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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Effects of Telfairia Occidentalis Seeds on the Serum Lipid Profile and Atherogenic Indices of Male Albino Wistar Rats

Anaelechi J. Onuegbu[1], Japhet M. Olisekodiaka[1], Tochukwu Ude[2], Ubuo K. Amah[1], John E. Okwara[1], Chigozie Ifeadike[3] and Olubunmi G. Ayelagbe[4]
[1]Department of Chemical Pathology, [3]Department of Community Medicine, Nnamdi Azikiwe Univerisity, Nnewi Campus, Anambra State, Nigeria
[2]Chemical Pathology Unit, Department of Medical Laboratory Science, Faculty of Health Science and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
[4]Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Abstract: The effects of T. occidentalis on serum lipid profile, non-HDL cholesterol and atherogenic indices were investigated in male wistar rats. The rats were fed for 28 days on diet formulated to contain 12.5, 37.5 and 75 g by weight of T. occidentalis seeds while the control group was fed on standard rat diet. Total cholesterol, Triglyceride, LDL, HDL and VLDL cholesterol were determined on blood samples collected on the 28th day using standard methods while atherogenic indices and non-HDL cholesterol were calculated using internationally accepted formula. The supplemented diet led to significant decrease in serum total, LDL, VLDL and Non-HDL cholesterol relative to control ($p<0.05$) while inducing a significant increase in HDL cholesterol though the effect appeared more pronounced at 37.5 g/weight. The diet supplementation also led to significant decrease in CRR, AC and AIP ($p<0.05$). These results suggest a possible dose dependent protective role of T. occidentalis seed against development of cardiovascular disease and in the management of dyslipidaemic conditions.

Key words: Telfairia occidentalis, wistar rats, cardiovascular disease

INTRODUCTION

People living in developing countries are also becoming more vulnerable to varied forms of dyslipidaemia, a major risk factor for cardiovascular disease. These forms are common in obese, diabetics and hypertensives (Kavey *et al.*, 2006, Oguejiofor *et al.*, 2012). Dyslipidaemia usually involve elevated plasma levels of triglycerides, total, LDL and VLDL cholesterol and a low level of HDL cholesterol (Franz *et al.*, 2002; Shen, 2007). The current therapeutic regimen for management and treatment of dyslipidaemia are expensive and exert economic burden on the patients. Hence attention has been turned to use of locally available medicinal plants seeds as a cost effective agent with minimal or no side effects. In Nigeria, a great number of plants and plant seeds are currently in use by traditional medicine practitioners in the management and treatment of a wide range of illnesses (Ikewuchi and Ikewuchi, 2009). T. occidentalis belongs to the family of curcubitaceae. It has been reported to contain nutrients like proteins, carbohydrates, vitamins, minerals, fibre, oxalates, saponins, glycosides, flavonoids, alkaloids and resins (Fasuyi, 2006; Iweala and Obidoa, 2009). The consumption of the leaf as part of diet or for use as a medicinal property is encouraged due to its reported characteristics as an antianemic (Olaniyan and Adeleke, 2005), antidiabetic (Nwozo *et al.*, 2004) and hepatoprotective effects (Olorunfemi *et al.*, 2005). Attention has been turned particularly to hitherto underutilized oil seeds. The seeds are said to contain as much as 41.59% fat and 25.4% protein (Ardabili *et al.*, 2011). The oil contains high levels of unsaturated fatty acids (67.87%) and lower levels of saturated fatty acids (30.32%) (Fagbemi *et al.*, 2007). Despite reported varied application, there appears to be dearth of literatures relating the effect of the plant seed on serum lipid profile and atherogenic indices. Therefore, the present study was designed to evaluate the effect of the seed produced in Nigeria on serum lipids, lipoproteins and atherogenic indices of rats fed graded doses with a view to find any therapeutic benefit in the management of dyslipidaemia a risk factor for development of cardiovascular disease and a common characteristic of diabetes mellitus and hypertension.

MATERIALS AND METHODS

Feed formulation: The fluted pumpkin fruits were bought from local markets in Anambra and Enugu States,

Corresponding Author: Anaelechi J. Onuegbu, Department of Chemical Pathology, Nnamdi Azikiwe Univerisity, Nnewi Campus, Anambra State, Nigeria

Nigeria. The botanical identification and authentication were confirmed at the Department of Botany, Nnamdi Azikiwe University, Awka. The fruits were sliced open and the pulp and seeds removed. The seeds were cleaned and freed from unwanted materials before they were shelled manually. Bad seeds were promptly removed. The good seeds were washed thoroughly and boiled for 1 h as described by Bakebain and Giami (1992). Thereafter, the seeds were dried in an oven at 50°C until a constant weight was achieved. The boiled and oven-dried seeds were then milled in a laboratory miller and used for the study.

Experimental design: Forty adult male albino rats (wistar strain) eight weeks old, that weighed between 101-136 g obtained from the Animal Breeding Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, were used as the experimental animals. The rats were kept in cages for two weeks, allowed to acclimatize to Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus and were allowed free access to food and water *ad libitum*. The protocol was in line with the guidelines of the National Institute of Health (NIH) (NIH Publication 85-23, 1985) for laboratory animal care and use. Each animal was randomly assigned to four groups of ten animals each: A, B, C and D. Rats in group A served as control and received 250 g/day normal rat chow (Vital feed Ltd, Jos, Plateau State, Nigeria). Those in group B, C and D received normal rat chow supplemented with 12.5, 37.5 and 75 g/day boiled T. occidentalis seeds, respectively. The supplemented diet were homogenized, manually pelletized and oven dried at 50°C to a constant weight and stored in air-tight container, then dispensed to the rats daily. The study lasted for 28 days. During this period each animal received 25 g of feed daily and had unrestricted access to water. After 28 days, the rats were fasted overnight and anaesthetized with chloroform and bled by cardiac puncture. The blood was transferred to serum separator tubes, allowed to clot, thereafter, centrifuged for 10 min at 2500 g. The sera was carefully removed and placed into clean and appropriately labeled sample containers and stored frozen until the time of analysis.

Determination of serum lipid profile/indices: The lipid profiles were determined using Cobas reagent kits manufactured by Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim, Germany. Serum total cholesterol was determined by the method of Allain *et al*. (1974). HDL-cholesterol by Sugiuchi *et al*. (1995). LDL-cholesterol by Rifai *et al*. (1992) and triglycerides by Wahlefeld and Bergmeyer (1974). The concentrations of the biochemical parameters were measured using Roche/Hitachi 902 analyser.

Serum non-HDL cholesterol concentration was determined as reported by Brunzell *et al*. (2008):

1: Non-HDLC = TC-HDLC

The atherogenic indices were calculated as described by Dobiasova (2004):

1: Cardiac Risk Ratio (CRR) = TC/HDLC
2: Atherogenic Coefficient (AC) = TC-HDLC/HDLC
3: Atherogenic Index of Plasma (AIP) = Log(TG/HDL)

The biochemical analyses were carried out using the facilities of APIN/PEPFAR Laboratory University of Nigeria Teaching Hospital Ituku-Ozalla, Enugu State, Nigeria.

Statistical analysis: Data collected were subjected to analysis of Variance (ANOVA). In order to test whether or not significant differences existed between groups. Pair-wise comparisons were made using the Post-hoc test. The mean±SD of each parameter was taken for each group. Test probability value of $p<0.05$ was considered significant. The analyses were carried out on SPSS for Windows version 16.0.

RESULTS

The effects of T. occidentalis diet on serum lipid profile and non-HDL cholesterol concentration is shown in Table 1. From the results, there was a significant decrease in the serum total, LDL, Non-HDL and VLDL cholesterol concentrations relative to control ($p<0.05$) while there were no significant difference in the serum triglyceride levels ($p>0.05$). The results also showed that there was a significant increase in the serum HDL cholesterol levels relative to control ($p<0.05$). All these effects were more pronounced at 37.5 g/day level of supplementation.

Table 2 depicts the effect of T. occidentalis supplemented diet on cardiac risk ratio (CRR), atherogenic coefficient (AC) and atherogenic index of plasma (AIP). The results showed that there was a significant decrease in CRR, AC and AIP levels relative to control ($p<0.05$), though this effects is dosage dependent.

DISCUSSION

Cholesterol is an essential substance involved in many cellular functions, including the maintenance of membrane fluidity, production of vitamin D on the surface of the skin, production of hormones and possibly helping cell connections in the brain (Adaramoye *et al*., 2005; Daniels *et al*., 2009). It is important that the body cells should have adequate supply of cholesterol. However, when cholesterol levels rise in the blood, they can have deleterious consequences, particularly its role in atherosclerosis,

Table 1: Effects of Telfairia occidentalis supplemented diet on serum lipid profile and Non-HDL cholesterol

Parameters (mmo/L)	A (Control)	B (12.5 g)	C (37.5 g)	D (75 g)	p-value
T/Chol	2.00±0.24	1.85±0.19 (0.134)	1.77±0.15 (0.030)	1.76±0.24 (0.024)	0.095
HDL-C	1.26±0.12	1.33±0.11 (0.245)	1.52±0.12 (0.000)	1.53±0.14 (0.000)	0.000
NonHDL	0.74±0.13	0.51±0.21 (0.004)	0.25±0.10 (0.000)	0.23±0.12 (0.000)	0.000
LDL-C	0.34±0.17	0.17±0.02 (0.000)	0.22±0.06 (0.009)	0.27±0.05 (0.124)	0.002
VLDL	0.20±0.05	0.21±0.05 (0.630)	0.12±0.06 (0.010)	0.14±0.05 (0.046)	0.004
TG	0.99±0.30	1.11±0.30 (0.379)	0.87±0.26 (0.342)	0.75±0.18 (0.076)	0.040

p-value is significant at $p<0.05$; significant increases in the serum concentration of the biochemical parameters when the control group was compared with the treated groups ($p<0.05$). One-way ANOVA and Post Hoc test

Table 2: Effects of Telfairia occidentalis supplemented diet on cardiac Indices

Parameters	A (Control)	B (12.5 g)	C (37.5 g)	D (75 g)	p-value
CRR	1.53±0.15	1.39±0.17 (0.028)	1.16±0.06 (0.000)	1.14±0.07 (0.000)	0.000
AC	0.71±0.40	0.39±0.17 (0.003)	0.16±0.06 (0.000)	0.14±0.07 (0.000)	0.000
AIP	-0.05±0.06	-0.08±0.13 (0.615)	-0.24±0.09 (0.001)	-0.31±0.12 (0.001)	0.000

p-value is significant at $p<0.05$; significant increases in the levels of the cardiac Indices when the control group was compared with the treated groups ($p<0.05$). One-way ANOVA and Post Hoc test

the leading cause of death in developed countries around the world (Stamler *et al*., 2000; Daniels *et al*., 2009). On this basis, efforts have been put into reducing the risk of cardiovascular diseases through the regulation of cholesterol, thus the therapeutic benefits of plant foods have been the focus of many extensive dietary studies (Yokozawa *et al*., 2006; Zhang *et al*., 2007). Traditional plant remedies have been used for centuries in the treatment of diseases (Akhtar and Ali, 1984), but only a few have been scientifically evaluated. Therefore, the effects of the diet preparations of T. occidentalis seeds on the serum lipid profile and atherogenic indices of rats were studied and compared. From the results obtained in the study, it is apparent that the diet preparations lowered the serum cholesterol levels of rats. Interestingly, the effect is dose dependent. Adaramoye *et al*. (2007) had shown that T. occidentalis has hypolipidaemic effect in rats fed cholesterol rich diet. Hypoglycaemic effect of the seed extract has also been reported (Eseyin *et al*., 2007). Ahmed-Raus *et al*. (2001) suggested that the mechanism of this hypocholesterolaemic action may be due to the inhibition of the absorption of dietary cholesterol in the intestine or stimulation of the biliary secretion of cholesterol and cholesterol excretion in faeces. Growing evidence suggests that high density lipoprotein cholesterol (HDL-C) is a good predictor of cardiovascular disease (CVD). However, epidemiological, mechanistic and intervention studies suggest that low HDL-C is a major CVD risk factor and that increasing HDL-C plasma levels may be beneficial, particularly to patients with low HDL-C levels (Philip, 2007). The results from the study showed that the treatment with T. occidentalis diets led to a significant increase in serum HDL-C, showing their protective role in CVD. The comparison of the effects showed that the diet preparation induced a significant dosage dependent increase in serum HDL-C i.e., at 37.5 g (15%) and 75 g (30%) concentration. The protective role of HDL-C against CVD has been suggested to occur in several ways (Nofer *et al*., 2002). High HDL exerts a protective effect by decreasing the rate of entry of cholesterol into the cell via LDL and increasing the rate of cholesterol release from the cell (Marcel *et al*., 1980), i.e., by promoting reverse cholesterol transport through scavenging excess cholesterol from peripheral tissues and subsequent esterification using lecithin cholesterol acyltransferase and deliverance to the liver and steroidogenic organs for synthesis of bile acids and lipoproteins and eventual elimination from the body (Assmann and Gotto, 2004; Ademuyiwa *et al*., 2005) and inhibiting the oxidation of LDL as well as the atherogenic effects of oxidized LDL by virtue of its antioxidant (Brunzell *et al*., 2008) and anti-inflammatory property (Ademuyiwa *et al*., 2005). Barakat and Mahmond (2011) suggested that increase in HDL-C might be due to stimulation of Pre-β HDL-C and reverse cholesterol transport. Moreover, flavonoids have been reported to increase plasma HDL cholesterol in both normolipidemic and hyperlipidaemic rats (Middleton *et al*., 2000). The results agree with Feoli *et al*. (2003), who stated that the increase in HDL-C is one of the most important criteria of anti-hypercholesterolemic agent. Brunzell *et al*. (2008) has shown that non-HDL cholesterol is a better predictor of cardiovascular disease risk than is LDL cholesterol. Therefore, the significantly lower serum non-HDL cholesterol observed in the test groups relative to control indicates the ability of the seeds to reduce cardiovascular risk. High serum levels of LDL and VLDL cholesterol is a risk factor for cardiovascular disease (Ademuyiwa *et al*., 2005; Lichtennstein *et al*., 2006) and often accompanies hypertension (Zicha *et al.*, 1995) and diabetes mellitus while decreases in plasma LDL cholesterol have been considered to reduce risk of coronary heart disease (Shen, 2007). In this study, we observed a significantly lower serum LDL and VLDL cholesterol in the animals supplemented with 37.5 g/ body weight of T. occidentalis seed, indicating the likely cardio-protective effect of the seed at that dose. This effect may be attributable to the flavonoid content of the plant. Middleton *et al.* (2000) had earlier reported that flavonoids lower plasma LDL cholesterol in both normolipidemic and hyperlipidemic rats. Plant sterols are also known to lower plasma LDL cholesterol (Garoufi *et al.*, 2014). The result is in conformity with the findings of Ejike *et al.* (2010), who stated that excessive consumption of the seed could lead to hypolipidaemia. Fibres have been reported to decrease plasma LDL-C by interrupting the cholesterol and bile acid absorption and increasing LDL-receptor activity (Venkateson *et al.*, 2003). Barakat and Mahmond (2011), suggested that the fibre content of the seed could contribute to the decrease in serum LDL-C. A high plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases (McBride, 2007) and is often associated with metabolic syndrome. The diet had no effect on the plasma levels of triglyceride. Atherogenic indices are strong indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and vice versa (Dobiasova, 2004; Usoro *et al.*, 2006). According to Usoro *et al.* (2006), low atherogenic indices are protective against coronary heart disease. From the result obtained it's apparent that supplementation with T. occidentalis significantly lowered the atherogenic indices; CRR, AC and AIP indicating the likely cardio-protective properties. The anti-lipidaemic and anti-atherogenic effects of T. occidentalis seeds found in this study could be multifactorial. The observed effect might be due to presence high levels polyunsaturated fatty acids, flavonoids, phytosterols and vitamins (ElAdawy and Taha, 2001; Fagbemi, 2007).

Conclusion: In this study, we observed that the T. occidentalis seeds administered produced a significantly lower lipid profile. This effect is comparable to that reported for T. occidentalis leaves on albino wistar rats.

In conclusion, all of these results indicate a likely potential dose dependent cardio-protective mechanism of the seeds against the development of atherosclerosis and coronary heart disease. It implies that the seeds may help in the management of dyslipidemic conditions.

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