

Hypolipidemic and Antioxidant Effects of *Tetrapleura tetraptera* Fruits, Including Seeds, in Hypercholesterolaemic Rats

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ABSTRACT

In hypercholesterolemia, lipid profile and biomarkers of oxidative stress are known to fluctuate. In this study, the hypolipidaemic and antioxidant effects of the fruits (including seeds) of *Tetrapleura tetraptera*, a spice used in the treatment of various ailments in Nigeria was investigated in cholesterol-fed rats. The ability of Questran[®] (a standard hypolipidaemic drug), to attenuate hypercholesterolemia in the rats was also examined. Rats were given by oral gavage cholesterol (40 mg/0.3 ml), *T. tetraptera* (250 mg/kg) and Questran[®] (0.26 g/kg), five times a week for 8 consecutive weeks. The hypolipidemic effect was assessed by measuring total cholesterol, LDL-cholesterol, triglyceride and HDL-cholesterol while the extent of oxidative stress was assessed by measuring thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities. The integrity of liver was assessed by determining the aspartate and alanine aminotransferases (ALT and AST) activities. *T. tetraptera* significantly ameliorated ($P < 0.05$) the cholesterol-induced body weight gain and, there was an over 50% decrease in serum and post mitochondria fraction (PMF) total cholesterol compared to untreated hypercholesterolaemic rats. LDL-cholesterol was significantly decreased in the extract-treated animals when compared to hypercholesterolaemic rats. In hypercholesterolaemic rats, a remarkably increase in lipid peroxidation (LPO) and a concomitant decrease in the enzymatic antioxidant status was observed; however, these indices were significantly attenuated in hypercholesterolaemic rats treated with the extract. These results indicate that the methanolic extract of *T. tetraptera* exerts a hypolipidemic effect, reduces body weight gain and increases the body's antioxidant defense system in hypercholesterolemic rats.

Keywords: antioxidant, hypercholesterolemia, LDL-c, lipid peroxidation

INTRODUCTION

Atherosclerosis is the root cause of the biggest killer of the 21st-century and it is the culprit behind coronary artery disease, cerebral vascular disease and peripheral vascular diseases (Malika *et al.* 2007). Elevated level of blood cholesterol increases the risk of heart disease and atherosclerosis (Lu *et al.* 2010; Rupp *et al.* 2010). Metabolic and epidemiological studies have shown that serum cholesterol is strongly governed by dietary intakes, especially of saturated fats (Rennison and van Wagoner 2009). Genetic factors are also involved in the interplay of regulation of cholesterol and triglyceride concentrations in the plasma (Phillips *et al.* 2010). Polyunsaturated fatty acids in the diet, lowers serum cholesterol by redistribution between plasma and tissue (Hu *et al.* 2001). An increase in cholesterol consumption may induce fatty liver, hepatic steatosis or hypertrophy of the liver (Schaefer *et al.* 1995). A high cholesterol diet is an important factor in the development of hyperlipidemia, atherosclerosis and ischemic heart disease (Hancock *et al.* 2008; Rennison and van Wagoner 2009). Abnormal deposition of cholesterol in the tissues is associated with several conditions including atherosclerosis, hypertension and diabetes mellitus.

A diet rich in cholesterol tends to induce free radical production, followed by hypercholesterolemia, which is a major risk factor in atherosclerosis. A number of theories such as dyslipidemia, hypercoagulability, oxidative stress, endothelia dysfunction, inflammation, and infection by certain pathogens have been propounded from time to time to explain this complex phenomenon (Malika *et al.* 2007). Reactive oxygen species have been involved in the physiological and pathological events such as inflammation, aging, mutagenicity and carcinogenicity and in the last decade evi-

dences abound to the role of free radical-mediated lipid peroxidation in the pathogenesis of atherosclerosis (Namiki 1990), neurodegenerative disorders (Bonannome *et al.* 1992), carcinogenesis and aging (Halliwell and Gutteridge 1992).

Much work has been done on the development of hypolipidemic agents and, some of such agents are Colestid[®], Niacin, Cholestyramin (Questran[®]) and they function by lowering high blood cholesterol. Hypolipidemic agents have lots of side effects such as formation of gall stones, gall bladder diseases, diarrhea and even death (Farmer 2003). Consequently plant foods and phytochemicals are being promoted to consumers as cardioprotective, antihypercholesterolemic and have been widely accepted as a healthy antiatherogenic diet. Plant sterols and stanols have proven effective in combination with lipid-lowering drugs (Ames *et al.* 1993).

Tetrapleura tetraptera (Taub) (Fabaceae) locally known as Aridan (South Western Nigeria), is widely distributed on the fringes of West Africa, especially the secondary forest of the Savannah woodland and rain forest. The tree is deciduous, about 20-25 m high and a girth of 1.5-3 m. The dark brown indehiscent pod (ribbed fruit) is about 15-25 cm long, approximately 5 cm across with 4 longitudinal wings like ridges nearly 3 cm broad, two of the wings are woody and the others are not with black seeds about 8 mm long. The plant has been used therapeutically in the management of schistosomiasis, asthma, epilepsy and hypertension (Ojewole 2005). Aqueous extracts of the fruits have analgesic and anticonvulsant properties (Ojewole 2005). In eastern Nigeria, the fruits are used to prepare pepper soup from first day of delivery to prevent postpartum contraction (Odukoya 2005) and are used as multivitamins in Ghana. *T. tetraptera* has shown antibacterial, antiulcer, anticonvulsant (Noamesi

et al. 2007; Okonkon *et al.* 2007), birth control (El-Izzi *et al.* 1990) and specific hypotensive effects in anaesthetized rats. Leaves, leaf stalks, root bark and fruits of this herb have shown molluscicidal activity (El Izzi *et al.* 1990; Adewumi 1991a); these parts are not genotoxic (Adewumi 1991b; Ngassapa 1993).

To our knowledge, there are no reports on the effect of *T. tetraptera* fruits on hypercholesterolemia, hence this study was designed to investigate the effect of methanolic extract of the dried fruits on lipid profile and antioxidant status of rats rendered hypercholesterolaemic by feeding dietary cholesterol.

MATERIALS AND METHODS

Chemicals

Adrenaline, thiobarbituric acid (TBA), Ellman's reagent (DTNB), glutathione (GSH) and bovine serum albumin (BSA) were purchased from Sigma Chemical (St Louis, MO, USA). Dietary cholesterol was procured from Aldrich Chemical (Milwaukee, WI, USA). Questran® (Bristol-Myers Squibb, Hounslow, UK) was obtained locally from a Chemist in Ibadan, Nigeria. The Randox diagnostic kit was used for cholesterol and high density lipoprotein cholesterol (HDL-c). Other reagents used were of purest quality available.

Plant material

Dried fruits of *T. tetraptera* were purchased locally from Bodija market, Ibadan, Nigeria and were identified at the Herbarium of Botany Department, University of Ibadan, Nigeria. Dried fruits, including seeds, were powdered using hammer mill and the powder was Soxhlet extracted using of methanol (1: 2.5) for 72 h. Dark brown concentrated methanol fraction obtained was used at a concentration of 250 mg/kg body weight.

Animals

Thirty male albino rats (Wistar strain) weighing between 120 and 137 g were obtained from the Institute of Advanced Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria and were housed in the Animal house, Biochemistry Department, University of Ibadan, Ibadan at normal room temperature. The rats were acclimatized for two weeks on standard diet (palletized Guinea feed, purchased from Guinea Feed, Ibadan, Nigeria). The animals were allowed free access to food and water *ad libitum*. Rats were randomly placed into six groups of five rats each. Group A served as control and received only corn oil. Group B served as positive control and received only Questran®. Animals in group C (treatment group) had cholesterol and extract of *T. tetraptera*. Groups E, F and G received cholesterol only, [cholesterol + Questran®] and plant extract, respectively. Corn oil was used as vehicle for the administration of extract, Questran® and cholesterol. Dietary cholesterol and Questran® were given at doses of 40 mg/0.3 ml/animal and 0.26 g/kg body weight, respectively (Adaramoye *et al.* 2005), while methanol extract of *T. tetraptera* was administered at a dose of 250 mg/kg body weight. All drugs were administered by oral gavage, five times a week for eight consecutive weeks.

Sample collection

Animals were fasted for 24 h after the last dose of drugs and were sacrificed by cervical dislocation. Blood was obtained using a 2-ml syringe by cardiac puncture into clean bottles and allowed to clot. These were spun at 3000 rpm for 10 min; the supernatant (serum) was removed and stored at 4°C. The various organs (liver, kidney and heart) were quickly removed, weighed, washed with 1.15% KCl, homogenized in 56 mM Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride and the homogenate was centrifuged at 10,000 rpm for 15 min to obtain post mitochondrial fraction (PMF) at 4°C.

Biochemical assays

Microsomal catalase (CAT) (EC 1.11.1.6) activity was determined by using hydrogen peroxide, briefly, the reaction mixture contained phosphate buffer (0.01 M, pH 7.0), tissue homogenate and 2 M H₂O₂. The reaction was stopped by the addition of dichromate-acetic acid reagents (5% potassium dichromate and glacial acetic acid were mixed in a ratio of 1:3).

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was assayed using the method of Misra and Fridovich 1972. The tissue homogenate (0.5 ml) was mixed with ethanol and chloroform mixture, and then centrifuged. To the supernatant, 0.025 M sodium pyrophosphate buffer (pH 8.3), phenazine methosulphate, nitro-blue tetrazolium and NADH were added and incubated at 30°C for 90 sec. The reaction was stopped by the addition of glacial acetic acid and mixed with *n*-butanol. The intensity of the chromogen in the *n*-butanol was measured at 560 nm.

Reduced glutathione (GSH) (EC 1.11.1.9) was determined at 412 nm using the method of Jollow *et al.* (1974), briefly, 10% trichloroacetic acid was added to the homogenate and centrifuged. 1.0 ml of the supernatant was treated with 0.5 ml Ellman's reagent and 3.0 ml of 0.2 M phosphate buffer (pH 8.0). The absorbance of the colour formed was read at 412 nm.

The extent of lipid peroxidation was determined by estimating the thiobarbituric acid reactive substances (TBARS) formed. Cholesterol was determined using Randox kit. The lipoproteins, measured using enzymatic colorimetric method; Very low density lipoprotein (VLDL) and Low density lipoprotein (LDL) were precipitated using phosphotungstic acid and magnesium chloride. After centrifugation at 3000 × *g* for 10 min at 25°C, the clear supernatant contained high density lipoprotein (HDL)-c which was assayed for cholesterol using Randox kit. LDL-cholesterol (LDL-c) was calculated using the formulae of Friedewald *et al.* (1972). The concentration of protein in the serum and PMF was determined using the method Lowry *et al.* (1951), with BSA as standard. Serum alanine aminotransferase (ALT) (EC 2.6.1.2) and aspartate aminotransferase (AST) (EC 2.6.1.1) were assayed using the Randox kit.

Statistical analysis

Results were expressed as the mean ± S.D. (n = 5). A one-way analysis of variance (ANOVA) was used for the data analysis with GraphPad Prism Version 4 as Statistical Package. Significant differences among groups were detected in the ANOVA using Dunnett's Multiple Range Test at *P* ≤ 0.05.

RESULTS

Results in **Table 1** imply that administration of cholesterol for 8 consecutive weeks caused a significant increase in the body weight of hypercholesterolaemic animals compared with the control. Simultaneous administration of *T. tetraptera* (250 mg/kg) with dietary cholesterol significantly decreased the cholesterol-induced body weight gain. Feeding cholesterol to rats produced a significant decrease in serum and hepatic HDL-cholesterol levels (**Table 2**). However, co-treatment with *T. tetraptera* and Questran® significantly attenuated the cholesterol-induced decrease in serum and hepatic HDL-cholesterol levels. Dietary cholesterol intake also caused a significant decrease in hepatic PMF glutathione level (GSH) in rats. Administration of *T. tetraptera* and Questran® produced a significant increase in the GSH level compared with hypercholesterolaemic group (**Table 2**). Specifically, there were 68 and 54% increases in hepatic PMF reduced glutathione levels following treatment with *T. tetraptera* and Questran®, respectively compared with hypercholesterolaemic rats. In **Table 3**, lipid peroxidation (LPO) increased by 119% in hypercholesterolaemic rats. The elevated LPO levels were significantly ameliorated in rats treated with *T. tetraptera* and Questran®. Furthermore, hepatic catalase and superoxide dismutase activities were significantly reduced in hypercholesterolaemic rats and, these were ameliorated upon treatment with the spice and Questran® (**Table 3**).

Table 1 Effect of *Tetrapleura tetraptera* fruit (including seed) on body weight and the relative weight of visceral organs in cholesterol-fed rats.

Grouping	Initial weight (g)	Final weight (g)	Weight gained (g)	Kidney weight (g)	Liver weight (g)	Heart weight (g)	Relative weight (as % body weight)		
							Kidney	Liver	Heart
Control	137.50 ± 7.39	218.50 ± 11.97	81.00 ± 7.96	0.97 ± 0.05	6.72 ± 0.78	0.78 ± 0.80	0.45 ± 0.14	3.08 ± 0.05	0.36 ± 0.04
Qu	120.00 ± 3.20	211.25 ± 9.58	91.25 ± 6.58	0.93 ± 0.07	7.14 ± 1.03	0.76 ± 0.14	0.44 ± 0.04	3.38 ± 0.07	0.36 ± 0.04
CH + Tt	142.50 ± 6.92	203.00 ± 8.57	60.50 ± 10.81**	0.87 ± 0.04	6.24 ± 0.96	0.68 ± 0.10	0.43 ± 0.02	3.07 ± 0.05	0.34 ± 0.02
CH	115.50 ± 7.62	238.00 ± 10.43	122.5 ± 8.37*	0.90 ± 0.07	7.24 ± 0.80	0.79 ± 0.06	0.38 ± 0.02	3.04 ± 0.07	0.33 ± 0.03
CH + Qu	130.00 ± 6.39	185.00 ± 8.200	55.00 ± 19.72**	0.88 ± 0.08	5.91 ± 0.58	0.76 ± 0.09	0.48 ± 0.05	3.20 ± 0.04	0.41 ± 0.05
Tt	126.25 ± 7.22	224.38 ± 11.97	98.13 ± 62.5	0.77 ± 0.05	7.18 ± 0.56	0.68 ± 0.16	0.34 ± 0.05	3.20 ± 0.06	0.30 ± 0.05

Data are the means ± S.D. (n = 5).

* Significantly different from control ($P < 0.05$).** Significantly different from CH group ($P < 0.05$).QU - Questran, CH - cholesterol, Tt - *Tetrapleura tetraptera*.**Table 2** Effect of *Tetrapleura tetraptera* fruit (including seed) on protein, high density lipoprotein (HDL)-cholesterol and glutathione levels in cholesterol-fed rats.

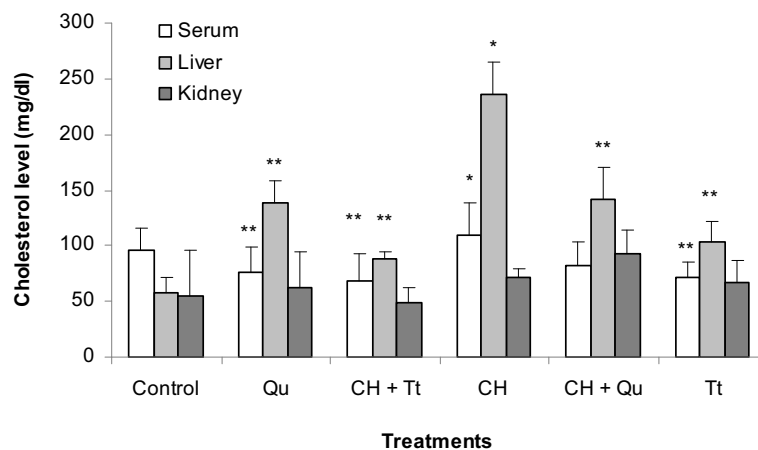
Grouping	Protein concentration				HDL-cholesterol			GSH	
	Serum (mg/100 ml)	Kidney (mg/100 ml)	Liver (mg/100 ml)	Heart (mg/100 ml)	Serum (mg/dl)	Kidney (mg/dl)	Liver (mg/dl)	Kidney (µg/ml)	Liver (µg/dl)
Control	2.11 ± 0.97	0.27 ± 0.06	1.05 ± 0.27	0.19 ± 0.03	59.20 ± 8.82	40.80 ± 8.71	45.42 ± 7.32	13.00 ± 2.03	12.82 ± 4.59
Qu	2.06 ± 0.41	0.30 ± 0.05	1.00 ± 0.41	0.18 ± 0.04	51.60 ± 9.74	39.20 ± 7.22	46.04 ± 5.77	13.72 ± 1.88	12.13 ± 4.07
CH + Tt	1.96 ± 0.87	0.28 ± 0.04	1.04 ± 0.49	0.17 ± 0.03	46.13 ± 9.05**	41.00 ± 9.21	38.93 ± 6.03**	12.16 ± 1.90	10.48 ± 2.31**
CH	2.02 ± 0.68	0.27 ± 0.03	1.08 ± 0.21	0.21 ± 0.05	31.80 ± 6.83*	38.67 ± 9.64	21.52 ± 4.94*	12.30 ± 2.94	6.24 ± 1.23*
CH + Qu	2.13 ± 0.75	0.24 ± 0.05	0.98 ± 0.66	0.20 ± 0.03	41.40 ± 8.15**	39.73 ± 7.34	36.87 ± 4.65**	11.67 ± 2.12	9.63 ± 1.31**
Tt	2.01 ± 0.81	0.28 ± 0.06	1.02 ± 0.41	0.19 ± 0.04	57.60 ± 9.45	40.13 ± 8.32	42.20 ± 6.23	12.05 ± 1.91	11.35 ± 4.21

Data are the means ± S.D. (n = 5).

* Significantly different from control ($P < 0.05$).** Significantly different from CH group ($P < 0.05$).QU - Questran, CH - cholesterol, Tt - *Tetrapleura tetraptera*.**Table 3** Effect of *Tetrapleura tetraptera* fruit (including seed) on the activities of superoxide dismutase, catalase, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lipid peroxidation in cholesterol-fed rats.

Grouping	Superoxide dismutase activity (U/ mg protein)		Catalase activity (µmol/mg protein)		AST	ALT	LPO
	Liver	Kidney	Liver	Kidney	Serum (U/l)	Serum (U/l)	Liver (µmol/mg protein)
Control	0.79 ± 0.21	0.04 ± 0.02	4.35 ± 0.83	3.01 ± 0.81	34.05 ± 2.23	9.60 ± 2.11	0.26 ± 0.01
Qu	0.81 ± 0.19	0.03 ± 0.03	4.53 ± 0.79	2.63 ± 0.62	37.20 ± 3.18	9.78 ± 1.85	0.24 ± 0.01
CH + Tt	0.57 ± 0.13**	0.03 ± 0.02	2.65 ± 0.63**	3.07 ± 0.72	38.68 ± 3.54	9.04 ± 2.09	0.31 ± 0.02**
CH	0.16 ± 0.05*	0.03 ± 0.03	1.04 ± 0.27*	2.53 ± 0.68	35.42 ± 4.27	10.03 ± 2.45	0.57 ± 0.02*
CH + Qu	0.63 ± 0.14**	0.04 ± 0.02	3.01 ± 0.59**	2.84 ± 0.71	35.08 ± 3.66	9.54 ± 2.08	0.35 ± 0.02**
Tt	0.74 ± 0.13	0.04 ± 0.03	3.97 ± 0.98	2.81 ± 0.76	32.65 ± 2.80	9.60 ± 2.13	0.30 ± 0.01

Data are the means ± S.D. (n = 5).

* Significantly different from control ($P < 0.05$).** Significantly different from CH group ($P < 0.05$).QU - Questran, CH - cholesterol, Tt - *Tetrapleura tetraptera*.**Fig. 1** Effect of a *Tetrapleura tetraptera* on serum, liver and kidney total cholesterol levels in cholesterol fed rats. Data are the means ± S.D. (n = 5).* Significantly different from control ($P < 0.05$). ** Significantly different from CH group ($P < 0.05$). QU - Questran, CH - cholesterol, Tt - *Tetrapleura tetraptera*.

Feeding cholesterol caused a significant increase in serum and hepatic PMF total cholesterol in the rats. However, hypercholesterolaemia induced by cholesterol intake was significantly ameliorated by co-treatment with *T. tetraptera* and Questran® (Fig. 1). Likewise, dietary cholesterol caused a significant increase in hepatic PMF triglycerides in the rats (Fig. 2). The hepatic hypertriglyceridaemia

induced was significantly ameliorated in rats co-treated with *T. tetraptera* and Questran®. Precisely, there was 69% decrease in hepatic PMF triglycerides following treatment with *T. tetraptera*. Also, significant reduction in serum triglyceride levels was observed in rats co-treated with *T. tetraptera* and Questran® (Fig. 2). In hypercholesterolaemic rats, significantly elevated serum and hepatic PMF LDL-

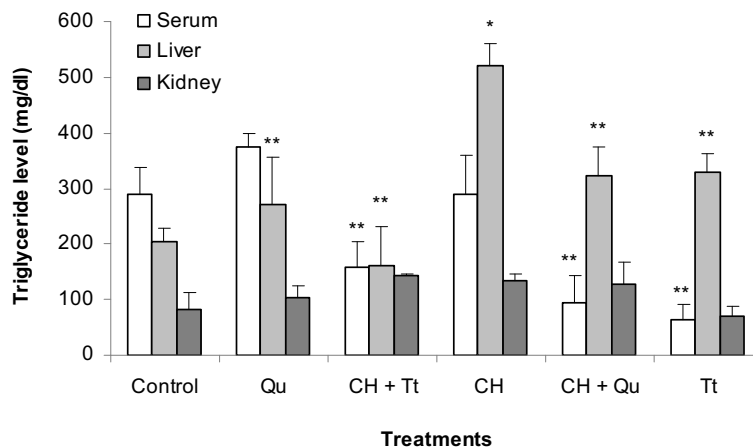


Fig. 2 Effect of *Tetrapleura tetraptera* on serum, liver and kidney triglyceride levels in cholesterol-fed rats. Data are the means \pm S.D. (n = 5). * Significantly different from control ($P < 0.05$). ** Significantly different from CH group ($P < 0.05$). QU - Questran, CH - cholesterol, Tt - *Tetrapleura tetraptera*.

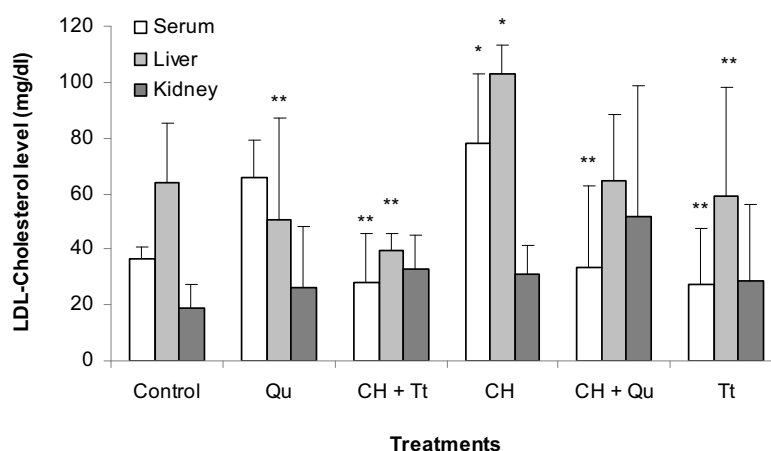


Fig. 3 Effect of *Tetrapleura tetraptera* on serum, liver and kidney low-density lipoprotein levels in cholesterol fed rats. Data are the means \pm S.D. (n = 5). * Significantly different from control ($P < 0.05$). ** Significantly different from CH group ($P < 0.05$). QU - Questran, CH - cholesterol, Tt - *Tetrapleura tetraptera*.

cholesterol levels were observed. Treatment with *T. tetraptera* and Questran[®] decreased both serum and hepatic PMF LDL cholesterol levels in the rats (Fig. 3). Precisely, there were 71 and 79% decreases in serum LDL-cholesterol levels, and 61% and 37% decreases in hepatic PMF LDL cholesterol levels following treatment with *T. tetraptera* and Questran[®], respectively.

DISCUSSION

Cholesterol is a component of cell membranes and, functions in the regulation of membrane fluidity and permeability (Ahmad-Raus 2001; Adaramoye *et al.* 2005). Cholesterol acts as a precursor for the synthesis of bile acid, steroid hormones and vitamin D, hence it is of critical importance that the cells of major tissues of the body be assured a continuous supply of cholesterol. However, hypercholesterolemia from either dietary indiscretion or genetic factors can cause cardiovascular disease (CVD), and precipitate oxidative stress.

Much attention has been drawn recently to the prospective biochemical function of naturally occurring antioxidants in biological system and these can either be nutritive or non-nutritive (Balkan *et al.* 2004; Amarowicz *et al.* 2004; Song *et al.* 2006; Su *et al.* 2008). Natural extracts with proven antioxidant activity usually contain compounds with phenolic moiety, such as coumarin, flavonoids, tocopherols and catechins (Cook and Samman 1996; Choi *et al.* 2002; Havsteen 2002). In the present study *T. tetraptera* and Questran[®] significantly produced over 50% reduction in dietary cholesterol induced body weight gain in the treated

animals. Also, feeding cholesterol to rats caused over 20% and 300% increases in the serum and hepatic total cholesterol levels, respectively. Dietary cholesterol has been reported to contribute significantly to elevation of plasma cholesterol in animal models (Adaramoye *et al.* 2005; Rennison and van Wagoner 2009). The presence of increased cholesterol levels in the diet has been demonstrated to elevate serum and aortic tissue cholesterol and as such cause an increase in atherosclerosis (Ahmad-Raus *et al.* 2001). Similarly, previous studies have established a linear correlation between dietary cholesterol intake and mortality of coronary heart disease (Li *et al.* 1994; Verschuren 1995). In this study, *T. tetraptera* was more effective than Questran[®] in reducing serum and hepatic total cholesterol level when compared with untreated hypercholesterolaemic rats. The mechanism of this hypocholesterolaemic action may be due to inhibition of the absorption of dietary cholesterol in the intestine or its production by the liver (Ahmad-Raus *et al.* 2001) or stimulation of the biliary secretion of cholesterol and cholesterol excretion in the faeces (Fungwe 1993).

Dietary cholesterol has been shown to reduce fatty acid oxidation, which in turn, increases the levels of hepatic and plasma triglyceride (Miettinen and Tarpillar 1977; Jody *et al.* 2009). There were 46 and 67% reduction in serum triglyceride levels following treatment with *T. tetraptera* and Questran[®], respectively. Likewise, administration of *T. tetraptera* and Questran[®] caused 71 and 79% decreases in serum LDL cholesterol levels and, 61 and 37% decreases, respectively in hepatic LDL cholesterol levels. LDL are the major transporters of cholesterol in the blood stream and are considered "bad cholesterol" because they carry fats out of

the liver to the blood vessels and seem to encourage the deposition of cholesterol in the arteries (Ruby *et al.* 2010). Plant sterols could cause decrease in total cholesterol, LDL cholesterol, triacylglycerol and as such would thereby increase high density lipoprotein (HDL) cholesterol levels (Lees *et al.* 1997; Jones *et al.* 2000). The higher the ratio of plant sterols to cholesterol in serum, the greater is the efficiency of cholesterol absorption (Noakes *et al.* 2002). β -sitosterol inhibits cholesterol production in the liver by acting on specific liver enzymes (Ostlund 2002). The liver actually manufactures more cholesterol than is typically absorbed from food. However HMG CoA reductase, an important enzyme for the manufacture of cholesterol in the liver, is broken down rapidly in the presence of β -sitosterol resulting in lower cholesterol production (Lichtenstein and Deckelbaum 2001). We can then believe that *T. tetraptera* elicited hypocholesterolaemic effect by promoting the re-distribution of cholesterol with more on the HDL particle, as well as decreasing HMG CoA reductase activity (Adaramoye *et al.* 2005).

An overabundance of free radicals in the cell leads to uncontrolled chain reactions and lipid peroxidation, resulting in various pathological conditions that may include atherogenesis and cancer. It has reported that hypercholesterolaemic atherosclerosis is associated with an increase in the level of the LPO product (malondialdehyde) which is an index of oxidative stress (Prasad 1999). Thus, a decrease in LPO level may lead to a reduction in the arterial wall cholesterol content (Yokazawa *et al.* 2003). In this study, there was over 100% increase in LPO level of hypercholesterolaemic rats, which was attenuated in rats treated with *T. tetraptera* and Questran[®]. Flavonoids are widely recognized as naturally occurring antioxidants that inhibit lipid peroxidation in biological membrane. Hypolipidemic activity of flavonoids from various sources has been reported by several workers (Chan *et al.* 1999; Adaramoye *et al.* 2005). Lipid peroxidation contributes to the development of CVDs, such as pre-eclampsia and atherosclerosis. Also, peroxidation causes impairment of biological membrane functions e.g. decreases fluidity, inactivates membrane bound enzymes receptors, etc. (Seviand and McLeod 1997). Animal bodies are equipped with endogenous enzymic and non-enzymic antioxidants to neutralize or combat the negative effect of oxidative stress. The enzymic antioxidants (Superoxide dismutase, SOD, Catalase, CAT and Glutathione peroxidase, etc.), and non-enzymic antioxidants (tocopherols, carotenoids and ascorbic acid, etc.) can neutralize free radicals and thus reduced LPO generation (Halliwell and Gutteridge 1992). In this study, feeding cholesterol to rats caused significant decrease in hepatic SOD and CAT activities. Co-treatment with *T. tetraptera* and Questran[®] induced the activities of these enzymes to combat the oxidative stress caused by dietary cholesterol. It is obvious that the depletion of hepatic SOD and CAT must be due to the generation of reactive oxygen species resulting from dietary cholesterol fed to the animals (Prasad 1999; Chan *et al.* 1999; Yokazawa *et al.* 2003). The resultant oxidative stress will affect functional and structural integrity of several membrane bound enzymes. Also, crude extracts of *T. tetraptera* was able to restore the hepatic reduced glutathione to normal levels (Chan *et al.* 1999; Adaramoye *et al.* 2005).

Evidence from the present study indicates that crude methanol extracts of *T. tetraptera* fruit, including seed, has hypolipidemic and antioxidant effects. The extract was found to be highly effective in reducing the levels of serum and hepatic total cholesterol, triglyceride and low density lipoprotein cholesterol, thereby exhibiting a hypocholesterolaemic effect in the studied animals. Therefore *T. tetraptera* fruits show therapeutic promise in preventing the development of atherosclerosis and possible related cardiovascular pathologies.

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