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Methanol Extract of Unfermented Theobroma Cacao Promotes Normal Lipid Profile of Wistar Rats

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ABSTRACT

Abnormal lipid metabolism is a major pathogenic factor for various cardiovascular diseases. The present study investigated the lipid profile of methanol extract of unfermented Theobroma cacao (TC) in Wistar rats. 24 Male Wistar rats were divided equally into four groups. Group 1 was the control group, and was administered 0.9% normal saline. Groups 2, 3 and 4 were administered 200mg/kg, 400mg/kg and 800mg/kg methanol extract of unfermented TC. Administration was via oral gavage and lasted for 21 days. The rats were sacrificed under chloroform anaesthesia. Blood was collected through cardiac puncture, allowed to clot, and later centrifuged to get serum. Laboratory assays were done for serum concentrations of total cholesterol (Tc), triacylglycerides (TAG), high density lipoprotein (HDL-c), and low density lipoprotein (LDL-c). Administration of TC extract resulted in an increase ($p<0.05$) serum concentration of HDL-c, with a consequent reduction in the serum concentrations of Tc, TAG, and LDL-c when compared with the control. The observed results showed that consumption of unfermented Theobroma cacao within the experimental dose promotes normal lipid profile of Wistar rats. Thus, if these results are extrapolated to man, consumption of unfermented Theobroma cacao is encouraged.

Keywords: *Theobroma Cacao, Total cholesterol, Triacylglycerides, High Density Lipoprotein*

INTRODUCTION

Cocoa (*Theobroma cacao*) belongs to the genus *Theobroma*, a group of small trees which grow in the Amazon basin and other tropical areas of South and Central Africa (fig. 1). They are classified under the subfamily Sterculioidea of the mallow family Malvaceae. The medicinal value of cocoa plants have assumed a more important dimension in the past few decades owing largely to the discovery that, extracts from cocoa plants contain not only minerals and primary metabolites, but also a diverse array of secondary metabolites with antioxidant potentials (Akinmoladum *et al.,* 2007).

Cocoa bean and its product (cocoa liquor, cocoa powder, and dark chocolate) are food sources rich in phenolic compounds (Arts *et al*., 1999). The pharmacologically active ingredients of cocoa seeds include amines, alkaloids, fatty acids, polyphenols, magnesium, phenylethylamine, theobromine, caffeine and N-acylethanolamines (Bruinsma and Taren, 1999). The health promoting properties of cocoa beans are attributed to their phenolic compounds, catechins and flavonols (Kelm*et al*., 2006), which are potent antioxidants that can attenuate inflammatory processes and other disease states.

Cocoa products also contain high levels of biologically active polyphenols that exert both acute and chronic antioxidant-associated health benefits (Kris-Etherton and Keen, 2002). Cocoa and cocoa products have also been shown to suppress the development of atherosclerotic lesions (Kurosawa *et al*., 2005), decrease platelet function (Murphy *et al*., 2003), increase dermal blood flow (Neukan, *et al*., 2007), inhibits the proliferation of human breast cancer cells (Ramljak*et al*., 2005), possess hypoglycaemic properties and decrease oxidation of LDL cholesterol (Tomaru*et al*., 2007). Due to its wide consumption, especially in food industries, with attendant increase in cardiovascular disorders, evaluating the effect of *Theobroma cacao* on lipid profile becomes very necessary.

Fig 1: *Theobroma cacao* tree with mature pods

MATERIALS AND METHODS

Plant Material

Unfermented *Theobroma Cacao* (cocoa beans) was obtained from a farm in Cross River State, Nigeria. The seeds were de-coated, ground and sieved into fine powder (cocoa powder). The methanol extract of *Theobroma Cacao* was obtained by macerating 1500 g of the cocoa powder in 2000 ml of methanol (BDH Ltd Poole, England) for 24 hours. The suspension was thereafter filtered with Whatman No.1 filter paper. The filtrate was concentrated under reduced pressure and the yield recorded.

Animals

Twenty-four (24) adult male Wistar rats (140 - 200g) were used for the study. They were divided into 4 groups of 6 rats each. Group 1 served as the normal control placed on normal saline. Groups 2, 3, and 4 were administered 200mg/kg, 400mg/kg, and 800mg/kg of methanol extract of unfermented *Theobroma cacao* respectively. Administration was via oral gavage, and lasted for 21 days. Approval was sought and consent granted by the Faculty of Biological Sciences Animal Research Ethics Committee, University of Nigeria, Nsukka, with Approval No: 019BCM20317. The animals were kept in plastic cages and controlled environment (12h light/dark cycles at 27 ± 2°C) one week for acclimatization before commencement of the study. The rats had free access to normal rat chow and tap water *ad libitum*.

Estimation of Lipid Profile

Blood samples of the rats were collected into sterilized centrifuge tubes on day zero, 14, and 21 of the experiment and were immediately spurned at 4000 rpm for 10 minutes. Serum was collected into clean sample bottles for biochemical assays. Lipid profile was evaluated by assessing Total cholesterol (Tc) [Siedel et al., 1983], Triacylglycerides (TAG) [Sullivan et al., 1985], High Density Lipoprotein (HDL-c), and Low Density Lipoprotein (LDL-c) [Friedewald et al., 1992]. All analysis was performed using commercially available kits based on the references and Manufacturer's instructions using analyzer.

Statistical Analysis

The results are presented as mean ± standard error of the mean. Data were analyzed using GraphPad prism software version 6.00 for Windows (GraphPad Software, San Diego, CA, USA). One-way analysis of variance with Turkey's post-test was performed, and probability level of $p<0.05$ was considered statistically significant.

Results

Effect of methanol extract of unfermented *TC* on serum total cholesterol

The mean serum total cholesterol in the control, 200mg/kg, 400mg/kg, and 800mg/kg of *TC* extract treated groups was 3.53 ± 0.12mmol/L, 3.10 ± 0.06mmol/L, 2.93 ± 0.07mmol/L and 2.57 ± 0.09mmol/L respectively on the 14th day of feeding, and 3.47 ± 0,03mmol/L, 2.80 ± 0.12mmol/L, 2.73 ± 0.09mmol/L, and 2.33 ± 0.03mmol/L respectively after the 21st day of feeding. There was a significant ($p<0.05$) decrease serum total cholesterol in the *TC* treated group when compared with the control group. This is presented in table1.

Effect of methanol extract of unfermented *TC* on serum low density lipoprotein (LDL-c)

The LDL-c levels in the control, 200mg/kg, 400mg/kg and 800mg/kg extract treated groups was 1.60 ± 0.06mmol/L, 1.10 ± 0.57mmol/L, 1.06 ± 0.06mmol/L, and 1,13 ± 0.008mmol/L respectively on the 14th day of feeding, and 1.53 ± 0.003mmol/L, 1.20 ± 0.10mmol/L, 1.10 ± 0.10mmol/L, and 1.10 ± 0.06mmol/L after the 21st day of the feeding period. The result showed decrease ($p<0.05$) levels of LDL-c in the *TC* treated groups compared with the control. This is presented in table 2.

Effect of methanol extract of *TC* on serum triacylglyceride (TAG) level

The serum triglyceride level in the control, 200mg/kg, 400mg/kg, and 800mg/kg extract treated groups was 1.27 ± 0,07mmol/L, 1.20 ± 0.06mmol/L, 1.27 ± 0.03mmol/L, and 1.03 ± 0.09mmol/L respectively on the 14th day of feeding, and 1.30 ± 0.06mmol/L, 1.17± 0.07mmol/L, 0.97 ± 0.09mmol/L, and 0.77 ± 0.09mmol/L respectively after the 21st day of the feeding period. Significant reductions ($p\leq0.05$) in triacylglycerol (TAG) were only observed in the group administered 400 and 600mg/kg of the extract after 21 days. This is presented in table 3.

Effect of methanol extract of *TC* on serum high density lipoprotein

HDL-c level in the control, 200mg/kg, 400mg/kg, and 800mg/kg extract treated groups after the 14th and 21st days of feeding was 1.33 ± 0.09mmol/L, 1.63 ± 0.13mmol/L, 1.67 ± 0.15mmol/L, 1.83 ± 0.07mmol/L, and 1.47 ± 0.07mmol/L, 1.87 ± 0.07mmol/L, 2.07 ± 0.03mmol/L, and 1.83 ± 0.19mmol/L respectively. The result showed a significant ($p<0.05$) increase level of HDL-c in the *TC* treated groups compared with the control. This is presented in table 4.

Table 1: Effect of methanol extract of unfermented *Theobroma cacao* on total cholesterol concentration (mmol/L) of Wistar rats

Days	Control	200mg/kg b.w	400mg/kg b.w	800mg/kg b.w
Day 0	$3.40 \pm 0.06_a$	$3.30 \pm 0.06_a$	$3.40 \pm 0.12_a$	$3.40 \pm 0.06_a$
Day 14	$3.53 \pm 0.12_a$	$3.10 \pm 0.06_b$	$2.93 \pm 0.07_b$	$2.57 \pm 0.09_b$
Day 21	$3.47 \pm 0.03_a$	$2.80 \pm 0.12_b$	$2.73 \pm 0.09_b$	$2.33 \pm 0.03_b$

Means with different lower case subscripts ($_{a, b, c}$) between and across groups are significantly different at $P<0.05$.

Table 2: Effect of methanol extract of unfermented *Theobroma cacao* on LDL-c (mmol/L) concentration of Wistar rats

Days	Control	200mg/kg b.w	400mg/kg b.w	800mg/kg b.w
Day 0	1.67 ± 0.09	1.47 ± 0.13	1.60 ± 0.12	1.60 ± 0.06
Day 14	$1.60 \pm 0.06_{a}$	$1.10 \pm 0.57_{b}$	$1.06 \pm 0.06_{b}$	$1.13 \pm 0.08_{b}$
Day 21	$1.53 \pm 0.03_{a}$	$1.20 \pm 0.10_{b}$	$1.10 \pm 0.10_{b}$	$1.10 \pm 0.06_{b}$

Means with different lower case subscripts ($_{a, b, c}$) between and across groups are significantly different at P<0.05.

Table 3: Effect of methanol extract of unfermented *Theobroma cacao* on TAG (mmol/L) concentration of Wistar rats

Days	Control	200mg/kg b.w	400mg/kg b.w	800mg/kg b.w
Day 0	1.30 ± 0.06	1.47 ± 0.09	1.43 ± 0.03	1.40 ± 0.06
Day 14	$1.27 \pm 0.07_{a}$	$1.20 \pm 0.06_{a}$	$1.27 \pm 0.03_{a}$	$1.03 \pm 0.09_{b}$
Day 21	$1.30 \pm 0.06_{a}$	$1.17 \pm 0.07_{a}$	$0.97 \pm 0.09_{b}$	$0.77 \pm 0.09_{b}$

Means with different lower case subscripts ($_{a, b, c}$) between and across groups are significantly different at P<0.05.

Table 4: Effect of methanol extract of unfermented *Theobroma cacao* on HDL-c (mmol/L) concentration of Wistar rats

Days	Control	200mg/kg b.w	400mg/kg b.w	600mg/kg b.w
Day 0	1.17 ± 0.03	1.27 ± 0.03	1.20 ± 0.06	1.23 ± 0.07
Day 14	1.33 ± 0.09	$1.63 \pm 0.13_{a}$	$1.67 \pm 015_{a}$	$1.83 \pm 0.07_{b}$
Day 21	1.47 ± 0.07	$1.87 \pm 0.07_{b}$	$2.07 \pm 0.03_{b}$	$1.83 \pm 0.19_{b}$

Means with different lower case subscripts ($_{a, b, c}$) across and between groups are significantly different at P<0.05.

Discussion

The present study investigated the effect of unfermented *Theobroma cacao* on lipid profile of Wistar rats. Administration of *TC* resulted in a decrease serum concentration of total cholesterol, low density lipoprotein, triacylglycerides, and an increase in the serum concentration of high density lipoprotein. Abnormal lipid metabolism is a major pathogenic factor for various cardiovascular diseases. Cholesterol for many years is reported to have a direct relationship with cardiovascular prognosis. An increase by 1% in total cholesterol, results in 2-3% increase in coronary heart disease (Carlson, Bottiger and Ahfeldt, 1979). Recent studies have shown that total cholesterol increase by 10% results in coronary-related mortality risk of about 38% (Law, Wald and Thompson, 1994). LDL on the other hand, is emphasized to be highly implicated in coronary heart disease (ILIB, 2003) and forms a discriminating criterion for cardiovascular risk.

Reports also showcase a relationship between LDH-cholesterol and triglycerides, indicating that in men, there is a 13% increase in the risk of cardiovascular disease, while women have a 37% increase risk, all resulting from increased levels of triglycerides (Criqui, *et al.*, 1993; Hokanson and Austin, 1996; Assman, Schulte and Eckardstein, 1996).

On the contrary, HDL-cholesterol has an inverse relationship with the risk of coronary heart disease. There is a 2-3% decrease in the risk of cardiovascular disease for every 1mg/dl elevation of HDL-Cholesterol (Gordon *et al.*, 1989). HDL-cholesterol is involved in cholesterol reversal transport, possesses anti-inflammatory capacity and protects against LDL-cholesterol oxidation (Ansell *et al.*, 2004). When the levels of HDL-cholesterol are low, the risk of cardiovascular diseases becomes prominent (Cui, *et al.*, 2001).

The ability of *TC* to promote normal lipid profile could be due to the presence of flavonoids and polyphenols known to have antioxidant and anti-inflammatory properties (Kelm*et al.*, 2006).

CONCLUSION

Our study suggests that *Theobroma cacao* promotes normal lipid profile. This is due to its ability to raise the serum concentrations of HDL-c, with a consequent decrease in the levels of LDL-c, TC, and TAG. This study therefore provides a basis for the use of *TC* as an alternative in the prevention, management, or control of dyslipidemia.

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