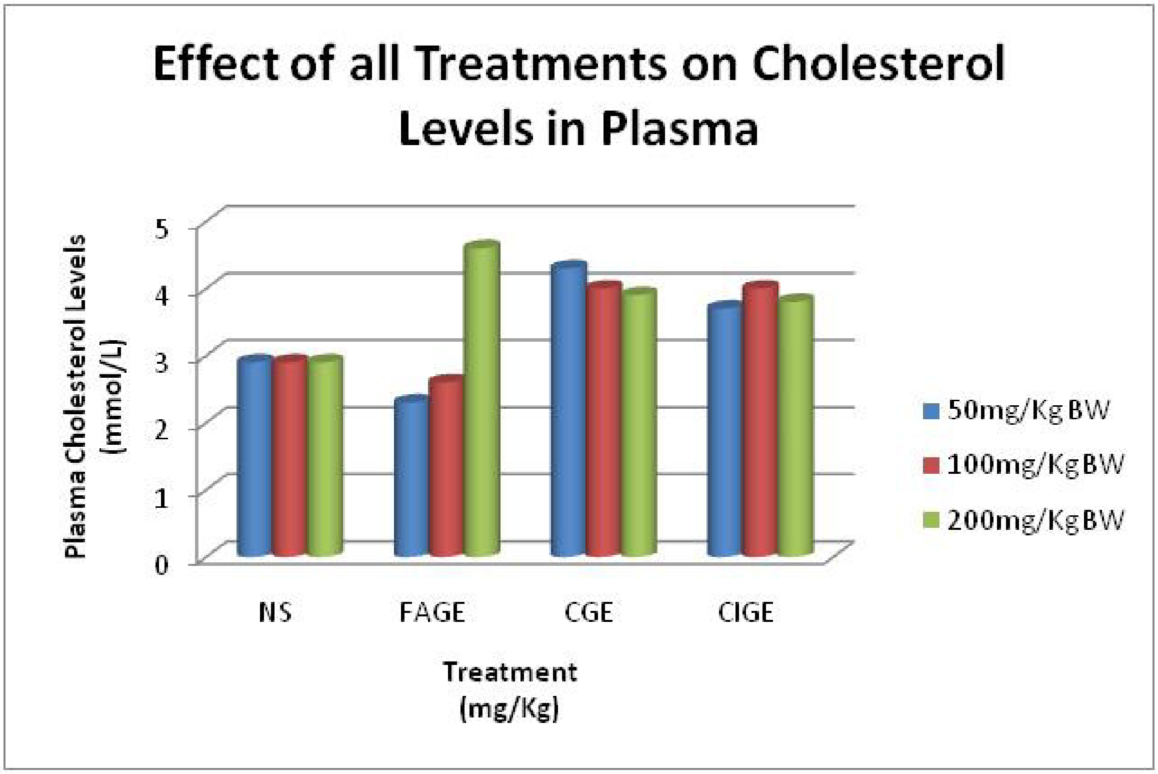

Figure 1. Mean plasma levels of Total Cholesterol following treatments of various concentrations of *Allium sativum* extracts

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-hock 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 *Allium sativum* extract treatment on 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 and treatment group T2 at CGE 200mg/kg body weight (ΔM=-0.809, p≤0.05). Mean differences in Plasma total cholesterol levels were however highly significant in treatment group T3 at CIGE 50mg/kg body weight (ΔM =-1.209, p≤0.05); CIGE 100mg/kg body weight (ΔM = -1.209, p≤0.05) and CIGE 200mg/kg body weight (ΔM = -1.318, p≤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.

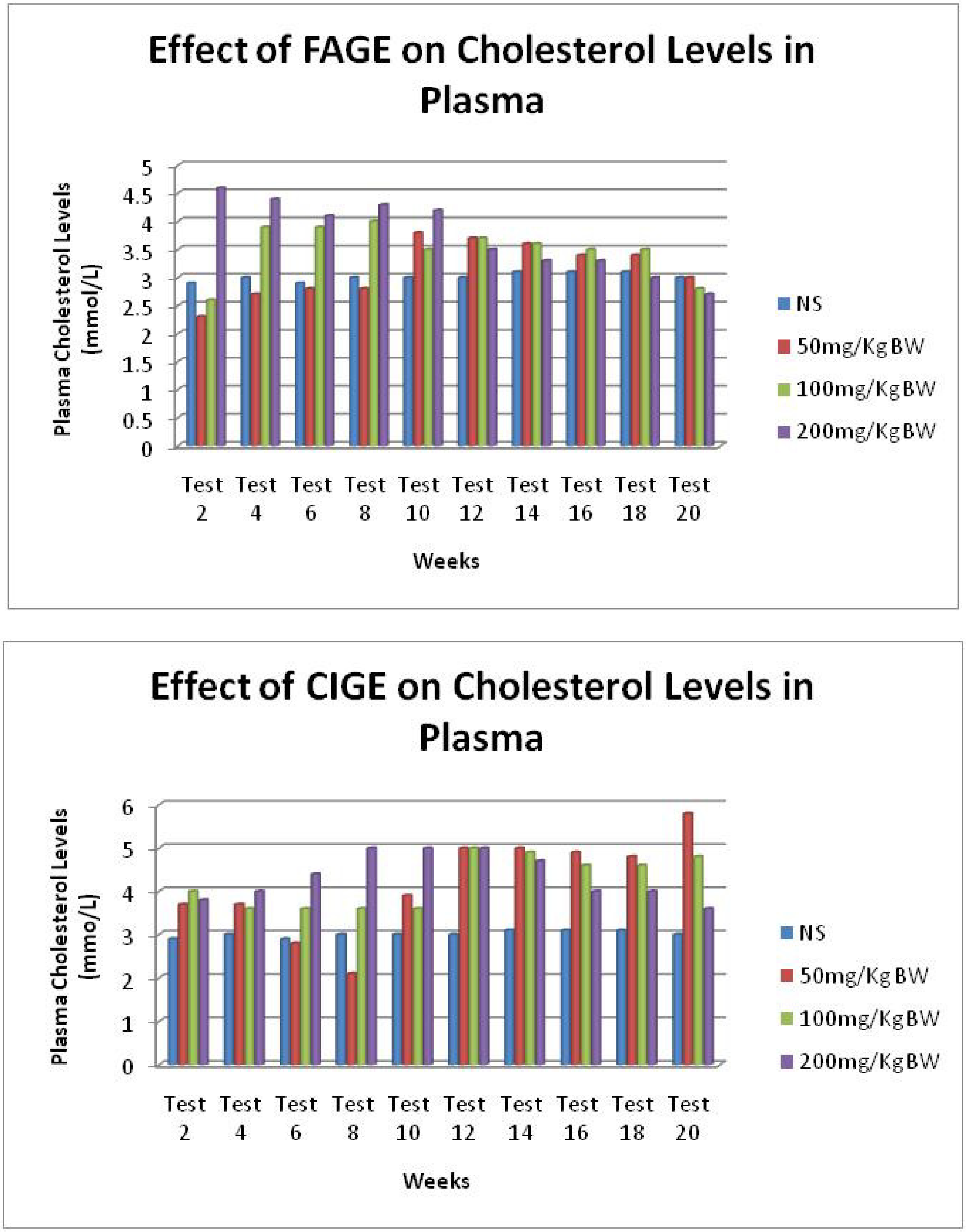

Figure 2. A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of Total Cholesterol for 20 weeks analyzed as 10 tests

DISCUSSION AND CONCLUSION

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 (Kamanna and Chandrasekhara, 1982). This results are similarly supported by the data collected in the current study that supports the fact that *A. sativum* is lipolytic and hypolipidemic. *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 (>200mg/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 meta-analysis study concluded that garlic should be considered as an alternative option with a higher safety profile than conventional cholesterol-lowering medications in patients with slightly elevated cholesterol.

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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