Evaluation of the Anti-diabetic Potential of *Rumex vesicarius* L. in normal and Streptozotocin induced Diabetic Rats

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Abstract
The development of effective and safe anti-diabetic drugs is urgent need of today which could be achieved from natural sources like plants and herbs. This study had been carried out to evaluate anti-diabetic activities of 95% ethanol extract of R. vesicarius macerated with aqueous gum acacia (2% w/v) suspension in normal fasted, fed, glucose loaded and streptozotocin induced diabetic male albino rats.

Treatment of experimental models with single dose of 250mg/kg body weight ethanolic extract of plant caused significant reduction in blood glucose level, along with the marked degranulation in insulin producing pancreatic β-cells. The results of the present study demonstrated anti-diabetic activity of R. vesicarius extract which could help in prevention of diabetic related complications.

Keywords: Anti-diabetic, Streptozotocin, Hyperglycemia, Pancreatic β-cells.

Introduction

Diabetes Mellitus (DM) is a disorder characterized by hyperglycemic condition caused by defect in the insulin secretion from the β-cells of pancreas, insulin sensitivity, or both[2]. It is linked to acute and chronic complications which contribute the most of DM-related mortality and morbidity as well as poor quality of life. Diabetic complications caused by persistently high blood sugar impact a number of organs, primarily the eyes, kidneys, blood vessels and nerves[14]. Generally, diabetes is treated with the use of synthetic drugs but due to its side effects and certain limitations, a search for new class of compounds is essential to overcome the diabetic related problems and therefore the attention has been drawn towards the utilization of plants in the area of pharmacology.

Medicinal plants have been used as a source of treatment for at least 4,000 years and are rich source of pharmaceuticals, binders, flavouring compounds, food additives, lubricants and colorants[10,17]. Plant-derived chemicals account for 25% of all prescribed medicines today. As a result, the importance of research on medicinal plants cannot be underestimated. Modern scientific methods have been employed to study a wide range of therapeutic plants and they have proven to be quite useful[17]. A number of plants have been employed in various herbal treatments of diabetes but only a few of them have been scientifically validated[10].

Rumex vesicarius L., a member of the Polygonaceae family, is a medicinal plant used to treat hepatic diseases, poor digestion and as a diuretic, laxative, tonic, analgesic and antibacterial agent. The plant is reported to control cholesterol levels and reduce biliary disorders[1,3,5,7,11,12] *R. vesicarius* is also used for the management of diabetes related complications, but requires scientific study to support its traditional claim. Therefore, the primary goal of this study is to investigate the anti-diabetic effect of the ethanolic crude extract of the whole aerial part of *R. vesicarius* plant.

Material and Methods

All the chemicals, solvents and reagents used for the work are of analytical grade.

Collection and Authentication of Plant Materials: The plant specimen for the proposed study was collected in the month of July-August from a local nursery by following intensive care[8]. Plant samples were authenticated by Dr. Lal Babu Chaudhary, Senior principal scientist and curator of herbarium plant diversity at CSIR - National Botanical Research Institute (NBRI) Lucknow, Uttar Pradesh. A sample specimen has been deposited at CSIR-NBRI herbarium (LWG) with accession no. LWG-108275 as a record.

Preparation of crude extract and its phytochemical analysis: The whole plant of *R. vesicarius* was washed thoroughly and subjected to shade dried. Around 500gm of coarsely powdered plant sample was subjected to successive extraction using ethanol by continuous hot perculation process in Soxhlet apparatus. The extract was concentrated by using the rotatory evaporator at 40ºC and evaporated to dryness in a water bath under controlled conditions. This dried extract was kept in air tight containers for further analysis. Using standard methods described by Suryavanshi et al[15], the extract and fractions were subjected to various qualitative chemical analyses to determine the components present.

Experimental Animals: Healthy male rats of *Charles Foster* strain weighing 120-160gm, bred in the animal house of CSIR- Central Drug Research Institute (CDRI), Lucknow, Uttar Pradesh, India were procured. The animals were kept in controlled conditions: temperature 25-26ºC, relative

humidity 60-70% and 12/12 hour light / dark cycle. These animals were housed in polypropylene cages with free access to pellet laboratory diet and water *ad libitum*. The animals were acclimatized to laboratory environment one week prior to the study as per OECD guidelines[9]. The protocol was approved by Institutional Animal Ethical Committee of Hygia Institute of Pharmaceutical Education and Research, Lucknow with reference No. HIPER/IAEC/67/21/04 and the study was conducted according to the CPCSEA guidelines. At the time of experiment, animals were divided into 4 different experimental groups of 5 animals each.

The blood glucose lowering effect of the extract was examined following four different experimental models while the control group was given only 2% gum acacia suspension to increase the sensitivity of the experimental procedure.

1. Fasted Model: The animals of this group were fasted overnight (18 hours) and blood was drawn from the tail vein (at 0 hour) to measure the blood glucose concentration using a glucometer (Accu-Chek Active Glucometer; Roche, Germany). The plant extract was suspended in 2% gum acacia and given to animals in a single dose of 250mg/kg body weight through metal canula. Blood samples were obtained at intervals of 1, 3 and 4 hours after feeding extract and blood glucose levels were calculated.

2. Fed Model: On the previous evening, an excessive amount of pellets was provided in the cages, resulting in some pellets being left over the next morning. Blood glucose levels were measured from the tail vein blood before (at 0 hours) and after administration of the *R. vesicarius* ethanolic crude extract at 1, 3 and 4 hours.

3. Diabetic Model: Streptozotocin (STZ) was dissolved in 0.1 M of freshly prepared, chilled citrate buffer of pH 4.5. A single intraperitoneal injection of streptozotocin (35 mg/kg body weight) was used to induce diabetes in overnight fasting rats of this group. Food and water were accessible to the animals. Three days after receiving an intraperitoneal STZ injection, blood glucose levels were tested and rats with fasting blood glucose levels greater than 200 mg/dl were considered diabetic. The experiment was then carried out using the same approach as the fasted model group to calculate the blood glucose concentration at different intervals of time.

4. Glucose loaded Model: This group's animals were fasted for 18 hours before blood was drawn (at 0 hour) to determine blood glucose levels. The animals were then fed the plant extract at the dose of 250mg/kg body weight. After half an hour, a glucose solution (1.5gm/kg b.w.) was given orally and blood samples were again collected at 1/2, 1 and 3 hours intervals to assess the concentration of glucose in the blood.

Histology of Pancreas: The anti-diabetic effect was verified by pancreas histology. After dissection, the pancreas of the extract-treated and control groups of rats were taken and fixed in alcoholic Bouin's solution for histological analysis. Haematoxylin and eosin dye were used to stain paraffin sections of the pancreas. The granularity of pancreatic β-cells was then examined[6].

Statistical Analysis of Data: Statistical analysis was performed using GraphPad Prism software (version 6.04). The data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test with multiple comparisons to determine the source of significant difference between the groups.

Results

Results of phytochemical analysis: The presence of flavonoids, Glycosides and steroids was determined during preliminary phytochemical screening of *R. vesicarius* ethanolic extract.

Effect of crude ethanolic extract on blood glucose: The crude extract of *R. vesicarius* had a significant blood glucose lowering impact in normal and diabetic treated groups as compared to the control group as shown in the table 1.

Table 1
Effect of 95% ethanol extract of *Rumex vesicarius* plant at a single dose of 250mg/kg body weight on blood glucose level of fasted, fed and streptozotocin-induced diabetic male albino rats.

Group	Treatment	Blood glucose level mg/dl (Mean ± S.E.) at time (hours)				Maximum % of blood glucose lowering from initial value
		0 hour	1 hour	3 hour	4 hour	
Fasted Model	Control	98.4 ±4.21 (5)	96.6 ±5.7 (5)	94 ±7.71 (5)	89.2 ±4.52 (5)	9.34% at 4h
	R.vesicarius	99.4 ±3.74 (5)	96.6 ±2.84 (5)	87.2 ±3.72 (5)	81 ±1.7 (5)	18.51% at 4h
Fed Model	Control	110.6 ±8.96 (5)	110.4 ±3.39 (5)	106.4 ±3.48 (5)	98.8 ±1.93 (5)	10.66% at 4h
	R.vesicarius	123 ±5.8 (5)	118.4 ±2.79 (5)	99.8 ±1.69 (5)	108 ±3.2 (5)	18.86% at 3h
Diabetic Model	Control	214.8 ±5.15 (5)	240.2 ±8.82 (5)	238.4 ±10.1 (5)	227.6 ±8.24 (5)	No lowering
	R.vesicarius	214.2 ±4.31 (5)	251±9.98 (5)	213 ±3.62 (5)	186.6 ±5.67 (5)	12.88% at 4h
In parenthesis the number of rats used is given						

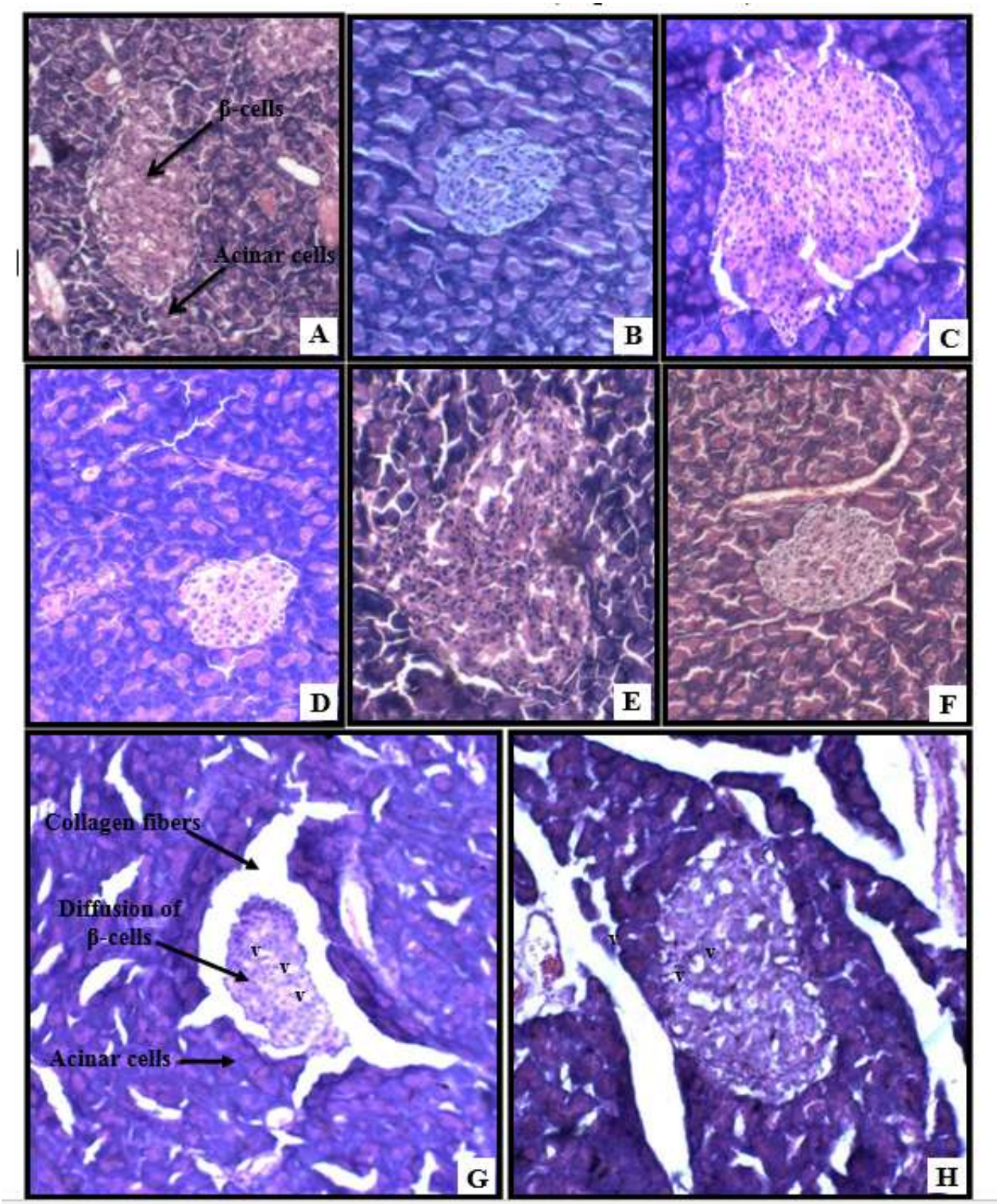

Figure 1: Histological examination of the pancreas of control (2% gum acacia-treated) and *R. vesicarius* extract-treated rats (Magnification-40X).

A, C and E showing pancreatic sections of control groups of Fasted, Fed and Glucose loaded respectively, closely packed lobules of pancreatic acini can be seen along with embedded Islets of Langerhans within the exocrine portion. B, D and F representing the pancreatic sections of *R. vesicarius* treated group of Fasted, Fed and Glucose loaded respectively showing degranulation in the islets of Langerhans.

G showing section of Diabetic control group which reveals the pathological changes in both exocrine and endocrine part of the pancreas represented by vacuolation (v), diffusion in the β-cells and atrophy in islets of langerhans which develops collagen fibres around it. H represents the section of *R. vesicarius* extract treated group.

After supplementation with plant extract the pancreas appeared similar to the control except for a few vacuoles, the majority of the islets of Langerhans cells were unaltered.

Table 2
Effect of 95% ethanol extract of *Rumex vesicarius* plant at a single dose of 250mg/kg body weight on blood glucose level of fasted, fed and streptozotocin-induced diabetic male albino rats.

Group	Treatment	Blood glucose level mg/dl (Mean ± S.E.) at time (hours)				Maximum % of blood glucose rise from initial value
		0 hour	1/2 hour	1 hour	3 hour	
Glucose Loaded Model	Control	91.8 ±2.06 (5)	99.8 ±1.77 (5)	105.2 ±3.01 (5)	99.6 ±1.6 (5)	14.59% at 1h
	R.vesicarius	92.8±3.48 (5)	95.2 ±2.91 (5)	98.6 ±3.22 (5)	94.8 ±1.98 (5)	6.25% at 1h
In parenthesis the number of rats used is given						

Effect of extract on pancreatic β-cells: Photomicrographs of sections of pancreas of *Rumex vesicarius* extract treated group rats were clearly showing prominently degranulated β-cells as compared to their respective control group of untreated rats. During examination of histological sections of pancreas in glucose loaded model, marked degranulation in β-cells of islets of Langerhans in extract treated rats can be observed as compared to control.

Discussion

The anti-diabetic potential of the ethanolic crude extract of *R. vesicarius* was investigated in this study by using normoglycemic and diabetic rats. In the light of the results (Table 1 and 2), treatment of all 4 groups rats with the 95% ethanol extract of *R. vesicarius* with dosage of 250mg/kg body weight significantly decreased the blood glucose level. The percentage reduction in fasting blood glucose level showed a greater reduction of 9.17% at 4th hour when compared to its control group from its initial value and 12.88% blood glucose lowering effect in diabetic treated group as compared to control group.

R. vesicarius extract was observed to decline the blood glucose levels in glucose loaded grouped rats (Table 2). This could be due to the restoration of a delayed insulin response, restriction of glucose absorption in the intestine, or an increase in glucose utilization. Degranulation in β-cells suggested that the rate of extract-induced insulin release was greater than the rate at which β-cells replenished their insulin storage and resulted in a reduction in blood glucose. In this context, other researchers have also reported that *Syzygium cimini* (13), *Securigera securida* (15) *Allium cepa* and *Ocimum sanctum* (4) have significant anti-diabetic and glucose tolerance effects in experimentally induced diabetic rats.

Conclusion

Eventually, the results of this study show that a 95% ethanolic extract of *R. vesicarius* had significant anti-diabetic activity in STZ-induced diabetic rats and suppression of postprandial hyperglycemia in normoglycemic mice, attempting to prove the traditional use of this plant for diabetes mellitus treatment.

The considerable degranulation of β-cells in treated rats suggests that the extract promoted insulin release from the β-cells of pancreas. Thus, it is crucial to conduct further studies including safety and characterization of bioactive compounds responsible for its anti-diabetic activity and suggest the mechanism of action.

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