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Antiatherogenic, Hypolipidemic and Antiinflammatory Benefits of Black Tea and *Zanthoxylum Zanthoxyloid*

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAS designed the study, wrote the protocol, involved in writing the first draft. Authors AO and BLJ managed the literature search and analyses of the study. Author KJP performed the statistical analysis. Author AA was actively involved in reading the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The use of medicinal plants in the treatment of ailments is increasing around the globe. This study assessed the antihyperlipidemic and antiatherogenic benefits of black tea (BT) and *Z. zanthoxylum (Zz)* in rats. Thirty two albino rats were randomly divided into eight groups each containing four animals. Group 1 normal control; animals in groups 2, 3 and 4 were fed standard diet supplemented with BT or *ZZ* or a combination of both at equal amounts (3% each), respectively. Animals in group 5 are control rats, fed diet supplemented with cholesterol and groundnut oil at a dose level of 100 g and 300g/25 kg diet respectively. Rats in groups 6, 7 and 8 were fed the same high lipid diet but supplemented with BT, Z.z or mixture of both respectively. Lipid enriched diet caused a significant increase in total cholesterol, total lipids, and triacylglycerols in both serum and liver. Serum phospholipids, LDL-C, and atherogenic index significantly increased compared with normal control group. BT and *Zz* significantly increased fecal total lipids,

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total cholesterol and triacylglycerol levels as well as significantly increased serum malondialdehyde, interleukin-2 and tumour necrosis factor-alpha compared to healthy control. Consumption of black tea, *Zanthoxylum zanthoxyloid* or a combination mixture of them by healthy and hyperlipidemic hypercholesterolemic rats resulted in significantly decreased lipid parameters in serum and liver and significant reduced lipid peroxidation and inflammation. These results suggest that both black tea and *Zanthoxylum zanthoxyloid* had anti-atherogenic and hypolipidemic effects and reduced oxidative stress via inhibition of inflammation and lipid peroxidation.

Keywords: Natural products; antiatherogenic; hypolipidemic; cardiovascular disease; anti-inflammatory; antioxidation.

1. INTRODUCTION

Atherosclerosis leading to coronary artery disease has assumed a virulent disease ratio and is the principal cause of death the world over in the developed as well as in the developing countries. Epidemiological studies have demonstrated a positive significant relationship between plasma cholesterol concentrations and coronary artery disease [1]. Clinical manifestations include plaque formation in the artery walls. In many cases, plaques protrude into the lumen of the artery and if sufficiently large, compromise the flow of blood [2].

In epidemiological studies of hypercholesterolemia, high levels of plasma cholesterol and LDL-C are significantly associated with the development of premature atherosclerosis [3]. Cholesterol accumulation in the arterial wall is the main sign of atherosclerosis. It has been suggested that LDL is the major source of cholesterol deposited in the vessel wall. Accumulation of cholesterol and other lipids is the most prominent manifestation of atherosclerosis at the arterial cell level. In addition to lipid accumulation, increased proliferative activity of vascular cells and enhanced synthesis of the extracellular matrix are characteristics of cellular atherogenesis.

Synthetic anti-hyperlipidaemic drugs like statins and synthetic antioxidants like probucol are widely used to treat atherosclerosis. Unfortunately, these drugs have side effects [4]. Hence, for treatment or management of atherosclerosis, much attention has been focused on the use of natural products that have very few side effects [5]. For instance, [6] reported antiatherogenic properties of *alpinia zerumbet* (a perennial ginger growing widely in the subtropics) seeds. Also, [7] reported antihyperlipidemic effects of flavonoids from *prunus davidiana*. Black tea and *Zanthoxylum zanthoxyloid* are some plants and herbs with antioxidative properties confirmed to have phenolic and flavonoid bioactive constituents. They have been employed in the management and treatment of series of ailments. For instance, *Zanthoxylum zanthoxyloid* is been used traditionally for the treatment of infections such as skin infection, gonorrhoea, dysentery [8]; elephantiasis, toothache, sexual impotence [9,10], while black tea is being used orally as a slimming remedy. The two have been shown to contain phenols and flavonoid which are good antioxidant agents. Because of their antioxidative potentials, this study is objectively designed to determine and compare the antilipidemic and antiatherogenic properties of black tea, ethanolic extract of *zanthoxylum zanthoxyloid* and combination of the two.

2. MATERIALS AND METHODOLOGY

2.1 Chemicals and Quantitative Assay Kits

All chemicals used including solvents are of analytical grade. Thiobarbituric acid (TBA), hydrochloric acid (HCL), trichloroacetic acid (TCA), Tris KCL buffer, chloroform, methanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The ELISA kits for the determination of rat interleukin-2 (IL-2) and tumor necrosis factor-alpha (TNF-α) were products of RayBiotech, Inc. USA, while those for total cholesterol, triacylglyceride and high density lipoprotein cholesterol (HDL-C) were products of LABKIT, CHEMELEX, S.A. Pol. Canovelles-Barcelona, Spain.

2.2 Plants Extract and Extraction Procedure

Stem of *Z. zanthoxyloide* was obtained from Ogbomoso, Nigeria in 2011 and was identified by Dr. Ogunkunle of the Botany Unit, Department of Pure and Applied Biology and were confirmed with a plant name index. The stem was grinded into a powdery form and the powder of *Z. zanthoxyloide* (1 kg) was macerated at room temperature with ethanol (70%) and extracted for 72 hours. On the third day, the combined ethanolic extract was filtered and the solvent was fully evaporated under reduced pressure to give a yellow solid. The black tea used was in powdery form and is a product of RUCHIM LTD, Nigeria..

2.3 Animals and Diet

All experiments were performed on albino rats weighing 105–115 g obtained from commercial breeder. Animals were housed in metal cages and kept in a room maintained at an average temperature of 25◦C ± 3◦C and with 12 h darkness and 12 h light. They were fed with standard pellet diet (Adom Animal Feeds, Nigeria) for a week before start of experiment. The rats were allowed to have free access to food and tap water *ad libitum*. From then on (t = 0) until the end of the experiment, rats were fed the feed (Table 1) which we prepared in our laboratory. The animals were kept in cages with raised floors of wide wire mesh to prevent the animals from consuming their feaces. The protocol conforms to the guidelines of the National Institute of Health (NIH) (NIH publication 85–23, 1985) for laboratory animal care and use.

2.4 Experimental Design

Preceeding the study, all rats consumed the same diet for one week. Thirty two albino rats were randomly divided into eight groups each containing four animals of both sexes (1:1). Group 1 (normal control) consisted of animals given standard diet alone while animals in groups 2, 3 and 4 were fed on standard diet supplemented with black tea (BT), *zanthoxylum zanthoxyloid (ZZ)* and a combination of black tea and *zanthoxylum zanthoxyloid* (BT+Zz) at equal amounts (3% each), respectively. Animals in group 5 were control hypercholesterolemic-hyperlipidemic rats, fed the high fat diet (standard diet supplemented with cholesterol and groundnut oil at a dose level of 100 g and 300g/25 kg diet respectively). Groups 6,7 and 8 consisted of animals that were fed high fat diet supplemented with black tea (G6), *Zanthoxylum zanthoxyloide* (G7) and combination of the black tea and *Zanthoxylum zanthoxyloid* at the same level (3% each)(G8). The rats were weighed every week. At the end of experimental period (60 days), and after overnight fasting (12 hours), the rats were scarified by cervical dislocation and blood samples were collected directly from the

heart. The sera were prepared in the laboratory stored in freezer until analyzed. Three major organs (heart, liver and kidney) were harvested, washed with ice-cold isotonic saline and weighed.

2.5 Serum Lipid Profile

The concentrations of the parameters were measured using spectrophotometric methods. The serum concentrations of total cholesterol (TC), triglyceride (TG), and high density lipoprotein cholesterol (HDL-C) were determined by methods described by [11,12,13] respectively. Low density lipoprotein cholesterol (LDL-C) was calculated using Frieldwald formula [14]. Very low density lipoprotein cholesterol (VLDL-C) was equally calculated. Lipid peroxidation was estimated in serum by measuring the malondialdehyde (MDA) production during the thiobarbituric acid reaction [15].

2.6 Assay Procedure for the Cytokines

All reagents and samples were brought to room temperature (18-25^{0}C) before use. According to the recommendation of the manufacturer, all standards and samples were run at least in duplicate. Hundred microlitres (100 μ L) of each standard and sample were added into appropriate wells. These wells were covered and incubated for 2.5hours at room temperature with gentle shaking. The solution was discarded and the well washed 4 times with 1x wash solution. The wells were washed by filling each well with wash buffer (300 μ L) using a multi-channel pipette or auto washer. The liquid was completely removed at each step as this is essential for good performance. After the last wash, remaining wash buffer was removed by aspirating. The plates were inverted and blotted against clean paper towels. One hundred microlitres (100 μ L) of 1x prepared biotinylated antibody was added to each well. This was incubated for 1 hour at room temperature with gentle shaking. The solution was discarded and washed 4 times with 1x wash solution.

One hundred microlitres (100 μ L) of prepared streptavidin solution was then added to each well. This was incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded and washed 4 times with 1x wash solution. One hundred microlitres (100 μ L) of TMB one-step substrate reagent was added to each well and incubated for 30minutes at room temperature in the dark with gentle shaking. Finally, fifty microlitres (50 μ L) of stop solution was added to each well read at 450nm immediately.

2.7 Atherogenic Index

The atherogenic index serum (AIS) which is the measure of the extent of atherosclerotic lesions based on serum lipids is determined in all eight groups. The atherogenic index is calculated using the formula AIS = TC/HDL [16].

Table 1. Compositions of both standard and atherogenic diets

Compositions	Standard diet (SD)(kg)	High fat diet (HFD) (kg)
Maize	4.485	4.485
Soya bean meal	1.870	1.870
Brewery dry grain (BDG)	8.750	8.750
Wheat bran (WB)	2.500	2.500
Rice bran (RB)	6.250	6.250
Oyster shell (OS)	0.500	0.500
Bone meal (BM)	0.250	0.250
Common salt (CS)	0.065	0.065
methionine	0.250	0.250
Fish meal	0.250	0.250
Groundnut oil	-	0.300
Cholesterol	-	0.100

2.8 Statistical Analysis

Results were expressed as means±SD. and analyzed for statistical significance by two-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Values were considered statistically significant at $p < 0.05$.

3. RESULTS

Table 2 Effect of black tea, zanthoxylum zanthoxyloid and combination of black tea and *Z.zanthoxyloid* on Body Weight and Relative Organs Weight:

As shown in Table 2, induced hypercholesterolemia and hyperlipidemia(HFD diet) caused a significant increase in body weight gain and a significant increase in the organs weight as compared with healthy control group (G1). Supplementation with BT, Z.z and combination of BT and Z.z to either normal or hyperlipidemic, hypercholesterolemic rats caused a significant decrease in body weight gain and in the organs weight as compared with their corresponding control rats.

Table 2. Effect of supplementing black tea, *Zanthoxylum zanthoxyloid* and combination of black tea and *Zanthoxylum zanthoxyloid* on body weight gain and organs weight in hypercholesterolemic rats

Groups	Body weight gain (g)	weight of liver (g)	weight of heart (g)	weight of kidney(g)
G1 (control)	69.60±2.88	8.05±0.22	0.66±0.04	0.84±0.02
G2 (SD+BT)	63.00±4.09	4.91±0.1	0.60±0.01	0.74±0.02
G3(SD+ZZ)	61.20±2.60	6.86±0.11	0.66±0.02	0.78±0.03
G4(SD+BT+*Z.Z*)	62.81±3.15	6.25±0.09	0.58±0.06	0.80±0.02
G5(HFD)	89.64±6.41	8.20±0.16	0.80±0.06	0.94±0.04
G6(HFD+BT)	67.98±9.20	6.93±.22	0.66±0.03	0.82±0.05
G7(HFD+ZZ)	73.61±4.18	8.03±0.35	0.70±0.04	0.88±0.06
G8(HFD+BT+ZZ)	75.47±3.81	6.82±0.38	0.56±0.04	0.90±0.06

Level of significance was picked at p<0.05; G1, G2, G3,G4,G5,G6, G7 and G8 indicated groups 1,2,3,4,5,6,7 and 8 respectively.

3.1 Effect of black tea, *Zanthoxylum zanthoxyloid* and combination of black tea and *Zanthoxylum zanthoxyloid* on Serum, Liver and Fecal Lipid Contents

In tables 3,4,5 and 6 feeding rats with 0.2 % cholesterol and 0.6% fat-enriched diet for 60days resulted in a significant elevation of serum and liver total cholesterol (130.09%, 81.11%), total lipids (56.08%, 99.57%) and triacylglycerols (55.34%, 40.73%). Serum phospholipids, LDL/HDL ratio and atherogenic index were also significantly increased in HFD control group .Fecal lipids were also significantly increased and influenced by the amount of fat in the diet. Supplementation with black tea, *Zanthoxylum zanthoxyloid* and combination of black tea and *Z.zanthoxyloid* to either standard diet or high fat diet caused a significant decrease in the levels of serum and liver total lipids, total cholesterol and triacylglycerols, however, fecal fat excretion was significantly increased by these supplementation.

Table 3. Effect of supplementing black tea and *z.zanthoxyloid* on lipid parameters and atherogenic index in healthy rats given standard and high fat diet

Groups	Total Cholesterol (mg/dL)	Total lipids (mg/dL)	Triacylglycerol (mg/dL)	Phospholipids (mg/dL)	Atherogenic index(TC/HDL-C)
G1 (control)	101.48±1.35	340.6±0.79	70.81±0.80	140.8±0.91	2.49±0.10
G2 (SD+BT)	91.88±0.51	262.49±15.04	59.04±0.71	115.79±2.6	2.15±0.11
G3(SD+ZZ)	95.51±0.59	300.66±6.07	66.24±1.01	125.21±1.01	2.37±0.06
G4(SD+BT+*Z.Z*)	94.61±0.95	280.01±14.98	64.90±0.53	120.20±2.98	2.31±0.05
G5(HFD)	234.4±5.01	531.62±2.2	110.1±2.01	236.72±2.41	8.72±0.45
G6(HFD+BT)	152.07±2.81	340.53±7.6	80.20±2.13	180.51±4.1	3.29±0.22
G7(HFD+ZZ)	176.68±2.75	390.67±8.10	92.00±2.34	208.01±4.97	4.88±0.31
G8(HFD+BT+*ZZ*)	155.68±2.24	350.02±7.87	82.46±2.04	178.05±9.73	3.41±0.18

TC= total cholesterol; HDL-C= high density lipoptotein cholesterol

Table 4. Effect of supplementing black tea, *Z.zanthoxyloid* and combination of black tea and *Z.zanthoxyloid* on VLDL-C, HDL-C, and LDL-C in healthy rats given standard and high fat diet

Groups	VLDL-C (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	LDL-C / HDL-C ratio
G1 (control)	14.27±0.09	40.8±0.67	49.00±2.0	1.20±0.01
G2 (SD+BT)	14.18±0.20	42.67±1.79	40.11±2.00	0.94±0.02
G3(SD+ZZ)	14.98±0.19	40.32±0.89	44.40±1.20	1.10±0.04
G4(SD+BT+*Z.Z*)	14.61±0.14	41.03±0.89	42.02±0.70	1.02±0.03
G5(HFD)	26.88±0.30	29.23±0.89	190.04±5.11	6.50±0.29
G6(HFD+BT)	20.91±0.52	46.21±2.01	96.01±1.99	2.08±0.2
G7(HFD+ZZ)	23.1±0.32	36.21±0.21	130.01±2.1	3.59±0.1
G8(HFD+BT+*ZZ*)	21.41±0.42	45.64±0.66	100.80±4.01	2.21±0.15

Table 5. Effect of supplementing black tea, *Z. zanthoxyloid* and combination of black tea and *Z. zanthoxyloid* on hepatic total cholesterol, total lipids and triacylglycerols in healthy rats given standard and high fat diet

Groups	Total Cholesterol Mg/g tissue	Total lipids Mg/g tissue	Triacylglycerol Mg/g tissue
G1 (control)	4.87±0.06	40.10±0.67[k]	7.12±0.20
G2 (SD+BT)	3.41±0.18	37.10±0.87	6.22±0.71
G3(SD+ZZ)	4.60±0.03	38.76±2.00	7.21±0.05
G4(SD+BT+*Z.Z*)	4.51±0.01	38.1±0.95	7.02±0.07
G5(HFD)	8.82±0.13	80.03 ±2.42	10.02±0.31
G6(HFD+BT)	5.93±0.32	63.32±2.12	7.41±0.20[j]
G7(HFD+ZZ)	7.12±0.11	71.02±2.0	8.70±0.53
G8(HFD+BT+*ZZ*)	6.11±0.13	65.2±2.01	7.32±0.32

Table 6. Effect of supplementing black tea, *Z. zanthoxyloid* and combination of black tea and *Z. zanthoxyloid* on fecal total cholesterol, total lipids and triacylglycerols in healthy rats given standard and high fat diet.

Groups	Total Cholesterol Mg/g	Total lipids Mg/g	Triacylglycerol Mg/g
G1 (control)	11.03±0.1	62.54±0.21	10.12±0.34
G2 (SD+BT)	11.51±0.12	68.1±0.39	11.56±0.18
G3(SD+ZZ)	11.20±0.19	66.02±0.23	10.46±0.5
G4(SD+BT+*Z.Z*)	11.12±0.20	67.52±0.65	11.02±0.64
G5(HFD)	21.22±0.7	105.56±0.98	18.66±0.45
G6(HFD+BT)	23.11±0.24	115.42±0.24	20.03±0.31
G7(HFD+ZZ)	22.12±0.4	110.1±1.03	19.31±0.67
G8(HFD+BT+*ZZ*)	23.12±0.22	112.31±0.65	19.34±0.27

Effect of black tea, *Zanthoxylum zanthoxylum* and combination of black tea and *Zanthoxylum zanthoxylum* on malondialdehyde, interleukin-2 and tumour necrosis factor-alpha:

In table 7, supplementing both standard diet and high lipid diet with black tea, *Z.zanthoxyloid* and combination of the 2 reduced oxidative stress as evident by reduction in formation of malondialdehyde which is one of the biochemical markers of lipid peroxidation. Also, these supplementations caused reduction in inflammation as indicated by reduced inflammatory responses as shown by reduced serum concentrations of interleukin-2 and TNF-alpha.

Table 7. Effect of supplementing black tea, *Z. zanthoxyloid* and combination of *Z. zanthoxyloid* on malondialdehyde, tumour necrosis factor-alpha and interleukin-2 in healthy rats given standard and high fat diet

Groups	MDA(Mg/dl)	IL-2(µmol/l)	TNF-α(pg/ml)
G1 (control)	4.70 ±0.14	55.33±6.22	340.55±24.32
G2 (SD+BT)	4.1 ±0.21	42.23±4.34	276.45±9.32
G3(SD+ZZ)	4.6 ±0.10	45.33±4.50	289.49±11.41
G4(SD+BT+*Z.Z*)	4.3 ±0.08	50.22±5.32	296.51±8.34
G5(HFD)	9.3±0.12	80.19±11.41	451.88±36.53
G6(HFD+BT)	6.00±0.21	65.53±7.45	362.65±12.45
G7(HFD+ZZ)	7.61±0.10	67.44±7.61	369.66±13.1
G8(HFD+BT+*ZZ*)	6.9±0.2	68.34±6.56	381.72±13.11

Table 8. Comparisons of Mean Values between Different Groups

	Comparisons of body and organs weights				Comparisons of serum biochemical parameters											Comparisons of hepatic concentrations of biochemical parameters			Comparisons of fecal concentrations of biochemical parameters		
	BW	LW	HW	KW	TC	TL	TG	PLP	AI	VLDL	HDL	LDL	MDA	IL-2	TNF-α	TC	TL	TG	TC	TL	TG
G5VSG1	S	NS	S	NS	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
G5VSG6	S	S	S	NS	S	S	S	S	S	NS	S	S	S	NS	S	S	S	S	NS	NS	NS
G5VSG7	S	NS	NS	NS	S	S	S	NS	S	NS	S	S	NS	NS	S	S	NS	NS	NS	NS	NS
G5VSG8	S	S	S	NS	S	S	S	S	S	NS	S	S	S	NS	S	S	S	S	NS	NS	NS

G1 VS G2	S	S	NS	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	NS	NS	NS	NS	NS
G1 VS G3	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
G1 VS G4	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

BW= body weight; LW= liver weight; HW= heart weight; KW= kidney weight; TC= total cholesterol; TL= total lipids; TG= triglyceride; PLP= phospholipids, AI= atherogenic index; VLDL= very low density lipoprotein; HDL= high density lipoprotein; LDL= low density lipoprotein,; MDA= malondialdehyde; IL-2= interleukin-2; TNF-α= tumour necrosis factor-alpha; S= significant; NS= not significant; Significance at p<0.05.

4. DISCUSSION

Dietary fat intake is the most important factor that modulates serum levels of cholesterol, and this modulation figured prominently in the early scientific study of cholesterol metabolism.

Portman and Stare [17] wrote a review on evaluated dietary factors in the regulation of serum cholesterol and described how every species studied exhibited a rise in serum cholesterol levels after a high-fat dietary challenge. Thus, fat intake has always been considered key in the control of blood lipid levels and associated cardiovascular diseases. Diets with increased lipid content, loosely termed high-fat diets, have been used successfully to increase serum cholesterol levels experimentally and have become valuable tools in the study of hypercholesterolemia.

In this study, consumption of lipid-rich diet caused increased body and relative weights of visceral organs. Here, the body and organs weight gain in hypercholesterolemic rats were decreased significantly upon treatment with black tea, *Z. zanthoxyloid* or mixture of black tea and *Z. zanthoxyloid*. These results suggest that among the herbs used, black tea has the most pronounced effect on the nutritional status of animals. These results are in agreement with [18] who reported that body weight gain and liver weight of rats fed with high fat diet significantly increased as compared with rats fed normal basal diet. [19] also reported that body weight gain differed significantly between hypercholesterolemic rats and normal control rats. This increase was probably due to the high lipid diet.

The body and liver weights of the rats fed black tea were significantly lower than those fed the control diet. The reduced rate of growth of the rats given diet supplemented with black tea was undoubtedly due to a reduced food intake. Black tea contains caffeine, which is one of the alkaloids that act as stimulants in the body. Caffeine suppresses appetite and equally stimulate thermogenesis, a physical reaction that increases the amount of calories the body burns. Caffeine may also cause temporary weight loss due to its diuretic effect. The caffeine in black tea might have caused the loss of weights observed in the body and organ weights of the rats given black tea. This supports its use as weight-loss agent for obese humans. Furthermore, the loss of body and relative organs weight in LRD-group given *Z. zanthoxyloid* may be due to its contents of phenolics and flavonoids. In our previous study, we reported presence of phenolic (chlorogenic and caffeic acids) and flavonoids (rutin, quercetin and kaempferol) in *Z. zanthoxyloid* [20]. The tannins and flavones are involved in free radical scavenging mechanism and may prevent atherogensis. Natural products extracts of therapeutic relevance are of paramount importance as reservoirs of structural and chemical diversity [21]. Epidemiological studies have reported a reduced risk of coronary heart disease in subjects with high flavonoid intake [22,23].

Accordingly, in this study consumption of lipid rich diet led to increased serum, hepatic and fecal concentrations of total cholesterol, total lipids and triglycerol. Hyperlipidemic effects of consumption of hypercholesterolemic diet in rats have been reported by [24]. Evidence suggested that increased intake of saturated fat increases all serum lipid levels. Diet induced hypercholesterolemia has health significance in the face of the increasing consumption of high fat fast food and the associated increase in the incidence of coronary heart disease, obesity in the general population, particularly in western countries. Supplementation of lipid rich diet with black tea, *Z. zanthoxyloide* or combination of the two reduced the hyperlipidemic effect of the lipid rich diet (LRD) by causing a reduction in the serum and hepatic concentrations of total cholesterol, total lipids and triglyceride. These properties of the two herbs/natural products are significant particularly in reducing risk of predisposition to

obesity and other associated lipid disorders. Use of medicinal plants and other natural products in the management of metabolic diseases is on the increase as they provide alternative, cheap and very potent means of alleviating ailments due to dietary factors. Supplementation of high fat diet with black tea, *Z. Zanthoxyloid* or their combination caused increased concentration of serum HDL-C. There is an inverse relationship between serum concentration of HDL-C and incidence of coronary heart disease (CHD). High density lipoprotein play a prominent role in the reverse cholesterol transportation thus reducing excessively high levels of cholesterol in the extrahepatic tissues, thus reducing the risk of CHD.

Rats fed lipid-rich diet showed high serum concentration of low density lipoprotein cholesterol. An increased serum LDL-C and TG-C concentrations has direct proportionality to occurrence of cardiovascular disorders. Supplementation of fat-rich diet with black tea, *Z. Zanthoxyloid* or combination of both caused reduction in the serum concentrations of LDL-C and TG. Previous studies have demonstrated that lowering plasma total cholesterol, LDL-C, and increasing HDL-C are beneficial in preventing risk of cardiovascular diseases (CVD) [25,26]. The data in this study indicated that both black tea and *Z. Zanthoxyloid*, either individually or in combination had potent hypolipidemic, hypocholesterolemic and hypotriglyceridemic properties. This implies that either of them or their combination may be useful in the control of serum lipid profiles.

Increased serum and hepatic cholesterol could be due to the high fat diet and this indicated that the dietary lipid obviously disturbed hepatic lipid metabolism. A rise in the liver and plasma cholesterol concentrations of rats fed HFD had been reported [27]. It has been suggested that the rise could be due to subsequent deposition and decreased cholesterol catabolism as indicated by reduction in bile production and turnover of bile acids [28]. Supplementation with black tea and *Z. Zanthoxyloid* caused reduction in the hepatic concentrations of total cholesterol, total lipids and triglyceride. That was an indication that the two herbs used in this study improved the lipid metabolism.

The accumulation of hepatic triglyceride in this study could be due to the enhancement of hepatic triglyceride synthesis and reduction of fatty acid beta-oxidation. Hepatic TG level is controlled mainly by TG synthesis, beta-oxidation and secretion in form of lipoprotein [29]. Increased lipogenesis, decreased oxidation of fatty acids and decreased secretion of VLDL has been suggested as causes for the accumulation of TG in the liver in the cholesterol- fed rats [30].

The atherogenic index (TC/HDL-C and LDL-C/HDL-C) was significantly reduced in groups fed diets supplemented with black tea*, Z. Zanthroxyloid* or their combination. The data from this study agreed with [31] who reported that a reduction in TC: HDL-C and LDL-C: HDL-C ratio is an important requirement of an antilipidemic and antiatherogenic agent.

In this study, supplementation of high lipid diet with BT and *Z. z* caused increased fecal excretion of cholesterol (8.91%, 4.24%), total lipids (9.34%, 4.30%) and triglyceride (7.34%, 3.48%) when compared with HFD control. The increased fecal excretion could be that both BT and *Z.z* caused reduction in the intestinal absorptibility/digestibility of these lipids. Though the percentage increase in fecal excretion may appear small, but it corresponds to weight loss and this may be responsible for reduced body and visceral organ weight observed in the rats given diet supplemented with BT and *Z.z*. The increased fecal excretion of lipids may be one of the hypolipidemic properties of BT and *Z.z*. Observation in this study showed higher fecal excretion of lipids in rats given high lipid diet supplemented with BT and

Z.z when compared with rats given standard diet alone. This may be an implication that supplementation with either of BT and *Z.z* may be effective in the prevention and treatment of hypercholesterolemia and hyperlipidemia.

High fat diet induced increased lipid peroxidation causing elevated serum concentration of malondialdehyde. This agreed with the findings of [32] who reported induction of lipid peroxidation by high saturated and unsaturated fat diets in rats. Long-term feeding of a high fat diet acts as an inducer of oxidative stress, since it significantly attenuates the hepatic enzyme antioxidant system [33], and increases the levels of lipid peroxidation products in the liver [34] and plasma [35]. There is good evidence that oxidative damage contributes to the pathology of atherosclerosis and vascular dysfunction generally, and that free radicals are involved in myocardial ischemia-reperfusion injury [36].

However, supplementation of diet with black tea, *Z.zanthoxyloid* or combination of both resulted in reduction of malondialdehyde, a biochemical marker of lipid peroxidation. The implication of this is that black tea and *Z. zanthoxyloid* possessed anti-oxidative factors thus inhibiting lipid peroxidation. This agrees with our previous study where we reported presence of phenolic (chlorogenic and caffeic acids) and flavonoids (rutin, quercetin and kaempferol) in *Z. zanthoxyloid* (20). Indeed, foods and beverages derived from plants are chemically complex, and cardiovascular protective effects could also arise from many other components or mixtures of components present, including fibre, immunostimulatory agents, monounsaturated fatty acids, agents that modulate cholesterol synthesis, B-vitamins, folic acid, agents modu-lating nitric oxide production, and even the humble ethanol molecule itself [37].

The profile of the interleukin-2 and tumour necrosis factor alpha obtained in rats fed high lipid diet (table 7) is an indication of occurrence of inflammation which is linked with hyperlipidemic condition observed in this study. We previously reported enhancement of inflammation due to atherogenic diet i.e. high fat and cholesterol diet [38]. Evidence abound that lipid and lipoprotein abnormalities as well as cytokines play significant roles in the pathogenesis and progression of atherosclerosis and cardiovascular diseases [39,40]. The elevations of these proinflammatory cytokines could be due in part, to an increase in oxidation of low density lipoprotein caused by high fat diet-induced hyperlipidemia. Addition of black tea and/or *Z. zanthoxyloid* significantly reduced inflammation as evident in reduction in serum concentrations of the cytokines (IL-2 and TNF-α). The mechanism of its anti-inflammatory property could be adduced to be similar to its anti-oxidative potential.

While the mechanism of the antiatherogenic property of both black tea and *Z. zanthoxyloid* may not be largely understood, the possible mode of action could be due to the interactions between the biomolecules present in them and the pro-oxidant molecules present either in the high fat diet or those produced from normal body physiological processes. Studies have shown that populations that incorporate herbs and medicinal plants into their diet have low incidence of chronic diseases [41]. Studies have suggested that oxidative stress-related chronic diseases including Type-2 diabetes mellitus, cardiovascular disease and obesity are all linked to excessive intake of calories, causing an imbalance of pro-oxidants and anti-oxidants in cellular systems, which impairs normal biological functions [42]. One benefit herbs and medicinal plants is that they contain bioactive ingredients called ‘phytochemicals’ that can reduce oxidative stress and moderate harmful biological pathways, therefore ameliorating these chronic diseases.

CONCLUSION

Conclusively, *Zanthoxylum zanthoxyloid* and black tea are good sources of health improving biomolecules. Although, black tea is already being taking as a remedy, extensive study should be carried out on suitability of inclusion of *Zanthoxylum zanthoxyloid* in our diet either as a tea or any other suitable form.

CONSENT

This is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Moarreaf R, Risk factor modification of coronary artery disease, Shiraz E-Medical Journal. 2004;5:(1):1–7.
2. Braunwald E, Heart Disease, Saunders, Philadelphia, Pa, USA. 1997;(3).
3. Martin SS, Blumenthal RS, Miller M. LDL cholesterol. The lower the better. The Medical Clinics of North America, 2012;96(1):13-26.
4. Lankin VZ, Tikhaze AK, Kukharchuk VV, et al. Antioxidants decreases the intensification of low density lipoprotein *in vivo* peroxidation during therapy with statins. Molecular and Cellular Biochemistry. 2003;249(1-2):129– 140.
5. Cooke JP. Nutriceuticals for cardiovascular health. American Journal of Cardiology. 1998;82(9).
6. Jamnian C, Atul U, Shinichi G, Masakazu F, Shinkichi T. Antiatherogenic properties of *alpinia zerumbet* seed. Molecules. 2012;17:6237-6248.
7. Choi JS, Yokozawa T, Oura H. Antihyperlipidemic effect of flavonoids from *Prunus davidiana.* J Nat Prod. 1991;54:218–224.
8. Noumi E. Les plantes a` e´pices, a` condiments et a` aromates du Cameroun. The`se de Doctorat en Sciences biologiques, Universite´ de Yaounde´. 1984;117.
9. Amvam Zollo PH, Biyiti L, Tchoumbougnang F, Menut C, Lamaty G, Bouchet Ph. Aromatic plants of tropical Central Africa. Part XXXII. Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon. Flavour and Fragrance Journal. 1998;13:107–114.
10. Odebiyi OO, Sofowora EA. Antimicrobial alkaloids from a Nigerian chewing stick (*Fagara xanthoxyloides*). Planta Medica. 1979;36:204–207.
11. Allain CC, Poon LS, Chan CSG. Enzymatic determination of total serum cholesterol. Clin. Chem. 1974;20:470-475.
12. Buccolo G, David H. Quantitative determination of serum triglyceride by the use of enzymes. Clin. Chem. 1973;19:476-482.

13. Assmann G, Schriewer H, Schmitz G, Hagele EO. Quantification of HDL-C by precipitation with phosphotungstic acid and MgCl2. Clin. Chem. 1983;29(12):2026-2030.
14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
15. Mihara M, Uchiyama M. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Analyt. Biochem. 1978;86:271–278.
16. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rats liver fractions in vitro. General features of the system used. Bioch. J. 1971;123(5):865-874.
17. Portman OW, Stare FJ. Dietary regulation of serum cholesterol levels. Phys Rev. 1959;3(39):407–442.
18. Vijaimohan K, Jainu M, Sabitha K, Subramanifam S, Anandhan CC. Devi,. Beneficial effects of alpha linolenic acid rich flaxseed oil on growth performance and hepatic cholesterol metabolism in high fat diet fed rats. Life Science. 2006;79:448-454.
19. Makni M, Fetoui H, Gargouri N, Jaber H, Boudawara T, Zeghal N. Hypolipidemic and hepatoprotective effects of flaxseed and pumpkin seed mixture in ω-3 and ω-6 fatty acids in hypercholesterolemic rats. Food Chem. Toxicol. 2008;46:3714-3720.
20. Adekunle AS, Kamdem JP, Aline AB, Rocha JBT. Antioxidant Activity and HPLC Analysis of *Zanthozylum zanthoxyloide.* Report and Opinion. 2012;4:(3).
21. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. Ayurveda and traditional Chinese medicine: a comparative overview. Evidence-Based Complementary and Alternative Medicine. 2005;2(4):465–473.
22. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. The Lancet. 1993;342(8878):1007–1011.
23. Hertog MGL, Kromhout D, Aravanis C, et al. Flavonoid intake and long-termrisk of coronary heart disease and cancer in the Seven Countries Study. Archives of Internal Medicine. 1995;155(4):381–386.
24. Ishikawa Y, Sugiyama H, Stylianou E, Kitamura M. Bioflavonoid quercetin inhibits interleukin-1-induced transcriptional expression of monocyte chemoattractant protein-1 in glomerular cells via suppression of nuclear factor-*κ*B. Journal of the American Society of Nephrology. 1999;10(11):2290–2296.
25. Csokay N, Prajda G. Weber, E. Olah. Molecular mechanisms in the antiproliferative action of quercetin. Life Sciences. 1997;60(24):2157–2163.
26. Gordon T, Castelli WP, Hjortland MC. High density lipoprotein asa protective factor against coronary heart disease. The Framingham Study. Am J Med. 1977;62:707–14.
27. Vijaimohan KM, Jainu K, Sabitha S, Subramanifam C, Anandhan C. Devi. Beneficial effects of alpha linolenic acid rich flaxseed oil on growth performance and hepatic cholesterol metabolism in high fat diet fed rats. Life Science. 2006;79:448-454.
28. Davis RA, McNeal MM, Moses RL. Intrahepatic assembly of very low density lipoprotein. Competition by cholesterol esters for the hydrophobic core. J Biol Chem. 1982;257:2634-2640.
29. Liu CH, Huang M, Huang PC. Sources of triacylglycerol accumulation in livers of rats fed a cholesterol-supplemented diet. Lipids. 1995;30(6):527-531.
30. Saravanan Subramaniam, Ramachandran Subramaniam, Suja Rajapandian, SubasiniUthrapathi, Victor Rajamanickam Gnanamanickam, and Govinda Prasad Dubey. Anti-atherogenic activity of ethanolic fraction of terminalia arjuna bark on hypercholesterolemic rabbits. Evidence-Based Complementary and Alternative Medicine. 2011;1-8.

31. Marczuk-Krynicka D, Hryniewiecki T, Paluszak J, Krauss H, Nowak D. High Fat Content in Diets and Oxidative Stress in Livers of Non-Diabetic and Diabetic Rats. Polish J. of Environ. Stud. 2009;18(2):249-253.
32. Vijayakumar RS, Surya D, Nalini N. Antioxidant efficacy of black paper (*Piper nigrum* L.) and piperine in rats with high fat diet induced oxidative stress. Redox Rep. 2004;9(2):105.
33. Oliveros LB, Videla AM, Gimenez MS. Effect of dietary fat saturation on lipid metabolism, arachidonic acid turnover and peritoneal macrophage oxidative stress in mice. Braz. J. Med. Biol. Res. 2004;37(3):311.
34. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine, ed. 3, Oxford: Oxford University Press, Oxford; 1999.
35. Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willett WC. Vegetable, fruit and cereal fiber intake and risk of coronary heart disease among men. J Am Med Assoc. 1996;275:447–451.
36. Adekunle AS, Adedeji AL, Oyewo EO, Adedosu OT, Omotoso AT. Hyperlipidemia induced by atherogenic diet enhanced oxidative stress in the kidney and inflammatory responses: an in-vivo study. Asian Journal of Natural & Applied Sciences. 2013;2(1):82-93.
37. Ginsberg HN. Lipoprorein metabolism and its relationship to atherosclerosis. Med Clin North Am. 1994;78:1-20.
38. Glew RH, Kassam HA, Bhanji RA, Okorodudu A, VanderJagt DJ. Serum lipid profiles and risk of cardiovascular disease in three different male populations in northern Nigeria. J Health Popul Nutr. 2002;20:166-74.
39. Quinn MT, Parthasarathy S, Fong LG, Steinberg D. Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/ macrophages during atherogenesis. Proc Natl Acad Sci USA 1987 84:2995–8.
40. Janabi M, Yamashita S, Hirano K, Sakai N, Hiraoka H, Matsumoto K, et al. Oxidized LDL-induced NF-kappa B activation and subsequent expression of proinflammatory genes are defective in monocyte-derived macrophages from CD36-deficient patients. Arterioscler Thromb Vasc Biol. 2000;20:1953–60.
41. Duthie GG, Gardner PT, Kyle JA. Plant polyphenols: are they the new magic bullet? Proc. Nutr. Soc. 2003;62(3):599-603.
42. Droge W. Free radicals in the physiological control of cell function. Physiol. Rev. 2002;82:47-95.

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