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Evaluation of Antidiabetic Activity of *Tecoma stans* in Streptozotocin – Nicotinamide Induced Diabetic Wistar Rats

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Abstract

Tecoma stans is a flowering perennial shrub or small tree, 5-7.6 m in height. The leaves bark, and roots contain many biologically active chemicals, and extracts from those tissues have been used in traditional folk medicine to treat many diseases and conditions. The aim of the present study is to evaluate its antidiabetic potential in animals. The dried stem bark of the plant was extracted using ethanol and acute a toxicity study of ethanolic extracts was investigated which did not show any toxic symptoms in doses up to 2000 mg/kg over 14 days. The oral antidiabetic activity of the ethanolic extract (250 and 500 mg/kg) was screened against streptozotocin (50 mg/kg; i.p.) + nicotinamide (120 mg/kg; i.p.) induced diabetes mellitus in rats. The investigational drug was administered for 21 consecutive days, and the effect of the extract on blood glucose levels was studied at regular intervals. At the end of the study, the blood samples were collected from all the animals for biochemical estimation, and the animals were sacrificed and the liver and pancreatic tissues were collected for histopathologic analysis. Ethanolic extract showed significant antidiabetic activity at 250 and 500 mg/kg, respectively, and this effect was comparable with that of glibenclamide.

Keywords: *Tecoma stans*, Bark, Ethanolic Extract

Introduction

Humanity can benefit much from plants. A large number of them are only utilised for medical purposes. "A medicinal plant is a plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis," states the World Health Organisation (WHO). Pharmaceutical corporations are highly interested in these plants because of their active components. Diabetes mellitus is a prevalent ailment that impacts nearly 6% of the global population. In low- and middle-income nations, the disease's dynamics are fast evolving. [1]

The International Diabetes Federation (IDF) projects that by 2030, low- and middle-income nations will account for 80% of the world's diabetes population. The number of diabetics in China, India, and the United States is 90.0, 61.3, and 23.7 million, respectively, according to the IDF 2011 study. By 2030, that number might rise to 129.7, 101.2, and 29.3 million.One of the six leading causes of death worldwide, diabetes also has a number of systemic side effects. Treatment options for diabetes mellitus include giving glucose-lowering medications including biguanides, thiazolidinediones, sulfonylureas, and alpha-glucosidase inhibitors, or using hormone therapy (insulin). The occurrence of adverse events is a challenge in the treatment of any systemic condition. For this reason, numerous research institutes and pharmaceutical corporations are engaged in the drug development process to identify molecules with low adverse events and strong therapeutic potential. [2]

Tecoma stans used traditionally for the treatment of several diseases including diabetes [3], hence the for work was undertaken to investigate the anti-diabetic activity of the plants.

Material and Methods

Collection of the plant

Taxonomically identified stem bark of *T. stands* were collected from the local area of the Bhopal, MP. The collected plants were authenticated by Dr. S.N. Dwiedi, Retd. Professor & Head & Visiting Professor, APS University, Rewa, MP. The voucher specimen of the plant was deposited inthe Department for further reference.

Animals

Adult Wistar rats (180 ± 10 g) of either sex were obtained and animals were housed in large, spacious polyacrylic cages at an ambient room temperature with 12-h light/12-h dark cycle. Rats had free access to water and rodent pellets diet (Hindustan Lever Ltd, Bangalore, India). The study was approved by the Institute Animal Ethics Committee of the College.

Preparation of ethanolic extract of *T. stans*

The shadow-, air-dried stem bark of *T. stans* were powdered and extracted with ethanol using Soxhlet apparatus for 6 h. The extracts were evaporated to dryness (resinous material) under reduced pressure at 60°C and stored at 4°C until use. [4]

Acute oral toxicity

Acute oral toxicity of the ethanolic extract of T. stans stem bark (EETSSB) was carried out as per the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423. Theprinciple involves a stepwise procedure with the use of a minimum number of animals per step to obtain sufficient information on the acute toxicity of the test substance to enable its classification. Healthy Wistar rats (3 animals/dose) of either sex were used for the experiment. Overnight fasted rats were orally fed with the plant extracts and polyherbal formulation in increasing dose levels of 5, 50, 300, and 2000 mg/kg body weight, respectively. The animals were observed for their behavioral (alertness, restlessness, irritability, and fearfulness), neurological (spontaneous activity, reactivity, touch response, pain response, and gait), and autonomic (defecation and urination) profiles continuously for 24 h. After a period of 24 h, the animals were observed for 14 days for mortality. [5]

Antidiabetic effect of Extract in streptozotocin- and nicotimanide-induced diabeticrats [6-8]

The male Wistar rats were divided into five different groups of six animals each as follows.

Group I: Normal control; Group II: Diabetic control; Group III: Diabetic rats treated with EETSSB (250 mg/kg) ; Group IV: Diabetic rats treated with EETSSB (500 mg/kg); Group V: Diabetic rats treated with glibenclamide (0.25 mg/kg).

Diabetes was induced in overnight-fasted rats by administering single intraperitoneal (i.p.) injection of freshly prepared streptozotocin (STZ) 50 mg/kg b.w. followed by 120 mg/kg of nicotimanide (NIC) in 0.1 M citrate buffer (pH 4.5) in a volume of 0.5 ml/kg b.wt. Diabetes was confirmed in the STZ + NIC treated rats by measuring fasting blood glucose levels after 48 h of induction. After 24 h of STZ +NIC injection, the rats were given 5% w/v of glucose solution (2 ml/kg b.w.) to prevent hypoglycemic mortality. Rats with fasting blood glucose of more than 200 mg/dl were considered as diabetics and they were divided randomly into four different groups. The standard (glibenclamide) and herbal formulation were suspended in 1% w/v carboxymethyl cellulose (CMC) and administered once daily through oral gavage for 21 consecutive days. The blood samples were collected on 1^{st}, 7^{th}, 14^{th}, and 21^{st} days of the treatment, through the tail vein of rats by pricking and were immediately used for the estimation of blood glucose with a glucometer. Weekly body weight variations were monitored for all the experimental animals.

At the end of the experiment, the blood sample was withdrawn from all the experimental animals through retro-orbital plexus puncture/posterior vena cava in plain and sodium ethylenediaminetetraacetic acid (EDTA) tubes for biochemical analysis. Finally the animals were sacrificed by diethyl ether anesthesia, and liver and pancreatic tissues were excised and used for biochemical and pathological analysis. Part of the tissue sample was preserved in an ice-cold container for biochemical analysis and the remaining was stored in 10% formalin solution for histopathologic analysis.

Statistical analysis

All the data were expressed as mean ± SEM. Statistical significance between the groups were tested using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test post-hoc test. A P less than 0.5 were considered significant.

Results and Discussion

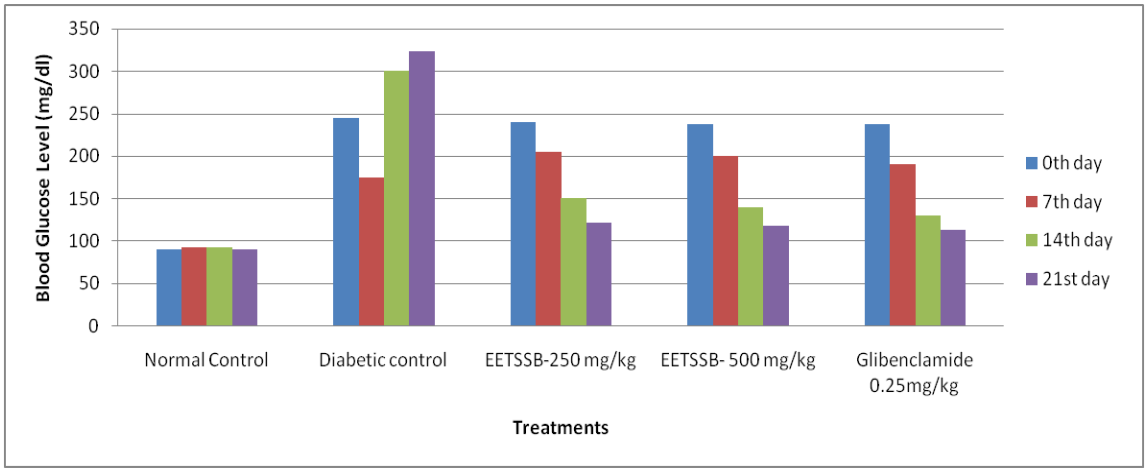
Acute toxicity studies did not show any mortality up to 2000 mg/kg given as single oral administration.Hence, the study was carried out at the dose levels of 250 and 500 mg/kg.

Throughout the study, the diabetic animals showed significant reduction in body weight when compared to the control animals. However, the EETSSB and glibenclamide inhibited the diabetes-induced body weight reduction. Diabetic control animals showed severe hyperglycemia compared to normal animals. It was observed that the standard drug glibenclamide lowered the blood glucose level significantly, bringing it back to near normal level, whereas the EETSSB at 250 mg/kg and 500 mg/kg significantly ($P < 0.001$) decreased the fasting blood serum glucose level in the diabetic rats on 7^{th}, 14^{th}, and 21^{st} days, as compared to the diabetic control group. The results are presented in Table 4.

Table 1: Effect of fasting blood glucose level (mg/dl) in STZ-and NIC induced diabetes rats

Treatments	Blood glucose level (mg/dl)			
	0th day	**7th day**	**14th day**	**21st day**
Normal Control	90.1±0.10	93.2±0.02	93.5±0.65	91.10±0.70
Diabetic control	245.32±1.10	175.29±1.21	300.28±1.11	323.11±1.21
EETSSB-250 mg/kg	240.72±0.16***	205.10±0.32***	150.38±0.01***	122.11±0.11***
EETSSB- 500 mg/kg	238.10±0.19***	200.88±0.16***	140.34±0.11***	118.45±0.12***
Glibenclamide 0.25mg/kg	237.39±0.11***	190.39±0.26***	130.29±0.09***	113.68±0.33***

Note: All reading are Mean±SEM, n=6; statistically significant ***$P < 0.001$



Graph 1: Effect of fasting blood glucose level (mg/dl) in STZ-and NIC induced diabetes rats

The histopathologic analysis of pancreas revealed severe congestion, huge decrease in the number of islets of Langerhans and β cells, and fibrosis and inflammatory cell infiltration into the islets of Langerhans in STZ- and NIC-induced hyperglycemic rats. While the ethanolic extract at the dose of 250 mg/kg and 500 mg/kg showed mild congestion and mild decrease in the number of islets of Langerhans with normal β cell population, indicating significant amount of recovery. Glibenclamide treatment showed moderate congestion with moderate decrease in the number of islets of Langerhans and β cells and mild lymphocytic infiltration.

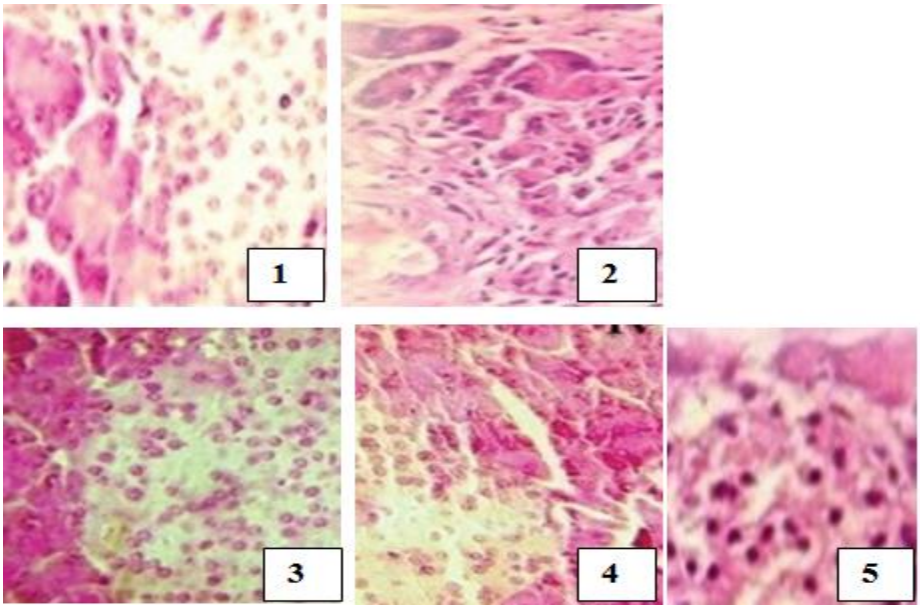


Fig. 1: Histopathology of pancreas in rats

[1=NC; 2=DC; 3=EETSST-250; 4=EETSST-500; 5=Glibenclamide]

Conclusion

Thus, our study findings demonstrate the antidiabetic effect of the EETSSB at the dose levels of 250 and 500 mg/kg. The antidiabetic potential of the polyherbal formulation is comparable with that of glibenclamide.

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