

Effect of Methanolic Extract of Cocoyam (*Colocasia Esculenta*) Leaves on Lipid Profile of Albino Rats

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Abstract

Despite the availability of statins and other lipid lowering drugs, a significant number of patients with hypercholesterolemia do not achieve adequate cholesterol reduction without side effect; thus treatment options with drugs possessing different mechanism of action, improved therapy and improved safety profile is required. This study is aimed at authenticating the claims about the potency of cocoyam (*Colocasia esculenta*) leaves in traditional medicine and a particular attention has been given to the effects of the plant on the lipid profile of albino rats. A total of 16 albino rats which were obtained from Nigerian Institute of Trypanosomiasis Research (NITR), Kaduna were used in this study, comprising of 4 in each group. The plants were shade dried and the extraction was carried out. Each group of the rats were administered with 1000mg/kg body weight, 1,500mg/kg body weight and 2000mg/kg body weight of the extract except for the control group which were administered with normal saline, after which lipid profile analysis (which include cholesterol, LDL, HDL and triglycerides tests) was carried out on the rats using Randox lipid analysis kit. The data obtained were analyzed using ANOVA. The findings of this work showed that cocoyam leaves is able to lower the levels of cholesterol and that of low density lipoprotein (LDL) significantly ($p < 0.05$), a feature it shares with other hypolipidemic drugs. This study recommends that consumption of vegetables rich in fiber and also possessing hypolipidemic effect such as cocoyam leaves should be increased so as to counter the prevalence of hyperlipidemia.

Key Words: Hypolipidemic Effect, *Colocasia esculenta*, Lipid Profile, albino rats.

Introduction

Plants have played a significant role in maintaining human health and improving the quality of human lives. The popularity of herbal medicine in recent times is based on the premise that plants contain natural substances that can promote health and alleviate illness; therefore the focus on plant research has increased all over the world and a large body of evidence shows immense potential of medicinal plants used in various traditional systems (Arulmozhi, Mazuumber, Ashok and Narayan 2007).

Cocoyam is scientifically known as *Colocasia esculenta*. It is a herbaceous perennial plant belonging to the family Araceae and constitutes one of the six

most important roots and tuber crops worldwide (Jennings, 1987). Cocoyam is the most underutilized root crop (Adane, Shimelis, Tilahun and Gulilat 2013) this is because cocoyam is cultivated majorly for consumption in most parts of the world. The common name of *Colocasia esculenta* is taro, It is also known as dasheen or elephant ears when grown as ornamental plant (Sheth, 2005). Cocoyam is an important crop in many parts of the world, mainly for small scale farmers and also a cash crop and foreign exchange earner. The crop plays a major role in the lives of many as a food security crop and has rich economic and socio-cultural connotations (Onwueme, 1999).

Cocoyam leaves contain various organic compounds that are important in combating diseases we are exposed to, but it must be properly cooked before consumption. The comprehension of the phytochemicals in plants and their usage goes a long way in treating diseases (Enwuru, Ogbonnia, Onyeniennera, Oyedele and Enwuru 2008). Cocoyam leaves contain calcium oxalate, fibres, minerals such as calcium, phosphorus, zinc, iron, magnesium, potassium, starch, vitamins A, B and C, e.t.c. (Sheth, 2005). Phytochemically, it contains apigenin, luteolin, tannins, flavonoids, phenol, alkaloids, and anthocyanin (Khare, 2007). The use of cocoyam leaves as a medicinal plant in traditional medicine is common in developing nations. However, there is little scientific evidence to justify its potency. Although *C. esculenta* has been reported to possess hypoglycemic activity and that may be due to its cyanoglucoside content (Grindley, Omoruyi and Morrisona 2002).

Yang and Yeh, (2005) also reported the antifungal activity of the plant. Lipids are hydrophobic organic substances that are insoluble in water but soluble in non-polar solvents such as ether, chloroform and benzene.

The main biological functions of lipids include energy storage, structural components of cell membranes and as important signaling molecules. (Anthea, 1993). Lipoproteins are biochemical assemblies that contain proteins and lipid molecules. These water soluble proteins combine with lipids and transport lipids in the blood (Madan and Sankaran, 2002). The low density lipoprotein (LDL) transports cholesterol from the liver to peripheral tissues. It is part of the variables used in the lipid profile analysis and it is referred to as the "bad cholesterol" as opposed to the High

density lipoprotein (HDL) which is referred to as "good cholesterol" or "healthy cholesterol" (Segrest, Jones, De Lorf and Dashti 2001). HDL transports cholesterol from arteries to the liver for excretion. Cholesterol is a waxy, fat-like substance found in every cell of the body. It is used to help digest fat and strengthen cell membranes in the body. Too much cholesterol can be dangerous to the body because it builds up on artery walls, increasing risk of blood clots, heart attack and stroke. Some medications can alter the levels of cholesterol, however it is most frequently caused by a combination of diet and genetic factors built in a person's make up.

In addition, significant number of people diagnosed with diet related ailments usually have increased amount of lipids in their body. The excessive amount of body lipids pre-disposes the subjects to obesity which is one of the leading causes of death worldwide. The percentage of obese children in United States increased from 7% in 1980 to 18% in 2012, while in adults it increased from 5% to nearly 21%. Obese individuals are more likely to have risk factor for cardiovascular diseases, type 2 diabetes mellitus, stroke, hypertension among others and for 70% youths, they have at least one risk factor (Ogden, Carroll, Kit and Flegal 2014). To this effect, there has been renewed interest in the exploration of medicinal plants in a bid to complement the conventional scientific procedures in combating nutritionally related health disorders. Conventional therapy has focused mainly on dietary modification and consumption of drugs that could help breakdown the lipids that accumulate within the body. However, very little has been done to verify the potency of traditional herbs including *C. esculenta*. This particular study therefore,

aims to investigate the effect of methanolic extract of *Colocasia esculenta* leaves in regulating the lipid profile of albino rats and this can give us an insight and a scientific proof for the potency of *C. esculenta* leaves on lipid profile of humans.

Objectives of the study:

To evaluate the effect of *Colocasia esculenta* leaves extract on key components involved in lipid profile analysis which are:

- i. Total cholesterol
- ii. High density lipoprotein (HDL)
- iii. Low density lipoprotein (LDL)
- iv. Triglycerides of albino rats.

Materials

Plant materials: Fresh *C. esculenta* leaves were obtained from Samaru, Zaria local Government of Kaduna State, Nigeria. The samples were identified and tested at the herbarium of Department of Biological Sciences, Faculty of Science A.B.U Zaria, Kaduna, Nigeria.

Animals: Male Swiss Albino rats were used for the study. They were obtained from Nigerian Institute for trypanosomiasis Research (NITR), Kaduna. The rats of 8 to 10 weeks old weighing between 90 to 130g, and were housed in wired mesh cages and fed with growers feed and water for a period of 14 days before the experiment and during the entire period of experimentation.

Chemicals/Reagents: The chemicals used for the study includes: Methanol 96%, Distilled water chloroform and Randox lipid analysis kit (cholesterol and triglyceride reagents)

Apparatus/equipments: Centrifuge, Hot air oven, Electronic weighing balance, Spectrophotometer, Water Bath, Incubator, Rotary Evaporator, Blender, Beakers, measuring cylinder, Spatula, Whattman filter paper, Dessicator, Surgical blades,

EDTA bottles.

Methods

The leaves were shade dried until ready to be milled. The dried leaves were grinded to powder by pounding using a clean mortar and pestle before blending in an electric blender (MC-BL1999). The methanolic extraction of *Colocasia esculenta* was carried out by weighing 275g of the powdered leaves and soaked in 1,375mls of methanol for 48hours before it was filtered using a clean muslin cloth and then whattman filter paper. Methanol extraction was carried out using the rotary evaporator (NYC, R-205D). At the end of extraction, the resulting residue was transferred into a clean beaker and dried in a water bath.

Experimental Animals: The 16 male albino rat weighing between 90-130g were distributed randomly into 4 groups (A-D) comprising of four (4) animals each. The animals were acclimatized for two weeks on a regular feed (Grower mash – Guinea feed) and water. Three groups were administered with different doses of the extract and the feed. Among the three groups administered with the extract, groundnut was added to the feed of one group so as to increase the fat intake of that particular group and also make comparison at the end of the experiment how fat dense foods can affect lipid levels of the experimental animals. The fourth group (control) were fed with the regular feed and water only as illustrated below:

1. Group A (control): feed alone
2. Group B: 1000mg/kg body weight +Feed
3. Group C: 1500mg/kg body weight + Feed
4. Group D: 2000mg/kg body weight + Feed + Ground nut

Administration of plant extract and blood sample collection

The different animal groups were administered with their respective doses of the extract orally for a period of seven days on varying individual weight, after which they were dissected by cardiac puncture with the aid of surgical blade. Blood samples were collected into EDTA bottles and centrifuged for further analysis.

Lipid Profile Test

The lipid profile analysis involves the following tests:

1. Total cholesterol
2. Triglyceride
3. High density lipoprotein

4. Low density lipoprotein

Determination of total cholesterol: After the collection of blood sample into EDTA bottles, the samples were centrifuged at 3000rpm for five minutes to separate the plasma from the whole blood. 1ml of cholesterol reagent was pipette into a clean test tube, then 0.01ml of the sample was added to the reagent and then it was shaken. The mixture was incubated for 10 minutes at 37°C. The absorbance of the sample against reagent blank was measured at 500nm within 60minutes. The concentration of cholesterol in the sample was calculated using the concentration of the standard of the reagent as shown below.

$$\text{Concentration of cholesterol} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Determination of triglyceride: The blood samples were collected into EDTA containers and then centrifuged at 3000rpm for five minutes to separate the plasma from the whole blood. 1ml of triglyceride reagent was pipette into a clean test tube, then 0.01ml of the sample was added to the reagent and then it was shaken. The

mixture was incubated for 10 minutes at 37°C. The absorbance of the sample against reagent blank was measured at 500nm within 60minutes. The concentration of triglyceride in the sample was calculated using the concentration of the standard of the reagent as shown below.

$$\text{Concentration of triglyceride} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Determination of high density lipoprotein (HDL): 0.5ml of cholesterol reagent was pipette into a clean test tube, 0.25 of the sample was added and it was shaken, then centrifuged at 4000 rpm for 10 minutes for deproteinization. Then 0.1ml of the supernatant was pipette and transferred into another clean test tube

containing cholesterol reagent; which was then incubated for ten minutes at 37°C. The absorbance was measured against the reagent blank at 500nm. The concentration of HDL was calculated using the concentration of cholesterol standard.

$$\text{Concentration of HDL} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Determination of low density lipoprotein (LDL): Low density lipoprotein is determined using the Friedwald formula (Friedewald, Levy, and Fredrickson 1972).

$$\text{LDL} = \frac{\text{Total cholesterol} - \text{triglyceride} - \text{HDL}}{2.2}$$

Data analysis: The results obtained were subjected to ANOVA ((analysis of variance) using SPSS 20.0. mean separation was done using the least significant difference ($p < 0.050$).

Results and Discussion

Table1. Lipid profile of albino rats after treatment with methanolic extract of *Colocasia esculenta* leaves

Parameters				
Treatment	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Group A (Control)	2.8700 a	1.5375	0.3575	1.7775a
Group B (1000mg/kg body weight)	1.4300b	1.2825	0.4450	0.4025b
Group C (1500mg/kg body weight)	1.5550b	1.2950	0.7650	0.5550b
Group D (2000mg/kg body weight)	1.4850b	1.2150	0.5275	0.4712b
Standard error	0.080	0.107	0.136	0.171

Values are means of triplicate measurements.

Values followed by different superscript letters are significantly different at 5% level of significance i.e. ($p < 0.05$).

Discussion

From the results obtained after carrying out the lipid analysis, there was a significant decrease in cholesterol level ($p < 0.05$) and this indicates that the extract was effective in lowering blood cholesterol level. One of the known mechanisms of action of *C. esculenta* is the inhibition of human lanosterol synthase (hoSc) activity (Sakano, Mutsuga, Tanaka, Suganmums, Inakuna and Tayoda 2005). Lanosterol synthase is more closely related to cholesterol biosynthesis than HMG-CoA reductase, therefore it is an attractive drug target (Telford, Lipson, and Barette 2005). Human lanosterol synthase inhibitors are thought to lower LDL and VLDL by dual

control mechanism (decrease in lanosterol formation and HMG-CoA reductase activity). Studies in which hoSc is partially inhibited have shown both a direct decrease in lanosterol formation and a decrease in HMG-CoA reductase activity. The 25epoxylanosterol, which is preferentially formed over lanosterol during partial hoSc inhibition, is believed to be responsible for this inhibition of HMG-CoA reductase activity (Panini, Gupta, Sexton, Parish and Rudney 1987).

This extract also contains fiber, (Boban, Nambisan and Narayan 2006) which reduced the cholesterol level; the mechanism of action of this dietary fiber in reducing cholesterol level is by decreasing the hepatic production of VLDL (Boban *et al.*, 2006). Likewise, there was significant decrease ($P < 0.05$) in the level of low density lipoprotein (LDL) and this also indicates that the extract is effective because LDL is known to be a

“bad cholesterol” while HDL is known as “good cholesterol”, this is due to the variation in the composition of both lipoproteins especially cholesterol content which is more than 50% in LDL and just about 20% in HDL, while the protein content in LDL is 20% in LDL and 50% in HDL. Therefore, decrease in LDL is an indication of the potency of extract in lowering of blood lipids.

Dietary cholesterol suppresses the biosynthesis of cholesterol especially in tissues other than the liver (Mary and Shawn, 2006); another effect of presence of free cholesterol is the inhibition of synthesis of LDL receptors (Mary and Shawn, 2006). The synthesis of LDL receptor is itself subject to feedback regulation, studies of cultured fibroblast showed that, when cholesterol is abundant in the cell, new LDL receptors are not synthesized, and so the uptake of cholesterol from plasma LDL is blocked. In familial hypercholesterolemia, cholesterol is deposited in various tissues because of high concentration of LDL in the plasma (Pamela, Richard and Denise 2007). The excess LDL is oxidized and the oxidized LDL is taken up by immune system cells called macrophages and become engorged to form foam cells (Pamela *et al.*, 2007). These foam cells become trapped in the walls of the blood vessels and contribute to the formation of atherosclerotic plaques that cause arterial narrowing and lead to heart disease (Pamela *et al.*, 2007). However, the values obtained for triglyceride and high density lipoprotein (HDL) are numerically different. There was decrease in level of triglycerides concentration and increase in level of HDL, this further corroborates the effectiveness of the extract and indicates that the extract is effective in increasing the HDL. Since HDL is referred to as

“good cholesterol” its increase in the blood is required. However, these differences in level of triglyceride and HDL were not statistically significant ($P < 0.05$).

One of the of the goals of using orthodox hypolipidemic drugs (e.g statins) is to lower blood concentration of total cholesterol, triglycerides, LDL and to increase HDL. Despite the availability of statins and other lipid lowering drugs, a significant number of patients with hypercholesterolemia do not achieve adequate cholesterol reduction; thus treatment options with drugs possessing different mechanism of action, improved therapy and improved safety profile are required. Statins act by competitively inhibiting the HMG-CoA reductase activity, the first and rate limiting enzyme of cholesterol biosynthesis (the rate limiting step) in the conversion of HMG-CoA to mevalonate (Brunton, Blurachal, Murri, Dandan Kaollmann 2011). Also, statins may negatively influence the amounts of intermediates required for other biosynthetic pathways e.g. synthesis of isoprenoids and co enzyme Q (Brunton *et al.*, 2011).

From Table 1, it was observed that the extract was more effective for treatment groups administered with 1,000 and 1,500 mg/kg body weight respectively (groups B and C). However, the decrease in the group administered with 2000mg/kg body weight (group D) was not as much as those of groups B and C. This could be due to the diet modification of the rats in group D. The diets of all rats in group D was supplemented with fatty food (groundnut) in order to increase the lipid level, and this shows that diet plays an important role in the outcome of the experiment. The weight of the experimental rats reduced after treatment, although this change was not significant

($p < 0.05$). This may be due to the lipid lowering effect of the extract.

Conclusion

This study shows that the methanolic extract of *C. esculenta* is able to significantly lower the levels of cholesterol and LDL, a feature it shares with other hypolipidemic drugs. The hypolipidemic property of *C. esculenta* leaves extract makes it a good candidate for the production of hypolipidemic drugs that will supplement the existing ones.

Recommendation

This study recommends that:

1. Individuals with tendency of obesity and people battling with hypercholesterolemia should take more vegetables possessing hypolipidemic effect like *Colocasia esculenta* leaves.
2. Individuals with tendency of obesity should avoid or reduce the consumption of fatty foods, especially saturated fats as it is a great contributor to the increase in unhealthy fats in the body which may affect the efficacy of lipid lowering drugs.
3. Apparently healthy looking individuals should check their cholesterol levels regularly to ensure it is within normal range, as healthy individuals will translate to healthy society and better economic performance.
4. The availability, accessibility and affordability of cocoyam leaves has made it an excellent candidate that should be used by all.

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