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Cardio-and-Hepatoprotective Benefits of Some Spices in Wistar Rats Induced with Metabolic Syndrome

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ABSTRACT

The increasing reliance on foods sweetened with fructose is of great concern due to its leading role in development of obesity and other related metabolic disorders. Since oxidative stress is one of the pathogenic processes for development of these disorders, this research evaluated the inclusion of antioxidant-rich spices in diet as means of ameliorating dyslipidaemia and liver dysfunction in metabolic syndrome-induced Wistar rats. Thirty rats were induced with metabolic syndrome using 55.43% high-fructose diet, divided into six groups and fed on diets supplemented with 2 % inclusions of ginger, black pepper, garlic, turmeric and a mixture of the spices. Serum lipid profiles, atherogenic indices and indices of liver function were determined. Compared with the control, metabolic syndrome-induced rats fed the spices-supplemented diets had significantly ($p<0.05$) higher levels of serum cholesterol, triglyceride and low-density lipoprotein cholesterol which were significantly lower in comparison with the metabolic syndrome-induced rats fed standard diet. The atherogenic index and coronary risk index as well as activities of aspartate aminotransaminase, alanine aminotransaminase, alkaline phosphatase and gamma glutamyl aminotransaminase were significantly ($p<0.05$) higher in the groups treated with spices-supplemented diets compared with the control, but lower in comparison with the metabolic syndrome-induced rats fed standard diet. There were no significant ($p>0.05$) variations among groups in levels of serum bilirubin. It is evident that including these spices in diets could alleviate dyslipidaemia and lower serum enzyme activities in metabolic syndrome-induced rats, and could therefore, enhance restoration of heart and liver damages caused by consuming metabolic syndrome-causing diets.

Keywords: Heart, Liver, Metabolic syndrome, Spices, Supplemented diet.

Introduction

The increasing reliance on foods and beverages sweetened with fructose is of great concern due to its leading role in development of obesity,[1] insulin resistance[2] and other consequent disorders referred to as metabolic syndrome[3]. Also known as cardiometabolic syndrome,[4] the syndrome is characterised by a cluster of any three or more of central obesity, hyperglycaemia, hypertension, dyslipidaemia and insulin resistance. Each of these components can alone or in synergy with any other one(s) cause cardiometabolic disorders such as atherosclerosis.[5] Other lifestyles that promote the syndrome include sedentary habits,[6-7] smoking[8] and alcoholism.[9] Although, the pathophysiology and pathogenesis of metabolic syndrome are not clearly illustrated, it is increasingly becoming clearer that the syndrome may be a disorder of energy utilization and storage.[10] One major pathologic process of this disorder seems to be generation of free radicals.[11-12] These radicals can directly cause cardiovascular damage and damage to other vital organs of the body such as the liver, or stimulate other pathways that lead to cardiovascular diseases.[13] Changes in lifestyle such as dietary modifications that involve improving food quality and altering the distribution of macromolecules and inclusion of antioxidant-rich plant products are promising in the treatment and management of the syndrome.[14]

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Fortifications of foods with plants rich in beneficial phytochemicals have been used in the management of various disease conditions.[15] Turmeric, garlic, ginger and African black pepper spices have been reported to possess health benefits due to their antioxidant properties. In a review by Oluyori *et al.*[16] garlic and turmeric extracts are variously reported to stimulate activities of antioxidant enzymes and as such prevent oxidative damage to organs. Idoko *et al.*[14] reported that 2 % inclusion of garlic and/or turmeric in metabolic syndrome-causing diet could be hepato- and nephroprotective in *Wistar* rats. Similarly, Imam *et al.*[10] reported that 2% inclusion of turmeric, garlic, ginger and African black pepper decrease weight gain and improve insulin sensitivity in rats induced with metabolic syndrome, but came short of establishing if such inclusion could ameliorate damages done to the heart and liver due to metabolic syndrome. This research was therefore conducted to study changes in lipid profile and liver functions in metabolic syndrome-induced *Wistar* rats fed diets supplemented with spices.

Materials and Methods

Chemicals and reagents

Chemicals and reagents were of analytical grades. The assay kits were procured from representatives of Randox Laboratories Ltd. UK in Nigeria. The feed ingredients; bone meal, soy bean meal, palm oil, methionine, premix minerals and vitamins were purchased from reliable vendors in Dutsinma central market of Katsina State. Corn starch was prepared from corn after a 72-hour soaking in clean water.

Plant identification and preparation

The spices; turmeric, ginger, garlic and West African black pepper were bought in March, 2021 from Dutsinma Central Market in Katsina State of Nigeria and were respectively identified as *Curcuma Longa*

(FUDMA/PSB/0143), *Zingiber officinale* (FUDMA/PSB/0042), *Allium sativum* (FUDMA/PSB/0019) *and Piper guineense* (FUDMA/PSB/0138) at the Herbarium in the Department of Biological Sciences of Federal University Dutsin-Ma (FUDMA). The spices were screened for spoilt ones, washed and pulverized after air drying for 72 hours. The pulverized spices were mixed in equal ratio to obtain mixed spices.

Experimental rats
About 12-week old Wistar rats (128.08±5.29 g) numbering thirty-five (35) were purchased from the Experimental Animal Unit of University of Jos, Nigeria and kept in wooden cages in the Department of Biochemistry and Molecular Biology of Federal University DutsinMa for 7-day acclimatization period before the commencement of the experiment.

Formulation of the experimental diets
The feed ingredients were appropriately mixed in accordance with the method of Idoko [14] and Imam *et al*. [10] to formulate the experimental diets (Table 1).

Table 1: Components of formulated diet

Feed ingredient	Standard diet g/100 g	High-fructose g/100 g
Corn starch	55.45	-
Fructose	-	55.45
Soybean meal	32	32
Cellulose	4.5	4.5
Palm oil	6	6
Bone meal	1.25	1.25
Salt mix	0.3	0.3
Vit/mineral mix	0.25	0.25
Methionine	0.25	0.25
Total	100	100

Mineral mix (g/kg): $CaCO_3$ (15.258), $CoCl_2.6H_2O$ (0.001), $ZnCl_2$ (0.001), $CuSO_4.5H_2O$ (0.019), $FeSO_4.7H_2O$ (1.078), $MgSO_4$ (2.929), $MnSO_4.2H_2O$ (0.178), KI (0.032), KH_2PO_4 (15.559) and NaCl (5.573)
Vitamin mix (g/kg diets): thiamine (0.02), riboflavin (0.03), pyridoxine (0.01), P-aminobenzoic acid (0.20), myo-inositol (2.00), biotin (0.001), menadione (0.01), ergocalciferol (0.4), choline-HCl (2.0), and cellulose (3.31), α–tocopherol acetate (50), retinal palmitate (0.4), calcium pantothenate (0.0016) and folic acid (0.0002).

Supplemented diet formulation
The spices were thoroughly mixed with the standard diet and high-fructose diets in a ratio of 2:98 to formulate the following diets[10]
A. Standard diet
B. High-fructose diet
C. 2% turmeric-supplemented standard diet
D. 2% ginger-supplemented standard diet
E. 2% garlic-supplemented standard diet
F. 2% black pepper-supplemented standard diet
G. 2% mixed spices-supplemented standard diet

Experimental design
Inducing metabolic syndrome
The inducement was as presented in our previous report[10] where the rats were grouped into two of 5 rats (control) and 30 rats (test group) with the control and test groups being fed standard diet and high-fructose diet respectively for seven weeks, the point at which metabolic syndrome was confirmed in them. The metabolic syndrome-induced group was subdivided into six (6) groups of 5 rats per group and randomly assigned to the 6 formulated spices-supplemented diets as follows;
Group A. Normal rats maintained on the standard diet (normal control)
Group B. Metabolic syndrome-induced rats fed standard diet (positive control)
Group C. Metabolic syndrome-induced rats fed 2% turmeric-supplemented diet
Group D. Metabolic syndrome-induced rats fed 2% West African black pepper-supplemented diet
Group E. Metabolic syndrome-induced rats fed 2% ginger-supplemented diet
Group F. Metabolic syndrome-induced rats fed 2% garlic-supplemented diet
Group G. Metabolic syndrome-induced rats fed 2% mixed spices-supplemented diet
The rats were maintained on their respective diet *ad libitum* for additional eight (8) weeks.

Blood sample collection
On the 105th day of the experiment, the rats were subjected to 12-hour fasting and weighed. They were then anesthetized and sacrificed by cutting off the jugular veins. Blood was collected into plain sample bottles and then centrifuged at 1500 g for 15 minutes to obtain serum.[14]

Determination of lipid profiles and atherogenic indices
Commercial Randox assay kits were used in evaluating the high-density lipoprotein (HDL), serum total cholesterol (TC) and triglyceride (TG) which respectively followed the methods of Tietz,[17] Abell *et al.*[18] and Tietz.[17] Low density lipoprotein (LDL) was computed as follows; LDL=Total cholesterol-(Triglycerides/2.2) – HDL.[19]
Both the coronary risk index (CRI) and atherogenic index (AI) were computed from the following relationships;
Atherogenic Index (AI) = LDL-C/HDL-C
Coronary risk index (CRI) = (TC/HDL-C).[20]

Assessment of changes in hepatic functions
Determination of serum enzymes activities and serum concentrations of bilirubin
The activities of alanine transaminase (ALT) and aspartate transaminase (AST) were estimated using respective Randox assay kits based on the modified method[21] of Reitman and Frankel. [22] Gamma glutamyl transaminase (GGT) determination followed method described by Szasz[23] while alkaline phosphatase (ALP) was based on the recommendation of Deutsche Gesellschaft für Klinische Chemie.[24]
The total and conjugate bilirubin concentrations were also estimated with the respective Randox kits in accordance with the method of Jendrassik and Grof [25]

Ethical consideration
The rats were maintained according to approved standards for keeping experimental animals as approved by the Federal University Dutsin-Ma Ethical Committee on the use of experimental animals and human subjects FUDMA/IEC/2021/198.

Statistical analysis
Results are presented as means± SEM. The obtained data during inducement of metabolic syndrome were subjected to student t-test while analysis of variance (ANOVA) and Ducan's new multiple range test were performed using SPSS version 16 (IBM Corp.) software package at 95% confidence interval on the obtained post-inducement data.

Results and Discussion

Lipid profiles and atherogenic indices in metabolic syndrome-induced rats fed spices supplemented diets
All groups induced with metabolic syndrome had significantly ($p<0.05$) higher levels of serum cholesterol, triglyceride, LDL-C but lower levels of HDL-C which also translated into significantly ($p<0.05$) higher atherogenic index (AI) and coronary risk index (CRI) when compared with the normal control. However, metabolic syndrome-induced rats respectively maintained on ginger, garlic and mixed spices-supplemented diets had significantly ($p<0.05$) lower cholesterol, triglyceride and LDL but higher HDL compared with the metabolic

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syndrome-induced rats maintained on the standard diet. The atherogenic indices were also significantly ($p<0.05$) lower in these groups when compared with the metabolic syndrome-induced rats maintained on the standard diet (Tables 2 & 3).

De novo synthesis of lipids is stimulated by high fructose diet by providing large amounts of hepatic triose-phosphate, the precursors of both the glycerol- and the fatty-acyl parts of VLDL-triglycerides.[26-27]. Fructose also activates HMG-CoA reductase and fatty acid synthase (FAS) by inducing expression of hepatic sterol regulatory element binding protein (SREBP), allowing lipogenesis to proceed even in the absence of insulin.[28-29] The results suggest that inclusion of ginger, black pepper, garlic, turmeric and a mixture of the spices enhances alleviation of dyslipidaemia resulting from consumption of high-fructose diet, and this could be by antagonizing the pathways of lipid dysregulation. Generally, the observed improved dyslipidaemia could have partly resulted from decrease in weight gain and improvement in insulin sensitivity in the groups fed the spices supplemented diets as previously reported by Imam *et al*.[10] Ginger had long been reported to decrease the hepatic expression of glycerol phosphate acyltransferase (GPAT), a rate limiting enzyme in tryglyceride synthesis and to suppress over production of Carbohydrate-Responsive Element-Binding Protein (ChREBP),[30] a protein that is activated by fructose administration.[31-32] This protein suppresses fatty acid oxidation[33] and promotes lipogenesis by coupling intermediates of carbohydrate metabolism to lipid synthesis via stimulation of lipogenic enzymes.[34] The finding is in agreement with Saravanan *et al*.[35] who reported anti-dyslipideamic effects of ginger supplements in rats induced with metabolic syndrome related diseases. Having lowered serum cholesterol, triglyceride and coronary risk index in dyslipidaemia-induced rats in comparison with non-supplemented diet, turmeric supplemented diet also showed strong tendency to normalize dyslipidaemia, and therefore a tendency to prevent development of heart damage. This is still consistent with the lower weight gain and improvement in insulin resistance and level of blood glucose in this group. Curcuminoids are the biological active compounds in turmeric and had been reported by Altobelli *et al*.[36] and Panahi *et al*.[37] to improve lipid profiles in objects with T2DM. Johnston *et al*.[38] and Sahebkar[39] also reported anti-cardiometabolic risks effects of curcuminoids, which according to Si *et al*.[40] involves improvement on serum lipid panels. Furthermore, curcumin decreases the hepatic expression of lipogenic transcription factors LXR-α and SREBP1c.[41-42] and decreases lipogenic gene expressions in diet-induced metabolic syndrome.[43] From the results, we can report that long term consumption of diets supplemented with turmeric powder could attenuate dyslipidemia like its bioactive component, curcuminoid. In the present study, 2% inclusion level of black pepper did not improve the serum concentrations of HDL and triglyceride compared with metabolic syndrome-induced rats fed normal diet. However, the anti-dyslipidemia and anti-atherogenicity of the black pepper is evident in the lower total cholesterol, LDL-C and atherogenic index (AI) in metabolic syndrome-induced rats fed black pepper-supplemented diet in comparison with the metabolic syndrome-induced rats fed normal diets. Piperine, an alkaloid is the major constituent of black pepper[44] and is believed to be responsible for most of its reported health benefits including lipid lowering effect in rats fed high fat diet.[45-46] Vijayakumar and Nalini[47] reported that Lecithin-cholesterol acyltransferase (LCAT) and Lipoprotien lipase, both of which are lipid metabolism enzymes are modulated by piperine. The result shows that inclusion of black pepper in the diet could produce similar anti-dyslipidemic and anti-atherogenicity effects like its bioactive component, piperine. Alliin, allicin, ajoene, diallyl disulfide (DADS), diallyl trisulfide (DAT) and diallyl sulfide (DAS) are among the bioactive components of garlic.[48-49] Garlic and its diallyldisulfide inhibit 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase).[50-51] Garlic extract is found to respectively inhibit expression of LXRα in the liver and stimulate its expression in the intestine.[52] LXRα is lipogenic transcription factors and this could therefore be one of the anti-dyslipidemia mechanisms seen in the diet supplemented with garlic in this research.

Table 2: Lipid profile of metabolic syndrome induced rats fed spices supplemented diets

	CHO	TG	HDL	LDL
Group A	3.21 ± 0.02^{a}	1.09 ± 0.01^{a}	1.87 ± 0.00^{a}	1.19 ± 0.00^{a}
Group B	7.02 ± 0.11^{d}	2.17 ± 0.02^{d}	1.61 ± 0.05^{c}	2.87 ± 0.17^{b}
Group C	5.68 ± 0.12^{c}	1.70 ± 0.05^{c}	1.57 ± 0.01^{c}	2.92 ± 0.09^{b}
Group D	6.31 ± 0.19^{e}	2.14 ± 0.05^{d}	1.56 ± 0.01^{c}	1.99 ± 0.13^{c}
Group E	5.73 ± 0.05^{c}	1.79 ± 0.01^{c}	1.59 ± 0.01^{c}	2.01 ± 0.03^{c}
Group F	5.50 ± 0.27^{c}	1.76 ± 0.05^{c}	1.58 ± 0.02^{c}	1.96 ± 0.09^{c}
Group G	4.94 ± 0.04^{b}	1.40 ± 0.10^{b}	1.70 ± 0.01^{b}	1.51 ± 0.01^{d}

Values are presented as mean ± SEM for 5 determinations. Values with different superscripts along the same column are significantly different ($P<0.05$).
Group A. Normal rats maintained on the standard diet (normal control)
Group B. Metabolic syndrome-induced rats fed standard diet (positive control)
Group C. Metabolic syndrome-induced rats fed 2% turmeric-supplemented diet
Group D. Metabolic syndrome-induced rats fed 2% West African black pepper-supplemented diet
Group E. Metabolic syndrome-induced rats fed 2% ginger-supplemented diet
Group F. Metabolic syndrome-induced rats fed 2% garlic-supplemented diet
Group G. Metabolic syndrome-induced rats fed 2% mixed spices-supplemented diet
CHO- Cholesterol
TG-Triglyceride
HDL- High density lipoprotein cholesterol
LDL- Low density lipoprotein cholesterol

Table 3: Atherogenic indices in metabolic syndrome induced rats fed spices supplemented diets

	AI	CRI
Group A	0.63 ± 0.00^{a}	1.71 ± 0.01^{a}
Group B	1.79 ± 0.15^{b}	4.37 ± 0.19^{b}
Group C	1.86 ± 0.07^{b}	3.61 ± 0.05^{c}
Group D	1.28 ± 0.09^{c}	4.04 ± 0.12^{b}
Group E	1.26 ± 0.02^{c}	3.60 ± 0.03^{c}
Group F	1.24 ± 0.04^{c}	3.47 ± 0.19^{c}
Group G	0.89 ± 0.01^{d}	2.90 ± 0.03^{d}

Values are presented as mean ± SEM 5 8 determinations. Values with different superscripts along the same column are significantly different ($P<0.05$).
Group A. Normal rats maintained on the standard diet (normal control)
Group B. Metabolic syndrome-induced rats fed standard diet (positive control)
Group C. Metabolic syndrome-induced rats fed 2% turmeric-supplemented diet
Group D. Metabolic syndrome-induced rats fed 2% West African black pepper-supplemented diet
Group E. Metabolic syndrome-induced rats fed 2% ginger-supplemented diet
Group F. Metabolic syndrome-induced rats fed 2% garlic-supplemented diet
Group G. Metabolic syndrome-induced rats fed 2% mixed spices-supplemented diet
CHO- Cholesterol
AI- Atherogenic index
CRI- Coronary risk index

By decreasing LXRα expression in the liver, SREBP1c will be decreased leading to decreased expression of lipogenic enzymes. Stimulation of LXRα in the intestine reduces absorption of cholesterol in the intestine.[53]

The significantly lower plasma total cholesterol, LDL-C, triglyceride, AI and CRI but higher HDL in the group of metabolic syndrome-induced rats fed diet supplemented with a mixture of turmeric, ginger, garlic and black pepper when compared with all other groups of metabolic syndrome-induced rats fed individual spices supplemented diets shows that the spices could act in synergy against dyslipidemia. Curcumin according to Rodrigues *et al*.[54] and Dei Cas and Ghidoni[55] has low solubility as well as fast metabolism which limit its gastrointestinal tract absorption. The consequent of this is low bioavailability of curcumin in the serum and less potential for positive health benefits. In the presence of piperine, curcumin´s absorption and bioavailability increase.[56] The causative role of dyslipidemia in development of cardiovascular disorders are well established. Atherogenic lipoproteins such as LDL, VLDL and triglycerides readily undergo oxidation and some changes in structures that cause their being trapped in the arteries. The build-up can eventually clog the arteries and shorten flow of blood to heart, resulting in heart problem. Further, low level of HDL limits removal of LDL from the blood and thereby increase the blockage of the arteries by LDL.[57-60] Also, myocardial membrane damage causes ester cholesterol to accumulate[61] and atherogenic index increases with increasing myocardial infraction.[62] Taken together, our findings show that supplementation of diets with any of these spices (turmeric, black pepper, ginger and garlic) could significantly reduce the risks of cardiovascular disorder caused by eating metabolic syndrome-causing diets. However, a combination of the four in the diet may be more effective.

Assessment of hepatic functions in metabolic syndrome-induced rats fed spices supplemented diets

Metabolic syndrome-induced rats fed spices supplemented diets had significantly ($p<0.05$) higher activities of the studied liver enzymes when compared with the normal control. Compared with the metabolic syndrome-induced rats maintained on normal diet however, the groups maintained on spices supplemented diets had significantly ($p<0.05$) lower AST, ALT, ALP and GGT (Table 4). There were no significant ($p>0.05$) variations among the groups in levels of serum bilirubin (Table 5). Levels of serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (GGT) are good indicators in evaluating liver damage.[63-64] Although we did not measure the levels of the liver enzymes (AST, ALT, GGT and ALP) in rats fed high fructose diet prior the commencement of treatment with spices supplemented diets, it is evident from our result that fructose caused liver damage in the rats leading to escape of the enzymes into the plasma. This is seen in the significantly higher activities of AST, ALT, ALP and GGT in the groups that received high fructose diets and then fed on standard diet. As observed in this study, fructose consumption results in dyslipideamia. We had also reported that it caused insulin resistance and hyperglycemia.[10] Furthermore, it also causes oxidative stress, inflammation and high blood pressure.[65] A combination of these health disorders can cause serious liver damage. Moreso, reports have shown that high-fructose increases the risk for non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH).[66-67] By causing hyperglycemia-induced insulin resistance, fructose consumption can cause organ damages that are not readily reversible.[68] Interestingly however, our findings show that consumption of diets supplemented with any of turmeric, ginger, garlic, black pepper or a combination of the four could attenuate fructose induced liver damage as shown by the lower serum activities of ALT, AST, GGT and ALP in comparison with the metabolic syndrome-induced rats fed standard diet.

Xiong *et al*.[69] and Salman *et al*[70] reported the reducing effect of curcumin, the bioactive compound of turmeric on serum activities of liver enzymes such as ALT, ALP, GGT and ALP. The effect of turmeric-supplemented diet in ameliorating liver damage in metabolic syndrome-induced rats is consistent with our earlier observed decreasing effect on obesity, insulin resistance, hyperglycemia[10] and dyslipideamia since these conditions invariably lead to liver injury. It had been reported that curcumin improves liver health and function by preventing fatty liver via inhibiting lipogenesis.[71-72] Since fructose causes oxidative stress and inflammation[54] which are the major culprits in liver injury,[73-75] the improvement in the cellular architecture of the liver as seen in the decreased serum enzyme activity could also be partly due to reported antioxidant properties of turmeric compound.[76-77] From the finding, it is evident that supplementation of diet with turmeric, like curcumin, its main bioactive component could reduce damage done to the liver resulting from consumption of metabolic syndrome-causing diets.

Table 4: Serum hepatic enzymes activities in metabolic syndrome induced rats fed spices supplemented diets

	AST(u/L)	ALT(u/L)	ALP(u/L)	GGT(u/L)
Group A	56.33 ± 2.19^{a}	28.67 ± 0.33^{a}	95.33 ± 0.88^{a}	38.00 ± 1.15^{a}
Group B	97.33 ± 0.33^{e}	45.33 ± 0.88^{e}	137.33 ± 1.33^{b}	76.00 ± 1.53^{b}
Group C	82.67 ± 1.45^{d}	34.23 ± 1.54^{bc}	124 ± 0.58^{c}	60.33 ± 0.88^{c}
Group D	81.33 ± 0.33^{d}	38.33 ± 2.19^{d}	124.33 ± 0.88^{c}	59.00 ± 1.53^{c}
Group E	70.67 ± 1.45^{c}	37.00 ± 1.20^{bd}	122.00 ± 1.15^{c}	57.67 ± 1.45^{c}
Group F	72.67 ± 0.67^{c}	37.53 ± 0.88^{bd}	122.33 ± 1.76^{c}	58.67 ± 1.76^{c}
Group G	63.33 ± 0.88^{b}	33.33 ± 0.33^{c}	123.00 ± 1.53^{c}	58.33 ± 1.20^{c}

Values are presented as mean ± SEM for 5 determinations. Values with different superscripts along the same column are significantly different ($P<0.05$).

Group A. Normal rats maintained on the standard diet (normal control)
Group B. Metabolic syndrome-induced rats fed standard diet (positive control)
Group C. Metabolic syndrome-induced rats fed 2% turmeric-supplemented diet
Group D. Metabolic syndrome-induced rats fed 2% West African black pepper-supplemented diet
Group E. Metabolic syndrome-induced rats fed 2% ginger-supplemented diet
Group F. Metabolic syndrome-induced rats fed 2% garlic-supplemented diet
Group G. Metabolic syndrome-induced rats fed 2% mixed spices-supplemented diet
CHO- Cholesterol
AST-Aspartate aminotransaminase
ALT-Alanine aminotransaminase
ALP- Alkaline phosphatase
GGT- Gamma glutamyl aminotransaminase

Table 5: Serum bilirubin concentrations of metabolic syndrome-induced rats fed spices supplemented diets

	CB (mg/dl)	TB (mg/dl)
Group A	3.10 ± 0.21[a]	14.62 ± 0.56[a]
Group B	3.40 ± 0.26[a]	16.94 ± 2.88[a]
Group C	3.70 ± 0.21[a]	13.30 ± 0.71[a]
Group D	3.50 ± 0.35[a]	13.03 ± 0.21[a]
Group E	3.73 ± 0.42[a]	13.25 ± 0.77[a]
Group F	3.47 ± 0.23[a]	12.44 ± 0.26[a]
Group G	3.30 ± 0.26[a]	12.87 ± 0.37[a]

Values are presented as mean ± SEM for 5 determinations. Values with different superscripts along the same column are significantly different (P<0.05).
Group A. Normal rats maintained on the standard diet (normal control)
Group B. Metabolic syndrome-induced rats fed standard diet (positive control)
Group C. Metabolic syndrome-induced rats fed 2% turmeric-supplemented diet
Group D. Metabolic syndrome-induced rats fed 2% West African black pepper-supplemented diet
Group E. Metabolic syndrome-induced rats fed 2% ginger-supplemented diet
Group F. Metabolic syndrome-induced rats fed 2% garlic-supplemented diet
Group G. Metabolic syndrome-induced rats fed 2% mixed spices-supplemented diet
CHO- Cholesterol
CB- Conjugated bilirubin
TB-Total bilirubin

Beneficial effect of ginger on metabolic diseases due to poor diet[78] and amelioration of fructose-induced fatty liver and hypertriglyceridemia[26] had been reported. Apart from its antimicrobial activity, Sagita *et al*,[79] and Badawi[80] reported the decreasing effect of ginger on the elevated levels of liver enzymes (ALT, AST and ALP) which is in agreement with our result. Recently, 6-gingerol which is a major active constituent of ginger was shown to protect liver from oxidative-induced damage.[81] Shogaol and gingerol both of which are among the active biological components of ginger are reported to have both antioxidant and anti-inflammatory activities.[82] our result shows that ginger powder may, like its extract significantly alleviate liver damage resulting from consumption of unhealthy diets.
In this study, it has been demonstrated that long term consumption of diet supplemented with garlic powder could, like its extracts alleviate liver damage caused by consumption of high fructose diet.
Although less effective than ginger, garlic and turmeric-supplemented diets in hepatoprotection, black pepper consumption in food showed hepatoprotection as could be seen in the lower activities of liver enzymes in the serum of metabolic syndrome-induced rats fed black pepper-supplemented diet as compared with the metabolic syndrome-induced rats fed normal diet. This result is also consistent with the improvement in insulin sensitivity, lipid metabolism and decrease in weight gain in metabolic syndrome induced-rats fed black pepper supplemented diet presented earlier. The aqueous seed extract of black pepper was shown by Uhegbu *et al.*[83] to decrease significantly the serum activities of ALT and ALP in the rat, suggesting hepatoprotective potential. In another research by El-Halawany *et al.*[84] methanol and chloroform extracts of black pepper reasonably reversed liver damage induced by tetrachloride. Furthermore, it had been demonstrated that piperine, the most bioactive compound in black pepper ameliorates symptoms of metabolic syndrome in high carbohydrate and high fat-fed rats via reduction of inflammation and oxidative stress[85-86] which according to Ogbunugafor *et al.*[87] could be due to presence of appreciable level of vitamin E in the plant. Some other researchers also reported hepatoprotective effects of piperine.[88-90] Piperin is not just hepatoprotective but also a bioenhancer[91]. It could be deduced from our work that black pepper powder could equally offer hepatoprotection against diet-induced damage like its most active phyto-molecule. The synergistic effect of piperin could partly be responsible for the seemingly better result obtained from the group of metabolic syndrome-induced rats fed diet supplemented with a mixture ginger, garlic, turmeric and black pepper as indicated by lower serum levels of ALT, AST when compared to metabolic syndrome-induced rats fed the various spices supplemented diets.
Although not specific marker of liver damage,[92] the elevation of bilirubin is regarded among indicators of liver injury which could be due to disruption at any stage of bilirubin metabolism such as defects in conjugation or biliary excretion, increased production or decreased uptake by the liver.[93] The reason for the nonsignificant difference among the groups is not clear. However, it could be that the metabolism of bilirubin was not affected by the consumption of high fructose diet and by the subsequent treatments. Jai *et al.* [68] equally observed no effect on bilirubin in experimental animals fed high-fructose and/ or high-fat diets. The nonsignificant difference could also mean that simply reverting to normal diet after being on high fructose diet was enough to normalize bilirubin metabolism. Recent report by Nehal *et al*. [94] showed that high-fructose intake- associated metabolic syndrome could be cured by reverting to regular diet, though with alternate day fasting. Hyperbilirubinemia may therefore not be a reliable criterium in monitoring metabolic syndrome.

Conclusion

The study evaluated the changes in lipid profiles, atherogenic indices and some indices of liver function. It is evident from the results that inclusion of ginger, garlic, black pepper, turmeric powder in the diets individually and synergistically alleviate dyslipidemia and lower serum enzyme activities in metabolic syndrome-induced rats. Therefore, the inclusion of the spices could enhance restoration of heart and liver damages caused by consumption of metabolic syndrome- causing diets.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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