OPEM
www.opem.org

Oriental Pharmacy and Experimental Medicine 2005 **5(3)**, 201-208

KHUP

Comparative antidiabetic activity of different fractions of methanolic extract of *Zingiber officinale* Roscoe in streptozotocin induced NIDDM rats

Sanjay V Kadnur and Ramesh K Goyal*

Department of Pharmacology, L.M. College of Pharmacy, Navarangpura, Ahmedabad - 380009, India

SUMMARY

Earlier we have reported the antidiabetic activity of fresh juice of rhizomes of *Zingiber officinale* (*Z. officinale*) and its correlation with 5-HT receptor antagonism. Since 6-gingerol the marker compound of *Z. officinale* is reported to posses 5-HT anatgonistic activity, the present investigation, was undertaken to find out the concentration of 6-gingerol present in methanolic extract of *Z. officinale* and its different fractions (petroleum ether, toluene and chloroform). We also evaluated these fractions for antidiabetic activity in streptozotocin (STZ)-induced neonatal type 2 diabetic rats. Fasting glucose and insulin levels in non insulin dependent diabetes mellitus (NIDDM) rats were found to be significantly ($P < 0.05$) higher than control rats and these were significantly decreased by treatment with methanolic extract of *Z. officinale* and its fractions. The results of oral glucose tolerance test (OGTT) showed that methanolic extract and its fractions significantly ($P < 0.05$) decreased both STZ-induced increase in $AUC_{glucose}$ and $AUC_{insulin}$ values in NIDDM groups. Treatment with petroleum ether fraction produced a greater reduction in elevated glucose and $AUC_{glucose}$ levels as compared to treatment with other fractions. Treatment with methanolic extract of *Z. officinale* and its fractions also produced significant reduction in the elevated lipid, serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) levels in NIDDM rats. The effect of petroleum ether fraction on elevated lipid, SGOT and SGPT levels was significantly greater as compared to treatment with other fractions. The concentration of 6-gingerol was found to be maximum in petroleum ether fraction (11.430%) and minimum in chloroform fraction (0.973%). The methanolic extract and toluene fraction was found to contain 3.080% and 2.191%, 6-gingerol respectively. In conclusion, our data suggest that methonolic extract and its fractions possess significant antidiabetic activity in NIDDM rats. The extent of activity appears to be dependent on the concentration of 6-gingerol present in the extract or its fractions.

Key words: *Zingiber officinale*; Streptozotocin; NIDDM

INTRODUCTION

Zingiber officinale Roscoe is a perennial herb with a subterranean, digitately branched rhizome belonging to the family Zingiberaceace. *Zingiber officinale (Z. officinale)* is commonly known as ginger. The plant is probably native to south east Asia and is cultivated in the tropical regions in both the eastern and western hemispheres. Ginger is one of the commonly used spices in Indian kitchen known by several names like Ardharakam, Adrak, Adu, Ala, etc.

Z. officinale has been reported to produce various pharmacological effects such as anti-emetic, antiulcer, antioxidant, anxiolytic, anti-inflammatory and

*Correspondence: Ramesh K Goyal, Department of Pharmacology, L. M. College of Pharmacy, Navarangpura, Ahmedabad - 380 009, India. Tel: +91-79-6302746; Fax: +91-79-6304865; E-mail: goyalrk@hotmail.com

antipyretic activity (Yamahara *et al.*, 1988; Yamahara *et al.*, 1989a; Reddy and Lokesh, 1992; Sharma *et al.*, 1998; Vishwakarma *et al.*, 2002). *Z. officinale* has also reported to reduce cholesterol levels and atherogenesis in rabbits fed with high cholesterol diets (Bhandari *et al.*, 1998). It also stimulates bile acid biosynthesis from cholesterol (Srinivasan and Sambaiah 1991). *Z. officinale* inhibits the contractile response of isolated guinea pig ileum to serotonin. Galanolactone, a diterpenoid and gingerol, the pungent principle isolated from *Z. officinale* is reported to be competitive antagonists predominantly at 5-HT receptors (Huang *et al.*, 1991). The aqueous extract of *Z. officinale* inhibited platelet aggregation, induced by ADP, epinephrine, collagen and arachidonic acid *in vitro*. These actions were correlated to 5-HT receptors, which are involved in platelet aggregation (Srivastava *et al.*, 1984).

Various studies have shown that 5-HT levels are high in streptozotocin (STZ) diabetic rats (Martin *et al.*, 1985). 5-HT produces hyperglycemia in normoglycemic rats involving specific 5-HT_{2A} and 5-HT_3 receptors (Goyal *et al.*, 2003). 5-HT modulators ondensetron and sarpogrelate are reported to possess significant antidiabetic activity in STZ-induced diabetic rats (Goyal *et al.*, 2003). Alcoholic extract of ginger produces blood glucose lowering effect in rabbits (Mascolo *et al.*, 1989) and in rats (Ahmed *et al.*, 1997). Fresh juice of *Z. officinale* has recently been reported from our laboratary to possess antidiabetic activity in STZ-induced type I diabetic rats (Akhani *et al.*, 2004). The antidiabetic activity of fresh juice of *Z. officinale* was proposed to be correlated through 5-HT receptor antagonism. Since 6-gingerol the chemical and biological marker substance present in *Z. officinale* is reported to possess 5-HT antagonistic activity (Yamahara *et al.*, 1989b), the present investigation was undertaken to study the effect of methanolic extract and its fractions in STZ-induced NIDDM rats and to correlate with concentrations of 6-gingerol present therein.

MATERIALS AND METHODS

Plant material

Dried rhizomes of *Z. officinale* was collected from local market. The plant was identified and authenticated by Prof. O. P. Saxena, Head, Botany Department, Gujarat University, Ahmedabad, India and a voucher specimen was deposited.

Extraction and fractionation

Four kilogram of shade-dried rhizome of *Z. officinale* was powderd and refluxed in round bottom flask containing methanol for 6 h. The methanolic extract was concentrated under reduced pressure (yield = 172.0 g). Methanolic extract (150.0 g) was further fractionated using solvents of varying polarity *viz.*, petroleum ether [60 - 80°C] (29.36 g), toluene (14.34 g), chloroform (6.01 g). The extracts and fractions were concentrated under reduced pressure and air dried to remove the solvent completely.

Standardization and dose selection

Standardization of methanolic extract and its fractions for 6-gingerol content, a biological and chemical marker substance was carried out by HPLC analysis using 8-methyl-n-vanillylnonamide as reference standard. The analysis was carried as per the method of He *et al.* (1998). Earlier our laboratory had studied various concentrations of methanolic extract of *Z. officinale* (100 - 750 mg/kg *p.o*) in diabetic rats and found 500 mg/kg *p.o.* dose of methanolic extract of *Z. officinale* to produce optimum anti-diabetic effect (Unpublished data), based on these data and on standardization of 6-gingerol concentration the dose of the fractions of methanolic extract were taken for the study.

NIDDM rat model and treatment protocol

Healthy albino rats of Sprague Dawley strain were kept for breeding. To induce NIDDM a single dose of injection of STZ (90 mg/kg: *i.p.*) [Sigma

Chemical Co., St. Louis, MO, USA] was given to 2 day old pups. Another group of pups received only saline. The animals were weaned at 30 days and after a period of 3 months, checked for fasting glucose levels to confirm the status of NIDDM. The animals showing fasting glucose levels of > 140 mg/dl were considered as diabetic. The pups that received saline were considered as control animals. The experimental animals were divided into six groups, six animals in each group. GP I: Normal control, GP II: NIDDM control, GP III: NIDDM treated with methanolic extract of *Z. officinale* (0.5 g/kg), GP IV: NIDDM treated with petroleum ether fraction (0.1 g/kg) ,GP V: NIDDM treated with toluene fraction (0.1 g/kg), GP VI: NIDDM treated with chloroform fraction (0.1 g/kg). Treatment was given daily for three weeks. The control groups received an equal volume of the vehicle. During the study standard food and water were provided *ad libitum*. Changes in body weight, food intake and water intake were recorded. 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, Goverment of India, New Delhi, India.

Oral glucose tolerance test (OGTT)

At the end of three weeks study rats were subjected to an OGTT. Glucose (1.5 g/kg) was administered to 12 h fasted rats and the blood samples were collected from tail vein at 0, 30, 60, 120 min. Serum was analyzed for glucose by GOD-POD method by using diagnostic reagent kits (Bayer Diagonstics Ltd, India) and insulin by RIA kits (Bhabha Atomic Research Center, Bombay, India). The results were expressed as integrated area under the curve for glucose ($AUC_{glucose}$) and insulin ($AUC_{insulin}$), that was calculated by trapezoid rule $AUC = (C_1 + C_2) / 2 \times (t_2 - t_1)$.

Effect on other biochemical parameters

At the end of three week treatment blood samples were collected from tail vein from 12 h fasted rats. Serum was separated and analyzed spectrophotometrically for cholesterol, triglyceride, HDL-Cholesterol, creatinine and urea using diagnostic reagent kits (Bayer Diagonstics Ltd, India). SGPT and SGOT were estimated using diagnostic reagent kits (Span Diagnostics, Ltd, India). VLDL-Cholesterol and LDL-Cholesterol were calculated as per Friedevald's equation:

$$\text{VLDL - Cholesterol} = \frac{\text{Total serum triglycerides}}{5}$$

$$\text{LDL - Cholesterol} = \text{Total serum cholesterol} - \frac{\text{Total serum triglycerides}}{5} - \text{HDL - Cholesterol}$$

Statistical analysis

All the experimental values were expressed as mean ± SEM. Results were analyzed statistically using analysis of variance (ANOVA) followed by Tukey's test ($P < 0.05$).

RESULTS

6-gingerol content in methanolic extract and its fractions

Standardization of the methanolic extract of *Z. officinale* and its fractions for 6-gingerol content showed presence of following concentrations of 6-gingerol, methanolic extract (3.080%), petroleum ether fraction (11.430%), toluene fraction (2.191%) and chloroform fraction (0.973%). This clearly shows enrichment of 6-gingerol content in petroleum ether fraction and reduction of the same in other fractions.

Effect on general parameters

STZ treated rats exhibited significant polydypsia and polyphagia as compared with the control animals. Treatment with methanolic extract and its

Table 1. Effect of methanolic extract of *Z. officinale* and its fractions on general and biochemical parameters in STZ diabetic rats.

Parameters	Normal control	NIDDM control	NIDDM treated with methanolic extract 500 mg/kg	NIDDM treated with petroleum ether fraction 100 mg/kg	NIDDM treated with toluene fraction 100 mg/kg	NIDDM treated with chloroform fraction 100 mg/kg
Body weight (g/rat)	203.3 ± 2.1	195.0 ± 4.2	196.7 ± 4.2	188.3 ± 4.7	188.3 ± 3.0	201.7 ± 3.0
Water intake (ml/rat/day)	38.8 ± 0.7	53.0 ± 0.5*	45.2 ± 0.5*	45.8 ± 0.5**	45.5 ± 0.5**	44.7 ± 0.6**
Food intake (g/rat/day)	18.3 ± 0.4	27.5 ± 0.3*	23.6 ± 0.7**	22.5 ± 0.3**	22.5 ± 0.3**	25.0 ± 0.4
SGOT (units/ml)	35.0 ± 1.4	56.3 ± 1.4*	44.6 ± 1.7**	46.3 ± 0.9**	47.3 ± 1.52**	50.3 ± 2.0
SGPT (units/ml)	25.0 ± 1.1	35.3 ± 1.2*	31.0 ± 0.8**	32.6 ± 0.8**	30.6 ± 0.4**	31.6 ± 0.6**
Urea (mg/dl)	34.3 ± 1.1	75.1 ± 1.1*	70.3 ± 1.9	74.1 ± 1.1	73.3 ± 1.0	70.7 ± 1.8
Creatinine (mg/dl)	0.45 ± 0.01	0.84 ± 0.02*	0.80 ± 0.02	0.81 ± 0.008	0.80 ± 0.007	0.70 ± 0.02

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

fractions significantly reduced polydypsia and polyphagia in these animals (Table 1).

Effect on glucose and insulin levels

Fasting glucose levels were significantly higher in NIDDM rats as compared with those in normal control animals which was associated with hyperinsulinemia in NIDDM control animals (Fig. 1B). Treatment with methanolic extract produced decrease in glucose levels.Treatment with fractions also significantly lowered both glucose and insulin levels (Fig. 1A and 1B).

Both $AUC_{glucose}$ and $AUC_{insulin}$ were significantly higher in NIDDM control as compared with normal control animals. Treatment with methanolic extract and its fractions significantly lowered both $AUC_{glucose}$ and $AUC_{insulin}$ values (Fig. 2A and 2B) in NIDDM rats. All these changes were significantly greater with 0.5 g/kg methanolic extract and its petroleum ether fraction. Toluene and choroform fractions also produced decrease in $AUC_{glucose}$ and $AUC_{insulin}$ values, however the reduction in these values were less as compared to methanolic extract and its petroleum ether fraction.

Effect on other biochemical parameters

STZ-induced NIDDM rats showed significant elevation in serum lipid levels as compared with normal control animals (Table 2). Treatment with methanolic extract and its fractions significantly lowered serum lipid levels as compared to NIDDM control (Table 2).

NIDDM control rats showed a significant increase in SGPT and SGOT levels as compared with normal control animals (Table 1). Treatment with methanolic extract and its fractions significantly prevented STZ-induced increase in these levels (Table 1). NIDDM control rats showed a significant increase in serum creatinine and urea levels as compared with normal control animals (Table 1). Treatment with methanolic extract and its fractions showed no changes in these levels.

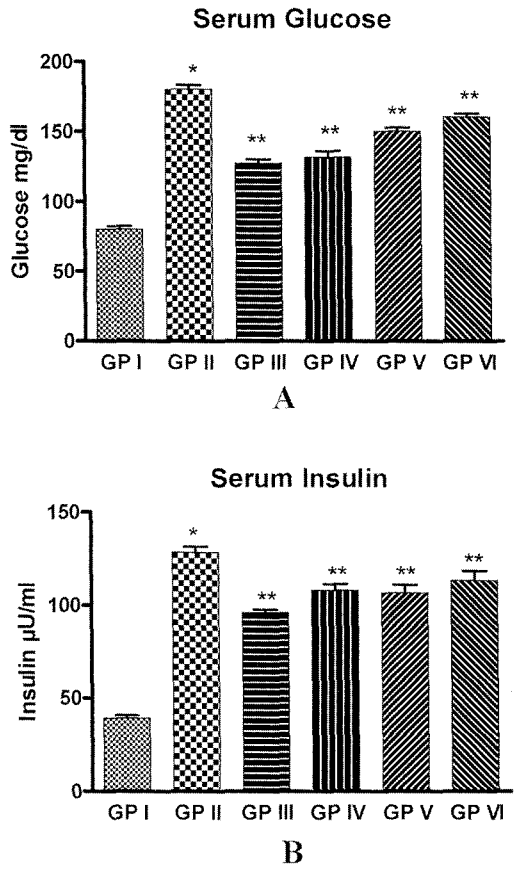

Fig. 1. (A) Effect of methanolic extract of *Zingiber officinale* and its fractions on serum glucose levels in STZ-induced type II diabetic rats. Each bar represents Mean ± SEM number of animals in each group = 6. GP I = normal control, GP II = NIDDM control, GP III = NIDDM treated with methanolic extract of *Z. officinale* (500 mg/kg), GP IV = NIDDM treated with petroleum ether fraction (100 mg/kg), GP V = NIDDM treated with toluene fraction (100 mg/kg), GP VI = NIDDM treated with chloroform fraction (100 mg/kg). *Significantly different from non-diabetic control ($P < 0.05$). **Significantly different from diabetic control ($P < 0.05$). (B) Effect of methanolic extract of *Zingiber officinale* and its fractions on serum insulin levels in STZ-induced type II diabetic rats. Each bar represents Mean ± SEM number of animals in each group = 6. GP I = normal control, GP II = NIDDM control, GP III = NIDDM treated with methanolic extract of *Z. officinale* (500 mg/kg), GP IV = NIDDM treated with petroleum ether fraction (100 mg/kg), GP V = NIDDM treated with toluene fraction (100 mg/kg), GP VI = NIDDM treated with chloroform fraction (100 mg/kg). *Significantly different from non-diabetic control ($P < 0.05$). **Significantly different from diabetic control ($P < 0.05$).

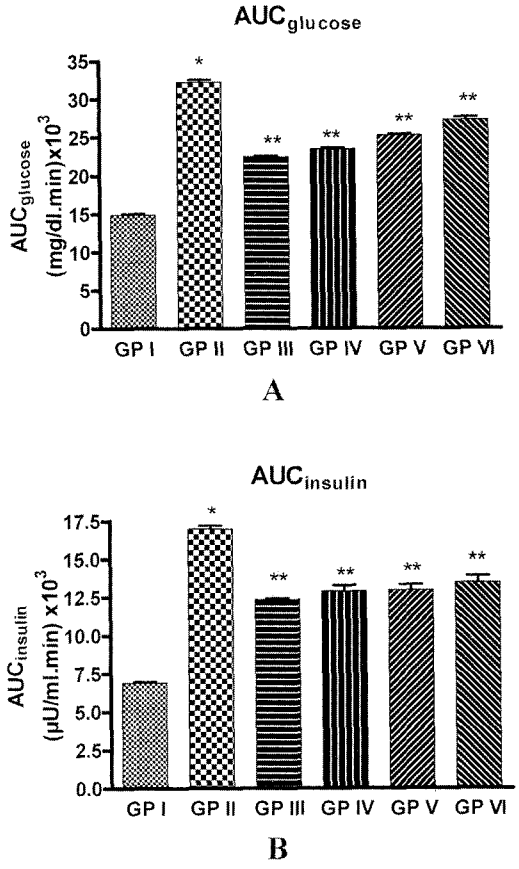

Fig. 2. (A) Effect of methanolic extract of *Zingiber officinale* and its fractions on $AUC_{glucose}$ levels in STZ-induced type II diabetic rats. Each bar represents Mean ± SEM number of animals in each group = 6. GP I = normal control, GP II = NIDDM control, GP III = NIDDM treated with methanolic extract of *Z. officinale* (500mg/kg), GP IV = NIDDM treated with petroleum ether fraction (100 mg/kg), GP V = NIDDM treated with toluene fraction (100 mg/kg), GP VI = NIDDM treated with chloroform fraction (100 mg/kg). *Significantly different from non-diabetic control ($P < 0.05$). **Significantly different from diabetic control ($P < 0.05$). (B) Effect of methanolic extract of *Zingiber officinale* and its fractions on $AUC_{insulin}$ levels in STZ-induced type II diabetic rats. Each bar represents Mean ± SEM number of animals in each group = 6. GP I = normal control, GP II = NIDDM control, GP III = NIDDM treated with methanolic extract of *Z. officinale* (500 mg/kg), GP IV = NIDDM treated with petroleum ether fraction (100 mg/kg), GP V = NIDDM treated with toluene fraction (100 mg/kg), GP VI = NIDDM treated with chloroform fraction (100 mg/kg). *Significantly different from non-diabetic control ($P < 0.05$). **Significantly different from diabetic control ($P < 0.05$).

Table 2. Effect of methanolic extract of *Z. officinale* and its fractions on lipid profile in STZ diabetic rats.

Parameters	Normal control	NIDDM control	NIDDM treated with methanolic extract 500 mg/kg	NIDDM treated with petroleum ether fraction 100 mg/kg	NIDDM treated with toluene fraction 100 mg/kg	NIDDM treated with chloroform fraction 100 mg/kg
Cholesterol (mg/dl)	49.6 ± 1.0	90.9 ± 1.2*	60.7 ± 1.4**	66.8 ± 0.6**	74.7 ± 0.9**	78.6 ± 1.2**
Triglyceride (mg/dl)	43.5 ± 1.0	117.5 ± 2.6*	76.5 ± 2.3**	86.8 ± 1.6**	98.3 ± 1.6**	98.1 ± 2.4**
HDL-Cholesterol (mg/dl)	12.0 ± 0.5	12.1 ± 0.6	11.9 ± 0.5	13.5 ± 0.3	12.6 ± 0.5	12.2 ± 0.4
LDL-Cholesterol (mg/dl)	28.8 ± 1.2	55.3 ± 2.3*	33.7 ± 1.8**	35.9 ± 1.8**	42.5 ± 1.2**	46.7 ± 1.1**
VLDL-Cholesterol (mg/dl)	8.6 ± 0.2	23.5 ± 0.5*	15.3 ± 0.4**	17.3 ± 0.3*	19.6 ± 0.3**	19.6 ± 0.41**

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

DISCUSSION

The results of the present study show that the treatment with methanolic extract of *Z. officinale* and its fractions produce significant reduction in elevated glucose and insulin levels in STZ-induced type II diabetic rats. Earlier our laboratory reported antidiabetic activty of fresh juice of *Zingiber officinale* in STZ-induced type I diabetic rats (Akhani *et al.*, 2004). Treatment with fresh juice of *Z. officinale* in STZ-induced type I diabetic rats showed reduction in elevated glucose levels and increase in insulin levels (Akhani *et al.*, 2004). In type I diabetes insulin levels are lower whereas in type II diabetes they are higher. The increase in insulin in type I diabetes is due to the actions on pancreas. Treatment with sarpogrelate and extracts of ginger produced insulin release from islets of pancreas (our unpublished results). 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 I diabetic rats and reduction in insulin levels in type II diabetic rats (Goyal *et al.*, 2003). 5-HT produces hyperglycemia in normoglycemic rats involving specific 5-HT_{2A} and 5-HT_3 receptors. Anti-diabetic activity of fresh juice of *Z. officinale* was proposed to be correlated through 5-HT receptor antagonis. The results of the present study support our earlier findings (Goyal *et al.*, 2003; Akhani *et al.*, 2004).

In the present study, NIDDM control rats showed significantly higher levels of fasting glucose levels as compared with non-diabetic control rats. This is consistent with earlier reports (Weir *et al.*, 1981; Gokhale *et al.*,1998). We also found increase in insulin levels and $AUC_{insulin}$ after oral glucose load in neonatal STZ-diabetic rats. This finding is similar to earlier reports that hyperinsulinemia in neonatal STZ-diabetic rats could be due to either decreased hepatic clearance of insulin or decreased number of insulin receptors, resulting in decreased insulin binding and lowered insulin degradation (Olefsky, 1981).

The results of OGTT further support the insulin sensitivity effect of *Z. officinale*, the $AUC_{insulin}$ of NIDDM control rats was significantly greater as compared with the non-diabetic rats. That was significantly decreased by methanolic extract and its fractions. This suggests that treatment with methanolic extract of *Z. officinale* and its fractions

does not alter the release of insulin, in conditions like hyperinsulinemia, but increases the insulin sensitivity for effective glucose disposal.

Reaven (1988) reported that insulin resistance in diabetic subjects leads to compensatory hyperinsulinemia, which is associated with increased LDL and reduced HDL concentrations. In our study also NIDDM rats showed hypercholesterolemia and hypertriglyceridemia and elevated levels of LDL and VLDL levels. Treatment with methanolic extract of *Z. officinale* and its fractions significantly decreased cholesterol, triglyceride, LDL and VLDL levels without significant changes in HDL levels. These findings also support the hypothesis that *Z. officinale* causes improvement in insulin sensitivity and control elevated levels of glucose and lipid levels in conditions of insulin resistance associated with hyperglycemia and hyperlipidemia.

In NIDDM rats there is increase in SGOT and SGPT levels (Vishwakarma *et al.*, 2003). In the present study also NIDDM rats showed increase in SGOT and SGPT levels and treatment with methanolic extract and its fractions significantly decreased both SGOT and SGPT levels.

All above results supports the hypothesis that activity of *Z. officinale* is similar to that of 5-HT_{2A} antagonist sarpogrelate. Further we also found that the anti-diabetic activity observed on treatment with methanolic extract and its petroleum ether fraction was greater as compared to toluene and chloroform fractions. Difference in the activity profile of different fractions can be correlated well with the concentration of 6-gingerol in these fractions. Standardization of the methanolic extract of *Z. officinale* and its fractions for 6-gingerol content showed the concentration of 6-gingerol was maximum in petroleum ether fraction (11.430 %) and minimum in chloroform fraction (0.973%). The concentration of 6-gingerol was found to be higher in methanolic extract (3.080%) and its petroleum ether fraction (11.430%). In other two fractions, that is in toluene and chloroform fractions, wherein the antidiabetic activity was lower, the content of 6-gingerol was also found to be lower 2.191% and 0.973% respectively. 6-gingerol is reported to possess 5-HT antagonistic activity (Yamahara *et al.*, 1989b). Thus, if 6-gingerol is considered to be responsible for anti-diabetic activity of *Z. officinale*, our data clearly indicate that the anti-diabetic activity appears to be dependent on the concentration of 6-gingerol present in the methanolic extract or its fractions.

In conclusion, our data suggests that methanolic extract of *Z. officinale* and its fractions produces potential antidiabetic activity. In addition to decreasing the serum glucose and lipids, it can also prevent liver dysfunction in STZ-diabetic rats. The extent of activity appears to be dependent on the concentration of 6-gingerol present in the extract or its fractions.

ACKNOWLEDGEMENTS

Authors would like to acknowledge University Grants Commission, New Delhi for financial aid under Major Research Project No. F-7-40/2001(SR-I) to Dr.Goyal with Mr. Kadnur as the Project Associate.

REFERENCES

Ahmed RS, Sharma SB. (1997) Biochemical studies on combined effects of garlic (*Allium sativum* Linn) and ginger (*Zingiber officinale* Roscoe) in albino rats. *Indian. J. Exp. Biol.* **35,** 841- 843.

Akhani SP, Vishwakarma SL, Goyal RK. (2004) Anti-diabetic activity of *Zingiber officinale* in streptozotocin-induced type I diabetic rats. *J. Pharm. Pharmacol.* **56,** 101-105.

Bhandari U, Sharma JN, Zafar R. (1998) The protective action of ethanolic *Z. officinale* (*Zingiber officinale*) extract in cholesterol fed rabbits. *J. Ethnopharmacol.* **61,** 167-171.

Gokhale MS, Shah DH, Hakim Z, Santani DD, Goyal RK. (1998) Effect of chronic treatment with amlodipine in non-insulin dependent diabetic rats. *Pharmacol. Res.* **37,** 455-459.

Goyal RK, Umrani DN, Bodiwala ND, Dhalla NS. (2003) Usefulness of 5-HT_{2A} receptor antagonists for the treatment of cardiovascular complications in diabetes. In: Atherosclerosis, hypertension and diabetes, edited by Pierce GN, Nagano M, Zahradka P, Dhalla NS, p. 317 Kluwer Academic Publisher, Boston

Hasenohrl RU, Nichau CH, Frisch CH, De Souza Silva, MA, Huston JP, Mattern CM, Hacker R. (1996) Anxiolytic-like effect of combined extracts of *Z. officinale* and *Ginkgo biloba* in the elevated plus-maze. *Pharmacol. Biochem. Behav.* **53,** 271-275.

He X, Bernart M, Lian L, Lin L. (1998) High-performance liquid chromatography electrospray mass spectrometric analysis of pungent constituents of ginger. *J. Chromatography A.* **796**, 327-334.

Huang Q, Iwamoto M, Aoki S, Tanaka N, Tajima K, Yamahara J. (1991) Anti-5-hydroxytryptamine effect of galanolactone, diterpenoid isolated from *Z. officinale*. *Chem. Pharm. Bull* (Tokyo). **39,** 397-399.

Lumb AB. (1993) Mechanism of antiemetic effect of *Z. officinale*. *Anaesthesia* **48,** 1118.

Martin FJ, Miguez JM, Aldegunde M, Atienza G. (1985). Platelet serotonin transport is altered in streptozotocin-induced diabetic rats. *Life Sci.* **56,** 1807-1815.

Mascolo N, Jain R, Jain SC, Capasso F. (1989) Ethnopharmacologic investigation of *Z. officinale* (*Zingiber officinale*). *J. Ethnopharmacol.* **27,** 129-140.

Olefsky JM. (1981) Insulin resistance and insulin action. An *in vitro* and *in vivo* respective. *Diabetes* **30**, 118-162.

Reaven GM. (1988) Role of insulin resistance in human disease. *Diabetes* **37**, 1595-1607.

Reddy AC, Lokesh BR. (1992) Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol. Cell. Biochem.* **111,** 117-124.

Sharma SS, Gupta YK. (1998) Reversal of cisplatin-induced delay in gastric emptying in rats by ginger (*Zingiber officinale*). *J. Ethnopharmacol.* **62,** 49-55.

Sharma JN, Srivastava KC, Gan EK. (1994) Suppressive effects of eugenol and *Z. officinale* oil on arthritic rats. *Pharmacology* **49,** 314-318.

Srinivasan K, Sambaiah K. (1991) The effect of spices on cholesterol 7 alpha- hydroxylase activity and on serum and hepatic cholesterol levels in rat. *Int. J. Vit. Nutr. Res.* **61,** 364-369.

Srivastava KC. (1984) Aqueous extracts of onion, garlic and *Z. officinale* inhibit platelet aggregation and alter arachidonic acid metabolism. *Biomed. Biochem. Acta* **43,** S335-346.

Vishwakarma SL, Rajani M, Goyal RK. (2003) Comparative antidiabetic activity of different fractions of *Enicostemma littorale* Blume in streptozotocin induced NIDDM rats. *Orient. Pharm. Exp. Med.* **3,** 196-204.

Vishwakarma SL, Pal SC, Kasture VS, Kasture SB. (2002) Anxiolytic and antiemetic activity of *Zingiber officinale*. *Phytother. Res.* **16,** 621-626

Weir GC, Clore ET, Zmachinski CJ, Bonnerweir S. (1981) Islet secretion in a new experimental model for non-insulin dependent diabetes. *Diabetes* **30**, 590-595.

Yamahara J, Mochizuki M, Rong HQ, Matsuda H., Fujimura, H. (1988) The anti-ulcer effect of *Z. officinale* constituents in rats. *J. Ethnopharmacol.* **23,** 299-304.

Yamahara J, Rong HQ, Iwamoto M, Naitoh Y, Kitani T, Fugumura H. (1989a) Inhibition of cytotoxic drug-induced vomiting in suncus by a *Z. officinale* constituents. *J. Ethnopharmacol.* **27,** 353-355.

Yamahara J, Rong HQ, Iwamoto M, Kobayashi G, Matsuda H, Fujimura H. (1989b) Active components of ginger exhibiting antiserotoninergic action. *Phytother. Res.* **3,** 70-71.