

## Insulin sensitizing actions of fenugreek seed polyphenols, quercetin & metformin in a rat model

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**Background & objectives:** Plant polyphenols have been known to exert anti-diabetic action and promote insulin action. The present study was carried out to compare the effects of administration of fenugreek seed polyphenolic extract (FPet), quercetin and metformin (a positive control) in an acquired model of insulin resistance (IR).

**Methods:** Adult male Wistar rats divided into seven groups (n=12). IR was induced in groups (groups 2, 3, 4 and 5) by feeding a high fructose diet (FRU) (60 g/100 g diet) for 60 days. From day 16, FRU rats were administered either FPet (200 mg/kg bw) (group 3), quercetin (50mg/kg bw) (group 4) or metformin (50 mg/kg bw) (group 5) for the next 45 days. Group 1 served as normal control while groups 6 and 7 served as FPet and quercetin controls respectively. Oral glucose tolerance test (OGTT) was done on day 59 to assess glucose tolerance. At the end of 60 days, the levels of glucose, insulin, triglycerides (TG) and free fatty acids (FFA) were measured in the blood and the activities of insulin-inducible and suppressible enzymes in cytosolic and mitochondrial fractions of liver and skeletal muscle. The extent of tyrosine phosphorylation of proteins in response to insulin was determined by assaying protein tyrosine kinase (PTK) and protein tyrosine phosphatase (PTP) in liver.

**Results:** Fructose caused increased levels of glucose, insulin, TG and FFA, alterations in insulin sensitivity indices, enzyme activities and reduced glycogen content. Higher PTP activity and lower PTK activity suggest reduced tyrosine phosphorylation status. Administration of FPet or quercetin improved insulin sensitivity and tyrosine phosphorylation in fructose-fed animals and the effect was comparable with that of metformin.

**Interpretation & conclusions:** Our findings indicated that FPet and quercetin improved insulin signaling and sensitivity and thereby promoted the cellular actions of insulin in this model.

**Key words** Fenugreek seeds - fructose - insulin resistance - metformin - polyphenols - quercetin

Insulin resistance (IR) is known to be associated with dyslipidaemia, hypertension and type 2 diabetes. Feeding a high fructose diet (60g/100g) to rodents induces IR. The fructose-fed rat displays a constellation of abnormalities that includes hyperglycaemia, hyperinsulinaemia, hypertriglyceridaemia and hypertension and is suggested

to mimic the human insulin resistance syndrome/metabolic syndrome<sup>1</sup>.

Recent evidences demonstrate that reversible tyrosine phosphorylation of the insulin receptor and its cellular substrate proteins play a crucial role in the mechanism of insulin action. Insulin signaling is

initiated by binding of insulin to the tyrosine kinase receptor which in turn stimulates autophosphorylation. This enhances the intrinsic tyrosine kinase activity of the receptor and evokes a series of phosphorylation events mediating insulin action. Protein tyrosine kinases (PTK) are the cellular enzymes that phosphorylate the tyrosine residues in proteins while protein tyrosine phosphatases (PTP) are the enzymes responsible for the selective dephosphorylation of tyrosine residues. The extent of tyrosine phosphorylation on a given protein is controlled by reciprocal actions of PTP and PTK activities.

Several studies have established that many Indian plants possess hypoglycaemic activity. It has been reported that medicinal herbs and spices improve glucose metabolism and insulin action<sup>2</sup>. In India, the seeds of fenugreek (*Trigonella foenum graecum*) have been used traditionally as a treatment for diabetes. Beneficial effects of the seeds have been evidenced in experimental diabetic animals and in both type 1 and type 2 diabetic subjects<sup>3,4</sup>. It has been reported that alcoholic extract of fenugreek seeds has an antidiabetic efficacy in streptozotocin-induced diabetic rats<sup>5</sup>. Besides, fenugreek seeds are also known to be antihyperlipidaemic<sup>6</sup>.

Fenugreek seeds are a rich source of polyphenols (>100mg/kg) and quercetin (3, 5, 7, 3', 4'-pentahydroxy flavone) is one of the constituents of the seeds<sup>7</sup>. Anti-diabetic actions of quercetin in streptozotocin-induced diabetic rats have been reported previously<sup>8</sup>. However the role of fenugreek seeds polyphenols or quercetin has not been tested in the fructose-induced model of IR in which multiple metabolic abnormalities are clustered.

This study was therefore designed to compare the effect of fenugreek seed polyphenolic extract (FPEt) and quercetin individually on the metabolic components of the IR syndrome and on the insulin sensitive enzymes in tissues of rats fed high fructose diet. A well known insulin sensitizer, metformin, used alone or as a combination drug in the treatment of type 2 diabetes was chosen as a reference drug.

### Material & Methods

**Chemicals:** Quercetin dihydrate and phosphoenol pyruvate, kits for the assay of PTP and PTK were purchased from Sigma-Aldrich Chemicals Pvt Ltd, Bangalore, India. All other chemicals and solvents used were of analytical grade and were purchased from Sisco Research Laboratories (SRL) Mumbai, India.

**Animals:** Male adult Wistar rats of body weight 150-180g were obtained from the Department of Experimental Medicine, Central Animal House, Rajah Muthiah Medical College, and the experiments were carried out in Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar, Tamil Nadu, India. The rats were housed under controlled conditions (22-25°C) on a 12 h light/12 h dark cycle, and received the standard pellet diet (Karnataka State Agro Corporation Ltd., Agro feeds division, Bangalore, India) and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC), Annamalai Nagar.

**Extraction of the polyphenols from fenugreek seeds:** Polyphenols were extracted from fenugreek seeds by the method of Xia *et al*<sup>9</sup>.

**Experimental groups:** After acclimatization for a period of one week, the animals were divided into seven groups consisting of 12 rats each. The following groups were maintained for the total period of 60 days:

Group 1- received the control diet containing starch (60g/100g) and tap water.

Group 2- received the high fructose diet (60 g/100 g) and tap water.

Group 3 - received the high fructose diet and tap water; FPEt (200 mg/kg) was administered from the day 16 onwards for the next 45 days.

Group 4- received the high fructose diet and tap water; quercetin dihydrate (50 mg/kg in 0.05% dimethyl sulphoxide) was administered from day 16 for the next 45 days.

Groups 5- received the high fructose diet and tap water; metformin (50 mg/kg) was administered from day 16 for the next 45 days.

Group 6- received control diet and tap water; FPEt (200 mg/kg) was administered from day 16 for the next 45 days.

Group 7 - given control diet and tap water; quercetin dihydrate (50 mg/kg in 0.05% dimethyl sulphoxide) was administered from day 16 for the next 45 days.

FPEt, quercetin and metformin were administered by oral gavage. The dosage of FPEt<sup>10</sup>, quercetin<sup>11</sup> and metformin<sup>12</sup> used in this study are based on the previous reports. The composition of the diet and the duration of the experiment were selected based on our earlier study<sup>12</sup>.

Body weight, food intake and fluid intake were measured regularly till 60 days. On day 59, an oral glucose tolerance test was performed in each group (n=6). For this, the animals fasted overnight had access to only water. Fasting blood samples were drawn by sinoocular puncture and each animal was given an oral glucose load of 2 g/kg body weight from a 30 per cent (w/v) solution of glucose by oral gavage. Additional tail blood samples were drawn at 60 and 120 min after the glucose load. All the samples were collected in heparinised test tubes and were immediately deproteinised and used for glucose and insulin assays. Oral glucose tolerance test (OGTT) curves were drawn by plotting blood glucose (mg/dl) against time (min). Area under curve (AUC) for glucose ( $AUC_{\text{glucose}}$ ) and insulin ( $AUC_{\text{insulin}}$ ) were determined using Graph pad prism version 5.01 Software Inc, CA, USA.

On day 60, the animals were anaesthetized with ketamine hydrochloride (35 mg/kg, ip) and sacrificed by cervical dislocation. Blood was collected from jugular vein and plasma was separated by centrifuging (Remi Instruments, Mumbai, India) the blood samples at 1500 g for 10 min. Organs liver and skeletal muscle were removed and washed in ice-cold saline. Homogenates were prepared using 0.1M Tris HCl buffer at pH 7.4 and used for various biochemical assays.

*Assay of glucose, insulin, triglycerides and free fatty acids:* Plasma glucose and insulin were measured using kits obtained from Agappe Diagnostic Pvt Ltd, Kerala and Accubind microwells, Monobind Inc, CA, USA, respectively. Plasma triglycerides (TG) and free fatty acids (FFA) were measured by spectrophotometry<sup>13</sup>. Insulin sensitivity was assessed by computing insulin sensitivity index ( $ISI_{0,120}$ ), quantitative insulin sensitivity check index (QUICKI) and the homeostatic model assessment (HOMA). The formulae used were as follows:

$$ISI_{0,120} = \frac{MCR}{\text{Log MSI}}$$

Where, metabolic clearance rate

$$MCR = \frac{m}{MPG}$$

MPG= Mean plasma glucose;

MSI= Mean plasma insulin

$$m = \frac{75,000\text{mg} + (0\text{min glucose} - 120\text{min glucose}) \times 0.19 \times \text{body weight}}{120\text{min glucose}}$$

$$\text{QUICKI} = \frac{1}{\text{Log insulin (mU/l)} + \text{log glucose (mg/dl)}}$$

$$\text{HOMA} = \frac{\text{Insulin } (\mu\text{U}) \times \text{glucose (mmol/l)}}{22.7}$$

*Activities of glucose and glycogen metabolizing enzymes:* The activities of hexokinase and glucose-6-phosphatase were determined by the methods described elsewhere<sup>14</sup>, pyruvate kinase<sup>15</sup>, glycogen phosphorylase<sup>16</sup>, and fructose1, 6-bisphosphatase<sup>17</sup>. The content of glycogen in liver and skeletal muscle was measured by the standard method<sup>13</sup>. Liver and muscle mitochondria were isolated and assayed for the activities of succinate dehydrogenase<sup>13</sup> and isocitrate dehydrogenase<sup>18</sup>. Protein content in the homogenate and mitochondria was measured by the method of Lowry *et al*<sup>19</sup>.

*Assay for PTK and PTP activity:* In a set of six animals in each group, the liver tissue was stimulated with insulin by opening the abdomen and exposing the portal vein by injecting  $10^{-5}\text{M}$  insulin. Liver tissue was removed within 30 sec, weighed and used for PTK and PTP assays.

*Statistical analysis:* Significance of differences between mean values were determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.  $P < 0.05$  was considered significant.

## Results

After the oral glucose challenge at all time points, the blood glucose levels were higher in animals fed fructose diet compared to control. Fructose-fed rats treated with FPET or quercetin showed a normal response which was comparable to metformin treated rats (data not shown).

At the end of 60 days, fasting glucose and insulin levels were higher (50 and 80%) in fructose diet fed rats. IR in fructose-fed rats is indicated by a higher HOMA values and lower QUICKI and  $ISI_{0,120}$  values as compared to normal. Rats treated with FPET, quercetin or metformin showed improved insulin sensitivity. FPET reduced the levels of plasma glucose and insulin by 20 per cent, quercetin by 25 per cent and metformin by 35 per cent as compared to rats fed only fructose (Table I).

Both area under the curve (AUC) for glucose ( $AUC_{\text{glucose}}$ ) and insulin ( $AUC_{\text{insulin}}$ ) were significantly higher in fructose-fed rats as compared to that of control rats. FPET, quercetin or metformin supplementation to fructose-fed rats significantly reduced both the  $AUC_{\text{glucose}}$  and  $AUC_{\text{insulin}}$  values (Table II).

The activities of hexokinase and pyruvate kinase were reduced while the activities of glucose-6-phosphatase and fructose1, 6-bisphosphatase were

**Table I.** Levels of glucose, insulin and insulin sensitivity indices in plasma of experimental animals

Particulars	CON	FRU	FRU + FPEt	FRU + Quer	FRU + MET	CON + FPEt	CON + Quer
Glucose (mM)	4.51 ± 0.21 <sup>d</sup>	7.15 ± 0.15 <sup>a</sup>	5.66 ± 0.21 <sup>b</sup>	5.22 ± 0.37 <sup>c</sup>	4.69 ± 0.27 <sup>d</sup>	4.50 ± 0.18 <sup>d</sup>	4.47 ± 0.22 <sup>d</sup>
Insulin (µU/ml)	46.58 ± 3.87 <sup>d</sup>	83.10 ± 6.37 <sup>a</sup>	65.38 ± 3.88 <sup>b</sup>	60.03 ± 4.60 <sup>c</sup>	50.06 ± 4.20 <sup>d</sup>	45.88 ± 4.22 <sup>d</sup>	44.78 ± 2.85 <sup>d</sup>
HOMA	9.32 ± 0.76 <sup>d</sup>	26.44 ± 1.39 <sup>a</sup>	15.63 ± 0.96 <sup>b</sup>	13.23 ± 0.83 <sup>c</sup>	10.48 ± 0.81 <sup>d</sup>	9.44 ± 0.56 <sup>d</sup>	9.56 ± 0.48 <sup>d</sup>
QUICKI	0.283 ± 0.017 <sup>d</sup>	0.248 ± 0.019 <sup>a</sup>	0.257 ± 0.012 <sup>b</sup>	0.267 ± 0.021 <sup>c</sup>	0.275 ± 0.019 <sup>d</sup>	0.281 ± 0.024 <sup>d</sup>	0.282 ± 0.02 <sup>d</sup>
ISI <sub>0,120</sub>	129.03 ± 9.87 <sup>d</sup>	60.87 ± 3.56 <sup>a</sup>	84.06 ± 4.29 <sup>b</sup>	105.11 ± 5.88 <sup>c</sup>	121.47 ± 7.35 <sup>d</sup>	128.65 ± 9.19 <sup>d</sup>	129.53 ± 8.98 <sup>d</sup>

Values are means ± SD of 6 rats from each group.

HOMA= Homeostatic model assessment; QUICKI= quantitative insulin sensitivity check index; ISI<sub>0,120</sub>= insulin sensitivity index at 0 and 120 mins. CON - control rats; FRU - fructose-fed rats; FRU +FPEt - fructose fed rats treated with FPEt (200mg/kg bw); FRU +Quer - fructose fed rats treated with quercetin (50mg/kg bw); FRU +MET- fructose-fed rats treated with metformin (50mg/kg bw); CON +FPEt - Control treated with FPEt (200mg/kg bw); CON +Quer – Control rats treated with quercetin (50mg/kg bw). Values not sharing common superscript are significant with each other at  $P < 0.05$ , [ANOVA followed by Duncan's multiple range test (DMRT)].

**Table II.** Area under the curve (AUC) for glucose and insulin of control and experimental rats

Particulars	CON	FRU	FRU + FPEt	FRU + Quer	FRU + MET	CON + FPEt	CON + Quer
AUC <sub>glucose</sub> (mg/ml/min)	159.50 ± 11.23 <sup>d</sup>	271.94 ± 21.60 <sup>a</sup>	203.47 ± 12.45 <sup>b</sup>	193.50 ± 11.30 <sup>c</sup>	175.70 ± 9.30 <sup>d</sup>	158.50 ± 10.97 <sup>d</sup>	157.00 ± 10.97 <sup>d</sup>
AUC <sub>insulin</sub> (µU/ml/min)	10021 ± 823 <sup>d</sup>	16652 ± 1060 <sup>a</sup>	12990 ± 993 <sup>b</sup>	11904 ± 1030 <sup>c</sup>	10649 ± 956 <sup>d</sup>	9892 ± 653 <sup>d</sup>	9833 ± 530 <sup>d</sup>

Values are means ± SD of 6 rats from each group.

CON - control rats; FRU - fructose-fed rats; FRU +FPEt - fructose fed rats treated with FPEt (200 mg/kg bw); FRU +Quer - fructose fed rats treated with quercetin (50 mg/Kg bw); FRU +MET- fructose-fed rats treated with metformin (50 mg/Kg bw); CON +FPEt - Control treated with FPEt (200 mg/kg bw); CON +Quer – Control rats treated with quercetin (50mg/kg bw).

Values not sharing common superscript are significant with each other at  $P < 0.05$ , [ANOVA followed by Duncan's multiple range test (DMRT)].

increased in rats fed high fructose. All the three treatment procedures, FPEt, quercetin or metformin restored the activities of these enzymes to near normal (Table III). The activity of this glycogen phosphorylase enzyme and the content of glycogen were reduced in fructose fed rats. FPEt, quercetin and metformin treatment restored the activity of this enzyme and the glycogen content (Table IV). High fructose fed rats showed reduced activities of isocitrate dehydrogenase and succinate dehydrogenase enzymes. Rats fed high fructose treated with FPEt and quercetin and metformin showed normal activities of these enzymes (Table IV).

The activity of PTP was increased whereas the activity of PTK was decreased in liver homogenate of fructose-fed rats. Treatment with FPEt or quercetin restored the activities indicating a rise in tyrosine phosphorylation which was comparable to the activity of metformin treated rats (Table V).

Fructose-fed rats showed increased levels of both TG and FFA by about 2- and 2.5-fold respectively. Rats treated with quercetin showed a better reduction than FPEt and the effect was comparable to metformin (Table VI).

## Discussion

High fructose diet has been shown to induce insulin resistance, hyperinsulinaemia, and dyslipidaemia in rats, hamsters and dogs<sup>1,20</sup>.

Increased plasma concentrations of glucose in fructose-fed rats observed in this study may be attributed to decreased glucose disposal in tissues. Hyperinsulinaemia could be caused by increased insulin secretion results from hyperactivity of the pancreatic cells to glucose and an activation of sympathetic nerves<sup>21</sup>. Further, fructose feeding decreases the efficacy of insulin extraction by the liver, which retards insulin clearance from the circulation<sup>22</sup>.

The increase in two gluconeogenic enzyme activities and reduction in two glycolytic enzymes with depletion of glycogen reserve in fructose-fed rat liver observed in this study were indicative of the liver being in the gluconeogenic state. Accelerated gluconeogenesis in fructose fed rats has been reported earlier and is attributed to insulin resistance observed in these rats<sup>23</sup>. Insulin can inhibit gluconeogenesis by repressing the activities of one or both of these enzymes. It has been reported that glucose-6-phosphatase mRNA

**Table III.** Activities of hexokinase and pyruvate kinase, glucose-6-phosphatase (G6Pase) and fructose 1, 6-bisphosphatase (F16BPase) in liver and skeletal muscle of experimental animals

Parameters	CON	FRU	FRU + FPEt	FRU + Quer	FRU + MET	CON + FPEt	CON + Quer	F-value
<i>Liver:</i>								
Hexokinase (A)	0.839 ± 0.02 <sup>d</sup>	0.392 ± 0.01 <sup>a</sup>	0.656 ± 0.03 <sup>b</sup>	0.701 ± 0.009 <sup>c</sup>	0.815 ± 0.04 <sup>d</sup>	0.837 ± 0.03 <sup>d</sup>	0.831 ± 0.009 <sup>d</sup>	439.90
Pyruvate kinase (B)	113.27 ± 6.53 <sup>d</sup>	69.83 ± 4.39 <sup>a</sup>	80.88 ± 5.05 <sup>b</sup>	92.06 ± 8.42 <sup>c</sup>	106.32 ± 8.27 <sup>d</sup>	109.43 ± 6.60 <sup>d</sup>	112.87 ± 6.17 <sup>d</sup>	37.97
G6Pase (C)	4.21 ± 0.24 <sup>d</sup>	7.94 ± 0.21 <sup>a</sup>	6.31 ± 0.22 <sup>b</sup>	5.32 ± 0.25 <sup>c</sup>	4.53 ± 0.34 <sup>d</sup>	4.26 ± 0.27 <sup>d</sup>	4.25 ± 0.31 <sup>d</sup>	132.15
F1, 6BPase (C)	4.75 ± 0.19 <sup>d</sup>	8.84 ± 0.52 <sup>a</sup>	6.18 ± 0.29 <sup>b</sup>	5.60 ± 0.24 <sup>c</sup>	5.02 ± 0.21 <sup>d</sup>	4.76 ± 0.20 <sup>d</sup>	4.78 ± 0.29 <sup>d</sup>	297.85
<i>Skeletal muscle:</i>								
Hexokinase (A)	4.28 ± 0.06 <sup>d</sup>	2.25 ± 0.22 <sup>a</sup>	3.43 ± 0.25 <sup>b</sup>	3.93 ± 0.23 <sup>c</sup>	4.14 ± 0.11 <sup>d</sup>	4.28 ± 0.13 <sup>d</sup>	4.21 ± 0.25 <sup>d</sup>	109.27
Pyruvate kinase (B)	91.63 ± 5.46 <sup>d</sup>	44.95 ± 3.80 <sup>a</sup>	60.33 ± 5.59 <sup>b</sup>	71.10 ± 2.65 <sup>c</sup>	86.75 ± 5.48 <sup>d</sup>	90.92 ± 7.21 <sup>d</sup>	87.44 ± 7.68 <sup>d</sup>	46.01
G6Pase (C)	4.47 ± 0.26 <sup>d</sup>	7.85 ± 0.24 <sup>a</sup>	5.38 ± 0.28 <sup>b</sup>	5.05 ± 0.19 <sup>c</sup>	4.59 ± 0.21 <sup>d</sup>	4.46 ± 0.28 <sup>d</sup>	4.42 ± 0.25 <sup>d</sup>	173.73
F1, 6BPase (C)	4.96 ± 0.31 <sup>d</sup>	8.56 ± 0.27 <sup>a</sup>	6.06 ± 0.21 <sup>b</sup>	5.72 ± 0.29 <sup>c</sup>	5.08 ± 0.30 <sup>d</sup>	4.97 ± 0.25 <sup>d</sup>	4.90 ± 0.22 <sup>d</sup>	269.62

Values are means ± SD of 6 rats from each group.

CON - control rats; FRU - fructose-fed rats; FRU + FPEt - fructose fed rats treated with FPEt (200mg/kg bw); FRU + Quer - fructose fed rats treated with quercetin (50 mg/kg bw); FRU + MET- fructose-fed rats treated with metformin (50 mg/kg bw); CON + FPEt - Control treated with FPEt (200 mg/kg bw); CON + Quer - Control rats treated with quercetin (50 mg/kg bw).

Values not sharing common superscript are significant with each other at  $P < 0.05$ , [ANOVA followed by Duncan's multiple range test (DMRT)].

A- μmoles of glucose phosphorylated/h/mg protein; B- μmoles of pyruvate formed /min/mg protein; C- μg of Pi liberated/min/mg protein.

G6 Pase, glucose-6-phosphatase; F1, 6B Pase, fructose 1, 6-bisphosphatase

**Table IV.** Activity of glycogen phosphorylase (GP) and the content of glycogen in cytosolic fraction and the activities of isocitrate dehydrogenase (ICDH) and succinate dehydrogenase (SDH) in mitochondria liver and skeletal muscle of experimental animals

Parameters	CON	FRU	FRU + FPEt	FRU + Quer	FRU + MET	CON + FPEt	CON + Quer	F-value
<i>GP (A):</i>								
Liver	4.11 ± 0.21 <sup>d</sup>	7.44 ± 0.30 <sup>a</sup>	6.43 ± 0.29 <sup>b</sup>	5.47 ± 0.45 <sup>c</sup>	4.40 ± 0.27 <sup>d</sup>	4.15 ± 0.22 <sup>d</sup>	4.06 ± 0.20 <sup>d</sup>	136.56
Skeletal muscle	5.29 ± 0.23 <sup>d</sup>	8.55 ± 0.33 <sup>a</sup>	7.32 ± 0.24 <sup>b</sup>	6.47 ± 0.22 <sup>c</sup>	5.57 ± 0.15 <sup>d</sup>	5.27 ± 0.23 <sup>d</sup>	5.31 ± 0.22 <sup>d</sup>	174.92
<i>Glycogen (B):</i>								
Liver	54.78 ± 5.21 <sup>d</sup>	32.14 ± 3.12 <sup>a</sup>	39.91 ± 3.85 <sup>b</sup>	45.54 ± 3.93 <sup>c</sup>	51.59 ± 5.00 <sup>d</sup>	55.10 ± 4.65 <sup>d</sup>	53.78 ± 4.96 <sup>d</sup>	22.81
Skeletal muscle	72.21 ± 4.97 <sup>d</sup>	44.54 ± 3.68 <sup>a</sup>	58.58 ± 2.06 <sup>b</sup>	62.64 ± 3.51 <sup>c</sup>	69.85 ± 3.18 <sup>d</sup>	72.56 ± 2.64 <sup>d</sup>	71.25 ± 2.62 <sup>d</sup>	53.90
<i>ICDH (A):</i>								
Liver	741.2 ± 28.8 <sup>d</sup>	538.8 ± 26.1 <sup>a</sup>	628.5 ± 20.5 <sup>b</sup>	668.5 ± 29.8 <sup>c</sup>	710.3 ± 15.6 <sup>d</sup>	740.8 ± 29.0 <sup>d</sup>	732.3 ± 21.2 <sup>d</sup>	55.00
Skeletal muscle	541.0 ± 29.8 <sup>d</sup>	381.6 ± 21.4 <sup>a</sup>	435.7 ± 26.8 <sup>b</sup>	464.6 ± 31.4 <sup>c</sup>	514.9 ± 30.9 <sup>d</sup>	542.1 ± 22.8 <sup>d</sup>	540.3 ± 25.9 <sup>d</sup>	50.17
<i>SDH (B):</i>								
Liver	28.74 ± 2.56 <sup>d</sup>	11.49 ± 0.96 <sup>a</sup>	15.74 ± 0.99 <sup>b</sup>	18.91 ± 1.58 <sup>c</sup>	26.23 ± 1.94 <sup>d</sup>	28.54 ± 2.45 <sup>d</sup>	28.58 ± 2.12 <sup>d</sup>	77.15
Skeletal muscle	25.26 ± 1.98 <sup>d</sup>	8.92 ± 0.84 <sup>a</sup>	13.34 ± 1.22 <sup>b</sup>	15.85 ± 1.41 <sup>c</sup>	22.95 ± 1.54 <sup>d</sup>	25.39 ± 2.01 <sup>d</sup>	25.17 ± 1.97 <sup>d</sup>	66.44

Values are means ± SD of 6 rats from each group.

CON - control rats; FRU - fructose-fed rats; FRU + FPEt - fructose fed rats treated with FPEt (200 mg/kg bw); FRU + Quer - fructose fed rats treated with quercetin (50 mg/kg bw); FRU + MET- fructose-fed rats treated with metformin (50mg/kg b.w); CON + FPEt - Control treated with FPEt (200 mg/kg bw); CON + Quer - Control rats treated with quercetin (50 mg/kg bw); ICDH, isocitrate dehydrogenase; SDH, succinate dehydrogenase; GP, glycogen phosphorylase. Values not sharing common superscript are significant with each other at  $P < 0.05$ , [ANOVA followed by Duncan's multiple range test (DMRT)].

A=μmoles of Pi liberates/h/mg protein; B= mg of glucose/g tissue; c=n moles of α-ketoglutarate liberated /h/mg protein; D= μ moles of succinate oxidized /min/mg protein.

**Table V.** Activities of insulin signaling enzymes such as protein tyrosine phosphatases (PTP) and protein tyrosine kinases (PTK) in liver of experimental animals

Parameters	CON	FRU	FRU + FPEt	FRU + Quer	FRU + MET	CON + FPEt	CON + Quer	F-value
PTP ( $A_{620}$ )	0.458 ± 0.02 <sup>d</sup>	0.731 ± 0.04 <sup>a</sup>	0.595 ± 0.03 <sup>b</sup>	0.567 ± 0.02 <sup>c</sup>	0.475 ± 0.02 <sup>d</sup>	0.463 ± 0.03 <sup>d</sup>	0.460 ± 0.04 <sup>d</sup>	374.17
PTK ( $A_{492}$ )	0.672 ± 0.04 <sup>d</sup>	0.335 ± 0.01 <sup>a</sup>	0.597 ± 0.04 <sup>b</sup>	0.637 ± 0.03 <sup>c</sup>	0.659 ± 0.02 <sup>d</sup>	0.670 ± 0.04 <sup>d</sup>	0.674 ± 0.03 <sup>d</sup>	479.64

Values are means ± SD of 6 rats from each group.  
 CON, control rats; FRU, fructose-fed rats; FRU + FPEt, fructose fed rats treated with FPEt (200 mg/kg bw); FRU + Quer - fructose fed rats treated with quercetin (50 mg/kg bw);  
 FRU + MET- fructose-fed rats treated with metformin (50 mg/kg bw); CON + FPEt - Control treated with FPEt (200 mg/kg bw); CON + Quer - Control rats treated with quercetin  
 (50 mg/kg bw).  
 Values not sharing common superscript are significant with each other at  $P < 0.05$ , [ANOVA followed by Duncan's multiply range test (DMRT)].

**Table VI.** Levels of triglycerides (TG) and free fatty acids (FFA) in plasma of control and experimental animals

Parameters	CON	FRU	FRU + FPEt	FRU + Quer	FRU + MET	CON + FPEt	CON + Quer	F-value
TG (mg/dl)	89.01 ± 4.20 <sup>d</sup>	163.42 ± 5.39 <sup>a</sup>	128.41 ± 6.08 <sup>b</sup>	117.47 ± 8.63 <sup>c</sup>	94.30 ± 3.34 <sup>d</sup>	89.71 ± 4.36 <sup>d</sup>	87.12 ± 5.94 <sup>d</sup>	98.16
FFA (mg/dl)	25.68 ± 2.42 <sup>d</sup>	72.24 ± 6.35 <sup>a</sup>	58.12 ± 4.69 <sup>b</sup>	47.99 ± 2.56 <sup>c</sup>	31.17 ± 2.67 <sup>d</sup>	26.84 ± 1.95 <sup>d</sup>	24.29 ± 1.54 <sup>d</sup>	63.54

Values are means ± SD of 6 rats from each group.  
 CON, control rats; FRU, fructose-fed rats; FRU + FPEt, fructose fed rats treated with FPEt (200 mg/kg bw); FRU + Quer - fructose fed rats treated with quercetin (50 mg/kg bw);  
 FRU + MET- fructose-fed rats treated with metformin (50 mg/kg bw); CON + FPEt - Control treated with FPEt (200 mg/kg bw); CON + Quer - Control rats treated with quercetin  
 (50 mg/kg bw).  
 Values not sharing common superscript are significant with each other at  $P < 0.05$ , [ANOVA followed by Duncan's multiply range test (DMRT)].

levels increase in liver after exposure to fructose even in the presence of physiological concentrations of glucose<sup>24</sup>.

Protein tyrosine phosphatases play an integral role within the insulin signaling cascade by negatively regulating signal transduction. Decreased PTK activity and increased PTP activity in liver was observed in rats fed with high fructose. PTP functions in concert with PTK to balance the cellular level of phosphotyrosine. Increased PTP and decreased PTK activity has been reported in several rodent models of insulin resistance<sup>25</sup>. Researchers have reported the reduced insulin receptor kinase in sucrose-fed rats<sup>26</sup> and reduction in phosphorylation of pp185, insulin receptor substrate-1/2 (IRS-1/2) in liver and skeletal muscle of fructose-fed rats<sup>27</sup>.

An overload of fructose to the liver perturbs the glucose metabolism and glucose uptake pathways leading to the enhanced rate of *de novo* lipogenesis and TG synthesis ultimately inducing insulin resistance. Elevated FFA has a stimulatory effect on glucose-6-phosphatase, the terminal enzyme of glycogenolysis by allosteric mechanism which in turn increases hepatic glucose production resulting in hyperglycaemia<sup>28</sup>.

The antihyperglycaemic effects of fenugreek seeds and quercetin in animal models have been recorded. Raghuram *et al*<sup>4</sup> reported that the hypoglycaemic action of fenugreek seeds was associated with an increase in molar insulin binding sites of erythrocytes, which might enhance glucose utilization. Quercetin at 10-50 mg/kg normalizes blood glucose level, augments liver glycogen content in alloxan-induced diabetic rats<sup>29</sup>.

In this study, the dysregulation of glucose metabolism observed in fructose-fed rats was overcome when treated with FPEt or quercetin. This could be possible because of the improved insulin action as suggested by insulin sensitivity indices, changes in both gluconeogenic and glycogenolytic enzymes in these two groups reflect that both FPEt and quercetin could reduce basal hepatic output through an effect on hepatic gluconeogenesis and glycogen breakdown. In this regard, the effects resemble the well known actions of metformin<sup>12</sup>. Reduction in plasma FFA levels in these animals may contribute to insulin action since elevated levels of plasma FFA also inhibit insulin-stimulated glucose transport/glucose uptake, glucose oxidation and glycogen synthesis in human skeletal muscle<sup>30</sup>. Activation of PTK and reduction in PTP by FPEt and

quercetin show that both have the ability not only to increase the extent of tyrosine phosphorylation but also to prolong the signaling. It has been reported that fenugreek seed aqueous extract activated the tyrosine phosphorylation of insulin receptor- $\beta$  subunit (IR- $\beta$ ), subsequently enhancing tyrosine phosphorylation of IRS-1 and the p85 subunit of phosphatidylinositol-3-kinase (PI3-K)<sup>31</sup>.

In conclusion, the present data show that the insulin signaling and sensitizing effects of polyphenolic portion of fenugreek seeds and quercetin in an *in vivo* model of insulin resistance were comparable with that of metformin. The molecular mechanisms such as activation of downstream signaling events including insulin receptor substrate activation and glucose uptake need to be elucidated.

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