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Evaluation of anti diabetic activity of methanolic extract of *Rivea ornata* (ROXB.) Leaves

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ABSTRACT

Objective: The current study was aimed to evaluate the Antidiabetic activity of methanolic extract of *Rivea ornata*.

Method: Plant extract was prepared by using Soxhlet method and the antidiabetic activity was evaluated by using Alloxan induced diabetes in rats.

Result: Treatment with methanolic extract of leaves of *Rivea ornata* (200 mg/kg) significantly decreased the level of blood glucose.

Conclusion: The methanolic extract of *Rivea ornata* at a dose of 200mg/kg exhibited significant diabetic activity in streptozotocin induced diabetes in rats.

Keywords: Diabetes Induction; Islet cells; Streptozotocin.

INTRODUCTION

As per the WHO, diabetes mellitus (DM) is defined as a heterogeneous metabolic disorder characterised by common feature of chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism. The number of individuals with diabetes is rising rapidly throughout the world. Both genetic and environmental factors contribute to its pathogenesis, which involves insufficient insulin secretion, reduced responsiveness to

endogenous or exogenous insulin, increased glucose production, and/or abnormalities in fat and protein metabolism. The resulting hyperglycemia may lead to both acute symptoms and metabolic abnormalities.

DM is a leading cause of morbidity and mortality world over. It is estimated that approximately 1% of population suffers from DM. The incidence is rising in the developed countries of the world at the rate of about 10% per year, especially of type 2 DM, due to rising incidence of obesity and reduced activity levels. DM is

expected to continue as a major health problem owing to its serious complications, especially end-stage renal disease, IHD, gangrene of the lower extremities, and blindness in the adults. It is anticipated that the number of diabetics will exceed 250 million by the year 2010.

Diabetes mellitus is a metabolic disorder of multiple etiologies. It is characterized by chronic hyperglycemia together with disturbances of carbohydrate, fat and protein metabolism resulting from defects of insulin secretion, insulin action or both. The relative contribution of these varies between different types of diabetes. These are associated with the development of the specific microvascular complications of retinopathy, which can lead to blindness, nephropathy with potential renal failure, and neuropathy. The latter carries the risk of foot ulcers and amputation and also autonomic nerve dysfunction. Diabetes is also associated with an increased risk of macrovascular disease.

Rivea ornata is a medicinal plant which traditionally and tropically used to treat several ailments. It is classified under the family of Convolvulaceae. In Tamil it is also known as Machutai or Musuttai. The plant is acrid, pungent, sweetish, oleaginous, cooling, tonic causes “kapha” and cures “vata”, biliousness, diseases of Heart, Bronchitis and effect of fatigue. In the Konkan region the juice of this plant added into the composition of an ointment for phthiasis and a preparation to be applied to piles.

Rivea ornata is climbing shrub, stems stout, terete, finely silky – pubescent (especially when young) with white appressed hairs. Leaves are 3-8 cm long, usually broader, orbicular or reniform, obtuse often apiculate or very shortly acuminate, glabrous above more or less densely white silky beneath, base cordate; petioles 2.5 – 7.5 cm long, densely silky, peduncles stout.

Aerial parts of this plant have anti-inflammatory effect, anti-bacterial effect, anti-oxidant effect, analgesic and anti-pyretic effect. Current study had been aimed to investigate the antidiabetic activity of Methanolic Extract of *Rivea ornata* (MERO) by streptozotocin induced hyperglycemia model using as reference standard.

MATERIALS AND METHODS

Plant material

The whole plants of *Rivea ornata* (Roxb.) were collected from the kolli hills at November 2021 and authentication was done by Prof. Dr. V. Nandagopalan, Dean of Science & Head, Department of Botany,

National College of Arts and Science (Autonomous), Tiruchirappalli, Tamil Nadu, India.

Preparation of plant extract

The fresh leaves of *Rivea ornata* (Roxb.) were collected and dried in the absence of sunlight and coarsely powdered. About 500 g of the dried powder was defatted with petroleum ether and extracted with methanol continuously in soxhlet apparatus for 1 day, after that, the solvent was evaporated to obtain the crude extract. The extract was then dried under vacuum and suspended in water before use.

Experimental animals

Healthy wistar albino rats (200 – 250g) were procured from Venkateshwara enterprises, Bangalore. The animals were kept in well ventilated and temperature controlled ($30^{\circ}\text{C} \pm 1^{\circ}\text{C}$) animal room for 7 days prior to the experimental period and provided with food and water *ad libitum*. The animals were allowed to acclimatize in laboratory conditions before the test. The protocol of this study was approved by the Institutional Animal Ethical Committee (IAEC) with approval number PCP/IAEC/004/.

Antidiabetic activity

Streptozotocin is an acidic compound obtained by the oxidation of uric acid and isolated as an efflorescent crystalline hydrate. It is a toxic glucose analogue, which selectively destroys insulin-producing cells (beta cells) in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus in these animals, with characteristics similar to type I diabetes in humans.

Wistar Albino Rats weighing 150-200g were divided into 4 groups and kept under the temperature of 25°C and the relative humidity of 50-60% with 12 hours dark and light cycle. Animals were fasted for 12 hours before and after injection of streptozotocin.

Group I: Normal saline (5mL/kg, b.w., *p.o.*)

Group II: Streptozotocin (150mg/kg, b.w., *p.o.*)

Group III: Streptozotocin (150mg/kg, b.w., *p.o.*) + Glibenclamide 10mg/kg, b.w., *p.o.*)

Group IV: Streptozotocin (150mg/kg, b.w., *p.o.*) + Methanolic Extract of *Rivea ornata* (200mg/kg, b.w., *p.o.*)

Experimental Design

Streptozotocin is used to induce diabetes. The dose

of Streptozotocin for diabetes control group is 150mg/kg of body weight. It was prepared by dissolving in normal saline and administered intraperitoneally. Rats were fasted 12 hours before and after injection of Streptozotocin, unfed animals are more susceptible for Streptozotocin induction diabetes. One week after treatment, animals with moderate diabetes (blood glucose level above of 150 mg /dl) were used for the experiment. Test and standard drugs were administered to the respective group in predetermined dose for 14 days.

Evaluation

Blood Glucose level

After 0 minutes, 1 hour, 2 hours, 4 hours, 7th day and 14th day from the administration of standard and test

drug, the blood glucose level was estimated by glucometer.

Histopathology of Pancreas

After the Blood glucose estimation was over the animals were sacrificed for histopathology studies. The pancreas of each animal from all four groups were isolated for histopathology studies. The isolated pancreas were carefully kept in 10% formalin solution in order to prevent the damage.

Statistical analysis

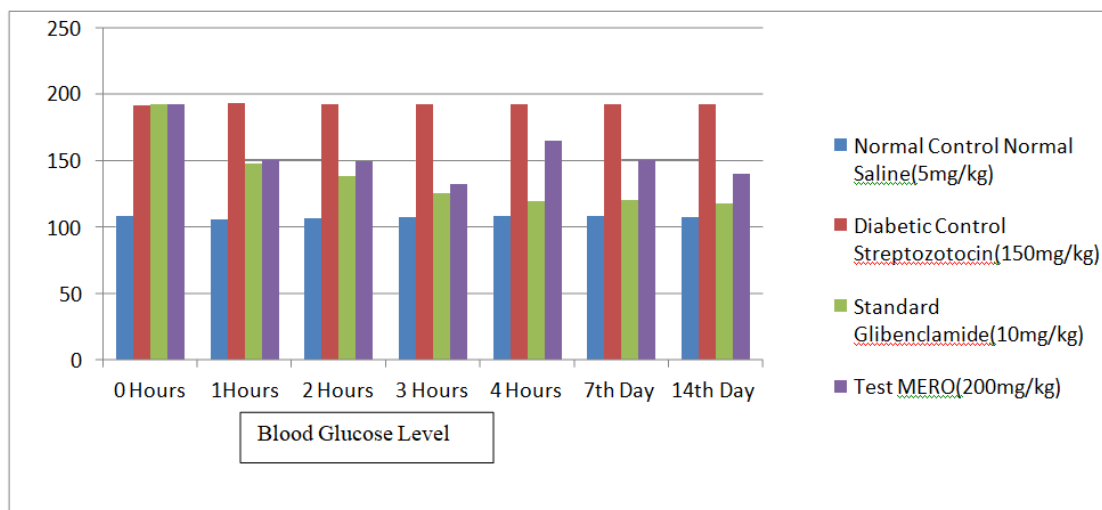
The results are expressed as Mean \pm SEM (n=6) two way ANOVA using a Graph pad and PRISM software version 8.2.1 (441). *** P<0.001, ** P<0.01 and *P<0.05 were considered as statistically significant.

RESULTS

Table 1: Antidiabetic activity of Methanolic Extract of *Rivea ornata*

S.No.	Drug/Dose	Blood Glucose Level						
		0 Hour	1 Hour	2 Hours	3 Hours	4 Hours	7th day	14thday
1	Normal Control Normal saline (5ml/kg)	108.33 \pm 1.58	105.67 \pm 2.29	106.00 \pm 1.29	107.50 \pm 2.14	108.33 \pm 2.20	108.33 \pm 1.89	107.17 \pm 1.54
2	Diabetic Control Streptozotocin (150mg/kg)	191.17 \pm 1.54	193.50 \pm 1.91	192.00 \pm 2.17	192.17 \pm 1.56	192.33 \pm 1.41	191.83 \pm 1.54	191.83 \pm 1.92
3	Standard Glibenclamide (10mg/kg)	192.5 \pm 1.26***	147.17 \pm 1.01***	138.17 \pm 1.08***	125.5 \pm 0.85***	119.00 \pm 0.73***	120.33 \pm 0.88***	117.33 \pm 0.49***
4	Test MERO (200mg/kg)	192.50 \pm 2.04***	150.17 \pm 0.70***	149.17 \pm 0.95***	132.00 \pm 0.73***	164.50 \pm 1.28***	150.33 \pm 0.99***	139.50 \pm 0.67***

n = 6 Values are expressed as \pm S.E.M. Values are Mean \pm SEM (n=6) two way ANOVA. Where, *** P<0.001, ** P<0.01 and * P<0.05



Graph 1: Antidiabetic activity of Methanolic Extract of *Rivea ornata*

Histopathological Study in Antidiabetic activity of MEROHistopathology of Pancreas

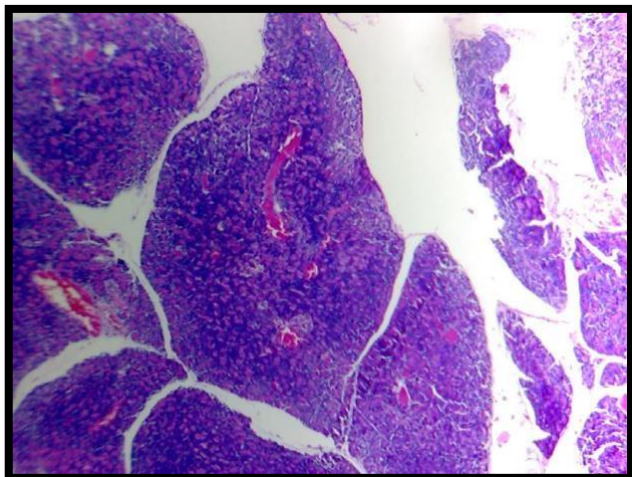


Fig 1: Normal Control

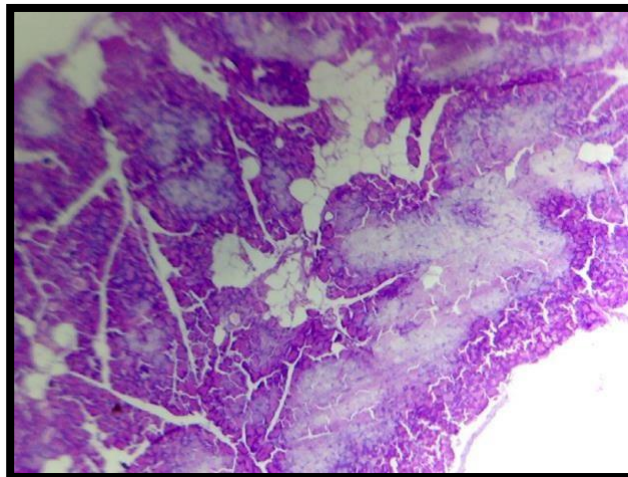


Fig 2: Toxic Control

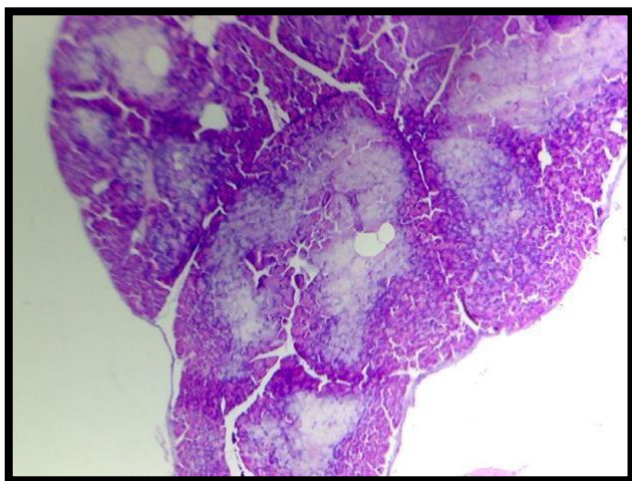


Fig 3: Standard

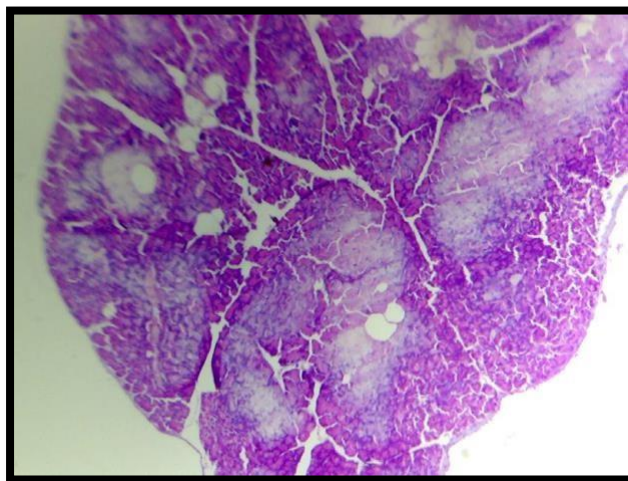


Fig 4: Test drug

Normal Control	- Normal Saline (5ml/kg)
Toxic Control	- Streptozotocin (150mg/kg)
Standard	- Streptozotocin (150mg/kg) + Glibenclamide (10mg/kg)
Test	- Streptozotocin (150mg/kg) + Methanolic Extract of <i>Rivea ormate</i> (200mg/kg)

Histopathological Study in Antidiabetic activity of MERO

Table 2: T.S of pancreas

S. No	Group	Description
1	Normal Control	Normal architecture of pancreatic islet cells
2	Toxic Control	Expanded and dilated architecture of pancreatic islet cells
3	Standard	Absence of dilatation, prominent hyperplastic pancreatic islets.
4	Test	Prominent hyperplastic pancreatic islets with moderate expansion and slight dilatation.

DISCUSSION

Streptozotocin Induced Hyperglycemia

Intraperitoneal administration of Streptozotocin (150mg/kg, b.w.) increased the blood glucose level by degrading the beta cells of pancreas. Treatment with methanolic extract of leaves of *Rivea ornata* (200mg/kg) was decrease the blood glucose level and shows antidiabetic index upto 3 hours. The blood glucose level was measured at 0 hour, 1 hour, 2 hour, 3 hour, 4 hour, 7th day and 14th day.

The pancreas was collected on 14th day and transverse sections were obtained from various groups suchas normal control, toxic control, standard and test

Streptozotocin has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase (The glucose sensor of the beta cell), and it causes a state of insulin- dependent diabetes. Streptozotocin also induce ROS (Reactive Oxygen Species) formation, resulting in the selective necrosis of beta cells.

The preliminary phytochemical analysis showed

that the methanolic extract of *Rivea ornata* contains well known antioxidant phytochemicals such as alkaloids, glycosides and polyphenols which act as free radical scavengers. The presumed mechanism of action of these antioxidants was because of insulin mimetic effect onthe peripheral tissues by either stimulation of regeneration process or release of pancreatic secretion of insulin from existing beta cells.

CONCLUSION

The methanolic extract of *Rivea ornata* at a dose of 200mg/kg showed significant antidiabetic activity in Streptozotocin induced hyperglycemic rats. This is showed by the reduction of blood glucose level in the group treated with 200mg/kg of methanolic extract of *Rivea ornata*

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