COMIBINATORIAL EFFICACY OF GTF ON ALTERED HEMATOLOGICAL AND LIPID PROFILE IN TYPE-2 DIABETIC WISTAR RATS

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ABSTRACT

Objective

To investigate the combinatorial effect of polyherbal formulation of GTF on altered haematological indices in high fat diet and high fructose fed Type-2 diabetic rats.

Methods

A total of 24 rats, 18 diabetic and 6 normal rats were used for this study. Diabetes was induced in male Wistar rats by high fat diet and high fructose feeding. After being confirmed diabetic, animals were orally treated with GTF polyherbal formulation in the ratio of 2:3:1 (100 mg, 150mg and 50mg/kg body weight) daily for 30 days. The haematological parameters including red blood and white blood cells and their functional indices were evaluated in diabetic, diabetic treated groups compared with the controls.

Results

GTF significantly reduced the blood glucose and lipid levels except HDL in HFD and HF fed diabetic rats. Similarly, the levels of red blood, white blood cells and their functional indices were significantly restored after GTF administration.

Conclusions

It can be concluded that the GTF possesses antihyperglycemic and anti-hyperlipidemic properties. In addition, the extract can prevent various complications of diabetes and improve some haematological parameters. Further experimental investigation is needed to exploit its relevant therapeutic effect to substantiate its ethnomedicinal usage.

Keywords - Gymnemic acid, Ferulic acid, Trigonelline, Type-2 diabetes.

1. INTRODUCTION

The burden of diabetes is increasing globally, particularly in developing countries. The causes are rapid increases in overweight, including obesity and physical inactivity.Diabetes is predicted to become the 7th leading cause of death in the world by the year 2030, Total deaths from diabetes are projected to rise by more than 50% in the next 10 years (WHO report, 2016).Type 2 diabetes mellitus is the world's largestendocrine disorder which is characterized by decrease ininsulin secretion, defect in glucose uptake in skeletalmuscle and fat and increased glucose production in theliver (White MF, 2002; Taniguchi CM et al., 2006).The long-term hyperglycemia is an important factor in the development and progression of micro- and macro-vascular complications, which include neuropathy, nephropathy, cardiovascular and cerebrovascular diseases (Altan, 2003 and Strojek, 2003).

Several hematological changes affecting the red blood cells (RBCs), white blood cells (WBCs), and the coagulation factors are shown to be directly associated with DM.(Mbata*et al.,*2015) Other hematological abnormalities reported in the DM patients include RBCs, WBCs, and platelet dysfunction(Mirza*et al.,*2012;Gkrania*et al.,*2010). Epidemiological study has indicated a close relationship between the WBC count and components of metabolic syndrome(Chen*et al.,*2006).These abnormalities have been shown to markedly increase blood viscosity that unfavourably affects the microcirculation, leading to microangiopathy(Cho *et al.,*2008).Studies revealed that higher WBC count, as one of the major components of inflammatory process, contributes to atherosclerotic progression and CVD(Chen*et al.,*2006; Nakanishi*et al.,*2002;Karthikeyan*et al.,*2006;Maitra*et al.,*2010). Haematological indices are important indicators for the evaluation of variations in size, number, and maturity of different blood cells (Belete*et al.,*2016).

A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of NIDDM (Marles *et al.,* 1995). Among these are

alkaloids, glycosides, galactomannan gum, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions such as Trigonelline, Ferulic acid and Gymnemic acid. The use of herbal products as polyherbal formulation dramatically increased throughout the world over the past two decades and majorpharmaceutical companies are currently conductingextensive research on plant materials for their potentialmedicinal value (Bhatt N, 1998). In the present study we havetried to evaluate the anti-diabetic properties and its effect on altered haematological indices of GTF in high fat diet and high fructose feeding induced Type-2 diabetic rats.

2. MATERIALS AND METHODS

2.1 Source of Chemicals

Gymnemic acid (Standardized to 75% gymnemic acid IV) was procured from Sant Clare herbals, Hungary. Trigonelline and Ferulic acid were procured from Sigma-Aldrich, USA.

2.2 Animals and Groupings

Twenty Four male Wistar*Albino* rats with a body weight between 150 and 180 g were obtained from Central Animal House Facility, University of Madras, Taramani, Chennai. Therats were housed in temperature-controlled (20–22°C) roomon a 12:12 h dark-light cycle. The ratswere acclimatised for 15-day period to the environment with free access tofood and water. The experimental protocols were approvedby the Institutional Animal Ethical Committee, University of Madras (IAEC No. 01/20/14).The rats were randomly divided into four groups.

Group 1: Rats fed with normal standard rat chow and water ad libitum, served as healthy control

Group 2: Rats fed with high fat diet/high fructose (25%) in drinking water for 90 days served as diabetic control

Group 3: Rats fed withhigh fat diet and high fructose (25%) in drinking water for 90 days and supplemented with Gymnemic acid (100mg) + Trigonelline (150 mg) + Ferulic acid 50mg/kg body weight orally for the last 30 days.

Group 4: Rats fed withHigh fat diet/High fructose (25%) in drinking water for 90 days and supplemented with Metformin 50mg/Kg body weight orally for the last 30 days.

Each group consisted of equal numberof rats (n = 6).

2.3 Preparation of High Fat diet

High fat diet comprised of Cholesterol (4%), deoxy Cholic acid (1.5%) and coconut oil (30%) and 655.5g of powdered standard rat chow per kilogram.

2.4 Preparation of High Fructose Water

The fructose thatwas used was D-fructose >99%.Fructose drinking water was freshly prepared every alternateday and the bottle covered using aluminium foil to prevent fermentation and was based on weight/volume formula. To prepare fructose 25% drinking water, 25 g of fructose was diluted in 100mL of R/O water. The HFWwas administered every day for 90 days.

2.5 Plasma Blood Glucose:

Blood samples were collected subsequently at 60, 120, and 180 minutes and centrifuged for 10 minutes at 800 x g at 4°C within 30 minutes to prevent autoglycolysis by leukocytes. Plasma glucose was estimated by Glucose Oxidase–Peroxidase method (Randox Laboratories Ltd.). Results are expressed as mg/dL.

2.6 Lipid profile

Analysis of serum enzymes assays were performed using Randox Diagnostic kits from Randox, UK in a Randox chemistry analyzer from Thermo Scientific Company. The serum lipid profile was assessed by assaying the serum levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) high density lipoprotein (HDL) and very low density lipoprotein (VLDL).

LDL mg/dl = Total cholesterol-HDL-TG/5

VLDL mg dl = Triglycerides/5

2.7 Hematological Evaluation

Hematology profile, which covers hemoglobin level (HGB), packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC), platelets (PLT), lymphocytes (LYMP), and neutrophils (NEU), was determined using a Sysmex KX21 Hematoanalyzer according to the manufacturer's protocol.

2.8 HbA1C

Glycosylated haemoglobin (HbA1c)levels were analyzed by HPLC and Ion exchange method. From the blood samples collected Glycosylated haemoglobin was separated from total haemoglobin. HbA1c content was calculated based on the ratio of HbA1c peak area to the total hemoglobin peak areas. Results are expressed in % Glycation.

2.9. Statistical analysis

To address the biological variability, each set of experiments was repeated at least six times, n = 6(experimental rats). Differences between the groups were analyzed by one-way analysis of variance (ANOVA) with the aid of SPSS software (SPSS Inc., Chicago, IL, USA) standard version 20. The p values of <0.05 were considered statistically significant for differences in mean using the least of significance difference, and data were reported as mean ± standard deviation.

3. Results

Figure-1 shows the effect of polyherbal formulation GTF on blood glucose. Administration of polyherbal formulation to Type 2 diabetic rats significantly ($p<0.05$) reversed the blood glucose levels to normalcy, level of reversion was better in group 3 than metformin administered group 4 rats. The HFD and HF fed group showed significant increase in blood glucose level when compared to other groups ($p<0.05$).

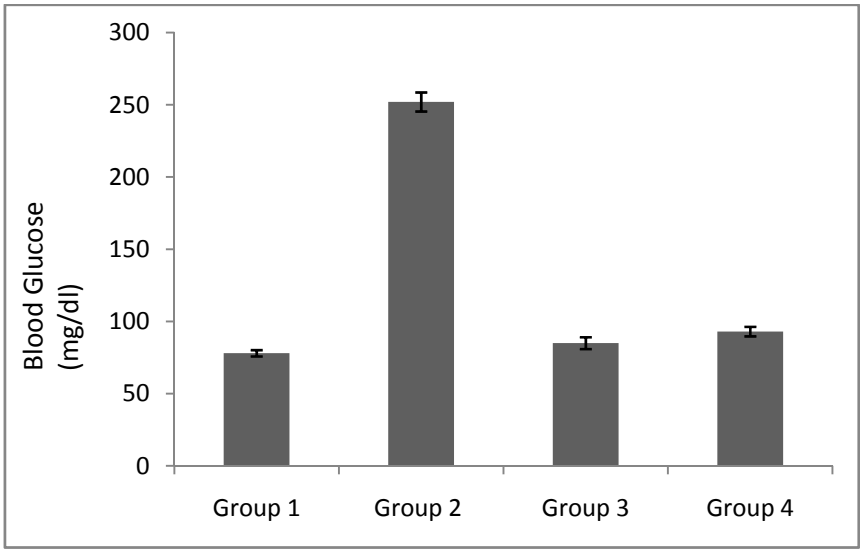

Figure 1 displays the plasma glucose levels at the end of experimentation period. Values are expressed as mg/dl. Values are expressed as Mean ± S.E.M of six animals in each group where 'a' denotes group 1 compared with all other groups, 'b' denotes group 2 compared with group 3 and group 4. Values are statistically significant at $p<0.05$.

Effect of GTF combinatorial therapy on serum lipid levels. The effects of GTF on lipid levels in treated rats are shown in the table-1. The results showed that the levels of TG, TC, LDL and VLDL-c in the HFD and HF fed group were significantly higher ($p<0.05$) when compared to control group I. GTF administered group 3 rats showed significantly decrease in the levels when compared with group 2 rats. Whereas HDL which reduced on T2D condition increased to near normalcy on GTF therapy for 30 days.

Table-1 displays the serum lipid profile of control and experimental animals. Values are expressed as mg/dl. Values are expressed as Mean ± S.E.M of six animals in each group where 'a' denotes group 1 compared with all other groups, 'b' denotes group 2 compared with group 3 and group 4. Values are statistically significant at $p<0.05$.

PARAMETERS	GROUPI	GROUP II	GROUP III	GROUP IV
Cholesterol (mg/dl)	128.1 ± 2.9	195.3 ± 4.1[a]	121.4± 3.9[b]	111.2 ± 2.9[b]
Triglycerides (mg/dl)	120.5 ± 3.2	198.4 ± 2.9[a]	128.2 ± 2.7[b]	115.7 ± 3.4[b]
LDL (mg/dl)	55.2 ± 2.8	113.1± 2.3[a]	59.7 ± 3.2[b]	44.4 ± 3.7[b]
VLDL (mg/dl)	22.6 ± 2.9	62.4± 3.2[a]	21.9 ± 2.8[b]	25.2 ± 1.7[b]
HDL (mg/dl)	49.8 ± 3.4	21.8± 1.9[a]	38.6± 2.7[b]	42.1± 1.2[b]

Table-2 displays the effect of GTF administration on altered Hematological indices. Significant decrease in the levels of Hb, RBC, Platelets and increase in the levels of WBC, Basophils, Neutrophils and Eosinophils are seen in high fat diet and high fructose fed group II rats. GTF administration showed significant ($p<0.05$) restoration of altered haematological indices when compared with group 2 rats.

Hemoglobin (Hb %)

Parameters	Group I Control	Group II Dia Control	Group III Dia + Com	Group IV Metformin
Hemoglobin (%)	13.2±0.8	8.5±0.3a	12.8±0.8b	13.3±0.7b
RBC X 10^6/cu.mm	7.98±0.3	6.16±0.2a	7.67±0.3b	7.96±0.4b
WBC x 10^3/cu.mm	7.21±0.4	10.58±0.2a	8.22±0.4b	8.18±0.5b
Platelets (Lakhs X cu.mm)	6.52±0.2	5.27±0.3a	6.59±0.4b	6.75±0.78b
Reticulocytes (%)	0.87±0.09	0.92±0.11a	1.11±0.16b	0.91±0.14b
Neutrophils (%)	21.10±2.29	25.67±2.35a	22.15±2.17b	23.22±2.55b
Lymphocytes (%)	76.35±6.8	75.16±7.27a	76.52±7.10b	73.39±7.12b
Monocytes (%)	4.21±0.3	4.32±0.4a	3.97± 0.3b	3.95±0.4b
Basophils (%)	1.12±0.15	0.94±0.09a	1.22±0.15b	1.18±0.10b

Table-1 displays the haematological indices of control and experimental animals. Values are expressed as Mean ± S.E.M of six animals in each group where 'a' denotes group 1 compared with all other groups, 'b' denotes group 2 compared with group 3 and group 4. Values are statistically significant at $p<0.05$.

Figure-2 displays the levels of glycosylaytion of hemoglobin (HbA1c) in control and experimental animals. Markedly increased levels ($p<0.05$) were seen in group 2 diabetic rats which on treatment with GTF reverted back to normalcy in group 3 animals.

Figure-2 displays the glycosylaytion levels of hemoglobin of control and experimental animals. Values are expressed as % of glycosylaytion. Values are expressed as Mean ± S.E.M of six animals in each group where 'a' denotes group 1 compared with all other groups, 'b' denotes group 2 compared with group 3 and group 4. Values are statistically significant at $p<0.05$.

4.Discussion:

In the present study, the beneficial effect of GTF combinatorial therapy wasinvestigated using high fat diet and high fructose induced obese Type-2 diabetic rat model. Type 2 Diabetes mellitus is metabolic syndrome which is associated with decreased insulin secretion, insulin action and subsequently decline in insulin sensitivity (Polonsky KS et al., 1998; Taylor SI et al., 1994). Hyperglycemia and hyperlipidemia are two important characteristics of type 2 diabetes. Previous studies have reported that the rats fed with HFD and HF developed significant hyperglycemia and hyperlipidemia which mimics human type-2 diabetes (Nampurath et al., 2008). Microvascular and macrovascular complications are mainly or partly dependent on hyperglycemia like increased formation of advanced glycation end products HbA1c.

Our present findings show a highly significant increase in blood glucose levels and impaired glucose tolerance upon high fat diet and fructose feeding for 90 days in Group 2 rats. Our results are supported by the studies of Nampurath *et al.* (2008) and Woods*et al.* (2003), which demonstrate that feeding rats with high fat diet and fructose leads to increased blood glucose. Administration of GTF to diabetic rats resulted in significant ($p<0.05$) decrease of blood glucose levels. Our results are coherent with the work of Sugihara *et al.* (2000) who have established the anti-diabetic activity of gymnemic acid IV in STZ induced diabetic rats. Administration of fenugreek alkaloids trigonelline to diabetic rats reduced blood glucose and this was accompanied by an increase in plasma insulin concentration has been reported by Wani and Kumar (2016). Ferulic acid has known to reduce hepatic glucose production by decreasing the activity of gluconeogenic enzymes (Yoshinari and Igarashi, 2010). Our results were coherent with the studies Prasath*et al.* (2014) that administration of polyherbal formulation significantly lowered blood glucose levels in HFD/STZ induced Type-2 diabetic rats.

Our study displays increased levels of serum lipids and lipo-proteins except HDL in HFD and HF fed T2D rats (Group 2) on comparison with that to control rats. Our results are in corroboration with the previously available literature (Buettner *et al.*,2006; Poudyal *et al.*,2010; Woods *et al.*,2003; Zhang *et al.*,2014). This marked increase in the lipid accumulation may be due to increased expression of adipogenic genes. Increase in the serum lipids on feeding High fat diet and High fructose has been well documented by the studies of (De Castro *et al.*2013). Increase in circulatory cholesterol may be due to the presence of cholic acid in the diet. Cholic acid enhances absorption of cholesterol into the blood stream (Senthil kumaran *et al.*,2008). Significant restoration of altered lipid profile and circulatory free fatty acids are seen after administration of GTF to type-2 diabetic rats.

The current study authenticates the anti-hyper-lipidemic activity of combination therapy in alleviating HFD and HF induced lipotoxicity mediated Type2 diabetes progression and attenuates dysregulation in the lipid metabolisms as evidenced by literature.

Our present findings displays the altered hematological indices in Type-2 diabetic group 2 rats on comparison with control rats. Erukainure and his colleagues (2013) have reported altered haematological indices in alloxan and high fibre diet induced T2D rats. Decreased levels of Hb (Kwon and Ahn, 2012), RBC (Jiang *et al,* 2003), platelets (Vinik *et al,* 2001) and infection markers like WBC (Vozarova*et.al.*2002), Neutrophils and monocytes (Van *et al.,*2004), lymphocytes were increased in high fat diet and high fructose feeding induced diabetes condition was found in our study. On GTF supplementation the altered haematological indices were reverted to normalcy, attributing the anti-inflammatory, anti-diabetic and anti-hyperlipidemic properties of the drug (Prasath *et al.,* 2014).

Glycosylated Haemoglobin (HbA1c), a key bio-marker for diabetes, shows glycemic control over the past three month period. Our result shows a marked increase in the levels of glycosylated haemoglobin in group 2 rats. Increased glucotoxicity induced oxidative stress is the prime reason for glycosylation (Hayden and Tiyagi, 2004; Giaccari *et al.,*2009). Metformin by increasing insulin sensitivity (Giannarelli *et al.,*2003) reduces blood glucose levels, which in turn might have been responsible for the observed reduction in the levels of glycosylation in group 4 rats. Oral supplementation of GTF formulation effectively lowered the levels of glycosylation in group 3 rats when compared to group 2 rats. This affirms the inhibitory action of combinatorial drug GTF against RAGE formation.

Conclusion:

The present study points out that hyperlipidemia and hyperglycemia induced alteration of haematological indices occurs in Type-2 diabetes condition. Polyherbal formulation of GTF significantly counteracted against Type-2 diabetes induced alterations of haematological indices, which play a crucial role in vascular complications associated with the disease, by reducing hyperlipidemia and hyperglycemia. However further studies are needed to evidence its mechanism of action as an anti-diabetic combinatorial drug.

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