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NARINGENIN ALLEVIATED METABOLIC SYNDROME EXPRESIONS IN THE HIGH-FAT DIET INDUCED RATS

Uthukam Venkatesham[1], Lalitha Repudi[2*]

[1]Research Scholar, Department of Pharmacy, Chaitanya Deemed to be University, Himayathnagar, Moinabad, Ranga Reddy (Dist), Hyderabad, Telangana, India.
[2*]Associate Professor, Department of Pharmacy, Chaitanya Deemed to be University, Himayathnagar, Moinabad, Ranga Reddy (Dist), Hyderabad, Telangana, India. Email: repudilalitha@gmail.com

ABSTRACT

Aim. To assessing the metabolic syndrome marker expressions and therapeutic effect of flavonoid Naringenin (NGN) in rats.

Materials and Methods: In this study the wistar rats with diabetes mellitus, a combination of a high-fat diet (HFD) and a low dose of streptozotocin (STZ) at 40 mg/kg was used to induce the metabolic syndrome in rats, then estimated the metabolic syndrome marker protein expressions and treated with the flavonoid Naringenin 50, 100 mg/kg for six weeks.

Results. The STZ was used for the study to cause diabetes and the developed model of metabolic syndrome co-existing with diabetes mellitus mimicked several aspects of metabolic syndrome, including dyslipidemia (increased triglyceride, total cholesterol, LDL cholesterol, and decreased HDL cholesterol), diabetes mellitus (blood glucose, HbA1c, serum insulin, and C-peptide), when compared to the Normal group with the HFD-STZ group, atherogenic index, inflammation (hs-CRP), GLUT4, and Adiponectin, PPAR-γ, SREBP-1 expression and TNF- alpha and deterioration in hepatic and renal function, and also the histological evaluation of the HFD-STZ group's heart, pancreas, liver, and kidney revealed the presence of edema, necrosis and inflammations. Treatment with the flavonoid Naringenin 50, 100 mg/kg alone and metformin for six weeks drastically and significantly ($p<0.001$) reduced, all these conditions of the metabolic syndromes.

Conclusion. The current study concluded that there was a metabolic syndrome induced by HFD in rats and treatment with the flavonoid Naringenin and metformin for six weeks, which were significantly reduced the metabolic syndrome indicators, lipid content, and inflammation.

KEYWORDS: HFD, Metabolic Syndrome, Lipid profiles, GLUT4, Adiponectin, TNF- alpha, PPAR-γ Flavonoids, Naringenin (NGN) and Metformin

INTRODUCTION

Metabolic syndrome encompasses cluster of risk factors for cardiovascular disease which includes abdominal obesity, dyslipidemia, hypertension, and hyperglycemia[1]. The incidence of metabolic syndrome is on the rise globally [2].World Health Organization (WHO), International Diabetes Federation (IDF), and National Cholesterol of Adult Treatment Panel III (NCEPATPIII) have laid down specific criteria for metabolic syndrome. Later, these organizations jointly developed a new definition of metabolic syndrome known as “harmonized criteria” which included central obesity, raised blood pressure, elevated triglyceride levels, low high-density lipoprotein (HDL), and raised glucose levels. Metabolic syndrome increases the risk of developing type II diabetes by impeding the critical regulatory influence of insulin on glucose, lipid, and protein metabolism. Patients developing type II diabetes have often gone through a state of obesity associated with reduced insulin sensitivity along with an activated beta cell compensatory mechanism, such as excess basal insulin secretion and hyperinsulinemia, as a part of their metabolic profile [3–5]. Thus, metabolic syndrome and diabetes mellitus are interrelated metabolic disorders which may co-exist on several occasions.

Diabetes mellitus co-existing with metabolic syndrome has become a common predicament in society due to change in lifestyle and dietary habits. The number of diabetics with metabolic syndrome is substantial and the prevalence is increasing throughout the world [6, 7]. Efforts should be directed to treating both the diseases together as a whole rather than independently by finding better treatments and novel prevention strategies for type II diabetes co-existing with metabolic syndrome. These viable animal models should address all the aspects of this human disease, developing all major signs of diabetes as well as metabolic syndrome, especially obesity, dyslipidemia, hypertension, and possibly fatty liver disease and kidney dysfunction. The streptozotocin and the Alloxan models of chemically induced diabetes are commonly used to screen antidiabetic drugs [8, 9]. However, these methods cause marked destruction of the pancreatic mass and may therefore mimic changes closer to type I diabetes rather than type II diabetes mellitus. Recently, it has been reported that rats fed with High-Fat Diet and combination of streptozotocin developed type II diabetes more closely to humans. High-Fat Diet will cause insulin resistance in peripheral tissues due to lipotoxicity; meanwhile, low dose of streptozotocin will induce mild defect in insulin secretion [10]. Combination of High-Fat Diet with low dose streptozotocin model has therefore successfully mimicked natural progress of diabetes development as well as metabolic features in human type II diabetes [11-13].

Phytoconstituents are more effective with less toxic effects, widely used as traditional medicine since ancient times and play a crucial role in obesity-related dyslipidemia, hepatotoxicity, oxidative stress, lipolysis of lipid, and appetite management. Flavonoids are plant secondary metabolites widely spread in citrus fruits, vegetables, seeds, and nuts showing antioxidant properties via reducing lipid peroxidation levels. Naringenin is a flavone glycoside found in grapefruit; naringin, when consumed orally, it hydrolyzes into its aglycone naringenin Naringin is a potent flavonoid with enormous therapeutic efficacy: antioxidant, anti-obesity, hypolipidemic, and hepatoprotective properties that prevent lipid oxidation, oxidative stress, inhibit lipoxygenase, and LDL-C oxidation, which in turn impairs the differentiation of adipocytes, inhibits lipid accumulation, promotes lipolysis, and mitigates inflammation [14-16]. Aside, naringenin serves as a therapeutic potential agent in the cardioprotective [17-19], hepatoprotective [20,21], anti-cancer [22,23], anti-inflammatory [24], metabolic syndrome and anti-bone regeneration fields [25-28]. Nowadays high-fat diet (HFD) ingestion is increasing day by day with our unhealthy dietary practice, and for prolonged intake eventually leads to the hepatotoxicity by induction of NASH/ NAFLD. Therefore, we like to bring attention to this global epidemic non-communicable disease and highlight the promising treatment with the bioactive flavonoid naringin. The main objective of the current study is to investigate the efficacy of naringin as a potential hepatoprotective and hypolipidemic agent through antioxidant defense as well as SREBP-1C and AMPK signaling pathways in liver injury caused by chronic high-fat diet feeding in Wistar rats.

The present study was designed to develop a unique animal model that will mimic the pathological features seen in a large pool of individuals with long term diabetes and metabolic syndrome, suitable for pharmacological screening of drugs beneficial in this condition. Such a model should replicate the components of metabolic syndrome such as hyperlipidemia, hypertension, and obesity along with type II diabetes mellitus.

MATERIALS AND METHODS

Experimental animals of adult male wistar rats, 10 to 12 weeks old, weighing 150 to 200 gm, were used in the study. They were maintained under standard laboratory conditions in the animal house. The study protocol was approved by the Institutional Animal Ethics Committee and conforms to the Committee for the Control and Supervision of Experiments on Animals of IAEC VBES, Hanamkonda. Rats were kept in polyacrylic cages with not more than four animals per cage, housed in an air-conditioned room, and kept under natural light-dark cycles. The animals were allowed free access to standard diet or High-Fat Diet as the case may be and water ad libitum. The High-Fat Diet (HFD) was prepared indigenously in our laboratory by using normal pellet diet, raw cholesterol, and mixture of vanaspati ghee and coconut oil (2 : 1). Normal rat pellet diet was powdered by grinding and mixed with 2.5% cholesterol and mixture of vanaspati ghee and coconut oil (5%). The mixture was made into pellet form and put into freezer to solidify. In addition 2% raw cholesterol powder was mixed in coconut oil and administered to the rats by oral route (3 mL/kg).

The HFD along with 2% liquid cholesterol (3 mL/kg) was orally fed to rats for 3 weeks to induce metabolic syndrome. A pilot study was carried out with dose of STZ (40 mg/kg) in order to select the appropriate dose of STZ for induction of diabetes. Therefore, a single STZ injection (40 mg/kg body wt, i.p., dissolved in 0.01 M citrate buffer, pH 4.5) was standardized to induce diabetes mellitus. Serum glucose estimations (blood sugar >200 mg/dL) were undertaken periodically (days 0, 2,4 and 6 weeks) from the retroorbitol vein to confirm the production of diabetes mellitus. Metabolic Syndrome: After one week of dietary manipulation, rats were injected intraperitoneally with STZ (40 mg/kg). The body weight and biochemical parameters (blood glucose, total cholesterol) were estimated 7 days after the vehicle or STZ injection; that is, on 6 weeks of dietary manipulation in rats. The rats with blood glucose (>200 mg/dL), total cholesterol (>110 mg/dL), triglyceride (>150 mg/dL), change in body weight (8% of initial weight), systolic blood pressure (>130 mm Hg), and reduced HDL levels (<35 mg/dL) confirmed presence of metabolic syndrome with diabetes. Thereafter the rats were either fed normal diet or HFD as per the protocol for 6weeks. Blood samples were collected from the retroorbital plexus at 0, 2, 4, and 6 weeks for estimation of biochemical parameters.

Experimental Groups

Group 1: Normal Control (NC). In Normal Control group, rats were administered distilled water orally using a feeding cannula for study period of 6 weeks.

Group 2: High Fat Diabetic Control (HFD). The HFD were fed to rats for 6weeks to produce metabolic syndrome.

Group 3: High Fat Diabetic Control (HFD-STZ). The HFD were fed to rats for 6weeks to produce metabolic syndrome. At the end of 1 weeks diabetes was induced by a single STZ injection (40 mg/kg body wt, i.p., dissolved in 0.01 M citrate buffer, pH 4.5).

Group 4: HFD-STZ+ treated with Low dose of flavonoid Naringenin (NGN 50mg/kg) for 6weeks (po).

Group 5: HFD-STZ+ treated with High dose of flavonoid Naringenin (NGN 100mg/kg)for 6weeks (po).

Group 6: HFD-STZ+ treated with Metformin (60mg/kg)for 6weeks (po).

Biochemical parameters (metabolic, cardiac, liver, and kidney function markers): the rat blood samples of all experimental groups were collected from the retroorbital plexus for 6 weeks for estimation of blood glucose, TC, TG, and CPK-MB. In addition, after the completion of the experimental duration (6 weeks), serum was used for the determination of the parameters like lipid profile, serum insulin, C-peptide, creatinine, SGPT, and hs- CRP , GLUT4, and Adiponectin, PPAR-γ, SREBP-1 expression and TNF- alpha by autoanalyzer or ELISA kits in the Pathology and Histopathological study.

RESULTS

Standardization of HFD and Standardization of HFD and dose of STZ at 40 mg/kg to induce metabolic syndrome co-existing with diabetes mellitus was undertaken in the laboratory. The 40 mg/kg of STZ produced desired increase in blood glucose levels (>200mg/dl) in HFD fed and HFD-STZ rats that was maintained through the study period. The HFD fed and HFD-STZ groups showed significant ($p < 0.001$) increase in body weight on 2nd, 4th and 6th week as compared with Normal group rats. The highly increase in body weight of HFD and HFD-STZ group rats at the end of 6th week. Similarly, the weights of the HFD, HFD-STZ group rats were increased significantly only at 4th and 6th week as compared to the Normal rats at similar time points. The weight difference between Normal and HFD-STZ in baseline weight and 6th week weight is very less in Normal than HFD-STZ group. The results shows weight loss at at 2nd, 4th and 6th week in HFD, HFD-STZ groups After treatment with Naringenin (50, 100mg/kg and Metformin 60mg/kg) alone and combinations (Figure 1).

The blood glucose, total cholesterol, and triglycerides levels in the HFD,HFD-STZ group rats were significantly higher ($p < 0.001$) as compared to Normal group rats at 0, 2nd 4th , and 6th week. Glycosylated hemoglobin, total cholesterol, low-density lipoprotein, and atherogenic index were significantly increased in HFD-STZ groups as compared with Normal groups at the end of 6 weeks. HOMA-IR increased in HFD but the results shows reduced all biochemical parameters at 2nd, 4th and 6th week in HFD, HFD-STZ groups After treatment with Naringenin alone and combinations (50, 100mg/kg and Metformin 60mg/kg), while serum insulin and HOMA-β are significantly reduced in HFD-STZ group as compared with normal. High-density lipoprotein was significantly decreased in HFD-STZas compared with normal but increased the insulin, HDL-C levels at 2nd, 4th and 6th week in HFD, HFD-STZ groups After treatment with Naringenin (50, 100mg/kg and Metformin 60mg/kg). The C-peptide levels in HFD-STZ were increased though statistically not significant as compared to normal rats (Figures 2, Table 2).

There was a significant ($p < 0.001$) increase in serum CPK-MB levels in HFD-STZ rats at 6th week as compared with Normal. The other cardiac markers hs-CRP and systolic blood pressure were measured on 6th week of study and were found to be significantly raised ($p < 0.01$) in HFD, HFD-STZ groups as compared with Normal but The results shows reduced the CPK-MB, Hs-CRP at 2nd, 4th and 6th week in HFD, HFD-STZ groups After treatment with Naringenin (50, 100mg/kg and Metformin 60mg/kg) (Table 2). The HFD, HFD-STZ group rats showed a significant ($p < 0.01$) increase in the level of SGPT (U/L) and Serum creatinine (mg/dL) when compared to Normal group rats at 2nd, 4th and 6th week but the results shows reduced the SGPT, Serum creatinin at 2nd, 4th and 6th week in HFD, HFD-STZ groups After treatment with Naringenin (50, 100mg/kg and Metformin 60mg/kg) (Table 1). The histopathology of liver was observed that there is a inflammation, steatosis and vasodilatation effects of HFD, HFD-STZ groups compared to treatment groups (HFD-NGN low dose, HFD-NGN High dose, Metformin) and normal control (fig.4). Adipogenic genes PPAR-γ, SREBP-1 were significantly up regulated in the HFD, HDF-STZ group as compared to normal controls, these genes were markedly down regulated by Naringenin and Metformin treatment, especially in the high-dose group. In a similar obese animals had significantly higher levels of inflammatory mediators (TNF-α), treatment with Naringenin significally reduced these inflammatory genes' expression. On the other hand, in rats fed a high-fat diet, metabolic genes linked to fatty acid oxidation and energy homeostasis (GLUT4, and Adiponectin) were markedly down regulated. But Naringenin administration restored these genes' expression in a dose-dependent manner, with the high-dose group exhibiting levels that were comparable to those of the metformin-treated and normal control groups (Table-1).

Table1: Gene expression proteins in rats fed with HFD, HFD-STZ groups, treatment with Naringenin (50mg/kg, 100mg/kg), Metformin (60mg/kg) and Normal group.

Gene proteins	Normal	HFD	HFD-STZ	NGN-50mg/kg	NGN-100/kg	Metformin-60mg/kg
Adiponectin (ug/ml)	15.2	8.3	6.4	9.5	13.6	14.2
GLUT4 (ng/mL)	1.6	0.45	0..36	0.71	0.87	1.2
PPAR-γ	1.56	2.43	3.65	1.98	1.59	1.46
SREBP-1c (ng/mL)	0.062	2.89	3.69	2.11	1.58	0.92
TNF-α (ng/mL)	0.17	3.54	4.67	2.54	1.33	0.47

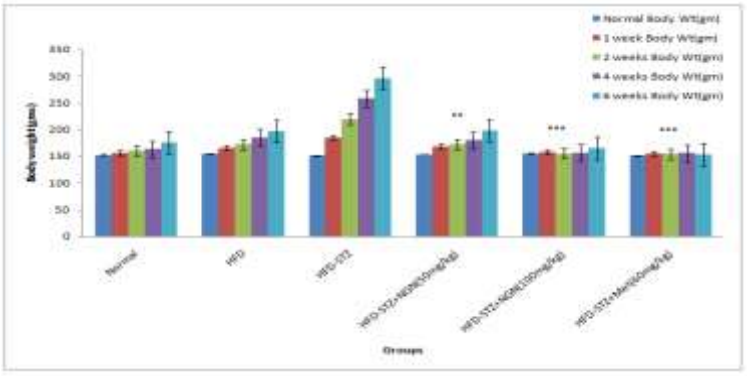

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Fig.1.Comparison of Body weights of rats with HFD, HFD-STZ groups and treatment with Naringenin (50mg/kg, 100mg/kg), Metformin (60mg/kg) and Normal group

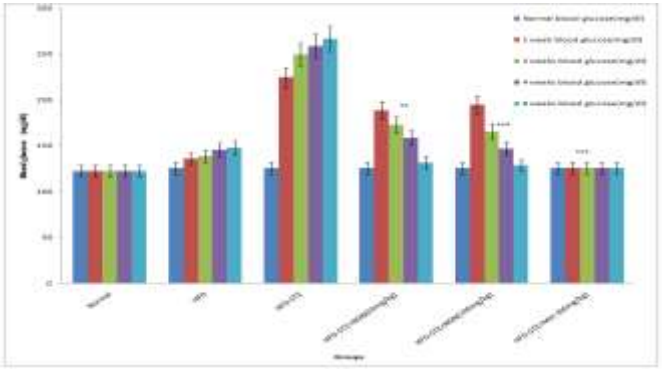

Fig.2.Comparison of blood glucose levels in rats with HFD, HFD-STZ groups and treatment with Naringenin (50mg/kg, 100mg/kg), Metformin (60mg/kg) and Normal group.

Table2: Biochemical parameters in rats with HFD, HFD-STZ groups and treatment with Naringenin (50mg/kg, 100mg/kg), Metformin (60mg/kg) and Normal group.

Parameters/Groups	Normal	HFD	HFD-STZ	HFD-STZ+ NGN (50mg/kg)	HFDSTZ+ NGN(100mg/kg)	HFD-STZ+Metf (60mg/kg)
TG(mg/dL)	112.75±11.4	312.8±62.2	373.2±32.1	197.75±44.23∗∗	152.53±32.11∗∗	132.33±22.16∗∗
HDL(mg/dL)	48.32±2.54	36.17±3.63	26.3±3.11	32.11±2.19∗∗	38.13±2.26∗∗	41.18±3.22∗∗
LDL(mg/dL)	60.6±2.3	128.76±15.1	162.1±22.5	87.32±13.24∗∗∗	68.32±11.14∗∗∗	62.12±16.19∗∗∗
HbA1c(%)	5.27±0.46	9.78±1.50	12.2±1.1∗	8.8±1.5∗∗∗	6.77±1.12∗∗∗	5.88±1.3∗∗∗
Insulin (μU/mL)	6.46±0.63	3.56±0.29∗	2.13±0.24	3.99±0.51∗	4.47±0.39∗∗	5.91±0.44∗∗
C-Peptide (ng/mL)	1.1±0.04	2.5±0.02	3.1±0.31	1.6±0.02.1∗	1.4±0.021∗∗∗	1.2±0.013∗∗∗
HOMA-IR	1.31±0.18	2.11±0.33	2.81±0.26	1.56±0.21∗	1.12±0.91	1.1±0.55
Atherogenic index	0.12±0.013	0.21±0.011	1.27±0.44	1.13±0.87∗	0.12±0.019∗∗∗	0.11±0.014∗∗∗
Serum Creatinine(mg/dl)	0.25±0.012	1.35±0.12	1.7±0.012	1.2±0.011∗∗	1.4±0.014∗∗∗	1.0±0.010
SGPT (7-57 U/L)	32.7±1.13	45.7±2.23	65.7±4.21	51.7±3.43∗	42.6±2.4∗∗∗	38.7±2.8∗∗∗
HS-CRP(mg/L) <1	0.96±0.06	1.2±0.017	4.3±0.022	2.6±0.08∗∗	1.6±0.041∗∗∗	1.21±0.05∗∗∗
CK-MB (ng/ml)	2.5±0.019	7.4±0.052	12.5±0.1.1	6.1±0.042∗∗	4.9±0.021∗∗∗	3.5±0.031∗∗∗

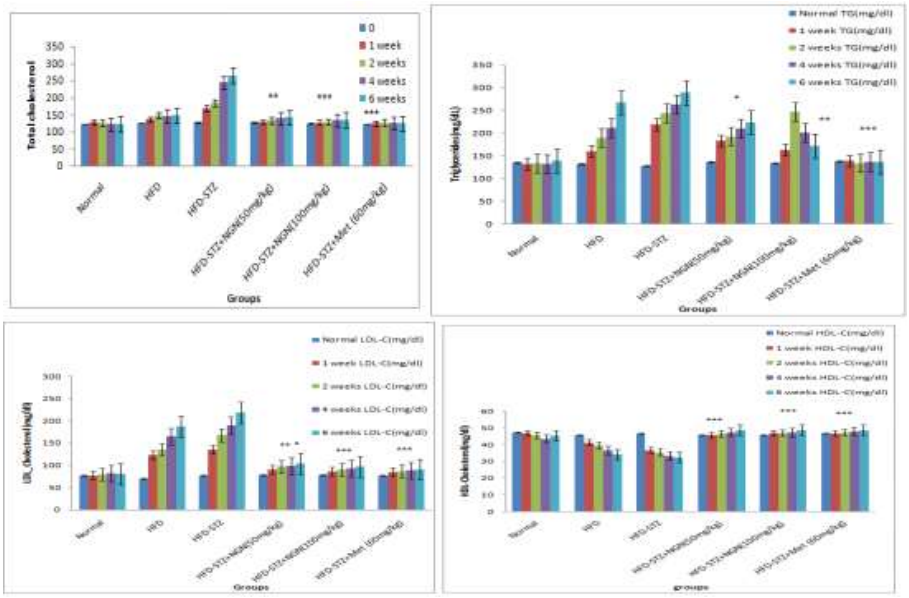

Fig.3.Comparison of lipid profiles levels in rats with HFD, HFD-STZ groups and treatment with Naringenin (50mg/kg, 100mg/kg), Metformin (60mg/kg) and Normal group.

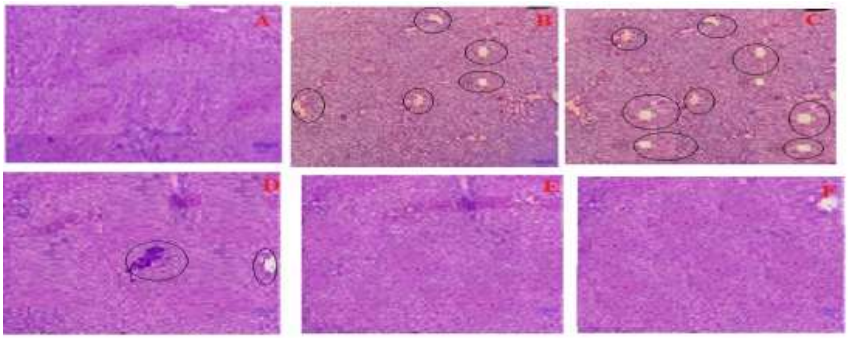

Fig. 4. The histological alterations in liver (100x) in different groups. A (CON), B (HFD) and C (HFD+STZ) D (HDF+NGN LD) E (HDF+NGN HD) F(HDF+MET) were examined under 100xmagnification. HFD,HDF-STZ groups showed Inflammatory cells (IC), steatosis (S), massive dilation of sinusoids (SD) than other four groups.

DISCUSSION

Metabolic syndrome includes central obesity, insulin resistance, elevated blood pressure, impaired glucose tolerance, and dyslipidemia. The number of adults with metabolic syndrome is substantial and the prevalence is increasing throughout the world. In the Indian subcontinent, 45% of males and 38% of females are diagnosed with metabolic syndrome. Majority of individuals diagnosed as metabolic syndrome are also diabetics. With the increase in proportion of such individuals with both the disease conditions co-existing, diabetes and metabolic syndrome need to be addressed not as independent diseases or separate entities but as a unique disease combination that requires urgent attention. There are several animal models of diabetes as well as metabolic syndrome. However, there is no experimental model where both diabetes and metabolic syndrome co-exist. Hyperglycemia, hypertension, hyperlipidemia, and low grade inflammation confer combined architecture of metabolic derangements which may initiate changes in heart, pancreas, liver, and kidney. It is therefore essential to establish models to target all these risk factors for the treatment and reduction of clustering factors of diabetes with metabolic syndrome as such unique pathogenesis cannot be adequately studied in either the diabetes or metabolic syndrome animal models alone. In the search to combat these risk factors together, efforts were directed to develop a suitable animal model that would mimic all the symptoms of human metabolic syndrome as well as diabetes to screen the potential target compounds [30].

Metabolic syndrome includes central obesity, insulin resistance, elevated blood pressure, impaired glucose tolerance, and dyslipidemia. Patients of metabolic syndrome may not have overt diabetes. However the objective of the present study was to develop an animal model which was essentially diabetic and in addition should possess the other components of metabolic syndrome such as dyslipidemia, central obesity, and hypertension. The present study standardized different doses of STZ (30, 35, and 40 mg/kg) to be used for induction of diabetes after HFD was fed to the experimental rats. Diabetes co-existing with metabolic syndrome was successfully established with 40 mg/kg dose of STZ in the present study.

Anthropometric Parameters. Various anthropometric parameters such as body weight, were evaluated in the healthy normal control and High Fat Diabetic (HFD-STZ) groups. The HFD-STZ group showed significant ($p < 0.001$) increase in body weight on 2nd 4th and 6th week as compared with Normal group rats.

The increase in body weight of HFD-STZ group rats was sustained till the end of 6th week. Similarly, the AC and TC of the HFD-STZ group rats also increased significantly at 4th and 6th week as compared to the Normal rats at similar time points.

These anthropometric findings are in contrast to other study results in published literature. A previous study by Novelli et al. [31] showed an increase in all anthropometric parameters till the end of the study duration in rats induced with metabolic syndrome.

The difference observed in the anthropometric results may be attributed to the diabetic state that is known to cause weight as shown by Kuate et al. [30]. Therefore this unique model of metabolic syndrome co-existing with diabetes would differ in the anthropometric results which are typically seen in various models of metabolic syndrome.

The present study evaluated several metabolic parameters such as blood glucose, glycosylated hemoglobin (HbA1c), decreased serum insulin, and C-Peptide.

The blood glucose levels in the HFD-STZgroup rats were significantly higher as compared to Normal group rats at 2nd, 4th and 6th week. Results were coinciding with the observations made in various animal models of metabolic syndrome as overt diabetes is an essential requirement for this syndrome. Kuate et al.

The previous study by Mansor et al. [32] and Wang et al. [33] has studied the diabetes related changes 3-4 weeks subsequent to streptozotocin administration. However in the present study, long term effects of diabetes, that is, 6 weeks after streptozocin administration, were studied. It may be hypothesized that the metabolic changes of diabetes are manifested after a longer duration. Therefore the present model is more suitable to study the metabolic syndrome in the setting of diabetes. In addition to hyperglycemia, the present study results also found poor glycemic control as indicated by increased HbA1c levels in HFD-STZ group as compared with Normal. Kehkashan and Waseem [34] also determined HbA1c in diabetic rats and showed elevated glycosylated hemoglobin levels, similar to present study results.

However hyperglycemia and glycemic control have been established so far in an animal model of metabolic syndrome. A deficiency of insulin and a decline in pancreatic function were evidenced by a reduced level of serum insulin, C-peptide, and HOMA-β, respectively, in HFD-STZ group as compared to Normal group rats. The HOMA-IR was raised in HFD-STZgroup as compared with Normal at the end of the study though the results were statistically significant. The C-peptide determined by El-Sheikh [35] and Kamal et al. [36] showed reduced level in diabetic rats similar to present study. Results showed reduced level of serum insulin as seen in present study. These results concur with the various models of metabolic syndrome induced with High- Fat Diet. In contrast to our results, Shahraki et al. [37] demonstrated that the High-Fat Diet causes increase in the insulin levels as it causes insulin resistance.

However, in the present study, administration of STZ causes reduced level of Insulin secretion because it damages the pancreas. Thus, deficiency of insulin rather than insulin resistance was the hallmark of this animal model developed to study metabolic syndrome in the setting of diabetes mellitus. Thus, the biochemical results showed increase in blood glucose with concomitant decrease in insulin and the pancreas HFD-STZ group of rats demonstrated damaged islets of Langerhans, atrophy of beta cells, and reduced beta cell mass as compared to Normal.

Insulin showed increased secretion of insulin in the Normal as compared to HFD-STZgroup. This suggests that those beta cells are functional in the normal group, secreting insulin as compared to HFD-STZ group. HFD-STZ caused beta cell dysfunction and loss of beta cell mass resulting in decreased insulin. The triglycerides and cholesterol levels in the HFD-STZ group rats were significantly higher as compared to Normal group rats at 2nd,4th and 6th week. Dyslipidemia is a hallmark of metabolic syndrome. These results concur with studies undertaken.

The present study also determined total cholesterol (TC), low-density lipoprotein (LDL), Triglycerides(TG) and high-density lipoprotein (HDL) at the end of the 6th week., Abnormal lipid profile as reflected by raised LDL, TC, TG and reduced HDL levels was observed in the HFD, HFD-STZ group as compared with Normal group, Naringenin and Metformin treated groups. Individuals with metabolic syndrome and diabetes have a twofold elevated risk of having a heart attack or stroke. Thus, it is critical that the cardiovascular complication of metabolic syndrome and diabetes are also replicated and HFD-STZ suggesting that the HFD-STZ rats are more prone to developing coronary artery diseases. CPK-MB, an enzyme found primarily in the myocardium, is widely used to evaluate the existence and extent of myocyte injury [38-40].

The present study determined the CPK-MB levels increased to confirm the myocardial injury induced by High-Fat Diet and HFD-STZ in rats. The CPK-MB levels reduced significantly at 4th and 6th week in the HFD, HFD-STZ groups as compared to Normal group, Naringenin and Metformin treated groups. Similar findings Renna et al. [41] also found raised hs-CRP in fructose fed hypertensive rats as compared with normal rats; similar results are shown by present study. The other cardiac markers hs-CRP and systolic blood pressure were measured at 6th week of study and were found to be significantly raised in HFD, HFD-STZ groups as compared with Normal, Naringenin and Metformin treated groups. Adipogenic genes PPAR-γ, SREBP-1 and TNF-α were up regulated in the HFD, HDF-STZ group as compared to normal controls, these genes were markedly down regulated by Naringenin and Metformin treatment, On the other hand, in rats fed a high-fat diet, metabolic genes linked to fatty acid oxidation and energy homeostasis (GLUT4, and Adiponectin) were markedly down regulated. Naringenin administration restored these genes' expression in a dose-dependent manner, comparable to those of the metformin-treated and normal control groups [42-43].

This study aimed at evaluating the potential therapeutic action of the Naringenin alone and Metformin in experimentally induced obese and type 2 diabetic rats (T2DM) with characteristic metabolic syndrome (MetS). As rats were fed high-fat diet, for 6 weeks; subsequently STZ (40 mg/kg) was administered to produce diabetes. The test drug Narigenin(50mg,100/kg) was administered for 6 weeks. The experimental parameters were evaluated 5 weeks after induction of diabetes. Absolute insulin deficiency (significant fall in serum insulin levels) in the HFD, HFD-diabetic

groups as compared to normal control group rats was observed in the present model but elevated insulin in 6 weeks of Naringenin and Metformin treated rats against HFD, HFD-STZ groups. The biochemical results showed increase in blood glucose with concomitant decrease in insulin. The pancreas of HFD, HFD-STZ groups of rats demonstrated damaged islets of Langerhans, atrophy of beta cells, and reduced beta cell mass as compared to Normal. It may be hypothesized that the beta cell mass was decreased in the HFD, HFD-STZ groups rats resulting in decrease in secretion of insulin and C-peptide because of necrosis of pancreatic cell mass but controlled the metabolic syndrome in 6 weeks treatment of Naringenin and Metformin treated rats against HFD, HFD-STZ groups. Therefore, studying the inflammatory changes, subsequent to diabetes as well as metabolic syndrome as undertaken in the present model has major clinical implications and 6 weeks tread with Naringenin and Metformin were controlled the atherogenic index in rats of HFD, HFD-STZ groups.

CONCLUSION

Thus, the present study has been concluding to develop a rodent model of metabolic syndrome in the setting of diabetes mellitus and the comorbidities, the present study has specific attributes of dyslipidemia, obesity, hypertension and diabetes that makes them useful for the mechanisms and potential therapies of metabolic syndrome in the setting of diabetes and controlled the metabolic syndrome in 6 weeks treatment with Naringenin and Metformin rats against HFD, HFD-STZ groups.

Conflict Of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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