

Antidiabetic activity of *Randia dumetorum* against streptozotocin (STZ) induced diabetics in rats

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Abstract

In the present investigation the antidiabetic activity of *Randia dumetorum* against streptozotocin (STZ) induced diabetics in rats. The rats treated with Streptozotocin showed a significant increase in glucose level and altered level of lipid profile, hemoglobin and insulin were observed. The mechanism underlying STZ hyperglycemia in diabetes mellitus involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues. Fifteen days administration of ethanolic extract of leaf of the *Randia dumetorum* (500mg/kg b.wt.) and standard as glibenclamide (0.25mg/kg) to diabetic rats resulted in significant ($p < 0.001$) reduction in blood glucose level, restored hemoglobin, and lipid profile as compared to diabetic rats. The present study suggests that the *Randia dumetorum* leaves extract had synergistic hypoglycemic and hypolipidemic effect revealed by decreased glucose, lipid levels and hemoglobin therefore attribute to therapeutic value of the *Randia dumetorum* extract of leaves to combat the diabetic condition in rats. The effect of *Randia dumetorum* leaves was better than glibenclamide. The potential activity of *Randia dumetorum* leaves may be due to the presence of phytochemicals.

Keywords: *Randia dumetorum*, Diabetic, Streptozotocin, Lipid profile, Glucose.

1. Introduction

Diabetes mellitus is an endocrine metabolic disorder characterized by hyperglycemia, altered lipids, carbohydrates, protein metabolism and it increases the risk of cardiovascular disease complications¹. The two forms of diabetes, type 1 and 2, differ in their basic mechanisms of development and in physiologic characteristics such as associations with obesity, age, and insulin. But, both types of diabetes share the common characteristics of hyperglycemia, microvascular and macrovascular complications. Moreover, the alterations of lipoprotein metabolism are involved in the pathogenesis of the cardiovascular disease in both forms of diabetes in a similar way². Diabetes has a considerable impact on the health, life style, life expectancy of patients and its related complications are major healthcare problems.

Currently, diabetes is controlled by a handful of available drugs such as oral hypoglycemic agents and insulin, but they have their own limitations. Traditionally, many herbal medicines and medicinal plants have been used for the treatment of diabetes as an alternative medicine³. Presence of various phytoconstituents in medicinal plants is thought to act on a different series of targets by multiple modes and mechanisms. Hence, plants have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications⁴. Screening of medicinal plants is one of the alternative and valid approaches in the drug development process because they contain diverse phytoconstituents which may give new drug leads and may be effective and safe in diabetes. In India, traditionally numbers of plants are used to manage the diabetic conditions and their active principles were isolated but few plants have been scientifically studied. In the present study, the chosen plant *Randia dumetorum*. The fruits of *Randia dumetorum* were reported to possess antidiabetic activity⁵. The antidiabetic activity of leaf of *Randia dumetorum* is not evaluated. Therefore, the present study was carried out to evaluate the antidiabetic activity of *Randia dumetorum* in STZ induced diabetes and to probe into the mechanism of its antidiabetic property.

2. Materials and Methods

2.1 Chemicals

Streptozotocin (STZ), Ethylene Diamine Tetra Acetic Acid (EDTA), Glibenclamide (Prudence Pharma Chem, India), Chloroform were purchased from Sigma chemical company, Mumbai. All other chemicals and reagents used in this

study was of analytical grade with high purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

2.2 Animals

Male albino rats of Wistar strain approximately 3-4 months young rats (weighing approximately 140-160g) and 24-26 months old rats (weighing approximately 380-410g) were used in this study. They were healthy animals procured from Sri Venkateswara enterprises, Bangalore, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature $27\pm 2^\circ\text{C}$ and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with experimental diet and water *ad libitum*. Diets were freshly mixed in small amounts every 2-3 days. They were acclimatized to the environment for 1 week prior to experimental use.

2.3 Plant materials

The fully mature *Randia dumetorum* leaves were collected in April 2013 from Tamil University, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Botanist, Dr. SoosaiRaj, M.Sc., Ph.D., Department of Botany, St. Josephs College, Tiruchirappalli, Tamil Nadu, India. A Voucher specimen has been deposited at the Rapinat Herbarium, St. Josephs College, Tiruchirappalli, Tamil Nadu, India.

2.4 Preparation of alcoholic extract

The collected *Randia dumetorum* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Randia dumetorum* leaves extract (RDLE) was stored in refrigerator until used.

2.5 Streptozotocin (STZ) Induced Diabetic rats

The animals were divided into four groups of six animals each. Diabetes was induced in all groups except normal control following overnight fasting (deprived of food for 16h allowed free access to water) by a single intraperitoneal injection of 65mg/kg of streptozotocin (STZ) dissolved in a freshly prepared 0.1M citrate buffer (pH4.5)⁶. The animals of normal control (Group I) were injected with saline alone. Diabetes was confirmed 72 h after induction by measurement of tail vein blood glucose levels by glucose oxidase-peroxidase method using strips. Group II served as diabetogenic rats (Control). Group III rats treated with *Randia dumetorum* at a dose of 500mg/kg was orally given once a day for 15 days after hyperglycemia was confirmed. Group IV rats treated with Glibenclamide as a standard at dose of 0.25mg/kg⁷. After complete the experimental period, the animals were killed cervical dislocation after an overnight fasting. The blood sample was collected. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000 rpm for 10minutes and then the serum (supernatant) was isolated and stored at refrigerated until required for analysis.

2.6 Biochemical estimations

Serum glucose was estimated by the oxidase method⁸. The total cholesterol (TC) was estimated by the method of Allain et al⁹. Triglyceride was estimated by the method of Werner *et al*¹⁰. High Density Lipoprotein (HDL) cholesterol was separated by adding phosphotungsti magnesium chloride to the fresh samples to precipitate other lipoproteins and the HDL cholesterol was estimated by the method. The concentration of Low Density Lipoprotein (LDL) cholesterol was calculated by using the Friedwald formula¹¹ and Very Low Density Lipoprotein (VLDL) cholesterol was calculated by dividing the triglycerides value (in mg/dl) by 5. Hemoglobin estimated by the method of Dacie and Lewis¹².

2.7 Statistical Analysis:

Values were expressed as mean \pm standard deviation for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for post-hoc multiple comparison tests. Statistical Package for Social Studies (SPSS) 9.0 version was used and $p < 0.001$ was considered to be significant.

3. Results

The ethanolic extract of *Randia dumetorum* was administered orally in an aqueous solution at a dose of 500mg/kg body wt. to diabetic rats to assess the synergetic impact of the plant extract. The plant extract were fed with normal and diabetes induced rats. The blood glucose levels was significantly ($P < 0.001$) reduced when compared to the specific diabetic control animals (Table 1).

Table 1: shows the effect of *Randia dumetorum* on glucose, Hb, cholesterol and triglycerides in experimental rats

Treatment Groups	Glucose (mg/dl)	Hb (g/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
Group I	101.69 \pm 5.08	13.76 \pm 0.68	90.96 \pm 4.54	114.28 \pm 5.71
Group II	206.32 \pm 10.31 ^a	9.03 \pm 0.45 ^a	236.36 \pm 11.81 ^a	188.57 \pm 9.42 ^a
Group III	96.61 \pm 4.83 ^b	15.49 \pm 0.77 ^b	76.36 \pm 3.81 ^b	82.85 \pm 4.14 ^b
Group IV (S)	98.30 \pm 4.91 ^b	13.35 \pm 0.66 ^b	82.74 \pm 4.13 ^b	87.14 \pm 4.35 ^b

Values were expressed as mean \pm SD for six rats each.

^a Compared with group I ($p < 0.001$) ^b Compared with group II ($p < 0.001$)

The lipid profile such as TC, TG, LDL and VLDL levels were significantly increased in diabetic control animals (DC) where as HDL levels were decreased when compared to the control rats. The plant extract were administered orally at a dose of 500mg/kg body wt., to diabetic rats significant ($P < 0.001$) depletion in the total cholesterol, TG, LDL, and VLDL levels and increment of HDL levels were recorded in the diabetic animals (Table 1 and 2). The depleted high density lipoprotein (HDL) in the diabetic rats, increased significantly ($P < 0.001$) after the administration of the plant extract. The plant extract possesses significant antidiabetic activity and close proximity to standard.

Table 1: shows the effect of *Randia dumetorum* on HDL, VLDL, and LDL- cholesterol in experimental rats

Treatment Groups	HDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)
Group I	24.57 ± 1.22	28.85 ± 1.44	37.54 ± 1.87
Group II	18.03 ± 0.98 ^a	37.71 ± 1.88 ^a	180.26 ± 8.18 ^a
Group III	27.11 ± 1.35 ^b	16.57 ± 0.62 ^b	37.76 ± 0.08 ^b
Group IV (S)	25.86 ± 1.29 ^b	17.42 ± 0.57 ^b	43.86 ± 2.19 ^b

Values were expressed as mean ± SD for six rats each.

^a Compared with group I ($p < 0.001$) ^b Compared with group II ($p < 0.001$)

4. Discussion

Streptozotocin (STZ) (2-deoxy-2-(3-methyl-3-nitrosourea)-D-glucopyranose) is commonly used for experimental induction of type-I diabetes mellitus, which causes selective pancreatic islet β -cell cytotoxicity mediated through the release of nitric oxide (NO). This results in rapid reduction in pancreatic islet pyridine nucleotide concentration and subsequent β -cell necrosis. The action of STZ on mitochondria generates SOD anions, which leads to diabetic complications¹³. Based on the above perspectives, in the present study, the antidiabetic activity has been assessed in rats made diabetic by STZ. Sulfonylureas such as glibenclamide are often used as a standard antidiabetic drug in STZ-induced diabetes to compare the efficacy of variety of antihyperglycemic compounds¹⁴.

In diabetes the increased blood sugar levels might be due to either insulin resistance of the body cells or decreased secretion of insulin from beta cells manifest in the decreased serum insulin levels¹⁵. Administration of *Randia dumetorum* extract to diabetic rats restored the levels of glucose. Present finding is in agreement with¹⁶ studies.

Diabetes affects both glucose and lipid metabolism¹⁷. The insulin deficiency depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes¹⁸.

The lipoprotein levels in the STZ induced diabetic rats of the present study reveal a significant alter in lipoprotein metabolism. The serum total cholesterol content increased significantly in diabetic animals. The elevated hypertriglyceridemia was increased in the synthesis of triglyceride rich lipoprotein particles (very low density lipoprotein, VLDL) in liver diminished catabolism in diabetic rats¹⁹. Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver^{20,21}. The increased levels of low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) in the diabetic animals might be due to over production of LDL and VLDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx. The high density lipoprotein (HDL) was significantly reduced in the diabetic rats which indicate a positive risk factor for atherosclerosis²². Supplementations of *Randia dumetorum* to diabetic rats restored the lipid profile. Our results concord with the earlier work done by Kesari *et al*²³. where it has been reported that lipid profile level in the plasma is restored with the treatment of *Aegle marmelos* seed extract in diabetic rats.

The blood glucose level of *Randia dumetorum* extract fed animal was significantly ($P < .001$) reduced. The levels of serum TC, TG, LDL, and VLDL were found to be significantly reduced in the plant extract treated diabetic animals. This might be due to the reduced hepatic triglyceride synthesis and or reduced lipolysis that might be due to the increase in serum insulin levels in the plant extract treated rats. The HDL increased significantly in the plant extract treated rats indicating a reversed atherogenic risk.

In uncontrolled or poorly controlled diabetes there is an increased glycosylation of a number of proteins including haemoglobin and α -crystalline of lens²⁴. Glycosylated haemoglobin (HbA1) was found to increase in patients with diabetes mellitus to approximately 16%²⁵ and the amount of increase is directly proportional to the blood glucose level²⁶. During diabetes the excess glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin. Therefore, the total haemoglobin level is decreased in STZ diabetic rats. So the total haemoglobin level is lowered in alloxan diabetic rats. Administration of *Randia dumetorum* reversed the total haemoglobin levels in alloxan diabetic rats.

The present study suggests that the *Randia dumetorum* extract had synergetic hypoglycemic effect revealed by decreased serum lipid levels, restored haemoglobin and therefore attribute to therapeutic value of the plant extract of *Randia dumetorum* to combat the diabetic condition in rats.

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