Full Length Research Paper

Anti-hyperglycaemic, lipid lowering and anti-oxidant properties of [6]-gingerol in db/db mice

Amar Bahadur Singh[1], Akanksha[2], Nilendra Singh[3], Rakesh Maurya[2] and Arvind Kumar Srivastava[1]*

[1]Biochemistry Division, Central Drug Research Institute, Lucknow-226001, India.
[2]Medicinal and Process Chemistry Division, CDRI, Lucknow-226001, India.
[3]Pharmacology Division CDRI, Lucknow-226001, India.

In the present study, we investigated the blood glucose lowering, lipid lowering and antioxidant effect of [6]-gingerol in type 2 diabetic db/db mice. Treatment of db/db mice with [6]-gingerol (100 mg/kg bw) for 12 days significantly ($p<0.05$) lowered fasting blood glucose and improved the glucose tolerance in db/db mice. Oral administration of [6]-gingerol also significantly ($p < 0.05$) decreased plasma triglycerides (TG), total cholesterol (TC), free fatty acid (FFA), low-density lipoprotein cholesterol (LDL-C) and plasma insulin concentration. In addition, [6]-gingerol significantly ($p < 0.05$) reduces the content of hydrogen peroxide or suppresses the reactive oxygen species (ROS) generation and restores the enzyme activity of catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in db/db mice. These findings suggest that [6]-gingerol exhibits a significant potential as an anti-hyperglycaemic, lipid lowering and anti-oxidant agent for the treatment of type 2 diabetes.

Key words: Antihyperglycaemic, antioxidant, antilipidemic, reactive oxygen species, db/db mice, [6]-gingerol.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease which now afflicts approximately 3% of the world population. It is classified into two types (type 1 and type 2) based on individual etiologies. Around 95% of diabetic patients are diagnosed with type 2 diabetes. A major feature of type 2 diabetes is insulin resistance and/or insulin deficiency which can cause hyperglycemia (Laakso et al., 2001). Therefore, a key strategy in treating patients with type 2 diabetes is to maintain blood glucose level in physiological range. Persistent hyperglycemia in diabetes causes increased production of free radicals through auto-oxidation of glucose and non-enzymatic protein glycation; these highly reactive free radicals exert their cytotoxic effects on membrane phospholipids and cause a wide spectrum of cell damage, including lipid peroxidation, inactivation of enzymes, alteration of intracellular oxidation-reduction state and DNA damage (Halliwell et al., 1984; Slater et al., 1987). This also results in oxidative stress, which is believed to be a pathogenetic factor in the development of diabetic complications. It is now apparent that elevation of plasma free fatty acids mentioned in abstract plays a pivotal role in the development of type 2 diabetes by causing insulin resistance. Type 2 diabetes develops because pancreatic β-cells eventually fail to produce enough insulin to compensate for the ongoing insulin resistance. There is a tight asso-ciation between type 2 diabetes and dyslipidemia. The latter is characterized by raised small, dense LDL levels, elevated levels of triglycerides, and low levels of HDL. Individually, the latter two factors increase the risk of cardiovascular disease, and the combination of the two is a risk factor for cardiovascular heart disease that is at least as strong as a high level of LDL cholesterol (Jeppesen et al., 1997).

Current oral anti-diabetic agents, which include insulin releasers, insulin sensitizers and α-glucosidase inhibitors, have modest efficacy and limited modes of action. In addition, current anti-diabetic drugs usually have adverse side effects, decreased efficacy over time, ineffectiveness against some long-term diabetic complications and low cost-effectiveness (Grover et al., 2002). Therefore, discovery and development of novel drugs for diabetes is still

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*Corresponding author. E-mail: drarv1955@yahoo.com. Tel: +91-522-2612411, ext 4346. Fax: +91-522-2623405.

needed.

Plants are recognized as a wonderful source of medi-cines. It is estimated that 1200 species of plants are used as folk medicines for diabetes (Marles et al., 1995). Most of them lack scientific evidence for their alleged benefits. Among them, *Zingiber officinale* Roscoe, which belongs to the Zingiberacae family and is commonly known as ginger, is a medicinal plant that has been widely used in Ayurvedic, Chinese and Tibb-Unani system of herbal medicines all over the world since antiquity, for a wide array of unrelated ailments that include arthritis, rheuma-tism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases. The oleoresins from rhizhome of ginger contain pungent ingredients including gingerol, shoagol, and zingerone, and have been found to possess many interesting pharmacological and physiological activities (Park et al., 1998; Surh et al., 1999). Recently a report showed the hypoglycaemic po-tentials of ginger in streptozotocin (STZ)-induced diabetic rats treated with an aqueous extract of raw ginger (500 mg/kg, intra-peritoneally) daily for a period of 7 weeks (Al-Amin et al., 2006). Additionally, it showed that raw ginger is effective in reversing the diabetic proteinuria and loss of body weight observed in the diabetic rats (Al-Amin et al., 2006). In this study, we investigated the anti-hyperglycaemic, lipid lowering and antioxidant effects of [6]-gingerol in db/db mice, a leptin receptor deficient mouse model for type 2 diabetes study (Cefalu et al., 2006).

MATERIALS AND METHODS

Animals

Male db/db mice (12 week old, 35-45 g body weight) and their lean heterozygote litter-mates db/+ mice were procured from National Animal Laboratory Center (NALC) of Central Drug Research Institute (CDRI), Lucknow, India and all procedures were conducted in strict accordance with care and use of Laboratory animals after necessary approval by Institutional Animal Ethical Committee (IAEC) vide no. 65/08/Biochem/IAEC dated11/06/2008. Animals were housed in polypropylene cages, in controlled environment (temperature, 25 ± 2ºC; humidity, 50-60%; light, 300 lux at floor level with regular 12 h light-12 h dark cycle; noise level, 50 decibel; ventilation, 10-15 air changes per h). Animals were provided with a standard laboratory diet and had free access to food and drinking water, unless stated otherwise.

Extraction and isolation of [6]-gingerol

The fresh rhizomes of *Z. officinale*, with voucher specimen number, 4735 was purchased from local market. Its specimen is kept in the laboratory of the investigator. Rhizomes (20.0 kg) were crushed and percolated in ethanol (20 l) for 48 h at room temperature. The percolate was collected and this process of extraction was repeated four times. The combined ethanolic extract was filtered and concentrated under reduced pressure at 55ºC, afforded brown liquid (7 l). The extract obtained was successively fractionated with hexane, chloroform, and n-butanol. The solvents were removed un-der reduced pressure to furnish corresponding fractions of hexane
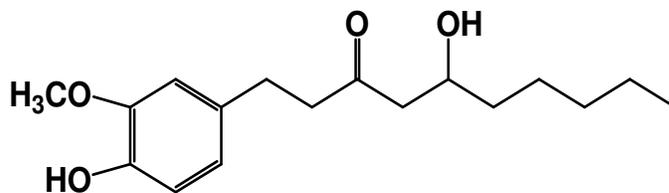

Figure 1. Chemical structure of [6]-gingerol.

hexane (200 g), chloroform (40 g), butanol (80 g) and aqueous (500 g). Chloroform fraction (35 g) was subjected to column chromato-graphy over silica gel (230-400 mesh) using hexane-ethyl acetate (9:1) mixture afforded fraction rich in [6]-gingerol (Figure 1). This fraction was further subjected to reverse phase column, using water: methanol (2:3) mixture, afforded [6]-gingerol (10 g). The identity of [6]-gingerol was confirmed by comparison of the spectra with the reported data (Connell et al., 1969).

Fasting blood glucose level and oral glucose tolerance test (OGTT)

Diabetic db/db mice showing blood glucose values from 250 to 400 mg/dl were selected for this study and randomly divided into groups of six mice in each. Group one served as diabetic control and was given vehicle (1% gum acacia), while the other experimental groups were orally administered with [6]-gingerol or rosiglitazone (rosiglitazone is a new thiazolidinedione that has been shown to be highly effective in reducing insulin resistance and improving glycaemic control in both animal models of diabetes and human Type 2 diabetes) at a dose 100 mg/kg body weight once daily for a period of 12 days. The fasting blood glucose level was measured after animals fasted for 4 h (starting from 9:00 a.m.) on day 0 (before treatment), day 5 (during treatment), and day 12 (last day of treatment). Blood glucose levels were measured by glucometer (ACCU-CHEK II; Roche Diagnostics) as per manufacturer's instructions.

An oral glucose tolerance test (OGTT) was performed on the last day of treatment after overnight fasting. Blood was sampled from the tail vein of mice at time 0 min (baseline) and at 30, 60, 90 and 120 min after an oral glucose load of 3.0 g/kg of body weight. Food, but not water was withheld from the cages during the course of experiment. The area under curve (AUC) of experimental animals was compared with that of vehicle-treated control group and the percent anti-hyperglycemic activity was determined as follows.

$$\text{Anti-hyperglycaemic activity \%} = \frac{\text{AUC of experimental group}}{\text{AUC of vehicle-treated group}} \times 100$$

Collection of blood samples and separation of plasma for biochemical studies

After the end of the experiment, blood samples of overnight fasted db/db mice of various groups were collected from retro-orbital venous plexus, using 5 µl heparinized glass capillary. Plasma was separated from the collected blood and used for the estimation of the levels of plasma lipids, total cholesterol (TC), triglycerides (TG), free fatty acid (FFA) and low-density lipoprotein cholesterol (LDL-C) by respective assay kits (Roche Diagnostics). Plasma insulin was measured by Mercodia-Mouse Insulin ELISA Kit (Mercodia AB Uppsala, Sweden). Animals were sacrificed at the end of experiment by cervical dislocation and tissues from liver and kidney were removed, rinsed with normal saline, frozen immediately in liquid nitrogen, and stored at –70ºC for various assays.

Determination of thiobarbituric acid-reactive substances (TBA-RS)

Measurement of MDA by TBA reactivity is the most widely used method for assessing lipid peroxidation (Colado et al., 1997). Kidney and liver tissues were washed with 1% (w/v) potassium chloride and subsequently homogenized in the same. The homogenized tissue was centrifuged at 1000 rpm for 15 min and supernatant was used for the estimation of MDA. In brief, the supernatant (0.5 ml) was added to a reaction mixture consisting of 30% TCA, 5 M HCl and 2% (w/v) thiobarbituric acid in 0.5 M NaOH. The reaction mixture was heated in a water bath at 95ºC for 15 min and centrifuged at 12000 rpm for 15 min. Absorbance of the supernatant was read at 532 nm with spectrophotometer (Perkin Elmer's, USA). The amount of MDA produced was calculated by using 1, 3, 3-tetra methoxy propane (TMP) as a standard and the result of lipid peroxidation was expressed as mM of MDA formed per mg of protein. Protein was estimated by the method of Lowry et al. (1951).

Isolation of hepatocytes from the liver of C57BL/KsJ-db/db mice

Liver cells were isolated by an adaptation of the two step perfusion method (Seqlen et al., 1973). Briefly, db/db mice were anesthetized with barbital (30 mg/kg, b.w, intraperitoneally) and their livers were removed intact. The liver was first perfused *in vitro* with cold normal saline solution, washed twice in HEPES buffered Hanks' (HBH) solution (pH 7.4) and gently chopped with a sterile blade into ~1 mm3 pieces in HBH buffer containing 0.5 mg/ml collagenase (type IV, 312 U/mg) (Sigma-Aldrich) and supplemented with 5 mM CaCl₂. The tissue suspension was incubated at 37ºC for 30 min in water bath with frequent shaking and then suspension was filtered through a 30-μm-nylon mesh filter to remove cell clumps and undissociated tissues. Single cell suspension obtained was centrifuged at 500 g for 5 min and resuspended in HBH buffer for further studies. Hepatocyte populations so obtained was intact and homogenous. Viability assay for hepatocyte was performed by trypan blue exclusion and 90% of viable hepatocytes were observed.

Measurement of reactive oxygen species (ROS)

Intracellular ROS was determined by 2,7-dichlorofluorescein diacetate which fluorescence was determined in a flow cytometer (Becton Dickinson, UK). H2DCFDA (100 μmol/L of 10 mM stock, Molecular Probes) was loaded into 1×10^6 cells, suspended in 0.5ml fresh DMEM (pH 7.2), and incubated at 37ºC for 30 min. For each sample, 10,000 cells (events) were acquired. Each experiment was performed in duplicate. DCF fluorescence was examined at 530 nm. Data were obtained and analyzed with the Cell Quest software from a PI negative cell population on a BD FACScan (Liao et al., 2003)

Hepatic and renal anti-oxidant enzyme assays

Catalase (CAT)

Catalase was estimated by the method of Aebi et al. (1984). The reaction mixture contained 0.1 M sodium phosphate buffer (pH 7.2); 4 mM H_2O_2 and suitably diluted enzyme (165 μl). Reaction was started by addition of H_2O_2 and the rate of the change in H_2O_2 concentration was followed by observing the decrease in optical density at 240 nm for 3 min. One unit of catalase is defined as the amount of enzyme that reduces one μmol of H_2O_2 per minute. Values of this and other enzymes are expressed as specific activity (units/mg protein).

Glutathione peroxidase (GPx)

GPx activity was assayed by the method of Forstrom et al. (1978). The reaction mixture contained 25 mM Tris-HCl buffer (pH 7.6); 0.12 mM NADPH; 1unit/ml Glutathione reductase; 1 mM reduced glutathione; 0.1 mM Hydrogen peroxide, and 160 μl of suitably diluted enzyme. The reaction was started by the addition of hydrogen peroxide and depletion of NADPH was measured spectrophotometrically at 340 nm for 3 min. One unit of GPx is defined as the amount of enzyme that oxidizes one μmol of NADPH per minute.

Superoxide dismutase (SOD)

SOD activity was determined according to the method of Fridovich et al. (1989). The reaction mixture contained 100 μl tissue homogenate, 880 μl (0.05 M) of carbonate buffer (pH 10.2). 20 μl of 30 mM epinephrine in 0.05% acetic acid was added to the mixture and absorbance was measured for 4 min at 480 nm with spectrophotometer. One unit of SOD is defined as the amount of enzyme that results in 50% inhibition in rate of epinephrine auto-oxidation.

Cytotoxicity assay

Cytotoxic effect of *in-vivo* active pure compounds on 3T3-L1 pre-adipocytes was evaluated by MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay (Mosmann et al., 1983). 3T3-L1 cells were maintained in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 200 μg/ml streptomycin and 0.25 μg/ml amphotericin B. Cells were seeded at 1×10^4 cells/well in 96 well culture plate in the same medium for 24 h. compound solution was added into each well at different concentration (1 ng/ml to 100 μg/ml) in triplicate and incubated at 37ºC for 24 h. 20 μl of MTT solution (5 mg/ml in PBS) was added into each well and incubated at 37℃ for 4 h. The absorbance was measured at 540 nm using ELISA plate reader (Molecular Devices, USA).

Statistical analyses

Student's t-test or one way ANOVA was used where ever appropriate. All the statistical analyses were performed with Graph Pad Prism software (Version 3.03). Statistical significance was considered at $p < 0.05$.

RESULTS

Effect of [6]-gingerol on fasting blood glucose

Blood glucose levels after 4 h of fasting were measured on days 0, 5 and 12 after daily oral administration of [6]-gingerol (100 mg/kg b.w). Figure 2A showed significant decrease in fasting blood glucose level on day 5 of 28.5% ($p < 0.05$) and day 12 of 54.8% ($p < 0.01$) compared to vehicle-treated control group, whereas rosiglitazone treatment reduced fasting blood glucose level by 21.4% ($p < 0.05$) on day 5 and 43.3% ($p < 0.01$) on day 12 respectively. In addition, [6]-gingerol treatment also moderately but significantly ($p < 0.05$) reduced body weight body weight (from 50.4 ± 8.9 g on day 0 to 43.5 ±7.8 g on day 12) in *db/db* mice. In a similar experiment, rosiglitazone showed significant ($p < 0.05$) weight gain(from 50.9 ± 8.0 g on day 0 to 58.9 ± 10.5 g on day 12) in
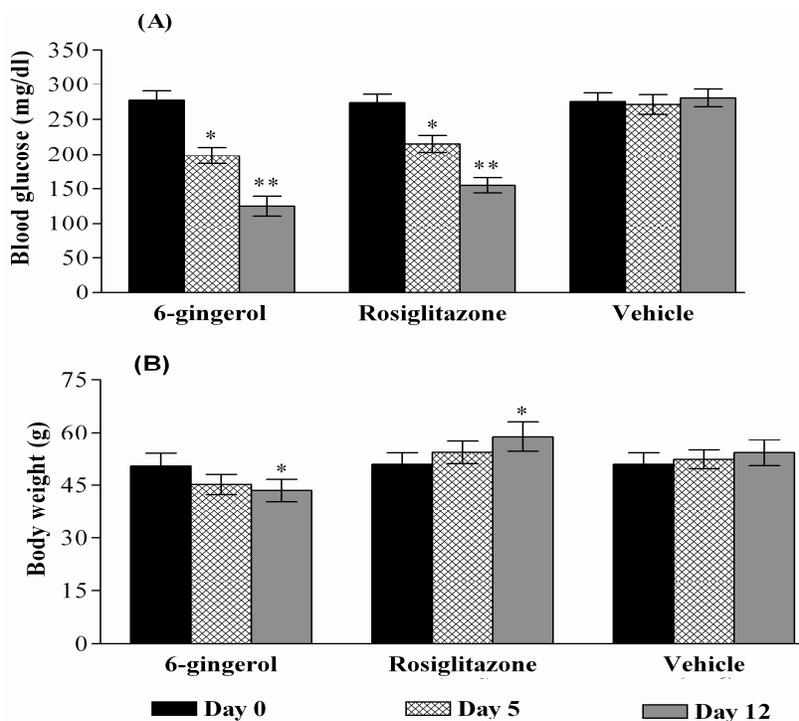
Figure 2. Effect of [6]-gingerol (100 mg/kg) treatment on (A) fasting blood glucose level (B) body weight in *db/db* mice. Data were expressed as mean ± SD. N=6, *p < 0.05, **p < 0.01 vs. control *db/db* mice group.

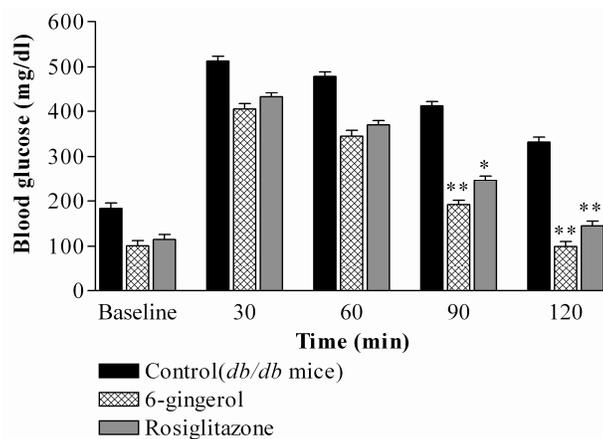**Figure 3.** Impact of [6]-gingerol (100 mg/kg) treatment on oral glucose tolerance in db/db mice. Data were expressed as mean ± SD. N=6, *p<0.05 & **p<0.01 vs. control db/db mice group.

db/db mice.

Effect of [6]-gingerol on oral glucose tolerance test

Prior to treatment, *db/db* mice demonstrated basal hyperglycemia and this hyperglycemia was exacerbated by the oral glucose load (3.0 g/kg b.w) and failed to return to fasting level after 120 min, indicating glucose intolerance (data not shown). After 12 days treatment, [6]-gingerol significantly (p<0.01) improved the glucose tolerance and inhibited rise in postprandial glucose level at time interval of 90 and 120 min after glucose load in *db/db* mice (Figure 3). For the [6]-gingerol *db/db* mice group, the area under the curve (AUC) of blood glucose decreased by approximately 40.5% compared to vehicle

Table 1. Effects of [6]-gingerol (100 mg/kg) on plasma insulin and plasma lipids in C57BL/KsJ-*db/db* mice. Values are expressed as mean ± S.E. (N=6), * p<0.05 and ** p<0.01 vs. control group; p value was determined by one-way ANOVA test.

Parameters	Control	[6]-gingerol	Rosiglitazone
Insulin (ng/ml)	14.5 ± 3.70	7.80 ± 0.78**	9.90 ± 0.80**
Triglyceride (mg/dl)	169.6 ± 7.30	99.8 ± 3.70**	109 .0 ± 2.20**
Total cholesterol (mg/dl)	160.5 ± 8.60	110.3 ± 4.68**	127.2 ± 5.18*
LDL-cholesterol (mg/dl)	133.8 ± 2.68	96.4 ± 9.00**	101.5 ± 9.90*
Free fatty acid (mg/dl)	36.8 ± 1.29	27.8 ± 1.20**	30.6 ± 2.00*

Table 2. Impact of [6]-gingerol (100 mg/kg) on the hepatic and renal anti-oxidant enzymes (U/mg protein) and MDA concentration (mmol/l) in C57BL/KsJ-db/db mice.

	db/+ mice	db/db mice #	[6]-gingerol *
Liver			
Catalase	10.5 ± 1.80	6.20 ± 2.50 ##	8.98 ± 2.40***
GPx	65.4 ± 3.50	48.5 ± 2.90 ##	60.1 ± 2.70*
SOD	20.5 ± 2.50	14.2 ± 1.90 ##	18.2 ± 2.30**
MDA	6.50 ±1.40	10.5 ± 1.50 ##	7.20 ± 1.80**
Kidney			
Catalase	8.5 ± 2.10	5.70 ± 2.50 ##	7.50 ± 2.40*
GPx	52.4 ± 3.50	38.5 ± 3.10 ##	47.0 ± 3.70**
SOD	23.0 ± 2.90	18.2 ± 2.30 #	21.3 ± 1.90*
MDA	8.22 ± 1.80	12.6 ± 1.20 ##	9.60 ± 1.50*

Data are expressed as mean ± S.D. (N= 6), # p<0.05 and ## p<0.01 vs. non-diabetic lean control group (*db/+* mice) as determined by one-way ANOVA test. Significance, *p<0.05, **p<0.01 and ***p<0.001 vs. diabetic control group (*db/db* mice) as determined by one-way ANOVA test.

treated group. In similar experiments, rosiglitazone also improved the glucose tolerance and showed 31.8% reduction in AUC of *db/db* mice.

Effect of [6]-gingerol on plasma lipids and plasma insulin

Diabetic *db/db* mice orally treated with [6]-gingerol (100 mg/kg b.w) for 12 consecutive days showed significant (< 0.05) reduction in the level of plasma triglycerides by 41.1%, total cholesterol by 31.2% and low-density lipoprotein cholesterol by 27.9%, free fatty acid by 24.4% as well as also significantly (p<0.05) lowered the plasma insulin concentrations by 46.2% in *db/db* mice (Table 1). In a similar experiment rosiglitazone at the same dose showed significant (*p<0.05*) reduction in plasma triglyceride by 35.7%, total cholesterol by 20.4%, LDL-cholesterol by 24.1%, free fatty acid by 16.8% and plasma insulin concentrations by 31.7% compared to vehicle treated control group.

Effect of [6]-gingerol on intracellular ROS generation

Isolated hepatocytes from liver of diabetic *db/db* mice

showed an increased level of ROS content by 262.8% (3.6 fold) as compared to vehicle treated non-diabetic lean control *db/+* mice as assessed by mean fluorescence of DCFDHA-DA. Diabetic *db/db* mice treated with [6]-gingerol for 12 consecutive days showed significant decrease in ROS content by 64.0% (2.8 fold) (**p < 0.01) (Figures 4a and b) as compared to diabetic control *db/db* mice group.

Effect of [6]-gingerol on anti-oxidative enzymes and lipid peroxidation

The activity of CAT, GPx and SOD decreased in liver of hyperglycemic *db/db* mice by 40.9, 25.8 and 30.7% respectively, when compared to non-diabetic lean control (*db/+*) mice. Similar reduction was also observed in kidney showing 32.9, 26.5 and 20.8% in CAT, GPx and SOD activity, respectively. *Db/db* mice treated with [6]-gingerol significantly (*p<0.05*) increased CAT activity by 44.8%; GPx activity by 23.9% and SOD activity by 28.1% in liver of *db/db* mice. Similar increment was also observed by 24.0, 22.1 and 17.0% activities of CAT, GPx and SOD in the kidney, respectively (Table 2).
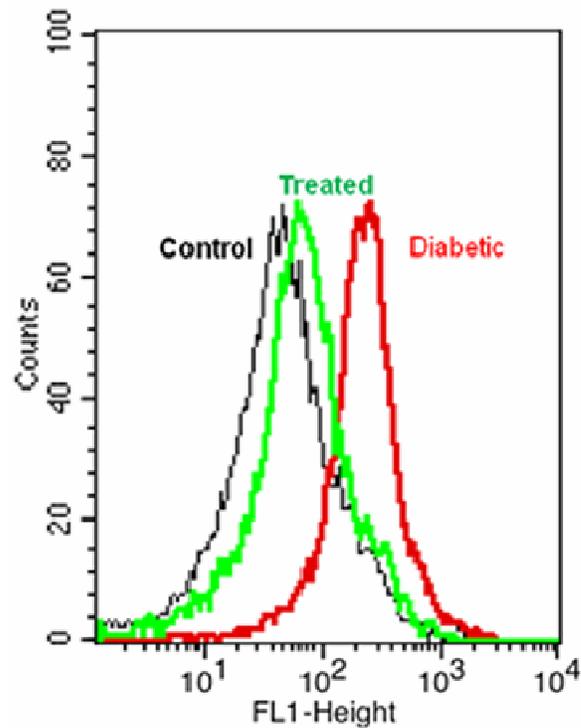
Lipid peroxidation was significantly (p < 0.05) higher

Figure 4a. Outlay histogram representation of mean fluorescence of DCFDHF DA for ROS in normal control (*db/+* mice), diabetic control (*db/db* mice), and treated with [6]-gingerol.
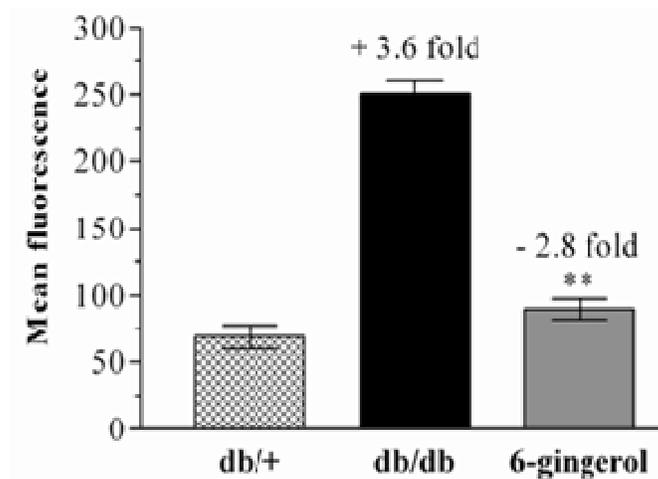

Figure 4b. Mean fluorescence of DCFDHF-DA for ROS estimation. ROS was measured in isolated hepatocytes of 6 animals in each group. Data were expressed as mean fluorescence ± SD. ***p<0.01* was considered significant when comparison was made among different groups by one way ANOVA followed by Dunnett's test. (+ indicates increase in mean fluorescence of DCFDHF-DA in diabetic *db/db* mice group as compared to non-diabetic control *db/+* mice. - indicates decrease in mean fluorescence of DCFDHF-DA in [6] gingerol treated *db/db* mice group as compared to diabetic *db/db* mice group).
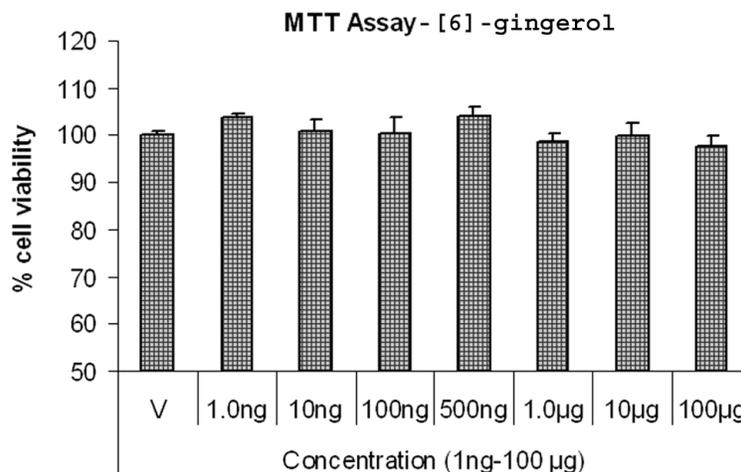
Figure 5. Cytotoxic effect analysis of [6]-gingerol isolated from rhizome of *Z. officinale* on 3T3-L1 pre-adipocytes using MTT assay (Mean ± SE, n=3).

in the liver and kidney tissues of diabetic *db/db* mice by 61.5 and 53.2% in comparison to the non-diabetic control (*db/+*) mice. In the present study, it was observed that the treatment of [6]-gingerol conferred significant ($p < 0.05$) reduction in the lipid peroxidation by 31.4% in liver and by reduction in the lipid peroxidation by 31.4% in liver and by 23.8% in kidney, when compared to the diabetic control *db/db* mice group (Table 2).

Cytotoxic effect of [6]-gingerol

In order to evaluate any cytotoxic effect of the [6]-gingerol isolated from rhizome of *Z. officinale*, the [6]-gingerol was incubated with 3T3-L1 pre-adipocyte cell lines for 24 h. The effect was monitored by MTT assay. The compounds at various concentrations (1 ng/ml to 100 μg/ml) did not show any effect on cell viability of 3T3-L1 cells and found safe at this concentration range (Figure 5).

DISCUSSION

Diabetes mellitus is the most crucial disease involving the endocrine gland pancreas. Its major manifestations include disordered metabolism and inappropriate hyperglycemia. In diabetes, oxidative stress has been found to occur mainly due to increased production of oxygen free radicals and a sharp reduction of antioxidant defenses (Fridlyand et al., 2005). Hence, compounds with both hypoglycemic and anti-oxidative properties would be useful anti-diabetic agents (Cemek et al., 2008). So, the aim of this study was to investigate the anti-diabetic and anti-oxidative effects of [6]-gingerol, isolated from *Z. officinale*, using type 2 diabetes models (*db/db* mice).

The results of the present study demonstrate that [6]-gingerol is an effective anti-hyperglycaemic, lipid lowering and free radicals scavenging agent in *db/db* mice. Insulin

resistance profoundly contributes to the pathophysiology of type 2 diabetes and induces reduced glucose utilization and increased glucose production in the liver, leading to hyperglycaemia (McGarry et al., 1994). Our results clearly demonstrated that treatment with [6]-gingerol significantly improved glucose homeostasis in *db/db* mice. It has been shown that the ability of insulin to mediate tissue glucose uptake is a critical step in maintaining glucose homeostasis and in clearing the postprandial glucose load (DeFronzo et al., 1985; Kruszynska et al., 1996). In addition, mice treated with [6]-gingerol showed significant ($p < 0.05$) improvement in fasting blood glucose levels and glucose tolerance. Interestingly, statistically significant weight loss in *db/db* mice was also observed. It is possible that the observed antihyperglycaemic effect of [6]-gingerol is not dependent on the body weight reduction. Insulin resistance is very often accompanied by obesity and the obesity not only increases the chance of developing type 2 diabetes, it is also independently associated with insulin resistance and other morbidity (Kruszynska et al., 1996). Thus, the insulin resistance in obese patients with type 2 diabetes is significantly worse than the insulin resistance in non-obese diabetic individuals (Seely et al., 1993). Past studies have shown that insulin sensitivity in type 2 diabetes patients improves with weight loss (DeFronzo et al., 1991), possibly due to an improvement in insulin-stimulated glucose transport into muscle (Friedman et al., 1992). Thus, anti-diabetic medications that also reduce body weight are particularly beneficial. This report suggests that [6]-gingerol can be used to treat diabetes, especially in obese diabetic patients.

In addition, administration of [6]-gingerol significantly ($p < 0.05$) lowered fasting plasma insulin concentration and plasma triglycerides, total cholesterol, free fatty acid and LDL-cholesterol level in *db/db* mice. There are growing evidences suggesting that hepatic insulin resistance is

associated with an increased production of free fatty acid and triglycerides. The circulating free fatty acid and triglycerides that are commonly elevated in obese and diabetic subjects, lower the ability of insulin to suppress hepatic glucose production by activating gluconeogenesis and inhibiting glycolysis (Hawkins et al., 2003; Shah et al., 2003). Therefore, a reduction in the circulating free fatty acid and triglycerides would be expected to improve hyperglycemia and strengthen the insulin response by suppressing glucose production, while increasing glucose utilization (Santomauro et al., 1999). Diabetes is known to increase ROS production and oxidative stress probably as a result of glucose auto-oxidation and non-enzymatic glycation (Gupta et al., 2007). Prolonged exposure of hyperglycemic condition creates predominance of oxidative stress over antioxidative defense systems, leading to oxidative DNA damage, which possibly contributes to pancreatic beta-cell dysfunction (Song et al., 2007). Our study suggests that *db/db* mice treated with [6]-gingerol had increased anti-oxidant enzyme activities and decreased hydrogen peroxide concentration in liver and kidney, respectively. Furthermore, the hepatic and renal MDA levels were also significantly ($p < 0.05$) lowered in the [6]-gingerol treated *db/db* mice, indicating a decreased rate of lipid peroxidation. As a result, enhanced antioxidant enzyme activities in the liver and kidney by [6]-gingerol may have a protective role against ROS, thereby decreasing the formation of hydrogen peroxide and lipid peroxidation. Thus, it seems reasonable that [6]-gingerol was effective for preventing hepatic and renal damage. In cytotoxic evaluation on 3T3 L-1 pre-adipocytes, [6]-gingerol showed no adverse effect on cell viability in a concentration range of 1 ng/ml to 100 µg/ml and was found to be safe at this concentration gradient.

In conclusion, the data obtained in this study suggest that [6]-gingerol is an effective anti-diabetic agent via its ability to enhance insulin sensitivity and to decrease hyperlipidemia in type 2 diabetic animals. Furthermore, it seems likely that [6]-gingerol is beneficial against oxidative stress, thereby being helpful in delaying or preventing complications of diabetes and aging.

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