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Effect of T*etrapleura tetraptera* fruit on plasma lipid profile and enzyme activities in some tissues of hypercholesterolemic rats

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ABSTRACT

Tetrapleura tetraptera has wide use in South East and Western part of Nigeria as a spice as well as plant to treat various medical ailments. This study was designed to investigate the effect of Tetrapleura-tetraptera dry fruit on the plasma lipid profile and enzyme activities in some tissues of hypercholesterolemic rats. Sixteen healthy white albino rats were divided into four groups A – D. Rats in group A served as the control and received cholesterol-free diet. Group B served as the hyper control and received 1% cholesterol in their diet. The group C and D which are the treated groups received 25g/kg and 50g/kg of the fruit of Tetrapleura tetraptera supplement in their diets respectively together with the 10g/kg cholesterol as with the group B. After feeding for a period of four weeks, the blood samples were collected via cardiac puncture and the organs were removed and assayed for enzyme activities and blood sample was also assayed for enzyme activities and the lipid profile. The result showed significantly higher levels of plasma total cholesterol, low density lipoprotein cholesterol triglycerides and the LDL/HDL ratio in the hyper control rats than the normal control ($P<0.05$) while feeding with 25g/kg and 50g/kg of the dry fruit supplement caused a decrease in the levels of total cholesterol, low density lipoproteins, triglycerides and LDL/HDL ratio with increase level of HDL when compared to the hyper control group ($P<0.05$). Feeding at 25 and 50g/kg caused a significant reduction in the creatinine and urea levels and also a reduction in the kidney enzyme activities when compared to the hyper control ($P<0.05$). However at 50g/kg, the AST and ALP levels were significantly increased in the liver and the heart. It appears that increased concentration of Tetrapleura tetraptera in the diet could cause damage to some of these organs. It can be concluded that even though the fruit of Tetrapleura tetraptera appears to have hypocholesterolemic effect on rats, feeding with excessive amount could be harmful to health.

Keywords: Hypercholesterolemia, *Tetrapleura tetraptera*, lipid profile and enzyme activities.

INTRODUCTION

Cholesterol is the major sterol in the human body. It is a structural component of cell membrane and plasma lipoproteins and it is also the starting material from which steroid hormones are synthesized [1].

Hypercholesterolemia is the presence of high levels of cholesterol in the blood [2]. It is not a disease but a metabolic derangement that can be caused by many diseases. It is closely related to the terms "hyperlipidemia" (elevated levels of lipid in the blood) and "hyperlipoproteinemia" (elevated levels of lipoprotein in the blood) [2]. Lipoprotein disorder is among the most common metabolic disease occurring in human. It may lead to coronary heart disease (CHD) [3].

In Nigeria, there seems to be a gradual shift from traditional foods consisting mainly of roots, cereals beans, tubers and vegetables to fatty foods, snacks and drinks which is evident by the increased number of eateries in our society [4]. These changes in dietary pattern toward a more westernized lifestyle could precipitate hyperlipidemia. Plants were the major source of materials used to combat different kinds of ailments in ancient times and quite a number of these plants are being explored in the treatment of various diseases [5]. Traditional use of any plant for medicinal purposes requires safety of such plants and hence needs to be screened for their toxicity level.

Tetrapleura tetraptera locally called '' Arindan'' in Yoruba belongs to the mimosaceae family. It is a specie of flowering plants in the pea family native to West Africa. The fruit consist of a fleshy pulp with small, brownish-black seeds. The dry fruit has a pleasant aroma and hence used to spice as a seasoning spices in the Southern part of Nigeria [6,7]. Research had shown that the plant have various pharmacological properties due to a variety of active constituents [8]. Pharmacological examination of aqueous extract of this plant also showed that it had little or no hypotensive effect on anaesthesized cats, dogs and rabbits, but significantly depressed the blood pressure of anaesthesized rats [9].

The aim of this study therefore is to assess the effect of dry fruit of *Tetrapleura tetraptera* on plasma lipid profile and some diagnostic enzymes namely Alkaline phosphatase (ALP) Aspartate and Alanine aminotransferase (AST and ALT) in the heart, liver, and kidney of hypercholesterolemic rats. Previous studies have shown that alteration in the activities of these enzymes was correlated with integrity of cellular systems.

MATERIALS AND METHODS

Dry fruit of *Tetrapleura tetraptera* was bought from the Oja-Oba market in Ado-Ekiti, cleaned, sundried, powdered and stored until required for diet composition.
All chemicals used for the study were of analytical grade (ANALAR).

Animal Groupings

Twenty four (24) male white albino rats (*Rattus novergicus*) with average weight of about 120g were used for the study. They were obtained from the Department of Biochemistry, University of Ilorin, Kwara –State, Nigeria. The rats were kept in good conditions and were given normal rat feed and water *ad libitum*. They were ramdomly divided into four experimental groups (A, B, C

and D). Group A served as the normal control fed with standard commercial diet, Group B were fed modified diet containing 20% fat and 1% cholesterol (Table 1) and after establishing hypercholesterolemia, this group was subdivided into Group C and D. Group B was still maintained on the hypercholesterolemic diet. In addition, Group C and D were fed 25mg/kg and 50mg/kg diet of *Tetrapleura tetraptera* respectively while the experimental period lasted for four weeks.

Preparation Of Serum and Tissue Homogenate

After an overnight fast, blood samples were collected into lithium- herparin bottles from the rats under chloroform anaesthesia via cardiac puncture. The rats were dissected to remove the heart, liver and the kidneys. The blood samples were centrifuged at 3000rpm for ten minutes . The clean plasma was collected and kept refrigerated until required for analysis.

Total Cholesterol (TC), HDL Cholesterol (HDL-C), LDL-Cholesterol, Triacylglycerol (TG) were estimated from the plasma. Plasma total cholesterol was estimated using Randox laboratory kit based on the enzymatic end point method. The HDL-Cholesterol was determined by the method of [10]. LDL-Cholesterol was calculated with the Friedeweld formula. [11].

The liver.kidney,and heart were removed quickly, drained of blood and weighed. It was then homogenized in sucrose buffer (0.25M) solution according to [12] and the homogenate kept frozen until required for analysis.

Enzyme and Protein measurements

Alkaline phosphatase (ALP)(E.C.3.1.3.1) activity was assayed using the method of [13] where p-nitrophenyl phosphate was hydrolysed and the absorbance read at 400mm.

Aspartate aminotransferase (AST) (E.C. 2.6.1.1) and alanine aminotransferase (ALT)(E.C 2.6.1.2) activities were determined using appropriate buffer systems by measuring the pyruvate resulting from transamination reactions at 546nm [14].

Table 1: Diet composition (g/kg)

	A	B	C	D
Soyameal	510	510	510	510
Vegetable oil	50	200	200	200
Cholesterol	_	10	10	10
Sucrose	100	100	100	100
Vitamin mineral mix	50	50	50	50
Cellulose	30	30	30	30
Corn starch	260	100	75	50
T. tetraptera fruit	_	_	25	50
A – Control group			C – Test group 1 (25g/kg T. tetraptera fruit)	
B – Hyper control group			D – Test group 2 (50g/kg T.tetraptera fruit).	

Protein concentration was measured by the Biuret method [15]. All measurements were done using Spectronic 20. Statistical evaluation was by analysis of variance (ANOVA). P values <0.05 were regarded significant statistically

Biochemical analysis: Total cholesterol, HDL-C, Triglyceride concentration as well as the activities of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were determined in the plasma using standard assay kits which were products of Randox Laboratories. The LDL-C was estimated by subtracting the value of HDL-C and triacyglycerol from total cholesterol.

Statistical analysis: The results are expressed as Mean ± standard deviation. Analysis of variance was used to test for differences in the groups.

All the values were expressed as mean± standard deviation (SD). Differences were considered to be statistically significant at P<0.05.

RESULTS

Table 2: Plasma Lipid Profile (µmol /l)

	CONTROL	HYPERCONTROL	25g/kg TEST	50g/kg TEST
TC	2.47±0.38^{a}	3.73± 0.96^{b}	3.17±0.40^{ab}	3.13±1.05^{a}
HDL	1.13±0.18^{a}	0.71±0.14^{b}	0.81±0.09^{ab}	0.84±0.25^{a}
TG	0.57±0.12^{a}	0.93±0.21^{b}	0.77±0.21^{a}	0.57±0.08^{a}
LDL	0.65±0.12	2.41±0.64^{b}	2.13±0.32^{ab}	1.55±0.24^{a}
LDL/HDL	0.58±0.14^{a}	3.39±0.20^{b}	2.63±0.16^{ab}	1.84±0.18^{a}

Results are mean± SD (P<0.05) from 4 observations. Values with the same superscript letter(s) along the same row are not statistically different.

LDL - Low density lipoprotein; HDL – High density lipoprotein; TG – Triglycerides; TC – Total cholesterol.

Table 3: Plasma Enzyme Activities (U/L)

	CONTROL	HYPERCONTROL	25g/kg TEST	50g/kg TEST
ALP	10.77±1.56^{a}	14.57±12.49^{a}	311.43±172.75^{b}	170.87±66.62^{ab}
ALT	154.37±19.36^{a}	181.13±12.61^{a}	50.23±22.29^{b}	41.33±10.26^{b}
AST	46.93±3.86^{a}	24.77±2.66^{a}	59.07±67.30^{a}	159.67±24.45^{b}

Results are mean± SD (P<0.05) from 4 observations. Values with the same superscript letter(s) along the same row are not significantly different.

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

Table 4: Heart Enzyme Activities (U/L)

	CONTROL	HYPERCONTROL	25g/kg TEST	50g/kg TEST
ALP	178.60±5.24^{a}	149.23±49.02^{b}	279.03±215.27^{c}	88.87±69.65^{ab}
ALT	100.53±1,99^{a}	209.57±92.07^{ab}	314.70±95.24^{b}	198.53±121.40^{ab}
AST	831.20±1.82^{a}	889.03±100.16^{ab}	957.67±43.24^{b}	683.33±57.30^{c}

Results are mean± SD (P<0.05) from 4 observations. Values with the same superscript letter(s) along the same row are not significantly different.

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

Table 5: Liver Enzyme Activities (U/L)

	CONTROL	HYPERCONTROL	25g/kg TEST	50g/kg TEST
ALP	148.70±63.87[a]	77.83±1.95[b]	177.80±31.17[ab]	249.23±52.33[ab]
ALT	881.93±161.25[a]	876.03±58.54[b]	967.03±450.08[a]	1205.08±84.00[a]
AST	875.37±5.04[a]	588.60±42.42[b]	937.90±237.04[ab]	743.83±84.33[ab]

Results are mean± SD (P<0.05) from 4 observations. Values with the same superscript letter(s) along the same row are not significantly different.

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Table 6: Kidney Enzyme Activities (u/L)

	CONTROL	HYPERCONTROL	25g/kg TEST	50g/kg TEST
ALP	842.47±118.13[a]	915.70±260.30[b]	625.13±216.16[a]	615.17±62.69[c]
ALT	163.10±10.97[a]	99.27±10.10[b]	115.47±32.42[b]	138.87±28.69[b]
AST	985.43±118.29[a]	924.77±131.687[a]	653.47±48.88[a]	821.60±33.10[ab]

Results are mean± SD (P<0.05) from 4 observations. Values with the same superscript letter(s) along the same row are not significantly different.

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

Table 7: Plasma Urea(mmol/l) and creatinine levels(µmol/l)

	CONTROL	HYPERCONTROL	25g/kg TEST	50g/kg TEST
CREATININE	416.67±378.73[a]	893.33±326.55[b]	730.00±113.58[a]	626.67±254.03[a]
UREA	6.53±1.55[a]	9.67±1.7[a]	8.23±1.65[ab]	8.13±1.19[ab]

Results are mean± SD (P<0.05) from 4 observations. Values with the same superscript letter(s) along the same row are not significantly different.

Table 2 shows the plasma lipid profile of the treated and control rats.The total cholesterol, triglycerides and LDL concentration increased significantly in the hypercontrol. The LDL/HDL ratio also increased with decreased HDL when compared to the normal control (P<0.05). In 25g/kg TET group, there was an insignificant decrease in the TC, TG, LDL and LDL/HDL ratio with an increase in HDL when compared to the hypercontrol. In the 50g/kg TET group, there was a significant decreased in TC, TG, LDL and LDL/HDL with a corresponding increase in the HDL level such that these values were not statistically different from that of the normal control (P<0.05).

Table 3 shows the plasma enzyme activities. ALP and ALT increased in the plasma when compared to the control and AST decreased (P<0.05). In the 25g/kg TET group, there was a significant decrease in the plasma level of ATL when compared to the control. Also, the activities of ALP and AST increased when compared to the normal control (P<0.05). In the 50g/kg TET group, the plasma levels of ALP and AST significantly increased.

Table 4 shows the activities of ALP, ALT and AST in the heart. The activities of ALT and AST significantly increased in the hypercontrol when compared to the normal control. ALP significantly decreased when compared to the normal control (P<0.05). In the 25g/kg TET group, the levels of ALP, ALT and AST increased significantly. In 5og/kg TET group, the levels of ALP and AST decreased significantly when compared to the normal control (P<0.05).

The enzyme activities in the liver are shown in the table 5. From the table, it was observed that the activities of ALP, ALT and AST reduced in hypercontrol when compared to the normal control. While the activities of ALP, ALT and AST in 25g/kg TET group insignificantly increased when compared to the normal control. Also, the activities of ALP and ALT in 50g/kg TET group insignificantly increased when compared to the control but the activity of AST insignificantly reduced when compared to the control.

Table 6 represents the kidney enzyme activities and from this table, it was shown that the activities of ALP significantly increased in the hypercontrol with a decrease in the 25 and 50g/kg TET group when compared to the normal control. ALT levels decreased significantly in the hypercontrol, 25 and 50g/kg TET when compared to the normal control. AST also decreased in the 50g/kg TET.

Plasma creatinine and urea levels is presented in Table 7. It shows that the levels of creatinine and urea increased in the hypercontrol when compared to the control ($P<0.05$) while feeding with 25g/kg and 50g/kg *T.tetraptera* dry fruit caused a significant reduction compared to hypercontrol group.

DISCUSSION

The increment in total cholesterol, LDL cholesterol, plasma triglycerides as well as LDL/HDL ratio in hypercontrol rats in this study might be as a result of cholesterol rich diet fed into the rats. Cholesterol rich diet has been demonstrated by several workers to increase plasma total cholesterol, LDL-cholesterol as well as plasma triglycerides concentration [16,17,18]. However, high concentration of blood cholesterol are associated with the development of diseases like cardiovascular, hypercholesterolemia and lithiasis vesicular. High cholesterol diets leads to cholesterol deposition in the arterial walls [19]. The reduction in total cholesterol, LDL-cholesterol as well as the triglyceride concentration on supplementation with 25g/kg and 50g/kg *T.tetraptera* fruit suggests the hypocholesterolemic potential of the plant. Also, the LDL/HDL cholesterol ratio which is thought to be the atherogenic index of lipoproteins [20] was lower in rats fed with the dry fruit of *T. tetraptera* than in the hypercholesterolemic rats. Although in the past, an increase in the serum total cholesterol level is associated with increased risk of atherosclerosis, however, recent reports indicated that the LDL/HDL ratio is a stronger index of atherogenicity of the lipoproteins rather than individual lipoprotein fraction i.e. the lower the ratio the less atherogenic the lipoprotein profile is thought to be [21,22].

This study agrees with the work of [23] where P_{407} was shown to increase triglycerides, total cholesterol and LDL-cholesterol level in the plasma. However, the higher triglyceride level was attributed to the inhibition of triglyceride degradation, due to direct inhibitory effect on lipoprotein lipase bound to capillary endothelium. Lipoprotein lipase is vital in the metabolism of triglycerides and is involved in several pathological disorders, including atherosclerosis and obesity. Hence, it is possible that the dry fruit of *Tetrapleura tetraptera* affects lipoprotein lipase activity since it was significantly effective in reducing triglyceride levels in this study.It was also reported that tomato lycopene could also prevent an increase in total and LDL serum cholesterol in high cholesterol fed rats[24]. Also previous studies have established an inverse relationship between HDL cholesterol and increase in cardiovascular disease [25]. Hypercholesterolemia has

been known through several studies to disturb the oxidant - pro-oxidant balance in favour of pro-oxidation hence weakening the efficiency of the antioxidant defense system, resulting in ineffective scavenging of free radicals which leads to tissue damage often associated with the development and progress of atherogenesis [26,27,28]. However, the presence of biological antioxidants in plants, which can prevent the uncontrolled formation of free radicals and activated oxygen species and or inhibit their reaction with biological structures may in part be responsible for the beneficial effects of the dry fruit of *T.tetraptera* suplement in hypercholesterolemic rats.

This study has demonstrated that, the dry fruit of *Tetrapleura tetraptera* is capable of reducing blood lipids significantly, it is important to state that the effectiveness of this plant appears to be related to the amount supplemented in the diet, hence *T.tetraptera* could be used for the treatment of elevated total cholesterol.

The decrease in the activity of ALP in the heart as observed in this study, with an increase in the plasma levels of this enzyme in the hypercontrol rats may suggest damage to the heart as a result of the hypercholesterol diet. In the group fed with 25g/kg body weight of the fruit of *T. tetraptera*, the heart activities of ALP, ALT and AST was observed to increase when compared with the normal control rats. This could be due to the increased synthesis of the enzyme in situ, and since there was no concomitant increase in plasma, it shows the heart appears to be recovering from the injury caused by the hypercholesterol diet.

The decrease observed in the activities of ALP and AST in the heart of the rats fed with 50g/kg of the plant fruit supplement with a concomitant increase in the plasma activities of these enzymes could be due to the damage to the membranes of the heart which results in the out flow of the enzymes from the heart into the plasma thereby increasing the activities of the enzymes in the plasma.

The liver is the largest solid organ in the body. It is the centre of all metabolic activities in the body. Drugs and other foreign substances are metabolized and inactivated in the liver. Essential functions of the liver tend to be lost in the development of hepatic disease or disorder [29]. The damage to the hepatocytes may lead to release of intercellular constituents into the plasma or circulation.

ALP is a marker enzyme of the plasma membrane and an indicator of liver cholestasis. The leakage of the enzyme into the plasma could be as a result of damage to the integrity of the liver. According to [30], AST and ALT are released into the plasma when there is severe hepatocellular injury. The presence of bioactive agents identified in the plant could also play a role in the selective toxicity observed. Saponins is present in large amounts in the plant [31] and its been reported to lyse red blood cells by destroying the erythrocyte membrane [32,33]) saponins could therefore serve as agent affecting the integrity of the membranes.

The decrease in the kidney enzyme activities of rats fed the dry fruit ot *T.tetraptera* shows that this organ may not be affected. ALP has been reported to be a very strong risk factor, taking third place for all forms of kidney diseases and a possible indicator for early development of renal damage. [34] reported a correlation between ALP and renal mortality indicating that the higher

the elevation of ALP the greater the risk of renal failure.This is further supported by the reduced levels of plasma urea and creatinine in the treated rats. In contrast to the observation made for the hypercholesterolemic rats, which suggest marked glomerular dysfunction.

The usual blood test which checks whether the kidneys are functioning properly measures the level of urea, creatinine and certain undissolved salts. Urea is a waste product formed from the breakdown of proteins; urea is passed out in the urine. A high blood level of urea (uraemia) indicates that the kidney may not be functioning properly, or dehydration. Creatinine is a waste product made by the muscles, creatinine passes into the blood stream and is usually passed out in urine. A high blood level of creatinine indicates that the kidneys are impaired. Creatinine is usually a more accurate marker of kidney function than urea.

This study has demonstrated that *Tetrapleura tetraptera* dry fruit could be protective against kidney disorders by decreasing urea and creatinine levels. A rise in serum creatinine is observed only with marked damage to functioning nephrons, a better estimation of kidney function is given by the creatinine clearance test. Creatinine clearance can be accurately calculated using serum creatinine concentration [35].

In conclusion, the result from this study suggests that supplements of dry fruit of *Tetrapleura tetraptera* in the diet could play a role in the reduction of the excessive levels of total cholesterol, LDL-cholesterol, triglycerides and as well decrease the LDL/HDL ratio in the body and hence prevents against cardiovascular diseases. However an excessive amount of the plant supplement could pose a risk to the heart and the liver. In otherwords, although dry fruit of *Tetrapleura tetraptera* is effective in lowering TC and LDL-C in rats, the plant should be administered in moderate amounts to prevent potential adverse effects. Further investigations would be required to know the mode of action in lowering cholesterol level.

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