Fenugreek Seed Extract Stabilize Plasma Lipid Levels in Type 2 Diabetes by Modulating PPARs and GLUT4 in Insulin Target Tissues

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ABSTRACT

Objective: Type 2 diabetes mellitus (T2DM) is a multifaceted disorder and the search for suitable drugs based on traditional phytotherapeutic agents continues among researchers worldwide. Fenugreek seed possess established antioxidant, hypoglycemic and lipid lowering properties but its mechanism of action at cellular level remains to be elucidated. The present study was taken up to explore the potential of fenugreek seed extract (FSE) to mediate its protective effects by modulating the expression of PPARγ, PPARα and GLUT4 genes in insulin responsive tissues of HFD fed and T2DM induced rats.

Method: Male Sprague Dawley rats in different groups were treated with FSE and their effects on biochemical markers as well as on the mRNA levels of PPARγ in adipose tissue, PPARα in liver and GLUT4 in muscle were assessed. Glibenclamide was used as positive control.

Results: HFD fed and T2DM rats showed discrepancy in blood levels of glucose, insulin, HbA1c, lipids and also in tissue levels of PPARγ, PPARα, and GLUT4 mRNA as compared to control animals. On the other hand, FSE treatment improved insulin, glucose and HbA1c levels along with regulated expression of PPAR and GLUT4 genes. Grippingly, these findings indicate the well pronounced protective effect of FSE at 300mg/kg b.w concentration and the effect was comparable to that of glibenclamide.

Conclusion: FSE exerted its effects through improvising the expression of PPARs and GLUT4 in insulin target tissues. Thus FSE offers further lead to study its effects by nutrigenomic approach and serves as a source for new antidiabetic and antiobesity drugs.

Keywords: Type 2 Diabetes mellitus, HFD, Fenugreek seeds extract,
INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a lifestyle disease and is characterized by elevated blood glucose levels, reduced insulin action or insulin deficiency. It is associated with abnormalities of glucose, lipid and protein metabolism\(^1\). As the incidence of diabetes mellitus is fast attaining epidemic proportions, successful management of this disease demands a multidisciplinary healthcare approach using a proper combination of diet, drug and exercise. An oral hypoglycemic agent stands as primary choice in the management of Type 2 diabetes and include sulfonylureas, biguanides, meglitinides, alpha-glucosidase inhibitors and thiazolidinediones are currently used. Available evidences indicate increased use of alternative medicines including plant preparations for the control of diabetes in many parts of the world\(^2,3\). The two primary approaches that could optimally control the development of T2DM are lifestyle changes and use of traditional medicinal herbs with less toxic effects. Plants have been used as traditional remedies for treating of diabetes in folklore medicine. Fenugreek (\textit{Trigonella foenum-graecum}), (Leguminaceae), a native of South East Europe and West Asia is also called as Methi in India and is one of the oldest medicinal plants whose parts have been used as a spice and vegetable in Indian culinary from time immemorial. Fenugreek seeds contain trigonelline, flavonoids, carotinoids, coumarins, saponins, galactomanan, 4-hydroxyisoleucine, proteins and lipids\(^4\). The hypoglycemic and hypolipidemic properties of fenugreek seed powder long been recognized and reported by several investigators in animal models and in humans\(^5,6,7\). The antidiabetic effect of fenugreek seeds and its phytoconstituents was reported to be mediated through improvement of insulin sensitivity in T2DM model\(^8\). Fenugreek seed powder (FSP) supplementation in diet has been shown to enhance the antioxidant potential in diabetic rats\(^9\). However further studies are required to elucidate the mechanism of action of fenugreek seed and its phytoconstituents at the cellular level. One possible mechanism may through altering the expression of Peroxisome Proliferator-Activated Receptors (PPARs). PPARs control the expression of genes related to lipid and glucose homeostasis, inflammatory responses, adipocyte differentiation and insulin action. The three isoforms of the PPAR family, i.e. PPAR\(\alpha\), PPAR\(\beta/\delta\), and PPAR\(\gamma\) have distinct tissue distribution patterns. PPAR\(\alpha\) is predominantly present in the liver and PPAR\(\gamma\) in adipose tissue, whereas PPAR\(\beta\) is ubiquitously expressed\(^10\). Altered PPAR expression is linked to the progression of diseases such as obesity, type 2 diabetes, cardiovascular diseases, cancer, hypertension, and chronic inflammation\(^11\). An intriguing link between the levels of expression of PPAR\(\gamma\) genes and development of insulin resistance has been established\(^12\).

Considering the role of fenugreek seeds in regulation of lipid metabolism\(^5,6,7\), we hypothesized that methanolic extract of fenugreek seed powder (FSE) may mediate its effects on lipid and insulin sensitizing effects by modulating the expression of PPAR\(\gamma\), PPAR\(\alpha\), and glucose transporter-4 (GLUT4) genes in insulin-responsive tissues like adipose tissue, liver, and skeletal muscle. With this inspiration, the present study was designed to assess the effect of FSE on the gene expression of PPAR\(\gamma\) in adipose tissue, PPAR\(\alpha\) in liver and GLUT4 in skeletal muscle in high fat diet fed and low dose STZ induced T2DM rats. The
levels of glucose, glycated hemoglobin (HbA1c), insulin, lipid profile in serum, glycogen content and hexokinase activity in skeletal muscle were also evaluated as markers of insulin sensitivity.

**MATERIALS AND METHODS**

High fat diet (HFD) was prepared with the following constituents – powdered NPD (365g), Lard (310g), casein (100g), Yolk(100g), Butter (100), vitamins (10g), mineral mix (10g), Methionine (3g), Yeast (1g) & NaCl (1g).

Streptozotocin was purchased from Sigma-Aldrich, All other chemicals and solvents were of the highest analytical grade.

**Preparation of plant extracts and chemoprofile of extract**

The methanolic extract of fenugreek seed powder (FSE) was prepared by mixing 100g of fenugreek seed powder (FSP) with 80% methanol and kept at room temperature for 5 days. After 5 days it was filtered and the residue was re-extracted twice under the same condition to ensure the complete extraction and the combined solvent was evaporated by rotary evaporator to get the residue. The residue was lyophilized and stored at -70°C and later this was used for feeding the experimental animals. HPTLC fingerprinting profile of FSE was developed using a mobile phase of Chloroform: Methanol: water (5:4:1) (v/v) which was standardized for the better separation and resolution of compounds.13.

**Experimental animals**

Male Sprague Dawley rats of age 8-10 weeks and with body weight 150-200g were supplied by National Centre for Laboratory Animal Sciences, National Institute of Nutrition, and Hyderabad, India. The animals were maintained in the animal house facility of Pondicherry University, in accordance with the guidelines of Committee for the purpose of Control and supervision of Experiments on Animals (CPCSEA), Govt. of India. The animals were housed in standard polypropylene cages (three rats/cage) and maintained under controlled room temperature (22±2°C) and humidity (55±5%) with 12:12 h lights and dark cycle. All rats were allowed for acclimatization for two weeks. The present work was carried out with the approval from Institutional Animal Ethical Committee, Pondicherry University (1159/C/07/CPCSEA).

**Induction of diabetes in rats**

T2DM was induced in male Sprague Dawley rats by feeding HFD for first four weeks and then administering streptozotocin (STZ) at a dose of 35mg/kg b.w by a single intraperitonial injection (ip) in 0.1 M citrate buffer (pH 4.5) to overnight fasted rats. The animals were continued on HFD for next four weeks. This would mimic natural incidence of the disease as reported earlier.14.

**Experimental Design**

The animals were divided into 8 groups of 6 rats each (n=6) and the experimental duration was for 8 weeks. Rats in Group 1 were given normal pellet diet (NPD) and they acted as control group; Group 2 rats were given FSE (300mg/kg b.w) for 8 weeks along with NPD to assess any toxic effect; Group 3 rats were given HFD alone for 8 weeks without induction of diabetes to assess the biochemical changes associated with obesity; Group 4 rats were given HFD along with FSE (300mg/kg b.w) from day one for 8 weeks; Group 5 rats were given HFD for first 4 weeks followed by STZ administration (35mg/kg b.w) and continued on HFD for the next 4 weeks; this group served as the T2DM model. Rats in Groups 6,7 and 8 were induced T2DM as in Group 5 and were given oral administration of 150mg FSE/ kg b.w, 300mg FSE/kg b.w and 10/mg of Glibenglamide /kg b.w respectively from the
3rd day after STZ administration till the end of the experimental period.

**In vitro** free radical scavenging activity of FSP and FSE by DPPH assay

The antioxidant potential of FSP and FSE was evaluated *in vitro* using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay. The samples were freshly prepared with methanol and used for *in vitro* assay. In the DPPH assay\(^{15-17}\), the decrease in absorbance of the test mixture (due to quenching of DPPH-free radical) was measured at 517 nm. The percentage of inhibition of FSP and FSE against DPPH was calculated and compared with the vitamin C standard. The percentage of inhibition was calculated according to the equation:

\[
I = (A_o - A_1/A_o) \times 100,
\]

Where \(A_o\) is the absorbance of the control reaction and \(A_1\) is the absorbance of the test compound.

At the end of the experimental period, the rats were euthanized by cervical dislocation after an overnight fasting and immediately opened surgically. Blood was collected from heart and stored at 4°C. Serum was separated by centrifuging (Remi Instruments, Mumbai, India) the blood samples at 1500 g for 10 min. Organs like liver, adipose tissue and skeletal muscle were removed, washed in ice-cold saline and homogenates were prepared using 0.1M Tris HCl buffer. Whole blood was used for estimating HbA1c by micro column method (AGGAPE-diagnostics) and hemoglobin by cyano-hemoglobin method. Serum glucose was estimated by GOD-PAP method (AGGAPE-diagnostics), serum cholesterol by CHOD-PAP (AGGAPE-diagnostics), triglycerides by GPO-PAP (AGGAPE-diagnostics), HDL-C Direct by Immuno-inhibition Mehtod (AGGAPE-diagnostics), and LDL-C Direct by Enzyme Selective Protection method (AGGAPE-diagnostics). The serum levels of creatine kinase (CK) and CK-MB were determined by using diagnostic kits (AGGAPE-diagnostics). Muscle glycogen and triglyceride levels were quantified respectively by Plummer method\(^18\) and GPO-PAP method (AGGAPE-diagnostics). Serum level of insulin was estimated by using ELISA kits as per manufacturer’s instructions (Invitrogen).

**Hexokinase activity**

Hexokinase activity in skeletal muscle was determined spectrophotometrically as follows: Tissue was homogenized in ice-cold 0.1M phosphate-buffered saline (pH 7.4). The assay mixture contained 25 mM D-glucose, 5 mM each of MgCl\(_2\), ATP and NADP in Tris glycine buffer (pH 7.6) and 150μl of the enzyme source from each of the prepared (10%) homogenate. The total volume of the assay cocktail was 2 ml in the cuvette the reaction was carried out at 37°C for 15 minutes and the absorbance noted at 340 nm. Unit of hexokinase was expressed as μU/mg of tissue protein.

**Analysis of relative gene expression of PPARα in liver, PPARγ in adipose tissue and GLUT4 in skeletal muscle by real-time quantitative PCR analysis**

Relative mRNA expression of PPARα in liver, PPARγ in adipose tissue and GLUT4 in skeletal muscle were analyzed using real-time quantitative PCR and the data was analyzed by the \(2^{-\Delta\Delta C_T}\) method\(^19\). The primers sequence used are as follows,

**PPARα**

- F5’-VTGTGGCTGCTATCATTTC
- TGTGG-3’
- R5’-VCTCCCCGCTCTCTTTTG
- AGTGC-3’
PPARγ
TTT TCA AGG GTG CCA GTT
TCA ATC C-3’
R5’-AAT CCT TGG CCC TCT GAG
AT-3’
GLUT4
F5’-GCTGTGGCCTGTTTCTC
CAA-3’
R5’-CCCATAGCCTCCGCAAC
ATA-3’
β-Actin
F5’-VGAGCGGAAATCTGTGC
GAC-3’
R5’-VGCCCTAGCATTGCG
TGGAC-3’

Statistical Analysis
Statistical analysis was performed by one way ANOVA followed by Tukey’s test using SPSS software version 7.5.

RESULTS
In the present study, we investigated the potential protective effects of FSE against changes in biochemical parameters as well as the expression of PPAR and GLUT4 mRNA in T2DM rats. We present two major results in this study: alterations in biochemical parameters including serum lipid profile, insulin levels, muscle glycogen content and hexokinase activity in HFD fed rats, T2DM induced rats and those treated with FSE; Relative expression of adipose tissue PPARγ, liver PPARα and muscle GLUT4 mRNA in HFD fed rats and T2DM rats and those treated with FSE.

In vitro free radical scavenging activity of FSP and FSE by DPPH assay
The free radical scavenging ability of FSP and the FSE was determined by DPPH assay. DPPH is a stable free radical having maximum absorbance at 517 nm that accepts an electron or hydrogen atom to become a stable diamagnetic molecule. In the presence of fenugreek seed extract the free radical nature of DPPH is lost that was determined by the decrease in its absorbance at 517 nm. In this study, the decrease in DPPH absorption in the presence of varying concentrations of FSP and the FSE was tested. FSE caused a maximum inhibition of 93.55% at 10mg/ml concentration which was comparable to that of vitamin C at 0.75 mg/ml concentration. On the other hand, the FSP caused a maximum inhibition of 33.6% (Fig.1). As FSE exhibited maximum free radical scavenging activity, its anti-diabetic potential in T2DM rats was evaluated in the in vivo studies.

The HPTLC fingerprinting of FSE
The HPTLC fingerprinting of FSE at three different concentrations (100, 200 and 300 in tracks 1, 2 and 3 respectively) indicated the presence various phytocompounds. The plate scanned at 254 and 366 nm (Fig. 2A) showed about 9 peaks with Rf ranging from 0.03 to 0.93 (Fig. 2B).

Glucose, insulin and glycated hemoglobin (HbA1c) levels
In the in vivo studies, the synergistic effect of various phytocompounds present in FSE against T2DM associated changes was evaluated by analyzing the levels of blood glucose, HbA1c and insulin. Group 3 animals that were fed on HFD alone for 8 weeks showed significant (p≤0.001) increase in serum glucose and insulin levels although Hb and HbA1c levels were not changed significantly (Table 1). On the other hand Group 4 animals that were given HFD along with FSE (300mg/kg b.w) from day one for 8 weeks showed significant (p≤0.001) reduction in blood glucose and insulin level. In Group 2 treated with FSE (300mg/kg b.w) for 8 weeks along with NPD (NPD+FSE) significant changes were not observed (Table 1). Conversely T2DM rats showed significantly (p≤0.001) elevated levels of
HbA1c and glucose with a simultaneous reduction in insulin levels as compared to control. On the other hand, treatment with FSE caused significant \((p \leq 0.001)\) reduction in blood glucose and HbA1c levels and a marked elevation in insulin levels (Table 1). Out of the two doses of FSE used in this study, the anti hyperglycemic effect of FSE was well pronounced at 300 mg/kg b.w and the values were comparable with those obtained with the standard drug glibenclamide.

Serum lipid profile

Group 3 rats fed on HFD showed significant increase in TG and LDL levels \((p \leq 0.001)\) as compared to control group and the group 4 fed on FSE along with HFD showed restoration of TG and LDL levels to normal range. Group 2 rats treated with FSE alone did not show any significant changes in the plasma lipid profile (Table 2). T2DM induced rats (group 5) displayed significantly \((p \leq 0.001)\) elevated levels of cholesterol, TG and LDL in serum while showing significant reduction in serum HDL levels. Interestingly, FSE treatment to T2DM rats (group 6 and 7) restored the levels of cholesterol, TG, LDL and HDL to normal range at both the doses used in this study. Particularly FSE at 300mg concentration was more effective than the standard drug Glibenclamide (Table 3).

Serum levels of CK and CK-MB

The serum levels of the enzymes CK and CK-MB were found to be significantly elevated \((p \leq 0.001)\) in HFD fed rats (group 3) and T2DM rats (group 5) and there was significant \((p \leq 0.001)\) reduction in CK and CK-MB in FSE treated rats (groups 4, 6, 7) and the effect was more pronounced with 300mg FSE. Rats treated with the standard drug glibenclamide (group 8) also showed similar effects (Table 3). Group 2, (NPD+FSE) significant changes were not observed (Table 3).

Hexokinase activity and glycogen content in skeletal muscle

Muscle glycogen levels and hexokinase activity are shown in Table 4. Rats fed on HFD (group 3) and T2DM rats (group 5) showed significant reduction in muscle glycogen content \((p \leq 0.001)\) as well as in hexokinase activity. In groups treated with FSE (groups 4, 6, 7) significant improvement in glycogen levels \((p \leq 0.05)\) and hexokinase activity \((p \leq 0.001)\) was observed (Table 4). The values obtained for glycogen levels and hexokinase activity in rats treated with 300mg FSE was comparable to that of standard drug glibenclamide (Table 3).

Relative expression of adipose tissue PPARγ, liver PPARα and muscle GLUT4

Prominent reduction in adipose tissue PPARγ, liver PPARα and muscle GLUT4 mRNA levels \((P \leq 0.001)\) was observed in T2DM rats as compared to control. Grippingly, in rats fed with 300mg of FSE /kg b.w after induction of diabetes the adipose tissue PPARγ \((P \leq 0.001)\) and liver PPARα \((P \leq 0.001)\) levels were significantly elevated and restored to control levels.(Fig. 3, 4). On the other hand, rats fed on HFD showed 4 fold increase in adipose tissue PPARγ \((P \leq 0.001)\) and 7 fold increase in liver PPARα \((P \leq 0.001)\) levels, while rats fed with 300mg FSE along with HFD showed significant reduction in adipose tissue PPARγ \((P \leq 0.001)\) as well as in liver PPARα \((P \leq 0.001)\) levels. In addition the muscle GLUT4 gene expression was significantly elevated in groups treated with FSE (group 4 and 7) (Fig. 5).

DISCUSSION

Under normal physiological condition, a wide range of antioxidant defenses protect the body from the adverse effects of free radicals produced in vivo but free radicals are generated more in diabetes through glucose auto-oxidation and protein glycation, developing oxidative stress. In
vitro DPPH (2, 2'-diphenyl-1-picrylhydrasyl hydrate) assay was done for screening the antioxidant activity of FSP and FSE which indicated that the water soluble fraction of FSE had more free radical scavenging activity than FSP. It may be due to abundant presence of antioxidant active principles in water soluble fraction of FSE than in crude FSP.

High fat diet has been shown to induce insulin resistance, hyperinsulinaemia and dyslipidaemia in rats and the risk of developing type 2 diabetes increases by 10 fold in obese cases. In the present study also, rats fed with HFD alone for 8 weeks showed significantly elevated serum glucose and insulin levels indicating high glucose induced pancreatic beta cell stimulation. They also displayed significant increase in serum TG and LDL levels, marked reduction in muscle glycogen levels and hexokinase activity and an associated increase in serum CK and CK-MB enzyme levels as compared to control group. Obesity is considered as one of the major contributing factors for the risk of developing insulin resistance and T2DM. In a recent report it has been shown that feeding of HFD along with administration of low dose STZ treatment can be used to induce T2DM in rats that mimic natural incidence of the disease in humans. Based on this report T2DM was induced in the present study in Sprague Dawley rats by a combination of HFD and low dose STZ treatment. In the early stage of T2DM, the predominant abnormality is reduced insulin sensitivity exhibited by peripheral tissues such as muscle and adipose tissue that contributes to hyperglycemia. In the present study also, T2DM rats showed significantly elevated levels of glucose, HbA1c and a simultaneous reduction in insulin levels. Also there was significant reduction in glycogen content and hexokinase activity in muscle. Of all the macromolecules that leak from damaged tissue, enzymes because of their tissue specificity and catalytic activity are the best markers of tissue damage. In this study, we observed significantly elevated levels serum enzymes CK and CK-MB in serum. These findings marked the onset of peripheral insulin resistance in T2DM group which can be correlated to the earlier report that feeding with HFD renders the animals insulin resistant and the slight insult by low dose of STZ compromise the β-cell function. The plasma lipid profile in T2DM group also indicated significant increase in cholesterol, TG, LDL levels along with low HDL levels. Treatment with fenugreek seeds has been shown to decrease the blood glucose levels in rats. In the present study treatment with FSE caused significant reduction in blood glucose and HbA1c levels and a marked elevation in insulin levels. FSE treatment also restored the levels of cholesterol, TG, LDL and HDL to normal range at both the doses used in this study. FSE also improved the glycogen content and hexokinase activity in muscle and caused a significant reduction in CK and CK-MB isoenzyme activity in serum suggesting the potential protective effects of FSE against peripheral tissue damage. Out of the two doses of FSE used in this study, the antihyperglycemic and antihyperlipidemic effect of FSE was well pronounced at the concentration of 300 mg/kg b.w and the values were comparable with those obtained with the standard drug glibenclamide.

PPARγ is highly expressed in white adipose tissue and induces primarily adipogenesis and lipid repartition. It regulates glucose metabolism and its expression enhances insulin sensitivity in rodent T2DM models. PPARγ is the master regulator of adipogenesis thereby stimulating the production of small-insulin sensitive adipocytes. PPARγ gene expression enhances fatty acid uptake in adipocytes and decreases lipotoxic damage to insulin-sensitive tissues. Adipose tissue specific PPARγ gene deletion studies in mice showed marked reduction of adipose tissue mass,
hypertrophy of the remaining adipocytes and associated hyperlipidemia, elevated free fatty acid levels and decreased leptin and adiponectin levels. The observations made in the present study on PPARγ mRNA levels suggested that the elevated PPARγ levels in HFD fed rats may due to increased activation of PPARγ genes which can be correlated with elevated TG and LDL levels in serum. The T2DM group on the other hand showed decreased levels of PPARγ mRNA along with dyslipidemia and decreased insulin levels. This may be explained as due to beta cell failure following streptozotocin action and subsequent PPARγ gene inactivation. Previous reports have indicated that the antidiabetic effects of plants like mulberry, Korean red ginseng, and Aegle marmelos are associated with upregulation of PPARγ expression. Similar effects are observed in the present study with FSE at 300 mg/kg b.w which increased the mRNA levels of PPARγ in adipose tissue (Fig. 3). PPARγ agonists such as thiozolidinediones activate fatty acid uptake and storage in adipose tissue while sparing other insulin sensitive tissues such as skeletal muscle, liver and beta cells from the detrimental effects of high concentrations of FFAs. FSE also acted in a similar manner in protecting insulin target issues as could be observed from the improved levels of muscle glycogen, hexokinase and CK and CK-MB activities in FSE treated groups.

PPARα which is expressed abundantly in liver activate cellular fatty acid uptake and utilization. In this study, expression of PPARα in liver and GLUT4 genes in skeletal muscle were assessed in diabetic and treated groups. Significantly elevated PPARα mRNA levels observed in HFD fed point to increased activation of PPARα genes by elevated circulating levels of serum lipids. The striking reduction in PPARα mRNA levels observed in T2DM group may be accounted with elevated plasma lipids and decreased insulin levels due to beta cell failure. FSE treatment on the other hand caused regulated expression of PPARα with significant elevation in T2DM rats treated with FSE. As skeletal muscle, accounts for >70% of the glucose removal from the serum, it is obvious that expression of GLUT4 is extremely important for overall glucose homeostasis. Our findings in the present study indicated decreased expression of muscle GLUT4 in T2DM and HFD groups that could also be correlated with the reduced muscle hexokinase activity, decreased glycogen content and elevated plasma glucose levels. In contrast, groups treated with FSE showed significant improvement in muscle GLUT4 expression. Taken together the improved expression of muscle GLUT4 gene, enhanced hexokinase activity and glycogen content in muscle as well as regulation of serum lipid levels are suggestive of a positive effect of FSE on carbohydrate and lipid metabolism in T2DM rats. Thus based on these observations the antihyperglycemic and lipid lowering effects of FSE may be considered as a combinatorial effect on different insulin target tissues. The insulin sensitizing effect of FSE may be due to its stimulating effect on insulin release, regulatory effect on lipid metabolism and maneuvering expression of PPAR and GLUT4.

CONCLUSION

From the experimental result, it can be concluded that the ameliorating effect of FSE against altered lipid and carbohydrate metabolism in T2DM animal model is mediated by its insulin sensitizing effect on peripheral tissues. With these observations, it is evident that FSE exert protective effect against T2DM associated changes specifically in adipose, liver and muscle. Thus Fenugreek seeds and its potential bioactive constituents can serve as a source of several new drugs.
REFERENCES


Table 1. Blood Hemoglobin, glycated hemoglobin, serum glucose and insulin

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hemoglobin (g/dl)</th>
<th>HbA1c (%)</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (μIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.05±0.16</td>
<td>6.4±0.26</td>
<td>87.37±5.89</td>
<td>45.52±3.39</td>
</tr>
<tr>
<td>NPD+FSE</td>
<td>15.9±1.81</td>
<td>5.9±1.12</td>
<td>91.48±8.71</td>
<td>52.73±3.89</td>
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<tr>
<td>HFD</td>
<td>14.14±1.2</td>
<td>6.92±0.75</td>
<td>137.82±9.5 *</td>
<td>87.23±5.92 *</td>
</tr>
<tr>
<td>HFD+FSE</td>
<td>15.16±0.78</td>
<td>6.04±0.43</td>
<td>93.78±4.65 **</td>
<td>60.47±2.56 **</td>
</tr>
<tr>
<td>T2DM</td>
<td>12.95±0.36 †</td>
<td>14.95±0.4 †</td>
<td>379.86±17.26 †</td>
<td>20.71±3.77 †</td>
</tr>
<tr>
<td>T2DM+FSE (150mg)</td>
<td>16.92±0.46 †</td>
<td>8.53±0.38 †</td>
<td>263.01±9.8 †</td>
<td>32.78±5.67 †</td>
</tr>
<tr>
<td>T2DM+FSE (300mg)</td>
<td>15.86±0.73 †</td>
<td>8.18±0.17 †</td>
<td>185.27±6.49 **</td>
<td>37.85±6.54 **</td>
</tr>
<tr>
<td>T2DM+ Glibenclamide</td>
<td>16.14±0.57 †</td>
<td>6.7±0.51 †</td>
<td>178.63±7.1 †</td>
<td>40.57±3.12 †</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD from 6 rats in each group. *p ≤0.05 and **p ≤0.001

Table 2. Serum lipid profile

<table>
<thead>
<tr>
<th>Groups</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
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<tbody>
<tr>
<td>Control</td>
<td>76.96±8.46</td>
<td>56.99±4.16</td>
<td>34.91±1.11</td>
<td>7.6±3.15</td>
</tr>
<tr>
<td>NPD+FSE</td>
<td>68.31±7.5</td>
<td>61.47±3.6</td>
<td>41.71±1.56</td>
<td>10.56±1.15</td>
</tr>
<tr>
<td>HFD</td>
<td>214.39±13.45 ‡</td>
<td>56.8±5.4</td>
<td>32.19±2.76</td>
<td>56.56±4.11 ‡</td>
</tr>
<tr>
<td>HFD+FSE</td>
<td>80.47±7.7 ‡</td>
<td>53.2±4.72</td>
<td>40.31±1.51</td>
<td>17.22±2.98 ‡</td>
</tr>
<tr>
<td>T2DM</td>
<td>451.21±47.89 ‡</td>
<td>154.88±10.91 ‡</td>
<td>23.93±1.81 ‡</td>
<td>76.24±1.4 ‡</td>
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<tr>
<td>T2DM+FSE (150mg)</td>
<td>74.57±9.68 ‡</td>
<td>53.64±8.09 ‡</td>
<td>47.58±2.45 ‡</td>
<td>11.04±6.09 ‡</td>
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<tr>
<td>T2DM+FSE (300mg)</td>
<td>60.67±7.5 ‡</td>
<td>52.49±4.79 ‡</td>
<td>46.75±1.87 ‡</td>
<td>6.4±0.43 ‡</td>
</tr>
<tr>
<td>T2DM+ Glibenclamide</td>
<td>81.32±3.79 ‡</td>
<td>57.81±3.91 ‡</td>
<td>35.22±2.63 ‡</td>
<td>7.78±4.29 ‡</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD from 6 rats in each group. **p ≤0.001
### Table 3. CK, CK-MB in serum, hexokinase activity and glycogen content in skeletal muscle

<table>
<thead>
<tr>
<th>Groups</th>
<th>CK U/L</th>
<th>CK-MB U/L</th>
<th>Hexokinase µU/ mg protein</th>
<th>Glycogen mg/100gm tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>557.39±28.52</td>
<td>156.8±26.75</td>
<td>0.185±0.011</td>
<td>34.64±0.53</td>
</tr>
<tr>
<td>NPD+FSE</td>
<td>466.35±43.45</td>
<td>99.52±21.43</td>
<td>0.179±0.023</td>
<td>39.75±1.73</td>
</tr>
<tr>
<td>HFD</td>
<td>1492±89.32</td>
<td>464.15±44.23</td>
<td>0.09±0.01</td>
<td>24.13±1.31</td>
</tr>
<tr>
<td>HFD+FSE</td>
<td>647.94±9.18</td>
<td>239.37±34.59</td>
<td>0.13±0.01</td>
<td>31.73±2.03</td>
</tr>
<tr>
<td>T2DM</td>
<td>1764.15±101.35</td>
<td>447.21±39.68</td>
<td>0.03±0.007</td>
<td>20.73±2.33</td>
</tr>
<tr>
<td>T2DM+FSE(150mg)</td>
<td>899.69±61.34</td>
<td>256.82±34.72</td>
<td>0.07±0.008</td>
<td>29.25±3.55</td>
</tr>
<tr>
<td>T2DM+FSE(300mg)</td>
<td>679.68±42.51</td>
<td>178.57±28.24</td>
<td>0.15±0.02</td>
<td>38.13±5.68</td>
</tr>
<tr>
<td>T2DM+ Glibenclamide</td>
<td>617.14±21.26</td>
<td>168.73±27.82</td>
<td>0.158±0.01</td>
<td>38.11±4.18</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD from 6 rats in each group. *p≤0.05 and ** p≤0.001

**Figure 1.** Free radical scavenging activity of FSP, FSE and standard antioxidant, vitamin C by DPPH assay.
Figure 2. HPTLC fingerprint profile of FSE. A. Developed HPTLC plate photographed at 254 and 366 nm showing bands which correspond to the existence of different phytoconstituents in FSE. B. HPTLC chromatogram in which each peak correspond to a various compounds .AU arbitrary unit, Rf retardation factor.
Figure 3. Relative expression of PPARγ gene in adipose tissue by real-time PCR analysis

Results are expressed as mean ± SD from 6 rats in each group. ** p≤0.001
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**Figure 5.** Relative expression of PPARα gene in Liver by real-time PCR analysis