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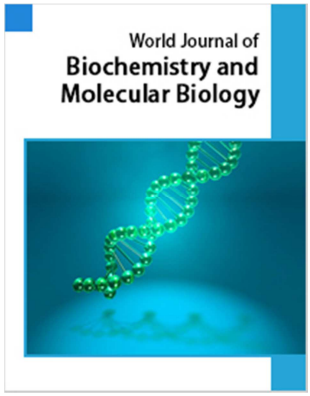

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Reducing Power Antioxidant Activity and Effect of Time-Dose Dependent Administration of *Allium sativum* Oil Extract on Lipid Profile of *Rattus norvegicus*

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Abstract

Reducing power antioxidant assay was carried out on garlic oil extract and ascorbic acid as standard. Effect of time and dose of garlic oil extract on Rattus novergicus was also studied. Obtained results revealed that there was an increase in reducing power values with increase in concentration of sample and standard (ascorbic acid) used. The sample extract (R^2 = 0.9993 for garlic oil) appears to have more reducing powers than the standard (R^2 = 0.978 for ascorbic acid). The lipid profile showed there was 4% reduction in total cholesterol from day 0 to day 7, an 8% reduction from day 7 to day 14 and 9% reduction from 14 to 21. There was a significant decrease in LDL-cholesterol by 42% from day 0 to 7; 7% decrease from day 7 to 14 and 29% decrease from day 14 to 21 and a decrease in triglyceride levels was also observed from day 0 to 7 by 3%, 51% from day 7 to 14 and 3% from day 14 to 21. Favourable and significant increase in HDL-cholesterol levels was noticed from days 0 to 7, 7 to 14 and 14 to 21 by 20%, 13% and 9% respectively. Garlic oil had a beneficial effect on the health of rats studied.

1. Introduction

The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body as a result of contact with excess of exogenous chemicals in our ambient environment and/or due to a number of endogenous metabolic processes involving redox enzymes. Under normal circumstances, the ROS generated are detoxified by the antioxidants present in the body and there is equilibrium between the ROS generated and the antioxidants present. However, owing to ROS overproduction and/or inadequate antioxidant defense, this equilibrium is interfered favoring the ROS upsurge that terminates in oxidative stress. The ROS easily affect and promote oxidative damage to various biomolecules including proteins, lipids, lipoproteins, and DNA [1]. This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases and also in the ageing process. The scientific interest in plant phenolics as chemopreventive and therapeutic agents against chronic and degenerative diseases has been increasing since the late 1990s, when the French paradox was associated

with the high intake of phenolics present in red wine [2] [3]

Based on the growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the usefulness of antioxidants in protection against these diseases is supported. Epidemiological studies have found that the intake of antioxidants, such as Vitamin C, reduce the risk of coronary heart disease and cancer [4]. The antioxidants may mediate their effect by directly reacting with ROS, quenching them and/or chelating the catalytic metal ions [5]. Several synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, are commercially available but are quite unsafe and their toxicity is a problem of concern. Natural antioxidants, especially phenolics and flavonoids, are safe and also bioactive [6]. Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant ability that may be used for human consumption.

Lipid profile is a panel of blood test carried out which serves as an initial medical screening tool in detecting abnormalities in lipids such as triglycerides and cholesterol. It usually includes the levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides and the calculated low-density lipoprotein (LDL) cholesterol [7] (Omale., 2014).

Allium sativum, garlic is a species of monocot, bulb-forming perennial whose relatives include Onions, Shallots and Leeks [8]. Garlic has been cultivated for over 5000 years, garlic has already been widely used for conditions such as parasites invasion, respiration problems, poor digestion, and low energy [9]. Garlic bulbs contain separate fleshy sections (Cloves), each covered with a papery skin (tunic). The plants produce a leafless flower stem (a scape), but the flowers are sterile and produce bulbis (small cloves) rather than seeds; the species is propagated clonally from cloves and bulbis [10].

Garlic has historically been grown for both culinary and medicinal purposes. Bulbs are most commonly used, but leaves, scrapes, and bulbis are also eaten. Garlic is frequent in the cuisines of Asia, the Middle East, the Mediterranean, and south and Central America [10]. Beneficial effects of garlic in lowering elevated serum cholesterol level for prevention of coronary heart diseases and hypertension is well established [11], and use as hypolipidemic and antihypertensive agents is common. It is believed that consumption of one clove of garlic everyday leads to significant reduction in cardiovascular complication [12].

The objective of this study is to evaluate the antioxidant potential of garlic (*Allium sativum*) oil using the reducing power assay method, and also to determine the effect of time and dose in the oral administration of the oil on lipid profile of *Rattus novergicus*.

2. Materials and Methods

2.1. Materials

The sample (Garlic) was obtained from Anyigba main market and was identified by the botanical unit of the department of Biological sciences of Kogi State University. It was then kept in a cold but dry environment for further analysis. n – Hexane, Methanol, Sulphuric acid, Sodium hydrogen phosphate and Ammonium molybdate were all products of BDH of England. Other reagents used in this study were of analytical grade and are available commercially. Wistar (albino) rats, *Rattus novergicus* was used for lipid profile study. Standard diagnostic kits for the determination of total cholesterol, HDL cholesterol, Triglyceride and LDL cholesterol available commercially from Agape Diagnostic (Switzerland GmbH) were used to analyze the serum from the rats [13].

2.2. Methods

Cloves were separated from the bulb, after which the bulb was pounded with the laboratory mortar and pestle to obtain a paste. The paste is dried in the oven at 50°C for 48 hours and pounded again to obtain a finer powder. The fine powder (50g) was processed for extraction of oil using n-hexane via the Sohxlet extraction techniques.

2.3. Antioxidant Assay

Sample solution in methanol (0.3ml: 1007gml^{-1}) was mixed with reagent solution (3ml; 0.6M sulphuric acid, 2.8mM sodium phosphate and 4mM ammonium molybdate). A blank composed of 3ml of reagent solution and methanol was also prepared. All tubes were capped and incubated in boiling water bath at 90°C for 90 minutes, absorbance of samples were read against blank at 695nM. The percent increase in reducing power was calculated using the following equation

$$\text{Increase in reducing power (\%)} = \frac{\text{A test} - \text{A blank}}{\text{A blank}} \times 100$$

Where;

A test = absorbance of the test solution, and

A blank = absorbance of blank.

2.4. Effect of the Oil Extracts on Rat Lipid Profile

The lipid profile was determined according to procedure described by [14] and with slight modification. Twelve (12) healthy, age-matched wistar rats weighing 100.12±20g were divided into four diet groups of three rats namely, A, B, C and D with A as the control. The animals were housed in individual wooden cages covered with net. Feeding was designed as follows:

Group A - Control rats took normal diet for zero days.

Group B - Test rats took 0.5ml oil extract with normal diet for 7 days.

Group C - Test rats took 0.5ml oil extract with normal diet for 14 days.

Group D - Test rats took 0.5ml oil extract with normal diet for 21 days.

On day(s) 0, 7, 14 and 21 of oil administration, three (3) rats were sacrificed by cardiac puncture. Blood samples were immediately collected by the aid of sterile syringe with needle

into plain sample bottle. Blood serum was separated with the aid of a centrifuge (Uniscope Laboratory centrifuge Model SM800B, UK), at 2000rpm for 15 minutes and used for analysis.

3. Results and Discussion

Table 1. *Physical properties of garlic oil obtained.*

Parameters	Results
Colour of oil	Light yellow
Oil yield	15.45%
Moisture content	61.20%

Some physical properties of extracted garlic oil are presented in Table 1. Natural antioxidant present in foods has become the object of greater interest in recent years. Such importance is based on studies that demonstrate the association between consumption of natural products and lower risk of degenerative disease [15]. Tables 2, 3 and figure 1 showed that there was an increase in absorbance values with increase in concentration of sample and standard (ascorbic acid) used.

Table 2. *Reducing power assay for garlic oil extract.*

Concentration (µg/ml)	Absorbance (695nM)	Reducing Power (%)
50	0.145	45
75	0.158	58
100	0.173	73
125	0.187	87

Results are mean±SD of three replicate measurements.

Table 3. *Reducing power assay for Ascorbic acid (Standard).*

Concentration (µg/ml)	Absorbance (695nM)	Reducing Power (%)
50	0.125	25
75	0.134	34
100	0.155	55
125	0.167	67

Results are mean±SD of three replicate measurements.

The sample extract (R^2 = 0.9993 for garlic oil) appears to have more reducing powers than the standard (R^2 = 0.978 for ascorbic acid) used as shown in figure 1.

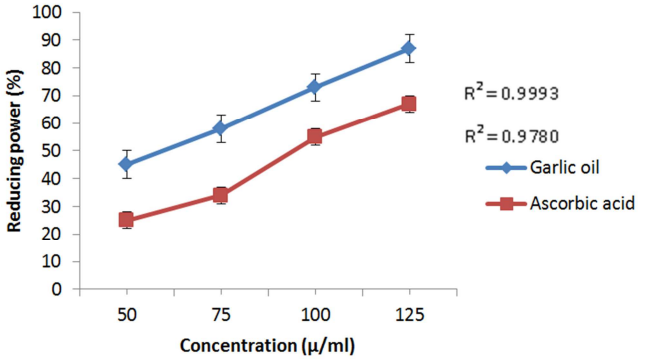

Figure 1. *Linear relationship between garlic extract and ascorbic acid reducing powers.*

This means that garlic will be a better/potent radical scavenger that ascorbic acid. Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases such as atherosclerosis, hypertension, thrombosis and diabetes [16]. Protective effects of garlic on atherosclerosis have been attributed to its capacity to reduce lipid content in the arterial wall. The results of this study have further confirmed this all important position.

The study on the effect of time, dose-dependent administration of garlic oil on the lipid profile of *Rattus novergicus* revealed a cholesterol lowering effect of the oil as observed in Table 4. There was 4% reduction in total cholesterol from day 0 to day 7, an 8% reduction from day 7 to day 14 and 9% reduction from 14 to 21. This may be attributed in part to reduced cholesterol synthesis by the liver [17]. It may also be due to decrease in hepatic activities involving cholesterogenic enzymes present in garlic oil [18].

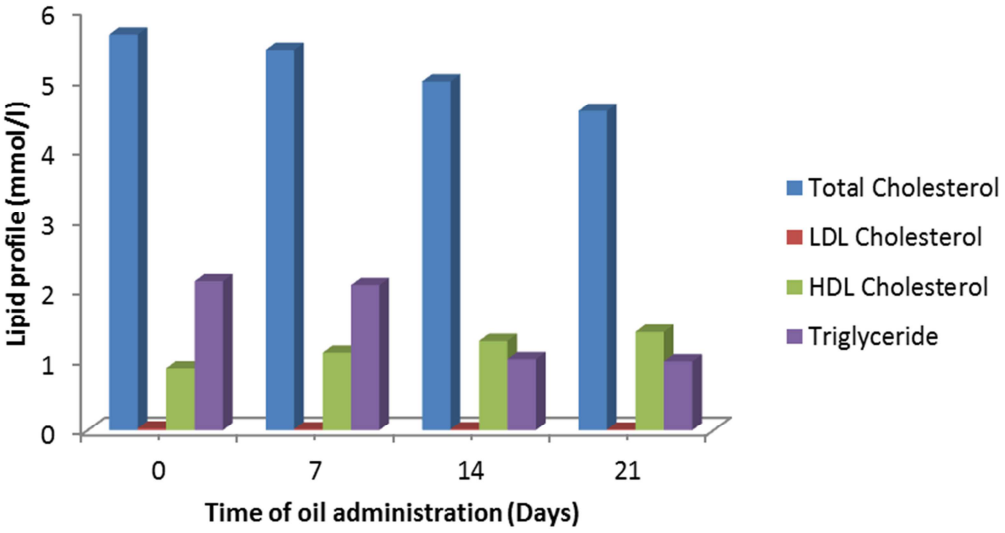

Figure 2. *Lipid profile of Rattus novergicus after oil administration.*

Table 4. *Lipid profile of Rattus novergicus before (day 0) and after (days 7, 14 & 21) oil administration.*

Groups	Test period (Days)	(mmol/l)			
		Total Cholesterol	**LDL Cholesterol**	**HDL Cholesterol**	**Triglyceride**
A	0	5.646±0.790	0.026±0.036	0.880±0.506	2.126±0.048
B	7	5.425±0.598	0.015±0.014	1.098±0.377	2.070±0.348
C	14	4.979±0.538	0.014±0.010	1.267±0.396	1.008±0.351
D	21	4.552±0.270	0.010±0.004	1.397±0.405	0.977±0.235

Results are mean±SD of three replicate measurements.

There was a significant decrease in LDL-cholesterol by 42% from day 0 to 7; 7% decrease from day 7 to 14 and 29% decrease from day 14 to 21 as shown in Table 4 and figure 2. This decrease may be as a result of suppression of LDL oxidation and lipogenic enzymes by active components in garlic such as s-allyl cysteine that are potent inhibitors of cholesterol synthesis [19] [20]. A decrease in triglyceride levels was also observed from day 0 to 7 by 3%, 51% from day 7 to 14 and 3% from day 14 to 21 which could be as result of the inhibitory effect of the oil extract on fatty acid synthesis.

Favourable and significant increase in HDL-cholesterol levels was noticed from days 0 to 7, 7 to 14 and 14 to 21 by 9%, 13% and 20% respectively again re-affirming the work of [17].

4. Conclusion

Garlic extract (oil) showed high reducing power activities. The results of this study show that the extract can be used as a source of natural antioxidant, as a possible food supplement and also in pharmaceutical industries. This is consistent with several reports that have shown close relationship between total phenolic contents and antioxidative activity of fruits, plants and vegetables [21]. However, work is still on-going in order to isolate and characterize the component that is responsible for the potent radical scavenging activity of garlic oil. These results confirm the use of natural plant materials in human lives not only for therapeutic purposes but also as food supplement for our general well being.

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