



Synergistic effect of Quercetin and 6-gingerol treatment in Streptozotocin induced type 2 diabetic rats and poloxamer P-407 induced hyperlipidemia

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Title: Synergistic effect of Quercetin and 6-gingerol treatment in streptozotocin induced type 2 diabetic rats and poloxamer P-407 induced hyperlipidemia

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Abstract

Ginger and quercetin has been reported to have significant antidiabetic effects. It has been proposed that 6-gingerol and quercetin are responsible for the antidiabetic activity and that a combination of the two produces a synergistic effect. However, the per se effect of 6-gingerol has not been reported. The objective of this study was to investigate the effects of 6-gingerol and quercetin *per se*, on streptozotocin induced non-insulin dependent diabetes mellitus (NIDDM) and hyperlipidemia. 6-Gingerol and quercetin reduced fasting glucose significantly with producing significant changes in the insulin levels of diabetic rats. The amelioration was significantly more with the combination, compared with either compound alone. The results of the oral glucose tolerance test (OGTT) indicated that 6-gingerol and quercetin reduced Streptozotocin (STZ) induced increases in both area under curve glucose (AUC_{glucose}) and area under curve insulin (AUC_{insulin}) in high fat feed type 2 diabetic rats. Treatment with 6-gingerol and quercetin reduced the levels of lipids in rats with NIDDM significantly. 6-Gingerol and quercetin had antihyperlipidemic effects in polaxamer P-407-induced hyperlipidemia. Quercetin and 6-gingerol show reduced serotonin induced hyperglycemia. This is the first study to demonstrate synergism of 6-gingerol and quercetin that has significant antidiabetic and

beneficial cardiac effects in type 2 diabetic rats and polaxamer-induced hyperlipidemic rats. The synergistic action is probably through modulation of the serotonergic system.

Key words: Quercetin, 6-gingerol, 5-HT receptor, Serotonin, diabetes, STZ.

1. Introduction

The prevalence of diabetes mellitus worldwide was estimated to have been 150 million people in 2000, making it a major non-communicable disorder, and the prevalence was expected to increase to 220 million people by 2010¹. Diabetes mellitus is characterized by hyperglycemia resulting from a lack of insulin secretion or insulin sensitivity or from both these conditions. The current treatment approaches for NIDDM includes diet, exercise and a variety of pharmacological agents, including insulin, biguanides, sulfonylureas and thiazolidinediones. The pharmacological agents available for treatment of diabetes are associated with significant adverse effects such as hypoglycemia, weight gain and edema²⁻³. In many cases monotherapy with pharmacological agents fails to improve blood glucose control, dyslipidemia and hypertension, and thus there is a need to develop novel therapeutic agents that can complement the existing agents. The development of new therapeutic agents will offer new choices to physicians and patients. One of the approaches is the use of herbal drugs and food supplements.

Various herbal drugs have been reported to possess anti-diabetic activity. *Zingiber officinale* exhibits significant antihyperglycemic and antihyperlipidemic properties in rats with streptozocin induced insulin dependent diabetes mellitus (IDDM)⁴. 6-Gingerol is a chemical constituent present in the rhizome of *Zingiber officinale*. Gingerols are reported to possess significant serotonin receptor modulatory activity⁵⁻⁷.

Serotonin is known to produce hypoglycemia in mice with 200-400 mg/kg administered intravenously⁸. In the 1990s, it was reported that 5-hydroxy tryptamine (5-HT) induces hyperglycemia at much lower doses (1–10 mg/kg, i.p.) in rats⁹⁻¹⁰. Time-dependent hyperglycemia with corresponding hypoinsulinemia was produced in fasted normoglycemic rats when they were injected with 5-HT (1 mg/kg) i.p. It has been suggested that 5-HT induces hyperglycemia in rats possibly due to the inhibition of release of insulin from the beta cells of the pancreas¹¹. *Z. officinale* is reported to contain compounds such as 6-gingerol that are likely to possess 5-HT-modulatory activity^{7, 12}.

Quercetin inhibits the formation of advanced glycation end products (AGEs) and results in secretion of insulin by opening L-type Ca²⁺ channels¹³⁻¹⁴. In human adipocytes, quercetin increases insulin sensitization by inhibiting the pro-inflammatory mediator tissue necrosis factor -alpha (TNF-alpha)¹⁵. Quercetin normalizes leptin signaling through Protein kinase B (Akt)/Forkhead box O1 (FoxO1) activation, which results in pancreatic beta cell mass protection. Previous studies have reported that quercetin reduces oxidative stress and inflammation nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinases (MAPK) pathway¹⁶⁻¹⁸.

However, the synergistic activity of 6-gingerol *and* quercetin has not been reported.

The aim of our investigation was to assess the possible beneficial effects of 6-gingerol and quercetin, in neonatal STZ-induced type 2 diabetic rats and polaxamer P-407 induced hyperlipidemic animal models.

2. Materials and methods

2.1 Induction of type 2 diabetes and treatment

Healthy wistar rats were maintained for breeding. A single dose (90 mg/kg, *i.p.*) of STZ (Sigma Chemical Co., St. Louis, MO, USA) was injected into 2-day-old pups. Another group of pups received only the vehicle. The animals were weaned at 30 days age. The rats that had fasting glucose levels higher than 140 mg/dl after 3 months were diagnosed as suffering from type 2 diabetes¹⁹. The pups that received the vehicle were used as control animals. The rats that had fasting glucose levels higher than 140 mg/dl after 1 month were diagnosed as suffering from type 2 diabetes. The experimental animals were divided into five groups, six animals in each group. Group I: Normal control, Group II: Disease or diabetic control, Group III: Diabetic treated with 6-gingerol (3 mg/kg, *i.p.*) purchased from Dalton Chemical Laboratories, Group IV: Diabetic treated with quercetin (10 mg/kg, *i.p.*) purchased from Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and Group V: Diabetic treated with combination of 6-gingerol (3 mg/kg, *i.p.*) and quercetin (10 mg/kg, *i.p.*). The drug treatment was administered daily for eight weeks. The control groups received the vehicle alone (the same volume as received by each treatment groups). Water was provided *ad libitum*. The guidelines of the NIH and the Animal Ethics Committee for animal experiments were followed. The study protocol was approved by the ethics committee (Protocol no FAH/NU 2014/ 17).

2.2 Blood sampling and biochemical analysis

At the end of the treatment, the rats were made to fast for 12 hours and blood samples were collected and serum was separated. The glucose, cholesterol and triglyceride levels in the serum were estimated using diagnostic reagent kits. Insulin levels were estimated by radioimmunoassay kit (Institute of Biological Production, Shanghai, China).

2.3 Oral glucose tolerance test (OGTT)

The rats were fasted for 18 hours and glucose administered at a dose of 1.5 g/kg orally. Blood samples were collected at 0, 30, 60 and 120 minutes. The serum was separated immediately and analyzed for glucose and insulin. The results of the OGTT were expressed as integrated areas under the curves (AUCs) over a period of 0–120 min.

2.4 Insulin Sensitivity Index (K_{ITT})

The insulin sensitivity index was calculated using the method of Alford et al²⁰. The rats were made to fast for 6 hours, and human insulin was injected through the tail vein at a dose of 0.2 IU/100 g body weight. Blood samples were collected after 10, 20, 30 and 60 minutes. The insulin sensitivity index was calculated as

$$K_{ITT} = (0.693/t_{1/2}) \times 100$$

2.5 Measurement of blood pressure and cardiovascular parameters

The blood pressure was recorded using the tail-cuff method with a Harvard blood pressure monitor (Kent, UK). In left ventricular hypertrophy resulted from accumulation of collagen and myocyte hypertrophy consequence in left ventricular dysfunction while cardiac hypertrophy includes accumulation of collagen and myocyte hypertrophy in heart²¹. The cardiac hypertrophy index was calculated as the ratio of the weight of the heart to the body weight, and the left ventricular hypertrophy index was calculated as the ratio of the weight of the left ventricle to the weight of the heart. The histopathology method of Prockop and Udenfriend was used to determine the collagen (hydroxyproline) concentration of the left ventricle²².

2.6 Histopathology of pancreas

The pancreas was removed, washed with phosphate buffer and fixed in formalin. A paraffin block of the tissue was prepared. Slides with 0.5 μ m sections were prepared and stained with hematoxylin and eosin.

2.7 Western Blotting

2.7.1 Membrane and cytosol preparation

Frozen cardiac tissue (approximately 700 g) was homogenized in a solution containing 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 1 mM ethylenediamine tetra acetic acid (EDTA) and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) using polytron centrifuge at 12,000 rpm. This homogenate was centrifuged at 1000g (Beckman, USA, rotor JA-20) at 0°C. The supernatant was centrifuged again at 10,000g at 0°C. The supernatant from this was finally subjected to centrifugation at 40,000g at 0°C. The pellets obtained from this final centrifugation were rich in the crude membrane fraction, and the supernatant was rich in cytosol. The pellets were resuspended in a solution containing 10 mM histidine and 250 mM sucrose (pH 7.0). Estimation of the protein in the suspension was performed using Lowry's method, and an equal amount of protein (2 mg/ml) was loaded by dilution with Laemmli buffer for the gel electrophoresis.

2.7.2 Western blotting

Samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with a 12% resolving gel. Each sample was run in duplicate. Gels were run at 200 V for approximately 45 minutes. Prestained molecular weight standards (Sigma, USA) were run in one corner well, and Laemmli buffer was added to another corner well to ensure the evenness of the electrophoresis. Electrophoretic transfer of proteins to PVDF membranes (Amersham) was performed using a Bio-Rad assembly at 100 V over approximately 2 hours. Then the membranes were incubated in Tris-buffered saline (50 mM Tris, 750 mM NaCl, pH 7.4) with 5% non-fat dry milk for 1 hour at room temperature. The membranes were washed with TBS, and GLUT 1 (antirabbit) or GLUT 4 (antigoat) antibody (Santa Cruz Biotechnology Inc, USA) diluted to

1:750 in TBS containing 5% milk. These were incubated overnight at 4°C. They were washed with TBS containing 0.1% Tween 20 (TBST) the next day. An appropriate second antibody (Santa Cruz Biotechnology Inc, USA) was in TBS (1:3000 dilution) with 5% milk. The membranes were incubated for 1 hour at room temperature, washed again with TBST and incubated finally with anti-rabbit horseradish-peroxidase-labeled antibody for 30 minutes at room temperature. The membranes were then dipped in chemiluminescence solution (Amersham) for 1 minute and immediately exposed on Kodak X-ray film for 1–2 minutes

2.8 5-HT induced hyperglycemia

Male Wistar rats (body weight 200–250 g) were injected with 5-HT (1 mg/kg dose, i.p.). Blood samples were collected from the tail vein prior to injection of 5-HT, and subsequent samples were collected 30, 60 and 120 minutes after the injection.

2.9 Polaxamer P-407 induced hyperlipidemia model

2.9.1 Antihyperlipidemic activity

The rats were divided into four groups, and all the animals except the normal controls were administered P-407 (400 mg/kg, i.p.) to induce hyperlipidemia. Blood samples were withdrawn from heparinized animals 1, 2, 3, 6, 12 and 24 hours after administration of poloxamer by cannulation of the jugular vein.

2.9.2 HMG-CoA reductase assay

The hepatic microsomal homogenate was used for determining 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity by the method of Rao and Ramkrishna²³. Homogenate samples (0.25 ml) were obtained from the poloxamer-treated animals, and the activity of HMG-CoA reductase was evaluated with drug concentrations of 0.5, 1.5, 5, 10 and 20 nM. The drug

solution was mixed with the homogenate stand for 15 minutes at room temperature. The substrate was then incubated at 37°C for 20 minutes, and finally the colorimetric method was used to evaluate the activity of the drug.

3. Statistical analysis

All the experimental values were expressed as the mean \pm S.E.M. Statistical analysis performed using one-way analysis of variance followed by Tukey's test. A value of $p < 0.05$ was considered statistically significant.

4. Results

4.1 Effect of quercetin and gingerol on biochemical parameters in type 2 diabetic rats

The fasting glucose level and the insulin level in the serum samples were significantly higher in the disease control animals compared with the animals of the vehicle control group. There was a significant reduction in the glucose and insulin levels of type 2 diabetic rats administered 6-gingerol and quercetin compared with the diabetic controls. Both the AUC_{glucose} and AUC_{insulin} values were significantly higher in the diabetic control animals compared with the diabetic animals treated with 6-gingerol and quercetin. The serum cholesterol and triglyceride were significantly higher in the diabetic control animals compared with the normal controls. Treatment with 6-gingerol and quercetin produced a significant reduction in the serum levels of cholesterol and triglyceride levels as compared with the diabetic control animals. The diabetic group treated with combination of quercetin and 6-gingerol shows significant amelioration as compared to quercetin or 6 gingerol alone in various biochemical parameters indicates synergism of drug combination (Table .1).

4.2 Effect of quercetin and gingerol on Insulin sensitivity index (K_{ITT}) in type 2 diabetic rats

significant differences were observed in the glucose disappearing rate between diabetic control and normal control rats. The insulin sensitivity index was reduced in diabetic control rats compared with normal rats. The sensitivity index of the rats treated with quercetin and gingerol treated rats was significantly increased compared with the diabetic control rats. The sensitivity index of rats treated with a combination of quercetin and 6-gingerol was significantly different compared with the values of rats treated with one drug alone (Table .1).

4.3 Effect of quercetin and gingerol on Cardiac hypertrophy index in type 2 diabetic rats

In the disease control group, the weight of the heart decreased compared with the normal controls. However, the cardiac hypertrophy index was significantly higher in the diabetic control rats compared with the treatment group. The value of this index was significantly reduced in rats treated with both quercetin and 6-gingerol (Table .1).

4.4 Effect of quercetin and gingerol on Left ventricular hypertrophy index in type 2 diabetic rats

The weight of the left ventricle was increased and the weight of the heart reduced in the animals treated with quercetin and gingerol. These weights were significantly different in the rats treated with one drug alone and the two drugs in combination compared with the diabetic control rats (Table .1).

4.5 Quercetin and gingerol treatment ameliorates pancreatic damage in type 2 diabetic rats

Pancreatic beta cell damage is a prominent feature of diabetic rats. Pancreatic sections of the treated rats showed marked signs of repair in animals treated with quercetin and gingerol, compared with type 2 diabetic rats (Figure 1).

4.6 Effect Quercetin and gingerol treatment on 5-HT induced hyperglycemia

Treatment with quercetin and gingerol significantly reduced 5-HT-induced hyperglycemia. The synergistic effect of quercetin and gingerol tends to normalize glucose levels (Table .2).

4.7 Antihyperlipidemic effects on P-407 induced hyperlipidemic rats

Quercetin and gingerol individually and synergistically reduced the total cholesterol, HDL, LDL and VLDL levels compared with the disease control (Table .2).

4.8 Effect Quercetin and gingerol HMG CoA reductase inhibition

The HMG CoA inhibition was evaluated from the HMG CoA to mevalonate ratio. Quercetin and 6-gingerol significantly inhibited HMG CoA reductase compared with the disease controls. Quercetin and gingerol were found to act synergistically as they significantly inhibited the enzyme involved in lipid anabolism in polaxamer-induced hyperlipidemic rats (Figure 2).

4.9 GLUT 4 immunoblot analysis

The immunoblot analysis of Glut 4 from cardiac myocytes of different groups showed increase in Glut4 expression in case of group treated with quercetin and combination of quercetin and 6-gingerol in the membrane while decrease in cytosol indicates GLUT 4 translocation (Figure 3).

5. Discussion

6-Gingerol and quercetin produced a significant reduction in elevated glycaemic parameters such as the glucose level and secretion of insulin in STZ-induced type 2 diabetic rats. Treatment of STZ-induced type 1 diabetic animals with the fresh juice of *Z. officinale* resulted in normalized glucose and insulin levels²⁴. The reduction in glucose and insulin levels obtained in the present study indicates extrapancreatic action of 6-gingerol and quercetin. These findings are similar to earlier findings with sarpogrelate, which increased insulin levels in type 1 diabetic rats and reduced insulin levels in type 2 diabetic rats²⁵. 5-HT produces hyperglycemia in normoglycemic rats through the involvement of specific 5-HT_{2A} and 5-HT₃ receptors. It is suggested that the antidiabetic activity of ginger is correlated with 5-HT receptor modulation.

The fasting glucose levels of diabetic control rats were significantly high compared with those of normal control animals. We also found increases in the insulin levels and AUC_{insulin} values after an oral glucose load in the diabetic control animals.

The OGTT results further support the insulin sensitivity effect of 6-gingerol and quercetin. The AUC_{insulin} values of the diabetic control animals were significantly greater compared with those of the normal control animals. Treatment with 6-gingerol and quercetin produced a significant reduction in the insulin levels and glucose levels compared with diabetic control animals. The results suggest that treatment with 6-gingerol does not enhance insulin release; rather, it reduces insulin release in conditions such as hyperinsulinemia and thereby indicates that treatment of type 2 diabetic animals with 6-gingerol increases the insulin sensitivity and effective glucose disposal. A synergistic effect was observed in the group treated with both quercetin and 6-gingerol.

Deranged lipid metabolism in diabetes mellitus has been well established both clinically and experimentally. It has been reported that rats treated with STZ have increased plasma levels of

triglycerides, cholesterol, free fatty acids and phospholipids²⁶. In accordance with these reports, in the present investigation also we observed elevated levels of serum lipids in polaxamer P-407 induced hyperlipidemic rats. Treatment with 6-gingerol and quercetin significantly brought down the serum cholesterol and triglyceride levels of hyperlipidemic rats, indicating an improvement of the lipid metabolism. The reduction of the lipid level brought about by 6-gingerol and quercetin is probably the result of the separate actions being improved by insulin regulation of lipoprotein lipase leading to enhanced degradation of triglycerides and concurrent suppression of lipolysis, resulting in decreased supply free fatty acids required for triglyceride biosynthesis²⁷. It has been reported previously that the insulin resistance in diabetic subjects results in hyperinsulinemia, with an increase in the LDL level and a reduction in the HDL level. In the present study, the diabetic animals had elevated LDL and VLDL levels²⁸. Treatment with 6-gingerol significantly reduced the LDL and VLDL levels without affecting the HDL levels. Reduction in LDL levels on treatment with quercetin and 6-gingerol has been reported previously. It has been shown in the previous studies that treatment with 6-gingerol dose-dependently inhibits LDL oxidation²⁹ while quercetin clearly inhibits triglyceride synthesis and thereby prevents atherosclerotic progression in animals³⁰. All the findings in the present study further support the hypothesis that 6-gingerol and quercetin cause the insulin action to be improved and control elevated glucose and lipid levels in insulin resistance associated with hyperglycemia and hyperlipidemia.

Quercetin decreased blood glucose, fasting and postprandial hyperglycemia serum cholesterol and LDL levels in alloxan induced diabetic animals³⁰⁻³². Quercetin, antioxidant reduced oxidative stress through NF-k β activation through hepatin iNOS increased expression STZ induced diabetic animals¹⁸.

Drug acting through insulin sensitivity at these sites need to be developed urgently. Further, the role of insulin resistance in the etiology of a wider spectrum of metabolic disorders such as obesity, hypertension and atherosclerosis, has been revealed^{27, 33}. The synergistic effects of 6-gingerol and quercetin in treatment of diabetes associated with insulin resistance and insulin secretion ameliorate pancreatic damage. The combination of these drugs can be considered as therapy that is useful in the treatment of metabolic disorders such as diabetes, obesity, hypertension and atherosclerosis.

Recent report suggest that the serotonergic system is involved in the progression of diabetes³⁴. Insulin exhibits antidepressant action in STZ-induced diabetic mice by increasing brain serotonin levels³⁵. Diabetic mice experiencing depression and anxiety benefit from ondansetron, a 5-HT₃ antagonist, through serotonin modulation³⁶. Platelets are activated in diabetic renal impairment through the release of serotonin. Serotonin controls insulin secretion through GTPases serotonylation in beta cells of pancreas^{11,37}. Sarpogrelate, a 5-HT_{2A} selective inhibitor, ameliorates ischemic blood perfusion by activating signaling of the endothelial 5-HT_{2B} receptor eNOS/Akt³⁸. Serotonin regulates PFK through PI3K and the PLC–PKC pathway³⁹. Arterial contraction through SERT activity was increased in NIDDM GK rats through the signaling of the p38 MAPK, PI3K and Rho kinases⁴⁰. Quercetin and 6-gingerol in combination ameliorate 5-HT-induced hyperglycemia through a synergistic effect. We have hypothesized the mechanism of action of quercetin and 6-gingerol to be through serotonergic modulation as shown in the figure. Quercetin and 6-gingerol increase cardiac Glut-4 expression in diabetic rats, and the serotonergic pathway is involved in this action(Figure 4)⁴¹.

To conclude, our data suggest that 6-gingerol and quercetin have a synergistic effect in the treatment of diabetic and hyperlipidemic animals through modulation of the serotonergic system.

Conflict of interest:

None.

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Abbreviations:

NIDDM: Non Insulin dependent diabetes mellitus

IDDM: Insulin dependent diabetes mellitus

AUC_{glucose} : area under curve glucose

AUC_{insulin} : area under curve insulin

5-HT : 5-hydroxytryptamine,

STZ : Streptozotocin

AGE : Advanced glycation end products

TNF- α : tissue necrosis factor - α

Akt : Protein kinase B

FoxO1: Forkhead box O1

NF- κ B: Nuclear factor- κ B

HMG-CoA: 3-hydroxy-3-methyl-glutaryl-CoA reductase

Glut 4: Glucose transporter type 4

PFK : phospho fructo kinase

PLC: phosphor lipase C

PKC: Protein kinase C

ERK 1/2 :extracellular signal-regulated kinase 1 and 2

JAK/STAT: janus kinases

p³⁸MAPK: phospho³⁸ mitogen-activated protein kinases

PI₃K : phosphoinositide 3-kinase

eNOS: endothelial nitric oxide synthase

Table 1: Effect of six week quercetin and 6-gingerol treatment on various biochemical parameters of STZ-induced type 2 diabetic rats

Parameters	Control	Disease control	Quercetin (10mg/kg)	6-gingerol (3mg/kg)	Quercetin (10mg/kg) + 6-gingerol (3 mg/kg)
serum glucose (mg/dl)	68.5±1.99	154.33±3.36**	134.5±3.31** ^a	139.16±2.35** ^a	115.5±2.34** ^{a, **b}
Serum insulin (μU/ml)	76.5±2.60	136.33±2.18**	111.5±2.66** ^a	114.0± 2.03** ^a	97.0±2.39** ^{a, **b}
AUC _{glucose} (mg/dl.min) × 10 ³	17.5±0.57	27±1.06**	21.66±0.66** ^a	24.5 ± 0.42* ^a	19.66±0.49** ^{a, **b}
AUC _{insulin} (μU/ml.min) × 10 ³	8.71±0.22	12.05±0.13**	11.7±0.16** ^a	11.35±0.20** ^a	10.75±0.24** ^{a, *b}
K _{ITT} (per min)	8.4±0.18	6.1±0.08**	7.38±0.18** ^a	7.21±0.15** ^a	7.75±0.07** ^{a, *b}
Serum cholesterol (mg/dl)	67.65±1.05	104.9±2.2**	95.41±0.9** ^a	93.91 ±0.91** ^a	86.56±0.87** ^{a, **b}
Serum triglyceride (mg/dl)	78.66±2.66	126.16±3.63**	102.66±2.51** ^a	110.33±2.01** ^a	94.5±1.25** ^{a, **b}
Blood pressure (mmHg)	96.33±2.6	143.50±4.13**	137.66±3.18** ^a	144.16 1.6** ^a	126.83±4.01** ^{a, **b}
Cardiac hypertrophic index (mg/g)	2.62±0.02	3.8±0.02**	3.36±0.03** ^a	3.42 ±0.04** ^a	2.99±0.05** ^{a, **b}
LV Hypertrophic index (mg/mg)	0.685±0.02	0.822±0.02*	0.74±0.01** ^a	0.71±0.02** ^a	0.67±0.03** ^{a, **b}
LV Collagen levels (mg/g LV tissue)	1.76±0.03	3.02±0.06**	2.66±0.04** ^a	2.66±0.05** ^a	2.03±0.09** ^{a, **b}

Data is shown as mean ± S.E.M. * significantly different as compared with vehicle control P<0.05, ** Significantly different as compared with vehicle control P<0.01, ^a vs Disease Control. ^b vs Quercetin treated One-way ANOVA followed by Tukey test. n=6.

Table 2: Effect of quercetin and 6-gingerol treatment on 5-HT induced hyperglycemia

Treatment \ Time (min)	0	30	60	120
5-HT (1mg/kg)	100.0±0.0	137.33±1.58	176.33±3.20	158.66±3.57
Quercetin	100.0±0.0	127.33±1.05*	168.5±4.16	146±2.93
6-gingerol	100.0±0.0	123.16±1.49*	167.5±4.18*	143.66±2.95
Quercetin (15mg/kg) + 6-Gingerol (1mg/kg)	100.0±0.0	115.83±1.07*	155.33±3.31*	128.16±2.15

Data is shown as % change in serum glucose after 5-HT injection, taking basal level as 100%. Data is shown as mean ± S.E.M. * significantly different as compared to 5-HT (1mg/kg) at corresponding time interval (n=6).

Table 3: Effect of quercetin and 6-gingerol treatment on polaxamer P-407 induced hyperlipidemia

Treatment	Time interval	Vehicle control	Disease control	Quercetin (10mg/kg)	6-Gingerol (3mg/kg)	Quercetin (10 mg/kg) + 6-Gingerol (3mg/kg)
Serum total cholesterol (mg/dl)	12 hr	83.16±3.27	164.83±4.02**	141±4.39** ^a	145.66±5.58** ^a	115.66±4.89** ^{a, **b}
	24 hr	76.83±5.67	196±6.70**	178.5±4.54** ^a	168.33±6.84** ^a	151.66±6.72** ^{a, **b}
HDL (mg/dl)	12 hr	45.16±0.7	24.66±0.66**	36.5±0.76** ^a	37.5±0.76** ^a	31.83±0.6** ^{a, *b}
	24 hr	50.66±0.88	33.16±1.13**	43.33±1.22** ^a	41.66±1.38** ^a	45.5±0.76** ^{a, **b}
VLDL (mg/dl)	12 hr	25.21±0.59	55.0±1.05**	44.65±0.75** ^a	48.16±0.70** ^a	39.58±0.71** ^{a, **b}
	24 hr	27.5±0.42	65.5±0.99**	54.66±1.35** ^a	58.33±0.66** ^a	49.16±0.94** ^{a, **b}
HDL/VLDL	12 hr	0.51±0.01	2.93±0.06**	1.76±0.05** ^a	1.99±0.03** ^a	1.64±0.05** ^{a, **b}
	24 hr	0.49±0.01	2.57±0.14**	1.35±0.04** ^a	1.37±0.08** ^a	1.01±0.05** ^{a, **b}

Data is shown as mean ± S.E.M. * significantly different as compared with vehicle control P<0.05, ** Significantly different as compared with vehicle control P<0.01, ^a vs Disease Control. ^b vs Quercetin treated One-way ANOVA followed by Tukey test. n=6.

Legends to figure

Fig. 1 Effect of quercetin, 6-gingerol and combination treatment on Pancreas histopathology (10X) in STZ-induced type 2 diabetic rats. NC = non diabetic control, DC = diabetic control, Q = diabetic treated with quercetin (10 mg/kg) *i. p.*, 6-G = diabetic treated with 6-gingerol (3 mg/kg) *i.p.* and Q+6-G = diabetic treated with quercetin (10 mg/kg) *i.p.* and 6-gingerol (3 mg/kg) *i.p.*

Fig. 2. Effect quercetin and 6-gingerol on microsomal HMG Co A reductase inhibition in poloxamer P-407 induced hyperlipidemic rats. # $P < 0.05$ as compared to NC rats

1= normal rats, 2= polaxamer P-407 induced rats, 3= polaxamer P-407 induced rats treated with quercetin (10 mg/kg) *i. p.*, 4= polaxamer P-407 induced rats treated with 6-gingerol (3 mg/kg) *i.p.* 5 = diabetic treated with quercetin (10 mg/kg) *i. p.* and 6-gingerol (3 mg/kg) *i.p.*

Fig. 3. Effect of treatment quercetin, 6-gingerol and combination of both on GLUT 4 protein levels in cardiomyocytes from control and diabetic rats. The upper panel indicates GLUT 4 protein levels in cell membrane and the lower panel in cytosol. 1 = non diabetic control, 2 = diabetic control, 3 = diabetic treated with quercetin (10 mg/kg) *i. p* and 6-gingerol (3 mg/kg) *i.p.*, 4 = diabetic treated with quercetin (10 mg/kg) *i. p.*

Fig 3.A Percentage change in GLUT 4 expression in membrane as compared to control

Fig 3.B Percentage change in GLUT 4 expression in cytosol as compared to control

Fig. 4 Quercetin and 6-gingerol modulates serotonergic pathway in diabetes and hyperlipidemia. Q= quercetin and G= 6-gingerol. (Source: Sonawane et al, 2015, Permission obtained from Elsevier Inc)

Figure. 1

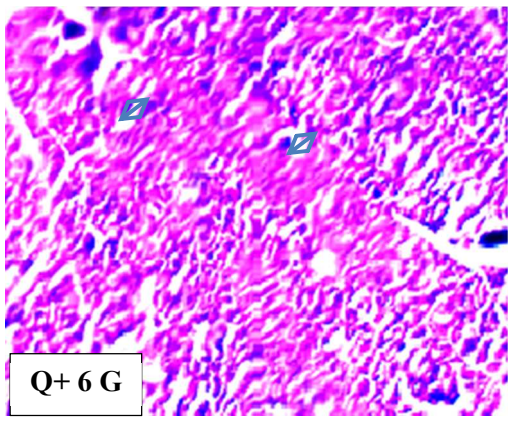
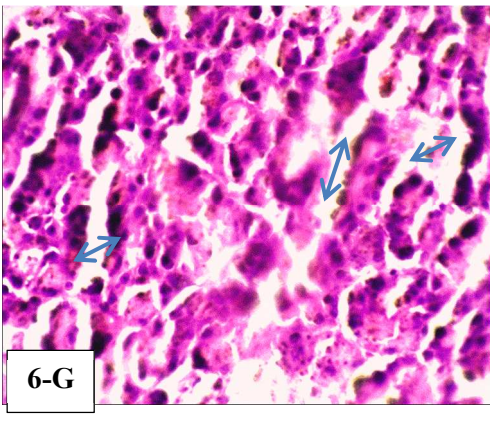
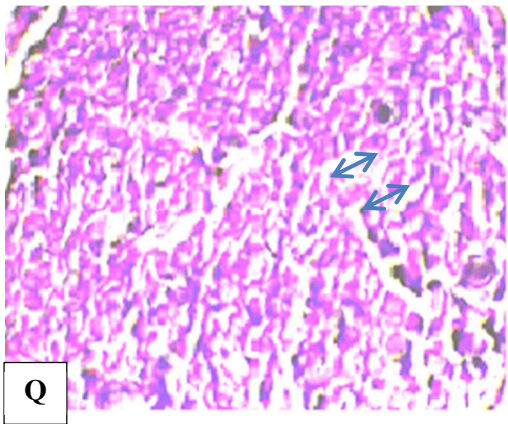
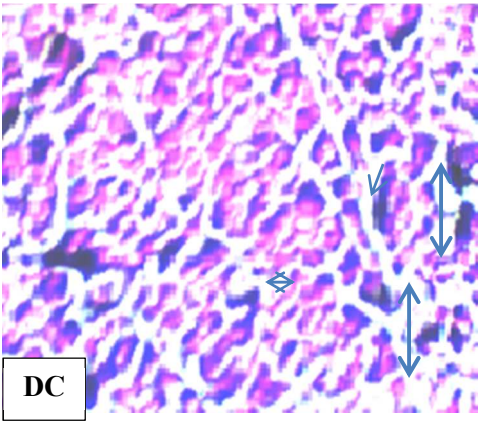
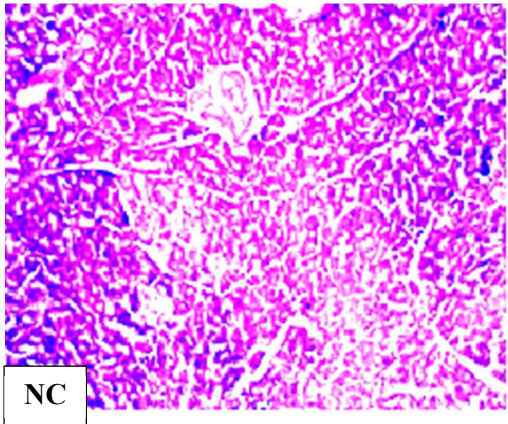
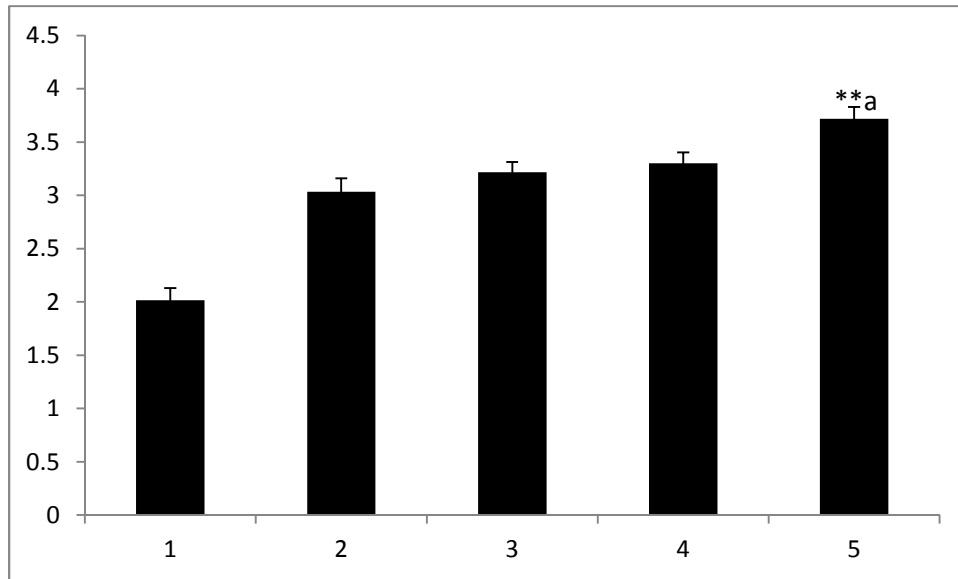


Figure 2

HMG CoA inhibitory activity



Data is shown as mean \pm S.E.M. * significantly different as compared with vehicle control $P < 0.05$, ** Significantly different as compared with vehicle control $P < 0.01$, ^a vs Disease Control.

Figure 3

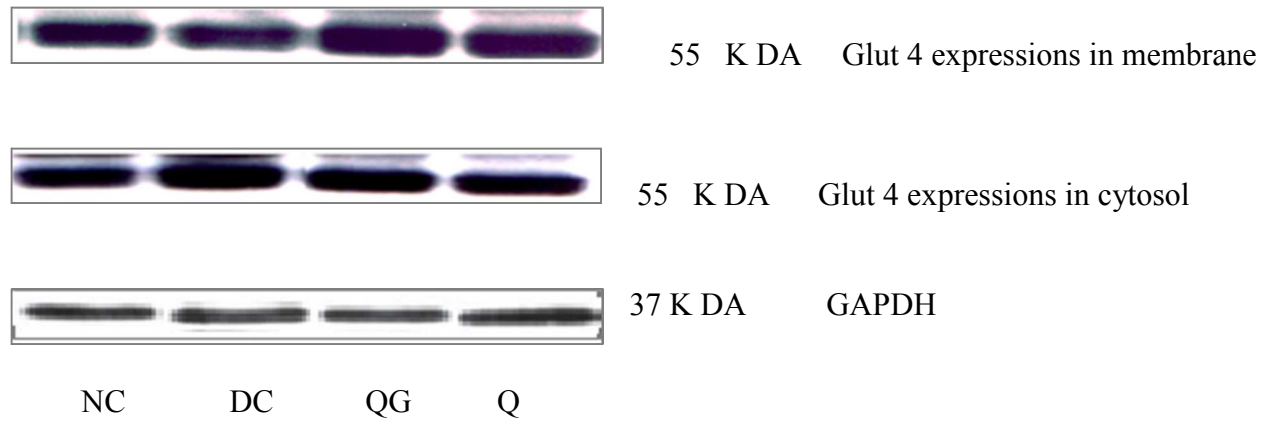
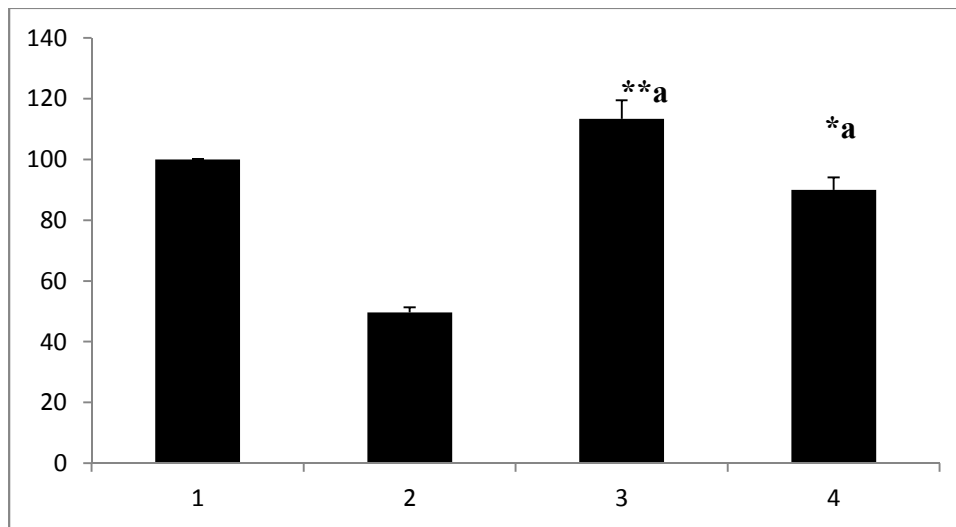
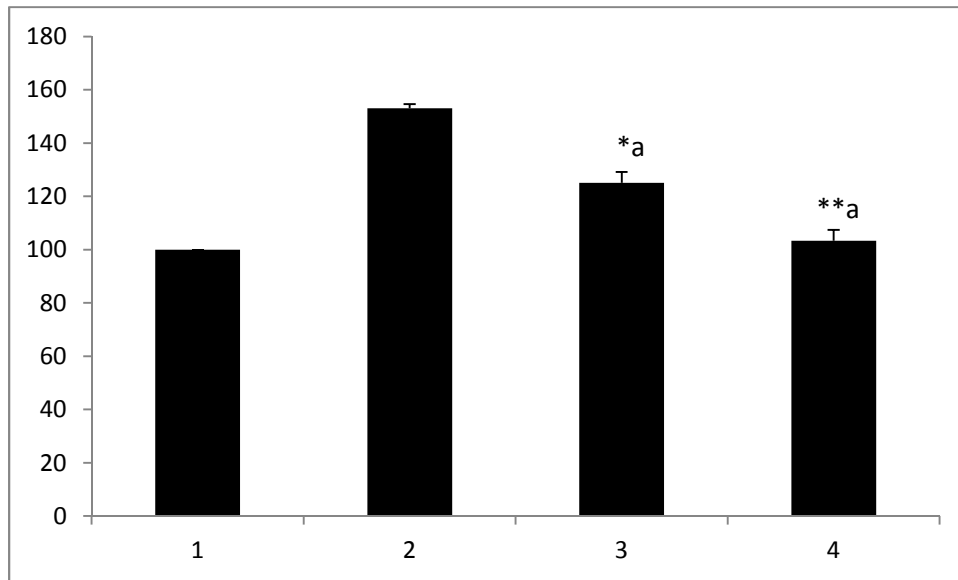


Figure 3.A



Data is shown as mean \pm S.E.M. * significantly different as compared with vehicle control $P < 0.05$, ** Significantly different as compared with vehicle control $P < 0.01$, ^a vs Disease Control.

Figure 3. B



Data is shown as mean \pm S.E.M. * significantly different as compared with vehicle control $P < 0.05$, ** Significantly different as compared with vehicle control $P < 0.01$, ^a vs Disease Control.

