

## LDL-cholesterol Lowering Effect of *Terminalia littoralis* Decoction: Hypothesized Mechanisms

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### ABSTRACT

Traditional medicines are administered mostly as decoctions. The mechanism of the LDL-cholesterol lowering effect of *Terminalia littoralis* decoction used in ethno-medicine is hypothesized. The decoction prepared from fallen dry leaves of *T. littoralis* was screened for its relevant phytochemical contents. The pH, concentration and fibre content were of the decoction determined. A feeding study using the decoction as the only source of fluid was carried out for 35 days using albino rates of the Wistar strain. Anthropometric measurements and serological examinations were also carried out. Hypothetical deductions were based on the presence of phytochemical and biochemical constituents with reported pharmacological activities in relation to pharmacological outcome. The decoction did not significantly ( $p > 0.05$ ) affect the liver function indices. It however significantly ( $p < 0.05$ ) increased body weight and conversion of feed mass to body mass but reduced serum LDL cholesterol concentration and LDL-cholesterol/ HDL-cholesterol ratio. The hypothesized mechanisms were that decoction's phytosterols competitively inhibited uptake of dietary cholesterol by intestinal cells and HMG-CoA reductase activity, the fibre content reduced enterohepatic bile acid cycle, the catechins increased LDL receptor activity and the tannin, flavonoid and saponin prevented the oxidation of LDL. In conclusion, the phytochemical and biochemical constituents of the *Terminalia littoralis* decoction lowered LDL-cholesterol through a combination of different biochemical mechanisms.

**Key words:** Decoction, ethno-medicine, mechanism of action, LDL-cholesterol, lowering effect

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### INTRODUCTION

The Leadwood tree family, Combretaceae, to which *Terminalia* belongs is made up of 250 species (Bep, 1986). The tropical almond tree (*Terminalia catappa* Linn.) is one of the most popular. It is also popularly referred to as the 'fruit' or 'umbrella' tree. The various extracts of the leaves and bark have been reported to have anti-diabetic, antioxidant and hepatoprotective values (Chen *et al.*, 2000; Nagappa *et al.*, 2003). *T. littoralis* Seem. is a look-a-like species of *T. catappa* with smaller leaves and fruits (Thomson and Evans, 2006). *T. littoralis* is used to treat eye complaints, thrush and rheumatism while *T. catappa* is used to treat eye complaints, infections of the oral cavity, thrush, sore throat, cough, intestinal complaints, diarrhoea, emetic, menstrual complaints, puerperal complaints, rheumatism and wounds in Samoa ([http://www.ctahr.hawaii.edu/adap/Publications/ADAP\\_pubs/1993-1.pdf](http://www.ctahr.hawaii.edu/adap/Publications/ADAP_pubs/1993-1.pdf); Thomson and Evans, 2006). Ram *et al.* (1997) have also reported the medicinal values of

*T. arguna*. In ethno-medicine, decoctions of the *Terminalia* species are administered as home-made prophylactic agents against sickle cell disease (SCD) crises as well as 'tea' for weight related ailments.

Traditional medicines are widespread throughout the world and is more accessible to most of the population in the Third World (Sofowora, 2002). They provides most of the health-care needs of most rural dwellers in Nigeria. This involves the use of local gin, oils and assorted local herbs (Ekwenye and Ijeomah, 2005). Medicines administered traditionally are pharmacologically decoctions (Sofowora, 2002). Most plant materials used in ethno-medicine are of no known biochemical constituents and modes of action other than that they are efficacious at treating the debilitating malady. Their phytochemical and biochemical constituents may be responsible for their acclaimed effects. This study analysed the decoction prepared from fallen dry leaves of *Terminalia littoralis* for its phytochemical constituents, conducted a rat feeding study using the decoction as the only source of fluid for

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35 days and conducted serological examinations. Hypothetical deductions of the biochemical mechanisms were made based on the presence of bioactive principles with reported pharmacological activities in the decoction.

## MATERIALS AND METHODS

**Procurement of leaf sample and feeds:** The fallen dried leaves of *Terminalia littoralis* Seem. (Herbarium number: IMSUH 127) were picked from under their trees at Nekede, Owerri, Nigeria. The leaves were authenticated by Prof. S.E. Okeke, a plant taxonomist, of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The guinea feeds used (growers mash, product of Bendel Feed and Flour Mill Limited, Sapele, Delta State, Nigeria), were purchased at the Relief Market, Owerri, Nigeria. All the chemicals used were of analytical-reagent grade and were purchased locally.

### Procurement and housing of experimental rats:

The thirty-two albino rats (*Rattus norvegicus*) used as models in the study were purchased from the animal facility of the Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria, on the 5th day of November, 2011. They were of the Wistar strain and weighed between 200 to 250 g. The rats were randomly distributed on weight basis into four groups of eight rats each. Their body weights were equalized as nearly as possible with mean weight of  $225.0 \pm 3.5$  g per group and were housed in wire- screened stainless steel cages with troughs for feed and fluid. They were placed under the same room temperature and circadian rhythm, and acclimatized for four days.

All procedures were conducted in accordance with the internationally accepted principles for laboratory animal use and care as encapsulated in the United States National Institute of Health guidelines of 1985 (NRC, 1985).

**Preparation of *Terminalia littoralis* decoction:** Fallen dry leaves of the *T. littoralis* were cleaned of debris and washed in distilled water. Each leaf was then mopped dry of water and chopped into bits. The leaf bits (100 g) were put in an aluminum pot and 4600 mL of distilled water was poured in. This was brought to boil and allowed to simmer for 20 min. The decoction produced was filtered off the leaf bits using muslin clothes.

**Determination of pH, concentration and crude fibre content:** The pH, concentration and crude fibre contents of the decoction were evaluated using the methods of AOAC (1984).

### Qualitative analysis for phytochemical constituents:

The presence of tannins, catechins and flavonoids in the decoction were determined according to the methods of Evans (2002). Saponins were detected using the frothing and red blood cell haemolysis tests described by Harborne (1973). Test for the presence of phytosterols (using the chloroformic cholesterol reactions with acetic anhydride and concentrated  $H_2SO_4$ ) was carried as described by Plummer (1971).

### Bioassay and collection of specimens:

Administration of the decoction was done according to the method of Pepato *et al.* (2001) who administered *Eugenia jambolana* leaf decoction in place of water on streptozotocin diabetic rats. The test rats drank the *T. littoralis* decoction in place of water as their only source of fluid. The test and control rats were placed *ad libitum* on the guinea feeds for 35 days, respectively. The control rats drank distilled water. The total feed consumed and total volume of fluid drunk by each rat for the duration of the experiment were also noted. The rats were euthanized in chloroform vapour, incisions were made into their abdominal and cardiac cavities and blood specimens were collected by cardiac puncture using a 5 mL hypodermic syringe and needles. The blood was allowed to clot and the sera collected by simple aspiration using Pasteur's pipette for serological examinations. The organs were excised, washed in 10% formosaline, blotted dry and weighed.

**Statistical analysis:** Data were analysed using the student's t-test of significance. Values were declared significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

Organic acids act as buffers in the vacuoles of plant cells (Lehninger *et al.*, 2000). Some of the metabolic reactions that take place in cells may have given rise to some of the phytochemicals and biochemicals detected or quantified in the decoction (Table 1). Ibegbulem *et al.* (2010) detected the same types of phytochemicals in *T. catappa* decoction. The organic acids which arise from the plethora of metabolic reactions that occur in plant

Table 1: Phytochemicals of interest, pH, concentration and fibre content of the decoction

Parameter	Result
Tannins	+
Flavonoids	+
Saponins	+
Catechins	+
Phytosterol	+
pH <sup>†</sup>	$5.95 \pm 0.01$
Concentration (mg mL <sup>-1</sup> ) <sup>†</sup>	$17.00 \pm 0.11$
Fibre content (%) <sup>†</sup>	$0.12 \pm 0.01$

Key: + = Detected, <sup>†</sup>Values are Mean  $\pm$  SD of triplicate determinations

Table 2: Effects of decoction on weight gained, total feed consumed and feed conversion ratio of test rat<sup>†</sup>

Group	Weight gained (g)		Total feed consumed (g)		Feed conversion ratio	
	M	F	M	F	M	F
Test (8)	62.78±11.33*	60.71±7.29*	338.64±6.35	337.86±7.91	5.39±0.56*	5.57±0.85*
Control (8)	41.94±9.14	38.27±13.41	335.74±19.71	338.02±16.80	8.01±0.46	8.83±0.47

<sup>†</sup>Values are Mean ±SD of the number of responses in parenthesis, M = Male rat, F = Female rat, \*Significantly different at p<0.05

Table 3: Effects of the decoction on liver function indices of test rats

Group	Parameter <sup>†</sup>											
	AST (U/l)		ALT (U/l)		AST: ALT		Total bilirubin (mg dL <sup>-1</sup> )		Total protein (g L <sup>-1</sup> )		Albumin (g L <sup>-1</sup> )	
	M	F	M	F	M	F	M	F	M	F	M	F
Test (8)	21.50±1.00	21.00±1.45	28.18±5.64	23.80±3.05	0.76±0.18	0.88±0.48	0.99±0.13	0.99±0.13	57.75±9.90	61.05±1.91	39.20±1.00	36.30±3.65
Control (8)	20.98±0.45	20.75±0.25	24.73±2.36	24.48±5.00	0.85±0.21	0.85±0.05	1.13±1.06	1.16±0.06	58.58±4.15	61.05±1.91	36.30±1.81	36.30±1.81

<sup>†</sup>Values are Mean ±SD of number of responses in parenthesis, AST = Aspartate aminotransferase; ALT = Alanine aminotransferase, M = Male rat, F = Female rat

Table 4: The effects of decoction on lipid profile of test rat serum

Group	Lipid (mg dL <sup>-1</sup> ) <sup>†</sup>							
	Triacylglycerol		VLDL-C		Total cholesterol		HDL-C	
	M	F	M	F	M	F	M	F
Test (8)	59.23±3.66	63.39±18.31	11.85±0.73	13.08±3.66	84.73±32.50	87.50±14.59	60.2±4.52	60.85±14.25
Control (8)	63.47±14.56	75.00±18.45	12.70±2.92	15.00±3.69	100.00±9.06	100.00±15.70	59.42±1.58	57.87±1.20

  

Group	Lipid (mg dL <sup>-1</sup> ) <sup>†</sup>					
	TC: HDL-C		LDL-C		LDL-C:HDL-C	
	M	F	M	F	M	F
Test (8)	1.40±0.24	1.53±0.02	26.39±8.33*	38.89±6.41*	0.45±0.08*	0.64±0.08*
Control (8)	1.68±0.07	1.73±0.13	63.89±3.21	65.28±2.78	1.08±0.01	1.13±0.11

<sup>†</sup>Values are Mean ±SD of responses in parenthesis, VLDL-C = Very low density lipoprotein-cholesterol, HDL-C = High density lipoprotein-cholesterol, TC = Total Cholesterol, LDL-C = Low Density Lipoprotein-cholesterol, M = Male rat, F = Female rat, \*Significantly different at p<0.05

cells may have been responsible for the acidity of the decoction. That means that all plant decoctions may be acidic since metabolic reactions occur in plants.

The test rats gained significantly (p<0.05) more weight than their control counterparts (Table 2). This indicated that the decoction presented them with extra nutrients other than those present in the feed. The nutrient content of the decoction is however suggested for further studies. There was no significant (p>0.05) difference between the feed consumption of the test and control rats. However, the feed conversion ratio of the test rats significantly (p<0.05) decreased relative to their control counterparts. This meant that the decoction increased the conversion of feed mass to body mass; by 32.71% for the male rats and 36.92% for female rats.

The liver function indices were not significantly (p>0.05) affected when the decoction was drunk in place of water (Table 3). This meant that the decoction was not toxic. Sex did not also influence the metabolism of the decoction, since there was no variation between the sexes. Ram *et al.* (1997) also reported that liver and renal functions parameters were not adversely affected when the tree bark extract of *T. arguna* was administered to rats.

Table 4 showed that the decoction did not significantly (p>0.05) reduce the serum triacylglycerol, VLDL-cholesterol, total cholesterol concentrations and

the total cholesterol/HDL-cholesterol ratio. It also did not increase significantly (p>0.05) the serum HDL-cholesterol concentration. The decoction however significantly (p<0.05) reduced the serum LDL-cholesterol concentrations and the LDL-cholesterol/HDL-cholesterol ratios of the test rats. The reduction in these indices were significantly (p<0.05) more in the male rats; indicating sex dependency. The reduction in these parameters also confirmed the LDL-cholesterol lowering effect of the decoction. Ram *et al.* (1997) however reported that the *T. arguna* tree bark extract reduced the total cholesterol, LDL-cholesterol, HDL-cholesterol, triacylglycerols and total cholesterol/HDL ratio as well as the LDL-cholesterol/HDL-cholesterol ratio of the rats. *T. catappa* fruit extract and *T. catappa* fallen dry leaf decoction had been also reported to have hypocholesterolemic effects in rats (Nagappa *et al.*, 2003; Ibegbulem *et al.*, 2011). The differences noted in this study may have been caused by the species of *Terminalia* used. It suggests that the degree of anti-atherogenic activity of the *Terminalia* species may be species dependent.

Tannins, saponins and flavonoids are the types of phytochemicals that had also been detected as constituents of plant extracts that have lipid lowering effects (Ram *et al.*, 1997; Adeneye *et al.*, 2008). These

phytochemicals also prevent the atherogenicity of LDL by preventing its oxidation (Wardlaw and Kessel, 2002).

The underlying biochemical mechanism of the decoction is largely unknown. Its LDL-cholesterol lowering effect may be due to its catechin, phytosterol, saponin and fibre contents (Table 1). Plant sterols are effective in blocking the absorption of cholesterol by intestinal cells (Garrett and Grisham, 1999). Potter (1995) had posited that the saponins contained in soybean promoted hypocholesterolemia by increasing LDL receptor activity and excretion of bile acids. Okoye (2011) reported that catechins are LDL receptor activators; phytosterols inhibit HMG-CoA reductase while fibres inhibit bile acid absorption. These constituents may have acted in synergy. The test rats clearly consumed more of these phytochemicals and fibre, as the water drunk by their control counterparts did not contain them. From the foregoing, we can then hypothesize that the *T. littoralis* decoction reduced enterohepatic bile acid cycle, increased LDL receptor activity and inhibited HMG-CoA reductase activity.

In conclusion, the constituents of *T. littoralis* decoction had serum LDL-cholesterol lowering effects.

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