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Biochemical Evaluation of Hypoglycemic Effect of GTF-231, a Polyherbal Preparation in High Fat Diet Fed- Low Dose STZ Induced Experimental Type 2 Diabetes in Rats

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ABSTRACT

The present study was aimed to evaluate the hypoglycemic properties of individual phytochemicals such as Gymnemic acid, Trigonelline and Ferulic acid as well as a polyherbal preparation (GTF-231) comprising of the above phytoingredients in the ratio of 2:3:1 in HFD fed-low dose STZ induced experimental type 2 diabetes in rats. Healthy, male Wistar rats weighing between 160-180g were used in the present study. The rats were divided into seven groups of six animals each. Group I served as control rats fed with normal pellet diet. Group II served as negative control and fed with a high fat diet for two weeks and intraperitoneally injected with a low dose of STZ (35mg/kg. b.w.) to induce experimental type 2 diabetes. Group III to V were induced with diabetes and treated with individual phytochemicals such as Gymnemic acid, Trigonelline and Ferulic acid for oral administration was fixed as 300 mg/kg.b.w./rat/day, respectively. Group VI was diabetic rats treated with polyherbal preparation at the concentration of 300mg/kg.b.w. Group VII was diabetic rats treated with metformin (200mg/kg.b.w.), a standard drug used for the treatment of type 2 diabetes. Body weight, food and fluid consumption were recorded periodically. At the end of the experimental period, the animals were starved overnight and OGTT was performed. After 30 days of experimental period, the animals were overnight fasted, anesthetized and scarified by cervical decapitation. Blood was collected with and without anticoagulants for the separation of plasma and serum, respectively. The biochemical analysis performed includes fasting blood glucose, HbA1c, plasma insulin, C-peptide and urine sugar. Insulin resistance, β-cell function and insulin sensitivity were also manipulated from HOMA-IR, HOMA β-cell function and QUICK-I index. The results of the present study clearly indicate that the polyherbal preparation as well as individual phytoingredients significantly decreased the levels of fasting blood glucose and HbA1c. Individual phytoingredients and GTF-231 improves the plasma insulin and C-peptide levels. From the arithmetic data, GTF and phytochemicals reduces the insulin resistance and enhances β-cell function and insulin sensitivity. The antidiabetic efficacy was more pronounced in rats treated with GTF-231. The results obtained clearly evidenced that the polyherbal preparation possess significant antidiabetic properties than the individual phytochemicals used in the present study indicating the synergistic effect of phytoingredients present in the polyherbal preparation.

Key words: Type 2 diabetes mellitus, High fat diet/STZ induced diabetes, Gymnemic acid, Trigonelline, Ferulic acid, Polyherbal preparation, Antidiabetic properties, HOMA-IR, HOMA-β cell function, QUICK-I index.

INTRODUCTION

Diabetes mellitus (DM) is a multifactorial, multisystemic metabolic disorder characterized by persistent elevation in both fasting and postprandial blood glucose levels over a prolonged period. The pancreatic β-cells or their secretary product, namely insulin is central in the pathophysiology of diabetes mellitus [1]. DM is either due to absolute lack of insulin secretion (T1DM) or its action (T2DM). T2DM accounts for more than 95% of the total diabetic population worldwide and it has become a global health issue in terms of its increasing prevalence and enormous economic burden [2]. The etiological factors associated with the increased prevalence of T2DM include increased

aging population, rapid cultural and social dynamics, sedentary life style, reduced physical activity and behavioural patterns [3]. According to International Diabetic Federation (IDF), more than 415 million people worldwide had diabetes mellitus in 2015 and are expected to rise to 642 million in 2040 unless otherwise immediate preventive measures are initiated. The debilitating effects of DM include various organ failures, progressive metabolic complications such as retinopathy, nephropathy, neuropathy and cardiovascular complications [4].

Life style and dietary changes are recommended in early stage of T2DM to control chronic hyperglycemia. However, most diabetics cannot escape from the value of pharmacotherapy to achieve desirable blood glucose levels. Most of the antidiabetic drugs cannot be used as a single therapy as T2DM is multifactorial and multisystemic in nature and hence a combinatorial approach is often preferred to maintain normoglycemia [5, 6]. However, most of the currently available drugs for the treatment of T2DM elicit undesirable side effects after prolonged use. As an important part of continued research in developing newer antidiabetic agents, several plant derived phytochemicals are being scientifically investigated for their beneficial as well as pharmacological properties.

Polyherbal preparations with various active principles and properties have been used in different regions and cultures to treat a wide range of human diseases [7]. Polyherbal formulations are collection of therapeutic entities that are formulated and blended properly on the basis of healing properties of individual phytochemicals with respect to the pathological conditions prevailing at the time of diagnosis [8]. The phytoingredients with diverse pharmacological properties principally and synergistically functions in a dynamic manner to elicit maximum therapeutic benefits with minimum side effects [9]. However, it is necessary to systematically investigate the role of individual phytochemicals present in the polyherbal preparations to ameliorate the concerns of *in vivo* safety and efficacy for successful treatment [10].

The polyherbal preparation used in the present study was formulated based on the earlier reports on the pharmacological properties of individual phytochemicals such as Gymnemic acid, Trigonelline and Ferulic acid which was originally isolated from the traditionally important medicinal plants such as *Gymnema sylvestre* R.Br. [11], *Trigonella foenum-graecum* [12] and *Ferula asafoetida* [13] respectively. Similarly, based on previous reports and the data obtained through dosage fixation studies conducted by us, the concentration of individual phytochemicals were fixed in the ratio of 2:3:1 for the preparation of a polyherbal preparation (GTF-231). In the present study, the effect of oral administration of individual phytoingredients as well as the polyherbal preparation in maintaining the glucose homeostasis in high fat diet fed-low dose STZ induced experimental type 2 diabetes in rats was systematically evaluated.

MATERIALS AND METHODS

Chemicals

Streptozotocin, Gymnemic acid, Trigonelline, Ferulic acid were procured from Sigma Chemicals Co., St. Louis, MO, USA, stored at 2–4 °C and protected from sunlight. All other chemicals were of analytical grade and were obtained from standard commercial suppliers.

Experimental rats

Male albino rats of Wistar strain weighing 160–180 g were procured from Tamilnadu Veterinary and Animal Sciences University, Chennai, India and maintained in clean, sterile, polypropylene cages under standard vivarium conditions (12 h light/dark cycle; temperature 22°C ± 3°C; relative humidity 55%) with *ad libitum* access to standard rat chow pellet (Hindustan Lever Ltd., Bangalore, India) and water. The animals were acclimatized to the laboratory environment for 2 weeks prior to the initiation of experiments. The experiments were designed and conducted in the current ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethical Committee guidelines (IAEC No.03/01/2014).

Preparation and dosage fixation studies

Gymnemic acid, trigonelline and ferulic acid were properly mixed in the ratio of 2:3:1 and the resultant complex, GTF-231, was stored in refrigeration. Acute oral toxicity of the individual phytoingredients as well as the polyherbal preparation was performed as per the guidelines prescribed by the Organization for Economic Cooperation and Development (OECD) revised draft guidelines 423. The OECD guideline involves a step wise procedure with the use of a minimum number of animals in each step to obtain sufficient data on the acute toxicity of the test compound to enable its classification. Briefly, the overnight fasted rats were orally administered with graded doses of individual phytochemicals as well as the polyherbal preparation. The rats were observed for their behavioural, neurological and autonomic characteristics for 24h and the animals were observed for the next 14 days for mortality. The acute toxicity studies of the individual as well as polyherbal preparation did not show any toxic symptoms in dose up to 2000 mg/k.g.b.w. over a period of 2 weeks.

Induction of T2DM in experimental rats

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 [14,15]. After 2 weeks of HFD fed, the overnight fasted rats of Group II to Group- VII were intraperitoneally injected with a single dose of freshly prepared solution of STZ (35 mg/kg.b.w/rat.; 0.1 mM cold citrate-phosphate buffer; pH 4.5) [16]. Control rats were injected with an equivalent volume of citrate buffer through the same route of administration. Since, streptozotocin is capable of inducing fatal hypoglycaemia as a result of massive pancreatic insulin release; the rats were supplied with 10% glucose solution after 6 h of STZ administration for the next 24 h to prevent hypoglycaemia [17]. After a week in time for the development and aggravation of diabetes, rats with moderate diabetes (i.e. blood glucose concentration ≥ 250 mg/dl) were considered as diabetic and chosen for further studies. Based on the previous reports for the antidiabetic properties of gymnemic acid [18], trigonelline [19] and ferulic acid [20], in the present study the dosage of the individual phytochemicals for oral administration was fixed as 300 mg/kg.b.w./rat/day for 30 days. Likewise, the dosage of GTF-2:3:1(Gymnemic acid-100mg: Trigonelline-150mg: Ferulic acid-50mg) was fixed as 300mg/kg.b.w/rat/day for 30 days [21].

Grouping of animals

The animals were divided into seven groups (n=6):

Group I - Control rats.
Group II - HFD fed - low dose STZ (i.p. 35mg/kg b.w.) induced diabetic rats.
Group III –Diabetic rats treated with Gymnemic acid (300 mg/kg b.w./rat/day orally for 30 days)
Group IV - Diabetic rats treated with Trigonelline (300 mg/kg b.w./rat/day orally for 30 days)
Group V - Diabetic rats treated with Ferulic acid (300 mg/kg b.w./rat/day orally for 30 days)
Group VI - Diabetic rats treated with Polyherbal preparation (GTF-2:3:1; 300 mg/kg b.w./rat/day orally for 30 days)
Group VII - Diabetic rats treated with a standard drug, Metformin (200 mg/kg b.w./rat/day) in aqueous solution orally for 30 days.

During the experimental period, body weight, food and fluid intake were monitored at regular time intervals. After 30 days of experimental period, the rats were fasted overnight and euthanized by anesthesia 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. The supernatants were collected, pooled and used for all estimations.

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 [22].

Determination of biochemical parameters

The fasting blood glucose level was determined by glucose oxidase diagnostic enzyme kit (Span Diagnostic Chemicals, India) and glycosylated hemoglobin was estimated by the method of Nayak and Pattabiraman (1981) [23]. The levels of plasma insulin and C-peptide were assayed using rat ELISA kit (Linco Research, St Charles, MO, USA). The presence of urine sugar was detected using urine strips (Diastix).

Assessment of insulin resistance and β cell function

Insulin resistance was assessed by QUICKI (Quantitative insulin check index) [24] and HOMA-IR. HOMA- **%**β score was calculated using blood glucose and plasma insulin concentrations according to the following formula [25];

HOMA-IR = [Blood glucose (mg/dl) × Insulin (μU/ml)] / 405
HOMA-β = [360 × Insulin (μU/ml)] / Blood glucose-63 %

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.

RESULTS

Figure 1 shows the effect of oral administration of gymnemic acid, trigonelline and ferulic acid as well as GTF-231 in body weight gain of experimental groups of rats. The raise in body weight was far less in diabetic control rats as compared to diabetic rats treated with individual phytochemicals as well as GTF-231. However, diabetic rats treated

with GTF-231 showed a significant increase in body weight gain when compared to the diabetic rats treated with individual phytoingredients which have showed a marginal increase in body weight and the efficacy of GTF-231 was comparable with metformin, a standard drug widely used for the treatment of T2DM.

The levels of food (Figure 2) and fluid (Figure 3) consumption were significantly increased in diabetic groups of rats when compared with control groups of rats. The increased crave of food and fluid utilization was normalized after oral treatment of individual phytochemicals as well as the polyherbal preparation which was comparable with metformin.

Figure 4 depicts the levels of blood glucose in control and experimental groups of rats after receiving an oral glucose load (2g/kg.b.w.). In control group of rats, the blood glucose level reached the maximum peak at 60 min after an oral glucose load and it was progressively declined to near normal level at 120 min representing the maintenance of normal glucose homeostasis. Conversely, 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. However, the diabetic rats treated with individual phytochemicals as well as the polyherbal preparation, showed a significant decrease in blood glucose levels at fasting, 30 and 60min time interval when compared with the diabetic control group of rats. In addition, the blood glucose levels returned to near basal level at 120 min after the oral glucose load in treated groups of rats. However, diabetic rats treated with GTF-231 showed a statistically significant improvement in glucose homeostasis than rats treated with individual phytochemicals during oral glucose tolerance test.

The levels of fasting blood glucose, glycosylated hemoglobin (HbA1c), plasma insulin, c-peptide and urine sugar in control and experimental groups of rats are depicted in table 1. The levels of fasting blood glucose and HbA1c% were found to be significantly elevated in diabetic group of rats when compared with control rats. Likewise, the levels of plasma insulin and C-peptide were moderately decreased in HFD-STZ induced diabetic rats. Urine sugar was observed in the diabetic group of rats. Oral administration of individual compounds as well as polyherbal preparation to experimental groups of rats showed improved levels of altered biochemical parameters.

The altered levels of HOMA-IR and HOMA-β indices (Figure 5 & 6) were reverted back to near normal in diabetic rats treated with individual phytochemicals as well as polyherbal preparation. Figure 7 depicts the quantitative insulin check index (QUICKI). Diabetic rats showed reduced insulin sensitivity which indicates the extent of insulin resistance. Individual phytoconstituents as well as polyherbal preparation restored the insulin sensitivity.

DISCUSSION

To combat the increasing prevalence of T2DM, there is an urgent need for more effective treatments as most of the currently available drugs for the treatment of T2DM elicit undesirable side effects after prolonged use. It is a fact that the type 2 diabetic patients are often treated with combinatorial drugs due to its multifactorial and multisystemic nature. The animal model used in the present study involves a combination of a diet high in fat to bring about insulin resistance, hyperinsulinemia and/or impaired glucose tolerance followed by treatment with a low dose of STZ, a specific β cell toxin which results in reduction in functional β cell mass [26, 27]. The pathological condition induced by the stressors namely the high fat diet and low dose STZ closely resembles the clinical features of T2DM, though on a shorter time scale than found in the human condition [14].

The transition from a metabolically healthy state to an obese and subsequent prediabetic state involves a vicious cycle comprising of hyperinsulinemia, insulin resistance, dyslipidemia and dysfunctional adipose tissue, ectopic fat accumulation in both liver as well as skeletal muscle and failure of insulin producing β cell function [28-30]. The composition and duration of high fat diet feeding timeframe greatly influence the onset of T2DM in experimental rats. The most commonly used approach is to feed rats with a diet high in fat but normal levels of carbohydrates rather than a diet high in carbohydrates to produce a 'high energy' feeding regimen [31].

STZ is a nitrosourea analogue, preferentially taken up by pancreatic β cells via GLUT2 glucose transporter and causes DNA alkylation followed by the activation of poly ADP ribosylation leading to depletion of cytosolic concentration of NAD^+ and ATP. Enhanced ATP dephosporylation after STZ treatment provides substrate for xanthine oxidase resulting in the generation of superoxide radicals. Further, nitric oxide (NO) moiety is liberated from STZ leading to irreversible destruction of β cells by necrosis [27]. It was observed that STZ administration at first abolished the β cell response to glucose. Temporary return to responsiveness then appears which is followed by its permanent loss and β cells are damaged [32]. Though, STZ is widely used to induce both type 1 and type 2 diabetes in animal models, the STZ dose will greatly affect the β cell mass remaining in the rats. Likewise, variation in the amount of β cell mass left in both type 1 and type 2 diabetes do exist in human [33, 34]. However, the loss of

β cell mass in the pathogenesis of type 1 diabetes occurs mainly as a result of an autoimmune reaction, which is not the case in T2DM.

The dose of STZ itself obviously has a significant impact on the phenotype of HFD-fed rats. STZ induces robust not absolute β cell ablation in a manner that depends on the dose, number of doses, time interval between the doses, route of administration, fed/fasted state upon STZ administration and the rat strain/vendor. However, several reports are available in the literature that a single low dose of STZ (<45mg/kg) induced experimental diabetes as a suitable model of experimental type 2 diabetes to screen the efficacy of lead molecules [15, 35].

The observed decrease in body weight of untreated diabetic group of rats may be due to excessive break down of tissue structural proteins which are known to contribute to body weight [36, 37]. Though, all the experimental groups of rats were continued to feed with a high fat diet, the untreated diabetic rats have shown a marginal increase in body weight gain when compared to other experimental groups of rats indicating the alteration in the major metabolic pathways due to decreased insulin sensitivity coupled with insufficiency. The significant improvement in body weight gain of other treated groups of rats evidenced the beneficial effects of the individual phytoingredients as well as the polyherbal preparation in maintaining normal homeostasis. However, diabetic rats treated with GTF-231 showed a relatively better improvement in body weight gain when compared to other experimental groups of rats indicating the synergistic effect of phytoingredients present in the polyherbal preparation over the individual phytoingredients and the efficacy of GTF-231 was comparable with metformin.

Increased food and fluid consumption was observed in diabetic group of rats. This indicates the polyphagic and polydipsic condition. The classic triad of diabetic symptoms are polyphagia, polydipsia and polyuria [38]. An increase in renal loss of glucose is accompanied by an excessive loss of water and electrolytes. Excessive loss of water leads to the activation of thirst mechanism, termed as polydipsia. An increased thirst may be due to hyperglycemia which raises the osmolarity of blood and makes it more concentrated. Glucosuria and increased tissue protein catabolism leads to crave excess amount of glucose for energy production, an increase in appetite and food intake called as polyphagia [39]. The food and fluid intake of diabetic groups of rats treated with individual as well as GTF-231 were significantly reduced after 30 days of treatment. From the data, it is clearly manifested that the individual phytochemicals as well as polyherbal preparation considerably reduced the pathophysiological symptoms through its significant hypoglycemic properties. Like, the effect on body weight gain, the diabetic rats treated with GTF-231 significantly improved the polyphagic and polydipsic condition than the experimental rats treated with individual phytochemicals.

Oral glucose tolerance test (OGTT) has been the mainstay for diagnosing diabetes for decades. It more efficiently detects prediabetics as well as patients with impaired glucose tolerance [40]. OGTT has the utility for evaluating insulin sensitivity and β-cell function during glucose administration via a physiological route [41]. OGTT is commonly used to evaluate the disease progression, outcome of treatments and to assess the physiological and pathophysiological conditions of diabetes mellitus [42]. Oral glucose tolerance test provide more physiological conditions for the estimation of β-cell function than does a test based on intravenous glucose administration. OGTT provides significant and valuable information for predicting the ensuing incidence of type 2 diabetes.

The routinely used imperative marker for estimating the degree of protein glycation in diabetes includes glycated haemoglobin levels. Extensive studies on HbA1c bring out the importance of HbA1c as a non-manipulatable and reliable biochemical parameter in assessing metabolic control as compared to one-point blood glucose estimation [43]. HbA1c is likely to be a more physiological assessment of glucose intolerance than the artificial conditions of the OGTT and hence it should be the preferred diagnostic test [44, 45]. During persistent hyperglycemia, glucose irreversibly binds to the N-terminal valine of the β chain of hemoglobin. The process of glycation at other positions such as lysine on the β-chain or at sites on the α-chain may be imperative at higher levels of glycation. The percent glycation of hemoglobin also depends on the average age of the erythrocytes in the sample and the percent of HbA1c is higher in older cells [46, 47]. HbA1c levels represent average glycemia over the entire 120 day life span of the red blood cell [48]. HbA1c measurement may also be used as a tool to stratify the risk of the patient developing microvascular complications because there was an exponential rise in the rate of secondary complications with increasing HbA1c values. Each 1% reduction in glycated haemoglobin was associated with a significant reduction in diabetes related mortalities [49]. HbA1c signifies the mean glucose levels maintained over the previous 6-8 weeks. Normoglycemia was achieved in diabetic groups of rats treated with individual as well as GTF-231 possibly through their ability to maintain glucose homeostasis. However, the diabetic rats treated with GTF-231 showed a significant decrease in the levels of fasting blood glucose as well as HbA1c and increased levels of plasma insulin next to diabetic rats treated with gymnemic acid and the efficacy of GTF-231 was comparable with metformin evidencing that the polyherbal preparation possesses significant insulin stimulatory and insulin sensitivity properties [50].

Patients with most depressed β-cell function had a higher HbA1c and higher baseline glucose, but lower baseline insulin and C-peptide [51]. The balance between the utilization and production of glucose is maintained by the hormone, insulin. It stimulates glucose uptake, utilization and storage while suppressing hepatic glucose production, thus reducing plasma glucose levels. Insulin inhibits the production and release of glucose by the liver, due to the blockage of gluconeogensis and glycogenolysis. C-peptide, a bioactive peptide plays a crucial role in the biosynthesis of insulin. It consists of 31 amino acids and has a half life of 30 min. Subsequent to the cleavage from the proinsulin molecule; C-peptide is released into the blood stream as equimolar concentrations to insulin [52]. The serum levels of insulin and C-peptide greatly increase in the early stage of T2DM because of insulin resistance. Although T2DM is a state of insulin resistance to relative insulin insufficiency, it may progress to a late-stage insulin and C-peptide-deficient state due to pancreatic β-cell demise [53]. Physiological concentrations of C-peptide activates extracellular signal regulated kinases, phosphatidyl inositol 3-kinase, protein kinase C, elevates intracellular calcium, and stimulates peroxisome proliferator activated receptor- γ [54]. The observed increased in the levels of C-peptide in diabetic rats treated with individual phytochemicals as well as GTF-231 may be due to the stimulation of molecular factors that produce c-peptide from the pancreatic β-cells, thereby ameliorate the secondary complications of diabetes. However, GTF-231 has more effective than the individual phytoingredients due to its synergistic mechanism.

Figure1: Effect of gymnemic acid, trigonelline, ferulic acid and GTF-231 on body weight gain in experimental groups of rats.

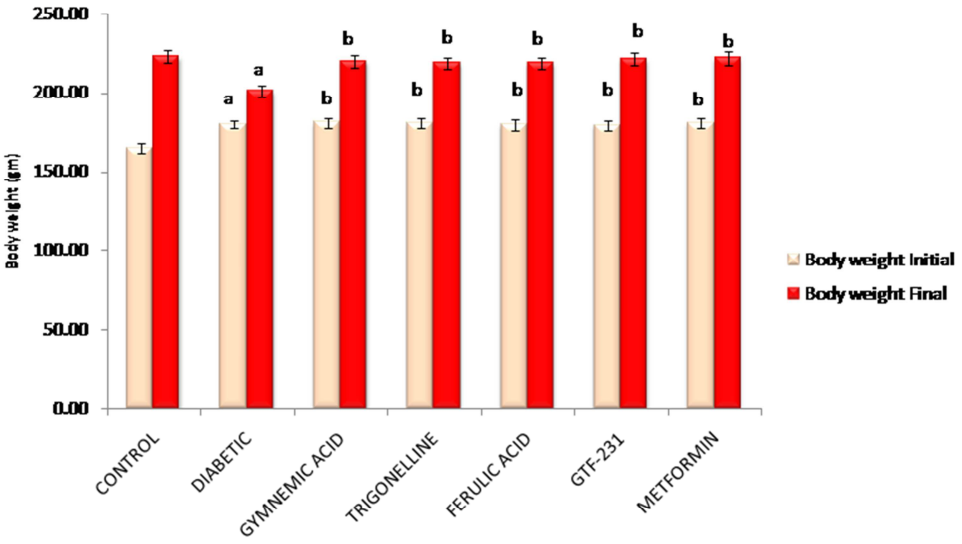

Figure1: Effect of gymnemic acid, trigonelline, ferulic acid and GTF-231 on body weight gain in experimental groups of rats.
Values are given as mean ±SEM for groups of six ra ts in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows
[a] compared with control
[b] compared with diabetic rats

The homeostasis model assessment of insulin resistance (HOMA-IR) developed by Matthews et al. (1985) [25] have been extensively used for the determination of insulin resistance. Insulin resistance is the primary metabolic disorder associated with obesity and appears to be the primary mediator of metabolic syndromes [55]. Likewise, QUICKI is also an empirically derived mathematical conversion of fasting blood glucose and plasma insulin concentrations. However, it provides a reliable, reproducible and accurate index of insulin sensitivity with a better optimistic prognostic power [56].

HOMA- β cell function is effectively used to measure the pancreatic β-cell function. Evidences suggested that hyperglycemia causes additional functional impairments in insulin release that go beyond the actual β-cell deficit

[57]. The majority of genes associated with type 2 diabetes have been linked to the β-cell dysfunction and impairments in β-cell mass. If β-cell mass is reduced by 50%, the secretory burden for the remaining β-cells increases by 100%, thereby leading to chronic β-cell stress [58]. The data obtained through the above three arithmetical indices suggest that the diabetic rats treated with GTF-231 significantly improved the β-cell function and insulin sensitivity than the individual phytochemicals indicating its synergistic efficacy in ameliorating chronic hyperglycemia.

Figure 2: The levels of food consumption in control and experimental groups of rats.

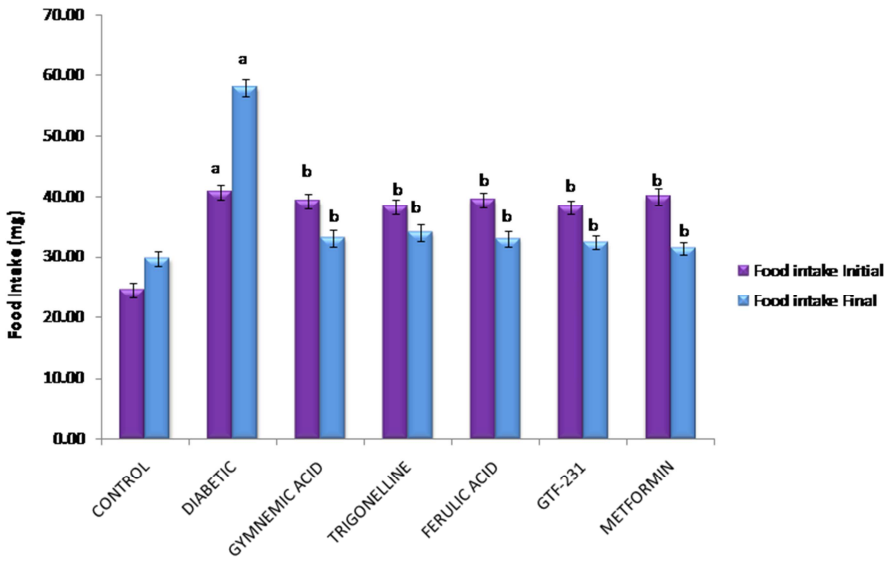

Figure 2: The levels of food consumption in control and experimental groups of rats.

Values are given as mean ±SEM for groups of six ra ts in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows

[a] compared with control

[b] compared with diabetic rats

Figure 3: The levels of fluid intake in control and experimental groups of rats.

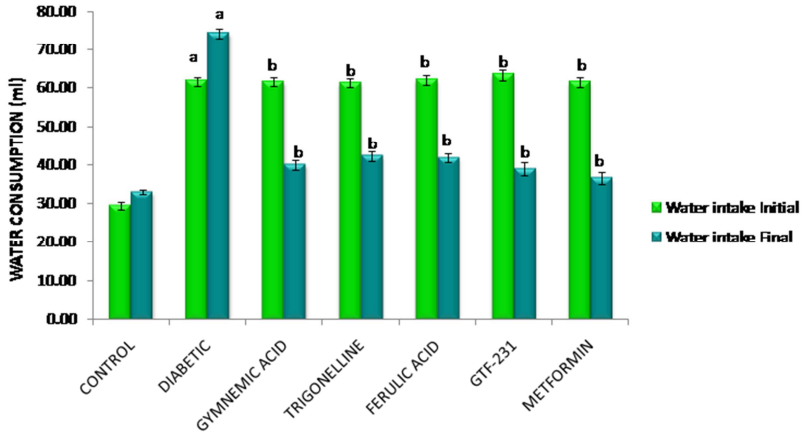

Figure 3: The levels of fluid intake in control and experimental groups of rats.
Values are given as mean ±SEM for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows
[a] compared with control
[b] compared with diabetic rats

Figure 4: The levels of blood glucose in control and experimental groups of rats after receiving an oral glucose load.

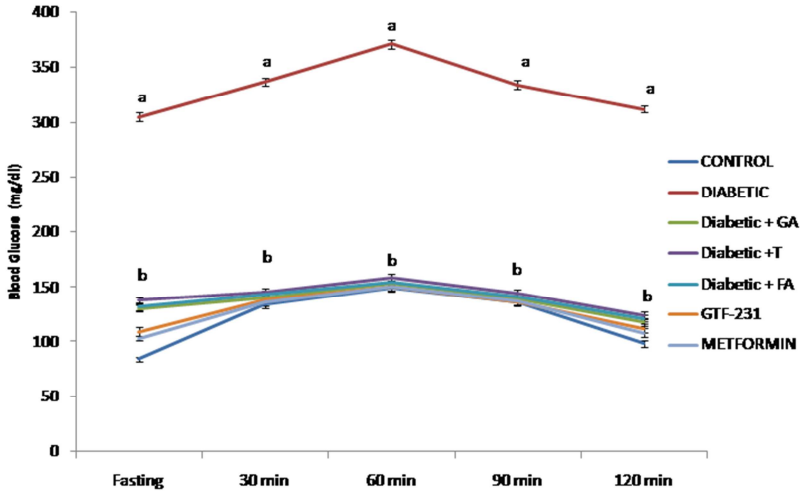

Figure 4: The levels of blood glucose in control and experimental groups of rats after receiving an oral glucose load.
Values are given as mean ±SEM for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows
[a] compared with control
[b] compared with diabetic rats

Figure 5: Effect of gymnemic acid, trigonelline, ferulic acid and GTF-231 on HOMA-IR.

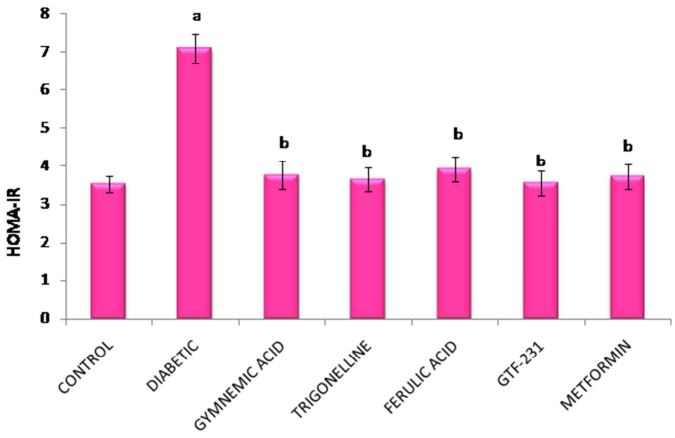

Figure 5: Effect of gymnemic acid, trigonelline, ferulic acid and GTF-231 on HOMA-IR

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

[a] compared with control

[b] compared with diabetic rats

Figure 6: Levels of β-cell function in control and experimental groups of rats.

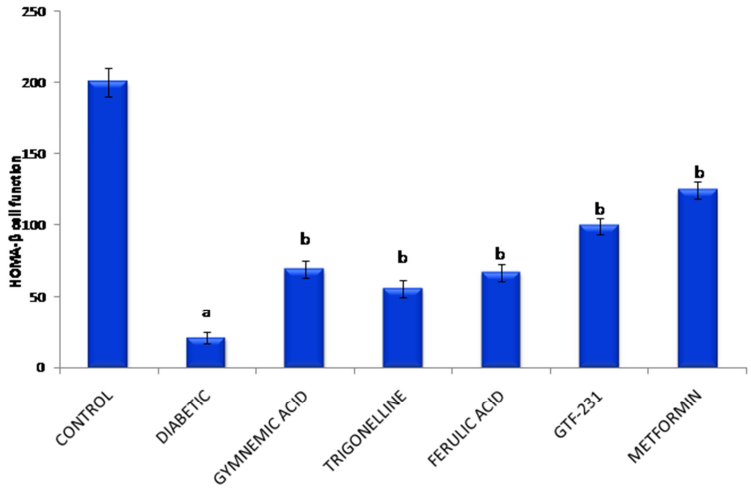

Figure 6: Levels of β-cell function in control and experimental groups of rats

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

[a] compared with control

[b] compared with diabetic rats

Figure 7: Effect of gymnemic acid, trigonelline, ferulic acid and GTF-231 on QUICK-I index in experimental groups of rats.

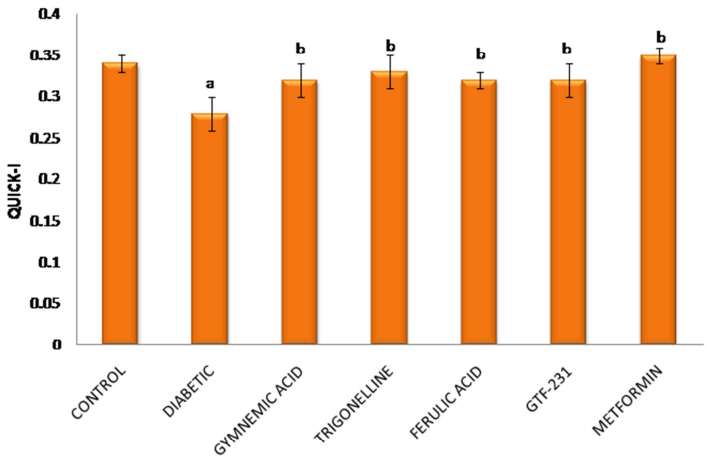

Figure 7: Effect of gymnemic acid, trigonelline, ferulic acid and GTF-231 on QUICK-I index in experimental groups of rats

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

[a] compared with control

[b] compared with diabetic rats

Table 1: Effect of gymnemic acid, trigonelline, ferulic acid and GTF-231 on fasting blood glucose, HbA1c, plasma insulin, c-peptide and urine sugar in experimental groups of rats

Groups	Fasting Blood Glucose (mg/dl)	HbA1c (% hemoglobin)	Plasma Insulin (µU/ml)	C-Peptide (pmol/mL)	Urine Sugar
Control	90.33±3.32	4.87±0.17	15.87±0.19	0.25 ± 0.022	Nil
Diabetic Control	305.22±8.32[a]	10.80±0.41[a]	9.30±0.13[a]	0.11 ± 0.010[a]	+++
Diabetic+Gymnemic acid	126.01±3.34[b]	6.03±0.49[b]	12.09±0.34[b]	0.20 ± 0.012[b]	Nil
Diabetic+Trigonelline	134.21±3.67[b]	6.91±0.53[b]	11.01±0.42[b]	0.18 ± 0.013[b]	Nil
Diabetic+ Ferulic acid	129.24±3.97[b]	6.46±0.52[b]	12.29±0.44[b]	0.19 ± 0.011[b]	Nil
Diabetic+GTF-231	110.28±3.01[b]	5.89±0.50[b]	13.01±0.64[b]	0.21 ± 0.011[b]	Nil
Diabetic+Metformin	104.71±4.14[b]	5.76±0.19[b]	14.42±0.75[b]	0.23 ± 0.012[b]	Nil

Results are expressed as mean ±SEM [n = 6]. One-way ANOVA followed by post hoc test LSD was done. The results were [a] compared to control rats and [b] compared to diabetic rats.

CONCLUSION

In conclusion, the results of the present study clearly evidenced that the polyherbal preparation (Gymnemic acid: Trigonelline: Ferulic acid - 2:3:1) possess significant antidiabetic properties than the individual phytoconstituents present in the polyherbal preparation which are evidenced from the decreased levels of fasting blood glucose, glycosylated haemoglobin and increased levels of plasma insulin and C-peptide. The arithmetic indicators also evidenced the better antidiabetic efficacy of GTF-231 over the individual phytoingredients. Though, several reports are available in the literature pertaining to the efficacy of the individual phytochemicals used for the preparation of polyherbal preparation in the study, the concentration of each phytochemical used in the preparation and the dosage used in the present study is much less when compared to the dosage used in other studies however efficacy was more pronounced due to the synergistic effect. Thus, GTF-231 may be considered as a safe and effective drug for the successful treatment of type 2 diabetes. Detailed studies are in progress to evaluate the effect of GTF-231 on lipid and protein metabolism as well as on oxidative stress in experimental type 2 diabetes.

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