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Full Length Research Paper

Biochemical effects of drinking *Terminalia catappa* Linn. decoction in Wistar rats

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The biochemical effects of drinking *Terminalia catappa* Linn. decoction in place of water using weanling wistar rat (*Rattus norvegicus*) models of both sexes was studied. In folklore, the decoction is taken as a medicine by sicklers. Some of the phytochemical and chemical constituents detected in the decoction included tannins, flavonoids, saponins, β-carotene, thiocyanates, cardiac glycosides, alkaloids, cyanogenic glycosides, vitamin C, ρ – hydroxybenzoic acid (ρ – HBA), alkaloids, catechins, free amino acids and monosaccharides. The decoction was acidic, pH 5.94 ± 0.01. It was administered to the rats in place of water, *ad libitum*. Test for its biochemical effects lasted for 35 days and was ingested at 18.3 mg/ml. Results of its effects on liver function parameters showed that most of the parameters were not significantly ($p > 0.05$) affected. However, it increased ($p < 0.05$) the alanine aminotransferase activity and serum total protein contents of the female rats and significantly reduced ($p < 0.05$) the serum total bilirubin levels of both male and female rats. It also significantly ($p < 0.05$) reduced the serum total cholesterol levels of the female rats and the serum LDL – cholesterol levels of both the male and female rats. The haematological indices were not significantly ($p > 0.05$) altered. Ingestion of the decoction in place of water significantly ($p < 0.05$) increased the body weight gained, fluid and feed intakes and did not interfere with the nutrient adequacy of the feed by reducing the feed conversion ratios (FCR's) of the test rats. In conclusion, the study established the safety of the decoction when drunk in place of water.

Key words: Biochemical effects, decoction, drink, rats, *Terminalia catappa*.

INTRODUCTION

The universal role of plants in the treatment of disease is exemplified by their employment in all major systems of medicine irrespective of the underlying philosophical premise. There is a great wealth of knowledge concerning the medicinal, narcotic and other properties of plants that is still transmitted orally from generation to generation by tribal societies, particularly those of Tropical Africa, North and South America and the pacific countries (Evans, 2002).

Information on the use of medicinal plants has been obtained from herbalists, herb sellers and indigenous people of Africa over many years (Sofowora, 2002). The United Nations Development Programme estimates the value of pharmaceutical products derived from developing world plants, animals and microbes to be more than $30 billion per year. Consider the success story of vinblastine and vincristine. These anticancer alkaloids are derived from the Madagascar periwinkle (*Catharanthus roseus*) (Cunningham et al., 2005). The use of single pure compounds, including synthetic drugs, is not without its limitations and in recent years there has been an immense revival in interest in the herbal and homoeopathic systems of medicine, both of which rely heavily on plant sources (Evans, 2002).

Terminalia species tree has a characteristic pagoda shape because it sends out a single stem from the top centre. Oliver-Bever (1986) reported that it is made up of 250 species. Oboh et al. (2008) also reported the phenotypic diversity of *Terminalia catappa* from southwestern Nigeria where variability in leaf shape and ripe fruit colour were observed. The fruit is a sessile, laterally compressed ovoid to ovate, smooth-skinned

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drupe. Ahmed et al. (2005) reported that the leaves contained several flavonoids, tannins, saponins, triterpinoid and phytosterols. Due to the above chemical richness, the leaves and bark are used in different traditional medicines for various purposes worldwide. They also reported the biochemical effects of administrating *T. catappa* Linn. aqueous and cold leaf extracts, intraperitoneally and showed that it caused the regeneration of the β-cells of the islets of Langerhans, decreased blood sugar, serum cholesterol, triglycerides, low density lipoprotein (LDL), creatinine, urea and alkaline phosphatase levels, while increasing the high density lipoprotein (HDL) level in diabetes mellitus (DM). Ram et al. (1997) who had earlier worked on a sister species (*Terminalia arguna*) reported that the oral administration of its aqueous tree bark extract did not have any effects on the liver, kidneys, lipid profile and haematological parameters of rats. The aqueous extracts of *T. catappa* leaves have been reported to have strong free radical scavenging activities (Kinoshita et al., 2007).

Moody et al. (2003) and Ibegbulem et al. (2010), respectively, reported the *in vitro* antisickling property of the extracts and decoction of *T. catappa*. The decoction is administered in folklore medicinal practice as a home-made prophylaxis against sickle-cell crises; without any reported side effect. Most of our traditional medicines are in forms of decoctions. This paper presents the biochemical effects of drinking *T. catappa* decoction in place of water in albino wistar rats.

MATERIALS AND METHODS

Preparation of decoction

Dried leaves of T. catappa Linn. (pink mesocarp fruited) were picked from under its tree situated at the Nekede Village, Owerri, Imo State. The leaves were authenticated by Professor S.E. Okere, a plant taxonomist, of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Imo State. They were cleaned of debris, washed in distilled water, mopped dry of water then chopped into bits. One 100 g of the chopped leaves was put in an aluminum pot and 4.6 L of distilled water was poured into the pot. This was brought to boil and allowed to simmer for 20 min. The decoction produced was filtered off the leaves using a muslin cloth and stored in a refrigerator at 4°C until used.

Analyses of decoction for constituents and property

Tests for the presence of catechins, β-carotene, cardiac glycosides, flavonoids and alkaloids were carried out using to the methods of Evans (2002). Test for the presence of cyanogenic glycosides was done using the method of AOAC (1990). Test for the presence of saponins was carried out using the method of Sofowora (2006). An aspect of the method used for the estimation of tannins by AOAC (1984) was adopted for the detection of tannins: 1.0 ml of the decoction was mixed with 0.5 ml of Folin - Denis reagent and 1.0 ml of 17% Na_2CO_3. The mixture was stood at room temperature (30°C) for 3 min for colour development. The decoction tested positive for the presence of tannins when it developed a blue colour (intensity varying with the concentration of tannins in the test sample). Test for the presence of thiocyanates was carried out by modifying the alkaline picrate paper method of Haque and Bradbury (1999). The modification made was that the orange/ brick-red paper strip was not washed in distilled water and the colour intensity measured spectrophotometrically.

Test for the presence of ρ -hydroxybenzoic acid (ρ – HBA) was run using the method of ASEAN (2005). Test for the presence of vitamin C was evaluated using the method of Lambert and Muir (1968). The presence of free amino acids and monosaccharides were detected using the methods of Plummer (1971). The pH and concentration of the decoction were determined using the methods of AOAC (1990).

Testing for the biochemical effects

A total of 32 weanling white albino rats (*Rattus norvegicus*) of the wistar strain (of both sexes) were used for the animal feeding experiment. They were purchased from the animal colony of the Department of Biochemistry, University of Port Harcourt, River State, Nigeria and were aged between seven (7) and eight (8) weeks. They weighed between 42 and 75 g. The rats were allotted to 4 groups of 8 rats each (2 groups for males and 2 groups for females). The groups, on sex bases, were equalized as nearly as possible on weight basis. Each rat was housed in a wire-screened cage with provisions for feed and fluid. Acclimatization of the rats to their new environment (at the laboratory of the Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria) lasted for 4 days and the test period lasted for 35 days. All the rats were maintained under the same conditions of light and dark cycles (circadian rhythm) and ambient room temperature.

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 induced diabetic rats. The duration of administration here was however for 35 days. Two groups of the test rats (of the respective sex) were respectively placed on the decoction from the fallen dried leaves of *T. catappa*. The decoction was served to the rats in place of water, *ad libitum*, for their respective rat groupings while distilled water served as the only source of fluid for the control group. All the rats had growers mash (guinea feed) (produced by Bendel Feed and Flour Mill Limited, Sapele, Delta State, Nigeria) as the only source of solid feed, *ad libitum.*

On day 35 of the experiment, each rat was re-weighed (and weight gained calculated) before being anaesthetized with chloroform ($CHCl_3$) vapour. Incisions were then made into their thoracic cavities. Blood samples were collected by heart aorta puncture using 10 ml hypodermic syringes. One millilitre of each blood sample was quickly transferred into sequestering bottle (containing EDTA as anticoagulant) and the rest put in a test tube and allowed to clot before the serum was collected. The whole blood samples and sera were used for the clinical assays. Diagnostic test kits for total protein, albumin, total bilirubin and the lipid profile parameters (with the exception of very low density lipoproteins, VLDL) were purchased from BioSystems® (S.A. Costa Brava of Barcelona, Spain) and diagnostic test kits for the estimation of the activities of alanine and aspartate aminotransferases (ALT and AST) were purchased from Randox® (Randox Laboratories Ltd., Antrim, United Kingdom). The assays were performed according to their manufacturers' instructions. All other chemicals were of good analytical grades. VLDL concentration was estimated using the methods of Burnstein and Sammaille (1960). Haemoglobin (Hb) concentration was estimated by the cyanmethaemoglobin method described by Bain and Bates (2002). Packed cell volume (PCV), mean cell haemoglobin concentration (MCHC) and visual white cell count (WBC) were estimated by the techniques of Baker et al. (2001).

Total fluid and feed intakes were also calculated and daily intakes evaluated. Feed conversion ratio (FCR) was calculated as a

Table 1. Phytochemical, chemical and property of the decoction*.

Parameter	Result
Tannins	+
Flavonoids	+
Saponins	+
Vitamin C	+
β-carotene	+
Catechins	+
Thiocyanates	+
ρ – HBA	+
Cyanogenic glycosides	+
Cardiac glycosides	+
Alkaloids	+
Amino acids	+
Monosacchrides	+
pH	5.94 ± 0.01
Concentration (mg/ ml)	18.3 ± 0.26

*Values are means of triplicate determinations. Key: + = present.

ratio of daily feed intake to daily weight gained as described by Church and Pond (1988). Results are presented as means ± SD of triplicate values for eight rats.

Statistical analysis

Data generated were evaluated by the use of the students' t – test of significance at 95% confidence limit. The p – values for the statistical analyses between the test and their control rats were reported while the statistical differences between the male and female rats for a parameter were shown using superscript letters. Ratios were not tagged.

RESULTS AND DISCUSSION

All the phytochemical and chemical constituents detected in the decoction (Table 1) are known to be beneficial. Their health benefits have been espoused by Ram et al. (1997), Wardlaw and Kessel (2002) and Adeneye et al. (2008). Balagopalan et al. (1988) reported the benefits of eating foods that contain thiocyanates and cyanates to sickle cell anaemia (SCA) patients while Akojie and Fung (1992) reported the antisickling activity of ρ–HBA in *Cajanus cajan*. Ibegbulem et al. (2010) attributed the antisickling property of the decoction to the presence of ρ–hydroxybenzoic acid, flavonoids, thiocyanates and some antisickling amino acids. The acidity of the decoction showed that most of our traditional medicines may be acidic.

The oral route of administration is the most popular route for administering decoctions. A decoction can also be referred to as a drug since it is a substance that is taken as a medicine. Zakrzewski (1991) reported that this route had the advantage that drugs could mix with food, acid, gastric enzymes and bacteria which could alter their toxicity either by influencing absorption or by modifying the compound. He added that sex, age and body weight were also contributing factors to how individuals reacted to drugs. Results of the liver function indices (Table 2) showed that the female rats were more sensitive ($p< 0.05$) to ingesting the decoction in place of water for the duration of the study. They were shown to have had higher serum AST activities and serum total protein levels and lower serum total bilirubin levels than their controls. Their serum AST activity and total bilirubin level did not however increase ($p > 0.05$) more than those of their male counterparts. The AST: ALT ratios of the rats showed that the pathologic condition, if any, was more of the liver. This may have been indicative of hypertrophy. The liver is normally the first port of call of nutrients and toxins alike that enter the body. In the course of trying to detoxify these foreign materials, there may be a hypertrophy of this organ as an adaptive measure.

Schoen (1999) said that hypertrophy was a compensatory mechanism for increased stress; even in increased physical exertion. It is the neutralization of noxious stimulus by the cells or one of its organelles (Cotran et al., 1999). However, the AST: ALT ratios of the test rats compared favourably with those presented by the control rats. The liver function indices also showed that the test rats were not jaundiced as shown by their total bilirubin levels. This meant that the decoction was not haemolytic. The test rats did not also develop oedema because they did not exhibit hypoalbuminemia (and hyperalbuminemia) as well as hypoproteinemia (and hyperproteinemia) as their albumin and total protein levels generally compared favourably with values presented by the control rats. The total protein and albumin levels of the test rats were by extension indicative of the maintenance of kidney integrity. The total serum protein levels of the female test rats were though higher ($p< 0.05$) than those of their male counterparts and control, indicating that they may have mobilized enzymes for detoxification, as the decoction was a xenobiotic. The decoction was found to have contained a wide spectrum of phytochemicals, chemicals and nutrient moieties that may have generally endangered the well-being of the liver. Antioxidants like tannins, flavonoids, vitamin C, β-carotene, saponins may have also contributed to the maintenance of the health of the liver. Ram et al. (1997) showed that liver and renal function parameters were not adversely affected when the tree bark extract of *T. arguna* was administered to rats.

Ingestion of the decoction generally reduced ($p< 0.05$) the susceptibility of the test rats to atherosclerosis (Table 3). The differences in the levels of some parameters noticed between the male and female rats may have been results of the interplay of sex hormones as they grew. Though lower values were noticed for some of the parameters on ingestion of the decoction, the reductions

Table 2. Effect of decoction on liver function indices of test rats.

Group	Parameter*											
	AST (U/l)		ALT (U/l)		AST: ALT		Total Bilirubin (mg/dl)		Total Protein (g/l)		Albumin (g/l)	
	M	F	M	F	M	F	M	F	M	F	M	F
T. catappa	21.00^{a}±2.83	21.08^{a}±0.30	27.48^{b}±8.88	29.45^{b}±5.16	0.76	0.72	0.97^{c}±0.15	1.07^{c}±0.33	59.40^{d}±5.39	66.83^{e}±5.64	35.35^{f}±4.53	36.80^{f}±1.55
Control	20.98^{b}±0.45	20.75^{b}±0.25	24.73^{c}±2.36	24.48^{c}±5.00	0.85	0.85	1.13^{d}±1.06	1.16^{d}±0.06	58.58^{f}±4.15	61.05^{f}±1.91	36.30^{g}±1.81	36.30^{g}±1.81
t_{cal}	0.02	2.39	0.85	1.96			2.81	6.40	0.34	2.74	0.55	0.59

*Values are means ± S.D of eight determinations. M = male rats, F = female rats. Values on the same row bearing the same superscript letter for a parameter are not significantly different (p>0.05).

Table 3. Effects of decoction on lipid profile of test rat serum.

Group	Parameter (mg/dl)*													
	Triacylglycerol		VLDL- Cholesterol		Total Cholesterol(TC)		HDL- Cholesterol		TC: HDL-C		LDL- Cholesterol		LDL-C:HDL-C	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
T. catappa	58.81^{a}±6.57	72.12^{b}±7.28	10.80^{c}±1.39	14.93^{d}±1.99	98.60^{e}±9.49	51.10^{f}±34.78	57.87^{g}±6.86	64.79^{g}±17.96	1.70	0.79	47.22^{h}±5.66	38.89^{h}±6.41	0.82	0.60
Control	63.47^{c}±14.56	75.00^{c}±18.45	12.70^{f}±2.92	15.00^{f}±3.69	100.00^{g}±9.06	100.00^{g}±15.70	59.42^{h}±1.58	57.87^{h}±1.20	1.64	1.73	63.89^{k}±3.21	65.28^{k}±2.78	1.08	1.13
t_{cal}	0.15	0.41	1.66	0.03	0.30	3.62	0.62	1.09			7.25	10.68		

*Values are means ± S.D of eight determinations. Values on the same row bearing the same superscript letter for a parameter are not significantly different (p>0.05).

were not significant (p> 0.05). The total cholesterol levels of the female rats and the LDL – cholesterol levels of both the male and female rats were significantly (p< 0.05) affected by the decoction when it was drunk in place of water. Gender differences also affected (p< 0.05) the levels of the triacylglycerol, VLDL – cholesterol, total serum cholesterol and LDL – cholesterol of the rats on ingestion of the decoction. The female rats seemed to have had their serum triacylglycerol, VLDL – cholesterol and HDL - cholesterol levels elevated (p< 0.05) after drinking the decoction, when compared with those of their male counterparts. This partly corroborated the findings of Kayali et al. (2009) who reported that aged female rats had reduced serum total cholesterol and elevated serum LDL – cholesterol levels than their aged male counterparts. This may have meant that the aged female rats were prone to atherosclerosis. The Kayali et al. (2009) type of elevation of serum LDL - cholesterol was noticed in the female control rats when compared with their male counterparts (though not significantly different, p> 0.05). Seidell et al. (1991) had earlier reported that men had higher serum triacylglycerol and total cholesterol and lower HDL – cholesterol compared to women. Our study showed that ingestion of the decoction made the male rats seem more (p< 0.05) prone to atherosclerosis, since their serum total cholesterol and serum LDL – cholesterol levels were raised when compared with their female counterparts.

However, most of the values presented by the test rats compared favourably with those presented by their controls. The LDL-cholesterol: HDL-cholesterol ratios of the test rats showed that their sera seemed to have contained more phospholipids than cholesterol. The decoction seemed to have engendered the production of HDL and phospholipids. This meant that it may have led to the reduction of the cholesterol contents of the sera; especially for the female rats. Berg et al. (2002) postulated that a serum esterase that degraded oxidized lipids had been found to be associated with HDL. They went further to say that the HDL-associated protein possibly destroyed the oxidized LDL, accounting for HDL's ability to protect against coronary

Table 4. Effect of decoctions on haematological indices of test rats*.

Group	Parameter							
	Hb (g/dl)		PCV (%)		MCHC (g/dl)		WBC (number/L)	
	M	F	M	F	M	F	M	F
T. catappa	9.83^{a}±1.77	8.50^{a}±2.05	32.25^{b}±6.13	27.25^{b}±4.79	0.31	0.31	4637.50^{c}±1978.37	3675^{c}±830.16
Control	8.98^{b}±0.46	8.05^{b}±1.00	34.50^{c}±1.73	31.75^{c}±6.24	0.26	0.25	4175.00^{d}±1074.3	5000^{d}±1802.31
t_{cal}	1.31	0.56	1.00	1.62			0.58	1.89

*Values are means ± S.D eight determinations.

disease. Glew (2006) said that the detergent properties of phospholipids, especially phosphotidylcholine, were important in bile to aid in solubilizing cholesterol. Quercetin, found in plants and related substances found in tea, as well as phenolics in wines act as antioxidants and reduce LDL oxidation (Wardlaw and Kessel, 2002). The lipid profile also showed that ingesting the decoction would discourage the development of atherosclerosis as shown by their LDL- cholesterol lowering effects and encourage the formation of the good cholesterol (HDL-cholesterol).

The risk of developing atherosclerosis is directly related to plasma LDL-cholesterol and inversely related to HDL-cholesterol levels (Glew, 2006). Pigments like tannins and flavonoids may have been responsible for this cholesterol lowering action. Phytochemicals like tannins, saponins and flavonoids are constituents of plant extracts that have lipid lowering effects (Ram et al., 1997; Adeneye et al., 2008). These phytochemicals also prevent the oxidation of LDL, preventing it from being atherogenic (Wardlaw and Kessel, 2002). Ram et al. (1997) showed that *T. arguna* tree bark extract reduced the total cholesterol, LDL-cholesterol, HDL-cholesterol, triacylglycerols and cholesterol: HDL ratio as well as the LDL-cholesterol: HDL-cholesterol ratio of the rats.

Ugonabo et al. (2007) and Essien (2008), respectively reported that SCA patients physiologically had lower total cholesterol, free cholesterol, cholesteryl esters, HDL-cholesterol, LDL-cholesterol, total phospholipids and triglycerides (except free fatty acids) than normal patients. Ugonabo et al. (2007) attributed this to increased synthesis of red cell membranes; hence cholesterol mobilization. Phospholipids are also major components of RBC membranes. The reduction in serum cholesterol by the decoctions may also be seen as a double edged sword. While it may discourage atherosclerosis, it may also deplete the cholesterol base needed for the synthesis of red cell membranes in SCA patients.

The haematological parameters were found not to have been affected ($p>0.05$) when the decoction was ingested in place of water (Table 4). Though the Hb levels were observed to have been elevated, this would seem beneficial to sicklers, as their Hb levels are normally low. PCV levels were also not found to have been altered ($p>0.05$). PCV levels are also low in SCA (Baker et al., 2001). The MCHC values were found to have been increased (though not significantly ($p>0.05$)). Increase in MCHC had been reported to be one of the conditions that precipitated sickling (Schechter et al., 1987; Cotran et al., 1999). Schechter et al. (1987) also reported that the MCHC, MCH and MCV values were not strickingly different between sickle and normal cells but for the existence of a significant number of very dense (corpuscular haemoglobin concentration >37 g/dl), small (corpuscular volume <80 μm^3) cells. In this study, the increase in MCHC may not necessarily result in the sickling of HbSS red blood cells because of the presence of the antisickling agents (like thiocyanates and ρ – HBA) that may always be present in the blood stream and/ or red cells at steady states. Intracellular polymerization leads to changes in the internal milieu of the cells. But when polymerization is supposedly inhibited by these agents, the homeostatic balance will be maintained. So, increase in MCHC may not be synonymous with sickling but may result in increased oxygen carrying capacity of the red blood cells. However, this is suggested for further studies. The WBC's were also found not to have been elevated ($p>0.05$). This may mean that the decoction did not present any immunologic challenge. Leucocytes are known to help man the body's immune system alongside macrophages and lymphocytes (Aster and Kumar, 1999; Nelson and Cox, 2000). These findings corroborated those of Ram et al. (1997).

Ingestion of the decoction (Table 5) generally increased ($p< 0.05$) the daily body weight gained, feed and fluid intakes leading to non interference with the feed's nutrient adequacy and improved their efficiency in feed utilization (for instance, its FCR) of the test rats. Church and Pond (1988) reported that lowering of FCR meant that less of total feed consumption was used for maintenance and more was available for gain. The test rats may also have gained more weights because of the added nutrients from the decoction like amino acids and monosaccharides that were detected in it (Table 1).

Conclusion

Our study showed that the decoction was safe when drunk in place of water.

Table 5. Effects of the decoctions on daily weight gained, fluid and feed intakes and feed conversion ratios of test rat*.

Group	Parameter							
	Weight gained (g/day)		Fluid intake (ml/day)		Feed intake (g/day)		Feed conversion ratio (FCR)	
	M	F	M	F	M	F	M	F
T. catappa	1.64^{a}±0.19	1.76^{a}±0.19	12.89^{b}±0.40	13.11^{b}±0.43	10.75^{c}±0.34	10.11^{d}±0.44	6.55	5.74
Control	1.20^{b}±0.26	1.09^{b}±0.38	11.57^{c}±0.17	11.46^{c} ±0.22	9.59^{f}±0.56	9.66^{f}±0.48	7.99	8.86
t_{cal}	3.86	4.46	8.52	9.59	5.04	1.95		

*Values are means ± S.D of eight determinations. Values on the same row bearing the same superscript letter for a parameter are not significantly different (p>0.05).

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