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Hypoglycemic and Antidiabetic Activity of *Glochidion velutinum* on Streptozotocin-Nicotinamide Induced Type 2 Diabetic Rats

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Abstract: The present investigation was aimed to study the antidiabetic property of leaves of *Glochidion velutinum* in Streptozotocin-Nicotinamide induced type 2 diabetic rats. Administration of ethanolic extract of *G. velutinum* leaves in the doses of 200 and 400 mg/kg to the STZ-Nicotinamide induced diabetic rats showed significant ($P<0.05$) reduction in blood glucose levels compared to diabetic control rats. Both the doses of EEGV treated diabetic rats showed significant ($P<0.05$) alteration in Lipid profile, SGOT and SGPT levels than the diabetic control rats. Administration of EEGV 400 mg/kg produced significant higher anti diabetic activity than EEGV 200 mg/kg dose. In conclusion ethanolic extract of *Glochidion velutinum* (EEGV) posses anti diabetic activity in type 2 diabetic rats.

Key words: Glochidion Velutinum · Streptozotocin · Nicotinamide · Type 2 Diabetes

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyerglycemia,glycosuria, nagetive nitrogen balance and sometimes ketonaemia resulting from the defects in insulin secreation, insulin action, or both. A chronic hyperglycemia condition in diabetes is associated with long term damage, dysfunction and failure of various organs such as eyes, kidneys, nerves, heart and blood vessels[1].There are two types of diabetes, Type I or Insulin dependent diabetes mellitus is an auto immune genetic disease resulting from an absolute deficiency of insulin due to destruction of pancreatic β-cells. Type 2 or Non insulin dependent diabetes mellitus is a multifactorial disease[2] which is characterized by insulin resistance associated not only with hyperinsulinaemia and hyperglycemia but also with atherosclerosis, Hypertension and abnormal lipid profile,collectively called syndrome X.

The dramatic increase in the prevalence of diabetes can be attributed to several factors. Globally, Diabetes has shadowed the spread of modern lifestyle and can be linked to an increasingly overweight and sedentary population [3]. The chronic metabolic disorder that affects 150 million people in 2000 and 221 million in 2010 and this number is projected to increase to 300 million by 2025 [4]. 90 percent of the present cases are type 2 diabetes and most of the increasing will be in type 2, paralleling the incidence of obesity [5]. The current treatment for control of DM includes diet, exercise, oral anti diabetic drugs and insulin therapy. However, insulin and other oral hypoglycemic drugs have characterstic profile of adverse effects. This has initiated the identification of novel drugs which might act in mechanistically distinct way compared to existing drug targets [6]. Hence, research is focused on medicinal plants which are used in the practices and development of newer drug leads from phytoconstituents with more potential and effective agents with lesser side effects than the existing hypoglycemic agents [1].

Traditionally, many medicinal plants are currently used in India for the treatment of diabetes and its efficacy has been proved scientifically [7].

Glochidion velutinum (Euphorbiaceae) is a small monoecious tree or large shrub upto 9 m with subcomplanate branches and leaves [8]. Several triterpenoids and triterpenoid glycosides and alkaloids are known to be the constituents of plant [9]. The stem bark of the plant is identified as a moderate bactericidal [10]. Traitionally the plant is used in the treatment of cancer, Diabetes, inflammation and for the healing of wounds [8]. Survey of current literature revealed that there is no scientific data documented for the effect of

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Glochidion velutinum leaves in the treatment of type 2 diabetes mellitus. Therefore, the present study was undertaken to investigate the antidiabetic activity of EEGV in type 2 diabetic rats.

MATERIALS AND METHODS

Plant Material: The fresh leaves of *Glochidion velutinum* were collected from the forest regions of Thalakona regions of Chitoor district andhra Pradesh, India. It was authenticated by expert taxonomist Dr.V.S.Raju, Department of Botany Kakatiya University, Warangal. The leaves were air dried under shade and powdered using mechanical grinder and stored in air- tight container.

Preparation of Ethanolic Extract of *Glochidion velutinum* Leaves (EEGV): Dried powdered plant leaves were extracted with ethanol using soxhlet apparatus for 72 hrs. The extract was concentrated under vaccum and stored for further pharmacological studies.

Drugs and Chemicals: Streptozotocin, Nicotinamide, Glibenclamide were procured from Sigma Aldrich Labs, GOD-POD Kits, SGOT, SGPT Kits, Total cholesterol and Triglyceride kits were procured from Excel Diagnostics Ltd, Hyderabad and all were of analytical grade.

Experimental Animals: Wistar albino rats of both sexes weighing 150-200g were used for study (Mahaveer Enterprises, Hyderabad). All animals were maintained under standard laboratory conditions [temperature (22 ± 2°C) and humidity 50 ± 15%] with 12 hours day: 12 hours night cycle. The animals were fed with normal laboratory diet and allowed to drink water *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics committee (2011/10/1/8) and by the regulatory body of government of India.

Acute Toxicity Studies: Acute oral toxicity study was performed as per Organisation for Economic Cooperation and Development (OECD) guidelines 423 [11]. After the oral administration of EEGV, animals were observed individually atleast once during the first 30 minutes and periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for total of 14 days.

Experimental Design for Hypoglycemic Activity and Oral Glucose Tolerance Test: The animals were divided into four groups (n = 6)

Group I: Rats served as normal- control and received 0.2 % Carboxy methyl cellulose (CMC)

Group II: Rats served as standard received Glibenclamide (10 mg/kg)

Group III: Rats were administered ethanolic extract of *G. velutinum* (200 mg/kg b.wt) in 0.2 % CMC as a fine suspension orally.

Group IV: Rats were administered ethanolic extract of *G. velutinum* (400 mg/kg b.wt) in 0.2 % CMC as a fine suspension orally.

All the animals were fasted for 18 h, before experimentation, but allowed free access to water.

Blood samples were collected for the measurement of blood glucose by puncture of retro-orbital puncture of retro-orbital plexus at 0, 1, 2, 4 and 6 h after feeding the plant extract. For oral glucose tolerance test the animals were loaded with glucose (3 g/kg p.o) [12] and the blood samples were collected on 0, 30, 60, 120 minutes time interval. The blood glucose levels were determined by using GOD-POD method [13].

Induction of Type 2 Diabetes: Sterptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. Non-insulin dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of 60 mg/kg streptozotocin, 15 min after the i.p administration of 120 mg/kg of nicotinamide.Hyperglycemia was confirmed by the elevated levels of blood glucose were determined at 72 h. then on day 7 after injection. Only rats confirmed to have permanent NIDDM were used for the antidiabetic study. The animals with blood glucose concentration more then 250mg/dl will be used for the study [14].

Experimental Design for Antihyperglycemic Activity: The rats were divided into five groups of six (n=6) each randomly

Group I: Rats served as normal- control and received 0.2% carboxy methyl cellulose(CMC)

Group II: Diabetic rats received 0.2 % CMC served as diabetic control

Group III: Diabetic rats served as standard received Glibenclamide (10 mg/kg)

Group IV: Rats were administered ethanolic extract of *G. velutinum* (200 mg/kg b.wt) in 0.2 % CMC as a fine suspension orally.

Group V: Rats were administered ethanolic extract of *G. velutinum* (400 mg/kg b.wt) in 0.2 % CMC as a fine suspension orally.

After an overnight fast, the plant extract suspended in 0.2 % CMC was fed by gastric intubations with the syringe. Blood samples were collected by puncture of retro-orbital plexus on at 0, 1, 2, 4, 6 and 8 h and the blood glucose levels were determined by using GOD-POD method [12].

Sub Acute Study: All the test standard substances are administered for 14 days.Blood samples were collected by retro-orbital puncture at 1st, 7th, 14th day at 0, 1, 2, 4, 6 and 8 h and the glucose levels were estimated by GOD-POD kit. On 14th day, plasma lipid profiles and liver enzyme levels were estimated using biochemical kits.

Statistical Analysis: The results are expressed as mean ± SD. Comparision between the groups was made by analysis of variance (ANOVA), followed by Dunnet's test as per suitability.

RESULTS

Acute Toxicity Study: From the acute toxicity studies no toxicity was found to doses of 2 g/kg and 5 g/kg and the doses selected are the low and high dose is 200 mg/kg and 400 mg/kg

Effect on Normal Rats: The effect of different doses of ethanolic extract of *G. velutinum* on fasting blood sugar level was assessed in normal rats at various time intervals (Figure1). It producesed significant ($p<0.01$) maximum reduction in blood glucose level of 16.37 ± 5.95 %, 16.31 ±6.48 % and 24.17 ± 6.00 % of normal rats treated with alcoholic extract of 200 and 400 mg/kg *G. velutinum* and 10 mg/kg of Glibenclamide respectively, after 4 h of treatment.

Effect on Oral Glucose Tolerance Test: Within 30 min of administration of glucose load, there was a progressive increase in postprandial blood glucose level of all the rats which peaked at 60 min, the EEGV treated groups (200 and 400 mg/kg) has shown significant reduction ($p<0.01$) in blood glucose levels (Figure 2).

Effect on Streptozotocin-Nicotinamide Induced Diabetic Rats: The antihyperglycemic effect of the extract on the fasting blood glucose levels on diabetic rats is shown in Figure 3. The alcoholic extract of *G. velutinum* at the dose of 400 mg/kg produced significant ($P<0.001$) maximum fall of 18.57 ± 3.23 on the blood glucose levels of diabetic rats after 6h of the treatment.

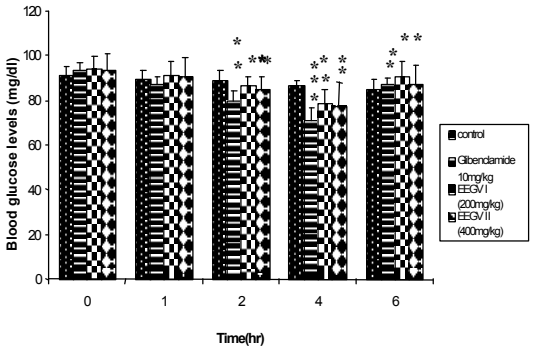

Fig. 1: Hypoglycemic effect of Ethanolic extract of *Glochidion velutinum* Values are mean ± SD, n = 6 in each group, *P<0.05 and ***P<0.001 when compared with vehicle treated group (Dunnet's test), Parenthesis indicate percentage increase in blood glucose levels.

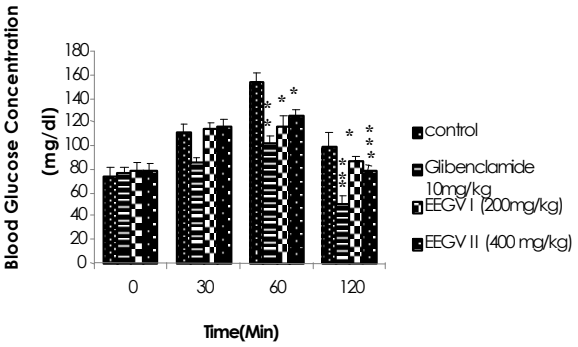

Fig. 2: Oral Glucose Tolerance Test of Ethanolic extract of *Glochidion velutinum* Values are mean ± SD, n = 6 in each group, *P<0.05, **P < 0.01 and ***P<0.001 when compared with vehicle treated group (Dunnet's test)

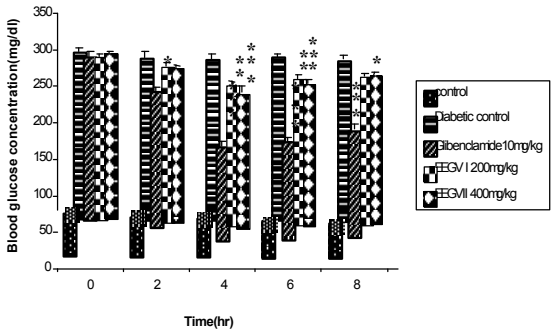

Fig. 3: Anti Hyperglycemic effect of Ethanolic extract of *Glochidion velutinum* Values are mean ± SD, n = 6 in each group,*P<0.05,**P < 0.01 and ***P<0.001 when compared with vehicle treated group (Dunnet's test).

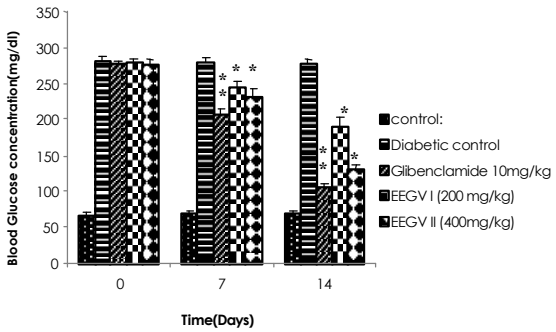

Fig. 4: Anti Diabetic effect of Ethanolic extract of *Glochidion velutinum* Values are mean ± SD, n = 6 in each group, *P<0.05, **P<0.01 when compared with diabetic control group (Dunnet's test)

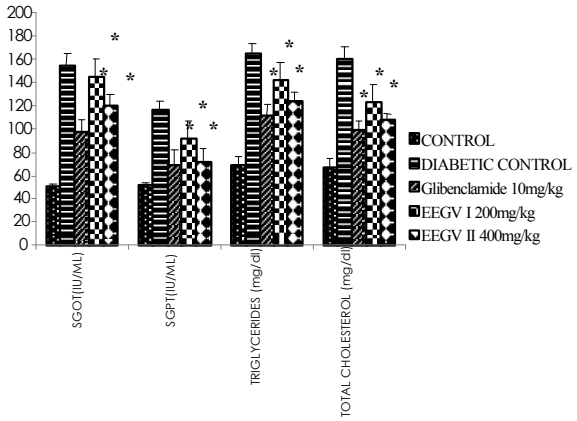

Fig. 5: SGOT, SGPT, triglycerides and total cholesterol levels in Diabetic rats and in normal rats (Mean ± SD) on 14th day(Sub acute study) Values are mean ± SD, n = 6 in each group, *P < 0.05 when compared with diabetic control group (Dunnet's test)

Figure 4 showed the anti diabetic activity of *G. velutinum* in the 14day study of different doses 200 and 400 mg/kg. The alcoholic extract of *G. velutinum* at the dose of 400 mg/kg produced significant (P<0.05) fall in blood glucose levels. The percentage reduction for the doses 200 and 400 mg/kg was found to be 31.90 ± 4.05 and 52.19 ± 2.71.

Effect on Sgot,sgpt and Lipid Profile: Figure 5 showsed the effect of alcoholic extract of *G. velutinum* on SGOT.SGPT and Lipid profile. It shows the significant reduction (p<0.05) in the levels of SGOT, SGPT, Triglycerides and Total cholesterol when compared diabetic control rats.

DISCUSSION

Streptozotocin induces diabetes by free radical generation, which causes a massive reduction of insulin secreting beta cells of the islets of langerhans, resulting in a decrease in endogenous insulin release [15]. The anti-diabetogenic effect of nicotinamide may be due, in part, to an increase in the pool size of NAD^{++} in beta-cells. NAD^{++} is the principal metabolite of nicotinamide. It appears that the pool size of NAD^{++} in beta-cells in pre-diabetics and diabetics is significantly reduced. Damage and destruction of beta-cells may occur via oxidative stress. Increased levels of reactive oxygen species in beta-cells may result in, among other things, oxidative damage to DNA resulting in DNA strand breaks.

The enzyme poly (ADP-ribose)polymerase or PARP is believed to play a role in DNA repair. PARP uses NAD^{++} as its substrate. In the context of a reduced level of NAD^{++}, PARP activity may essentially use most of the cellular NAD^{++}. This could result in cellular apoptosis. Nicotinamide is an inhibitor of PARP. It also has antioxidant activity and, of course, is metabolized to NAD^{++}. All of these effects may play some role in the possible anti-diabetogenic action of nicotinamide. Nicotinamide by the above mechanism opposes the effects of streptozotocin,helps in partial destruction of beta cells and helps in the development of type 2 diabetes [16-18].

The data obtained clearly indicate that the administration of *G. velutinum* extract exhibit antihyperglycemic effect and also has effect on the normal glucose levels, lipid profile, SGOT and SGPT levels.

On the basis of the current investigation it was noted that the ethanolic extract of *G. velutinum* has the antidiabetic activity and these results provide pharmacological evidence for its folklore claim as an antidiabetic agent.

CONCLUSION

From the present study, it is concluded that *Glochidion velutinum* may be useful in treating Diabetes mellitus with no visible signs or symptoms of toxicity in normal rats indicating a high margin of safety. The extracts exhibited anti-hyperglycemic activity comparable to that of a standard drug, glibenclamide. The traditional use of *Glochidion velutinum* to treat diabetes is supported by laboratory results from this study, suggesting a need to isolate and evaluate active constituents responsible for the exhibited biological acivity.

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