

**ANTIDIABETIC AND HYPOLIPIDEMIC EFFECTS OF METHANOLIC EXTRACT OF
VENTILAGO MADERASPATANA IN STREPTOZOTOCIN INDUCED DIABETIC RATS**SASIKALA C¹, YAJAMAN SUDHAKAR²¹Department of Pharmacy, Jawaharlal Nehru Technological University, Kakinada - 534 202, Andhra Pradesh, India. ², Government Polytechnic for Women, Kadapa - 516 002, Andhra Pradesh, India. Email: sasivenkee@gmail.com

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ABSTRACT

This study evaluated methanolic extract of *Ventilago maderaspatana* (MVM) on reducing hyperglycemia and hyperlipidemia in streptozotocin (STZ) - induced diabetic rats. MVM was orally administered once a day after 3 days of STZ - induction at 100, 200 and 400 mg/kg for 45 day and the results showed that serum fasting blood glucose, glucose-6-phosphatase, fructose 1, 6-phosphatase in hepatic tissues, total cholesterol, free fatty acids, and triglyceride in serum, hemoglobin A1c (HbA1c) in blood levels were significantly decreased, whereas Hb in blood and serum high density lipoprotein, glucokinase, glucose-6-phosphate dehydrogenase in hepatic tissues and liver and muscle glycogen level were increased. The dosage of 400 mg/kg is more effective than that of 100, 200 mg/kg. These results suggest that the MVM possesses antidiabetic and hypolipidemic effects in STZ - induced diabetic rats.

Keywords: *Ventilago maderaspatana*, Antihyperlipidemic, Antihyperglycemic and streptozotocin.**INTRODUCTION**

The plant *Ventilago maderaspatana* (Family: Rhamnaceae) is commonly known as Red creeper. It is large, much branched, woody climber with branches hanging down and dark grey bark having vertical cracks exposing the inner vermilion surface: Leaves simple, alternate, oblong-lanceolate or elliptic-ovate, obtuse or acute, entire or crenate, base rounded or acute, main nerves 4-8 pairs; flowers greenish with an offensive odor in drooping pubescent terminal panicles, petals much smaller than the sepals; fruits yellowish globular nuts, supported by the persistent calyx, wing linear-oblong, one-nerved, rounded at the apex. The bark is bitter, astringent, thermogenic, digestive, carminative, and stomachic, alexeteric, vulnerary, depurative, stimulant and tonic. It is useful in the treatment of dyspepsia, colic, flatulence, erysipelas, epilepsy, scabies, pruritus, skin diseases, fever and general debility [1]. The plant is constituted with isofuranonaphthaquinones, ventilone-C, ventiloquinones E,G,] eleuthrin, and enantiopure 1,3-dimethyl pyranonaphthoquinones [2]. Stem bark is used for gout [3]. The root bark is used as carminative, stomachic and stimulant and also has hepatoprotective properties as a natural antioxidant [4,5].

MATERIALS AND METHODS**Collection of leaves**

V. maderaspatana is a fern like tree belonging to family Rhamnaceae was obtained from Tirupathi in August and authenticated by Dr. Madhavachety (Department of Botany, SV University, Tirupathi).

Preparation of extract

The leaves were shade-dried at room temperature for 10 days, coarsely powdered and the powder was passed through sieve No. 60. The powdered material (300 g) was extracted with methanol (1000 mL) using Soxhlet apparatus. The extract was then dried under reduced pressure. The dried extract (15.7 g) was stored in desiccators.

Animals

Healthy female albino rats weighing 150-250 g were obtained from King's Institute, Chennai. Housed grouply in polypropylene cages, maintained under standard conditions (12 hrs light and 12 hrs dark cycle; 21 ± 3°C; 35-60% humidity), the animals were fed with standard rat pellet diet (Sai Durga Foods and Feeds, Bangalore) and provided

water ad libitum. The experimental protocols were approved by the Institutional Animal Ethical Committee in Shri Vishnu College of Pharmacy.

Streptozotocin (STZ) – induced diabetic rats

STZ purchased from Sigma Aldrich, was dissolved in ice-cold normal saline immediately before use. Diabetes was induced in rats by intra peritoneal injection of STZ at a dose of 45 mg/kg, dissolved in normal saline. 3 days after STZ administration, the blood samples were drawn from the tail veins and glucose levels determined to confirm the presence of diabetes. Those are rats exhibiting blood glucose levels higher than 250 mg/dl were selected for the studies. These diabetic rats were divided into five groups as follows. Group I, Normal control group received food and water. Group II untreated (diabetic control) received 0.5 mL of 5% tween 80. Group III, Group IV and Group V received 100, 200 and 400 mg/kg of methanolic extract of *V. maderaspatana* (MVM), respectively. The treatment was continued daily for 45 days. Blood was collected from the tail for glucose estimation just before drug administration on the 1st day and 1 hr after sample administration on day 45. The animals were killed after blood collection on day 45. Blood samples were collected and centrifuged to separate serum for estimation of diabetic and lipid marker. Blood glucose, serum total cholesterol (TC), free fatty acids (FFA), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and triglycerides (TG) were analyzed. Haemoglobin (Hb) and HbA_{1c} were determined using blood sample. Glucokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, fructose 1, 6-phosphatase in hepatic tissues and glycogen in liver and muscle were estimated.

Liver homogenate preparation

Liver tissue was sliced into pieces and homogenized in appropriate buffer (pH 7.0) in cold condition to give 10% homogenate (w/v). The homogenate was centrifuged at 1000 rpm for 10 minutes at 0°C in a cold centrifuge. The supernatant was separated and used to measure carbohydrate metabolic enzyme activities.

Biochemical analysis

Plasma glucose, HbA_{1c} level were measured by enzymatic method using commercial kit (Crest Biosystem, Goa) with a semi-autoanalyzer. Hb was estimated by the method of Drabkin and Austin [6]. TC, TG and HDL cholesterol levels in plasma were determined using commercially

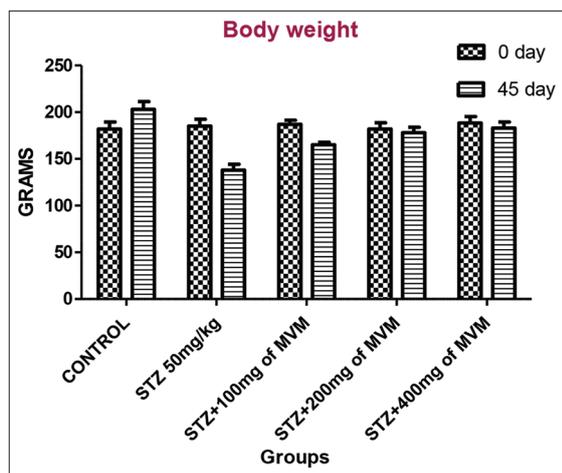


Fig. 1: Effect of methanolic extract of *Ventilago maderaspatana* on body weight of control and experimental rats

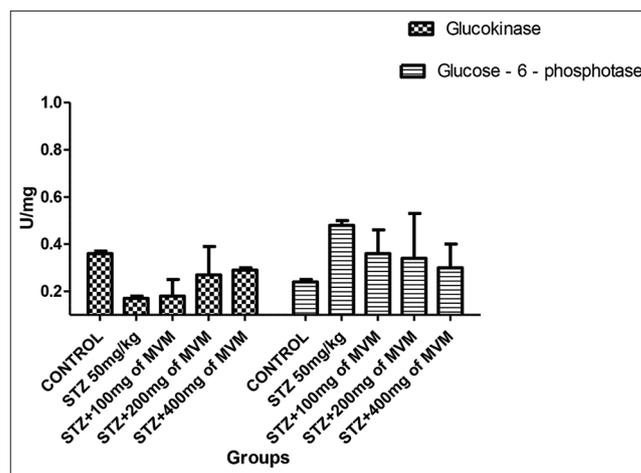


Fig. 4: Effect of methanolic extract of *Ventilago maderaspatana* on carbohydrate markers of control and experimental rats

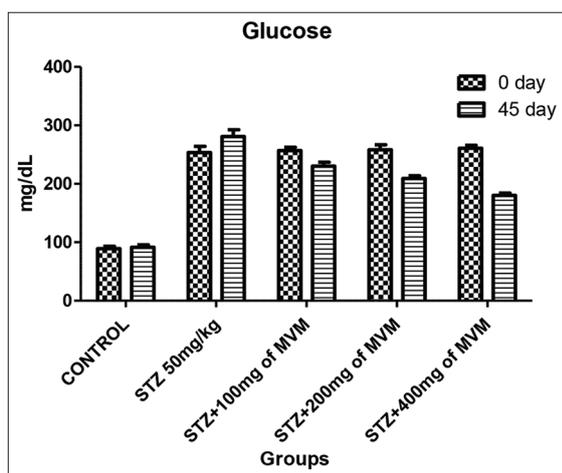


Fig. 2: Effect of methanolic extract of *Ventilago maderaspatana* on glucose level of control and experimental rats

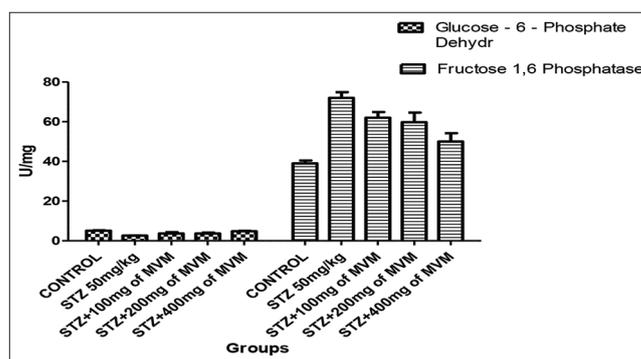


Fig. 5: Effect of methanolic extract of *Ventilago maderaspatana* on carbohydrate markers of control and experimental rats

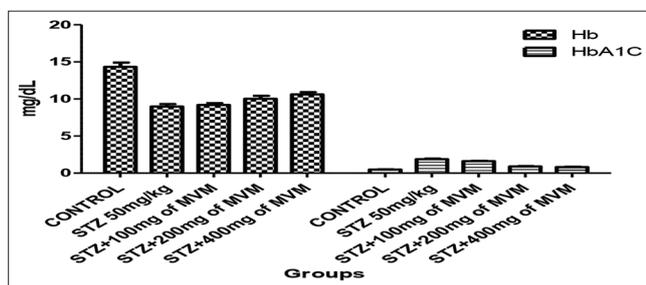


Fig. 3: Effect of methanolic extract of *Ventilago maderaspatana* on haemoglobin (Hb) and HbA1c of control and experimental rats

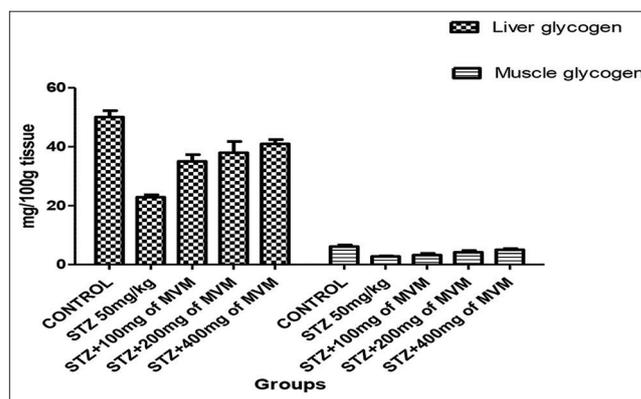


Fig. 6: Effect of methanolic extract of *Ventilago maderaspatana* on glycogen of control and experimental rats

available kits according to the instructions of the manufacturer (Crest Biosystem, Goa) with a semi-autoanalyzer. LDL cholesterol and VLDL cholesterol were calculated from TG, TC and HDL concentrations following Friedewald formula [7]. VLDL cholesterol = TG/5: LDL = TC-(HDL+VLDL). FFA in the serum were estimated by the method of Falholt *et al.* [8]. Assay of hexokinase D (glucokinase) was estimated by the O-toluidine method of Sasaki *et al.* [9]. Glucose 6-phosphate dehydrogenase was estimated by the method of Ells and Kirkman [10]. Assay of glucose 6-phosphatase (glucose 6-phosphate phosphohydrolase) was estimated by Fiske and Subbarow method [11]. Assay of fructose 1, 6-bisphosphatase was estimated according to the

method described by Fiske and Subbarow [11]. Glycogen was estimated by the method of Morales *et al.* [12].

Statistical analysis

The results were expressed as mean ± standard deviation statistical comparisons were made by means of one-way ANOVA and the results were considered as statistically significant when p<0.05.

RESULTS

The hyperglycemic animals showed significant decrease in the glucose level on long-term treatment for the 45-day model at the doses of 100,

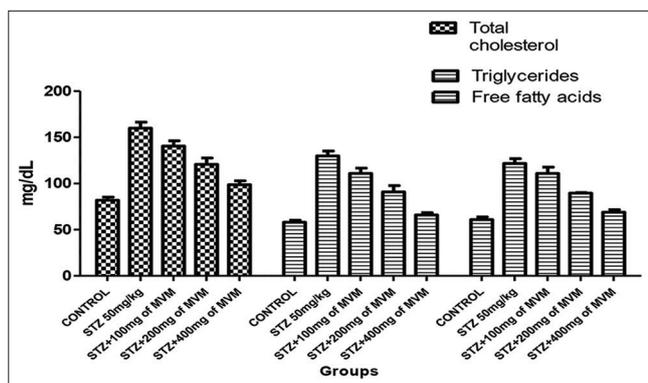


Fig. 7: Effect of methanolic extract of *Ventilago maderaspatana* on lipid level of control and experimental rats

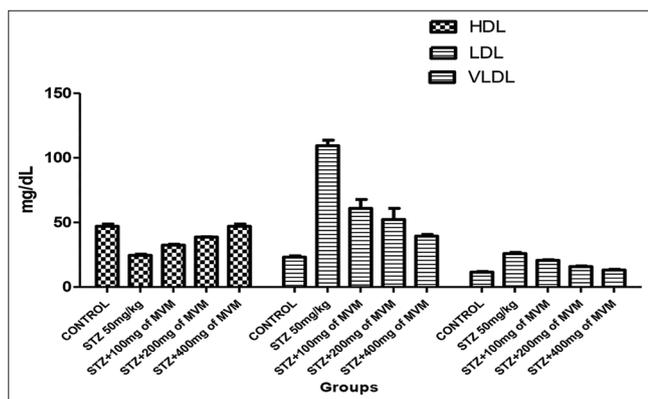


Fig. 8: Effect of methanolic extract of *Ventilago maderaspatana* on lipid level of control and experimental rats

200 and 400 mg/kg of MVM (Table 1). Body weight slightly increased in the normal control rats and extracted treated rats, compared with initial body weight (Table 1). The effect of MVM on glucose levels in STZ - induced diabetic rats is shown in Table 1. MVM (100, 200 and 400 mg/kg) treated rats, the blood glucose levels at three doses steadily decreased and were found to be 230, 209 and 180 mg/100 mL, respectively, and Hb level was significantly increased and HbA1c level was significantly decreased in MVM compared with untreated diabetic rats on the 45th day ($p < 0.05$).

The glucokinase, glucose-6-phosphate dehydrogenase and liver, muscle glycogen was significantly decreased in untreated diabetic rats compared to control group. After the treatment with methanolic extract glucokinase, glucose-6-phosphate dehydrogenase and liver, muscle glycogen was significantly increased in treated diabetic rats compared with untreated diabetic rats. Glucose-6-phosphotase and fructose 1, 6-phosphotase were significantly decreased compared to untreated diabetic groups (Table 2) ($p < 0.05$).

The serum TG, TC, FFA, LDL and VLDL levels were significantly higher in untreated diabetic rats compared to those in normal rats, while the HDL levels were significantly decreased in the diabetic rats compared to those in normal rats. After treatment with MVM in diabetic rats, a significant reduction in serum level of cholesterol, FFA, TG, LDL, VLDL and a significant increase in HDL was observed (Table 3) ($p < 0.05$).

DISCUSSION AND CONCLUSION

Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases [13]. STZ induced diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia [14]. STZ is a pancreatic cell toxin that induces rapid and irreversible necrosis of cells [15]. Methanolic extract of MVM at doses of 100, 200 and 400 mg/kg possesses significant anti-hyperglycemic activity on long-term (45-day) treatment in rats. The MVM showed maximum activity at 400 mg/kg. In the STZ-induced diabetic rats, the rise in blood glucose is accompanied by an increase in the serum cholesterol, TG, LDL, VLDL and the decrease in HDL, whereas the treatment with MVM reduced cholesterol, TG, LDL, VLDL and improved HDL in diabetic rats. On the basis of the current investigation it was noted that the MVM showed antihyperglycemic and antihyperlipidemic and it can be suggested that these results provide pharmacological evidence for its folklore claim as an anti-diabetic agent.

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