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ORIGINAL CONTRIBUTION

Naringenin enhances insulin-stimulated tyrosine phosphorylation and improves the cellular actions of insulin in a dietary model of metabolic syndrome

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Abstract
Background There is a growing interest in identifying putative insulin sensitizers from the herbal sources.
Aim of the study The present study explores the effects of naringenin, a bioflavonoid, in the high fructose-induced model of insulin resistance.
Methods Adult male Wistar rats were divided into two groups and were fed either a starch-based control diet or a high fructose diet (60 g/100 g) for 60 days. From the 16th day, rats in each group were divided into two, one of which was administered naringenin (50 mg/kg b.w.) and the other was untreated for the next 45 days. Oral glucose tolerance test (OGTT) was done on day 59. On day 60, the levels of glucose, insulin, triglycerides (TG), free fatty acids (FFA) in blood, and the activities of insulin-inducible and suppressible enzymes in the cytosolic and mitochondrial fractions of liver and skeletal muscle were assayed. The extent of protein tyrosine phosphorylation in response to insulin was determined by assaying protein tyrosine kinase (PTK) and protein tyrosine phosphatase (PTP) in liver. Liver histology with periodic acid-Schiff (PAS) staining was done to detect glycogen.
Results Fructose administration increased the plasma levels of glucose, insulin, TG, and FFA as compared to control rats. Insulin resistance was indicated by alterations in insulin sensitivity indices. Alterations in enzyme activities and reduced glycogen content were observed in fructose-fed rats. PTP activity was higher, while PTK activity was lower suggesting reduced tyrosine phosphorylation status. Administration of naringenin improved insulin sensitivity and enhanced tyrosine phosphorylation in fructose-fed animals, while it did not affect the parameters in control diet-fed rats.
Conclusions Naringenin improves insulin signaling and sensitivity and thereby promotes the cellular actions of insulin in this model.

Keywords Fructose · Insulin resistance · Rats · Naringenin · Tyrosine phosphorylation

Abbreviations

HOMA	Homeostatic model assessment
FFA	Free fatty acids
ISI	Insulin sensitivity index
NAD^+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide reduced
OGTT	Oral glucose tolerance test
PTP	Protein tyrosine phosphatase
PTK	Protein tyrosine kinase
PAS	Periodic acid-Schiff
QUICKI	Quantitative insulin sensitivity check index
STZ	Streptozotocin
TG	Triglycerides

Introduction

Flavonoids are found in many plant-based foods including fruits and vegetables, and comprise the major dietary group of plant polyphenols. Naringenin, (4,5,7-trihydroxy-flavanone-7 rhamnoglucoside), is a naturally occurring

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flavanone glycoside obtained from citrus fruits and grapefruit. Naringenin has been reported to be useful in treating gastric ulcer, cancer [49] and atherosclerosis [27]. Naringenin is one of the predominant flavonoids [46] in fenugreek seeds, the administration of which has therapeutic effects on animal models of insulin-deficient and insulin-resistant diabetes [17].

Insulin resistance is a condition wherein the hepatic and peripheral tissues become resistant to insulin resulting in defects in glucose and lipid metabolism. Studies in rats have demonstrated that a high dosage of fructose produces a decline in whole-body insulin sensitivity [2] and induces a constellation of abnormalities that includes hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and hypertension that parallel the human metabolic syndrome [43]. This model is widely used to identify putative insulin sensitizers from natural sources [31, 58].

Binding of insulin to the heterodimeric transmembrane receptor stimulates autophosphorylation of the receptor. This enhances its intrinsic tyrosine kinase activity and evokes a series of protein phosphorylation events. Protein tyrosine kinases (PTK) are the cellular enzymes that phosphorylate the tyrosine residues in proteins while protein tyrosine phosphatases (PTP) selectively dephosphorylate the tyrosine residues. The extent of tyrosine phosphorylation on a given protein is controlled by the reciprocal actions of PTP and PTK [45]. Agents which enhance tyrosine phosphorylation could act as effective insulin sensitizers [57].

Literature search revealed that naringenin exerts antihyperglycemic effects in streptozotcin (STZ)-induced diabetic rats as well as in db/db mice model [1, 20]. Type 2 diabetes is associated with cluster of abnormalities such as hypertension, endothelial damage, inflammation, cardiac hypertrophy, atherosclerosis, fibrosis, and complications. STZ induces type 1 diabetes that can be relevant only to the small proportion of the diabetic patients. Diet-induced models of type 2 diabetes rather than STZ-induced model of type 1 diabetes are suggested to serve as a better model to investigate the possible intervention of these complications [53]. Therefore, the current study was undertaken to examine the impact of naringenin on insulin sensitivity indices and tyrosine phosphorylation status in this rat model. The insulin-sensitive enzymes of glucose metabolism in tissues were also assayed.

Materials and methods

Animals

Male Wistar rats of body weight 150–180 g were obtained from the Department of Experimental Medicine, Central Animal House, Rajah Muthiah Medical College, Annamalai Nagar. They were housed under controlled conditions (22–25°C) on a 12-h light/12-h dark cycle. They all received a standard pellet diet (Karnataka State Agro Corporation Ltd., Agro feeds division, Bangalore, India) and water ad libitum. The animals were cared as per the principles and guidelines of the Institutional Animal Ethical Committee (IAEC) in accordance with the Committee for the Purpose of Control and Supervision on Experimental Animals guidelines on animal care. The study protocol was approved by the IAEC.

Experimental groups

After acclimatization for a period of 1 week, the animals were divided into four groups. Group 1 rats received the control diet containing 60% corn starch, 20% casein, 0.7% methionine, 5% ground nut oil, 10.5% wheat bran, and 3.5% salt mixture and received 0.5% carboxymethyl cellulose. Group 2 rats received the high fructose diet in which corn starch in control diet was replaced by fructose. Group 3 rats were fed the high fructose diet and from the 16th day onwards, they were fed naringenin (50 mg/Kg in 0.5% carboxymethyl cellulose). Group 4 rats received control starch diet and naringenin (50 mg/Kg b.w. in carboxymethyl cellulose) from the 16th day. Diet and water were provided ad libitum. Naringenin was administered by oral gavage and the dosage used in this study is based on the previous report in the literature [28]. The total duration of the experiment was 60 days.

Body weight, food intake, and fluid intake were measured regularly till the end of 60 days. On day 59, an oral glucose tolerance test (OGTT) was performed in rats ($n = 6$) from each group. 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% (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 assayed for glucose and insulin. OGTT curves were drawn by plotting blood glucose (mg/dl) against time (min). Area under curve for glucose ($AUC_{glucose}$) and insulin ($AUC_{insulin}$) were determined using Graph Pad Prism version 5.01 Software Inc, CA, USA.

On day 60, the animals in each group ($n = 6$) were anesthetized with ketamine hydrochloride (35 mg/Kg, i.p) and killed by cervical dislocation. Blood was collected from jugular vein and plasma was separated by centrifuging the blood samples at 1,500×g for 10 min. Liver and skeletal muscle were removed and washed in ice-cold saline. Homogenates were prepared using 0.1 M Tris–HCl buffer, pH 7.4.

Table 1 Body weight, food intake and levels of glucose, insulin, TG and FFA and insulin sensitivity indices in experimental animals

Particulars	CON	FRU	FRU + NAR	CON + NAR	ANOVA[2]		
					Diet	Treatment	Interaction
Final body weight (g)	220.56 ± 10.21	230.00 ± 10.4	225.45 ± 12.55	218.62 ± 15.35	NS	NS	NS
Food intake (g/day)	18.56 ± 0.56	16.95 ± 1.25	17.50 ± 0.95	18.00 ± 1.31	NS	NS	NS
Glucose (mM)	4.51 ± 0.21[c]	7.15 ± 0.15[a]	5.01 ± 0.37[b]	4.52 ± 0.21[c]	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Insulin (μU/ml)	46.58 ± 3.87[c]	83.10 ± 6.38[a]	56.89 ± 5.42[b]	45.95 ± 3.46[c]	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
HOMA	9.32 ± 0.76[c]	26.44 ± 1.39[a]	12.25 ± 0.87[b]	9.61 ± 0.67[c]	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
QUICKI	0.283 ± 0.017[c]	0.248 ± 0.019[a]	0.268 ± 0.015[b]	0.285 ± 0.016[c]	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
$ISI_{0,120}$	133.18 ± 9.87[c]	60.32 ± 3.56[a]	115.21 ± 6.56[b]	137.23 ± 9.65[c]	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
TG (mg/dl)	89.01 ± 4.20[c]	163.42 ± 5.39[a]	109.14 ± 9.55[b]	88.79 ± 4.58[c]	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
FFA (mg/dl)	25.68 ± 2.43[c]	72.24 ± 6.35[a]	41.49 ± 2.89[b]	25.79 ± 1.76[c]	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
$AUC_{glucose}$ (mg/min/ml)	159.50 ± 11.23	271.94 ± 21.60	203.47 ± 12.45	157.00 ± 10.97	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
$AUC_{insulin}$ (μU/ml/min)	10021 ± 823	16652 ± 1060	12990 ± 993	9833 ± 530	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Values are mean ± SD of six rats from each group

CON control rats received 0.5% CMC as vehicle, *FRU* fructose-fed rats, *FRU + NAR* fructose-fed rats treated with naringenin (50 mg/kg b.w.), *CON + NAR* control rats treated with naringenin (50 mg/kg b.w.). Values not sharing common superscript are significant with each other

$P < 0.05$, ANOVA followed by DMRT

Assay of glucose, insulin, triglycerides, and free fatty acids

Plasma glucose and insulin were measured using kits obtained from Agappe Diagnostics Pvt Ltd, Kerala and Accubind Microwells, Monobind Inc., CA, USA, respectively. Triglycerides (TG) [11] and free fatty acids (FFA) [10] were assayed in plasma. Insulin sensitivity was assessed by computing insulin sensitivity index ($ISI_{0,120}$) [16], quantitative insulin sensitivity check index (QUICKI) [21], and homeostatic model assessment (HOMA) [35].

Measurement of glucose and glycogen metabolizing enzymes activities and NAD^+/NADH ratio

The activities of hexokinase [7], pyruvate kinase [56], glycogen phosphorylase [9], glucose-6-phosphatase [24], and fructose 1,6-bisphosphatase [15] were assayed by standard procedures. Glycogen content in liver and muscle were determined by the method of Morales et al. [38]. Liver and muscle mitochondria were isolated according to the procedure of Johnson and Lardy [19] and assayed for isocitrate dehydrogenase [22] and succinate dehydrogenase [48] activities. The concentrations of NAD^+ and NADH in the liver were measured by the method of Matsumura and Miyachi [36]. Protein content in the homogenate and mitochondria was measured by the method of Lowry et al. [32].

Assay for PTK and PTP activity

The liver tissue was stimulated with insulin by opening the abdomen and exposing the portal vein by injecting 10^{-5} M insulin. Liver tissue was removed within 30 s, weighed, and processed for the assay of PTK [42] and PTP [33] using ELISA kits (Sigma–Aldrich, Inc., St. Louis, MO, USA).

Histology

A portion of the liver was placed in neutral buffered formalin (10% formalin in phosphate buffer) and later sectioned using a microtome of thickness 3–5 μm and stained with periodic acid-Schiff (PAS) reagent to detect glycogen.

Effect of naringenin on intestinal glucose absorption

The effect of naringenin on intestinal glucose absorption was measured by the method of Sugimoto et al. [52]. A set of 12 rats maintained on the starch diet were starved for 16 h and orally given naringenin (0, 10, 30, and 50 g/Kg body weight dissolved in 0.5 ml of 0.5% carboxymethyl cellulose, $n = 3$ for each dosage). Ten minutes later, the rats were orally administered glucose (2 g/kg body weight dissolved in 0.5 ml water). Blood (about 0.5 ml) was sampled from the portal vein under diethyl ether anesthesia at 30 min after the administration of glucose. The concentration of glucose in the portal vein was measured.

Statistical analysis

Values are mean ± SD for six rats. Statistical evaluation was done by two way analysis of variance and considering diet and treatment as two factors using Graph Pad Prism version 5.01 Software Inc, CA, USA. The results of

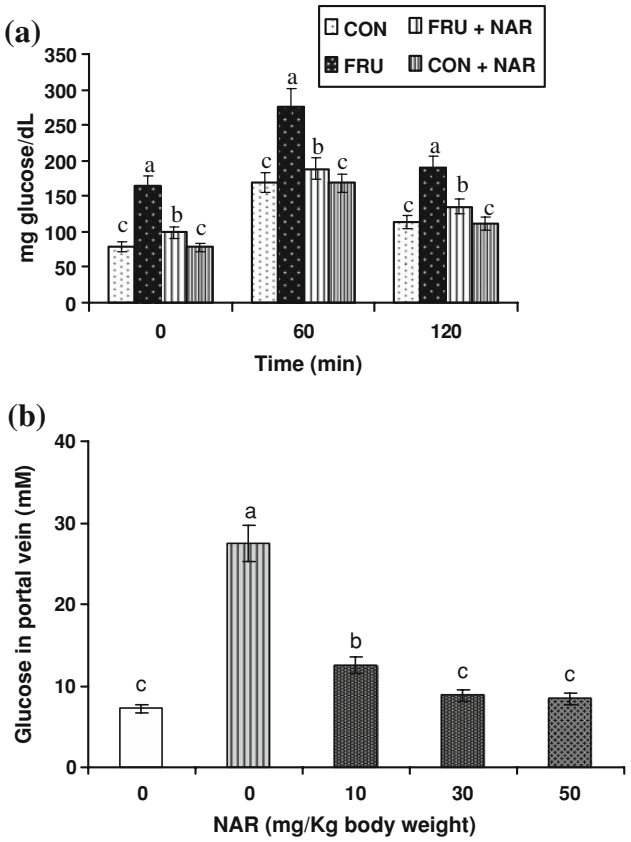

Fig. 1 **a** Oral glucose tolerance test curves of experimental animals. Values are mean ± SD ($n = 6$). *CON* control rats, *FRU* fructose-fed rats, *FRU + NAR* fructose-fed rats treated with naringenin (50 mg/kg b.w.), *CON + NAR* control rats treated with naringenin (50 mg/kg b.w.). Values not sharing common superscript are significant with each other at $P < 0.05$, ANOVA followed by DMRT. **b** Inhibition of intestinal absorption of glucose by naringenin. *Open bar* Rats did not receive glucose and naringenin. *Lined bar* Rats administered glucose alone. *Dotted bar* Rats administered glucose and naringenin (10, 30, 50 mg/kg b.w.). Values are means ± SD of three rats. Values not sharing common superscript are significant with each other at $P < 0.05$, ANOVA followed by DMRT

glucose absorption studies were mean ± SD of three measurements for which statistical evaluation was done by unpaired Student's t test. A value of $P < 0.05$ was considered to be significant.

Results

The daily food intake and mean final body weights of the animal groups are given in Table 1. No significant differences in the mean final body weight were found between the experimental groups. Daily food consumption was also found to be similar in the various experimental groups.

Figure 1a shows the blood glucose levels after the oral glucose load. At all time points, the levels were higher in naringenin-unsupplemented fructose-fed rats as compared to control. Fructose-fed rats treated with naringenin showed a normal response.

Figure 1b represents the effect of naringenin on the intestinal absorption of glucose. In rats treated with glucose alone, the glucose concentration was greatly elevated at 30 min, while in rats treated with glucose and naringenin, the glucose concentration was significantly lowered. The inhibitory effect of glucose absorption was found to be dose-dependent.

Both $AUC_{glucose}$ and $AUC_{insulin}$ were significantly higher in fructose-fed rats as compared to control rats. Naringenin supplementation significantly reduced both the $AUC_{glucose}$ and $AUC_{insulin}$ values (Table 1).

The levels of plasma glucose, insulin, TG, and FFA in experimental animals are shown in Table 1. Fasting glucose and insulin levels were higher (50 and 80%) in fructose diet-fed rats. Naringenin treated rats showed 50% reduction in both plasma glucose and insulin as compared to rats fed only fructose. Fructose-fed rats showed increased levels of both TG and FFA by about 2 and 2.5-fold, respectively. Rats treated with naringenin showed significant reduction compared to rats fed fructose diet alone (Table 1). The values of HOMA, QUICKI, and $ISI_{0,120}$ are given in Table 1. In fructose-fed rats, insulin resistance (IR) was evident from significant alterations in HOMA, QUICKI, and $ISI_{0,120}$ values as compared to control rats. Rats treated with naringenin showed improved insulin sensitivity.

Table 2 gives the activities of key enzymes of glycolysis and gluconeogenesis in liver and muscle. The activities of hexokinase and pyruvate kinase were reduced, while the activities of glucose-6-phosphatase and fructose 1,6-bisphosphatase were increased in rats fed high fructose. Treatment with naringenin restored the activities of these enzymes to near normal.

The activity of glycogen phosphorylase and the content of glycogen were reduced in high fructose-fed rats compared to control diet-fed rats (Table 3). The activities of isocitrate dehydrogenase and succinate dehydrogenase in liver and muscle were reduced in high fructose-fed rats as shown in Table 3. Naringenin treatment restored the activities of these enzymes and the glycogen content.

The total NAD^+/NADH ratio in liver and skeletal muscle is shown in the Table 3. The ratio was markedly lower in rats fed the high fructose diet than the control rats ($P < 0.05$). This reduction was prevented in the fructose-fed rats treated with naringenin.

Figure 2a and b present the absorbance of PTK and PTP in rat liver in response to insulin stimulation. The activity of PTP was increased, whereas the activity of PTK was decreased in liver homogenate of fructose-fed rats. Treatment with naringenin restored the activities indicating a rise in tyrosine phosphorylation.

Table 2 Activities of hexokinase and pyruvate kinase, glucose-6-phosphatase (G6Pase) fructose 1,6-bisphosphatase (F16BPase) in liver and skeletal muscle of experimental animals

Particulars	CON	FRU	FRU + NAR	CON + NAR	ANOVA		
					Diet	Treatment	Interaction
Liver							
Hexokinase (A)	0.839 ± 0.02^{c}	0.392 ± 0.01^{a}	0.720 ± 0.03^{b}	0.835 ± 0.05^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Pyruvate kinase (B)	113.27 ± 6.53^{c}	69.83 ± 4.39^{a}	96.89 ± 8.55^{b}	112.87 ± 6.14^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
G6Pase (C)	4.21 ± 0.24^{c}	7.94 ± 0.21^{a}	5.02 ± 0.27^{b}	4.25 ± 0.31^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
F16BPase (C)	4.75 ± 0.19^{c}	8.84 ± 0.52^{a}	5.45 ± 0.28^{b}	4.78 ± 0.17^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Skeletal muscle							
Hexokinase (A)	4.28 ± 0.06^{c}	2.25 ± 0.22^{a}	3.88 ± 0.23^{b}	4.24 ± 0.06^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Pyruvate kinase (B)	91.63 ± 5.46^{c}	44.95 ± 3.80^{a}	77.44 ± 3.61^{b}	89.11 ± 7.68^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
G6Pase (C)	4.47 ± 0.26^{c}	7.85 ± 0.24^{a}	4.90 ± 0.22^{b}	4.45 ± 0.25^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
F16BPase (C)	4.96 ± 0.31^{c}	8.56 ± 0.27^{a}	5.49 ± 0.29^{b}	4.95 ± 0.15^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Values are mean ± SD of six rats from each group

CON control rats received 0.5% CMC as vehicle. *FRU* fructose-fed rats, *FRU + NAR* fructose-fed rats treated with naringenin (50 mg/kg b.w.), *CON + NAR* control rats treated with naringenin (50 mg/kg b.w.)

Values not sharing common superscript are significant with each other. $P < 0.05$, ANOVA followed by DMRT

A micromoles of glucose phosphorylated/h/mg protein, *B* micromoles of pyruvate formed/min/mg protein, *C* microgram of Pi liberated/min/mg protein

Table 3 Content of glycogen and activities of glycogen phosphorylase (GP) in cytosolic, isocitrate dehydorgenase (ICDH) and succinate dehydrogenase in mitochondiral and NAD^{+}/NADH ratio in cytosolic fraction of liver and skeletal muscle of experimental animals

Particulars	CON	FRU	FRU + NAR	CON + NAR	ANOVA		
					Diet	Treatment	Interaction
GP (A)							
Liver	4.11 ± 0.21^{c}	7.44 ± 0.30^{a}	5.18 ± 0.41^{b}	4.09 ± 0.17^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Skeletal muscle	5.29 ± 0.23^{c}	8.55 ± 0.33^{a}	6.35 ± 0.20^{b}	5.28 ± 0.24^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Glycogen (B)							
Liver	54.78 ± 5.21^{c}	32.14 ± 3.12^{a}	46.21 ± 3.61^{b}	54.11 ± 4.96^{c}	$P < 0.0001$	$P < 0.0012$	$P < 0.0005$
Skeletal muscle	72.21 ± 4.97^{c}	44.54 ± 3.68^{a}	65.49 ± 3.55^{b}	71.75 ± 2.62^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
ICDH (C)							
Liver	743.6 ± 31.8^{c}	538.8 ± 26.1^{a}	673.0 ± 28.7^{b}	741.8 ± 31.3^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Skeletal muscle	541.0 ± 29.8^{c}	381.6 ± 21.4^{a}	473.7 ± 14.5^{b}	538.3 ± 25.8^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
SDH (D)							
Liver	28.74 ± 2.56^{c}	11.49 ± 0.96^{a}	21.11 ± 1.57^{b}	28.57 ± 2.26^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Skeletal muscle	25.26 ± 1.98^{c}	8.92 ± 0.84^{a}	18.01 ± 0.94^{b}	25.17 ± 2.40^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
NAD^{+}/NADH ratio							
Liver	7.45 ± 0.56^{c}	4.18 ± 0.61^{a}	6.89 ± 0.34^{b}	7.41 ± 0.66^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Skeletal muscle	4.28 ± 0.24^{c}	3.22 ± 0.27^{a}	3.98 ± 0.22^{b}	4.32 ± 0.25^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Values are mean ± SD of six rats from each group

CON control rats received 0.5% CMC as vehicle, *FRU* fructose-fed rats, *FRU + NAR* fructose-fed rats treated with naringenin (50 mg/kg b.w.), *CON + NAR* control rats treated with naringenin (50 mg/kg b.w.)

Values not sharing common superscript are significant with each other. $P < 0.05$, ANOVA followed by DMRT

A micromoles of Pi liberates/h/mg protein, *B* milligram of glucose/g tissue, *C* nanomoles of α-ketoglutarate liberated/h/mg protein, *D* micromoles of succinate oxidized/min/mg protein

Figure 3 shows the sections of liver from control and experimental animals stained with PAS. In Fig. 3a, the liver section from control rat shows normal distribution of glycogen in the hepatocytes. Figure 3b represents the liver section of a rat fed high fructose diet, which shows reduction in glycogen granules and macro- and micro-

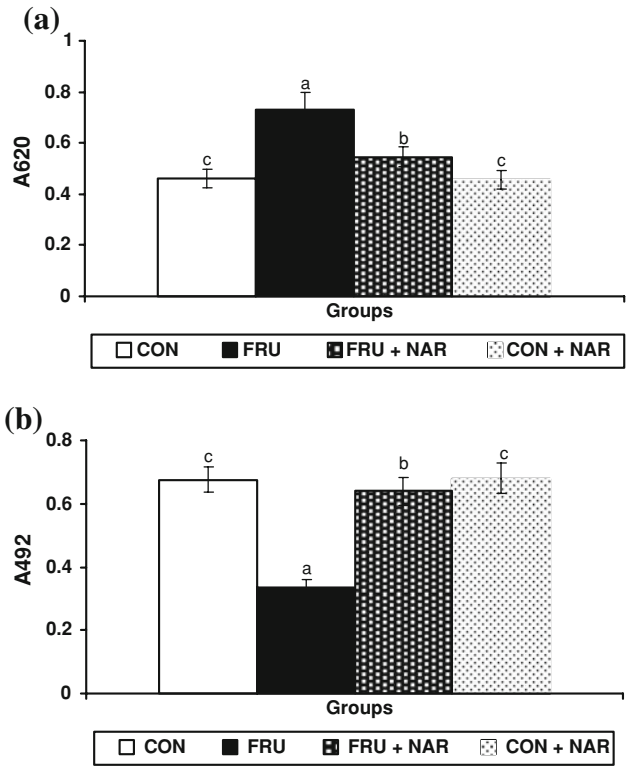

Fig. 2 Activities of PTP and PTK in liver of experimental animals. **a** Absorbance at 620 nm reflects the total amount of free inorganic phosphate in the samples generated in the dephosphorylation reaction of the phosphotyrosine peptide substrate quantified using malachite green/ammonium molybdate reagent. The mean absorbance values thus reflect the relative amount of tyrosine phosphatase activity for each group. **b** Absorbance at 492 nm reflects the relative amount of tyrosine kinase activity in the sample that is based on an ELISA using phosphorylation of a PTK-specific polymer substrate by the sample. The phosphorylated polymer substrate is probed with a horseradish peroxidase-conjugated phosphotyrosine specific monoclonal antibody and detected with a chromogenic substrate. Values are mean ± SD ($n = 6$). *CON* control rats, *FRU* fructose-fed rats, *FRU + NAR* fructose-fed rats treated with naringenin (50 mg/kg b.w.), *CON + NAR* control rats treated with naringenin (50 mg/kg b.w.). Values not sharing common superscript are significant with each other at $P < 0.05$, ANOVA followed by DMRT

vesicular steatosis. Figure 3c represents the liver section of a fructose-fed rat treated with naringenin, which shows the restoration of glycogen and the fatty changes. Liver section from normal rat treated with naringenin shows normal architecture (Fig 3d).

In all the estimations, control rats treated with naringenin did not show any significant alterations as compared to control rats.

Discussion

Rats fed high fructose diet serve as a reliable model for the investigation of insulin resistance [43]. High fructose feeding has been documented to induce insulin resistance, hyperinsulinemia, and dyslipidemia in rats, dogs, hamsters, and in humans [26, 29, 34, 59]. Fructose induces both hepatic and peripheral insulin resistance that occurs primarily due to the defects in the downstream events of the insulin signaling cascade [4].

Increased blood glucose level associated with hyperinsulinemia suggests impaired insulin action. Hyperglycemia may be attributed to decreased glucose disposal in tissues. Hyperinsulinemia could have resulted from a compensatory response by the pancreatic B-cells. Fructose feeding decreases the efficacy of insulin extraction by the liver, which retards insulin clearance from the circulation [51]. The disposal of ingested glucose into peripheral tissues was markedly reduced in rats fed fructose as evidenced by the OGTT curves and insulin sensitivity indices.

Altered activities of certain insulin-sensitive enzymes in liver and skeletal muscle together with a depletion of glycogen reserve in the liver indicate a gluconeogenic state of the liver induced by fructose. Accelerated gluconeogenesis and increased hepatic glucose output in fructose diet-fed rats have been reported and are attributed to insulin resistance observed in these rats [54]. Glucose-6-phosphatase mRNA level is shown to increase in liver after exposure to fructose even in the presence of physiological concentrations of glucose [5]. The breakdown of hepatic autoregulation of glycogen synthesis and degradation could be responsible for reduced glycogen. This could be related to reduced insulin function to suppress glycogenolysis. Decreased response of glycogen synthase to insulin has been observed in soleus muscle of sucrose-rich diet-fed rats [23]. Micro- and macro vesicular steatosis and reduction in glycogen were observed in fructose-fed rats.

PTPs are important enzymes in the insulin signaling cascade causing negative regulation of signal transduction. PTP functions in concert with PTK to balance the cellular level of phosphotyrosine [57]. Decreased PTK activity and increased PTP activity in liver were observed in rats fed high fructose in this study. Increased PTP and decreased PTK activity has been reported in other rodent models of insulin resistance [3, 37]. Researchers have reported the reduction in phosphorylation of pp185 (IRS-1/2) in liver and skeletal muscle of fructose-fed rats [55].

Hypertriglyceridemia and increase in FFA level, the other components of insulin resistance syndrome were also observed upon high fructose feeding. Fructose feeding stimulates the hepatic production of TG by promoting the reesterification of circulating non-esterified fatty acids and also by stimulating de novo fatty acid synthesis [50]. This increased delivery of TG and FFA to the muscle interferes with the utilization of glucose through the principles of Randle cycle and thereby interferes with insulin action. Elevated FFA stimulates glucose-6-phosphatase which

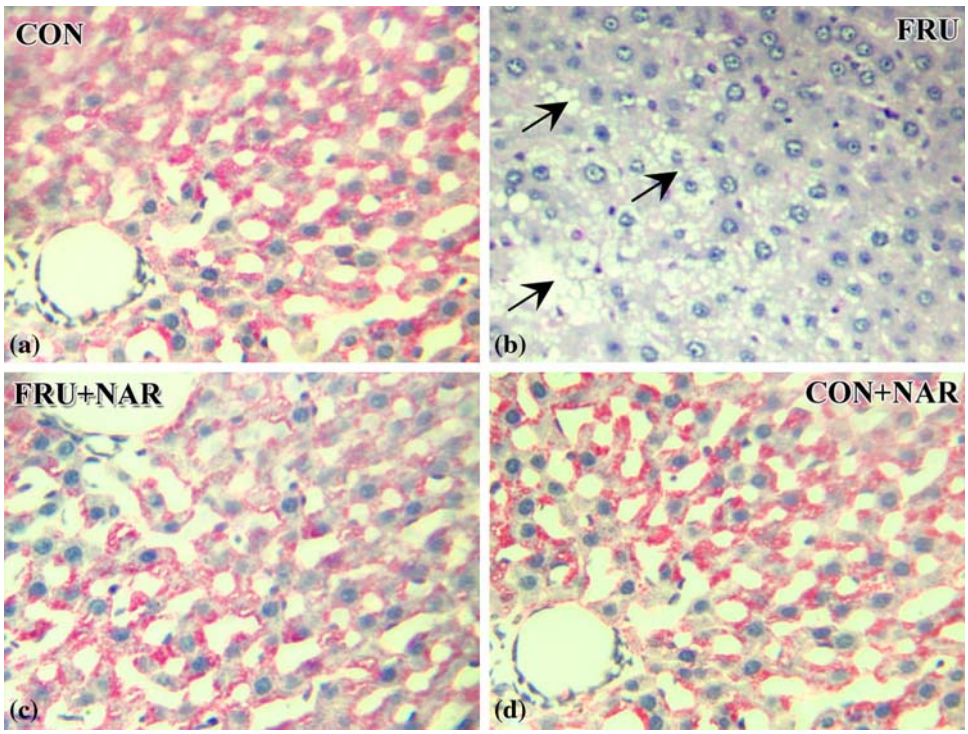

Fig. 3 Liver sections of experimental animals stained by periodic acid-Schiff staining (×200). **a** Control, **b** fructose-fed rats, **c** fructose-fed rats treated with naringenin (50 mg/kg b.w.), **d** control rats treated with naringenin (50 mg/kg b.w.)

inturn increases the hepatic glucose production resulting in hyperglycemia [25]. The lipogenic nature of fructose is responsible for dysregulation of adipocyte, secretion of inflammatory mediators, and activation of the stress-sensitive pathways, a condition called lipotoxicity that culminates in the aberrant regulation of insulin signaling pathways [41].

The decrease in total NAD^+/NADH ratio is a metabolic marker of poorly controlled diabetes [39]. Changes in redox ratio contribute to metabolic imbalance and diabetic complications by modulating the pathways of lipid and glucose metabolism and non-enzymatic glycation.

Improved insulin action in liver and skeletal muscle, reduction in plasma FFA levels and hepatic gluconeogenesis, and enhanced glucose oxidation by naringenin are the major findings of this study. Naringenin elicits insulin sensitivity by improving the proximal steps in insulin action. Activation of PTK and reduction in PTP by naringenin show that this flavonoid not only increases the extent of tyrosine phosphorylation but also prolongs the signaling process. Naringenin at the dosage could restore the glycogen and fatty changes to normal as revealed in the histological studies. The beneficial effect of naringenin in this model also provides the evidence for the antidiabetic effects exhibited by fenugreek seeds [17].

Lim et al. [30] observed that naringenin promotes glucose uptake in primary rat adipocytes by 163% compared to insulin (130%) at a concentration of 100 μM providing evidence for its insulin sensitivity effect at the adipocyte level.

Diets containing naringenin (1%) increased the hepatic fatty acid oxidation through upregulation of gene encoding the enzymes for peroxisomal β-oxidation in mice and this change may account for its ability to lower serum lipid levels [18]. Furthermore, the in vivo hypolipidemic action of naringenin has been attributed to increase in LDL-receptor expression via phosphatidylinositol 3-kinase-mediated upregulation of sterol regulatory element-binding protein [6].

Normalization of redox ratio could not only explain the improvement of metabolic milieu but is also suggestive of the antioxidant property of this flavonoid reported in the literature [44].

The intestinal absorption of glucose appears to be inhibited by naringenin in a dose-dependent manner in the current study. Naringenin has been shown to delay glucose uptake from intestinal lumen and lower the post-prandial glucose levels by inhibition of α-glucosidases [47].

Studies demonstrate that naringenin inhibits specific cytochrome P450 isoforms such as CYP1A2 [13] and CYP3A4 [14] in humans. However, in the current study, we have not tested such an inhibitory effect in the liver. Naringenin has been shown to have high bioavilability and low toxicity. Studies show that naringenin (the aglycone) and naringin (rhamnoglucoside) are efficiently absorbed after administration and have high bioavailability [12].

A preliminary toxicological study revealed that naringenin is nontoxic with a high LD_{50} (>5,000 mg/kg) [40].

The lack of effect of naringenin in control diet-fed rats suggests that the dosage is sufficient enough to normalize fructose-induced glucose and lipid changes without any impact on the normal metabolic functions in control rats.

Naringenin has insulin-like properties [8] and has been shown to prevent hyperglycemia and hyperlipidemia in STZ-treated rats as well as in genetic diabetic mice [1, 20]. Our findings with naringenin in this model are important for several reasons. First, this model closely reflects the natural history and metabolic characteristics of human type 2 diabetes rather than the STZ-model, which shows the features of type 1 diabetes. Second, epidemiological and biochemical studies show that high fructose intake has been shown to contribute to metabolic disturbances leading to insulin resistance and is causally related to the rise in the prevalence of metabolic syndrome. The effects of naringenin on downstream signaling events are currently underway in our laboratory.

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