
Antidiabetic Activity of *Zingiber officinale* Roscoe in Streptozotocin-Induced Non-insulin Dependent Diabetic Rats.

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The fresh as well as dried rhizome of ginger, *Zingiber officinale* Roscoe is widely used in the traditional system of medicine. We have studied the effect of fresh juice of *Z. officinale* (4 ml/kg) and methanolic extract (0.5 g/kg) administered orally daily for 6 w on streptozotocin induced non-insulin dependent diabetic rats (90 mg/kg, i.p. single dose). Diabetes produced a significant increase in fasting glucose levels that was associated with increase in insulin levels. Treatment with *Z. officinale* produced a significant decrease in fasting glucose and increase in insulin levels in non-insulin dependent type diabetes mellitus rats. In oral glucose tolerance test treatment with *Z. officinale* was found to significantly ($P < 0.05$) decrease Area Under the Curve_{glucose} and increase Area Under the Curve_{insulin} values in diabetic rats. Treatment with *Z. officinale* also produced decrease in serum cholesterol, serum triglyceride levels and decrease in blood pressure in diabetic rats. Our data suggest a potential antidiabetic activity of the *Z. officinale* in non-insulin dependent diabetes mellitus model of diabetic rats.

Indigenous herbs are used as remedies against various diseases in the traditional system of medicine or in ethnomedical practices. For the past few decades compounds from natural sources have been gaining importance because of the vast chemical diversity that they offer. This has led to phenomenal increase in the demand for the herbal medicine in the last two decades. Ginger is one of the commonly used spices in Indian kitchen known by several names like *Ardharakam*, *Adrak*, *Adu*, *Ala*. Ginger is an underground rhizome of the plant *Zingiber officinale* belonging to family Zingiberaceae. Ginger has been reported to produce variety of pharmacological effects such as anti-emetic, antiulcer, antioxidant, anxiolytic, anti-inflammatory and antipyretic¹⁻⁵. 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.

Ginger exerts its central anti-emetic effect via 5-HT₃

antagonism⁸. Ginger inhibits the contractile response of isolated guinea pig ileum to serotonin. Galanolactone, a diterpenoid and gingerols a pungent principle isolated from ginger are reported to be competitive antagonists predominantly at 5HT₃ receptors⁹. Srivastava *et al*¹⁰ found that aqueous extract of ginger inhibited platelet aggregation, induced by ADP, epinephrine, collagen and arachidonic acid *in vitro*. These actions can also be correlated to 5-HT receptors which are involved in platelet aggregation. Hastener *et al*¹¹ showed that anxiolytic activity of ginger involved specific 5-HT receptors. Various studies have shown that 5-HT levels are high in streptozotocin STZ diabetic rats¹². 5-HT produces hyperglycemia in normoglycemic rats involving specific 5-HT_{2A} and 5-HT₃ receptors¹³. It has further been shown that chronic treatment with 5-HT₂ antagonist sarpogrelate and 5-HT₃ antagonist ondansetron produce number of beneficial effects in diabetic rats¹³. Many of the activities of ginger are because of its 5-HT antagonistic activity. Alcoholic extract of ginger produces blood glucose lowering effect in rabbits¹⁴ and in rats¹⁵. In the light of these, we have studied the effect of 6-w treatment with ginger on blood glucose and other biochemical parameters in STZ-induced non-in-

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sulin dependent diabetic rats.

MATERIALS AND METHODS

The fresh rhizomes of ginger were obtained from local market. These rhizomes were authenticated the Botany Department, Gujarat University, Ahmedabad. House specimen was deposited at Botany Department, Gujarat University, Ahmedabad.

Preparation of juice:

Fresh rhizomes of *Z. officinale* (1 kg) were collected and cut into small pieces, homogenised in mortar and pestle and then blended in a blender. It was then squeezed in muslin cloth to obtain the juice. Sodium benzoate (0.5 %) was added as a preservative. The juice was stored at a temperature of -15 to -20° in a well closed glass container.

Preparation of methanol extract:

One kilogram of dried rhizomes of *Z. officinale* was obtained from commercial sources. The rhizomes were authenticated by comparing their microscopy with the reference standard of *Zingiber officinale* and extracted with methanol using a soxhlet extractor.

NIDDM 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 Non-insulin dependent diabetes mellitus, a single dose of injection of STZ (90 mg/kg; i.p., NIDDM Sigma Chemical Co., St. Louis, MO,) 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 NIDDM. 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 in four groups, six animals in each group (1) control, (2) NIDDM control, and (3) NIDDM treated with juice at *Z. officinale*. (4) NIDDM treated with methanol extract of *Z. Offcinale*(0.5g/kg) Treatment was given daily for 6 w. The control group received an equal volume of the vehicle (4 ml/kg).

Blood sampling and biochemical analysis:

At the end of 6 w treatment, the animals were kept on 12 h fasting and the blood samples were collected from the tail vein into centrifuge tubes and allowed to clot for 30 min at room temperature. Blood samples were centrifuged at 3000 rpm for 20 min. Serum was separated and stored at -20° until analysis was done. Serum samples were analyzed spectrophotometrically for glucose, cholesterol and triglycerides, (Bayer Diagnostics Kit, Vadodara). Serum insulin levels were estimated by radioimmunoassay method using the kit from Bhabha Atomic Research center, Mumbai, India.

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})

TABLE 1: EFFECTS OF JUICE AND METHANOLIC EXTRACT OF *Z. OFFICINALE* ON VARIOUS PARAMETERS IN NIDDM RATS

Parameters	Control	NIDDM control	NIDDM treated with juice	NIDDM treated with methanolic extract
Body weight (g)	267.3±15.12	200.8±7.5*	190.2±8.1	173.5±4.0
Food intake (g/rat/day)	29±2.32	53±6.2*	31±4.2**	30±2.3**
Water intake (ml/rat/day)	22±3.53	47±4.8*	38±4.5	36±4.1
AUC _{glucose} (mg/dl min)×10 ³	8.9±5.0	56.1±4.5*	29.8±5.2 **	38.3±2.0**
AUC _{insulin} (mU/ml.min)×10 ³	7.1±1.2	11.1±1.3*	14.8±1.2**	16.1±1.3**
Blood Pressure(mm Hg)	86.7±5.5	165.0±3.6*	116.6±4.9**	128.1±2.4**
Hear Rate (beats/min)	361±22.3	318±18.9	342±12.8	330±16.4

n=6 (* significantly different from control $P < 0.05$) (** significantly different from NIDDM control $P < 0.05$)

and insulin (AUC_{insulin}) over a period of 0-120 min.

Measurement of blood pressure:

Blood pressure was recorded by the tail-cuff method using the Harvard blood pressure monitor (Kent, UK). The rat was placed into a restrainer and its tail was introduced into the cuff. The initial gain set was established by means of a pulse sensor to get monitor deflection. The pressure was first raised to 200 mm Hg and then slowly released by means of a screw attachment. There was increase in the magnitude of the deflections recorded on the pulse analyzer with the decrease in the pressure. The point at which there was the highest magnitude of deflection was taken as

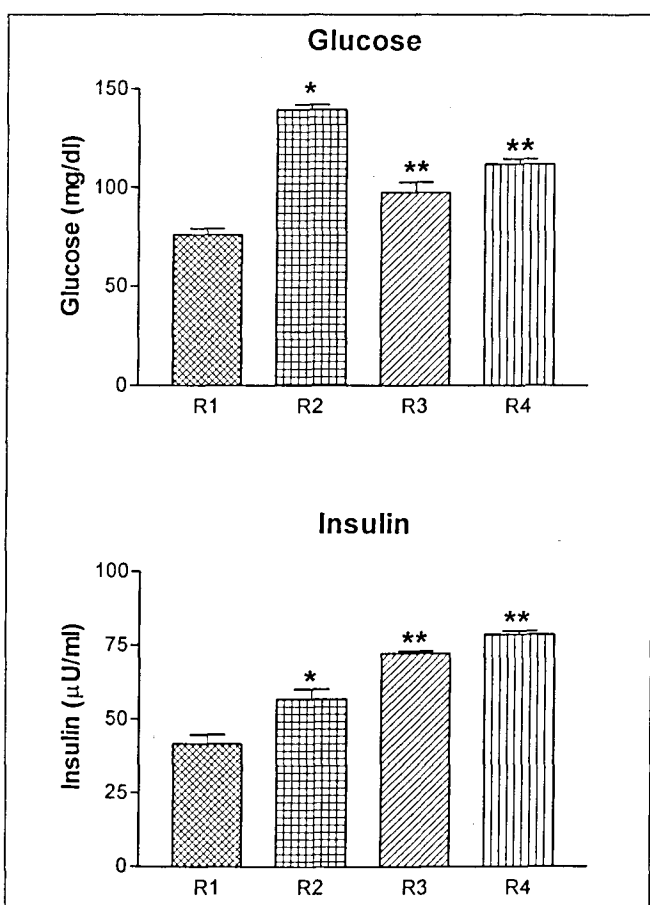


Fig 1: Effect of *Zingiber officinale* treatment on serum glucose and serum insulin in STZ-diabetic rats.

Each bar represents mean \pm SEM. of 6 animals in each group. R1=control, R2=diabetic control, R3=diabetic treated with juice of *Z. officinale* (4 ml/kg), R4=diabetic treated with methanolic extract of *Z. officinale* (0.5 g/kg). *Significantly different from non-diabetic control, ** significantly different from diabetic control $p < 0.05$.

the systolic blood pressure of the rat. At this point the heart rate was measured by increasing chart speed and recording the number of beats per min. Blood pressure recordings were repeated three times to obtain consistent results.

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

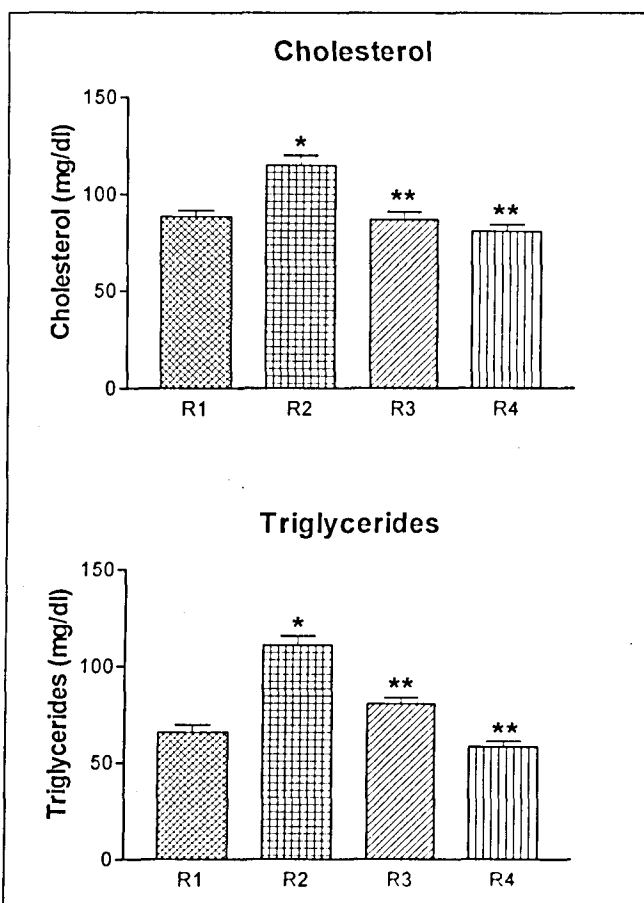


Fig. 2: Effect of *Zingiber officinale* treatment on serum cholesterol and serum triglycerides in STZ-diabetic rats.

Each bar represents mean \pm SEM. number of animals in each group=6. R1=control, R2=diabetic control, R3=diabetic treated with juice of *Z. officinale* (4 ml/kg), R4=diabetic treated with methanolic extract of *Z. Officinale* (0.5 g/kg). * Significantly different from non-diabetic control, ** significantly different from diabetic control $p < 0.05$.

Animals, which received STZ, showed a significant reduction in weight gain, increase in water intake and food intake as compared to control animals ($P < 0.05$, Table 1). 90% of pups administered STZ were found to have glucose level above 140 mg / after three months. During the study there was no mortality in the diabetic rats. STZ was administered by intraperitoneal route. STZ was prepared by weighing accurate weight of STZ and dissolving it in normal saline to get 100 mg/ml of STZ. Animals, which received STZ, showed a significant reduction in weight gain, increase in water intake and food intake as compared to control animals ($P < 0.05$, Table 1). Treatment with juice and methanolic extract of *Z. officinale* did not alter the body weight and water intake. However, both the juice and the extract of ginger produced a significant decrease in food intake (Table 1).

STZ injection produced hyperglycemia and hyperinsulinemia in rats. Treatment with *Z. officinale* significantly decreased fasting blood glucose level and significantly increased insulin level in diabetic rats (fig.1). AUC_{glucose} and AUC_{insulin} were significantly greater in diabetic rats as compared to control rats during oral glucose tolerance test. Treatment with juice and methanolic extract of *Z. officinale* significantly lowered AUC_{glucose} in diabetic rats, but AUC_{insulin} was significantly increased (Table 1). STZ produced hypercholesterolemia and hypertriglyceridemia in diabetic animals. *Z. officinale* treatment significantly lowered the total cholesterol as well as triglyceride in diabetic rats (fig. 2). The diabetic animals showed higher blood pressure and heart rate as compared to the control groups. Treatment with *Z. officinale* produced a significant decrease in blood pressure but no significant effect was observed on heart rate in diabetic animals.

DISCUSSION

In the present study STZ-treated rats significantly reduced weight gain, increase in water intake and food intake as compared with the control animals. Treatment with juice of *Z. officinale* failed to produce significant change in the body weight or water intake of these animals during the six-w of administration. Treatment with fresh juice and methanolic extract of *Z. officinale* not only decreased the fasting blood glucose levels but also caused an increase in serum insulin levels. This can be explained on the basis of involvement of 5-HT receptors and their possible modulators present in *Z. officinale*. Sarpogrelate, a 5-HT_{2A} antagonist has been reported to produce both decrease in glucose and increase in insulin levels in STZ-diabetic rats¹³.

The presence of galanolactone and gingerol have been reported in *Z. officinale* and both these compounds have been reported to act as competitive antagonist for 5-HT receptors⁹. 5-HT receptor stimulation causes an inhibitory effect on secretion of insulin (our unpublished data). The AUC_{glucose} levels in diabetic treated rats was significantly decreased and AUC_{insulin} was increased in rats treated with juice and methanolic extract of *Z. officinale*. Ethanolic extract of *Z. officinale* is reported to have hypoglycemic action in rabbits¹⁴.

STZ induced NIDDM rats showed significant increase in serum cholesterol and triglyceride levels. Durrington¹⁶ reported that insulin resistance or insulin deficiency was associated with hypercholesterolemia and hypertriglyceridemia. *Z. officinale* treatment significantly decreased both, serum cholesterol and triglycerides. *Z. officinale* is reported to decrease LDL-cholesterol, VLDL-cholesterol and triglycerides levels in apolipoprotein-E deficient mice¹⁷. Bhandari *et al*⁶. have 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-epoxylabeled-12-ene-15,16-dial, a compound isolated from *Z. officinale* interfered with cholesterol biosynthesis in homogenized liver of hypercholesterolemic mouse¹⁸. STZ-induced diabetes is reported to affect serotonergic system. Plasma concentration of 5-HT is high in diabetics than in normal subjects¹². 5-HT is reported to have lipolytic action on adipocytes increasing plasma levels of free fatty acids¹⁹. In our study, *Z. officinale* treatment showed significant decrease in triglyceride and cholesterol levels. It is possible that the mechanism of reduction of serum lipid levels with *Z. officinale* may be not only through insulin release as mentioned before but also some direct actions of 5-HT on lipids levels.

STZ-diabetic rats showed increase in blood pressure and bradycardia. Treatment with *Z. officinale* lowered blood pressure but did not alter STZ-induced bradycardia. These results can also be explained on the basis of involvement of insulin in blood pressure²⁰. In conclusion, our data suggest that *Z. officinale* possesses significant anti-diabetic activity. Presence of 5-HT modulators in *Z. officinale* may be responsible for this action. However, further studies are required to prove this hypothesis.

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