

**SERUM GLUCOSE, LIPID PROFILE AND OXIDATIVE STRESS
MARKERS OF SALT-INDUCED METABOLIC SYNDROME
RATS ADMINISTERED ANTIOXIDANT RICH NUTRACEUTICAL**

ABSTRACT

Metabolic syndrome is indeed a high risk condition involving obesity, dyslipidemia, hypertension and diabetes mellitus. The study aim to formulate an antioxidant rich nutraceutical from locally available foodstuff (onion, garlic, ginger, tomato, lemon, palm oil, water melon seeds) and investigate their effects on body weight, serum glucose, lipid profile, insulin and oxidative stress markers in salt induced metabolic syndrome rats. The rats were placed on 8% salt diet for 6 weeks and then supplementation and treatment with nutraceutical and nifedipine in the presence of salt diet for additional 4 weeks. Feeding rats with salt diet for 6 weeks increased body weight of the salt-loaded rats relative to control. Significant ($P<0.001$) increase in serum blood glucose and lipid profile, and decrease in high density lipoprotein-cholesterol (HDL-C) was observed in salt-loaded rats as compared with control. Supplementation with nutraceutical lowered body weight of the salt-loaded rats and significant ($P<0.001$) decrease in the serum blood glucose, lipid profile, malonyldialdehyde (MDA), insulin levels, insulin resistance, and increased HDL-C and antioxidant indices was observed. The study suggests that the nutraceuticals are useful in reversing most of the component of metabolic syndrome.

Key words: Metabolic syndrome, dyslipidemia, diabetes mellitus and nutraceutical

1. INTRODUCTION

Metabolic syndrome (MS) involved cluster of cardiovascular risk factors closely linked to insulin resistance, hyperglycaemia and dyslipidaemia whose prevalence is high and rapidly rising in the Western population [1]. In the category of over 50 years of age, MS affects more than 40% of the population in the United States and nearly 30% in Europe [2]. From the studies by [3, 4] and [5], incidence of full blow metabolic syndrome in Nigeria was 20.5% in 52 patients, about 72.4% were dyslipidaemic, and 54.3% were hypertensive. Concurrent hypertension and dyslipidaemia, obesity and dyslipidaemia, and hypertension and obesity occurred in 44.4, 42.5 and 33.1% of type 2 diabetes, respectively. MS is currently considered to confer an increased risk of cardiovascular events, in part, to a cluster of other factors such as hyperuricemia, a proinflammatory state, impaired fibrinolysis and oxidative stress [6, 7].

Many studies have used nutraceutical in the management of hypertension or diabetes, but to my knowledge no work has been reported in Nigeria for the use of nutraceutical in the management of metabolic syndrome. Thus, the disease continues to pose huge challenge and threat to the health and educational sectors as well as the socio-economic development of Nigeria. According to [8], priorities have not been given to components of metabolic syndrome and other non communicable diseases (NCDS) in Nigerian health sector [8]. Furthermore, epidemiological studies and clinical trials suggested that, diets known to contain significant amount of naturally occurring antioxidants appear to ameliorate most of the traits of metabolic syndrome and may reduce cardiovascular risk [9]. Some locally available foodstuff contains significant amount of antioxidants and may be use for formulation of an antioxidant rich nutraceutical.

The research was conducted to determine the effects of 8% salt diet on serum glucose and lipid profiles in albino rats at 6 week of salt loading and to evaluate the effects of antioxidants rich nutraceutical on lipid profile, oxidative stress markers, insulin resistance and glucose tolerance of salt-induced metabolic syndrome rats.

2. MATERIAL AND METHODS

2.1 EXPERIMENTAL ANIMALS

Wistar albino rats of both sexes weighing between 150-220g were used for the study. The animals were purchased from the Department of Biological Science, Usmanu Danfodiyo University, Sokoto, Nigeria and were allowed to acclimatize for two weeks before the commencement of the experiment. The animals were grouped into 6 groups of eight rats each and were fed with pelletized growers' feed (Vital feed, Jos, Nigeria) and allowed access to water *ad libitum* before and during the experimental period.

2.2 EXPERIMENTAL DESIGN

The animals were randomly divided into 4 groups of eight rats each.

Group I Salt-loaded, treated with 250mg/kg of nutraceutical

Group II Salt-loaded, treated with 500mg/kg of nutraceutical

Group III Salt-loaded, untreated

Group IV Normal, control

2.3 INDUCTION OF METABOLIC SYNDROME IN RATS

The rats were placed on 8% w/w salt diet [10] except the control group, for 6 weeks and treatment with nutraceutical for additional 4 weeks.

2.4 PREPARATION OF ANTIOXIDANT MICRONUTRIENTS SUPPLEMENTS

The nutraceutical was prepared from onions, garlic, tomatoes, ginger, water melon seeds, lemon and palm oil in ratio of 4:4:4:4:2:1:1. This was done by mixing 20g of onions; 20g of garlic, 20g of tomatoes and 20g of ginger in 100ml distilled water and blended using electric blender. 10g of ground water melon seeds were then added and blended once again. To this, 5g of lemon juice and 5g of palm oil were added, mixed. The nutraceutical solution was packaged into clean dry containers and stored frozen at -20°C until required.

The appropriate dosages of the nutraceutical were administered to the animals orally once daily by intubation using intravenous cannula tube for 4 weeks.

2.5 BIOCHEMICAL ANALYSES

The fasting serum glucose level was estimated by glucose oxidase method [11]. Serum total cholesterol by enzymatic method using Randox kit [12]. Serum HDL- C was estimated by enzymatic method of [13] while serum Triglyceride was by enzymatic method of [14]. Serum LDL- C was calculated using Friedewald formula [15].

Insulin was estimated by SPI bio rat insulin enzyme immunoassay kit according to [16]. The assay is based on the competition between unlabelled rat insulin and acetylcholinesterase linked to rat insulin (tracer) for limited specific guinea-pig anti-rat insulin antiserum sites. The plate was then washed and Ellman's reagent added to the wells and the

acetylcholinesterase tracer acts on the Ellman's reagent to form a yellow compound which was determined at 450nm.

Colorimetric method was used for the estimation of tissue malondialdehyde [17]. Cayman's Superoxide Dismutase Assay Kit was used for the estimation of SOD. The assay utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine at 450nm. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radicals.

The catalase activity was estimated using Cayman's Catalase Assay Kit. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H₂O₂. The formaldehyde produced is measured with 4- amino-3-hydrazino-5-mercapto-1, 2, 4-triazole (Purpald) as the chromogen at 540nm.

Glutathione Peroxidase was assayed using Cayman's Assay Kit. This assay measures glutathione peroxidase activity indirectly by a coupled reaction with glutathione reductase. Oxidized glutathione, produced upon reduction of hydrogen peroxide by glutathione peroxidase, is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to NADP⁺ is accompanied by a decrease in absorbance at 340nm. Serum vitamin E and C were assayed by the method of [18] and [19] respectively.

STATISTICAL ANALYSIS

Values are expressed as mean $\pm$ standard deviation of 8 rats per group. The biochemical parameters were analyzed statistically using one way analysis of variance (ANOVA), followed by Dunnett's and Tukey multiple comparison test using Graph pad instat software (version; San Diego, U.S.A). Differences were considered significant at P<0.05.

3. RESULTS

The effect of 8% w/w salt diet on serum glucose and lipid profile is presented in table 1. The result indicate significant (p<0.001) increase in the levels of serum glucose, total cholesterol, triglyceride, LDL-C and VLDL-C of the salt treated groups as compared with control group. The result also indicated significant (p<0.001) decrease in the level of HDL- C of the salt treated groups as compared with control group.

Table 1: Biochemical profile of salt- induced metabolic syndrome rat's before treatment.

Group	Glc (mg/dl)	TC (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
i-iii	153.30 $\pm$ 3.4 ^a	158.60 $\pm$ 9.7 ^a	160.60 $\pm$ 11.5 ^a	30.26 $\pm$ 0.4 ^a	96.22 $\pm$ 7.7 ^a	32.12 $\pm$ 0.3 ^a
Iv	86.40 $\pm$ 1.9	97.57 $\pm$ 1.9	98.83 $\pm$ 1.3	40.20 $\pm$ 0.6	37.60 $\pm$ 2.3	19.77 $\pm$ 0.2

Glc- glucose, TC- total cholesterol, TG- triglyceride, HDL-C- high density lipoprotein-cholesterol, LDL-C- low density lipoprotein- cholesterol, VLDL-C- very low density lipoprotein- cholesterol. Group I-III; are salt loaded and Group IV; normal untreated. Values are expressed Mean $\pm$ SD; n=8^aP< 0.001 when compared Group I-III with Group IV- control by Dunnett's multiple comparison test.

The effect of nutraceutical on lipid profile is presented in Table 2. The result indicated significant (P<0.001) decreased in the levels of total cholesterol, triglyceride, LDL-C and VLDL-C of the supplemented groups with nutraceutical as compared with salt-loaded non-supplemented. There was no significant (P>0.05) difference between groups supplemented with nutraceutical and control.

The result also show significant (P<0.001) increase in the levels of total cholesterol, triglyceride, LDL-C and VLDL-C of the salt-loaded non-supplemented group as compared with control.

The levels of HDL-C significantly ($P<0.001$) increased in the groups supplemented with nutraceutical as compared with salt-loaded non-supplemented groups. Significant ($P<0.05$) decrease was observed in the level of HDL-C of the salt-loaded non-supplemented group as compared with control.

Table 2: Effect of nutraceutical on lipid profiles in rats with metabolic syndrome

Groups	TC (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
I	106.0±7.3 ^{ac}	117.0±9.6 ^{ac}	42.0±3.4 ^{ac}	39.0±8.7 ^{ac}	24.0±1.9 ^{ac}
II	107.9±5.9 ^{bc}	114.0±4.8 ^{bc}	43.0±5.3 ^{bc}	42.0±1.4 ^{bc}	23.0±1.6 ^{bc}
III	182.0±15.8 ^{cd}	169.0±14.0 ^{cd}	30.5±4.4 ^{cd}	117.0±9.0 ^{cd}	34.3±2.9 ^{cd}
IV	102.4±4.9 ^d	110.5±9.6 ^d	42.8±4.6 ^d	37.7±10.2 ^d	22.8±1.9 ^d

TC- total cholesterol, TG- triglyceride, HDL-C- high density lipoprotein- cholesterol, LDL-C- low density lipoprotein- cholesterol, VLDL-C- very low density lipoprotein- cholesterol. Group I; salt loaded treated with 250mg/kg of nutraceutical, Group II; salt loaded treated with 500mg/kg of nutraceutical, Group III; salt-loaded untreated, Group IV; control. Values are expressed as Mean ±SD; n=8. ^a $P<0.001$ when compared with salt-loaded untreated, ^b $P>0.05$ when compared with control, ^c $P<0.001$ when compared with salt-loaded treated, ^d $P<0.05$ when compared with control by Tukey multiple comparison test.

The effect of supplementation with nutraceutical on serum glucose, insulin and insulin resistance in rats with metabolic syndrome is presented in Table 3. The result indicate significant ($P<0.001$) decrease in serum glucose of the supplemented groups as compared with salt-loaded non-supplemented groups. There was no significant ($P>0.05$) difference between supplemented groups and control. Significant ($P<0.001$) increase in serum glucose levels occurred in salt-loaded non-supplemented groups as compared with control. Significant ($P<0.001$) decrease in insulin levels and insulin resistance in supplemented groups occurred as compared with salt-loaded non-supplemented groups. There was no significant ($P>0.05$) difference between supplemented groups and control. Insulin levels and insulin resistance significantly ($P<0.001$) increase in salt-loaded non-supplemented groups as compared with control.

Table 3: Effect of supplementation with nutraceutical on serum glucose, insulin and insulin resistance in rats with metabolic syndrome.

Groups	Glucose (mmol/l)	Insulin (μU/ml)	HOMA-IR
I	5.43±0.1 ^{ac}	7.20±2.5 ^{ac}	1.74±0.0 ^{ac}
II	5.20±0.1 ^{bc}	6.50±0.3 ^{bc}	1.50±0.0 ^{bc}
III	8.01±0.1 ^{cd}	22.30±2.2 ^{cd}	7.94±0.0 ^{cd}
IV	5.33±0.1 ^d	6.70±1.2 ^d	1.59±0.0 ^d

HOMA-IR; Homeostasis Model Assessment- insulin Resistance. Group I; salt loaded treated with 250mg/kg of nutraceutical, Group II; salt loaded treated with 500mg/kg of nutraceutical, Group III; salt-loaded untreated, Group IV; control. Values are expressed as Mean ±SD; n=8. ^a $P<0.001$ when compared with salt-loaded untreated, ^b $P>0.05$ when compared with control, ^c $P<0.001$ when compared with salt-loaded treated, ^d $P<0.05$ when compared with control by Tukey multiple comparison test.

The effect of supplementation with nutraceutical on markers of oxidative stress in rats with metabolic syndrome is presented in Table 4. The result indicate significant ($P<0.001$) decrease in MDA of supplemented groups with nutraceutical as compared with salt-loaded non-supplemented groups. There was no significant ($P>0.05$) variation between supplemented groups with nutraceutical and control. Significant ($P<0.001$) increased in MDA level of salt-loaded non-supplemented groups as compared with control was observed. The result also indicate significant ($P<0.001$) increase in of supplemented groups with nutraceutical as compared with salt-loaded non-supplemented groups. There was no significant ($P>0.05$) difference between supplemented groups with nutraceutical and control group. The result also show significant ($P<0.001$) decrease in serum antioxidant enzymes and vitamin C, E of salt-loaded non-supplemented groups as compared with control.

Table 4: Effect of supplementation with nutraceutical on markers of oxidative stress in rats with metabolic syndrome.

Group	MDA nmol/mg	SOD u/ml	Catalase nmol/min/ml	GPX nmol/min/ml	Vit. E mg/dl	Vit. C mg/dl
I	0.3±0.1 ^{ac}	4.4±1.0 ^{ac}	7.1±0.7 ^{ac}	163.0±2.1 ^{ac}	0.7±0.1 ^{ac}	0.4±0.0 ^{ac}
II	0.4±0.0 ^{bc}	4.3±1.3 ^{bc}	14.1±2.9 ^{bc}	153.0±9.1 ^{bc}	0.9±0.2 ^{bc}	0.4±0.1 ^{bc}
III	2.4±0.4 ^{cd}	0.6±0.0 ^{cd}	2.5±0.0 ^{cd}	40.1±6.1 ^{cd}	0.2±0.0 ^{cd}	0.1±0.0 ^{cd}
IV	0.2±0.1 ^d	4.1±0.7 ^d	12.2±4.6 ^d	119.9±7.6 ^d	0.3±0 ^d	0.4±0.1 ^d

Group I; salt loaded treated with 250mg/kg of nutraceutical, Group II; salt loaded treated with 500mg/kg of nutraceutical, Group III; salt-loaded untreated, Group IV; control. Values are expressed as Mean± SD; n=8. ^a $P<0.001$ when compared with salt-loaded untreated, ^b $P>0.05$ when compared with control, ^c $P<0.001$ when compared with salt-loaded untreated with control, ^d $P<0.05$ when compared with control by Tukey multiple comparison test.

3.1 DISCUSSION

Metabolic syndrome (MS) is a condition involving obesity, dyslipidaemia, hypertension and diabetes mellitus as a consequence of the interaction between genetic and environmental factors [1]. MS is common worldwide, associated with target organ damage such as heart, kidney and brain [20]. Thus, development of therapeutic/management strategies that may delay the onset and prevent complications associated with MS is critical to improve the life of patients with MS.

Salt- loading to the rats for 6 weeks significantly ($P<0.001$) increase the hall marks of metabolic syndrome summarized as serum glucose, TC, TAG, LDL-C and VLDL-C. HDL-C was significantly ($P<0.001$) decreased. The mechanism of high salt diet-induced metabolic syndrome could be attributed to increase concentration of sodium in circulation which in turns activates sympathetic nervous system and renin-angiotensin-aldesterol-system (RAAS) [21] as well as increased signaling through the mineralocorticoid receptors (MR) [22]. These may lead to increase production of reactive oxygen species and oxidative stress, and finally contribute to aetiopathology of insulin resistance, impaired glucose homeostasis and dyslipidaemia [23]. Other possible mechanism is that salt diet is associated with the activation of adipokines (leptin, angiotensinogen, tumor necrosis factor α , transforming growth factor β and resistin etc) that may stimulate hepatic TAG synthesis, which in turned promotes the assembly and secretion of LDL, VLDL and reduction of HDL cholesterol [24]. Obesity, insulin resistance and diabetes may also be induced [25]; [26].

This present study found significant ($P<0.001$) decrease in serum Glu, TC, TAG, LDL-C, VLDL-C and significant ($P<0.001$) increase in HDL-C of all the rats supplemented with nutraceutical, compared with non-supplemented rats. This could be attributed to glycemic and hypolipidaemic effects of nutraceutical, in which the nutraceutical reduces LDL

cholesterol, particularly small dense LDL, which is associated with increased risk of atherosclerosis. The lowering effect of nutraceutical may be attributed to both inhibition of hepatic fatty acid synthesis and increase catabolism of TAG rich lipoproteins [27]. This increase in VLDL catabolism may results from up-regulation of lipoprotein lipase expression and increased lipoprotein lipase activity because of the reduction in serum apo C-III levels [28]. Increased in HDL cholesterol may be associated with increase expression of apo A-I and A-II genes [29] and all these mechanisms can influence insulin sensitivity in order to improve glucose homeostasis [30].

MDA is a reliable marker of lipid peroxidation and peroxidative tissue injury [31]. It has been shown to be elevated in salt induced experimental rats' model, suggesting that is a consequence rather than a cause of metabolic syndrome [32]. Significant decrease in MDA levels was observed in all rats supplemented with nutraceutical. This could be attributed to protective effects of nutraceutical against lipid peroxidation [33, 34]; [35]. Significant increase in MAD levels was observed in non- supplemented rats. This could be attributed to active lipid peroxidation which occurred in rats with metabolic syndrome as a consequence of the over production of ROS [32].

The significant increase in the levels of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase), vitamin C and E was observed in all rats supplemented with nutraceutical as compared with non- supplemented rats. This could be associated with the increase bioavailability of these micronutrients that are important components and co-factors of both antioxidant enzymes and vitamins [36].

Insulin resistance and hyperinsulinaemia are key factors in the development of metabolic syndrome [37]. Indeed, insulin resistance and hyperinsulinaemia are good predictors of metabolic syndrome [37]. Our findings indicated significant ($P<0.01$) decrease in insulin levels and insulin resistance in all the rats supplemented as compared with non-supplemented rats. Nutraceutical enhances tyrosine kinase activity on the insulin receptors and inhibit phosphotyrosine phosphatase (an enzyme that inactivates insulin receptors) in order to improve insulin sensitivity and peripheral glucose uptake [38] and this indicates the combine action of several micronutrients in the nutraceutical.

4. CONCLUSION

Antioxidant rich nutraceutical may have promising effect in the management of some of the traits of metabolic syndrome.

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