Alterations in Serum Lipid profile in Administration of *Cinnamomum cassia* and *Allium sativum* diets on High fat diet induced Hyperlipidemic rats

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ABSTRACT

This study assesses the effects of hyperlipidemia on serum lipid profile. These parameters were measured in forty-two albino wistar rats between (100g-120g). The animals were divided into 7 groups of 6 animals each. Group 1 received normal pellet for 60 days. Group 2 received high fat diet for 60 days. Group 3 received high fat diet for 30 days to induce hyperlipidemia and treated with standard drug (Atorvastatin 1.2 mg/kg) for 30 days. Group 4 received high fat diet with 10% garlic from 30-60 days. Group 5 received high fat diet with 10% cinnamon from 30-60 days. Group 6 received high fat diet with 30% garlic from 30-60 days. Group 7 received high fat diet with 30% cinnamon from 30-60 days. Administration of HFD significantly increased TC, TG, LDL and decreased HDL among induced groups except normal control. There was remarkable increase in the body weight of the animals after induction with high fat feed. It was discovered that the levels of TC, TG, LDL were significantly reduced while the level of HDL was significantly increased after treatment with 10% garlic, 10% cinnamon, 30% garlic and 30% cinnamon. While the weights of the hyperlipidemic induced untreated group increased significantly. From the results obtained, the formulations of garlic and cinnamon in their different percentages have hypolipidemic effects.

Keywords: Lipid profile, *Cinnamomum cassia*, *Allium sativum*, fat diet

INTRODUCTION

Hyperlipidemia is considered one of the major risk factors causing cardiovascular diseases (CVDs). CVDs accounts for one third of total deaths around the world, it is believed that CVDs will turn out to be the main cause of death and disability worldwide by the year 2020 [1]. Hyperlipidemia is a medical condition characterized by an elevation of any or all lipid profile and/or lipoproteins in the blood. It is also called hypercholesterolemia/hyperlipoproteinemia [2]. Hypercholesterolemia and hypertriglyceridemia are the main cause of atherosclerosis which is strongly related to ischemic heart disease (IHD) [3]. There is a strong relation between IHD and the high mortality rate. Furthermore, elevated plasma cholesterol levels cause more than four million deaths in a year [4]. Atherosclerosis is a process of arteries hardening due to deposition of cholesterol in the arterial wall which causes narrowing of the arteries. Atherosclerosis and atherosclerosis associated disorders like coronary, cerebrovascular and peripheral vascular diseases are accelerated by the presence of hyperlipidemia [5]. Hyperlipidemia relates to increased oxidative stress causing significant production of oxygen free radicals, which may lead to oxidative modifications in low-density lipoproteins, which present a significant function in the initiation and progression of atherosclerosis and associated cardiovascular diseases [6]. Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk, [7] dyslipidemia (abnormal amount of lipids in the blood) can also describe elevated
total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL). Human body is a complex machine for maintaining the homeostasis of various organ and organ system. Any undesirable change will disturb the balance resulting in a diseased state [8]. Lipids are fats in the blood stream, commonly divided into cholesterol and triglycerides. Cholesterol circulates in the bloodstream and is involved in the structure and function of cells. Triglycerides (TG) are best viewed as energy that is either used immediately or stored in fat cells. TG is manufactured in the liver from the foods or by being absorbed from the intestine [9]. Virchow in 19th century who identified cholesterol crystals in atherosclerotic lesion and stated that endothelial cell injury initiates atherogenesis [10]. In a modification of this hypothesis it was proposed that the endothelium normally influences the behaviour of arterial smooth muscle cells by providing a barrier to the passage of plasma proteins, and that the major effect of haemodynamic or other factors that injure endothelium is to reduce the effectiveness of the barrier [11]. Arteries are normally smooth and unobstructed on the inside, but in case of increased lipid level, a sticky substance called plaque is formed inside the walls of arteries. This leads to reduced blood flow, leading to stiffening and narrowing of the arteries. It has been proved that elevated plasma levels of cholesterol and of LDL are responsible for atherosclerosis in man, and epidemiological data suggests that elevated plasma levels of HDL have a protective effect [12].

Hyperlipidemia in general can be classified into two sub types: primary hyperlipidemia and secondary hyperlipidemia

Primary hyperlipidemia
This usually take place as a result of genetic problems i.e., mutation within receptor protein, which may be due to single (monogenic) gene defect or multiple (polygenic) gene defect. This type may occur as a result of change in dietary and lack of proper physical activities. See table below for summaries on the various classes of primary hyperlipidemia.

<table>
<thead>
<tr>
<th>TYPE</th>
<th>DISORDER</th>
<th>CAUSE</th>
<th>OCCURANCE</th>
<th>ELEVATED PLASMA LIPOPROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Familial lipoprotein lipase deficiency</td>
<td>Genetic</td>
<td>Very rare</td>
<td>Chyomicrons</td>
</tr>
<tr>
<td>Iia</td>
<td>Familial hypercholesterolemia</td>
<td>Genetics</td>
<td>Less common</td>
<td>LDL</td>
</tr>
<tr>
<td>Iib</td>
<td>Polgenic hypercholesterolemia</td>
<td>Multifactorial</td>
<td>Commonest</td>
<td>LDL</td>
</tr>
<tr>
<td>III</td>
<td>Familial dysbetalipoproteinemia</td>
<td>Genetic</td>
<td>Rare</td>
<td>IDL, Chyomicrons, Remnants</td>
</tr>
<tr>
<td>IV</td>
<td>Hypertriglyceridemia</td>
<td>Multifactorial</td>
<td>Common</td>
<td>VLDL</td>
</tr>
<tr>
<td>V</td>
<td>Familial combined hyperlipidemia</td>
<td>Genetics</td>
<td>Less common</td>
<td>VLDL, LDL</td>
</tr>
</tbody>
</table>

Secondary hyperlipidemia
This arises as a result of other underlining diseases like diabetes, hypothyroidism, nephritic syndrome, chronic alcoholism, and with use of drugs like corticosteroids, oral contraceptives, beta blockers [12]. Secondary hyperlipidemia together with significant hypertriglyceridemia can cause pancreatitis [12].
Causes of Hyperlipidemia

The main cause of hyperlipidemia includes changes in lifestyle habits in which risk factor is mainly poor diet i.e. with a fat intake greater than 40 percent of total calories, saturated fat intake greater than 10 percent of total calories; and cholesterol intake greater than 300 milligrams per day or treatable medical conditions [12]. The abnormal cholesterol levels are the result of an unhealthy lifestyle including taking high-fat diet and other lifestyle factors like being overweight, smoking heavy alcohol use and lack of exercise. Other factors include diabetes, kidney disease, pregnancy, and an underactive thyroid gland [13]. Other illnesses that may elevate cholesterol levels include polycystic ovarian syndrome and kidney disease.

The higher levels of female hormones like estrogen, have been noted to increase or change cholesterol levels. In addition, drugs like diuretics, beta-blockers and medicines used to treat depression have also been reported to raise cholesterol levels [14]. Another modifying factors in the development and progression of hyperlipidemia are age and gender. It has been shown that cholesterol levels rise as the person gets older [16, 17]. Heredity has also been a modifying factor for the progression of hyperlipidemia as it has been noted that the genes partly determine the amount of cholesterol body makes [18, 19]. It has also been noted that chronic renal failure, metabolic syndrome and nephrotic syndrome can predispose to hyperlipidemia [20, 21].

Materials and Methods

Apparatus and equipments

The following chemicals, reagents and drugs were used: Spectrophotometer (B. Bran Scientific &Instrument Company, England), Water Bath (Techmel & Techmel, Texas, USA), and Micropippete (Finnipipette® Labsystems, Finland).

Animals

A total of forty-two (42) male albino wistar rats, (weighting between 100- 120g) were purchased from the Laboratory Animal Facility of the Department of Veterinary, Physiology and Pharmacology, University of Nigeria, Nsukka and transferred to the animal House of the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Agulu Campus where the animals were used for the experiment. They were housed in clean metal cages, supplied with pelleted feed and water and handled in compliance with the National Institute of Health Guidelines for care and use of laboratory animals 8th edition (2011).

Collection of plant materials

Dried cinnamon barks (Cinnamomum cassia) and garlic cloves (Allium sativa L.) samples were obtained dry from Relief Market at Onitsha in Anambra State.
Preparation of cinnamon and garlic powder

Dried cinnamon and garlic were reduced to powder by crushing in a grinder machine.

Induction of experimental hyperlipidemia

Hyperlipidemia was induced by feeding the animals on high fat diet composed of 35% Vital grower feed, 10% egg yolk, 5% crayfish, 35% Palm kernel cake (P.K.C) and 15% Margarine for 30 days [18]. After four weeks, blood samples were collected from the animals by periorbital through capillary tubes and into blood sample collection tubes. It was centrifuged at 3000 rpm for 30 minutes in a centrifuge machine. The clear sera were collected and used for the biochemical analysis of the lipid profile: Total cholesterol (T.C), triglycerides (T.G) and high density lipoproteins using Randox reagent kits. Low density lipoproteins (L.D.L) was calculated using Friedewald formula.

Upon confirmation of hyperlipidemia on the other groups of animals apart from those in the normal control group, the animals were grouped into seven groups for treatment.

Experimental Design

Forty-two (42) male Wistar rats consisting of thirty-five (35) hyperlipidemic rats were randomized into six groups of seven rats each and seven (7) non-hyperlipidemic rats as follows:

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>RATS CONDITIONS</th>
<th>TREATMENTS</th>
<th>DOSAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1</td>
<td>Normal control</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>GROUP 2</td>
<td>Negative control (hyperlipidemic)</td>
<td>High fat feed</td>
<td>100 g</td>
</tr>
<tr>
<td>GROUP 3</td>
<td>Positive control (hyperlipidemic treated)</td>
<td>Atorvastatin + High fat feed</td>
<td>1.2 mg + 99.99 g</td>
</tr>
<tr>
<td>GROUP 4</td>
<td>Hyperlipidemic treated</td>
<td>Garlic powder + High fat feed</td>
<td>10 g + 90 g</td>
</tr>
<tr>
<td>GROUP 5</td>
<td>Hyperlipidemic treated</td>
<td>Cinnamon powder + High fat feed</td>
<td>10 g + 90g</td>
</tr>
<tr>
<td>GROUP 6</td>
<td>Hyperlipidemic treated</td>
<td>Garlic powder + High fat feed</td>
<td>30 g + 70g</td>
</tr>
<tr>
<td>GROUP 7</td>
<td>Hyperlipidemic treated</td>
<td>Cinnamon powder + High fat feed</td>
<td>30 g + 70g</td>
</tr>
</tbody>
</table>

Collection of blood samples

Daily administration of atorvastatin, cinnamon powder, garlic powder and high fat feed were given to the animals for another 30 days. After the final dosage, the animals were fasted overnight but left free access to water. The animals were sacrificed using chloroform anesthesia and the blood of each individual animal was collected immediately for determination of serum lipid profile of total cholesterol, triglycerides and high density lipoproteins cholesterol.

Biochemical assays

Determination of serum total cholesterol:

Serum total cholesterol (TC) was evaluated using Randox commercial assay kits following the methods described by Ezeigho (2016) [8]. One millilitre (1 ml) of the working cholesterol reagent was added into tubes labeled blank, standard and test groups. Ten microlitres of standard cholesterol reagent, and samples were added into their respective tubes. They were mixed and allowed to stand for 10 minutes at room temperature. Absorbance of samples and standard were read with the aid of a spectrophotometer at 500 nm. Total cholesterol level in sample was calculated using the formula:

\[
\text{Total cholesterol in sample (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard.}
\]
Determination of serum triglyceride

Serum triglyceride was evaluated according to the methods described by Tietz, (2014) [20]. One millilitre (1 ml) of the working triglyceride reagent was added into tubes labeled blank, standard and test groups. Ten microlitres of standard triglyceride reagent, and samples were added into their respective tubes. They were mixed and allowed to stand for 10 minutes at room temperature. Absorbance of samples and standard were read with the aid of a spectrophotometer at 500 nm. Total cholesterol level in sample was calculated using the formula:

\[
\text{Total triglyceride in sample (mg/dL) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}}
\]

Determination of serum high density lipoprotein cholesterol (HDL-cholesterol):

Serum HDL-cholesterol was evaluated according to the methods developed by National Institute of Health Consensus Development Conference Statement (NIHCDCS). One hundred microlitres (100 ul) of samples and standard cholesterol reagent were dispensed into test tubes containing 250 ul of HDL cholesterol precipitate (R1). The mixture was centrifuged at 4000 rpm for 10 minutes. Thereafter, 100 uL of samples and standard supernatants were added to another set of test tubes labeled samples and standard containing cholesterol reagent. The mixture was incubated for 10 minutes at room temperature and absorbance of standard and samples were measured against reagent blank at 500 nm within 60 minutes using Spectrophotometer. HDL-cholesterol level in sample was calculated using the formula below;

\[
\text{HDL cholesterol in sample (mg/dL) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}}
\]

Determination of serum low density lipoprotein cholesterol (LDL-cholesterol):

Low density lipoproteins (LDL) cholesterol in serum was calculated using the equation described by [9]. The Friedewald’s equation estimates the value of HDL-C using the values of total cholesterol, triglyceride and HDL-cholesterol [9].

\[
\text{LDL cholesterol (mg/dl) = Total cholesterol - \left(\frac{\text{Triglycerides}}{5} + \text{HDL cholesterol}\right)}
\]

Statistical analysis

Data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS-20). Results were presented as mean ± Standard error of mean (SEM) of sample replicates. Raw data were subjected to one way analyses of variance (ANOVA) followed by post hoc turkey’s test. p<0.05 was considered to be statistically significant.

RESULTS

PRESENTATION OF RESULTS

Effect of garlic and cinnamon on serum lipid profile

Fig 1: Represent the effect of administration of the various treatment on serum total cholesterol, triglycerides, high density lipoproteins and low density lipoproteins. The levels of triglyceride, total cholesterol and LDL-C were significantly elevated and the level of serum HDL-C was decreased in the hyperlipidemic control group as compared to normal control. After treatment with 10% garlic and cinnamon as well as 30% garlic and cinnamon there were alteration in lipid metabolism. Significantly there was decrease in serum triglyceride (TG), total cholesterol (TC) and LDL-cholesterol levels and increased HDL cholesterol concentration. TC 10% garlic (170.11 ±1.41<sup>b</sup>), TG30% garlic (112.40 ±0.74<sup>d</sup>) and LDL 10% garlic (78.08±5.11<sup>ae</sup>) displayed higher increase when compared with 10% and 30% cinnamon. TC 30% cinnamon (150.68
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±14.13*), TG 30% (88.80 ± 4.21*) and LDL 30% cinnamon (62.24±5.31*) had the highest reduction. In HDL, 30% cinnamon displayed the highest increase.

**Fig. 1:** Effect of garlic and cinnamon on serum lipid profile

Lipid profile significantly reduced (*b-*m p<0.05)] when compared to hyperlipidemic induced untreated and significantly increased (*p-*p3 p<0.05) when compared to hyperlipidemic induced untreated (*).

**Fig. 6:** There was remarkable increase in the body weight of the animals after induction with high fat feed. The weights of the animals in the treated groups were reduced after treatment with the various percentages of garlic and cinnamon. While the weights of the hyperlipidemic induced untreated group increased significantly. Before induction, Group 3 (10% garlic 158.02 ± 20.04) had the highest weight increase. After induction Group 1 (224 ± 11.31) displayed the highest weight which is the hyperlipidemic control group fed continuously with the high fat diet. Then after treatment Group 5 (30% garlic 159.32±20.12) showed the highest weight reduction.

**Fig. 1:** Effect of garlic and cinnamon on serum lipid profile

TCH= Total Cholesterol, TRIG= Triglycerides, HDL= High density lipoproteins, LDL= Low density lipoproteins, NC= Normal control, HYP-C= Hyperlipidemic control, STD-C= Standard drug control and H$_2$O= water
DISCUSSION

This work was carried out to investigate the possible effect of garlic and cinnamon on hyperlipidemic, high fat feed induced rats. Cholesterol, a vital part of all cell membrane, is an essential molecule which all living beings depend on for existence. Large amount of cholesterol are found in breast milk, where it is essential for infant nourishment and brain development. It helps in the healing and repair of blood vessels. Blood vessels have a delicate lining and in the event of turbulence, the lining becomes damaged and has to be repaired [4]. The result of serum lipid profile gotten from this study as displayed in Table 1 and figure 1 reveals that the administration of HFD significantly increased TC, TG, LDL and decreased HDL among induced groups except normal control. It was discovered that the levels of TC, TG, LDL were significantly reduced while the level of HDL was significantly increased after treatment with 10% garlic, 10% cinnamon, 30% garlic and 30% cinnamon [13]. These significant changes in serum lipid profiles were dose dependent. the elevations in serum TC, TG, LDL and HDL levels observed in this study are in agreement with those reported in several studies [16, 17, 18]. It has been reported that high serum abnormally levels of TC and LDL are associated with an increased risk for atherosclerosis [2, 3, 4, 6]. Hypercholesterolemia and hypertriglyceridemia are independent risk factors that alone or together can accelerate the development of coronary artery disease and the progression of atherosclerotic lesions [15]. In this study, a significant increase in HDL-c concentration indicates that HDL-c may play a protective role through reversing cholesterol transport, inhibiting the oxidation of LDL and neutralizing the atherogenesis effects of oxidized LDL.

CONCLUSION

From current study, after supplementation with Cinnamomum cassia and Allium sativum diet the alteration in lipid metabolism was significantly attenuated as evidenced by decreased serum triglyceride (TG), total cholesterol (TC), and LDL-cholesterol levels and increased HDL-cholesterol concentration in hyperlipidemic rats.
REFERENCES


