



## Hypolipidemic Activity of the Methanolic Extract of *Ficus thonningii* (Blume) Leaves in Hypercholesterolemic-Induced Rats

AHUR, V.M. <sup>1</sup>, UDEM, S.C. <sup>2</sup> and MADUBUNYI, I.I. <sup>2</sup>

<sup>1</sup>Department of Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria, <sup>2</sup>Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria\*Corresponding author. E-mail: leodemase@yahoo.com, Tel.: +234-806-5728084

### SUMMARY

*Ficus thonningii* has been reported to have nutritional and ethno-medical uses especially by the Igede-speaking people of Benue State, Nigeria. Therefore, this study was carried out to assess effects of methanolic leaf extract of *Ficus thonningii* (MEFT) against cholesterol-induced hyperlipidaemia in rats. Adult rats were divided into 6 groups of 5 rats each. Group 1 and 2 were orally administered 5 ml/kg/day of distilled water. Groups 3-6 received 10 mg/kg/day of simvastatin, 50, 100 and 200 mg/kg/day of extract respectively, for 30 days. Cholesterol (400 mg/kg/day) was also given to all the groups except group 1 for 30 days. On day 31, blood samples were collected for the determination of serum triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL-c), very low density lipoprotein (VLDL-c), and high density lipoprotein (HDL-c). MEFT (200 mg/kg) significantly lowered TG, TC, LDL-c and VLDL-c levels. At this dose the extract also significantly increased HDL-c levels when compared with the cholesterol treated group. These suggest the extract to possess preventive or therapeutic effects on diet-induced hyperlipidaemia.

**KEYWORDS:** hyperlipidaemia, lipoproteins, atherogenic index, cardiovascular diseases

### INTRODUCTION

In recent years much prominence has been given to the association of abnormal levels of lipids (e.g. total cholesterol, triglycerides, LDL and VLDL) with atherosclerosis and coronary artery diseases; which are the leading cause of death (Ross, 1993; Crowther, 2005). The World Health Organisation (WHO) estimates that every year, 12 million people worldwide die from cardiovascular diseases, with most of them being from the developing world (Kmietowicz, 2002). Hyperlipidaemia is characterized by elevated serum total cholesterol and low and very low density lipoprotein cholesterol and decreased high density lipoprotein levels. Standard treatment of dyslipidaemia with statins and other synthetic agents are available, although not without side effects (Jerzy and Anna, 2012). Many medicinal plants have been screened, evaluated and tested against various cardiovascular diseases including hyperlipidaemia, still the search is on for compounds that can safely and effectively control blood or tissue lipid (triglycerides, cholesterol, LDL-c) with minimal side effects.

*F. thonningii* Blume also known as the wild fig belongs to the Moraceae family and is widely used in local medicine for the treatment of sore throat, colds, diarrhoea,

jaundice, malaria fever, agalactia and gonorrhoea (Watt and Breyer, 1962; Ajayi, 2008). It has been observed to possess analgesic and anti-inflammatory properties (Otimenyin et al., 2004), antimicrobial activity (Ndukwe et al., 2007), bronchitis and urinary infection (Cousins and Huffman, 2002), reduced intestinal motility (Onwkaeme and Udoh, 2000). A survey of 100 households from two rural-settled groups in Fulani villages, Northeastern Nigeria, for dietary practices and the use of edible wild plants showed that *F. thonningii* was among those classified as 'good sources of protein and fat and excellent sources of calcium and iron or copper and zinc' (Lockett et al., 2000). The leaves of *F. thonningii* are prepared as vegetable (cuisine) by the Igede-speaking people of Benue State, Nigeria (Fadoju et al., 1991; Igoli et al., 2002). Phytochemical analysis of the plant revealed the presence of carbohydrates, soluble starch, glycosides, steroids, unsaturated steroids, aglycones, tannins, saponins, flavonoids, alkaloids, and anthraquinone glycosides (Trease and Evans, 1989; Ndukwe et al., 2007). Studies on the effect of *F. thonningii* on the serum lipid level are lacking. Hence, the present study was carried out to evaluate the effect of the methanolic extract of *F. thonningii* on serum lipid and electrolyte profiles.

## MATERIALS and METHODS

### Materials

#### Chemicals and Reagents

Assay kit for serum calcium, potassium and sodium were purchased from Teco Diagnostics, Anaheim, CA. The drugs used were cholesterol (BDH Chemicals Ltd, London) and simvastatin (Simvor 20® Ranbaxy, India). All reagent (n-hexane, methanol and tween 20) used were of analytical grade. Analytical equipment used were rotary evaporator (Buchi, Germany), autoanalyzer (Hitachi 902, Germany), spectrophotometer (UNICO

UV-2100 Spectrophotometer, US), analytical weighing balance (Metler-Toledo GmbH, Greifensee, Switzerland) and Hammer-mill (Lab mill Type-8, Christy and Norris Ltd, England). The different reagents used for the lipid profiles were procured from Rotche Ltd.

### Collection and identification of the plant material

Fresh leaves of *F. thonningii* were collected from Kyado village, Mbazum ward of Ukum Local Government Area of Benue State, Nigeria in May, 2009 and identified by a plant taxonomist in the Department of Botany, University of Nigeria, Nsukka. Voucher specimen has been deposited in the Department's herbarium for reference purposes.

### Preparation and extraction of plant material

The leaves of *F. thonningii* were air dried at room temperature and reduced to powder by grinding using a Hammer-mill with a 2 mm filter. The powder was stored in an air-tight container at room temperature until required for use. The dried powder (500 g) were measured out and put in a round bottom flask and exhaustively extracted with 800 ml of 80% methanol. The extraction was by cold maceration at room temperature (25-28°C) with intermittent shaking at 2 hr intervals for 48 hr. Whatman filter paper No 1 was used to filter the extract at the end of 48 hr. The filtrate was concentrated in vacuo using rotary evaporator connected to a cold water circulator at 40°C and 210 milibar yielded 31.78 g (6.4%) of methanol extract (MEFT) of *F. thonningii* leaves.

### Experimental animals

All the animals used in this study were obtained from a stock bred and maintained at the animal house of Olusegun Obansanjo College of Health Sciences, Benue State University, Makurdi, Nigeria. The animals

were kept in plastic cages and acclimatized for a period of 2 weeks at ambient temperature and cyclical diurnal changes in the animal house of the Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria. Standard commercial rat pellets (Vital feed®) prepared by Grand Cereal and Oil Company (GCOL) Jos, Nigeria and water were provided ad libitum. The laboratory animals were used in accordance with good laboratory practice regulation (TDR, 2001) and the principle of laboratory animal care as documented by Zimmerman (1983) and NIH (1985).

#### Median lethal dose (LD<sub>50</sub>) estimation of MEFT

Median lethal dose (LD<sub>50</sub>) was estimated using revised up-and-down procedure (UDP) as described by US EP (1998) and Gosh (1984). The upper limit test dose of 5000 mg/kg bw was used. Five healthy female rats (nulliparous and non-pregnant), aged 10 weeks and weighing between 118 and 138 g were fasted overnight before dosing. The limit dose of 5000 mg/kg bw of the methanolic extract of *F. thonningii* (MEFT) was administered to the 1st rat by gavage and the animal was observed for mortality and other signs of toxicity for a period of 48 hours following dosing (with special attention given during the first 4 hours) and thereafter for a period of 14 days. Since the 1st animal survived, four additional animals were sequentially dosed at approximately 48 hours interval making a total of 5 animals. The LD<sub>50</sub> was predicted to be above 5000 mg/kg bw if three or more rats survived the test for a period of 2 weeks.

#### Experimental procedure

Rats weighing between 100 -150 g were divided into six (6) groups of five (5) animals each (n = 5). Group 1 (normal

control) was administered a single dose of 2% tween 20 (5 ml/kg bw p.o). Groups 2-6 were fed a single dose of cholesterol (400 mg/kg bw p.o in 2% w/v tween 20 s.i.d). Simvastatin (10 mg/kg bw p.o in 2% w/v tween 20 s.i.d) was given to Group 3 (positive control) 6 hr post cholesterol administration. Groups 4-6 also received graded doses (50, 100, and 200 mg/kg bw p.o in 2% tween 20) of MEFT respectively 6 hr post-cholesterol administration. All the treatments lasted for 30 days. The weights of the all the animals were taken every week. At the end of the 30 day treatment (day 31st), blood samples were collected from the retro-orbital plexus after an overnight fast with the help of capillary tubes into non-anticoagulant test tubes. Serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) were analyzed by using a Hitachi system 902 automatic analyzer (Germany). Very low density lipoprotein cholesterol (VLDL-c) was calculated by using Friedewald formula (Friedewald et al., 1972). Atherogenic index (AI) was calculated using the formula of Abbot et al. (1988) and Coronary risk index (CRI) was calculated by the method of Alladi et al. (1989). Serum electrolytes (calcium, potassium and sodium) were determined by the method of Healy (1995) using commercial laboratory kits (Teco Diagnostics, Anaheim, CA). All the animals were sacrificed using ether and dissected to collect the liver, spleen, kidney, heart and lung for relative organ weight determination.

#### Statistical Analyses

The data on body weights, relative organ weight and biochemical parameters were analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 and expressed as mean±SEM. A test for significance at 5% level among groups was performed using One-way Analysis of

Variance (ANOVA). Least significance difference (LSD) post-hoc test was used to detect significant difference between the treatment and control groups.

## RESULTS

### Median lethal dose (LD50) determination

The methanolic extract of *F. thonningii* was found to be non-toxic at the test limit dose of 5000 mg/kg bw p. o. during the 14 days of observation. The LD50 of MEFT in rats was therefore estimated to be above 5000 mg/kg bw.

TABLE 1: The effect of MEFT leaves on serum triglycerides, cholesterol, HDL-c, LDL-c and VLDL-c in hypercholesterolemic-induced rats

Group	TGLY (mg/dL)	TCH (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)	FBS (mmol/L)
Normal Control	62.80±4.03	44.20±0.97	24.25±1.16	7.39±1.40	12.56±0.81	4.36±0.53
Cholesterol control	160.80±5.47 <sup>ac</sup>	139.60±4.99 <sup>ac</sup>	15.83±1.10 <sup>ac</sup>	91.60±5.00 <sup>ac</sup>	32.16±1.09 <sup>ac</sup>	4.26±0.34
Simvastatin control	72.20±3.02 <sup>b</sup>	62.20±4.45 <sup>a</sup>	27.43±2.87 <sup>b</sup>	20.33±2.73 <sup>ab</sup>	14.44±0.60 <sup>b</sup>	4.34±0.34
MEFT (50 mg/kg)	153.60±6.07 <sup>ac</sup>	136.60±2.62 <sup>ac</sup>	14.97±0.72 <sup>ac</sup>	90.92±3.23 <sup>ac</sup>	30.72±1.31 <sup>ac</sup>	3.48±0.36
MEFT (100 mg/kg)	151.80±5.48 <sup>ac</sup>	137.00±1.51 <sup>ac</sup>	15.05±1.56 <sup>ac</sup>	91.59±0.85 <sup>ac</sup>	30.36±1.10 <sup>ac</sup>	3.44±0.19
MEFT (200 mg/kg)	116.00±3.35 <sup>abc</sup>	102.00±5.51 <sup>abc</sup>	18.54±1.44 <sup>ac</sup>	64.06±5.97 <sup>abc</sup>	23.20±0.67 <sup>abc</sup>	3.46±0.33

Values are Mean±SEM, n=6. P<0.05 <sup>a</sup>=significantly different compared to normal control, <sup>b</sup>=significantly different compared to cholesterol control, <sup>c</sup>=significantly different compared to simvastatin control.

The effect of ethyl acetate extract of *F. thonningii* leaves on serum triglycerides, cholesterol, HDL-c, LDL-c and VLDL-c in hypercholesterolemic rats

Hypercholesterolemia was produced in rats by feeding a high cholesterol diet over a period of 30 days. Table 1 revealed significant (P<0.05) increase in serum TG, TC, LDL-c and VLDL-c and a significant (P<0.05) decrease in HDL-c levels in the hypercholesterolemic-induced rats with values of 160.80±5.47, 139.60±4.99, 91.60±5.00 and 32.16±1.09 and 15.83±1.10 mg/dL respectively. These values were significantly different (P<0.05) in comparison with the normal control (62.80±4.03, 44.20±0.97, 7.39±1.40 and 12.56±0.81 and 24.25±1.16 mg/dL). Treatment with MEFT at 50 and 100 mg/kg bw did not produce any significant (P>0.05) effects on hypercholesterolemic animals. But treatment with MEFT at a dose level of 200 mg/kg bw caused a significant (P<0.05) reduction in TG, TC, LDL-c and VLDL-c and further increased serum levels of HDL-

c significantly (P<0.05). There was no significant difference between the activity of the extract at this dose and that of simvastatin (Simvor 20®).

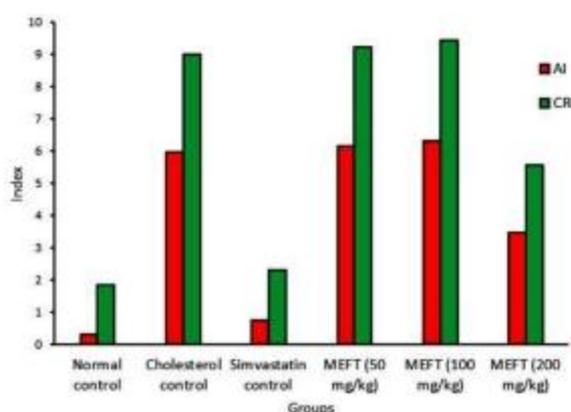
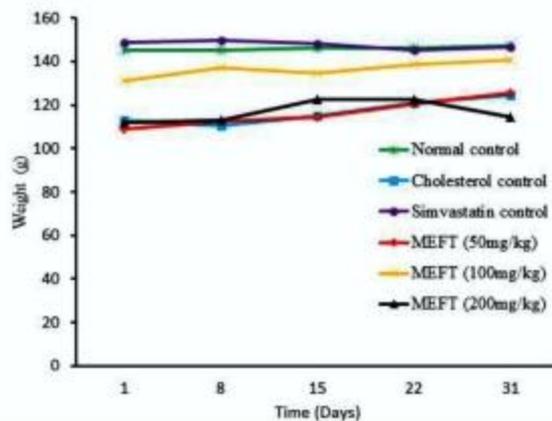
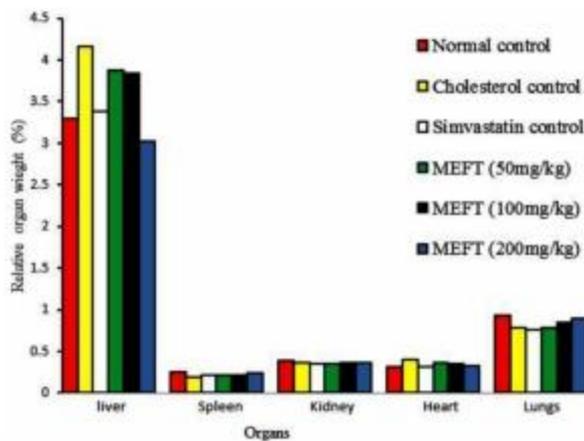


Figure 1: The effect of MEFT on atherogenic index (AI) and coronary risk index (CRI) in hypercholesterolemic-induced rats



**Figure 2:** Changes in mean body weight of rats fed with high cholesterol diet (n=5, values are mean of body weights,  $P < 0.05$ )



**Figure 3:** The effect of MEFT on relative organ weight of hypercholesterolemic rats (n=5, a=significantly different compared to the cholesterol control at  $P < 0.05$ )

Simvastatin at the dose of 10 mg/kg lowered serum triglycerides, cholesterols, LDL-c and VLDL-c significantly but did not bring them down to baseline values in comparison with the normal control that received only the diluent (2% tween 20) (TABLE I). Table 1 also depicts effects of 30 days of oral treatment with 400 mg/kg/day of cholesterol, 10 mg/kg/day of simvastatin and 50-200 mg/kg/day of MEFT on fasting blood glucose. There was

no significant difference in the values of FBS among the control and the treatment groups. However, MEFT at 50-200 mg/kg showed lower values of FBS compared to normal, cholesterol and simvastatin controls, though the differences were not significant ( $P > 0.05$ ).

### The effect of MEFT on atherogenic index (AI) and coronary risk index (CRI) in hypercholesterolemic rats

Hypercholesterolemic-induced rats without treatment (cholesterol control group) showed a significant ( $P < 0.05$ ) increase in the ratios of LDL-c:HDL-c (AI) and cholesterol:HDL-c (CRI). Cholesterol at 400 mg/kg bw p.o significantly increased ( $P < 0.05$ ) the AI and CRI of rats in the cholesterol control rats. However, rats treated with MEFT at the dose of 200 mg/kg caused a significant ( $P < 0.05$ ) reduction in cardiovascular risks index (i.e. AI and CRI) of experimental rats (Fig 1). The results also showed that MEFT at lower doses (50 and 100 mg/kg bw) did not reduce the AI and CRI index in hypercholesterolemic-induced rats.

### Effect of MEFT on mean body weight of rats fed with high cholesterol diet

Group 1 (normal control) and 3 (simvastatin control) showed relative constant mean body weights throughout 30 days of experimentation. However, body weight of control rats decreased in the first week from 113.0 g to 110.8 g in the second week of the experimentation and later increased significantly ( $P < 0.05$ ) to a value of 124 g in the 4th week (Fig 2). Rats in group 4 and 5 treated with 50 and 100 mg/kg of MEFT together with high cholesterol diet showed a progressive mean body weight gain throughout the 30 days of experiment. MEFT at 200 mg/kg bw reversed the progressive increase in mean body weight from 122.8 g in the 3rd week to 114.8 g in the 4th week of treatment (Fig 2).

#### Effect of MEFT on relative organ weight of hypercholesterolemic rats

The mean relative weights of spleen, kidney and lungs were not significantly different ( $P>0.05$ ) among the treatment and control groups. However, the relative weight of the liver, significantly increased ( $P<0.05$ ) in the cholesterol control group ( $4.20\pm0.13\%$ ) compared to the normal control ( $3.30\pm0.33\%$ ) and the simvastatin control ( $3.38\pm0.27\%$ ). Also, the relative weight of the heart, significantly increased ( $P<0.05$ ) in the cholesterol control group ( $0.40\pm0.02\%$ ) compared to the normal control ( $0.31\pm0.03\%$ ) and the simvastatin control ( $0.32\pm0.02\%$ ). MEFT at 200 mg/kg bw also showed a considerable reduction of the relative liver and heart weights ( $3.02\pm0.19\%$  and  $0.33\pm0.02\%$ ) similar to the normal and simvastatin control groups (Fig 3).

#### Effects of MEFT on serum calcium, potassium and sodium ions of hyperlipidaemia-induced rats

Serum concentrations of calcium ions were relatively similar in the all the groups except cholesterol control that have significantly lower calcium values compared to the normal and simvastatin controls (TABLE II). Serum potassium ions of the animals given MEFT at dose of 200 mg/kg bw were significantly ( $P<0.05$ ) lower compared to the control groups (TABLE II). However, the differences were not significant ( $P>0.05$ ) (TABLE II). All serum concentrations of sodium ions in the control groups were similar, but MEFT at all doses (50, 100 and 200 mg/kg bw) showed a decrease in sodium ion levels, but the difference were also not significant ( $P>0.05$ ) (TABLE II).

TABLE 2: Effects of MEFT on serum calcium, potassium and sodium of hyperlipidemia-induced rats

Group	Ca <sup>2+</sup> (mmol/L)	K <sup>+</sup> (mmol/L)	Na <sup>+</sup> (mmol/L)
Normal control	2.49±0.10	4.08±0.15	142.84±4.08
Cholesterol Control	2.22±0.12 <sup>ac</sup>	4.70±0.23	144.96±9.48
Simvastatin control	2.47±0.82	4.78±0.38	142.80±7.66
MEFT (50 mg/kg)	2.30±0.05	4.64±0.28	123.40±9.80
MEFT (100 mg/kg)	2.26±0.07	4.04±0.19	130.00±9.35
MEFT (200 mg/kg)	2.35±0.04	3.22±0.32 <sup>abc</sup>	124.26±4.96

Values are Mean±SEM, n=6.  $P<0.05$  <sup>a</sup>=significantly different compared to normal control, <sup>b</sup>=significantly different compared to cholesterol control, <sup>c</sup>=significantly different compared to simvastatin control.

#### DISCUSSION

The body supply of lipid energy in the form of fatty acids comes from the diet via the small intestine or through endogenous fatty acid synthesis, primarily in the liver. These dietary fatty acids are esterified to form triglycerides (TG) (Brites et al., 1998) and cholesterol, which are stored by fat

cells in lipid droplets (Sniderman and Cianflone, 1999). Upon demand, intracellular triglycerides and cholesterol are hydrolyzed by the action of hormones sensitive lipase to release free fatty acids in the form of lipoproteins. Lipoproteins are known to transport cholesterol and triglycerides in the form of chylomicron,

very low density lipoprotein (VLDL-c), low density lipoprotein (LDL-c) and high density lipoproteins (HDL-c) (She and Wang, 1999; Hesser et al., 1979; Mackness and Durrington, 1995). These fatty acids are oxidized during Krebs's cycle to generate energy in the liver (Murray et al., 2006). Fatty acid oxidation is one of the major sources of energy utilized by the body for metabolic purposes. But excessive consumption of foods rich in fatty acids might lead to hyperlipidaemia which predisposes the body to complications of vascular diseases (Saleh et al., 1999; Nakanishi et al., 2002). Oxidative modification of LDL-c appears to have an important role in initiation and progression of atherogenic changes in arterial walls especially the aorta (Esterbauer et al., 1993). Cardiovascular diseases have reached an alarming prevalence rate, and recent researches have shown that cardiovascular problems in patients could be reduced with cholesterol lowering agents (Mahmood et al., 2010). As a result of severe organ toxicity from available chemical therapeutics with hypolipidaemic properties, herbal medicines in many countries of the world have become potential sources of hypolipidaemic drugs that are less toxic, affordable and readily available.

In this study, feeding the animals with cholesterol supplement resulted in significant ( $P < 0.05$ ) increase in the levels of serum triglycerides, cholesterols, LDL-c and VLDL-c. The same finding has been made by Demacker et al (1991) where hypercholesterolemic diet fed to animals induced production of cholesteryl ester rich-VLDL-c by the liver and intestine. There was also reduction in the rate of cholesterol removal by the hepatic LDL receptors (Goldstein et al., 1983) resulting in increased levels of LDL-c and VLDL-c. In our study, MEFT at lower doses (50 and

100 mg/kg bw) did not exert significant lipid lowering effect, but at high doses (200 mg/kg bw) was able to improve the levels of HDL-c and reduced the levels of triglycerides, cholesterols, LDL-c and VLDL-c significantly ( $P < 0.05$ ). In effect, there was a significant ( $P < 0.05$ ) reduction in atherogenic risks (AI, CRI) (Kinosian et al., 1994; Hermansen et al., 2003). The mechanism of action of this lowering of levels of triglycerides, cholesterols, LDL-c and VLDL-c and increased levels of HDL-c is not known. One of the mechanisms of reducing the levels of triglycerides, LDL-c and VLDL-c is by making cholesterol unavailable since it is used for the synthesis of these constituents. In effect, hypolipidaemic agent will inhibit cholesterol absorption from GIT and also inhibit the activities of the enzyme, Acyl-CoA cholesterol acyltransferase (ACAT), a key enzyme responsible for the esterification, absorption and synthesis of cholesterol (Katsuren et al., 2010). This agent will also be capable of increasing the levels of HDL-c. HDL-c may be protective by reversing cholesterol transport, inhibiting the oxidation of LDL-c and has the capacity to neutralized atherogenic effects of oxidized LDL-c (Wilson, et al., 1988). Literature has reported the hypolipidaemic effects of flavonoids, alkaloids, saponins and tannins (Chapman, 1995). The presence of these phytochemicals in MEFT (Trease and Evans, 1989; Ndukwe et al., 2007) may be responsible for these observed pharmacological effects. Flavonoids in particular have been shown to possess hypolipidaemic effects by preventing LDL-c oxidation, a prerequisite for hyperlipidaemia (Bangham and Horne, 1962). Saponin is known to elicit serum cholesterol lowering activity by causing resin-like action, thereby reducing the enterohepatic circulation of bile acids (Topping et al., 1980); in the process, the conversion of cholesterol to bile acids is

enhanced in the liver resulting in concomitant hypocholesterolemia (Kritchevsky, 1977; Potter et al., 1979). These phytoconstituents may also have inhibitory effect on ACAT, by inhibiting the absorption and storage of metabolic fatty acids, thereby reducing the levels of plasma cholesterol and triglycerides levels. This will ultimately reduce the levels of VLDL-c and LDL-c in the liver and further prevent cardiovascular attacks. Literary works revealed a dearth of knowledge of *F. thonningii* with respect to diabetes mellitus, therefore a preliminary assessment of fasting blood sugar (FBS) in the 30 day antihyperlipidaemic study of the plant revealed the plant to lower blood glucose levels significantly as compared to the control groups. This result may point to the antidiabetic property of the plant but required further validation.

The relative organ weight is very important in the proper functioning of the organs of the body. The weight of the organs in the body increases in direct proportion with increase in the body weight of the animals. In diseased states, this may not be so. In the analysis of relative organ weight, average organ weights of spleen, heart, kidney and lungs at the end of the experiments were within the normal range. MEFT at 200 mg/kg bw significantly ( $P < 0.05$ ) inhibited the liver weight gain induced by high cholesterol diet compared to cholesterol control group. This could be suggested that MEFT might have blocked the accumulation of fatty acid in the hepatocytes. Similarly, MEFT (200 mg/kg bw) significantly reverses the progressive body weight gain (obese) in the last week of the experiment to almost the base line values comparable to the normal and simvastatin controls. This could be as a result of the inhibition of cholesterol absorption from the intestine as phytochemical analysis of *F. thonningii* elucidates the presence of saponins (Trease

and Evans, 1989; Ndukwe et al., 2007). Saponins are reported to prevent cholesterol absorption, interfere with its enterohepatic circulation and increase its faecal excretion (Chapman, 1995; Purohit and Vyas, 2006). Again, these hypotheses would require experimental validation. However, the prevention of body weight gain by MEFT at 200 mg/kg bw is in contrast with the report of Coker et al. (2009) who reported the weight gain effect of methanolic extract of *F. thonningii*. This could be as a result of the dietary value (having a crude protein value of 7 g/100kg) of the plant as reported by Ademosun et al. (1988) and Bamikole et al. (2001). Lockett et al. (2002) reported "chediya" (*F. thonningii*) to be an excellent source of dietary protein, calcium and iron or copper and zinc; therefore, serum analysis of some electrolyte showed a significant decrease in serum calcium ( $Ca^{2+}$ ) in the group treated cholesterol alone and also a significant decrease in potassium levels of the group treated MEFT at 200 mg/kg bw. Type of fat in diet plays an important role on serum calcium concentration and bone health (Amr et al., 2010). Feeding of experimental rats with high levels of cholesterol diet resulted in a significant decrease in serum calcium ions (TABLE II). This finding is in agreement with the findings of Watkin et al. (1996) who reported that growing animals fed saturated fat enriched diets had significantly greater absorption rate of calcium by bones, increased bone formation and subsequently low calcium plasma or serum concentration. Therefore, low calcium concentration encountered in our study (cholesterol control group) may be as a result of increased calcium uptake by bones induced by hypercholesterolemic diets. A number of drugs are reported to increase renal loss of serum potassium (hypokalaemia) including diuretics (non potassium sparing), cisplatin, aminoglycosides, amphotericin and foscarnet and acetazolamide (Crook,

2006), therefore, the observed hypokalaemia in the group treated MEFT (200 mg/kg bw) may be as a result of the extract inducing renal lost of serum potassium as the dose increases.

From this study, we can suggest that MEFT possess hypolipidaemic effects by lowering serum triglycerides, cholesterol, LDL-c and VLDL-c, but enhancing HDL-c values in hypercholesterolemic-induced rats. The possible mechanism of MEFT on prevention of hyperlipidaemia may be related to its ability to prevent oxidation of LDL-c or inhibition of intestinal absorption of dietary cholesterol. Further studies in other animal species and in other hyperlipidaemic model are required to elucidate its hypolipidaemic activity.

#### CONFLICT OF INTEREST STATEMENT

This is an original work done by us. There is no conflict of interest regarding financial, personals or the relationship with other people or organizations. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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