PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 26 (5) 893-901 May 2022

Obesity and Insulin Resistance Components of Metabolic Syndrome Induced by High-fructose Diet in Wistar Rats could be attenuated by Spices-Supplemented Diets

***IMAM, NU; IDOKO, AS; OSIBEMHE, M; LAWAL, N; ZAHARADDEEN, AS**

Department of Biochemistry and Molecular Biology, Federal University Dutsinma, Katsina State, Nigeria

**Corresponding Author Email: imamnasirusman@gmail.com*
Other Authors Email: asidoko1@gmail.com; osibeme@gmail.com; nlbatagarawa@gmail.com; zasman@rocketmail.com

ABSTRACT: Constant consumption of fructose-sweetened diets and beverages has led to increasing incidences of metabolic disorders such as metabolic syndrome (MS). This research was designed to evaluate the potentials of supplementing diets with some locally available spices in ameliorating obesity and insulin resistance in induced metabolic syndrome. Thirty out of thirty-five rats weighing 128.08±5.29g were induced with MS, divided into six groups and treated with diets supplemented with powders of garlic, ginger, turmeric, black pepper and equal mixture of the spices while the other 5 rats were maintained on normal diet throughout the experiment. The weight and blood glucose were recorded weekly, while serum insulin level was determined on the 56th day of the treatment after which HOMA-IR was computed. The group of MS-induced rats fed normal diet had significantly ($p<0.05$) higher weekly average weight gain of 11.43g±0.63g compared with other groups. Our data show that blood glucose level (mmol/L) ranged from 4.61±0.22 - 5.99±0.17; Insulin (μU/mL) 8.00±0.41 - 22.00±0.58 and HOMA-IR 1.65±0.14 - 5.47±0.07. The treated groups had significantly ($p<0.05$) higher percentage decrease in blood glucose and HOMA-IR when compared with the control but all the MS-induced rats maintained on supplemented diets had significantly ($p<0.05$) lower blood glucose and HOMA-IR when compared with MS-induced rats maintained on normal diet. From our finding, obesity and insulin resistance due to consumption of MS-causing diets could be ameliorated by consumption of diets fortified with any of the four spices, but fortification with a blend of the spices could give a better result.

DOI: https://dx.doi.org/10.4314/jasem.v26i5.17

Impact factor: http://sjifactor.com/passport.php?id=21082

Google Analytics: https://www.ajol.info/stats/bdf07303d34706088ffffbc8a92c9c1491b12470

Dates: Received: 02 April 2022; Revised: 30 April 2022; Accepted: 22 May 2022

Keywords: mechanical properties, wood species, density, sorption properties

Keywords: obesity, insulin resistance, metabolic syndrome, spices, fructose

Insulin resistance (IR) refers to a condition of inappropriate response of cells particularly those of the heart, muscle and adipose tissues to insulin action (Samuel and Shulman, 2016). Under this condition, target tissues cannot coordinate suppression of gluconeogenesis and lipolysis, cannot promote normal uptake of blood glucose and glycogen synthesis in the presence of normal level of insulin in the blood. In an attempt to overcome this, more insulin is secreted resulting in hyperinsulinemia (Saidur *et al*., 2021). There is a strong positive correlation between prevalence of obesity and insulin resistance. Obesity which is accumulation (in excess) of fat in the adipose tissues that could negatively interfere and lower the health status of the individual, causes the release of mediators of inflammation such as tumour necrosis factor α while reducing adiponectin productions. The inflammatory state in obesity is usually accompanied by increased reactive oxygen species (Mohammed *et al*., 2017) due to depletion of antioxidant sources such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) and vitamins A, E, and C (Amirkhizi *et al*., 2007) in addition to inducement of increased production of ROS by adipokines synthesized in excess by adipose tissues in obese individuals (Fernández-Sánchez *et al*., 2011). Inflammation is at the centre of the mechanism mediating and promoting insulin resistance (Qiang *et al*., 2014). Long-time exposure to inflammatory mediators leads to the stimulation of cytokine signalling proteins. The consequence of this is that insulin signalling receptors would be eventually blocked in the pancreatic β-cells (Li *et al*., 2015; Feve and Bastard, 2009). Researches have shown that oxidative stress impairs insulin signalling and therefore causes insulin resistance of the cell (Samantha and Walter, 2017; Justin and Sushil, 2011).

**Corresponding Author Email: imamnasirusman@gmail.com*

Overconsumption of unsatiating sweetened foods such as beverages has been a major contributing factor to the increasing prevalence of obesity (Max and Gerald, 2018) and insulin resistance (Softic *et al*., 2016). Insulin resistance plays a causative role in many metabolic disturbances such as metabolic syndrome (Shin-Hae *et al*., 2022; Habib *et al*., 2019; Perry *et al*., 2014) and it has been shown that fructose is the main component of dietary sugar that prompt metabolic perturbations (Softic *et al*., 2020). Metabolic syndrome is a consequence of poor diet, unhealthy lifestyle and sedentary habits and is commonly referred to as lifestyle disorders. The syndrome typically consists of the presence of obesity (mainly abdominal), insulin resistance, hypertension, and dislipidemia, and has been recognized to be at the it is at the root of the wide spread of the diseases such as type 2 diabetes mellitus cardiovascular related problems and other disabilities (Mohammad, 2018). Although thiazolidinediones and metformin are good candidates for treating insulin resistance, the cost, side effects and availability particularly in rural areas that are not spared of this disorder make searching for alternative a necessity. Any intervention that could result in weight loss and improved insulin sensitivity could be of importance in the management of metabolic disorders associated with insulin resistance. Spices such as *Zingiber officinale* (ginger), *Piper guineense* (West African black pepper), *Allium sativum* (garlic) and *Curcuma Longa* (turmeric) are severally reported to have many health benefits such as antioxidative and anti-inflammatory properties (Baselga-Escudero *et al*., 2017). Recently, Idoko *et al*. (2022) reported that 2% supplementation of high-fructose diet with garlic and/or turmeric could protect both the liver and kidney from damaging effect of high-fructose diet. Previously, Idoko *et al*. (2018) reported that reported that 2% inclusion level of a mixture of some spices in high salt diet could give protection against organ deteriorations and improve lipid metabolism in experimental Wistar rats. Consequent upon the foregoing, we evaluated the potentials of spices-supplemented diets in ameliorating obesity and insulin resistance in Wistar rats induced with metabolic syndrome by feeding them with high fructose meals

MATERIALS AND METHODS

Feed ingredients: The Spices (ginger, garlic, turmeric and West African black pepper) were bought from Dutsinma Central Market, Katsina State of Nigeria and authenticated at the Department of Biological Sciences Herbarium at federal university Dutsinma where they were identified as *Zingiber officinale* (FUDMA/PSB/0042), *Allium sativum*(FUDMA/PSB/0019), *Curcuma Longa* (FUDMA/PSB/0143) *and Piper guineense* (FUDMA/PSB/0138) respectively. Corn, soybeans mill and palm oil were also bought from Dutsinma Central market, while rice bran was collected from a rice milling factory in Dutsinma. Corn starch was prepared from yellow corn after soaking in water for 48 hours. Other ingredients as shown in table 1 were of analytical grade.

Experimental: Thirty-five (35) adult male albino rats (128.08±5.29g) were purchased from the Experimental animal unit of University of Jos, Nigeria.

Preparation of the spices: The spices; ginger, garlic, turmeric and West African black pepper were air-dried and pulverised. A blend of the spices was prepared by mixing the spices thoroughly in equal ratio.

Formulation of the experimental diets: The normal diet for rodents and high-fructose diet were prepared by appropriately mixing the feed ingredients (Table 1).

Table 1: Approximate compositions of the Feed

Feed component	**Control diet g/100g**	**High-fructose g/100g**
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
Vitamin and mineral mix	0.25	0.25
Methionine	0.25	0.25
Total	100	100

The spices were respectively mixed with the standard diet in a ratio of 2:98 to formulate 2% spices-supplemented diets (Idoko *et al*., 2022). The formulated experimental diets were:

A. Normal rodent diet (control)
B. High fructose diet
C. 2% turmeric supplement in standard diet
D. 2% ginger supplement in standard diet
E. 2% garlic supplement in standard diet
F. 2% black pepper supplement in standard diet
G. 2% blended spices supplement in standard diet

Proximate composition of the formulated diets: The crude protein, ether extract (fat), fibre, ash and moisture were determined following routine techniques described by AOAC (1995). Nitrogen Free Extract (NFE) and metabolizable energy (ME) of the formulated diets were respectively computed as follow;

NFE= 100 - (Values of Crude Protein + Fat + Crude Fibre + Ash + Moisture) (AOAC, 1995)

ME= Metabolizable Energy (KCal/Kg) = (37 X C.P) + (81.8 X Fat) + (35 X N.F.E) (AOAC, 1995)

Inducement of metabolic syndrome: The thirty-five Wistar male rats were divided into two groups of 5 rats (control) and 30 rats. The two groups had similar average weight. The control was placed on the formulated normal rodent diet, while the second group of thirty (30) rats was placed on high-fructose diet until metabolic syndrome was confirmed after seven (7) weeks (Silvia *et al*., 2018; Ibitoye *et al*., 2017; Ropelle *et al*., 2010). The inducement of the syndrome was confirmed by comparing the body weight and blood glucose levels of the two groups on weekly bases, and lipid profile fortnightly to follow up the development of overweight, hyperglycaemia and dyslipidaemia (Romeo *et al*., 2012) all of which are important components of the syndrome. Glucometer and Lipidplus were respectively used in monitoring the blood glucose level and lipid profile.

Experimental Design: The group on the control diet was sustained on the normal diet. Metabolic syndrome-induced rats were divided into six (6) groups of 5 rats each and randomly assigned to the 6 formulated spices-supplemented diets.

The groupings were as follow: Normal rats maintained on the control diet (Normal control)

A. Metabolic syndrome-induced rats fed on the control diet (positive control)

B. Metabolic syndrome-induced rats fed on 2% turmeric-supplemented diet

C. Metabolic syndrome-induced rats fed on 2% West African black pepper-supplemented diet

D. Metabolic syndrome-induced rats fed on 2% ginger supplemented diet

E. Metabolic syndrome-induced rats fed on 2% garlic-supplemented diet

F. Metabolic syndrome-induced rats fed on 2% blended spices-supplemented diet

The rats were maintained on their respective diet *ad libitum* for additional eight (8) weeks with weekly monitoring of the weight and blood glucose level.

Blood sample collection and treatment: At the end of the 15-week period of the experiment, the rats were made unconscious in a transparent bucket containing chloroform-soaked cotton wool and then sacrificed by cutting their jugular veins. In order to obtain serum for analysis, the blood was collected into plain sample containers and subjected to 15-minute centrifugation at 1500 RPM. The serum was frozen until it was required for analysis Silvia *et al*. (2018).

Determination of feed intake and body weight: The feed intake and change in weight were recorded weekly to monitor the feeding and growth patterns of each group.

Determination of blood glucose level, insulin and HOMA-IR: Blood glucose level was measured using glucometer. Insulin level was determined enzymatically using assay kits from Randox Laboratories Ltd. The homeostasis model assessment (HOMA) of insulin resistance index was calculated as follows: HOMA-IR = fasting insulin mU/L) × (fasting glucose mmol/L) / 22.5 (Jinhua *et al*., 2013)

Statistical Analysis: Results are means± SEM of three determinations. The statistical significance was assessed by subjecting the raw data during inducement to student t-test while the post-treatment data were subjected to one-way ANOVA. SPSS (16.0 version) was the statistical package program used for the analysis.

RESULTS AND DISCUSSION

Proximate composition of the formulated diets: The proximate compositions of the formulated diets varied significantly ($p<0.05$) with garlic supplemented diet and high fructose diet having the highest and lowest crude protein respectively. Diet containing black pepper had the highest ether extract level (fat) while the control and diet containing garlic had the lowest values. The high fructose diet had significantly higher moisture compared to the control and to all other formulated diets. The highest metabolizable energy is contained in black pepper-supplemented diet while high fructose diet had the least metabolizable energy. The full results are as presented in Table 2.

The inducement of metabolic syndrome in rats: The changes in blood glucose level, weight and lipid profiles presented in Table 3. All these parameters were significantly ($p<0.05$) increased in the group fed high fructose diet except HDL which did not vary significantly ($p>0.05$).

Table 2: Proximate composition of the formulated diets

	CP	EE	MOISTURE	FIBRE	ASH	NFE	ME
C	12.87±0.01^a	13.79±0.02^a	8.01±0.00^c	8.41±0.00^c	4.11±0.00^c	52.81±0.01^a	3452.3±1.02^a
FRUCTOSE	12.20±0.00^a	14.11±0.01^b	9.84±0.01^d	6.89±0.01^d	4.47±0.00^a	52.48±0.00^c	3442.4±0.50^a
BP	13.61±0.08^b	14.86±0.01^d	7.26±0.01^a	7.39±0.06^a	4.46±0.02^a	52.42±0.15^c	3554.1±1.58^f
T	13.74±0.01^b	14.75±0.01^c	7.81±0.00^b	7.64±0.00^b	3.85±0.00^b	52.20±0.02^b	3541.9±0.25^e
GINGER	13.93±0.00^b	14.05±0.01^b	7.40±0.01^e	7.76±0.00^e	4.65±0.00^d	52.87±0.01^a	3515.00±0.77^c
GARLIC	14.88±0.01^c	13.97±0.58^a	7.67±0.00^f	7.12±0.01^e	3.64±0.01^e	52.71±0.03^a	3538.00±1.18^d
MIX	14.05±0.01^d	14.20±0.00^c	6.85±0.01^g	8.79±0.01^f	3.98±0.01^f	52.12±0.02^b	3505.8±±0.43^b

Values are means ±S.E.M for 3 determinations. Different superscripts along the same column indicates significant difference (P<0.05). Same superscripts along the same column indicates non-significant difference (P>0.05).
C: Normal rodent diet (control); F: High fructose diet; T: Diet based on 2% inclusion level of turmeric; GI: Diet based on 2% inclusion level of ginger; GA: Diet based on 2% inclusion level of garlic; BP: Diet based on 2% inclusion level of black pepper; MIX: Diet based on 2% inclusion level of the blended spices; CP: Crude fibre; EE: Ether extract; NFE: Nitrogen Free Extract; ME: Metabolizable Energy

Table 3: Average initial and final values of blood glucose level, weight and lipid panels during metabolic syndrome inducement

		C	I
Blood(mg/dl)	IV	82.75±1.60	86.67±1.46
	EV	83.75±1.11	115.57±2.94
Weight (g)	IV	132.25±1.5	131.55±4.8
	EV	200.05±1.78	254.9±1.48
Tchol (mmol/L)	IV	3.13±0.12	3.2±0.06
	EV	3.19±0.03	9.70±0.12
TG (mmol/L)	IV	0.98±0.01	1±0.02
	EV	1.05±0.02	2.89±0.1
LDL (mmol/L)	IV	1.07±0.01	1.16±0.04
	EV	1.08±0.01	3.99±0.26
HDL (mmol/L)	IV	1.84±0.12	1.9±0.02
	EV	1.85±0.03	1.26±0.09
AI	IV	0.59±0.04	0.58±0.05
	EV	0.62±0.03	3.22±0.32
CRI	IV	1.71±0.05	1.7±0.13
	EV	2.76±0.09	7.87±0.54

C- Control group; I-Group being induced with metabolic syndrome; IV- Initial values; EV-Values at the end of induction period; Tchol- Total cholesterol; TG- Triglyceride; LDL-C- Low density lipoprotein cholesterol; HDL-C- high density lipoprotein cholesterol; AI- atherogenic index; CRI- coronary risk index

Changes in growth and insulin sensitivity in metabolic syndrome-induced rats fed spices supplemented diets: All the groups showed gradual but steady weight gain during the treatment period as depicted in figure 1. Although there were significant variations ($p<0.05$) in the weekly weight gained within all the groups, the overall weight gain in all metabolic syndrome induced rats fed spices supplemented diets did not significantly differ ($p>0.05$) from the weight gain in the control but were significantly lower ($p<0.05$) when compared with the group of metabolic syndrome induced rats fed the control diet (Table 4).

Indices of insulin resistance in metabolic syndrome induced rats fed spices supplemented diets: Except for the control whose blood glucose level did not vary significantly ($p<0.05$) throughout the treatment period, the post treatment blood glucose levels were significantly decreased in all the groups when compared with their respective pre-treatment levels. However, a sharp rise in blood glucose level was observed in the group fed black pepper supplemented diet on the 3rd week of the treatment period (Figure 3). Blended spices, ginger, garlic and turmeric supplemented diets caused significantly higher percentage decrease in blood glucose levels in the groups of metabolic syndrome induced rats as compared with the group of metabolic syndrome induced rats fed normal diet (Table 5). Although all the test groups had significantly ($p<0.05$) higher insulin resistance when compared with the control, all the metabolic syndrome induced rats maintained on supplemented diets had significantly ($p<0.05$) lower insulin resistance (as represented by the computed HOMA-IR) when compared with the metabolic syndrome induced rats maintained on non-supplemented diet (Table 6). High fructose diet intake has been associated with excess energy intake and development of features encountered in the metabolic syndrome (Isabelle *et al*., 2013).

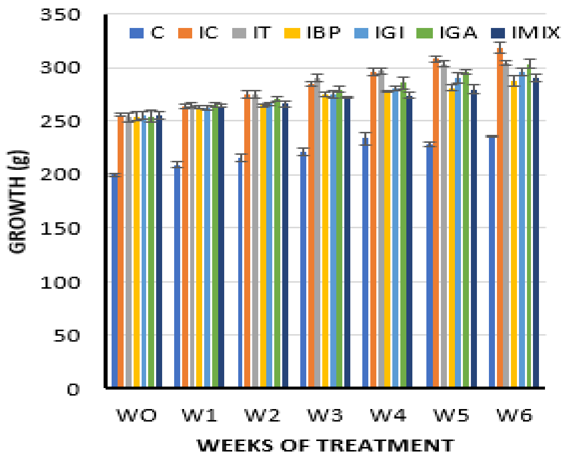

Fig 1: Growth pattern of metabolic syndrome induced rats fed spices supplemented diets

C-Normal rats maintained on the control diet; IC-Metabolic syndrome-induced rats maintained on the control diet; IT-Metabolic syndrome-induced rats maintained on diet based on 2% inclusion level of turmeric; IBP- Metabolic syndrome-induced rats maintained on diet based on 2% inclusion level of West African black pepper; IGI- Metabolic syndrome-induced rats maintained on diet based on 2% inclusion level of ginger; IGA-Metabolic syndrome-induced rats maintained on diet based on 2% inclusion level of garlic; IMIX- Metabolic syndrome-induced rats maintained on 2% inclusion level of blended spices.

Table 4: **Average weight gained in weight in metabolic syndrome induced rats fed spices supplemented diets**

C	**6.01±2.42[a]**
IC	11.43±0.63[b]
IT	8.55±1.98[a]
IBP	5.61±1.27[a]
IGI	6.78.58±0.81[a]
IGA	8.17±0.97[a]
IMIX	5.91±1.48[a]

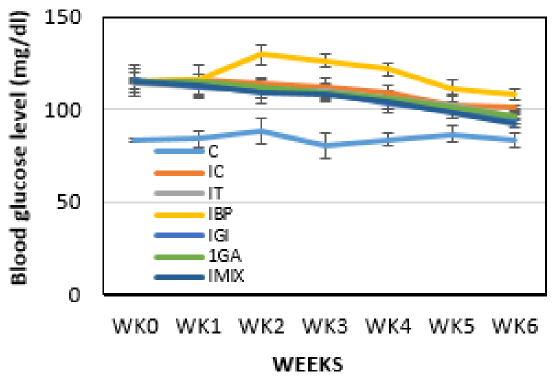

Fig 2: Blood glucose levels of metabolic syndrome-induced rats fed spices supplemented diets

The hyperglycemia, dyslipideamia, and significantly higher weight gain in the group fed high fructose diet (Table 3) might have arisen due to different metabolic abnormalities caused by fructose. Since fructose is mainly metabolized in the liver where it bypasses phosphofructokinase action (a key regulatory enzyme in the glycolytic pathway), it provides an unregulated source of acetyl coenzyme A for hepatic *de novo* lipogenesis (Samuel, 2011). High fructose diet intake has been associated with excess energy intake and development of features encountered in the metabolic syndrome (Isabelle *et al*., 2013). The hyperglycemia, dyslipideamia, and significantly higher weight gain in the group fed high fructose diet (Table 3) might have arisen due to different metabolic abnormalities caused by fructose. Since fructose is mainly metabolized in the liver where it bypasses phosphofructokinase action (a key regulatory enzyme in the glycolytic pathway), it provides an unregulated source of acetyl coenzyme A for hepatic *de novo* lipogenesis (Samuel, 2011).

Table 5: Percentage reduction in blood glucose level in metabolic syndrome induced rats fed spices supplemented diets

	ABBT	ABAT	PR
IC	115.25	100.75	12.58%
IT	114.50	96	16.16%
IBP	115.50	107.75	6.71%
IGI	115.75	94.75	18.14%
IGA	115.5	96.50	16.45%
IMIX	115.50	92.75	19.70%

ABBT-Average Blood glucose level before treatment (mg/dl); ABAT-Average Blood glucose level after treatment (mg/dl); PR-Percentage

Table 6: Index of insulin resistance in metabolic syndrome induced rats fed spices supplemented diets

	BGL (mmol/L)	Insulin(μU/mL)	HOMA-IR
C	4.61 ± 0.22^{a}	8.00 ± 0.41^{a}	1.65 ± 0.14^{a}
IC	5.60 ± 0.10^{bc}	22.00 ± 0.58^{e}	5.47 ± 0.07^{f}
IT	5.33 ± 0.22^{b}	15.75 ± 0.25^{d}	3.73 ± 0.13^{d}
IBP	5.99 ± 0.17^{c}	16.25 ± 0.75^{d}	4.33 ± 0.26^{e}
IGI	5.26 ± 0.15^{b}	13.75 ± 0.48^{c}	3.21 ± 0.07^{c}
IGA	5.36 ± 0.10^{b}	12.50 ± 0.29^{c}	2.97 ± 0.02^{c}
IMIX	5.15 ± 0.14^{b}	10.75 ± 0.63^{b}	2.45 ± 0.10^{b}

Different superscripts along the same column indicates significant difference ($P<0.05$). Same superscripts along the same column indicates non-significant difference ($P>0.05$).

Unusual accumulation of triglyceride in the muscle and liver has been postulated to be implicated in development of obesity (Fitsum *et al*., 2013). Softic *et al*. (2017) observed that fructose induces synthesis of enzymes of fatty acid synthesis, such as acetyl-CoA carboxylase 1(ACC1), ATP citrate lyase (ACLY), fatty acid synthase and stearoyl-CoA desaturase 1 (SCD1). Our findings are consistent with Malik *et al*. (2019) and Hannou *et al*. (2018) who reported that high intake of fructose is at the root of development of metabolic diseases such as obesity, hyperglyceamia/insulin resistance and hyperlipidemia. These conditions are risk factors for the development of diabetes, cardiometabolic disease and organ failures. It follows from the finding that supplementation of the diet (2%) with any of ginger, garlic, turmeric and black pepper could retard/reverse the progression of obesity in diet-induced obese rats (Table 4) which could be due to increase in thermogenesis, energy expenditure and lipolysis coupled with inhibition of the intestinal absorption of dietary fat; conditions that could have been mediated by some bioactive compounds contained in the spices. Seok *et al*. (2021) reported that ginger intake caused upregulation of fibroblast growth factor 21 (FGF21), acyl-CoA oxidase 1 (ACOX1), and carnitine palmitoyltransferase 1 (CPT1) all of which increase fatty-acid utilization and consequently cause decrease in weight. Previously, Sayed *et al*. (2020) and Wang *et al*. (2020) had demonstrated antiobesity properties of ginger which resulted into decrease in body weight and fat mass in models of animal. The main bioactive components of ginger, 6-gingerol and 6-shogaol have been reported to exhibit anti-obesity effects by altering the activities of some lipid metabolism marker enzymes and decreasing the expression of various lipogenic marker proteins in both rats and cell lines (Suk *et al*., 2016). Kim and Kim (2011) found that fat accumulating gene expressions were decreased by administration of garlic in mice which translated into decreased body weight gain and epididymal fat accumulation. They also reported increase in temperature and stimulation of AMP-activated protein kinase (AMPK), an indication that anti-obesity effects of garlic could be through AMPK activation, increased thermogenesis and decreased multiple gene expressions involved in adipogenesis. AMPK regulates energy balance by coordinating metabolic pathways thereby balancing nutrient supply with energy demand. This makes AMPK an important therapeutic target for controlling human diseases including metabolic syndrome (Joungmok *et al*., 2016). The decrease in weight gain in the metabolic syndrome induced rats fed turmeric supplemented diet indicates that turmeric has the capacity to regulate metabolism. Curcumin is the major constituent of turmeric and is reported to be responsible for its antiobesity activities such as inhibition of adipogenesis and activation of lipolysis. Liang-Yi *et al*. (2019) found that apart from inducing apoptosis of 3T3-L1 adipocytes, curcumin also modulated expression of adiponectin and modulated molecular signaling of AMPK. The decrease in weight gain in the metabolic syndrome induced rats fed black pepper supplemented diet could have been due the effect of its active ingredients on energy expenditure and other related physiological events. The major constituents of black pepper include piperine, eugenol, lipase enzyme and essential oils such as limonene, β-pinene, and β-caryophyllene (Tainter and Grenis, 2001; Majeed and Prakash, 2000). Piperine has been reported to increase the body's energy expenditure which is thought to be through the production of hormone-like chemicals involved in regulation of energy balance (Westerterp-Plantenga *et al*., 2006). The lipase enzyme could be involved in decreasing gain in weight by increasing the fat breakdown. Our finding shows that the studied spices (turmeric, black pepper, ginger and garlic) have synergistic anti-obesity activities as seen in higher decrease in the metabolic syndrome induced rats fed a blend of the spices. However, for the manifestation of the anti-obesity effect, long term consumption (minimum of 3 weeks) of the spices may be required. It is evident from our finding that inclusion of these spices in diets for a long period could decrease weight gain in obesity caused by consumption of metabolic syndrome causing diets.The lowering of blood glucose level and HOMA-IR in the groups of metabolic syndrome-induced rats fed spices supplemented diets (Tables 5 and 6) is indicative of improved glucose sensitivity in these rats. Diet supplementations with these spices could therefore ameliorate hyperglycemia and its accompanying consequences. The improved insulin sensitivity in the group fed ginger supplemented diet could be through up-regulation of FGF-21 as reported by Seok *et al*. (2021). In addition to increasing energy expenditure and fat utilization, FGF21 is reported to prevent development of metabolic derangement by normalizing glucose and lipid homeostasis (Wing and Po, 2016). In other researches with animal models, it was demonstrated that ginger extract has antihyperinsulinemia and lowers HOMA-IR in fructose-fed rats (Wang *et al*., 2013) and in sodium arsenite-induced hyperglycemia and glucose intolerance (Chakraborty *et al.,* 2012). These results suggest that ginger supplementation improves hepatic insulin resistance which could be one of the reasons for its antiobesity effect. One of the ways through which fructose causes obesity according to Uyeda and Repa (2006) is via activation of hepatic carbohydrate response element binding protein (ChREBP) which stimulates lipogenic gene expressions. It had been shown by Gao *et al.* (2012) that long term consumption of ginger exerts its antiobesity and antihyperinsulinemia by modulating hepatic ChREBP-mediated lipogenic pathway resulting in suppression of overexpressed ChREBP. The improvement in insulin resistance and level of blood glucose is in agreement with our finding that ginger causes decrease in weight gain in obese rats.

Curcumin is the most abundant of all (Perez-Torres *et al*., 2013; Jurenka, 2009) and is reported to be mainly responsible for most of the health benefits of the turmeric. The improvement in both the hyperglyceamia and insulin resistance as represented by lower HOMA-IR in the metabolic syndrome induced rats fed turmeric supplemented diet is an indication that turmeric in diet may improve glucose utilization and insulin sensitivity. Curcumin had been reported by Kang and Kim (2010) to decrease glucose level by activating pathways of glycolysis and glycogen synthesis while downregulating gluconeogenic pathway by inhibiting the activities of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. Interestingly, curcumin plays two seemingly opposing roles on insulin secretions. It reduces synthesis and secretion of insulin, and improves insulin sensitivity in non-hyperglycemic but insulin resistant models (Shao *et al.*, 2012). On the other hand, it increases insulin production in models induced with hyperglyceamia to maintain the normal blood glucose levels (Na *et al*., 2011). The improvement in insulin sensitivity seen in the group fed black pepper supplemented diet could be due to the anti-inflammatory property of piperine, a major constituent of the black pepper. Metabolic inflammation causes over secretion of pro-inflammatory cytokines such as TNF-α in obesity leading to resistance to insulin through impairment of insulin signaling (Lackey and Olefsky, 2016). Smilkov *et al*. (2019) report biological activities of piperine which include anti-oxidation and anti-inflammatory. So, by its anti-inflammatory activity, black pepper decreases secretion of TNF-α thereby acts to maintain the sensitivity of hepatocytes and adipocytes to insulin. Diet supplementation with garlic could improve insulin sensitivity individuals with metabolic syndrome as seen in results of insulin resistance indices in metabolic syndrome induced rats fed garlic supplemented diet. Jung *et al*. (2011) had also reported lowering effect of garlic preparations on blood glucose levels. As earlier discussed, inflammation exacerbates insulin resistance. Therefore, the improvement in insulin sensitivity in metabolic syndrome induced rats fed garlic supplemented diet may be due to the anti-inflammatory and anti-oxidative effects of garlic. Although, each of the spices (turmeric, ginger, garlic and black pepper) had positive effect on improving hyperglycemic and insulin resistance conditions in metabolic syndrome induced rats, the spices could act synergistically to achieve a better result as seen in the significantly lower HOMA-IR in the group of metabolic syndrome-induced rats fed the mixed spices supplemented diet as compared with other metabolic syndrome induced rats fed the individual spices supplemented diets. The effectiveness of combined ginger and curcumin in alleviating hyperglycemia and oxidative stress had been reported by Hala *et al*. (2013). It is evident from our finding that hyperglycemia and insulin resistance due to consumption of high-fructose diet or metabolic syndrome causing diets could be ameliorated by long term consumption of diets fortified with any of the four spices, but fortification with a blend of the spices could give a better result.

Conclusion: Having investigated the supplementation of diets with powders of ginger, garlic, black pepper, turmeric and a blend of the four as easy and affordable means of treating some components of metabolic syndrome, our findings show that the inclusion of the spices could decrease weight gain and improve insulin sensitivity in metabolic syndrome-induced rats with a blend of the four spices having better effects.

REFERENCES

Amirkhizi, F; Siassi, F; Minaie, S; Djalali, M; Rahimi, A; Chamari, M (2007). Is obesity associated with increased plasma lipid peroxidación and oxidative stress in women. *ARYA Atheroscler. J*. (2): 189–192.

Association of Official Analytical Chemists (AOAC) (1995). Official methods of analysis. Washington D.C.

Baselga-Escudero, L; Souza-Mello, V; Pascual-Serrano, A; Rachid, T; Voci, A; Demori, I.
Grasselli, E (2017). Beneficial effects of the Mediterranean spices and aromas on non-alcoholic fatty liver disease. *Trends Food Sci. Technol*, 61:141–159

Chakraborty, D; Mukherjee, A; Sikdar, S; Paul, A; Ghosh, S; Khuda-Bukhsh, A. R (2012). [6]-Gingerol isolated from ginger attenuates sodium arsenite induced oxidative stress and plays a corrective role in improving insulin signaling in mice. Toxicology Letters, 210(34–43).

Feve, B; Bastard, J.P (2009). The role of interleukins in insulin resistance and type 2 diabetes mellitus. *Nat Rev Endocrinol*, 5(6):305–11.

Fernández-Sánchez, A; Madrigal-Santillán, E; Bautista, M; Esquivel-Soto, J; Morales-González, A; Esquivel-Chirino, C; Durante-Montiel, I; Sánchez-Rivera, G; Valadez-Vega, C; Morales-González, J.A (2011). Inflammation, oxidative stress, and obesity. *Int. J. Mol. Sci.* 12: 3117–3132.

Fitsum, G; Pascaline, MA; Laetitia K; Caroline, CP; Emilie, K; Denis, F; Christophe, OS (2013). Ectopic lipid accumulation: A potential cause for metabolic disturbances and a contributor to the alteration of kidney function. Biochimie 95(2013):1971-1979

Gao, H; Guan, T; Li, C; Zuo, G; Yamahara, J; Wang, J; Li, Y (2012). Treatment with ginger ameliorates fructose-induced Fatty liver and hypertriglyceridemia in rats: Modulation of the hepatic carbohydrate response element-binding protein-mediated pathway. Evidence-Based Complementary and Alternative Medicine. Assessed from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3502023/ on 13/03/2022

Hala, AH; Nadia, SA; Al-Anood, A (2013). Effect of Ginger, Curcumin and their mixture on blood glucose and lipids in diabetic rats. Life Sci J. 10(4): 428-442.

Hannou, SA; Haslam, DE; McKeown, NM; Herman, A (2018). Fructose metabolism and metabolic disease. J Clin Invest, (128):545–555

Habib, Y; Farin, R. F; Alexandra, EB; Amirhossein, S (2019). Insulin resistance: Review of the underlying molecular Mechanisms. *J Cell Physiol*. 234:8152–8161.

Ibitoye, OB; Ghali, UM; Adekunle, JB; Uwazie, JN; Ajiboye, TO (2017). Antidyslipidemic, Anti-Inflammatory, and Antioxidant Activities of Aqueous Leaf Extract of *Dioscoreophyllum cumminsii* (Stapf) Diels in High-Fat Diet-Fed Rats. Hindawi Evidence-Based Complementary and Alternative Medicine Volume 2017.

Idoko, AS; Zaharaddeen, AS; Ilouno, LE; Ibrahim, M; Arinola, AM; Dabai, TA; Okereke, GE (2018). Physical and Biochemical Studies on Excess Weight-Induced Rats Maintained on Mixed Spices-Supplemented Diet. *Nigerian Journal of Basic and Applied Science*. 26(2): 09-16

Idoko, AS; Abdullahi, A; Maibulangu, BM; Nura, L; Imam, NU; Muhammed, F; Umar, S (2022). Allium sativum and Curcuma longa l Powder Protect Against Hepatotoxic and Nephrotoxic Effects of High-fructose Diet. FUOYE Journal of Pure and Applied Sciences, 7(1) In press

Isabelle, A; Michel, H; Philip, A; Gerber, LS; Stefanie, BM; Luc, T; Giatgen, AS; Kaspar, B (2013). Moderate Amounts of Fructose Consumption Impair Insulin Sensitivity in Healthy Young Men. A randomized controlled trial. Diabetes care.

Jinhua, Y; Ming, L; Lu, X; Ying, W; Hong, C; Xiaoyuan, Z; Jie, M (2013). Insulin resistance determined by Homeostasis Model Assessment (HOMA) and associations with metabolic syndrome among Chinese children and teenagers. Diabetology and Metabolic Syndrome, 5 (71)

Joungmok, K; Goowon, Y; Yeji, K; Jin, K; Joohun, H (2016). AMPK activators: mechanisms of action and physiological activities. Experimental & Molecular Medicine, 48(4):224

Jung, YM; Lee, SH; Lee, DS; You, MJ; Chung, IK; Cheon, WH; Kwon, YS; Lee, YJ; Ku, SK (2011). Fermented garlic protects diabetic, obese mice when fed a high-fat diet by antioxidant effects. Nutr Res. 31(3):87-96

Justin, L; Sushil, KJ (2011). Oxidative stress, insulin signaling, and diabetes. Free Radic Biol Med, 50(5):567-75.

Kang, C; Kim, E (2010). Synergistic effect of curcumin and insulin on muscle cell glucose metabolism. Food Chem. Toxicol. (48)2366–2373.

Kim, MJ; Kim, HK (2011). Effect of garlic on high fat induced obesity. Acta. Biol. Hung, 62(3):244-54

Lackey, DE; Olefsky, JM (2016). Regulation of metabolism by the innate immune system. Nat Rev Endocrinol.12(1):15–28.

Li, C; Rui, C; Hua, W; Fengxia, L (2015). Mechanisms Linking Inflammation to Insulin Resistance. International Journal of Endocrinology, 2015

Liang-Yi, W; Chien-Wei, C; Luen-Kui, C; Hsiang-Yun, C; Chih-Ling, C; Chi-Chang, J. (2019). Curcumin Attenuates Adipogenesis by Inducing Preadipocyte Apoptosis and Inhibiting Adipocyte Differentiation.Nutrients. 11(10): 2307.

Majeed, D; Prakash, L (2000). The medicinal uses of pepper. Int Pepper News. 2000;1:23Y31.

Malik, VS; Li, Y; Pan, A; De Koning, L; Schernhammer, E; Willett, W (2019). Long-term consumption of sugar-sweetened and artificially sweetened beverages and risk of mortality in US adults. *Circulation*, (139):2113–2125

Max, CP; Gerald, IS (2018). Mechanisms of insulin action and insulin resistance. *Physiol Rev* (98): 2133–2223.

Mohammad, GS (2018). The Global Epidemic of the Metabolic Syndrome. Curr Hypertens Rep. 20(2): 12.

Mohammed, SE; Ismail, P; Huzwah, K; Asmah, R; Yehia, A (2017). Obesity and inflammation: the linking mechanism and the complications. *Arch Med Sci*, 13(4): 851–863

Na, LX; Zhang, YL; Li, Y; Liu, LY; Li, R (2011). Curcumin improves insulin resistance in skeletal muscle of rats. Nutr. Metab. Cardiovasc. Dis. (21): 526–533.

Perry, RJ; Camporez, JG; Kursawe, R (2015). Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. Cell, 160(4):745–758.

Qiang, S; Jia, L; Feng, G (2014). New insights into insulin: The anti-inflammatory effect and its clinical relevance, World J Diabetes, 15; 5(2): 89–96.

Romeo, GR; Lee, J; Shoelson, SE (2012). Metabolic syndrome, insulin resistance, and roles of inflammation–mechanisms and therapeutic targets. *Arterioscler Thromb. Vasc. Biol.* 32(8): 1771–1776.

Ropelle, ER; Flores, MB; Cintra, DE; Rocha, GZ; Pauli, JR; Morari, J (2010) IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKKbeta and ER stress inhibition. PLoS Biol. 8(8):1–20.

Saidur, R; Khandkar, SH; Sharnali, D; Sushmita, K; Elikanah, OA; Ataur, R; Abdul, H; Jamal, U; Myung-Geol, P (2021). Role of Insulin in Health and Disease: An Update International Journal of Molecular Science, (22) 6403.

Samantha, H; Walter, HH (2017). The etiology of oxidative stress in insulin resistance. Biomed J. 40(5):257-262

Samuel, VT; Shulman, GI (2016). The pathogenesis of insulin resistance: Integrating signaling pathways and substrate flux. *The Journal of Clinical Investigation*, 126(1): 12–22.

Shin-Hae, L; Shi-Young, P; Cheol, SC (2022). Insulin Resistance: From Mechanisms to Therapeutic Strategies. *Diabetes Metab J.* 46(1):15-37.

Silvia, M; Marta, G; Gema, V; Julien, A; Jean, FL; Marta, M (2018). High Fat/High
Glucose Diet Induces Metabolic Syndrome in an Experimental Rat Model. Nutrients, 10(10): 1502.

Sayed, SM; Ahmed, MM; El-Shehawi, AM; Alkafafy, M; Alotaibi, SS; El-Sawy, H; Farouk, S; Elshazly, SA (2020). Ginger Water Reduces Body Weight Gain and Improves Energy Expenditure in Rats. Foods. 9:38.

Seok, HS; Feng, F; Inhae, K (2021). Ginger (*Zingiber officinale*) Attenuates Obesity and Adipose Tissue Remodeling in High-Fat Diet-Fed C57BL/6 Mice.Int J Environ Res Public Health. 18(2): 631

Shao, W; Yu, Z; Chiang, Y; Yang, Y; Chai, T; Foltz, W; Lu, H; Fantus, I.G; Jin, T (2012). Crcumin prevents high fat diet induced insulin resistance and obesity via attenuating lipogenesis in liver and inflammatory pathway in adipocytes. PLoS One. 7:e28784.

Smilkov, K; Ackova, DG; Cvetkovski, A; Ruskovska, T; Vidovic, B; Atalay, M (2019). Piperine: old spice and new Nutraceutical? Curr Pharm Des. 25(15):1729–39

Softic, S; Cohen, DE; Kahn, CR (2016). Role of dietary fructose and hepatic de novo lipogenesis in fatty liver disease. *Dig Dis Sci.* 61 (5):1282–1293.

Softic, S; Kimber LS; Jeremie B; Senad D; Miguel AL; Richard, JJ; Ronald, CK (2020). Fructose and hepatic insulin resistance. *Crit. Rev. Clinic. Lab. Sci.* 2019. 57(5):308-322

Suk, S; Seo, SG; Yu, JG; Yang, H; Jeong, E; Jang, YJ (2016). A bioactive constituent of ginger, 6-shogaol, prevents adipogenesis and stimulates lipolysis in 3T3-L1 adipocytes. *J Food Biochem*, (40):84–90.

Tainter, D; Grenis, A (2001). Spices and Seasonings, A Food Technology Handbook. 2nd ed. Hoboken, NJ: Wiley-IEEE; 2001

Uyeda, K; Repa, JJ (2006). Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metabolism*, (4)107–110.

Wang, J; Li D; Wang, P; Hu, X; Chen, F (2019). Ginger prevents obesity through regulation of energy metabolism and activation of browning in high-fat diet-induced obese mice. *J. Nutr. Biochem.* (70):105–115

Wang, J; Gao, H; Ke, D; Zuo, G; Yang, Y; Yamahara, J. Li, Y (2013). Improvement of liquid fructose-induced adipose tissue insulin resistance by ginger treatment in rats is associated with suppression of adipose macrophage-related proinflammatory cytokines. Evidence-Based Complementary and Alternative Medicine. Retreived from https://pubmed.ncbi.nlm.nih.gov/23533500/ on 09/11/2021.

Westerterp-Plantenga, M; Diepvens, K; Joosen, A; Berube-Parent, S; Tremblay, A (2006). Metabolic effects of spices, teas, and caffeine. *Physiol Behav.* (89):85-91

Wing, YS; Po, SL; (2016). Fibroblast Growth Factor 21 As an Emerging Therapeutic Target for Type 2 Diabetes Mellitus. *Med Res Rev*. 36(4):672-704.