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Research Article

Hypoglycemic and hypolipidemic activity of aqueous extract of *Ficus racemosa* seeds

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ABSTRACT: **Introduction:** The incidence of diabetes mellitus is on rise all over the world. Moreover the synthetic drugs are likely to give serious side effects. That is why the expert committee on diabetes at WHO recommends the screening of medicinal plants for the management of diabetes. **Objective:** The present study was designed to investigate the hypoglycemic and hypolipidemic potential of *F. racemosa* seeds in streptozocin (STZ) induced diabetic mice. **Materials and Methods:** Swiss albino mice of both sexes, aged 7-8 weeks, average weight of 20-30 gm were used for the experiments. Animals were treated with aqueous extract of *F. racemosa* seeds at a dose of 200 mg/kg body weight. Blood glucose, triglycerides, LDL, HDL and cholesterol was measured at the beginning and end of the experiment. **Results:** Blood glucose and other studied parameters were elevated in the diabetic mice and were brought about near to the control group (except HDL) by the aqueous extract of *F. racemosa* seeds (200 mg/kg body weight). The decrease in all the parameters (except HDL) were statistically significant ($P < 0.001$). **Conclusion:** The present study suggests that the aqueous extract of *F. racemosa* seeds can be used for further isolation and identification of active principles with hypoglycemic and hypolipidemic potential.

KEY WORDS: *F. racemosa* seeds, diabetes, streptozocin (STZ), hypoglycemic, hypolipidemic

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by abnormalities in carbohydrate, lipid and lipoprotein metabolism, which not only lead to hyperglycemia but also cause many complications, such as hyperlipidemia, hyperinsulinemia, hypertension and atherosclerosis.[1-3] The chronic hyperglycemia of diabetes is associated with dysfunction, damage and failure of various organs over the long term.[4] Despite the availability of many antidiabetic medicines in the market, diabetes and its related complications continue to be major medical problems. Plant derivatives with purported hypoglycemic properties are used in folk medicine and traditional healing systems around the world.[5]

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Over 400 traditional plant treatments for diabetes have been reported although only a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs should be further investigated.[6]

Ficus racemosa belonging to the family Moraceae is a medium tall tree with quite rich green foliage that provides good shade. It is popularly known as "country fig" in English and "Atti" in Tamil. Different parts of *F. racemosa* are traditionally used as fodder, as food and for ceremonial purposes.[7] All parts of this plant (leaves, fruits, bark, latex and sap of the root) are medicinally important in the traditional system of medicine in India.[8] The leaves, bark and fruits of *F. racemosa* are employed in native medicine to treat several diseases.[9] Several experimental studies have demonstrated the anti-inflammatory, hepatoprotective and hypoglycemic effects of the plant *F. racemosa*.[10-12]

However to the best of our knowledge no work has been reported previously on the hypoglycemic and hypolipidemic

activity of the seeds of *F. racemosa*. The present study was undertaken to investigate the hypoglycemic and hypolipidemic effect of *F. racemosa* seeds.

MATERIALS AND METHODS

Collection and Identification of the Plant

The fresh seed of *Ficus racemosa* was collected in February 2009 from the area of Purana Palton, Dhaka. The plant was identified by the National Herbarium where a voucher specimen was deposited having the accession number 34479.

Drying and Pulverization

The fresh seed was first washed with water to remove adhering dirt and then cut into small pieces and sun dried for 4 days. After complete drying, the entire portion was pulverized into a coarse powder with the help of a grinding machine and was stored in an airtight container at room temperature for further process.

Extraction of Plant Material

Ten grams of powdered seeds were mixed with 1000 ml distilled water, boiled for 10 min and then cooled for 15 min. Thereafter, the aqueous extract was filtered using a Millipore filter (Millipore 0.2 mm) to remove particulate matter. The filtrate was then freeze-dried from BCSIR (Bangladesh Council of Scientific and Industrial Research), Dhaka, Bangladesh.

Drugs, Chemicals and Reagents

Metformin was purchased from Square Pharmaceuticals Ltd. Dhaka, Bangladesh. All other reagents, assay kits and chemicals used in this work were purchased from Sigma Chemical Co. St Louis, MO, USA.

Experimental Animals and Their Management

Swiss-albino mice of both sexes aged 7-8 weeks and with an average weight of 20-30 gm were used for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental conditions for one week for acclimatization after their purchase and fed ICDDR; B formulated rodent food and water ad labium. They were housed individually in cages and were kept at constant room temperature (25.0 ± 3.0 ºC), humidity 35-60% and 12 hours light/dark cycle. Excreta were removed from the cages every day. The animals were divided into four groups having 6 mice in each group and named as following:

Group 1: Mice treated with 200 mg/kg extract
Group 2: Mice treated Metformin (500 µg/ kg)
Group 3: Diabetic Control
Group 4: Control

Induction of Diabetes Mellitus and Measurement of Plasma Glucose

Diabetes was induced by a single intraperitoneal (i.p.) injection of 100 ml of sterile phosphate buffered solution (PBS—pH 7.4) containing streptozotocin (STZ) (65 mg/kg), (Zanosar, Pharmacia & Upjohn, ON, Canada). After 4 days, the hyperglycemia was established.[13] Glucose concentration was measured in a blood sample obtained from tail puncture using glucometer (One Touch Ultra). Only animals that had a blood glucose concentration higher than 10 mM, 4 days after treatment with STZ, were used for the study.[13]

Blood Sample Collection and Preparation of Plasma

At the end of 14 days treatment, after 24 h fasting, blood samples were collected from post vena cava of the mice anaesthetizing with Ketamine (500 mg/kg body, intra peritoneal) and transferred into heparinised tubes immediately. Blood was then centrifuged at 4000 *g* for 10 min using a bench top centrifuge (MSE Minor, England). The supernatant serum samples were collected using dry Pasteur pipette and stored in the refrigerator for further analyses. All analyses were completed within 24 h of sample collection.

Determination of Lipid Profile

Triglycerides, total cholesterol and HDL concentration were evaluated according to the instruction of manufacturer of assay kits (purchased from Sigma Chemical Co, St Louis, MO, USA). According to Friedewald's formula,[14] VLDL and LDL were calculated as: VLDL cholesterol = TG/5 and LDL cholesterol = TC – (VLDL+HDL cholesterol).

STATISTICAL ANALYSIS

The value of glucose (mmol/l) and lipid profile parameters (mg/dl) were expressed as mean ± SEM (standard error of mean) and analyzed for ANOVA and post hoc Dunnet's t-test. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 18) was applied for the analysis of data. Differences between groups were considered significant at $P < 0.05$, 0.001 levels.

RESULTS

The hypoglycemic effect of *Ficus racemosa* seeds is shown in Table 1. It was found that the seed extract of *F. racemosa* reduced blood glucose level in streptozocin-induced diabetic mice and produced substantial hypoglycemic effects. In the first week after induction of diabetes, the blood glucose level was 15.98 ± 0.67 mmol/l and it was 6.57 ± 0.82 mmol/l in the third week after treatment and it was near to the control group 6.0 ± 1.03 mmol/l. Incase of lipid profile, the plant extract decreased the level of cholesterol, triglycerides and LDL (Table 2). The decrease in cholesterol,

Table 1: Effect of *F. racemosa* seeds extract on fasting blood glucose

Groups	1st week	3rd week	Decrease/increase (%)
	Glucose (mmol/l)	Glucose (mmol/l)	
Group 1 (200 mg/kg)	15.98 ± 0.67	6.57 ± 0.82(***)	–55.680
Group 2 Metformin (500 µg/ kg)	10.68 ± 0.11	5.85 ± 0.18(***)	–51.546
Group 3 (Diabetic Control)	11.52 ± 0.42	15.40.73 ± 0.67(***)	24.479
Group 4 (Control)	6.0 ± 1.03	6.8 ± 1.90	1.493

All the values (mmol/l) are expressed as mean ± SEM (standard error of mean). In each group 6 mice were taken. Level of significance was taken as *$P < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS = Not Significant.

Table 2: Effect of *F. racemosa* seeds extract on lipid profile parameters

Parameters	Control (n=6)	Diabetic control (n=6)	Standard control (metformin 500 µg/kg)	Extract, 200 mg/kg (n=6)
Triglycerides	54 ± 2.6	102 ± 6.6	66.13 ± 6.7	61.042 ± 10.20 (***)
Total cholesterol	120.7 ± 10.4	259.7 ± 19.4	218.01 ± 16.67	162.96 ± 22.91 (***)
LDL	180.3 ± 22.8	304.3 ± 22.8	255.328 ± 17.77	212.53 ± 4.22 (***)
HDL	9.6 ± 1.7	14.6 ± 1.7	18.13 ± 0.64	15.54 ± 0.095 NS

All the values (mg/dl) are expressed as mean ± SEM (standard error of mean). In each group 6 mice were taken. Level of significance was taken as *$P < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS = Not Significant.

triglycerides and LDL level was statistically highly significant ($p < 0.001$) in comparison with the control group. The reduction in the level of all studied parameters (cholesterol, triglycerides and LDL) almost near to the values of the control group in third week of treatment. In the case of cholesterol, it varied from 259.7 ± 19.4 to 162.96 ± 22.91 (mg/dl). For triglycerides it ranged from 102 ± 6.6 to 61.042 ± 10.20 (mg/dl) and for LDL, it ranged from 304.3 ± 22.8 to 212.53 ± 4.22 (mg/dl). The level of control was 120.7 ± 10.4, 54 ± 2.6 and 180.3 ± 22.8 (mg/dl) for cholesterol, triglycerides and LDL respectively. There was a slight increase in the HDL level in comparison with control. But it was statistically non significant.

DISCUSSION

Diabetes mellitus remains the most common chronic disorder of carbohydrate, fat and protein metabolism.[15] Apart from hyperglycemia, it is accompanied by hypercholesterolemia, hyperlipidemia and hepatic steatosis.[16]

Streptozotocin (STZ) is widely used to induce diabetes in experimental animals. As STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells, NO has been proposed to contribute to STZ-induced DNA damage.[17-18] The hypoglycemic effect of *F. racemosa* seeds may be due to the presence of leucocyanidin-3-O-β-D-glucopyrancoside, leucopelargonidin-3-O-β-D glucopyranoside, leucopelargonidin-3-O-α-L-rhamnopyranoside and all of which are known to reduce hyperglycemia.[21] These compounds are also isolated from *Ficus bengalensis* and the leucopelargonidin derivative isolated from *Ficus bengalensis* has been shown to decrease fasting blood sugar levels at a dosage level of 100 mg/kg/day in diabetic rats.[22-23]

The mechanism involved may be due to binding to the insulin receptors to act as insulin secretagogue, like biguanides. Other probable mechanisms by which the extracts of *F. racemosa* lowered blood glucose levels in diabetic mice may be by increasing glycogenesis, inhibiting gluconeogenesis in the liver, or inhibiting the absorption of glucose from the intestine or these might have improved insulin resistance. Further experiments are needed to determine the actual mechanism of action of the active constituents of the relative plant fractions.

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease. This abnormally high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots; thus, hypercholesterolemia and hypertriglyceridemia are known to occur in STZ induced diabetic mice. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyzes triglycerides. However, lipoprotein lipase is not activated in conditions of insulin deficiency, thus resulting in hypertriglyceridemia.[24] The observed hypocholesterolemic and hypotriglyceridemic effects of *F. racemosa* seeds therefore may be due to the activation of the enzyme, lipoprotein lipase. So the next step should be to isolate the compounds that are responsible for the observed antidiabetic activity and to elucidate the exact mechanism of action.

REFERENCES

1. Defronzo RA, Bondonna RC, Ferranini E. Pathogenesis of NIDDM: a balanced overview. Diabetes Care, 1992; 15:318–367.
2. Chait A, Brunzell JD. Diabetes, Lipids, and Atherosclerosis. In: Le Roith D, Taylor SI, Olefsky JM (Eds.), Diabetes Mellitus. Lippincott-Raven Publishers, Philadelphia, 1996; pp. 467–469.
3. Alberti KGMM, Zimmet P, DeFronzo RA. International Textbook of Diabetes Mellitus, 2nd ed. John Wiley and Sons, New York, 1997.

4. Lyra R, Oliveira M, Lins D, Cavalcanti N. Prevention of type 2 diabetes mellitus. Arq Bras Endocrinol Metab. 2006; 50:239-249.
5. Huang TH, Kota BP, Razmovski V, Roufogalis BD. Herbal or natural medicines as modulators of peroxisome proliferator-activated receptors and related nuclear receptors for therapy of metabolic syndrome. Basic Clin Pharmacol Toxicol. 2005; 96:3-14.
6. Shanmugasundaram ERB, Leela Gopinath K, Radha Shanmugasundaram K,. Rajendran VM. Possible regeneration of the islets of langerhans in streptozotocin-diabetic rats given gymnema sylvestre leaf extracts. J Ethnopharmacol. 1990; 30(3):265-269.
7. Manandhar NP. Fodder trees. The Rising Nepal. 1972; 7(330):1-2.
8. Kirtikar KR, Basu BD, Indian Medicinal Plants, 2nd ed, Vol. III, Dehra Dun. 1975; pp. 2327-2328.
9. Joshi SG. Oleaceae In: Medicinal plants. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi. 2000; pp 281-282.
10. Li RW, Leach DN, Myers SP, Lin GD, Leach GJ, Waterman PG. A new anti-inflammatory glucoside from *Ficus racemosa* (L.). Planta Med. 2004; 70:421-426.
11. Mandal SC, Maity TK, Das J, Pal M, Saha BP. Hepatoprotective activity of *Ficus racemosa* leaf extract on liver damage caused by carbon tetrachloride in rats. Phytother Res. 1999; 13:430-432.
12. Bhaskara RR, Murugesan T, Sinha S, Saha BP, Pal M, Mandal SC. Glucose lowering efficacy of *Ficus racemosa* barks extract in normal and alloxan diabetic rats. Phytother Res. 2002; 16:590-592.
13. Vianna RMJ, Ongali B, Regoli D, Calixto JB, Couture R. Upregulation of kinin B1 receptor in the lung of streptozotocin-diabetic rat: autoradiographic and functional evidence. *British Journal of Pharmacology,* 2003; 138:13–22.
14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972; 18(6):499-502.
15. Murray M, Pizzorno J. Encyclopedia of Natural Medicine, Rockling: Prima Health Publishing, 1997; pp: 401. ISBN: 0-7615-1157-1.
16. Harris RA, Crabb DW. Metabolic interrelationships. In: Textbook of Biochemistry with Clinical Correlations, Ed. Delvin TM, New York: John Wiley and Sons Inc., 1982; pp: 531-559.
17. Kröncke KD, Fehsel K, Sommer A, Rodriguez ML, Kolb-Bachofen V. Nitric oxide generation during cellular metabolization of the diabetogenic N-methyl-N-nitroso-urea streptozotocin contributes to islet cell DNA damage. Biol Chem Hoppe-Seyle. 1995; 376:179-185.
18. Morgan NG, Cable HC, Newcombe NR, Williams GT. Treatment of cultured pancreatic β-cells with streptozotocin induces cell death by apoptosis. Biosci Rep. 1994; 14:243-250.
19. Takasu N, Komiya I, Asawa T, Nagasawa Y, Yamada T. Streptozotocin- and alloxan-induced H_2O_2 generation and DNA fragmentation in pancreatic islets, H_2O_2 as mediator for DNA fragmentation. Diabetes. 1991; 40:1141-1145.
20. Bedoya FJ, Solano F, Lucas M. N-monomethyl-arginine and nicotinamide prevent streptozotocin-induced double strand DNA break formation in pancreatic rat islets. Experientia. 1996; 52:344-347.
21. Joy PP, Thomas J, Mathew S, Skaria BP. *Medicinal Plants.* Tropical Horticulture Vol. 2. Calcutta: Naya Prokash. 2001; p. 449-632.
22. Cherian S, Kumar RV, Augusti KT, Kidwai JR. Antidiabetic effect of a glycoside of pelargonidin isolated from the bark of *Ficus bengalensis* Linn. Indian J Biochem Biophys. 1992; 29:380-382.
23. Geeta BS, Mathew BC, Augusti KT. Hypoglycemic effects of Leucodelphinidin derivative isolated from *Ficus bengalenses* (Linn). Indian J Physiol Pharmacol. 1994; 38:220-222.
24. Pushparaj PN, Low HK, Manikandan J, Tan BK, Tan CH. Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. J Ethnopharmacol. 2007; 111:430-434.