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Evaluation of Antidiabetic Activity of *Embelia robusta* Seed Extract in Alloxan Induced Diabetic Wistar Rats.

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Abstract:

Objective:
To study the anti-diabetic activity of methanolic seed extract of *Embelia robusta* on Alloxan (100-130mg/kg body wt) induced diabetic rats.

Method:
The 30 adult wistar rats were randomly divided into 5-groups, 6 rats in each group. Group-I: normal healthy control, Group-II: diabetic control, Group-III: standard (treated with Gliclazide 50mg/kg body wt),Group-IV test group treated with methanolic seed extract of *E.robusta* at the doses of 50 mg/ kg, Group-V test group treated with methanolic seed extract of *E.robusta* at the doses 100mg/ kg of body wt orally for 28days.

Result:
The results were analysed by ANOVA. Reduction of blood glucose levels in Gliclazide (61.97%) in group-III were comparable to that of group-IV (41.94%) group-V (53.84%). P-value significantly <0.05.The histopathological study has shown regeneration of β-cells of pancreas in all treated groups.

Conclusion:
Antidiabetic activity of methanolic seed extract of *E.robusta* increased dose of 100mg/kg body wt has revealed more anti-diabetic effect.The effect is comparable to the standard drug.

Keywords: *Embelia robusta*, Fasting blood glucose (FBS), Histopathology, Gliclazide

INTRODUCTION:

Diabetes mellitus is the major metabolic disorder and is considered as one of the five leading causes of death in the world (1). Currently there are 150 million diabetic patients worldwide and estimated that this number will rise to around 57 million people by the year 2025(2).

Diabetes is a clinical syndrome characterized by hyperglycaemia due to absolute or relative deficiency of insulin. This can arise in many different ways, but is most commonly due to autoimmune type 1 diabetes or to adult onset type-2 diabetes. Lack of insulin affects the metabolism of carbohydrates, protein and fat, and can cause a significant disturbance of water and electrolyte homeostasis (3). The long standing diabetic condition affects the other organs like eye, skin, kidney, nervous system and macro, micro vascular complications (4).

Medicinal plants have richest sources of bioactive compounds which can be extracted and screened in the diabetic research. There are numerous traditional medicinal plants reported to have hypoglycaemic properties, such as *Gymnema sylvestre* (5), *Embelica officianalis* (6) *Momordica charantia*,*Carica papaya*,*Trigonella foenum, Curcuma longa* (7).

The present study has been taken up to evaluate the antidiabetic properties of *E.robusta*.It is an important species under the family of Myrsinaceae, commonly known as vidanga and vayavidang.

The plant is a climber with slender branches and long internodes. The leaves are elliptic broad and covered with minute glands. The fruits are berries and contain the benzoquinone compound, embelin [2,5-dihydroxy-3-undecyl,2-cyclohexadiene-1,4-benzo-quinone] as a major bioactive constituent.

Seed extract shows various biological activities such as anticancer, antifungal, antibacterial (8), free radical scavenging (9).Traditionally seeds are employed as a remedy for toothache, headache, snakebite. The plant extract is used to maintain healthy skin and to support the digestive function, treatment for the fever and to cure skin diseases.

The aim of the present study is to investigate the effects of *E.robusta* seed extract on blood glucose level and histopathological changes in alloxan induced diabetic rats.

MATERIALS AND METHODS:

E.robusta seeds were collected from local market, identified and authenticated by department of Botany, Osmania college for women Koti, Hyderabad.

Preparation of *E.robusta* seed extract:

E.robusta seeds of 100gm were purchased from local market and shade dried. The seeds were coarsely powdered and subjected to Soxhlet extraction with methanol for 72hr at 60°c. The solvent was removed under rotary evaporator and extract was isolated. Different concentrations of seed extracts 50 and 100 mg/kg body wt was made by dissolving in 1% Tween 80 in the normal saline.

Experimental Animals:

The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC), 0(Registration no. 428/01C/CPCSEA, 7th February 2015), Gandhi Medical College, Secunderabad.

Adult wistar rats (150-200g) were obtained from NIN, Hyderabad. All the laboratory studies were conducted at Central animal house, Gandhi Medical College, Secunderabad. The animals were maintained under standard laboratory conditions such as temperature (26+2°c), relative humidity (45-55%) and 12h light/dark cycle. Animals were fed with rodent pellet diet and water adlibitum.

Experimental Induction of diabetes in Rats:

Rats were acclimatized for a period of 7 days. After 18hours of fasting, the rats were injected intraperitonially with alloxan monohydrate, dissolved in sterile normal saline at a dose of 100-130mg/kg body wt. After induction animals were given 5% glucose solution to counter hypoglycaemic shock. Animals showing more than 200mg/dl blood glucose were considered as diabetic and selected for the experimentation.

Experimental Design:

a. Group- I : Normal healthy control (N=6);
b. Group- II: Diabetic Control (N=24); randomly divided into 4 groups of 6 rats each (diabetes induced by Alloxan monohydrate of 130 mg/kg body wt)
c. Group- III : Standard; (Gliclazide 50mg/kg body wt) drug was given daily using an intragastric tube for 28 days
d. Group -IV : Methanolic *E. robusta* seed extract (50 mg/kg body wt) was given daily using an intragastric tube for 28 days.
e. Group - V : Methanolic *E. robusta* seed extract (100 mg/kg body wt) was given daily using an intragastric tube for 28 days.

Collection of blood sample:

Fasting blood samples were collected from retro-orbital plexus using micro-capillary technique under mild ether anesthesia. Serum was separated by centrifugation at 2000 rpm for 20 minutes, blood glucose levels were measured on 0,7th,14th, 21st, 28th day and blood glucose was estimated by GOD-POD enzymatic method(10).

Histopathological studies:

The Pancreatic tissues samples were subjected to histopathological studies as described (11).The tissues were fixed using 10% formalin routinely processed and embedded in paraffin wax. Paraffin section were taken (5µm thick) and stained with hematoxylin and eosin (H & E). After dewaxing all the slides were examined under a light microscope for pathological studies.

Statistical analysis :

Statistical analysis was carried out using Graphpad Prism (5.0). The collected values of fasting blood glucose were expressed as mean ± standard deviation and the data analyzed using ANOVA followed by post-hoc tukey's multiple comparison test method, results were considered statistically significant at $P<0.05$.

RESULTS:

Blood glucose:

The methanolic seed extract of *E.robusta* dissolved in 1% tween 80 with normal saline was administrated orally to diabetic rats at dose of 50 and 100mg/kg body wt to evaluate the Antidiabetic effect of seed extract. Significant ($P<0.05$) reduction in glucose was observed in diabetic rats treated with two different concentrations of extracts. Both the doses reduced the blood glucose level on 7th,14th, 21st, 28th day, when compared with standard control . The percentage of glucose reduction was observed at dose 50mg/kg body wt was (41.94%) and 100mg/kg body wt (53.84%) on 28th day.

Histopathology:

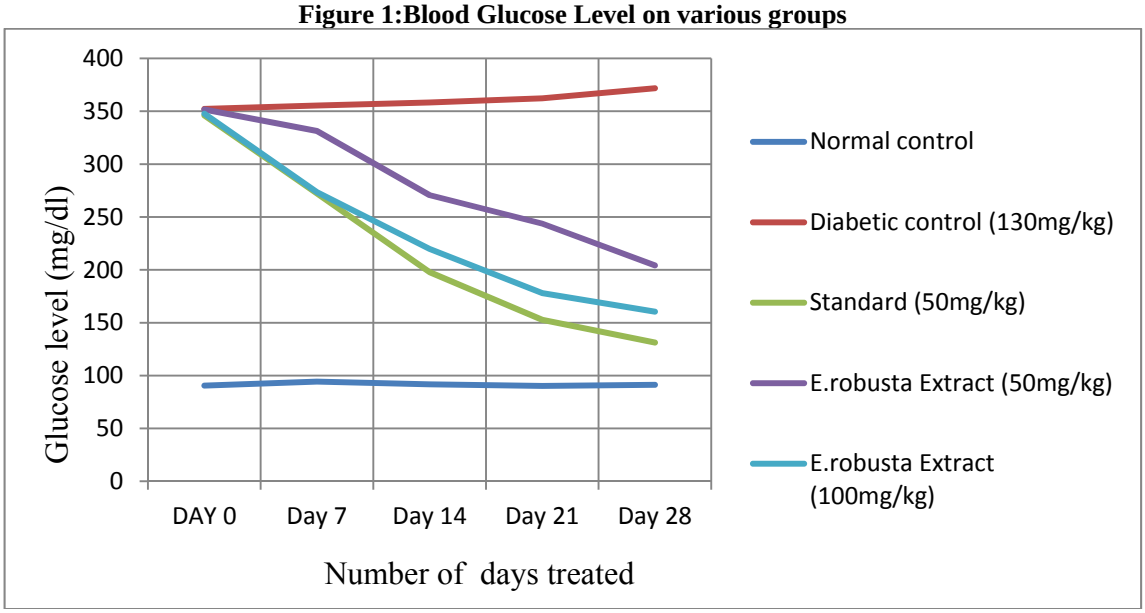
Histopathological studies (figure-II) were carried out, in which normal healthy control group rats showed normal acini (Group-I). Diabetic control group rats showed extensive damage of the islet of langerhans of pancreas(Group-II). Gliclazide (50mg/kg body wt) treated diabetic rats showed regeneration of β-cells (Group-III). *E.robusta* (50mg/kg body wt) treated diabetic rats showed restoration of pancreatic ß-cell (Group- IV). *E.robusta* (100mg/kg body wt) treated diabetic rats showed abundant regeneration of pancreatic ß-cell also enlarged size of ß cells (Group-V) .

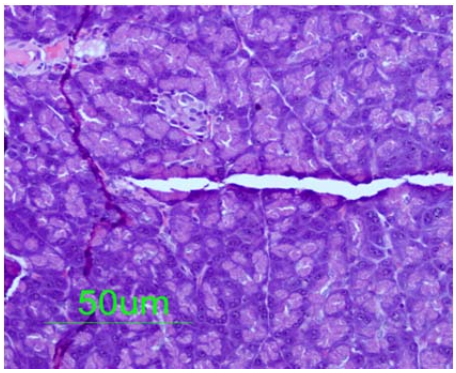
Table 1: Effect of Methanolic seed extract of *E.robusta* on blood glucose (mg/dl) level in Alloxan induced diabetic rats. [Values are mean ± SD from 6 animals in each group]

Blood Glucose (mg/dl)					
Groups	**Day0** (Mean±SD)	**Day7** (Mean±SD)	**Day 14** (Mean±SD)	**Day 21** (Mean±/SD)	**Day 28** (Mean±/SD)
Normal control	90.50±5.577	94.33±7.815	91.67±6.212	90.17±3.656	91.33±6.314
Diabetic control	352.3±6.121	355.5±6.058	358.3±4.412	362.2±5.811	371.7±4.033
Standard (Gliclazide) (50mg/kg)	346.3±6.947	272.3±7.005	197.8±5.981	152.8±7.468	131.7±3.559
E.robusta Extract (50mg/kg)	351.7±5.317	331.3±7.118	270.7±7.118	243.7±3.559	204.2±4.021
E.robusta Extract (100mg/kg)	347.7±6.186	273.5±7.662	219.8±6.014	178.0±6.066	160.5±6.025

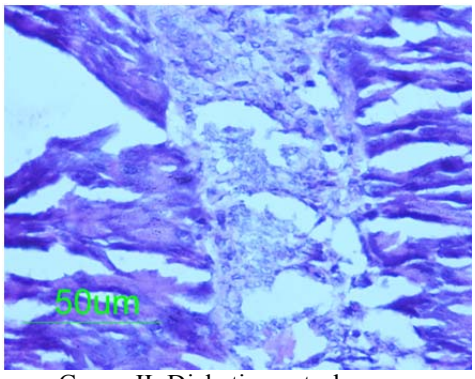
P values <0.05; when compared with normal control group, diabetic control group.

Figure 1:Blood Glucose Level on various groups

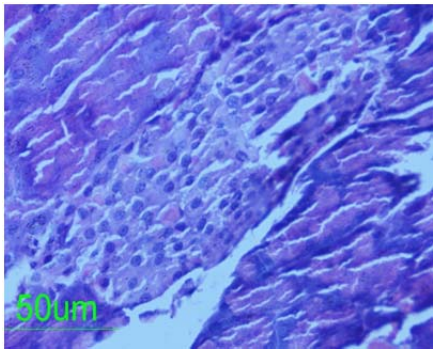




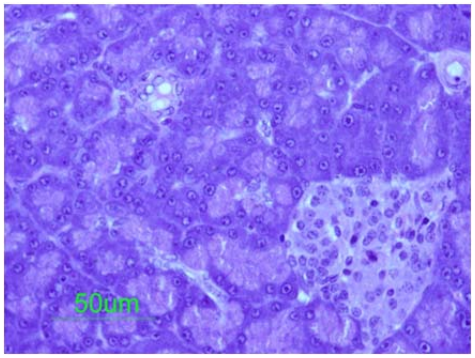
Group-I: Normal healthy control group;



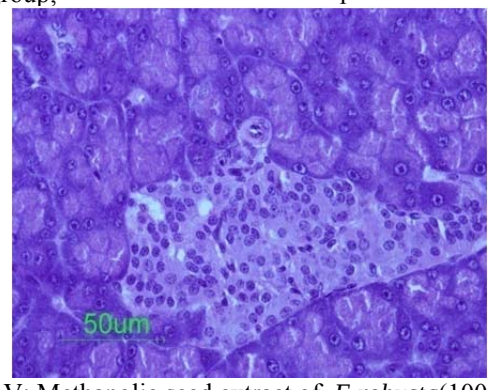
Group-II: Diabetic control group;



Group-III : Standard group;



Group-IV:Methanolic seed extract *E.robusta*(50mg/kg);



Group-V: Methanolic seed extract of *E.robusta*(100mg/kg).

Figure 2: Histological examination of Pancreas in experimental animals

DISCUSSION:

Antidiabetic activity of seed extract of *E. robusta* was screened using blood glucose level and histopathological studies.

The seed extract exhibited significant activity at the dose of 50mg/kg body wt (41.94%) and at the dose of 100mg/kg body wt (53.84%). In correlation with the present study earlier *E.ribes* seed extract was found to have antidiabetic activity at the doses of 100mg/kg and 200mg/kg body wt (12). Similarly antidiabetic activity of plant extract has been reported in many plants like *Berberis aristata* (13), *Pterocarpus marsupium* (14), *Allium sativum* (15).

The histopathological studies (figure-II) has shown regeneration of ß-cells by gliclazide (Group-III) in alloxan induced diabetic rats. A comparable ß-cell regeneration was also observed in methanolic seed extract of *E.robusta* at the doses of 50mg/kg body wt and 100mg/kg body wt (Group-IV & Group-V) after 28 days of treatment. Similar studies of regeneration of pancreatic ß-cells was observed by *Gymnema sylvestre* (16), *curcumin longa* (17), *Catharanthus roseus* (18).

CONCLUSIONS:

In the present study methanolic seed extract of *E.robusta* at dose (100mg/kg body wt) Group-V exhibited significant antihyperglycemic activity than at dose (50mg/kg body wt) Group-IV in alloxan induced diabetic rats.

E.robusta seed extract at dose (100mg/kg body wt) Group-IV has shown significant glucose lowering effect and the Percentage of reduction (53.84%), where as the percentage of reduction in glucose levels foe standard gliclazide was (61.97%). The percentage of reduction for Group-IV was almost comparable to standard. At the end of the study the Histopathological examination of pancreatic tissues shows ß-cell regeneration which could be the reason for its reduction of glucose levels.

These studies clearly showed that *E.robusta* seed extract has an excellent antidiabetic potential and can be used for the development of herbal formulations for control of diabetes mellitus and its clinical complication. Further studies on toxicity, lipid lowering properties and identification of bioactive principal of *E.robusta* seed extract will be explored in future work.

AUTHOR CONTRIBUTIONS:

Sharada seekonda performed experimental work, drafted and shaped the manuscript. A. Sabitha Rani conceived the work and gave valuable insights. We are thank full to Dr.T.S.Ushasree and Dr.K.Indira providing necessary facilities to carry out our work, gave critical inputs for drafting the manuscript and performed statistical analysis. Dr.M.D.Suleman helped in performing biochemical analysis**,** Dr.Padmaja gave good suggestions.

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