
Comparative Antidiabetic Activity of Methanolic Extract and Ethyl Acetate Extract of *Zingiber officinale* Roscoe

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Earlier we have reported the antidiabetic activity of fresh juice of rhizomes of *Zingiber officinale* and its correlation with 5-HT receptor antagonism. Since 6-gingerol the marker compound of *Z. officinale* is reported to possess 5-HT antagonistic activity, the present investigation was undertaken to find out the concentration of 6-gingerol present in methanolic extract and ethyl acetate extract of *Z. officinale*. We also evaluated these extracts for antidiabetic activity in streptozotocin-induced type 2 diabetic rats. Streptozotocin-induced type 2 diabetic rats showed a significant increase in fasting glucose levels that was associated with increase in insulin levels. Treatment with methanolic extract and ethyl acetate extract of *Z. officinale* produced a significant decrease in fasting glucose and insulin levels in type 2 diabetic rats. In oral glucose tolerance test, treatment with methanolic extract and ethyl acetate extract of *Z. officinale* was found to significantly decrease AUC_{glucose} and AUC_{insulin} values in type 2 diabetic rats. Treatment with methanolic extract produced greater reduction in elevated levels of glucose and AUC_{glucose} as compared to ethyl acetate extract. Treatment with methanolic extract and ethyl acetate extract of *Z. officinale* also produced decrease in serum cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol levels in type 2 diabetic rats. The concentration of 6-gingerol was found to be greater in methanolic extract (3.08 %) and lower in ethyl acetate extract (1.64 %). In conclusion our data suggest methanolic extract of *Z. officinale* to have better antidiabetic activity in type 2 diabetic rats as compared to ethyl acetate extract of *Z. officinale*. The extent of activity appears to be dependent on the concentration of 6-gingerol present in the extracts.

Zingiber officinale Roscoe is one of the commonly used spices in India. *Zingiber officinale* is commonly known as ginger. Ginger is an underground rhizome of the plant *Zingiber officinale* belonging to family Zingiberaceae. Ginger is known by several names like *Ardharakam*, *Adrak*, *Adu*, *Ala*, in Indian languages. Ginger has been reported to produce various pharmacological effects such as antiemetic, antiulcer, antioxidant, anxiolytic, antiinflammatory and antipyretic activity¹⁻⁵. Ginger has also reported to reduce cholesterol levels and atherogenesis in rabbits fed with high cholesterol diets⁶. It also stimulates bile acid biosynthesis from cholesterol⁷. Aqueous extract of ginger is reported to inhibit platelet aggregation, induced by ADP, epinephrine,

collagen and arachidonic acid *in vitro*, these actions were correlated to gingerols which inhibit platelet aggregation⁹.

5-Hydroxytryptamine (5-HT) produces hyperglycemia in normoglycemic rats involving specific 5-HT_{2A} and 5-HT₃ receptors¹⁰. It has further been shown that chronic treatment with 5-HT_{2A} antagonist sarpogrelate and 5-HT₃ antagonist ondansetron produce antidiabetic activity and many other beneficial effects in diabetic rats¹⁰. Many of the pharmacological activities of ginger are because of its 5-HT antagonistic activity. Alcohol extract of ginger is reported to produce blood glucose lowering effect in rabbits¹² and in rats¹³. The effect of alcohol extract of *Z. officinale* on rabbits was carried out in normal rats which were not diabetic¹². Earlier our laboratory reported, treatment with fresh juice of *Z. officinale* on streptozotocin (STZ) induced type 1 diabetes

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to produce significant antidiabetic activity. The antidiabetic activity of fresh juice of *Z. officinale* was proposed to be correlated through 5-HT receptor antagonism¹⁴. However the study with fresh juice of *Z. officinale* was not standardized for 6-gingerol content in the juice. 6-gingerol is the biological and chemical marker substance present in *Z. officinale*. 6-gingerol is reported to possess 5-HT antagonistic activity¹¹.

In light of these earlier observations, in the present investigation we have studied the effect of 3 w treatment with ginger extracts standardized for 6-gingerol content and the effects of extracts on blood glucose and other biochemical parameters in STZ-induced type 2 diabetic rats and thereby understand the significance of concentrations of 5-HT antagonistic substance 6-gingerol content in the extracts for the activity.

MATERIALS AND METHODS

Plant material:

The dried rhizomes of *Z. officinale* were obtained from local market, they were authenticated at Botany Department, Gujarat University, Ahmedabad. The authentication was done by comparing dried rhizomes of *Z. officinale* morphologically and microscopically as mentioned in different standard texts and floras.

Preparation of extracts:

Dried rhizomes of *Zingiber officinale* Roscoe were powdered and used for further extraction. Powdered dried rhizomes (500 g) of *Z. officinale* was taken in a round bottom flask and extracted with methanol (1.5 lx2) under reflux for 6 h each time. The extracts were filtered, pooled and concentrated to dryness under reduced pressure (yield: 4.3 %) and used for antidiabetic study. Powdered dried rhizomes (500 g) of *Z. officinale* was taken in a round bottom flask and extracted with ethyl acetate (1.5 lx2) under reflux for 6 h each time. The extracts were filtered, pooled and concentrated to dryness under reduced pressure (yield: 4.6%), which was used for antidiabetic study.

Standardization of extracts:

Standardization of methanolic extract and ethyl acetate extract of *Z. officinale* for 6-gingerol content, was carried out by HPLC analysis using 8-methyl-n-vanillynonamide as reference standard. The analysis was performed as per earlier reported method¹⁵.

Type 2 diabetic rat model and treatment protocol:

Healthy Sprague Dawley rats were kept for breeding under well controlled conditions of temperature (22±2°), humidity (55±5 %) and 12 h/12 h light-dark cycle. To induce type 2 diabetes a single dose of injection of streptozotocin (STZ, 90 mg/kg, i.p., Sigma Chemical Co., St. Louis, MO, USA) was given to the 2 d old pups. Another group of pups received only saline. The animals were weaned at 30 d and after a period of 3 mo, they were checked for fasting glucose levels to confirm the status of Type 2 diabetes. The animals showing fasting glucose levels >140 mg/dl were considered as diabetic. The pups that received saline were considered as control animals. The experimental animals were divided into four groups, six animals in each group, group 1: normal control, group 2: diabetic control, group 3: diabetic treated with methanolic extract (ME) of *Z. officinale* 0.5 g/kg, group 4: diabetic treated with ethyl acetate extract (EAE) of *Z. officinale* 0.5 g/kg. Treatment was given once daily per orally for 3 w. The control group received an equal volume of the vehicle. All the procedures were performed in accordance with the Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under Ministry of Animal Welfare Division, Government of India, New Delhi, India.

Blood sampling and biochemical analysis:

At the end of 3 w treatment, the animals were kept for overnight fasting, four hours after drug administration morning blood samples were collected from the tail vein into centrifuge tubes. The blood samples were allowed to clot for 30 min at room temperature and then centrifuged at 5000 rpm for 20 min. Serum samples thus obtained were stored at -20° until biochemical estimations were carried out. Serum samples were analyzed spectrophotometrically by using double beam UV/Vis spectrophotometer (Shimadzu UV-1601, Japan). Glucose, cholesterol, triglycerides, HDL-cholesterol, urea and creatinine, were estimated using respective diagnostic kits (Bayer Diagnostics Ltd, India). Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) were also estimated using respective diagnostic kits (Span Diagnostics, India). Serum insulin levels were estimated by radioimmunoassay method using the kit from Bhabha Atomic Research Center, Mumbai. VLDL-cholesterol and LDL-cholesterol were calculated as per Friedewald's equation, VLDL-cholesterol=total serum triglycerides/5 and LDL-cholesterol=total serum cholesterol-total serum triglycer-

ides/5-HDL-cholesterol.

Oral glucose tolerance test:

Rats were subjected to an oral glucose tolerance test (OGTT). Glucose (1.5 g/kg) was administered to 12 h fasted rats. Blood samples were collected at 0, 30, 60 and 120 min. Serum was separated immediately and analyzed for glucose and insulin. The results of OGTT were expressed as integrated areas under the curves for glucose (AUC_{glucose}) and insulin (AUC_{insulin}) over a period of 0-120 min.

Statistical analysis:

The results were analyzed statistically using one way ANOVA followed by Tukey's multiple tests to determine level of significance. Value of $P < 0.05$ was considered significant.

RESULTS

Standardization of the ME and EAE of *Z. officinale* for 6-gingerol content showed presence of 3.08 % w/w and 1.64 % w/w of 6-gingerol in ME and EAE respectively. This clearly shows higher concentrations of 6-gingerol content in ME as compared to EAE.

Animals, which received STZ, showed a significant reduction in body weight, increase in water intake and food intake as compared to control animals ($P < 0.05$) (Table 1). Treatment with ME and EAE of *Z. officinale* did not alter the body weight. However, both extracts of *Z. officinale* produced a significant decrease in food intake and water intake (Table 1).

STZ injection produced hyperglycemia and hyperinsulinemia in rats. Treatment with ME and EAE of *Z. officinale* significantly decreased fasting blood glucose levels (Table 2). Treatment with ME and EAE significantly reduced fasting serum insulin levels as compared to diabetic control rats (Table 2). AUC_{glucose} and AUC_{insulin} were significantly greater in diabetic rats as compared to diabetic control rats during oral glucose tolerance test. Treatment with ME and EAE of *Z. officinale* significantly lowered AUC_{glucose} levels as compared to diabetic control rats (Table 2). Treatment with ME and EAE of *Z. officinale* significantly lowered AUC_{insulin} levels as compared to diabetic control rats (Table 2).

Diabetic control rats showed significantly elevated levels of cholesterol, triglyceride, LDL-cholesterol, VLDL-cholesterol and no changes in HDL-cholesterol as compared to normal control rats. Treatment with ME and EAE showed significant reduction in cholesterol, triglyceride, LDL-cho-

lesterol, VLDL-cholesterol and no changes in HDL-cholesterol as compared to diabetic control rats (Table 3).

The diabetic control animals showed elevated fasting SGOT and SGPT levels as compared to normal control rats, treatment with ME and EAE showed significant reduction in elevated levels of fasting SGOT and SGPT levels as compared to diabetic control rats (Table 4).

The diabetic control animals showed elevated fasting serum urea and creatinine levels as compared to normal control rats, treatment with ME and EAE showed no significant changes in elevated levels of serum urea and creatinine levels as compared to diabetic control rats (Table 4).

TABLE 1: EFFECT OF *ZINGIBER OFFICINALE* ON VARIOUS PARAMETERS IN DIABETIC RATS.

Treatment	Body weight (g/rat)	Water intake (ml/rat/d)	Food intake (g/rat/d)
Control	228.3±4.01	39.72±0.79	17.22±0.55
Diabetic	205.0±4.28*	52.50±0.71*	25.55±0.70*
ME	205.0±4.28	44.72±0.66**	45.00±0.42**
EA	203.3±3.33	45.00±0.42**	21.66±0.42**

Each value is mean±SEM. (n=6). *Significantly different from control ($P < 0.05$). **Significantly different from diabetic control ($P < 0.05$).

DISCUSSION

The results of the present study show that the treatment with ME and EAE of *Z. officinale* produce significant reduction in elevated glucose and insulin levels in STZ-induced type 2 diabetic rats. Earlier our laboratory reported antidiabetic activity of fresh juice of *Zingiber officinale* in STZ-induced type 1 diabetic rats¹⁴. Treatment with fresh juice of *Z. officinale* in STZ-induced type 1 diabetic rats showed reduction in elevated glucose levels and increase in insulin levels¹⁴. In type 1 diabetes insulin levels are lower whereas in type 2 diabetes they are higher. The increase in insulin in type 1 diabetes is due to the actions on pancreas. The reduction in insulin levels obtained in the present study indicates extrapancreatic action of *Z. officinale*. These findings are similar to our earlier findings with sarpogrelate which showed increase in insulin levels in type 1 diabetic rats and reduction in insulin levels in type 2 diabetic rats¹⁰. 5-HT produces hyperglycemia in normoglycemic rats involving specific 5-HT_{2A} and 5-HT₃ receptors. Antidiabetic activity of fresh juice of *Z. officinale* was proposed to be correlated through 5-HT receptor antagonism. The results of the present study support our earlier findings.

TABLE 2: EFFECT OF ZINGIBER OFFICINALE ON VARIOUS PARAMETERS IN DIABETIC RATS.

Treatment	Glucose (mg/dl)	Insulin (μ U/ml)	AUC _{glucose} (mg/dl.min) $\times 10^3$	AUC _{insulin} (μ U/ml.min) $\times 10^3$
Control	75.60 \pm 1.8	43.00 \pm 1.5	13.90 \pm 0.1	6.80 \pm 0.1
Diabetic	157.8 \pm 3.9*	110.7 \pm 2.7*	30.10 \pm 0.3*	14.70 \pm 0.3*
ME	124.3 \pm 2.5**	89.30 \pm 2.4**	22.30 \pm 0.2**	11.90 \pm 0.3**
EA	142.1 \pm 1.9**	87.30 \pm 2.1**	25.70 \pm 0.1**	12.30 \pm 0.2**

Each value is mean \pm SEM. (n=6). *Significantly different from control (P<0.05). **Significantly different from diabetic control (P<0.05).

TABLE 3: EFFECT OF ZINGIBER OFFICINALE ON VARIOUS PARAMETERS IN DIABETIC RATS.

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-Cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)	VLDL-Cholesterol (mg/dl)
Control	43.9 \pm 1.2	41.2 \pm 1.1	12.4 \pm 0.5	23.3 \pm 0.9	8.2 \pm 0.2
Diabetic	79.6 \pm 2.1*	116.2 \pm 2.4*	12.0 \pm 0.3	44.3 \pm 1.9*	23.2 \pm 0.4*
ME	57.2 \pm 1.5**	71.0 \pm 2.6**	11.7 \pm 0.4	31.2 \pm 0.9**	14.2 \pm 0.5**
EA	65.3 \pm 1.7**	87.0 \pm 2.3**	12.0 \pm 0.3	35.8 \pm 1.3**	17.4 \pm 0.4**

Each value is mean \pm SEM. (n=6). *Significantly different from control (P<0.05). **Significantly different from diabetic control (P<0.05).

TABLE 4: EFFECT OF ZINGIBER OFFICINALE ON VARIOUS PARAMETERS IN DIABETIC RATS.

Treatment	SGOT (units/ml)	SGPT (units/ml)	Urea (mg/dl)	Creatinine (mg/dl)
Control	32.00 \pm 1.03	25.00 \pm 1.23	33.99 \pm 1.08	0.43 \pm 0.01
Diabetic	59.67 \pm 1.40*	42.00 \pm 1.46*	65.65 \pm 2.05*	0.75 \pm 0.03*
ME	43.67 \pm 1.40**	33.67 \pm 1.20**	61.58 \pm 1.75	0.66 \pm 0.01
EA	47.67 \pm 1.40**	33.67 \pm 0.95**	67.95 \pm 1.62	0.72 \pm 0.02

Each value is mean \pm SEM. (n=6). *Significantly different from control (P<0.05). **Significantly different from diabetic control (P<0.05).

The AUC_{glucose} and AUC_{insulin} levels in diabetic treated rats was significantly decreased in rats treated with ME and EAE of *Z. officinale*. Treatment with ME reduced fasting serum glucose levels by 21% and AUC_{glucose} by 26%, whereas treatment with EAE reduced fasting serum glucose by 10% and AUC_{glucose} by 14%. These observations clearly show that ME has better activity as compared to EAE. Earlier studies with ethanolic extract of *Z. officinale* is reported to have hypoglycemic action in rabbits¹². However, the study was done on normal rabbits which were devoid of diabetes and also the study were conducted using non standardized ginger extract for any marker substance nor they measured insulin levels to explain the condition. Hence this study

shows that the reduction in serum glucose and AUC_{glucose} levels in conditions of type 2 diabetes are associated with reduction in insulin levels. From this observation one can understand that the antihyperglycemic action produced by *Z. officinale* extracts in type 2 diabetes is by increase in peripheral glucose uptake and increased insulin sensitivity, because there was no increase in insulin levels rather there was reduction in elevated insulin levels.

In the present study STZ-treated rats significantly reduced body weight, increase in water intake and food intake as compared with the control animals. Treatment with ME and EAE of *Z. officinale* failed to produce significant

change in the body weight, however there was a significant reduction in water intake and food intake in the diabetic animals treated with ME and EAE of *Z. officinale* as compared to diabetic control rats. STZ-induced diabetic rats showed significant increase in serum lipid levels. Durrington¹⁶ reported that insulin resistance or insulin deficiency was associated with hypercholesterolemia and hypertriglyceridemia. *Z. officinale* treatment significantly decreased serum cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol levels. ME of *Z. officinale* reduced elevated lipid levels better as compared to reduction seen with EAE treatment. *Z. officinale* is reported to decrease LDL-cholesterol, VLDL-cholesterol and triglyceride levels in apolipoprotein-E deficient mice¹⁷. Earlier it has been reported that ethanolic extract of *Z. officinale* prevents hypercholesterolemia and development of atherosclerosis in cholesterol fed rabbits⁶. It is also reported that (E)-8 beta, 17- epoxy-labeled-12-ene-15,16-dial, a compound isolated from *Z. officinale* interfered with cholesterol biosynthesis in homogenized liver of hypercholesterolemic mouse¹⁸. Diabetes is considered to be a disease associated with liver dysfunction, the indicator of which is the elevated SGOT and SGPT levels in diabetic animals as compared to normal control rats. Earlier studies with *Z. officinale* as shown to possess good antioxidant activity¹⁹, the reduction in the elevated SGOT and SGPT levels on treatment with ME and EAE of *Z. officinale* shows that in diabetic conditions *Z. officinale* may be useful in not only reducing elevated glucose and lipid levels but also it can reduce the elevated hepatic enzyme levels by which liver dysfunction in diabetes can be prevented.

Standardization of ME and EAE of *Z. officinale* for 6-gingerol content showed the concentration of 6-gingerol to be greater in ME (3.08 %) and lower in EAE (1.64 %). 6-gingerol is reported to possess 5-HT antagonist activity¹¹. Thus if 6-gingerol is considered to be responsible for antidiabetic activity of *Z. officinale*, our data clearly indicate that the antidiabetic activity appears to be dependent on the concentration of 6-gingerol present in the ME and EAE.

In conclusion, our data suggest that both ME and EAE of *Z. officinale* are beneficial in reducing elevated levels of serum glucose, lipids and liver enzymes in STZ-induced type 2 diabetic rats, but treatment with ME of *Z. officinale* has better antidiabetic activity as compared to EAE in STZ-

induced type 2 diabetic rats. The extent of activity appears to be dependent on the concentration of 6-gingerol present in the extracts.

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