



Anti diabetic activity of *Dolichos lablab* (seeds) in Streptozotocin-Nicotinamide induced diabetic rats.

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Abstract

Plan: The present study was designed to investigate the antidiabetic activity of methanolic extract of *Dolichos lablab* seeds.

Preface: In traditional medicine plants are generally used for treatment of various acute and chronic disease and abnormalities in the body. Due to the present fast life of the human a drastic increase in chronic disease conditions mainly diabetes has been determined. The present study therefore justifies its use in the folklore remedies as an antidiabetic drug of natural origin.

Methodology: Antidiabetic activity was evaluated by Streptozotocin-Nicotinamide induced diabetic model. The methanolic extract of the seeds of *Dolichos lablab* was given by Oral route at doses of 200mg/kg and 400mg/kg b.wt.

Outcome: Treated diabetic rats with MEDL dose dependently ($P < 0.001$) reduced blood glucose levels, total cholesterol, triglycerides, SGPT, SGOT levels compared to untreated diabetic rats in STZ induced diabetic model. MEDL 400mg/kg b.wt was found to possess more promising antidiabetic activity compared to 200mg/kg b.wt

Key words: *Dolichos lablab* seeds, Streptozotocin, type 2 diabetes.

1. Introduction:

Diabetes mellitus (DM) is a chronic disease and major endocrine disorder caused by inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is a growing health problem in most countries and its incidence is considered to be high (4%-5%) all over the world¹. Chronic hyperglycemia causes complications linked to diabetes, such as heart disease, retinopathy, kidney disease and neuropathy. It is also a common cause of chronic morbidity and disability among the working population in the world. Several drugs, such as sulfonylureas, metformin and α -glucosidase inhibitors are used presently to reduced the hyperglycemia. The increased blood glucose levels in diabetes produce superoxide anions, which generate hydroxyl radicals via Haber-Weiss reaction, resulting in peroxidation of membrane lipids and protein glycation, leads to oxidative damage to cell membranes.



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These radicals further damage other important biomolecules including carbohydrates, proteins and DNA². Streptozotocin selectively destroy β -cells of pancreas by generating excess ROS and carbonium ion (CH³) leading to DNA breaks by alkylating DNA bases. The N nitroso-N methyl urea portion of the molecule exhibits diabetogenic activity. Glucose may act as carrier for this cytotoxic group³. Plants have been commonly used to treat diabetes since ancient times and have served as a good source of medicine⁴. We have recently reviewed the Indian plants that may have an antidiabetic potential. The treatment of DM in clinical practice has been confined to use of oral hypoglycemic agents and insulin, the former being reported to be endowed with characteristic profiles of serious side effects⁵. *Dolichos lablab* (Leguminosae) is a traditional medicinal plant recognized to be effective in the treatment of DM⁶.

Lablab purpurea is a climbing or erect perennial herbaceous crop often grown as an annual. It grows upto 40 inches tall with long stems in climbing types extending as much as 25ft(6m) from the base of the plant. The leaves are trifoliolate and the flowers are white in colour.⁷ The fruit is a flat, broad pod, with wavy margins 4-5 inches long. When immature, the pods and their nutritious seeds can be eaten. *Dolichos lablab* seed contain carbohydrates, sterols, alkaloid like trigonelline (which exhibits hypoglycemic activity), flavonoids like genestein, saponins, glycosides and proteins^{8,9}.

The whole plant, roots, leaves, stem, flowers are extensively used in traditional system of medicine for various ailments like anticholesterolemic, antidote, carminative, hypoglycemic. The stem is used in the treatment of cholera, vomiting, diarrhea. The juice from the pods is used to treat inflamed ears and throats. The seeds are used as an anthelmintic, antispasmodic, astringent, digestive, febrifuge and stomachic¹⁰. Survey of current literature revealed that there is no scientific data documented for the effect of *Dolichos lablab* seeds in the treatment of type 2 diabetes mellitus. Therefore, the present study was undertaken to investigate the antidiabetic activity of MEDL in diabetic rats¹¹.

2. Materials and Methods

2.1. Plant material: Dried seeds of *Dolichos lablab* were collected from local market and authenticated by an taxonomist, retired professor Dr.V.S.Raju, Department of Botany, Kakatiya University, Warangal. Dried seeds were powdered using mechanical grinder and stored in air tight container.

2.2. Preparation of methanolic extract of *Dolichos lablab* seeds (MEDL): Dried powdered seeds were extracted with methanol using soxhlet apparatus for 20-24 hrs. The extract was concentrated under vacuum and stored for further pharmacological studies.

2.3. Preliminary phytochemical screening:

Freshly prepared crude extracts were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts was performed using the following reagents and chemicals:

Alkaloids with Dragondroff's reagent, flavonoids with the use of Mg and HCl; Tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce stable foam and steroids with Libermann-Buchard reagent. These were identified by characteristic colour changes using standard procedure¹².

2.4. Drugs and chemicals:

Streptozotocin, Nicotinamide, Glibenclamide were procured from Sigma Aldrich Labs, GOD-POD Kits were procured from Renckon diagnostics, Total cholesterol and Triglyceride kits were procured from Excel diagnostics Pvt Ltd, Hyderabad.

2.5. Experimental Animals:

Wistar rats of both sexes weighing 150-200gm were used for study (Mahaveer Enterprises, Hyderabad). All animals were maintained under standard laboratory conditions (temperature 22±2⁰C 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*. All protocols were performed in accordance with the Institutional Animal Ethical Committee (IAEC) as per the directions of the CPCSEA (Committee for the purpose of Control and Supervision of Experiments on Animals).

2.6. Acute toxicity studies:

Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines 423¹³. Administration of stepwise dose of extract MEDL (50 mg/kg-2000mg/kg b.wt), animals were observed individually at least 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. The dose 2000mg/kg was found to be safe and no toxicity was observed. One-fifth and one-tenth of upper limit dose were selected as the label for examination of antidiabetic activity.

2.7. 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-received 0.5% Carboxy methyl cellulose (CMC).

Group-II: Rats served as Standard-received Glibenclamide (10 mg/kg b.wt).

Group-III: Rats were administered methanolic extract of Dolichos lablab (200mg/kg b.wt) in 0.5% CMC as a fine suspension orally.

Group-IV: Rats were administered methanolic extract of Dolichos lablab (400mg/kg b.wt) in 0.5% CMC as a fine suspension orally.

All animals were fasted for 18 hr, before experimentation, but allowed free access to water. Blood samples were collected for the measurement of blood glucose level by puncture of retro-orbital plexus at 0hr, 2hr, 4hr and 6hr from control and test group animals after feeding the plant extract.

For oral glucose tolerance test the animals were loaded with glucose (2gm/kg) and the blood samples were collected on 0, 30, 60,120 minutes time interval. The blood glucose levels were determined by using GOD-POD method¹³.The anti diabetic activity was conducted as per IAEC approval number IAEC/CPCSEA Approval number- 2012/11/3110.

2.8. Induction of diabetes.

Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. NIDDM was induced in overnight fasted Wistar strain albino 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 glucose levels in plasma, determined at 72 h after administration. Rats with fasting blood sugar levels around 160 to 300 mg/dl were selected for the study¹⁴.

2.8.1. Experimental design for Antihyperglycemic Activity:

The animals were divided into five groups (n=6)

- Group-I: Rats served as normal-control and received 0.5% Carboxy methyl cellulose(CMC).
- Group-II: Diabetic rats served as diabetic-control and received 0.5% Carboxy methyl cellulose (CMC)
- Group-III: Diabetic rats served as Standard-received Glibenclamide (10 mg/kg b.wt).
- Group-IV: Diabetic rats were administered methanolic extract of *Dolichos lablab* (200mg/kg b.wt) in 0.5% CMC as a fine suspension orally.
- Group-V: Diabetic rats were administered methanolic extract of *Dolichos lablab* (400mg/kg b.wt) in 0.5% CMC as a fine suspension orally.

Blood samples were collected for the measurement of blood glucose level by puncture of retro-orbital plexis at 0hr, 2hr, 4hr and 6hr from control and test group animals after feeding the plant extract¹³.

2.9. Sub Acute Study:

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

3. Statistical Analysis:

The results are expressed as mean \pm SD. Comparison between the groups was made by analysis of variance (ANOVA), followed by Dunnet's test as per suitability.

4. Results

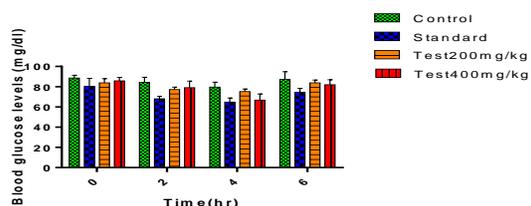
Phytochemical screening

Phytochemical analyses of the crude extract revealed the presence of flavonoids, steroids, alkaloids, glycosides and saponins.

Acute toxicity study:

From the acute studies no toxicity was found to dose of 2000mg/kg hence, 1/10th (200mg/kg b.wt) and 1/5th (400mg/kg b.wt) of this dose was selected for further study.

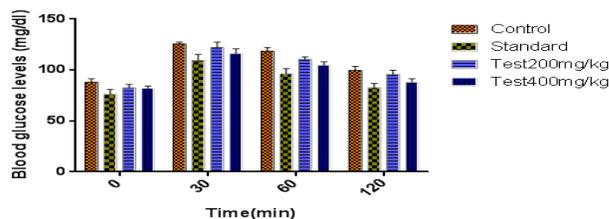
Figure .1: Hypoglycemic effect of methanolic extract of *Dolichos lablab* in normal rats.



Data represents mean \pm S.D.(n= 6). * p <0.05. ** p <0.01 significant compared to control, analysed by one-way ANOVA followed by Dunnett's test. Parenthesis indicates % reduction in blood glucose levels.

Effect on Normal Rats: The effect of different doses of methanolic extract of *Dolichos lablab* on fasting blood sugar level was assessed in normal rats at various time intervals (Figure.1). It produced significant (P <0.01) maximum reduction in blood glucose level of $9.92 \pm 6.54\%$ and $22.17 \pm 6.99\%$ at dose of 200mg/kg and 400mg/kg and effect was dose dependent.

Figure.2: Effect of Methanolic extract of *Dolichos lablab* on glucose tolerance test in normal rats.



Data represents mean \pm S.D.(n= 6). * p <0.05. ** p <0.01 significant compared to control, analysed by one-way ANOVA followed by Dunnett's test.

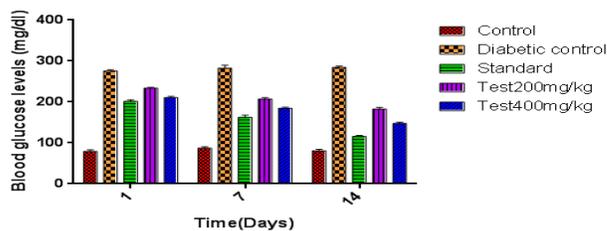
Effect on Oral glucose Tolerance Test:

Administration of glucose load, there was a progressively increase in postprandial blood glucose level of all the rats which peaked at 60 min, the MEDL treated groups (200 and 400mg/kg) has shown significant reduction ($P<0.01$) in blood glucose levels (Figure.2).

Effect on Streptozotocin-Nicotinamide Induced Diabetic Rats:

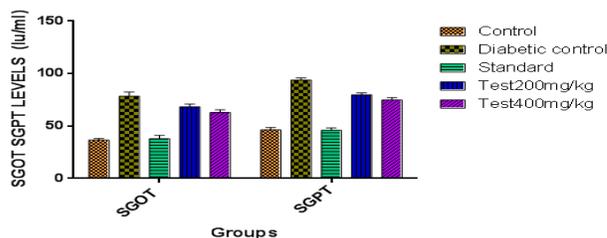
Figure.3 showed the antidiabetic activity of *D.lablab* in the 14th day study of different doses 200 and 400 mg/kg. Methanolic extract of *D.lablab* at the dose of 400mg/kg produced significant ($P<0.001$) fall in blood glucose levels than 200mg/kg. The percentage reduction for the doses 200 and 400mg/kg was found to be $10.16\pm 1.96\%$ and $25.11\pm 1.18\%$.

Figure.3: Anti diabetic effect of methanolic extract of *Dolichos lablab* in diabetic rats.



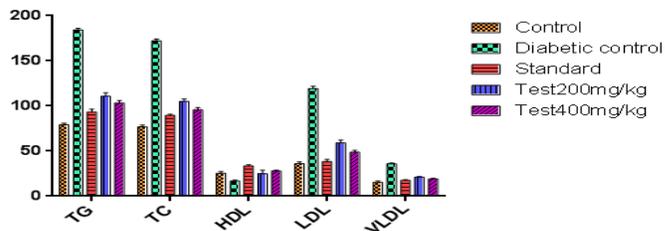
Data represents mean \pm S.D.(n= 6). * $p<0.05$, ** $p<0.01$, *** $P<0.001$ significant compared to diabetic control, analysed by one-way ANOVA followed by Dunnett's test. Parenthesis indicates % reduction in blood glucose levels

Figure.4: Effect of methanolic extract of *Dolichos lablab* on liver enzyme (SGOT,SGPT)levels in diabetic rats.



Data represents mean \pm S.D.(n= 6). * $p<0.05$, ** $p<0.01$, *** $P<0.001$ significant compared to diabetic control, analysed by one-way ANOVA followed by Dunnett's test. Parenthesis indicates % reduction in blood glucose levels.

Figure.5: Effect of methanolic extract of *Dolichos lablab* on lipid profiles in diabetic rats.



Data represents mean \pm S.D.(n= 6). * $p < 0.05$, ** $p < 0.01$, significant compared to diabetic control, analysed by one-way ANOVA followed by Dunnett's test.

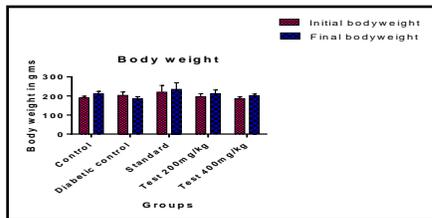
Effect on SGPT, SGOT and Lipid profile:

Figure.4, 5 showed the effect of treated diabetic rats with methanolic extract of *D.lablab* significant reduction ($p < 0.05$) in the levels of SGOT, SGPT, Triglycerides and Total cholesterol when compared to untreated diabetic control rats.

Body weight:

Administration of MEDL to diabetic rats resulted in an increased in the body weight compared to untreated diabetic rats. Results suggested that *Dolichos lablab* treatment has positive effect on maintaining body weight.

Figure.6: Effect of methanolic extract of *Dolichos lablab* on body weight in diabetic rats.



Data represents mean \pm S.D.(n= 6). * $p < 0.05$, ** $p < 0.01$, significant compared to diabetic control, analysed by one-way ANOVA followed by Dunnett's test.

5. Discussion

Phytochemical analysis of the crude extract revealed the presence of flavonoids, steroids, alkaloids, glycosides and saponins. In acute toxicity study, methanolic extract of *Dolichos lablab* seeds did not show significant toxicity signs when observed for the parameters during the first four hours and followed by daily observations for 14 days and no mortality was also observed, the drug was found to be safe at the tested dose level of 2000mg/kg b.wt. Methanolic extract of *Dlochos lablab* reduced elevated blood glucose levels in Streptozotocin-Nicotinamide induced diabetic rats.

Administration of methanolic extract of *Dolichos lablab* seeds to diabetic rats(Grps-IV,V) significantly recovered the levels of blood glucose, serum lipid profiles and liver enzymes, resulted in an increased in body weight compared to untreated diabetic rats(Grp-II)

6. Conclusion

The extracts exhibited significant antihyperglycemic activity in streptozotocin-induced diabetic rats. Further studies are necessary to elucidate in detail the mechanism based investigations has to be carried out. Methanolic extract also showed improvement in parameters like body weight, lipid profile and other biochemical parameters.

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