JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 101–105

Received February 20, 2003
Accepted August 21, 2003
DOI 10.1211/0022357022403
ISSN 0022-3573

# Anti-diabetic activity of *Zingiber officinale* in streptozotocin-induced type I diabetic rats

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## Abstract

The fresh and dried rhizome of *Zingiber officinale* Roscoe (commonly known as ginger) is widely used in traditional medicine. We have studied the effect of the juice of *Z. officinale* (4 mL kg$^{-1}$, p.o. daily) for 6 weeks on streptozotocin (STZ)-induced type I diabetic rats with particular reference to the involvement of serotonin (5-hydroxytryptamine; 5-HT) receptors in glycaemic control. In normoglycaemic rats, 5-HT (1 mg kg$^{-1}$, i.p.) produced hyperglycaemia and hypoinsulinaemia, which was significantly prevented by the juice of *Z. officinale*. STZ-diabetes produced a significant increase in fasting glucose levels that was associated with a significant decrease in serum insulin levels. Treatment with *Z. officinale* produced a significant increase in insulin levels and a decrease in fasting glucose levels in diabetic rats. In an oral glucose tolerance test, treatment with *Z. officinale* was found to decrease significantly the area under the curve of glucose and to increase the area under the curve of insulin in STZ-diabetic rats. Treatment with *Z. officinale* also caused a decrease in serum cholesterol, serum triglyceride and blood pressure in diabetic rats. Our data suggest a potential antidiabetic activity of the juice of *Z. officinale* in type I diabetic rats, possibly involving 5-HT receptors.

## Introduction

Ginger, a commonly used spice in the Indian kitchen, is known by several names – Ardharakam, Adrak, Adu, Ala, etc. Ginger is an underground rhizome of the plant *Zingiber officinale* Roscoe belonging to the family Zingiberaceae. *Z. officinale* possesses anti-emetic (Yamahara et al 1989), anti-inflammatory and antipyretic (Sharma et al 1994), anti-ulcer (Yamahara et al 1988), antioxidant (Reddy & Lokesh 1992) and anxiolytic activity (Vishwakarma et al 2002). *Z. officinale* has also been reported to reduce cholesterol levels and atherogenesis in rabbits fed with high cholesterol diets (Bhandari et al 1998). It stimulates bile-acid biosynthesis from cholesterol (Srinivasan & Sambaiah 1991).

*Z. officinale* exerts its central anti-emetic effect via serotonin (5-HT)$_3$ antagonism (Lumb 1993). *Z. officinale* inhibits the contractile response of guinea-pig isolated ileum to serotonin. Galanolactone, a diterpenoid, and gingerols, the pungent principles isolated from *Z. officinale*, are reported to be competitive antagonists predominantly at 5-HT$_3$ receptors (Huang et al 1991). Srivastava (1984) found that aqueous extract of *Z. officinale* inhibited platelet aggregation, induced by ADP, adrenaline (epinephrine), collagen and arachidonic acid in-vitro. These actions can also be correlated to 5-HT receptors, which are involved in platelet aggregation. Hasenohrl et al (1996) showed that the anxiolytic activity of *Z. officinale* involved specific 5-HT receptors. Various studies have shown that 5-HT levels are high in streptozotocin (STZ) diabetic rats (Martin et al 1985). 5-HT produces hyperglycaemia in normoglycaemic rats involving specific 5-HT$_{2A}$ and 5-HT$_3$ receptors (Goyal et al 2003). It has further been shown that chronic treatment with the 5-HT$_2$ antagonist sarpogrelate and 5-HT$_3$ antagonist ondansetron produces a number of beneficial effects in diabetic rats with respect to glucose and other biochemical alterations (Goyal et al 2003). Much of the activity of *Z. officinale* is because of its 5-HT antagonism. An alcoholic extract of *Z. officinale* produces a blood-glucose-lowering effect in rabbits (Mascolo et al 1989) and in rats (Ahmed & Sharma 1997). In the light of these findings, we have studied the effect of a 6-week treatment with *Z. officinale* on blood glucose and other biochemical parameters

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**Funding:** The authors acknowledge the University Grants Commission, New Delhi, for providing financial assistance.

in STZ-diabetic rats with particular reference to the involvement of 5-HT receptors in glycaemic control.

## Materials and Methods

### Plant material

The fresh rhizomes of *Z. officinale* were obtained from a local market and were authenticated by Professor O. P. Saxena (Botany Department, Gujarat University, Ahmedabad, India). A house specimen was deposited at the Botany Department, Gujarat University, Ahmedabad, India. The rhizome of *Z. officinale* was standardised according to USP 26, NF 21 and the sample passed the test of gingerol.

### Preparation of juice

Fresh rhizomes of *Z. officinale* (1 kg) were collected and crushed. These were then squeezed in muslin cloth to obtain the juice. Sodium benzoate (0.5%) was added as a preservative. The juice was stored at −15 to −20 °C in a well-closed glass container.

### 5-HT-induced hyperglycemia

Sprague-Dawley male rats, 200–250 g, were injected with 5-hydroxytryptamine creatinine sulfate (5-HT; Sigma, St Louis, MO) at a dose of 1 mg $kg^{-1}$ intraperitoneally. Blood samples were collected through the tail vein before 5-HT injection and at 30, 60 and 120 min after the injection of 5-HT. To study the effect of *Z. officinale* on 5-HT, we used 4 mL $kg^{-1}$ of fresh juice of *Z. officinale* by mouth. The selection of this dose was based on the report by Sharma & Gupta (1998) and our preliminary experiments carried out for the selection of this dose. The juice was given 60 min before 5-HT injection and the samples were collected as mentioned above. These samples were subjected to glucose and insulin analysis.

### Induction of diabetes

Diabetes was induced by single tail-vein injection of STZ (45 mg $kg^{-1}$; Sigma, St Louis, MO) to male Sprague-Dawley rats (200–250 g). Rats showing glucosuria more than 2% (Diastix; Bayer Diagnostics, India) or a blood glucose level of $>140$ mg $dL^{-1}$ 48 h after STZ injection were selected for the experiment. Rats were divided into four groups: non-diabetic control, non-diabetic treated, diabetic control and diabetic treated ($n = 6$–8 in each group). Treatment groups received *Z. officinale* at a dose of 4 mL $kg^{-1}$ by mouth daily for six weeks. Control groups received the vehicle (distilled water). During the study, standard food and water were freely available. Changes in body weight, food intake and water intake were recorded. All the procedures were performed in accordance with the Institutional Animal Care 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 6 weeks of treatment, 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 rev $min^{-1}$ for 20 min. Serum was separated and stored at −20 °C until analysis was performed. Serum samples were analysed spectrophotometrically for glucose, cholesterol and triglycerides using the kit from Bayer Diagnostics (India). Serum insulin levels were estimated by radioimmunoassay method using the kit from Bhabha Atomic Research Center (Mumbai, India).

### Oral glucose tolerance test

All groups were subjected to an oral glucose tolerance test (OGTT). Glucose (1.5 g $kg^{-1}$) was administered to 12-h fasted rats. Blood samples were collected at 0, 30, 60 and 120 min. Serum was separated immediately and analysed for glucose and insulin. The results of the OGTT were expressed as integrated areas under the curves for glucose ($AUC_{glucose}$) 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 mmHg and then slowly released by means of a screw attachment. During this decline of pressure, the point at which there is an increase in magnitude of deflection of the pulse analyser was considered as 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 recording was repeated three times to obtain consistent results.

### Statistical analysis

The results were analysed statistically using one-way analysis of variance followed by Tukey's multiple tests to determine the level of significance. A value of $P < 0.05$ was considered significant.

## Results

### Effect of 5-HT on blood glucose and insulin levels and its interaction with *Z. officinale* in normoglycaemic rats

5-HT produced a time-dependent increase in blood glucose level from its basal value and a decrease in insulin

levels in normoglycaemic rats after injection (Figure 1A). *Z. officinale* per-se had no effect on serum glucose (Figure 1A) or serum insulin (Figure 1B) levels. Pre-treatment with *Z. officinale,* however, caused inhibition of 5-HT-induced hyperglycaemia, and also prevented the decrease in insulin levels (Figure 1).

### General features of diabetic rats

STZ produced significant loss of body weight and an increase in food and water intake as compared with non-diabetic control rats (Table 1). Treatment with *Z. officinale* did not alter body weight, food intake or water intake (Table 1).

*Serum glucose, insulin and lipid levels of diabetic rats*

STZ injection produced severe hyperglycaemia and hypoinsulinaemia in rats. Treatment with *Z. officinale* significantly decreased the fasting blood glucose level and significantly increased the insulin level in diabetic rats (Table 1). The extent of the decrease in glucose or increase in insulin was not enough to bring it to the level of the non-diabetic control group. The $AUC_{glucose}$ was significantly greater and the $AUC_{insulin}$ was significantly lower in diabetic rats than in non-diabetic control rats during the OGTT. Treatment with *Z. officinale* significantly lowered the $AUC_{glucose}$ in diabetic rats and the $AUC_{insulin}$ was significantly increased (Table 1). STZ produced hypercholesterolaemia and hypertriglyceridaemia in diabetic rats. *Z. officinale* treatment significantly lowered the total cholesterol, as well as triglycerides, in diabetic rats (Table 1).

*Blood pressure*

The diabetic rats had higher blood pressure and bradycardia as compared with the non-diabetic control group. Treatment with *Z. officinale* lowered the elevated blood pressure in diabetic rats but the heart rate remained unaltered (Table 1). *Z. officinale* treatment did not have any effect on the above-mentioned parameters in non-diabetic control rats.

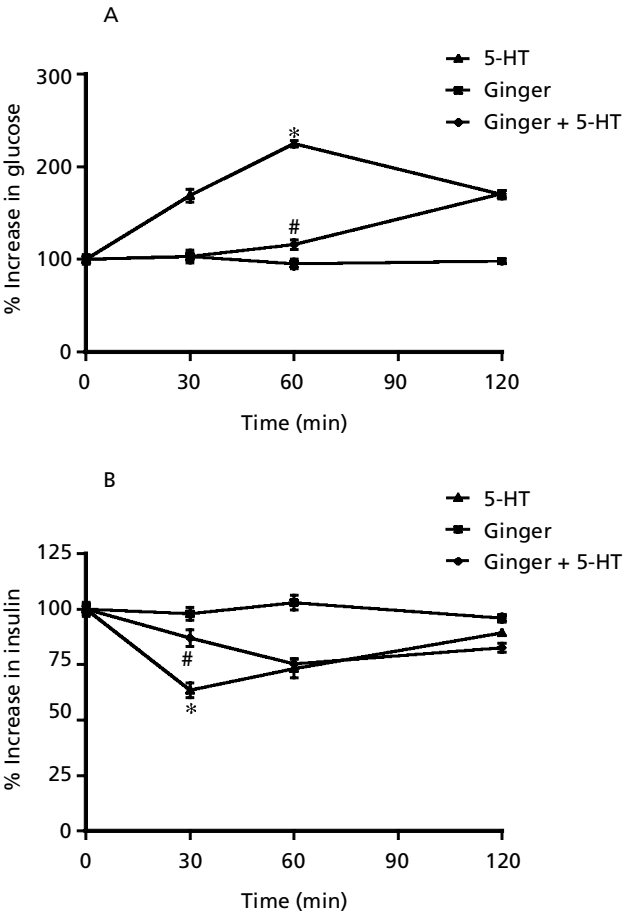


**Figure 1** Effect of 5-HT (1 mg kg$^{-1}$ i.p.) on blood glucose (A) and insulin (B) and its interaction with *Zingiber officinale* (4 mL kg$^{-1}$ p.o.) in type I diabetic rats. Each point indicates mean ± s.e.m. of six rats from each group. $P < 0.05$, compared with **Zingiber officinale* or #5-HT at the corresponding time interval.

**Table 1** Effects of Z. *officinale* juice on various parameters in type I diabetic rats.

| Parameters | Non-diabetic control | Non-diabetic treated | Diabetic control | Diabetic treated |
|---|---|---|---|---|
| Body weight (g) | 267.3 ± 15.1 | 247.1 ± 12.6 | 196.2 ± 7.8* | 187.1 ± 6.7 |
| Food intake (g/rat/day) | 29.0 ± 2.3 | 24 ± 3 | 79.1 ± 3.2* | 67.1 ± 5.4 |
| Water intake (mL/rat/day) | 22.0 ± 3.5 | 17.0 ± 2.1 | 107.0 ± 4.6* | 94.0 ± 3.5 |
| Glucose (mg dL$^{-1}$) | 76.1 ± 3.1 | 77.1 ± 4.6 | 231.1 ± 7.8* | 177.4 ± 7.1** |
| Insulin ($\mu$U mL$^{-1}$) | 41.8 ± 3.1 | 37.1 ± 1.5 | 16.2 ± 2.9* | 27.5 ± 1.5** |
| $AUC_{glucose}$ (mg dL$^{-1}$ min) × $10^3$ | 8.9 ± 0.5 | 8.7 ± 0.3 | 48.3 ± 0.7* | 37.8 ± 0.6** |
| $AUC_{insulin}$ ($\mu$U mL$^{-1}$ min) × $10^3$ | 7.1 ± 0.1 | 6.9 ± 0.1 | 3.4 ± 0.2* | 5.2 ± 0.1** |
| Cholesterol (mg dL$^{-1}$) | 88.4 ± 3.1 | 81.8 ± 3.7 | 129.8 ± 5.3* | 89.71 ± 3.6** |
| Triglyceride (mg dL$^{-1}$) | 65.8 ± 3.7 | 54.7 ± 3.6 | 137.3 ± 5.9* | 93.6 ± 3.7** |
| Blood pressure (mmHg) | 86.7 ± 5.5 | 83.4 ± 5.9 | 152.2 ± 4.7* | 122.2 ± 3.5** |
| Heart rate (beats/min) | 376.2 ± 12.2 | 362.4 ± 15.6 | 318.4 ± 12.8* | 330.8 ± 14.7 |

Data are means ± s.e.m., n = 6–8. *$P < 0.05$, compared with non-diabetic control; **$P < 0.05$, compared with diabetic control.

## Discussion

5-Hydroxytryptamine per-se (or its agonists) has been reported to induce hyperglycaemia in rats in the recent past (Chaouloff & Jeanrenaud 1987; Sugimoto et al 1992; Yamada et al 1999). In our study, 5-HT produced a time-dependent increase in glucose level and a decrease in insulin level. *Z. officinale* per-se did not produce any significant alteration in glucose and insulin levels; however, it inhibited 5-HT-induced hyperglycaemia and hypoinsulinaemia. Lumb (1993) reported the involvement of 5-$HT_3$ receptors in the anti-emetic action of *Z. officinale*. Earlier studies from our laboratory have reported that 5-HT-induced hyperglycaemia was inhibited by the 5-$HT_{2A}$ antagonist sarpogrelate and the 5-$HT_3$ antagonist ondansetron (Goyal et al 2003). The inhibition of 5-HT-induced hyperglycaemia by *Z. officinale* suggests the presence of a 5-$HT_{2A}$ or 5-$HT_3$ receptor antagonist in *Z. officinale*. Chronic treatment with sarpogrelate and ondansetron is reported to decrease blood glucose and to have beneficial effects on lipid profile in STZ-diabetic rats (Goyal et al 2003). If *Z. officinale* contains a 5-HT receptor antagonist, it is expected to produce beneficial effects in STZ-diabetic rats. In this study, STZ produced loss of body weight, hyperphagia and polydypsia in type I diabetic rats. *Z. officinale* treatment did not produce any change in body weight, hyperphagia and polydypsia in diabetic rats. However, it produced a significant decrease in serum glucose and $AUC_{glucose}$, as well as an increase in serum insulin and $AUC_{insulin}$ levels in diabetic rats treated with *Z. officinale*. The extent of alterations in these values by *Z. officinale* was not adequate to bring them to the level of non-diabetic controls. It is possible that higher dose or longer duration of treatment is required to bring about such changes. Further experiments are required to be done.

STZ-diabetic rats showed significant increases in serum cholesterol and triglyceride levels. *Z. officinale* treatment significantly decreased both serum cholesterol and triglycerides. *Z. officinale* is reported to decrease LDL-cholesterol, VLDL-cholesterol and triglycerides levels in apolipoprotin-E deficient mice (Fuhrman et al 2000). Bhandari et al (1998) have reported that an ethanolic extract of *Z. officinale* prevents hypercholesterolaemia and development of atherosclerosis in cholesterol-fed rabbits. It is also reported that (E)-8 beta, 17-epoxylabed-12-ene-15,16-dial, a compound isolated from *Z. officinale,* interfered with cholesterol biosynthesis in liver homogenates of hypercholesterolaemic mice (Tanabe et al 1993). STZ-induced diabetes is reported to affect the serotonergic system. The plasma concentration of 5-HT is higher in diabetic than in non-diabetic subjects (Martin et al 1985). 5-HT is reported to have lipolytic action on adipocytes, increasing plasma levels of free fatty acids (Martinez-Conde et al 1984). In our study, treatment with *Z. officinale* juice significantly decreased triglycerides and cholesterol levels in diabetic rats. It is possible that the reduction in serum lipid levels with *Z. officinale* may be due to its antagonistic action on 5-HT receptors, thereby increasing insulin levels.

STZ-induced diabetic rats showed an increase in blood pressure and bradycardia. Treatement with *Z. officinale* lowered the blood pressure and had no effect on the heart rate. This may be because of the improved glucose and lipid profile. In conclusion, *Z. officinale* is effective in partially controlling blood glucose by release of insulin and also shows improvement in STZ-induced metabolic alteration related to lipids.

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