Antidiabetic properties of GTF – 231, an ayurvedic formulation studied in high fat diet fed-low dose STZ induced experimental type 2 diabetes in rats

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ABSTRACT

Diabetes Mellitus (DM) is a multidimensional, multisystemic, metabolic disorder arises due to the deficiency (Type 1) or efficiency (Type 2) of insulin, a hormone synthesized by the pancreatic β cells. DM is recognized by persistent elevation in fasting as well as post prandial blood glucose levels. Though, drugs are plenty for the treatment of diabetes, none is found to be an ideal due to undesirable side effects and diminution associated with prolonged treatment. Further, excessive generations of free radicals have also been implicated in the onset and progression of diabetes and its secondary complications. Hence, search for novel drugs without side effects preferably from the plant origin continues. In the present study, an attempt has been made to scientifically evaluate the antidiabetic properties of GTF – 231, an ayurvedic formulation in high fat diet-low dose streptozotocin induced experimental type 2 diabetes in rats. GTF 231 contains plant secondary metabolites such as Gymnemic acid, Trigonelline and Ferulic acid in the ratio of 2: 3: 1. Oral glucose tolerance test (OGTT) was performed to study the effect of the GTF-231 on glucose homeostasis. Insulin tolerance test (ITT) was performed to evaluate the effect of GTF-231 on insulin sensitivity. Insulin resistance was also measured by HOMA-IR. Oral administration of GTF-231(300mg/kg.b.w./rat/day) for a period of 30 days significantly improved the glucose homeostasis in diabetic rats which is evidenced from the results of OGTT, ITT and HOMA-IR. Further, the treatment significantly improved the altered levels of fasting blood glucose, glycosylated hemoglobin (HbA1c), protein, urea, uric acid and creatinine. The altered activities of liver enzyme markers such as AST, ALT and ALP observed in the diabetic rats were reverted back to near normal after treatment with GTF-231. The altered activities of carbohydrate metabolizing enzymes such as glucokinase, pyruvate kinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase in hepatic tissues of diabetic rats were significantly reverted to near normalcy upon treatment with GTF-231. The efficacy of the drug was comparable with metformin, a standard drug widely used for the treatment of diabetes. In conclusion, the data obtained clearly established that the ayurvedic formulation has significant antidiabetic activity which in turn may be due to the synergetic effect of the individual phytochemicals present in the formulation.

Keywords: Diabetes mellitus, High fat diet, Streptozotocin, GTF formulation, Metformin.

INTRODUCTION

Diabetes Mellitus (DM) is a multidimensional, multisystemic metabolic disorder characterized by altered glucose homeostasis, due to a progressive decline in insulin secretion and its action leading to discomposure in the carbohydrate, protein and lipid metabolisms [1]. The global prevalence of DM is escalating as a result of increased ageing population, urbanization and associated lifestyle changes [2, 3]. According to an estimate, one person is detected with diabetes every 5 seconds somewhere in the World; while someone dies of it every 10 seconds. DM has attained a pandemic form of disease [4]. Hence, it is extremely imperative to control the prevalence of diabetes and its complications to alleviate the human distress. Researchers are excitedly trying to manage this crippling disorder. Plant based products provide various beneficial and pharmacological effects due to its abundant source of bioactive phytochemicals [5]. Various herbal remedies are used in traditional systems of medicine prevailing around the
world, although only some of them have been scientifically evaluated for their toxicity and efficacy. Combination therapies are employed for the treatment of various critical diseases, such as cancer, acquired immunodeficiency syndrome and pulmonary tuberculosis, in order to achieve enhanced therapeutic effects [6].

GA is the main constituent present in the leaves of Gymnema sylvestre (Asclepiadaceae). It is as low growing, perennial, medicinal woody climber found in central and peninsular India. Its leaves are commonly known as “Gurmar” in India which means ‘sugar destroying’[7] and the leaves are traditionally used for the treatment of diabetes mellitus [8-11].

Fenugreek seeds contain significant amounts of trigonelline (1-methylpyridin-1-ium-3-carboxylate) and most of the pharmacological properties of fenugreek are attributed to the presence of trigonelline [12-14]. Trigonelline is a metabolite of niacin in humans, which is a component of vitamin supplements and is used for its antihyperlipidemic and hypocholesterolemic activity [15].

Ferulic acid ((2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enolic acid) is a phenolic acid present in asafoetida which exhibits numerous pharmacological activities such as an anticancer, antioxidant and anti-inflammatory properties. Ferula asafoetida belongs to family Umbelliferae. It is a tall perennial plant which grows up to 2 m and requires moist soil. The dried latex, an oleo-gum-resin, known as asafoetida is obtained by making deep incision in the roots and rhizomes; and is widely preferred for culinary purpose as well as for medicinal use. It is found to be high concentrations in foods such as navy bean, corn bran, wheat bran, eggplant, artichokes and beets [16, 17].

Since, DM is a multifactorial and multisystemic metabolic disorder, the current trend in diabetes treatment is towards combinatorial therapy. Some ingredients are more powerful in combination than alone due to its synergistic effects. Hence, in the present study, an attempt has been made to study the effect of GTF-231 in HFD low dose STZ induced experimental type 2 diabetes in rats.

**MATERIALS AND METHODS**

**Chemicals**
Gymnemic acid, Trigonelline, Ferulic acid and Streptozotocin, were procured from Sigma Aldrich, stored at 2-4°C and protected from light. All other chemicals used were purchased from standard commercial suppliers and were of analytical grade quality.

**Animals**
Male albino rats of Wistar strain weighing about 160–180 g were procured from Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai. The rats were housed in spacious polypropylene cages lined with husk. The experimental rats were maintained in a controlled environment (12:12 ± 1 h light/dark cycle; temperature 22°C ± 3°C; relative humidity 55%). Animals were acclimatized to standard husbandry conditions for one week to eliminate the effect of stress prior to initiation of the experiments. The rats were fed with commercial pellet rat chow (Hindustan Lever Ltd., Bangalore, India), and had free access to water *ad libitum*. The experiments were designed and conducted in strict accordance with the current ethical norms approved by Ministry of Social justices and Empowerment, Government of India and Institutional Animal Ethical Committee guidelines approval (IAEC No.03/01/2014).

**High fat diet fed streptozotocin induced diabetes**
The rats were allocated into two dietary regimens by feeding either normal pellet diet (NPD) or high fat diet (HFD) for 2 weeks of dietary manipulation. The composition of HFD is powdered NPD – 365g/kg, Lard – 310 g/kg, Caseine – 250g/kg, cholesterol – 10g/kg, vitamin and mineral mix – 60g/kg, DL-methionine – 3g/kg, Yeast powder – 1g/kg, NaCl – 1g/kg [18, 19]. After 2 weeks of HFD, the Group II, Group III and Group IV rats were injected with a single dose of STZ (35 mg/kg b.w./rat), while the Group I rats fed with NPD was injected with 0.5 ml of freshly prepared cold citrate buffer (pH 4.5) in a same volume intraperitoneally. After one week of STZ injection, rats with fasting blood glucose levels ≥ 300 mg/dL were considered as diabetic and chosen for further studies. The animals were divided into four groups, each comprising of a minimum of six rats as follows:

**Experimental animals design**
The animals were divided into four groups, comprising a minimum of six animals in each group as follows:

- **Group 1** – Control rats.
- **Group 2** – HFD+STZ (i.p. 35mg/kg b.w.) induced rats.
- **Group 3** – Gymnemic acid, Trigonelline and Ferulic acid (GTF-2:3:1; 300 mg/kg b.w/rat/day) treated diabetic rats
- **Group 4** – Diabetic rats treated with metformin (200 mg/ kg b.w/rat/day) in aqueous solution orally for 30 days.
At the end of 30 days experimental period, rats were fasted overnight, anaesthetized, using ketamine (80 mg/kg b.w./rat, i.p.) and sacrificed by cervical decapitation. Blood was collected with and without anticoagulant for plasma and serum separation respectively.

**Oral glucose tolerance test (OGTT)**

Overnight fasted rats of all groups were subjected to oral glucose tolerance test on the last week of the experimental period. The blood glucose levels were monitored at 0, 30, 60, 90 and 120 min using One Touch glucometer (Life scan, Johnson and Johnson Company) after oral administration of 2 g/kg b.w. glucose as aqueous solution [20].

**Insulin tolerance test (ITT)**

At the end of the experimental period, rats were fasted for 6 h and injected with Insulin (0.75 IU/kg, ip) and then blood samples were collected at 0, 30, 60, and 120 minutes for the measurement of plasma glucose [21]. The values are presented as a percentage of initial plasma glucose level.

**Determination of homeostasis model of insulin assessment**

As the insulin abnormality cannot be accurately detected by a single determination of insulin or glucose levels, the insulin resistance was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR) Matthews et al., 1985 [22] as follows

\[
\text{HOMA-IR} = \frac{\text{Fasting insulin level (µU/mL)} \times \text{Fasting blood glucose (mg/dl)}}{405}
\]

**Biochemical parameters**

Fasting blood glucose, glycosylated hemoglobin, plasma protein, blood urea, uric acid and serum creatinine levels were estimated [23-28]. Plasma insulin level was assayed using ELISA kit (LincoResearch, St Charles, MO, USA) for rat insulin assay. The presence of urine sugar was detected using urine strips (Diastix). The activities of pathological marker enzymes such as Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) in serum were assayed [29, 30].

**Assay of key enzymes of carbohydrate metabolism**

A portion of the liver tissue was dissected out, washed immediately with ice-cold saline and was homogenized in 0.1M Tris–HCl buffer (pH 7.4) for the assay of key enzymes of carbohydrate metabolism. The homogenate was centrifuged at 10,000 rpm to remove the debris and the supernatant was used as the enzyme source for the assay of glucokinase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, glucose-6-phosphate dehydrogenase, glycogen synthase, glycogen phosphorylase[31-38] Another portion of wet liver tissue was used for the estimation of glycogen content [39].

**Statistical analysis**

The results were expressed as mean ± S.E.M of six rats per group and statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS (version 16) program followed by LSD. Values were considered statistically significant when p < 0.05.

**RESULTS AND DISCUSSION**

HFD fed-low dose STZ induced type 2 experimental diabetic rats involves a combination of a diet rich in fat to bring about hyperinsulinemia, insulin resistance and/or glucose intolerance followed by treatment with the β-cell toxin STZ, which results in a severe reduction in functional β-cell mass. Simultaneously, these two stressors are designed to mimic the pathology of type 2 diabetes in shorter timescale than found in the human condition[40, 41]. The effect of oral administration of GTF-231 on OGTT in control and experimental groups of rats is presented in Figure 1. The effect of GTF as well as metformin treatment on the levels of fasting blood glucose in the experimental groups of rats receiving an intraperitoneal insulin challenge is shown in Figure 2. The blood glucose level is significantly reduced in diabetic rats treated with GTF-231 as well as metformin.

In control rats, the blood glucose level reached the maximum peak at 60 min after an oral glucose load and it was gradually reverted back to near normal level at 120 min indicating the maintenance of normal glucose homeostasis. On the other hand, the blood glucose levels in HFD-STZ induced diabetic rats reached the maximum peak at 60 min and remained unsubsidized over the next 60 min. Oral treatment with GTF-231 as well as metformin resulted in a significant decrease in fasting 30 and 60 min compared with untreated diabetic rats. In addition, the blood glucose levels returned to basal level at 120 min after the oral glucose load in GTF-231 and metformin treated diabetic group of rats indicating the maintenance of normoglycemia in GTF treated rats.
OGTT, a test of immense value in favor of using fasting plasma glucose concentration was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes. Chronic hyperglycemia is an important factor in the development and progression of complications of diabetes mellitus [42].

Like OGTT, ITT monitor glucose concentration over time, but in response to a bolus of insulin rather than of glucose. Glucose concentration is monitored every 15 to 30 minutes for 60 to 90 minutes following a bolus of insulin administered via intraperitoneal injection. The degree to which glucose level falls following the insulin bolus is an indicative of whole body insulin action.

HOMA-IR of normal, diabetic and diabetic treated with GTF-231 is depicted in Figure 3. Diabetic rats showed a significant elevation of HOMA-IR that was decreased significantly upon the administration of GTF-231. Insulin resistance is a characteristic metabolic defect that precedes overt β cell dysfunction and is primarily associated with resistance to insulin-mediated glucose disposal at the periphery and compensatory hyperinsulinemia. HOMA-IR has proved to be a robust tool for the surrogate assessment of insulin resistance[43]. As a more convenient method to measure insulin resistance, the homeostasis model assessment of insulin resistance was developed and widely used in clinical and epidemiologic studies [44, 45].

The effect of GTF-231 on the levels of fasting blood glucose, glycosylated hemoglobin, and plasma insulin and urine sugar in HFD-STZ diabetic rats is depicted in Table 1. The levels of fasting blood glucose and HbA1c was found to be significantly elevated in diabetic rats as compared with normal control. Oral administration of GTF-231 to diabetic rats significantly improved the altered levels of FBG as well as HbA1c. The levels of plasma insulin were moderately decreased in HFD-STZ induced diabetic rats. Diabetic rats treated with GTF-231 as well as metformin showed improved insulin level. Urine sugar which was present in the diabetic group of rats was found to be absent in GTF-231 as well as metformin treated groups of rats.

Blood glucose is a reliable index for diagnosis and prognosis of diabetes mellitus. During diabetes, the blood glucose levels are considerably increased which results from reduced glucose utilization by various tissues, which is the typical condition of insulinopenic[46]. Increased secretion of insulin causes increase in the utilization of glucose from extra hepatic tissue that decreases the blood glucose level [47]. Elevations in plasma insulin levels are closely correlated with increases in degree of resistance to insulin mediated glucose uptake [48].

The non-enzymatic, irreversible covalent bonding of excessive glucose with hemoglobin in circulation results in the formation of HbA1C which is a critical parameter for assessing long term glycaemic control and predicting the incidence of diabetic complications [49]. HbA1c was introduced into clinical use in the 1980s and subsequently has become a cornerstone of clinical practice [50]. HbA1c reflects average plasma glucose maintained over the previous 8 to 12 weeks [51]. Glycosylated hemoglobin remains in the circulation for the rest of the lifetime of erythrocytes (120 days). For these reasons the determination of glycosylated hemoglobin has become an important reliable tool in the diagnosis and prognosis of diabetes mellitus.

The formation of HbA1c is a two step process in which glucose initially binds with the N-terminal valine of a β-chain to form an unstable aldimine which subsequently undergoes an Amadori rearrangement to form a stable ketoamine. The first reaction is rapid and reversible while the second is irreversible. There is rapid formation and dissociation of the aldimine dependent upon the ambient glucose concentration and a slower rate of ketoamine formation. The observed decrease in the levels of HbA1c in the GTF-231 treated rats indicates the maintenance of normoglycemia in diabetic rats.

The renal threshold for glucose is of great interest to physiologists in relation to kidney function and of equal importance to clinicians in the study and treatment of glycosuria in diabetes. Below renal threshold level of glucose, sugar is absent in the urine, while above this level, appreciable amounts of sugar appear in the urine and gradually increase both in total amount and in percentage concentration as the blood sugar becomes progressively higher. The appearance of glucose in urine is reflected in the concept of a renal threshold for glucose excretion. The concept of a renal threshold for glucose excretion is propagated with the threshold specified at ~10 mmol/L [52].

The effect of GTF-231 on the levels of plasma protein, blood urea, serum uric acid and serum creatinine in control and experimental groups of rats are presented in Table 2. The level of total protein was found to be decreased in STZ induced diabetic rats. The levels of blood urea, serum uric acid and serum creatinine were found to be elevated in STZ induced diabetic rats. These biochemical markers were reverted back to normalcy upon the oral administration of the GTF-231.
A decline in serum protein level in diabetic has been attributed to inhibition of oxidative phosphorylation which leads to decrease in protein synthesis, increase in catabolic processes and reduction of protein absorption [53]. Urea and creatinine are nitrogenous end products of metabolism. Urea is the primary metabolite derived from dietary protein and tissue protein turnover. Creatinine is the product of muscle creatine catabolism. It is produced in the muscles by the non-enzymatic changes of creatine and phosphocreatinine. The liver has a crucial role in the assembly of creatinine through methylation of guanidine aminoacetic acid [54]. Urea is an organic compound, playing a vital role in the metabolism of nitrogen-containing compounds [55].

Accumulation of purines is the main source for the production of uric acid by the activity of xanthine oxidase. This accumulated purines evidence the increased oxidative stress which is closely related to diabetes and its vascular complications. Thus, the elevated levels of circulating uric acid level may be an indicator that the body is trying to protect itself from the deleterious effects of free radicals by increasing the products of endogenous antioxidants, such as uric acid [56].

Figure 4 depicts the effect of GTF-231 on AST, ALT and ALP in the serum of control and experimental groups of rats. Activities of marker enzymes such as AST, ALT and ALP in the plasma can be used to indirectly assess the integrity of liver tissue and extent of damage after being exposed to certain pharmacological agents such as STZ and Alloxan. These enzymes are usually liver markers whose plasma concentration above homeostatic limit could be associated with various forms of disorders which affect the functional integrity of the liver tissue [57].

Liver ALP is mobilized most rapidly into blood and its levels in plasma may increase at early periods of liver damage. Increase in the levels of ALP in diabetes may be as a result of leaking out from the tissue into the blood stream as a result of the adverse effect in the liver [58]. The elevated activities of these physiological enzymes were restored to near normal levels in GTF-231 as well as metformin treated diabetic rats. It revealed that, GTF-231 treated diabetic rats showed the tissue protective and non-toxic nature of the compound.

Table 3 represents the levels of glycogen content and activities of glycogen synthase and glycogen phosphorylase in liver tissues control and experimental groups of rats. The levels of glycogen content and glycogen synthase were reduced in diabetic rats whereas treatment with GTF-231 as well as metformin to diabetic groups of rats restored the glycogen level in liver tissues. Elevated activity of glycogen phosphorylase is reverted back to the normal level in GTF-231 as well as metformin treated diabetic groups of rats.

Table 4 depicts the effect of extract on the activities of hexokinase, pyruvate kinase and lactate dehydrogenase in liver tissue of experimental groups of rats. The activities of hexokinase and pyruvate kinase were significantly diminished in liver tissue of streptozotocin induced diabetic rats. The effect of lactate dehydrogenase in liver of experimental groups of rats was increased whereas after oral treatment of GTF-231 revealed back to the normalcy. Oral administration of GTF-231 to diabetic rats altered the activities of these enzymes to near normalcy in liver tissue similar to metformin treated rats.

Table 5 depicts the activities of glucose-6-phosphatase, fructose-1, 6-bisphosphatase and glucose-6-phosphate dehydrogenase in liver tissue of control and experimental groups of rats. The liver tissue of diabetic rats showed a significant elevation in the activities of glucose-6-phosphatase, fructose-1, 6-bisphosphatase and glucose-6-phosphate dehydrogenase. The altered activities of these enzymes were reverted to near normalcy by treatment with GTF-231 as well metformin as in diabetic groups of rats.

Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances by altering carbohydrate-metabolizing enzymes and leads to diabetes [59].

Glycogen is the primary intracellular storage form of glucose and its level in various tissue are a direct reflection of insulin activity promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since STZ causes selective destruction of β-cells of pancreas resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues decrease as they depend on insulin for influx of glucose [60]. In general, increased hepatic glucose production, decreased hepatic glycogen synthesis and glycolysis, are the major symptoms of type 2 diabetes that result in hyperglycemia [61].

Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes. One such enzyme is hexokinase that catalyses the conversion of glucose to glucose-6-phosphate and plays a central role in the maintenance of glucose
homeostasis [62]. In the liver, hexokinase is an important regulatory enzyme in the oxidation of glucose [63]. Being an insulin-dependent enzyme, the hepatic hexokinase activity of diabetic rats is almost entirely inhibited or inactivated due to the absence of insulin [64]. This impairment results in a marked reduction in the rate of glucose oxidation via glycolysis, which ultimately leads to hyperglycemia. Oral administration of GTF-231 to HFD-STZ induced diabetic rats resulted in a significant reversal in the activity of hexokinase, thereby increased the oxidation of glucose.

Glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyses the ultimate biochemical reaction of both glycogenolysis and gluconeogenesis. Fructose-1, 6-bisphosphatase is one of the key enzymes of gluconeogenic pathway. Hepatic glucose production is raised in diabetic state and is associated with the impaired suppression of the gluconeogenic enzyme fructose 1, 6-bisphosphatase. Gluconeogenic enzyme activation is due to the state of insulin impairment because under normal conditions, insulin functions as a suppressor of gluconeogenic enzymes [65, 66].

Lactate dehydrogenase (LDH) is a terminal glycolytic enzymethat plays an indispensable role in the int erconversion of pyruvate lactate to yield energy under anaerobic conditions [67]. The decreased activity of LDH in tissues could be important to ensure that a high proportion of both pyruvate and NADH, supplied by glycolysis, is subsequently oxidized by mitochondria. Indeed, elevated LDH levels observed in the experimental diabetic animals are associated with impaired glucose-stimulated insulin secretion[68]. Thus, increased activity of LDH interferes with normal homeostasis [62]. In the liver, hexokinase is an important regulatory enzyme in the oxidation of glucose [63]. Being a dependent enzyme, the hepatic hexokinase activity of diabetic rats is almost entirely inhibited or inactivated due to the absence of insulin [64]. This impairment results in a marked reduction in the rate of glucose oxidation via glycolysis, which ultimately leads to hyperglycemia. Oral administration of GTF-231 to HFD-STZ induced diabetic rats resulted in a significant reversal in the activity of hexokinase, thereby increased the oxidation of glucose.

Table 1 Effect of GTF-231on the levels of fasting blood glucose, HbA1c, Plasma insulin and urine sugar in the experimental groups of rats after 30 days experimental period

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting blood glucose (mg/dl)</th>
<th>HbA1c (%)</th>
<th>Plasma insulin (µU/ml)</th>
<th>Urine sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>87.66 ± 4.74</td>
<td>5.17 ± 0.30</td>
<td>15.67 ± 0.19</td>
<td>Nil</td>
</tr>
<tr>
<td>Diabetic</td>
<td>298.09 ± 9.05 a</td>
<td>12.60 ± 0.46</td>
<td>10.29 ± 0.32 b</td>
<td>+++</td>
</tr>
<tr>
<td>Diabetic + GTF-231</td>
<td>134.18 ± 5.94 b</td>
<td>7.72 ± 0.29</td>
<td>12.45 ± 0.49 b</td>
<td>Nil</td>
</tr>
<tr>
<td>Diabetic + Metformin</td>
<td>120.27 ± 6.85 b</td>
<td>6.97 ± 0.19</td>
<td>14.50 ± 0.46 b</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM for groups of six rats in each. One way ANOVA followed by post hoc test LSD. Values with different superscript letter in the same parameter are significantly different, P<0.05.

Table 2 Effect of GTF-231and the levels of total proteins, blood urea, Serum uric acid, serum creatinine of control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Protein (g/dl)</th>
<th>Blood urea (mg/dl)</th>
<th>Serum uric acid (mg/dl)</th>
<th>Serum Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.16±0.28</td>
<td>21.94±0.52</td>
<td>2.34±0.24</td>
<td>0.42±0.01</td>
</tr>
<tr>
<td>Diabetic</td>
<td>5.84±0.47</td>
<td>47.68±2.19</td>
<td>5.82±0.45</td>
<td>1.03±0.01</td>
</tr>
<tr>
<td>Diabetic + GTF-231</td>
<td>7.62±0.25</td>
<td>27.55±1.54</td>
<td>2.28±0.03</td>
<td>0.56±0.01</td>
</tr>
<tr>
<td>Diabetic + Metformin</td>
<td>7.59±0.31</td>
<td>31.90±1.09</td>
<td>2.05±0.10</td>
<td>0.51±0.01</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. for groups of six rats in each. One-way ANOVA followed by post hoc test L.S.D. statistical significance was compared within the groups as follows.

Table 3 Level of glycogen content and activities of glycogen synthase and glycogen phosphorylase in liver tissues of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glycogen</th>
<th>Glycogen synthase</th>
<th>Glycogen phosphorylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65.83±2.12</td>
<td>818.30±7.59</td>
<td>611.33±2.77</td>
</tr>
<tr>
<td>Diabetic</td>
<td>26.67±2.45</td>
<td>468.17±5.10</td>
<td>874.33±5.09</td>
</tr>
<tr>
<td>Diabetic + GTF-231</td>
<td>46.67±2.24</td>
<td>733.08±9.58</td>
<td>673.17±4.93</td>
</tr>
<tr>
<td>Diabetic + Metformin</td>
<td>47.83±3.09</td>
<td>737.14±8.98</td>
<td>668.33±2.27</td>
</tr>
</tbody>
</table>

Units are expressed as: mg/g wet tissue for glycogen, µmol of UDP formed/h/mg protein for Glycogen synthase and µ mol of Pi liberated/h/mg of protein for Glycogen phosphorylase. Values are given as mean ± S.D. for groups of six rats in each. One-way ANOVA followed by post hoc test L.S.D. statistical significance was compared within the groups as follows.

Glucose-6-phosphate dehydrogenase, “housekeeping” enzyme, catalyzes the first and rate-limiting step of the hexose monophosphate shunt and produces NADPH needed for the maintenance of reduced glutathione and reductive biosynthesis [69]. Observed decrease is seen in glucose-6-phosphate dehydrogenase activity of the diabetic rats might also suggest a decrease in metabolism via the phosphogluconate oxidation pathway [70]. Therefore, the
present study suggests that decreased glucose-6-phosphate dehydrogenase activity is of consequence in the pathogenesis of diabetic complications and the activity of glucose-6-phosphate dehydrogenase is regulated to near normalcy by the treatment with GTF-231 in HFD-low dose STZ induced experimental type 2 diabetes in rats.

**Table 4** Effect GTF-231 on the levels of Hexokinase, pyruvate Kinase and Lactate dehydrogenase in liver tissues of control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hexokinase</th>
<th>Pyruvate Kinase</th>
<th>Lactate dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>265.30±4.56</td>
<td>220.33±4.65</td>
<td>215.0±2.63</td>
</tr>
<tr>
<td>Diabetics</td>
<td>134.50±4.15</td>
<td>117.67±4.62</td>
<td>545.33±39.44</td>
</tr>
<tr>
<td>Diabetic + GTF-231</td>
<td>213.83±10.19</td>
<td>181.17±3.88</td>
<td>302.67±5.03</td>
</tr>
<tr>
<td>Diabetic + Metformin</td>
<td>220.50±9.74</td>
<td>189.50±1.80</td>
<td>283.17±3.21</td>
</tr>
</tbody>
</table>

Units are expressed as: µmol of Glucose-6-Phosphate formed/h/mg of protein for Hexokinase, µU/mg of protein for Pyruvate Kinase, µmol of pyruvate formed/h/mg of protein for lactate dehydrogenase. Values are given as mean ± S.D. for groups of six rats in each. One-way ANOVA followed by post hoc test L.S.D. statistical significance was compared within the groups as follows.

1 Diabetic rats were compared with control rats.

2 Diabetic + GTF-231 and Diabetic + Metformin treated diabetic rats were compared diabetic rats

**Table 5** Effect of GTF-231 on the levels of Glucose-6-Phosphatase, Fructose-1,6-biphosphatase and Glucose-6-phosphate-dehydrogenase in liver tissues of control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose-6-Phosphatase</th>
<th>Fructose-1,6-biphosphatase</th>
<th>Glucose-6-phosphate-dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1040.50±4.62</td>
<td>464.01±4.26</td>
<td>512.33±46.23</td>
</tr>
<tr>
<td>Diabetics</td>
<td>1988.67±19.45</td>
<td>844.33±14.45</td>
<td>263.00±3.20</td>
</tr>
<tr>
<td>Diabetic + GTF-231</td>
<td>1230.33±16.82</td>
<td>505.50±5.80</td>
<td>384.33±4.40</td>
</tr>
<tr>
<td>Diabetic + Metformin</td>
<td>1355.17±129.59</td>
<td>560.57±6.24</td>
<td>410.00±5.07</td>
</tr>
</tbody>
</table>

Units are expressed as µ mol of Pi liberated/h/mg of protein for Glucose-6-Phosphatase and Fructose-1,6-biphosphatase and µ mol of NADPH/min/mg of protein for Glucose-6-phosphate-dehydrogenase. Values are given as mean ± S.D. for groups of six rats in each. One-way ANOVA followed by post hoc test L.S.D. statistical significance was compared within the groups as follows.

1 Diabetic rats were compared with control rats.

2 Diabetic + GTF-231 and Diabetic + Metformin treated diabetic rats were compared diabetic rats

**Figure 1:** The effect of oral administration of GTF-231 on OGTT in control and experimental groups of rats
Figure 2: The effect of oral administration of GTF-231 on ITT in control and experimental groups of rats

Figure 3: HOMA-IR on GTF-231 treated control and experimental groups of rats
CONCLUSION

Thus the results of the present study indicate the role of GTF-231 in maintaining normoglycemia through the modulation of insulin resistance and regulation of carbohydrate metabolizing enzymes. In addition, the data also evidenced the tissue protective nature of GTF-231. Further studies are in progress to understand the molecular mechanism involved in the regulation of normoglycemia by the administration of GTF-231.

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REFERENCES

[6] C. Che; Z. J. Wang; M.S.S. Chow; C.W.K. Lam; Molecules. 2013, 18, 5125-5141.