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Research Article

Effect of Three Forms of *Allium Sativum* Bioactive Compounds on Blood of Adult Wistar Albino Rats

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Abstract

The medicinal properties of *Allium sativum* have been known since the ancient times. Among its effects is a beneficial action on the development of atherosclerosis, hyperlipidemia, cholesterolemia, and hypertensive experimental paradigms both *in vivo* and *in vitro*. The present work compared the beneficial effects of three forms of garlic; raw Allium sativum (functional food), Allium sativum extract (nutraceutical), and synthetic *Allium sativum* (pharmaceutical) on lipoproteins and haematology of blood plasma. The study was carried out by feeding adult Wistar albino rats. Fourty adult Wistar albino rats divided into four groups of ten rats each. Group 1 was fed with functional food *Allium sativum*, group 2 with a nutraceutical *Allium sativum*, group 3 with nutriceutical *Allium sativum* and group 4 the control for six weeks.

The blood sample was collected by cervical decapitation into plain test tube and EDTA test tube using a 2ml syringe 24h after the last feed, and then lipid profile and haematological parameters were analyzed. The results showed that functional food group 1 garlic significantly ($p<0.05$) increased the RBC, haemoglobin concentration and platelet counts and decrease in the WBC compared to the group treated with nutraceutical group 2 and nutriceutical groups 3. The functional food group 1 administration produced a significant ($p<0.05$) decreased in CHOL, TG and LDL with a significant increase in HDL level compared to group 2 and 3. Thus, the the functional food group 1 garlic improved the lipid profile and hematological activities of the adult wistar rats.

Keywords: *Allium sativum*; Nutraceuticals; Nutriceuticals; Functional foods

Introduction

A member of the Liliaceae family, garlic (*Allium sativum*) is a cultivated food highly regarded throughout the world. Garlic has been reputed to possess medicinal and therapeutic effects originating from Central and Western Asia [1,2]. The bulbs (cloves) had been used to cure illnesses in ancient Egypt [3]. Garlic is one of the earliest of cultivated plants and the most widely investigated medicinal plant [4]. The Ebers Codex and Egyptian medical papyrus dating to about 1550BCE mentioned garlic as an effective remedy for variety of ailments [1,5]. Early men of medicine such as The Great Hippocrates, Aristotle and Pliny espoused a number of therapeutic uses for this plant [6]. Today it is commonly used in many cultures as a seasoning or spice [5]. According to the US Food and Drug Administration survey of 900 people, garlic is the second most utilized supplement (behind Echinacea), with almost 17% of the population using garlic supplement in the preceding 12 months [7]. High percentage of garlic eaten today comes from Republic of China, Republic of South Korea, India, Spain, and the United States of America [5].

In addition to its reputation as a healthy food, garlic has shown wide spectrum of pharmacological activities including antimicrobial and antioxidant capacities [2,8-12]. Additionally, anti-atherosclerotic and anti-cancer properties have also been demonstrated [13]. Garlic contain a rich oganosulfur compounds such as allicin, diallyl sulfide and diallyl trisulfide which are medicinally active and plays a vital role in therapeutic activity [14-16]. Recent studies from Korea have further elucidated novel sulfur containing nitrogenous compounds responsible for the greening process of crushed or bruised garlic [17]. These compounds are not released when the garlic is finely peeled and have been found to differ significantly from other green plant pigments [17]. It is clear that even with a plant medicine as well characterized as garlic, there is still much to be learned [18]. Garlic also reduces cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA.

Garlic has been shown to inhibit LDL oxidation, arterial plaque formation, platelet aggregation, decrease homocysteine, lower blood pressure, and increase microcirculation [19,20], which is important in diabetes, where microvascular changes increase heart disease and dementia risks [21]. Garlic may also help prevent cognitive decline by protecting neurons from neurotoxicity and neuronal death, hence preventing ischemia-or reperfusion-related neuronal death and by improving learning and memory retention [22]. Garlic may also possess anti-inflammatory abilities to suppress

the nuclear factor-kappa B activation pathway [23]. Gorenstein and collegues 2006 reported that consumption alone can decrease serum lipids and may be effective in normal cases, but not to be relied on as the main therapeutic agent for hyperlipidemia. Other recent animal work also corroborates the beneficial effect of using boiled or raw garlic, the forms most often used most commonly.

Garlic nutraceutical supplements are very expensive especially for developing countries like Nigeria, irrespective of being expensive but play a vital role in our lipid profile and hematological parameters. Thus, functional foods also play the same role and they are cheap, accessible and always available. Despite the medicinal importance of nutraceuticals (ever world garlic), nutriceuticals (synthetic garlic), functional foods (natural garlic) which are rich in allicin that plays an antioxidant role in the body and also nutraceuticals are very expensive supplement for a common man especially in the developing countries like Nigeria. Whereas most functional foods functional foods are indigenous, wide spread, affordable and can be reach at most seasons of the year. This study intends to determine the effect on antioxidant supplements on blood following nutraceuticals, nutriceuticals and functional foods thereby creating awareness of their antioxidant supplement that can positively influence lipid profile, some hematological parameters, serum malodialdehyde level and plasma nitric oxide concentration.

Materials and Methods

Procurement of garlic products and extract preparation

Bulbs of *Allium sativum* (Garlic) used for this work was purchased from Keffi Market, Nassarawa State, Nigeria, Nuticeutical garlic was purchased from a Pharmacy in Keffi, Nigeria and Nutraceutical-garlic was purchased from Green World Industry, Abuja, Nigeria. This raw garlic was removed from their cloves and was ground into juice every day before it was administered to the animals in that group.

Experimental animals

The study was carried out using 40 adult albino rats which were all male and was obtained from Bingham University, Nasarawa State. They were housed in a clean wooden cage at the animal house of Zoology Department, Nasarawa State University. It was fed with grower's mash (vital feed) and water.

Animal treatment

The animals were randomly allotted into four (4) groups of ten animals each; group 4 (Control) and Group 1 (raw garlic), Group 2 (Nutraceutical garlic) and Group 3 (Nutriceutical garlic). The rats were acclimatized for one week before commencing the dietary supplement to various groups 1, 2 and 3 to observe their effect on the lipid profile, hematological parameters, malondialdehyde, and plasma nitric oxide level in the rats. The fourth group severed as control, group 1 consists of ten rats housed in wooden cage and fed with natural garlic juice, vital mash and clean water. Group 2 consist of ten rats housed in wooden cage and fed with nutraceutical-Garlic, vital mash and clean water. Group 3 consist of ten rats housed in wooden cage and fed with nutriceutical-Garlic, vital mash and clean water. Group 4 consist of ten rats housed in wooden cage and fed with vital mash and clean water. The experimental period (feeding period) lasted for 12 weeks.

Animal sacrifice

Twenty-four hours after the administration of the last dose of dietary supplements on test groups and control respectively, the animals were sacrificed. Incision was made into the animal's cervical region with the aid of a sterile blade. Blood sample was collected by cervical decapitation into sterile bottles and lithium hyperinized tube using a 2ml syringe.

Biological essays

Serum was collected by centrifuging the collected blood in a bench centrifuge for 10 minutes, the serum was then used to run lipid profile and malondialdehyde test. The plasma was used to analyze plasma nitrite and nitrate concentration. The blood sample collected in sterile bottle was used to run some haematological test which includes platelet, haemoglobin, red blood cell and white blood cell. Serum lipoproteins were analysed using reflotron automated machine (model RB232 products of Roche Diagnostic GMbH, Sandhofer-Strasse, Mannhein, Germany) and reagent stripes. Total cholesterol was determined with lithium hyperinized serum on Reflotron system method described by CHOP-PAP NCEP, 2001. Triglycerides concentrations were determined with reflotron in Lithium hyperinized based on GPO-PAD, 2001 method. Low density lipoprotein cholesterol (LDL-C) was calculated in a program integrated in the reflotron system photometers by caluculation based on Friedewald 1972. The equation. LLC=Total cholesterol-(Triglyceride/2.2–HDL) mmol/L.

Reference values were taken from High density lipoprotein cholesterol was determined with reflotron based on homogenious HDLc plus NCEP, 2001 method. Serum malondialdehyde were determined using the method described by Wsowicz et al. [21]. Malondialdehyde was reacted with thiobarbituric acid by incubating for 1 hour at 100 °C. The reaction fluorescence intensity was measured in the n-butanol phase with a flouresence spectrophotometer (Hitachi, model F-4010) excitation at 525nm, emission at 547nm were expressed as µmol/L. Plasma nitrite plus nitrate concentrations as an index of plasma nitric oxide levels were determined by the method described by Cortas and Wakid. Quantification of nitrite and nitrate was based on the the Greiss reaction, in which a chromophore with a strong absorbance at 540nm is formed by reaction of nitrite with a mixture of naphtyl-ethylenediamine and sulphanilamide. The absorbance was read using a spectrophotometer (ultraspec plus, pharmacia LKB Biochrom Ltd, Cambridge, UK) to give the nitrite concentration.

For Nitrate detection the sample was treated with copporised cadmium in the glycerine buffer at pH 9.7 to reduce nitrate to nitrite,

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the concentration which represented the total nitrite plus nitrate. A standard curve was established with a set of serial dilutions (10^{-4}, 10^{-3}mol/L) of sodium nitrite. All samples were assayed in triplicate and result expressed in µmol/L. Haematological parameters were analysed using Abachus reagents kit and Abachus Junior 5 automated analyser (model HESC544 Ameisgasse Vienna, Austria and product of Diatron Messtechnik). White blood cells (WBC), Red blood cells (RBC) and blood platelets (blood Plt) were analysed based on operating principle of impedence method commonly known as Coulter metnod [24]. It counted and sized cells by detecting and measuring changes in electrical impedence when the particles in a conductive liquid passed through a small aperture that caused some changes in impedence of the conductive blood cell suspension. While haemoglobin (HB) measurement is that lysed 1:196 sample dilution is measured by a cyanmethemoglobin method [25]. The reagents lysed the red blood cells, which released haemoglobin.

Statistical Analyses

The data collected was statistically analyzed using SPSS for Windows version 17.0. Test of significance was carried out using ANOVA to check if garlic had any effect on lipid profile and haematological parameters and also check if there is any significant. A p-value of <0.05 was considered statistically significant.

Table 1: Effect of three forms of garlic on lipids and lipoproteins profile (mmol/L).

Group	Cholesterol	Triglyceride	Low Density Lipoprotein	High Density Lipoprotein
1	5.07±0.06	1.32±0.06	3.00±0.04	1.93-0.02
2	6.43±0.07	2.07±0.02	3.52±0.06	1.50-0.05
3	7.22±0.03	2.68-±0.05	3.01±0.05	1.10-0.04
4	8.55±0.04	2.97±0.04	3.63±0.07	0.90-0.02

Result

Effect of three garlic forms on red blood cells (x103cells/L (RBC)

A significant increase in catalytic activities of erythrocytes were found in the treatment group (p>0.05) (group 1 that is raw garlic, group 2 ever world garlic and group 3 synthetic garlic). When compared with healthy subjects (control group) (p>0.05). However, the significant increase was observed to be high in animals treated with raw garlic (group 1) (Table 1-3). The haemoglobin concentration of albino rats were increased in group 1 (fed with functional foods) and group 2 (fed with nutraceuticals) when compared with group 3 (fed with nutriceuticals) and group 4 (control group) the expected 3 (p<0.05) considered significant.

Table 2: Effect of three forms of garlic on Malondialdehyde and Nitrite plus nitrate concentrations (µmol/L).

Group	Malondialdehyde Concentration	Nitrite Plus Nitrate Concentration
1	1.01	40.22
2	1.75	31.06
3	2.03	20.47
4	2.89	18

Table 3: Effect of three forms of garlic on haematological parameter.

Group	RBC(×10^6cells/L)	WBC(×10^3cells/L)	Blood plts(×10^3cells/L)	HB(g/dL)
1	05.82±0.04	06.12±0.05	360.02±0.04	12.00±0.07
2	05.36±0.07	09.73±0.06	328.64±0.08	09.23-±0.04
3	05.45±0.03	08.47±0.02	341.39-±0.07	10.18±0.06
4	04.81±0.06	11.08±0.03	333.45±0.02	08.91±0.02

The platelet count increases significantly (p<0.05) in group 1 (fed with functional foods) when compared with group 2 (fed with nutraceuticals), group 3 (fed with nutriceuticals) and group 4 (control group). Variation among column mean was significantly greater than the expected by chance. The mean values of the platelet count increased in all treated groups significantly, that is group 1(fed with functional foods) when compared with group 2 (fed with nutraceuticals), group 3 (fed with nutriceuticals) and group 4 (control group).

White blood cell level in the group 1 (fed with functional foods) was significantly lower (p<0.05) compared to that of group 4 (control) and the mean was significantly (p=0.05) different compared to group 2 (fed with nutraceuticals), and group 3 (fed with nutriceuticals). Group 1 (fed with functional food) has lower concentration of Cholesterol followed by group 2 (fed with nutraceutical) and group 3 (fed with nutriceutical), the group 4 (control group) has the highest concentration of Cholesterol.

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Effects of the Three Garlic varieties on Triglyceride

Levels of the triglyceride of albino rats were increased in group 1 (fed with functional foods) and group 2 (fed with nutraceuticals) when compared with group 3 (fed with nutriceuticals) and group 4 (control group). Group 1 (fed with functional food) has higher concentration of High-density Lipoprotein (HDL) followed by group 2 (fed with nutraceutical) and group 3 (fed with nutriceutical), the group 4 (control group) has the lowest concentration of High-Density Lipoprotein. Group 1 (fed with functional food) has lower concentration of Low Density Lipoproteins followed by group 2 (fed with nutraceutical) and group 3 (fed with nutriceutical), the group 4 (control group) has the highest concentration of Low Density Lipoproteins.

Discussion

The medicinal properties of garlic have been known since the ancient Egyptian era, ancient Israel and the Romans [26-30]. Among its effects is a beneficial action on the development of atherosclerosis, hyperlipidemic, cholesterolemic, hypertensive, thrombotic and immunomodulatory experimental paradigms both *in vivo* and *in vitro* [31-38]. In the present work, we investigated the possible beneficial effects of three varieties of garlic (natural, ever world and synthetic) on the changes the plasma level of Red Blood Cell (RBC), Heamaglobin concentration (Hb), Platelet Count, White Blood Cell (WBC), Cholesterol, Triglyceride (TG), High Density Lipoprotein (HDL) and Low-density Lipoprotein (LDL) in Wistar albino rats. The result obtained in this study showed that functional foods (raw garlic), nutraceuticals (ever world garlic) and nutriceuticals (synthetic garlic) positively influences the lipid profile (Chol, TG, HDL and LDL) and hematological parameters (RBC, Hb, Platelet Count and WBC). However, a greatest effect was achieved in group fed with raw/natural garlic, followed by group fed ever world garlic and then group fed with synthetic garlic (see animal treatment at Materials and Methods section).

The assessment of haematological parameters could reveal the deleterious effect of foreign compounds such as toxins, drugs, chemicals and plant extracts on the blood constituent of animals [39]. It has also been used to determine the possible changes in the levels of biomolecules such as metabolites, haematology and histology of organs [40]. Present work showed that daily administration of animals with various doses of garlic significantly decreased the red blood cell level in group 4 (control) was shown to be lower compared to any other groups (1, 2, 3) fed with garlic. This might be due to oxidation properties of allicin which is capable of converting red pigment haemoglobin in the red blood cells to methaemoglobin which irreversibly cannot carry oxygen to the organs and tissues. Cases of anaemia have been seen from excessive consumption of some raw garlic preparation containing allicin and degraded compounds [41]. However, in this work, varieties of garlic used significantly increased haemoglobin concentration of albino rats were increased rats fed with functional foods and nutraceuticals and decreased the level of haemoglobin in rats fed with nutriceuticals and the control group.

This showed that raw/natural garlic (functional food) and nutraceutical form of garlic are not toxic to red blood cells. This report is in disagreement with the work of [42,43]. Also, in this study, doses of garlic varieties significantly increase the platelet count in group fed with functional foods compared to rats fed with nutraceuticals, nutriceuticals and the control group. However, *in vivo* bioavailable compounds may produce the antiplatelet effect because allicin does not adequately get into blood circulation [44]. There was a significant decreased in the white blood cell level in the group fed with functional foods compared to that of control and the two groups fed with nutraceuticals and nutriceuticals. This therefore suggests that after feeding with the supplement, the leukocytes decreased. Allicin led to a lower number of white blood cells compared to rats in the controls but elicited increased phagocytic activity.

The lipid profile of the three varieties of garlic was clearly demonstrated in the present study. These parameters were also positively influenced by the garlic supplementation compared with the control. The assessment of lipid profile in the present study showed that natural garlic is more capable of reducing the plasma lipid levels such as cholesterol rats than those fed with nutraceutical, nutriceutical and the control. This work is in agreement with previous works [45]. However, triglyceride showed opposite effect on rats fed with natural garlic and nutraceuticals. This high effect observed in natural garlic is in disagreement with the work of Aka et al. [14] but lowered in rats fed with nutriceuticals and the controls. The concentration of HDL in rats treated with natural garlic (functional food) is higher followed by nutraceutical and nutriceutical treated rats. However, the control group has the lowest concentration. Probably this result work in concur with other scientist like Slowing et al. [38] states that garlic is known for its pharmacologic and nutritional properties, garlic elicited a reduction in plasma levels of lipids by inhibiting hepatic cholesterol synthesis.

This form of cholesterol is commonly known as good cholesterol which helps in reducing the serum cholesterol. The concentration of LDL in rats fed with natural garlic (functional food) is lower followed by nutraceutical and nutriceutical fed rats. However, the control group has the highest concentration. Lehoux [32] reported that garlic supplementation reduce accumulation of cholesterol, triglyceride and low density lipoprotein but increase high density lipoprotein on the vascular walls of animals and in human. The anti-clotting effects of garlic possibly reduce plaque formation in blood vessels. This could possibly help protect against heart disease and stroke which may have be the reason for the reduced lipid profile (total cholesterol, triglyceride and LDL) and increases the HDL. Moreover, garlic prevents blood from clumping (aggregation) and sticking to blood vessels (adhesion). Allicin produces this effect in vitro during its brief and transient presence.

This work has established that functional foods (raw/natural garlic extract) has the highest concentration of allicin and also may be due to nutrient-nutrient interaction of other nutrients which lead to a positive result followed by nutraceuticals (ever

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world garlic) with nutriceutical (synthetic garlic) having the least concentration of this element. It has also established through comparative analysis that raw garlic has the highest concentration of allicin than already prepared garlic. Since raw garlic is more effective and even less expensive than other garlic supplementation it is recommended that government should create awareness for an increase consumption and utility of raw garlic irrespective of the bad breath. From the results it has been shown that garlic reduces plasma levels of lipids and also serves as anti-platelet but a research should be carried out on other hematological parameters and on humans. However, before this garlic can be recommended for use as a chemotherapeutic strategy for patients, their activity must be confirmed in rigorously designed clinical trials.

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