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Therapeutic Impacts of Garlic Varieties on Hyperlipidemia Induced In Male Wistar Rats

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Abstract

Background: Every year, cardiovascular diseases take the lives of 17.7 million people accounting for 31% of all global deaths with atherosclerotic cardiovascular disease being the leading cause of mortality. Interestingly, hyperlipidemia is an important modifiable risk factor contributing to atherosclerosis.

Methods: Qualitative phytochemical screenings was done according to standard protocols. For hyperlipidemia protocol, animals were divided randomly into 4 groups of 6 rats each. Group 1 (normal group) were fed with normal rat feed, Group 2 (control group) fed with a hyperlipidemic diet composed of 75% normal rat feed plus 20% reheated soya bean oil and 5% butter, Group 3 and 4 (Experimental groups) were fed as in Group 2 in addition to the daily administration of white and red garlic juice simultaneously and respectively. The experiment lasted for 6 weeks.

Results: Biochemical Parameters such as Triglycerides, Cholesterol, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) studied, revealed white garlic and red garlic to have significantly increased the serum concentration of HDL ($p<0.05$), and decreased the serum concentration of triglycerides, cholesterol while very significantly reducing LDL concentration ($P<0.01$). These activities may not be unconnected with flavonoids, total polyphenols content (TPC) and sulfur compounds present in the two varieties.

Conclusions: Consequently, the selected nutraceuticals could be useful against hyperlipidemic diet since they could increase the level of serum good cholesterol (HDL) and decrease LDL.

Keywords: Biochemical parameters; Total polyphenol content; Wistar rats; Atherosclerosis.

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Background

Lipids are fats in the blood stream and commonly divided into cholesterol and triglycerides.Hyperlipidemia is a condition when abnormally high levels of lipids i.e. fatty substances are found in the blood. This condition is also known as hypercholesterolemia [1]. Arteries are normally smooth and unobstructed on the inside, when the lipid level is high; a sticky substance called plaque is formed inside the walls of the arteries. This condition leads to the narrowing of the arteries and the consequences are stiffening of the arteries and reduced blood flow [2]. It has been proven that elevated plasma levels of cholesterol and of LDL (Low Density Lipoprotein) are responsible for atherosclerosis in man and epidemiological studies suggests that elevated plasma levels of HDL (High Density Lipoprotein) have a protective effect [3]. Arteriosclerotic Cardiovascular disease develops as a result of these risk factors. It's reported that at least two-thirds of cardiovascular deaths occur in low- and middle-income countries, bringing a double burden of disease to poor and developing world economies [4]. The incidence could be due to increased hyperlipidemic diet commonly consumed in these regions. However, a potential solution could lie in the use of medicinal plants which have served humanity for thousand of years.

WHO (World Health Organization) estimates that about 70 percent of the world's population relies on plants for their primary health care and some 35000 to 70000 species has been used as medicaments a figure corresponding to 14 to 28 % of the 250000 plant species estimated to occur around the world [5-7]. Their chemical and biological diversity represent a potential limitless renewable source for the development of new pharmaceuticals [8]. There is an ever increasing awareness of medicinal plants usage in recent years as result of many years of struggle against illnesses with contemporary science acknowledging their active action and potential. The reason they are today included in modern chemotherapy [9]. Actually, in today's global market more than 50 important drugs are originated from tropical plants and garlic being one of such plants [10]. Garlic (*Allium sativum* L.) belongs to the plant family Liliaceae, which is a genus of 500 species. It has been in use for many centuries, and was recognized for its therapeutic and medicinal value as far back as the era of ancient Egypt [11]. The garlic clove contains various nutrients such as water soluble nutrients which include vitamins, enzymes, amino acids natural sugars and fat soluble nutrients and other functional active components [12]. One of the most important compounds found in garlic is the amino acid, aromatic compound: alliin. When *Allium sativum* is injured, cut or crushed, an enzyme named allinase affects alliin and transforming it to allicin which is the main factor of the strong odour of garlic [13,14]. Epidemiological studies have shown that diets rich in fruits, herbs and spices are associated with a low risk of cardiovascular disease.Garlic is used for many conditions related to the heart and blood system and has been credited to prevent coronary heart disease, heart attack, reduced blood flow due to narrowed arteries, and "hardening of the arteries" (atherosclerosis**)** [14] as well as hyperlipidemia. In clinical studies, taking garlic capsules for 12 weeks, could decrease cholesterol and low density lipoprotein (LDL) significantly in 46 patients with high blood cholesterol [15]. The aim of this study was to determine the therapeutic benefits of two garlic varieties as potential preventive medicine for hyperlipidemia

Methods

This experimental study was carried out in the Medical Research and Applied Biochemistry Laboratory at the Faculty of Health Sciences, University of Buea, Cameroon.

Plant materials and Preparation of garlic juice

The plant materials: Nutraceuticals: *Allium sativum,* Red and White varieties were purchased from the local market in Buea, South West Region, Cameroon, identified and authenticated in the Department of Botany and Plant Physiology, Faculty of Science, University of Buea. They were cleaned from dirt, chopped into small pieces and weighed. For garlic juice, 100g of each: red and white varieties was weighed and blended into 250ml of distilled water separately and macerated for 24 hours. The filtrates were obtained using Whatman paper No.1. and the weight recorded. The slurry produced was stored in a clean container and kept in the refrigerator at 4^oC until further use [16].

Determination of Total Phenolic Content (TPC) and Qualitative Phytochemical Screening of the Garlic juice

The total phenolic content of the garlic juices was determined using the Folin-Ciocalteu colorimetric method as described by **Chlopicka *et al*., [17]**. Test for terpenoids (Salkowski test), Test for alkaloids (Dragendorf's test), Test for tannins (Ferric chloride test), Test for steroids (Lieberman-Burchard test), Test for flavonoids (Cyanidine test), Test for saponins (Frothing test and confirmatory test), test for cardiac glycosides (Keller-Killiani test) and Test for phenolics ($FeCl_3$ test) were used for Qualitative Phytochemical Screening [18-20].

Preparation of hyperlipidemic diet

Palm oil was purchased from the local market in Buea South West Region, Cameroon. The oil was used to fry ripe plantains in a stainless-steel container at 180°C for 20minutes. The hot oil was then allowed 2 hours cooling interval, and a fresh batch of ripe plantains was fried with no addition of fresh oil to replace any loss due to evaporation and absorption. The entire frying process was then repeated 4 times in order to increase its cholesterol content [21]. 20% of weighted heated palm oil was then mixed with 5% butter, and 75% weighted normal rat feed and Pellets were formed and allowed to air dry before feeding to the animals.

Acute toxicity testing

Four groups of three rats each were used for each extract. The rats were given red garlic and white garlic extract through oral route at doses of 1000, 2500 and 5000 mg/kg body weight in each group. The extracts were administered once every day for one week and the rats were observed for death and signs of toxicity after 30mins, 1hr, 24hrs, 48hrs and 1 week after administration [22]. The following parameters were observed, changes in skin, eyes, coma, convulsion, tremor, diarrhea, morbidity, mortality and bodyweight.

Grouping and Treatment of Rats

24 healthy adult male Wistar rats (150–280 g) were obtained from the Animal Resource Unit, Faculty of Science, University of Dschang. The rats were reared in plastic bowl cages at room temperature with 12 hours light and dark cycles. All rats were allowed to access food and tap water *Ad Libitum* [21].

Experimental design

Animals were selected randomly and assigned to 4 groups of 6 each. Group 1 (normal control) rats were fed with a control diet made up of the standard rat diet, group 2 (positive control); rats were fed with a hyperlipidemic diet, group 3 (white garlic treated); rats were fed with hyperlipidemic diet plus white garlic juice and group 4 (red garlic treated); rats were fed with hyperlipidemic diet plus red garlic juice. Garlic juice was administered daily at a dose of 10 $mlkg^{-1}$. [23]. At the end of the treatment period animals were sacrificed under light ether anesthesia after 12 hour fasting period, whole blood was collected by cardiac puncture using a 23 gauge syringe and stored in a dry tube. The blood collected was centrifuged at 1200rpm for 5 minutes and serum was separated from the whole blood and transferred to an Eppendorf tube and used for the estimation of total cholesterol, and triglycerides by adding 10 µL of rat's serum to 100 µl of working reagent and incubated for 5 minutes, then mixed and measured using a semi-automated spectrophotometer within 2 hours of sacrificing the animals. High density lipoprotein was estimated by using the precipitation properties of LDL and VLDL in buffered solution and quantification of the supernatant. LDL was estimated by using Friedewald formula:

$$LDL\text{-}C_{(mg/dl)} = TC - [HDL\text{-}C + (TG/5)]$$

Reagent kits

Total cholesterol (TC), triglyceride (Trig), high density lipoprotein cholesterol (HDL-C) reagent assay kits (CHON-LAB) were obtained from Biopharcam ltd, Buea, Cameroon. The lipid profile parameters were determined using the manufacturer's procedure. The concentration of low density lipoprotein (LDL-C) was calculated using the Friedewald formula, LDL-C=TC-(HDL-C+TG/5).

Data Analysis

The results were statistically analyzed using one way analysis of variance (ANOVA). The means were separated by the Duncan multiple test using SPSS. Statistical comparison of the serum lipid between different groups of rats were carried out through unpaired 't'- test for significance difference. The values were considered significant at ($p<0.05$) and very significant at ($p<0.01$).

Results

The results of phytochemical screening of the aqueous extract of the two varieties of garlic are represented on Table 1. The two garlic types tested showed the presence of flavonoids, saponins, tannins, cardiac glycosides, phenols, terpenoids in all extracts and the absence of alkaloids and steroids in both varieties. However, the quantity of saponins and cardiac glycosides seem to be higher in the red varieties Table 1.

Acute toxicity testing showed both red garlic and white garlic to have no toxic effect on the rats after dosing the rats with 1000 mg/kg, 2500 mg/kg and 5000 mg/kg. Rats were active, mobile and looked healthy Table 2. Death of experimental animals did not occur and therefore LD50 of the two garlic types could not be calculated.

The use of the upper dose of 5000 mg/kg body weight in daily extract administration was considered because garlic is not known to have any major effects on the consumers [22 -24]. After 6 weeks of feeding rats with hyperlipidemic diet, there was an increase in total body weight of animals in all groups through the study period as shown in Tables 3 and 4. Body weight increased in all groups with the control group increasing least and the red garlic group increasing most.

The biochemical parameters studied Table 5 revealed a decrease in the levels of TG in the treated groups, 187 and 200 mg/dl in red and white garlic respectively. While the control group 2 showed 266 mg/dl. For LDL, the treated groups revealed levels of 124 and 115 mg/dl for red and white garlic respectively as against 239 mg/dl in the treated group 2. The same trend was observed for cholesterol (CH). However, an inversed tendency was observed for High Density Lipoprotein (HDL). From 60 mg/dl in the treated group 2 up to 97, and 89 mg/dl (Red and White Garlic respectively).

Table 1. Total Phenolic Content (TPC) and phytochemical screening of *Allium sativum* (red and white garlic) aqueous extract.

Plants Phytochemicals	*Allium sativum* (Red garlic)	*Allium sativum* (white garlic)
Steroids	_	_
Terpenoids	+	+
Flavonoids	+	+
Saponins	++	+
Phenols	+	+
Cardiac glycosides	+++	++
Tannins	++	++
Alkaloids	_	_
TPC (mg GAE/100g))	25.16±0.64	29.05± 1.63

+ Present (the number of + indicates the extend of reaction). - Absent

Table 2. Acute toxicity effect of red garlic extract at different doses on weight and rat behavior.

Group (n=3)	Daily-Extract mg/kg	Initial-body weight day 1	Final-body weight day 2	Observation
Group A	1000	752	775	Strong, active, healthy.
Group B	2500	760	783	Strong, active, healthy.
Group C	5000	766	790	Strong, active, health

Table 3. Acute toxicity of white garlic extract at different doses on weight and rat behavior.

Group (n=3)	Daily-Extract mg/kg	Initial-body weight day 1	Final-body weight day 2	Observation
Group A	1000	752	775	Strong, active, healthy.
Group B	2500	760	783	Strong, active, healthy.
Group C	5000	766	790	Strong, active, health

Table 4. Change in body weight

Group	Initial body weight(g)	Final body weight (g)	Weight gained (g)
Group 1 (normal)	1306	1574	268
Group 2(control)	1417	1602	185
Group 3 (red garlic)	1554	1885	331
Group4 (white garlic)	1186	1420	234

Table 5. Concentration of lipid profile parameters in all rat groups

	Serum lipid profile of rats			
Group	**Triglyceride mg/dl**	**Cholesterol mg/dl**	**HDL mg/dl**	**LDL mg/dl**
Group 1 (normal)	239.10 ± 26.85	248.37 ± 54.34*	60.22 ± 20.19	107.56 ± 32.09**
Group 2 (control)	266.45 ± 21.62	360.82 ± 38.66	60.95 ± 21.56	239.51 ± 35.36
Group 3 (red garlic)	187.05 ± 23.02 *	254.15 ± 40.47 *	97.82 ± 11.01 *	124.20 ± 32.78**
Group 4 (white garlic)	200.43 ± 25.01*	240.22 ± 44.99 *	89.67 ± 7.76 *	115.66 ± 39.45**

* Significant (p<0.05) ** very significant (P<0.01) compared to group 2

Discussion

Phytochemical (qualitative) analysis of the major bioactive constituents of *Allium sativum* (white and red garlic), aqueous extracts was investigated Table 1. The results showed the presence of flavonoids, saponins, tannins, cardiac glycosides, phenols, terpenoids in all extracts and the absence of alkaloids and steroids in the two varieties of garlic, these results are important for the expression of activities. Literature shows that garlic contains 33 organosulfur compounds (OSC), 17 amino acids (including all essential amino acids), minerals such as phosphorus, calcium, iron, potassium, magnesium, selenium, zinc, and vitamins A, B, C, and E [25]. Many of these components may be responsible for the activities attributed to garlic in selectively influencing the lipid profile parameters.

TPC in white garlic was higher than that in red. Phenolic compounds are the major groups, contributing to the antioxidant activity of vegetables, fruit, cereals and other plant-based materials, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [26,27]. Variability of TPC of different varieties or cultivars could be attributed to various cultivar characteristics. This is in agreement with previous reports of Chen [28] although Sterling and Eagling [29] reported that the variety and origin of garlic were not important factors affecting the agriculture traits of this crop.

For acute toxicity testing of the extracts, different concentrations of the extracts were administered to animals and observed for a period of 1 week for any changes in the following: skin, eyes, sleep, coma, convulsion, tremor, diarrhea, morbidity and mortality (Table 3). After dosing the rats with 1000 mg/kg, 2500 mg/kg and 5000 mg/kg both Garlic varieties were found to have no toxic effect on the rats. The use of the upper dose of 5000 mg/kg body weight in daily extract administration was considered because garlic is not known to have any major toxic effects on the consumers [30-31]. Thus, garlic with its enormous documented medicinal potential can be used as a lifelong therapy or consumed daily and will pose little or no side effects. Natural products throughout history have been reported to be devoid of any side effect and are consumed daily in our society [30].

To study hyperlipidemia, six (6) weeks were enough to induce hyperlipidemia in control group of rats [23]. After 6 weeks of feeding the rats, the weight gain was least among group 2 fed with hyperlipidemic diet, while group 3 rats fed with hyperlipidemic diet plus red garlic treatment showed the most weight gain (Table 4). Our results are similar to those of *Adejoke et al*., who reported a significant increase in weight gain in the garlic-treated group fed with hypercholesterolemic diet [31]. Garlic is said to alter hormones associated with protein anabolism by increasing testicular testosterone. When testosterone levels are higher, more protein synthesis occurs. As a result, more muscle tissue and strength is developed leading to increase in body weight [31].

While studying the serum lipid profile, there was a significant increase in the mean serum lipid concentration of cholesterol, triglyceride and a very significant increase in LDL, no significant decrease in HDL in the control rats (group 2), fed with hyperlipidemic diet compared to those of normal rats (group 1) fed with a normal rat diet (Table 5). The very significant ($p < 0.01$) increase in the mean serum concentration of LDL and significant ($p < 0.05$) increase in cholesterol and triglycerides of the control rats, were similar to the results reported by Mostapha *et al*. [31] whose hyperlipidemic diets contained basal laboratory diet + 6% coconut oil +1% cholesterol for 8 weeks and hyper cholesterolemic diet (HPC) enriched with 25% soy bean oil and 1% cholesterol respectively [23]. However, the change in HDL cholesterol serum concentration was not significant ($p>0.05$). This is not in accordance with the findings of *Ahmed et al* and *El Gamal et al.,* [23, 24] works. This could be as a result of increase HDL precursor synthesis by the liver and small intestine in order to eliminate the increased cholesterol and LDL in the blood [24]. The observed increase of cholesterol, triglycerides and LDL, in the control group (group 2) shows the potential risk involved with poor feeding habits as the hyperlipidemic diet which in the present study was prepared using a mixture of reheated soya bean oil, and butter which are fat -food commonly consumed in our society. Increase concentration of LDL in the intima of blood vessels elicits an immune response which drives the pathogenic evolution of arteriosclerosis by releasing pro-inflammatory mediators leading to a chronic inflammatory reaction thereby oxidizing the LDL. The oxidized LDL induces the formation of foam cells and fatty streaks in the vessel wall which is the hallmark of the initiation of arteriosclerosis plaque formation [24,31]. Comparing the mean serum lipid parameters concentration in groups 3 and 4 with the control group 2, the result showed red and white garlic to have a significant effect on reducing triglycerides and cholesterol ($p < 0.05$) while increasing HDL. The significant increased in HDL observed was similar to the findings Mostapha *et al.* [23] who reported garlic to be very significant ($p < 0.01$) in reducing triglycerides, cholesterol, LDL and increasing HDL. The finding was also in line with that of Thomson et al. [32] who found significant decrease in total cholesterol and Triglycerides mean serum concentration in diabetic rats treated with garlic [32].

The difference between the two types of garlic used in this study was also determined and no significant difference was observed between the red and the white garlic in respect to the Phytochemical screenings. In general the results showed both garlic varieties to have hypolipidemic activities with its most effect seen in reducing serum LDL. Garlic is known to have antioxidant and hypolipidemic properties that are attributed to its organosulfur compounds allicin, which has been reported to be one of the most active ingredient of garlic [33]. Allicin is the main biologically active component of freshly crushed garlic (*Allium sativum*) cloves and it is produced by the interaction of the non-protein amino acid alliin with the enzyme alliinase. It's been well documented that, aged garlic extract (AGE) inhibits lipid peroxidation, reducing ischemic or reperfusion damage and inhibiting oxidative modification of LDL, thus protecting endothelial cells from the injury by the oxidized molecules, which contributes to atherosclerosis [34]. Another suggestion of the mechanism by which garlic reduces cholesterol is that garlic blocks HMG-CoA reductase, a key enzyme, which controls the rate of cholesterol synthesis in the liver [35]. From this study consumption of garlic could be beneficial in selectively influencing the lipid profile development, since it is associated with reduced macrophage-mediated oxidation of LDL.

Conclusions

In the present study, we found out that the selected nutraceuticals could act against hyperlipidemic diet common in our society, by decreasing the level of serum bad cholesterol (LDL) and increase the level of good cholesterol (HDL). The nature of the phyto-constituents and the quantity of the total polyphenols (TPN) with their inherent antioxidant potential are the culprits for such beneficial effects. However, it does not preclude more in-depth studiesby pharmaceutical companies for right formulation and adequate dosage.

Abbreviations

AGE = Aged Garlic Extract
HDL = High Density Lipoprotein
HMG-Co-A = 3-Hydroxy-3-Methyl-Glutaryl-Co -Enzyme A Reductase
LDL = Low Density Lipoprotein
TC = Total Cholesterol
TPC = Total Phenolic Contents
WHO = World Health Organization

Authors' Contribution

René NIA: The conception, design and supervision of the research work; Abia S. Njang: The supervisee: brought the idea and Carried out the work under directives; Ufuan A. ACHIDI: Supervision of the work; Bernard TIENCHEU: Contributed the quantitative part of the work and re-lecture; Germain S. Taiwe: Contributed in the animal experimentation part of the work and re-lecture

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Conflict of interest

The authors declare that they have no competing interests

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