



## Antidiabetic Activity of Ethanolic Extracts of *Rhyncosia beddomei* and *Glycosmis pentaphylla* in Alloxan-induced Diabetic Rats

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### ABSTRACT

The objective of the study is to evaluate the anti-diabetic activity of ethanolic extract of *Rhyncosia beddomei* and ethanolic extract of *Glycosmis pentaphylla* leaves in alloxan-induced diabetes in adult male wistar albino rats. The effect of ethanolic extract of *Rhyncosia beddomei* (EERB) and ethanolic extract of *Glycosmis pentaphylla* (EEGP) on change in blood glucose level, lipid metabolism and histology of fat were examined in the study. Alloxan-induced diabetic adult male wistar albino rats were administered ethanolic extract (200 and 400 mg/kg, p.o) of *Rhyncosia beddomei* (EERB) and *Glycosmis pentaphylla* (EEGP) or standard drug Glibenclamide 2.5 mg/kg or normal control (0.9% Saline) or diabetic control group (Alloxan-induced diabetic rats). The blood glucose levels of rats were recorded after single dose of test and standard drug administration at 0, 1, 2, 4 & 8 hrs and multi dose drug administration for 10 days in each group. Blood glucose levels were recorded at 0, 3, 7 and 10th day of test drug administration. After the last test drug administration i.e. on 11<sup>th</sup> day of study, animals were sacrificed and performed the evaluation activities. Alloxan-induced diabetes control group animals showed statistically significant increase in blood glucose levels, total cholesterol, triglycerides, LDL-C, VLDL-C and statistically significant decrease in HDL-C as compared to normal group animals. The alloxan-induced animals received EERB (200 & 400 mg/kg) and EEGP (200 & 400 mg/kg) showed decrease in glucose levels, total cholesterol, triglycerides, LDL-C, VLDL-C and statistically significant increase in HDL-C as compared to alloxan-induced diabetes control group animals along with standard group (Glibenclamide 5mg/Kg) animals. The body weight of EERB and EEGP 200 & 400 mg/kg rats showed significantly elevated. In conclusion, the results of diabetic study indicated that test extracts of *Rhyncosia beddomei* and *Glycosmis pentaphylla* have a beneficial effect on normalizing glucose level in alloxan-induced induced diabetic rats. This suggests the efficacy of *Rhyncosia beddomei* and *Glycosmis pentaphylla* in the maintenance of glucose homeostasis and may be used as a therapeutic agent in the management of diabetes mellitus.

**Keywords:** Anti-diabetic, *Rhyncosia beddomei*, *Glycosmis pentaphylla*, alloxan, Glibenclamide.

### INTRODUCTION

Diabetes mellitus (DM) is one of the oldest disease identified in humans. The symptoms or Diabetes were explained 3000 years ago by the ancient Egyptians. The term "diabetes" was first coined by Araetus of Cappodocia (81-133AD) and the word mellitus (honey sweet) was denoted by Thomas Willis (Britain) in 1675 after confirming the sweetness of urine and blood of patients (first noticed by the ancient Indians). In 1776, Dobson (Britain) firstly confirmed the presence of excess sugar in urine and blood as a cause of their sweetness<sup>1</sup>.

Diabetes mellitus (DM) is characterized by increase in blood glucose levels (hyperglycemia). Hyperglycemia is due to change in metabolism of carbohydrates, fat and protein, resulting from defects in insulin secretion by pancreatic b-cells and impairment of insulin action or both<sup>2,3</sup>.

Diabetes mellitus is divided into two types. They are type 1 and type 2 diabetes. Diet, lifestyle, obesity, fat distribution, gestational pre-disposition, hereditary factors and age are the main etiological factors of type 2 diabetes<sup>4</sup>. Type 1 diabetes is mostly due to genetic origin, however genetic predisposition with viral infection and

autoimmune disorders combination also cannot be ruled out.

The increased prevalence of type 2 diabetes is combined with significant increase in cardiovascular morbidity and mortality. Diabetes is presently predicted to influence beyond 150 million patients globally, and this number may double by 2025<sup>5,6</sup>. Patients with type 2 diabetes are more prone to progress angina and acute myocardial infarction. The mortality rate is also much higher in diabetics compared to non-diabetics suffering from acute myocardial infarction<sup>7</sup>. The expected four year survival rate is predicted as 50 %<sup>8</sup>.

There are two pathophysiological developments heading to type 2 diabetes related cardiac complications. They are ischemic heart disease (as a result of accelerated atherosclerosis) and a specific diabetic cardiomyopathy, characterized initially as diastolic dysfunction which progresses to symptomatic heart failure<sup>9</sup>. Although the pathogenesis of diabetic cardiomyopathy is multi factorial and complex, metabolic disturbances related to hyperlipidemia, hyperglycemia and insulin resistance, as well as myocardial fibrosis, oxidative stress, and proinflammatory cytokines are proposed to be contributing factors<sup>10</sup>.



Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative. It is existing as alloxan hydrate in aqueous solution. Alloxan is a toxic glucose analogue. Alloxan administration selectively destroys beta cells (insulin producing cells) in the pancreas in rodents and many other animal species. Alloxan is selectively toxic to pancreatic beta cells as it conversely accumulates in beta cells through uptake by the GLUT2 glucose transporter. Alloxan generates reactive oxygen species (ROS) in a cyclic reaction with dialuric acid (its reduction product) in the presence of intracellular thiols. The free radicals formed in this redox reaction triggers alloxan induced beta cell toxic action<sup>11</sup>.

Alloxan, selectively kills beta cells of pancreas is used to induce diabetes in laboratory animals. This activity of alloxan is due to selective uptake of the compound as its structural similarity to glucose along with highly efficient uptake mechanism of beta cell's (GLUT2).

However, alloxan is not toxic to the human beta cell, even in very high doses, probably due to differing glucose uptake mechanisms between humans and rodents<sup>12,13</sup>.

Ayurvedic medicine system is one of the oldest medicine system with a history of more than 3000 years. Various molecules derived from the herbal medicines are in use for several kind of diseases and disorders. Ayurvedic medicine system is called as Gold Mine as it gives new molecules and also with different mechanism of action. Various plant decoctions or infusions used in traditional medicine to reduce obesity could be used to eliminate the clinical side effects of the current chemically formulated antiobesity agents<sup>14-19</sup>. A large study of literature shows that the significant progress has been made concerning our knowledge of bioactive components in plant foods and their links to obesity.

In these recent days, there has been an increasing interest in hypoglycemic agents derived from plant sources. Plant sources are usually considered to be non-toxic, with fewer side effects than synthetic sources. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of organisms.

*Rhynchosia beddomei* is commonly known as Adavi-kandi. In telugu called as 'Vendiaku', belongs to the family Fabaceae. It is mainly found in Eastern Ghats of Andhra Pradesh, India. The leaves consist of flavonoids, alkaloids, glycosides, lignans, tri-terpenoids and noted to be useful as abortifacient, antibacterial, antidiabetic and hepatoprotective. Leaves are also used for wounds, cuts, boils and rheumatic pains by adivasi tribes<sup>20-24</sup>.

*Glycosmis pentaphylla* is a is a shrub or a small tree commonly known as Ban-nimbu in (Hindi) and Golugu, Gongipadu in (Telugu) belongs to the family Rutaceae. It is distributed almost in all districts, India, Sri Lanka to S. E. Asia and W. Malaysia. The plant is used in indigenous medicine for cough, rheumatism, anaemia and jaundice.

The juice of the leaves tastes bitter. Leaves juice is used in the treatment of fever, liver complaints and as vermifuge. The paste of the leaves along with ginger is used in the treatment of eczema and skin infections. The root decoction is used for the treatment of facial inflammations<sup>25</sup>.

## MATERIALS AND METHODS

### Animal Selection

Adult male Wistar albino rats weighing 150-200 g, procured from in-house animal breeding facility of SICRA labs and were housed in a clean polypropylene cage with not more than three animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C with dark/light cycle 12/12 h). They were fed with standard pellet diet and water ad libitum. The animals were acclimatized to laboratory conditions for 10 days prior to experiment. All experiments were conducted according to the Guidelines of Experimental Animal Care issued by the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) regulated by the Government of India.

### Collection and authentication of Plant Material

The plants of *Rhynchosia beddomei* baker and *Glycosmis pentaphylla* (Retz.) Correa were collected from Thirupathi, Andhra Pradesh, India and these plant species were authenticated by Dr. K. Madhava Chetty, assistant professor, department of Botany, Sri Venkateswara University, Thirupathi, Andhra Pradesh, India.

### Extraction method

The leaves of *Rhynchosia beddomei* were shade dried and crushed into a coarsed powder by using grinder. The powdered material was extracted by soxhlet apparatus and ethanol (95%) as solvent for 24 hours<sup>26</sup>. The solvent was evaporated by a rotavapor under reduced pressure at 40-50 degrees controlled temperature.

The same extraction procedure was repeated for *Glycosmis pentaphylla*.

### Test drug preparation

Both of the ethanolic extracts and standard drug were suspended in normal saline (0.9% NaCl in water). While preparation of test drug concentration precaution has been taken that all animal groups should receive approximate same volume of dose i.e. volume of the dose should be around 1000 µl. All the test drugs and standard were administered orally by intragastric catheter.

### Induction of diabetes by Alloxan

Diabetes mellitus was induced in overnight fasted male rats by a single intraperitoneal injection of Alloxan (120 mg/kg body weight)<sup>12</sup>. After 96 hours of alloxan induction, fasting blood glucose levels were obtained by tail snip method. The rats showing blood glucose level above 250 mg/dl were used for the present investigation.



### Acute toxicity

The acute toxicity of the ethanolic extract of *Rhynchosia beddomei* (EERB) and ethanolic extract of *Glycosmis pentaphylla* (EEGP) leaves were determined as per the OECD guideline no. 423 (Organization for Economic Cooperation and Development)<sup>27</sup>. It was observed that the test extracts showed no mortality even at a dose of 2000 mg/kg bodyweight. Hence, 1/10th and 1/5th of 2000 mg/kg (200 mg/kg and 400 mg/kg) doses were selected for this study.

### Estimation of Fasting Blood Glucose (FBG) levels

Fasting blood glucose was estimated for all the rats of all groups. All the animals were abstained from food overnight and water was permitted. The next morning, blood was collected by cutting tail vein of the rats. Blood glucose levels were estimated by glucose strips of Accu check.

### Animal grouping

The animals were randomly divided into following 9 groups; each group consists of six animals. Animal grouping and their treatment is as follows:

Group- I : Normal Control (0.9% Saline)

Group- II: Alloxan-induced diabetic control

Group- III : Alloxan-induced diabetic rats given Glibenclamide 2.5 mg/kg

Group- IV : Alloxan-induced diabetic rats given EERB (200mg/kg)

Group- V : Alloxan-induced diabetic rats given EERB (400mg/kg)

Group- VI : Alloxan-induced diabetic rats given EEGP (200 mg/kg)

Group- VII : Alloxan-induced diabetic rats given EEGP (400 mg/kg)

All the above groups received respective treatments at a single a single daily dose for a period of 10 days.

### Evaluation of Parameters

Anti-hyperglycemic effect of EERB and EEGP on blood glucose in alloxan induced Diabetic rats after single dose and after multi dose:

After single dose: After administering first dose of test and standard drug, the blood samples were collected from tail and glucose levels were estimated by using glucometer (Accu check) at 0, 1, 2, 4, and 8 hours .

After multi dose: The same study continued by administering different doses of ethanolic extracts of plants 200 mg/kg and 400 mg/kg

### Estimation of EERB and EEGP on lipid profile

On 11th day, all the animals were sacrificed and collected blood for the estimation of lipid profile and collected

pancreas for histopathological studies. The collected blood was centrifuged for about 05 min at 4000 G at 4o C and serum was separated out for the analysis of lipid profile. Serum samples were analyzed for Triglyceride and total cholesterol, using biochemical kits of Span diagnostics. Cholesterol: one step method<sup>28</sup>; Triglycerides: by end point method<sup>29</sup> and HDL-C<sup>30</sup>.

LDL cholesterol was estimated by using Friedwald's (1972) formula as follows:

$$\text{LDL in mg \%} = \text{Total cholesterol} - \text{HDL-C} - \text{Triglycerides}/5$$

VLDL cholesterol was estimated by using following formula: VLDL in mg % =TG/5

Estimation of EERB and EEGP on body weight:

Initial and final (11th day) body weights of each group rats were analysed.

### Statistical analysis

The results of the experiments were analysed by using one way analysis of variance (ANOVA) to the mean  $\pm$  SEM of groups and followed by Dunnett's multiple comparison test. P values less than 0.05 ( $p < 0.05$ ) were taken as statistically significant using GraphPad Prism. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

## RESULTS

### Effect of EERB and EEGP on acute toxicity study:

Acute toxicity studies showed that both the extracts were non-toxic in nature. There were no deaths or no toxic reactions were observed throughout the study period.

Anti-diabetic effect of EERB and EEGP on blood glucose levels after single dose study:

In alloxan-induced diabetic rats (control group) blood glucose levels were significantly high as compared to normal group rats in all time points like 0, 1, 2, 4, 8 hours.

Glibenclamide 2.5 mg/kg orally administered rats reduced glucose levels significantly on 1, 2, 4 & 8 hours as compared to control group rats.

In this study, EERB 200 & 400 mg/kg and EEGP 200 & 400 mg/kg orally administered group rats significantly reduced ( $p < 0.05$ ) blood glucose levels on 1, 2, 4 & 8 hours. EERB 200 & 400 mg/kg (47.63% & 58.51%) and EEGP 200 & 400 mg/kg (47.46% & 52.69%) effectively reduced blood glucose level of diabetic control group rats. These reduced values were comparable to the standard control Glibenclamide 2.5 mg/kg (61.78%).

### Anti-diabetic effect of EERB and EEGP on blood glucose levels after multi dose study

There was a significant difference between normal group blood glucose levels and control group blood glucose levels. EERB 200 & 400 mg/kg, EEGP 200 & 400 mg/kg & Glibenclamide 2.5 mg/kg significantly reduced blood glucose levels as compared to alloxan-induced diabetic control group.

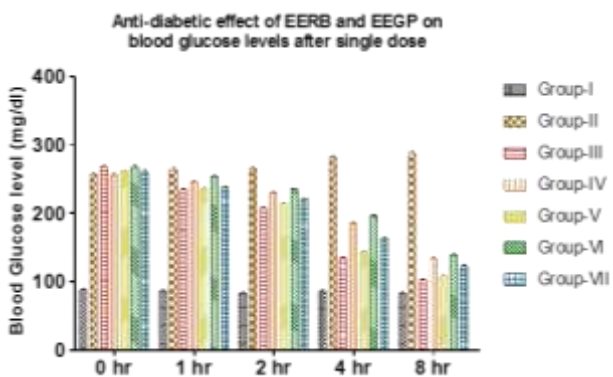
In this study, EERB 200 & 400 mg/kg (54.45% & 62.01%) and EEGP 200 & 400 mg/kg (53.95% & 57.54%) effectively ( $p < 0.05$ ) reduced blood glucose level of diabetic rats. EERB and EEGP were gradually decreased the blood glucose levels on 3rd, 7th and 10th day. These reduced blood glucose levels were comparable to standard control Glibenclamide 2.5 mg/kg (66.35%). The test extracts, EERB and EEGP showed dose-dependent activity.

**Effect of EERB and EEGP on lipid profile**

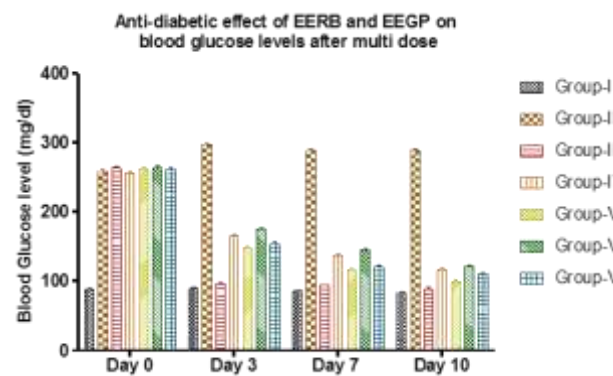
In alloxan-induced diabetic rats (control group), lipid profile parameters like TC, TG, LDL and VLDL were significantly ( $p < 0.05$ ) elevated and HDL levels were decreased ( $p < 0.05$ ) as compared to normal group. EERB 200 & 400 mg/kg, EEGP 200 & 400 mg/kg and Glibenclamide 2.5 mg/kg significantly ( $p < 0.05$ ) reduced TC, TG, LDL and VLDL and significantly ( $p < 0.05$ ) elevated HDL cholesterol as compared to alloxan-induced diabetic control group.

**Effect of EERB and EEGP on body weight**

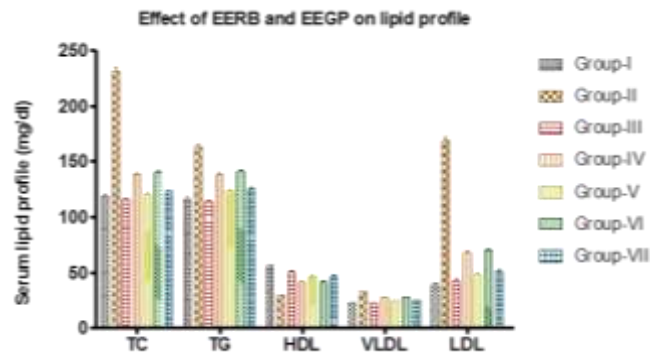
There was no significant difference in initial body weight rats of all groups. The body weights of alloxan-induced diabetic control group rats were significantly ( $p < 0.05$ ) reduced as compared to normal group rats. The body weights of EERB, EEGP and Glibenclamide treated group rats were significantly elevated ( $p < 0.05$ ) as compared to alloxan-induced diabetic control group rats.



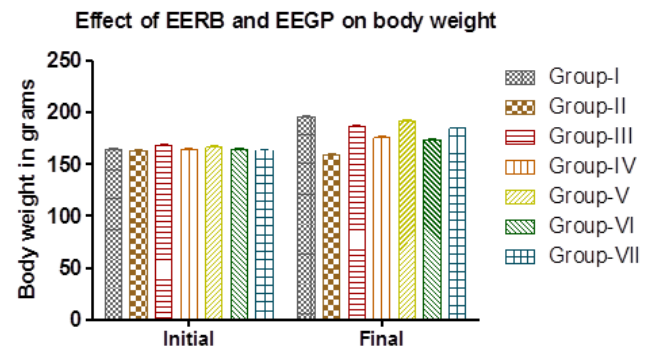
**Figure 1:** Anti-diabetic effect of EERB and EEGP on blood glucose levels after single dose:



**Figure 2:** Anti-diabetic effect of EERB and EEGP on blood glucose levels after multi dose:



**Figure 3:** Effect of EERB and EEGP on lipid profile:



**Figure 4:** Effect of EERB and EEGP on body weight:

**DISCUSSION**

Diabetes mellitus is a chronic disease having high blood glucose levels as symptom and is due to a low insulin levels in the blood. In the present study diabetic rats showed a significant elevation in plasma glucose concentration. All of these results are similar with other studies conducted in rats<sup>31</sup>.

Alloxan is widely used to induce diabetes in experimental animals by generation of reactive oxygen species that causes damage to  $\beta$ -cells<sup>32</sup>. Alloxan reduces to dialuric acid forms a redox cycle with superoxide radicals<sup>33, 34</sup>. These radicals undergo dismutation to hydrogen peroxide. There after highly reactive hydroxyl radicals are formed by Fenton reaction. The actions of reactive oxygen species with a concurrent increase in cytosolic calcium concentration results in rapid destruction of  $\beta$ -cells and thus increases the blood sugar level<sup>35</sup>.

Both the studies of single dose and multiple dose studies orally administered EERB and EEGP showed significant reduction in blood glucose levels in diabetic rats. The study results showed that highest reduction of blood glucose was observed 400 mg of the EERB and EEGP. The effect was comparable with standard control Glibenclamide 2.5 mg/kg. The anti-diabetic and anti-hyperlipidemic effects of EERB and EEGP may be associated with more than one mode of action. One of the possible mechanism of action is involving regularization of insulin secretion or increase in insulin sensitivity or both the mechanisms could be possible<sup>36, 37</sup>. Along with these, anti-oxidant effect was observed through increase in glucose metabolism<sup>38,39</sup>.



These results also suggesting that the control group rats showed elevations in TC, TG, LDL and VLDL and reduction in HDL. Because of increase in LDL and VLDL productivity and protein lipase unavailability showed insulin deficiency. Both the extracts significantly reduced TC, TG, LDL and VLDL and increased HDL levels. This effect is due to lipoprotein lipase act like insulin<sup>40</sup>.

The decrease in body weight of diabetic rats was due to muscle wasting. The test extracts showed significant increase in body weight by controlling muscle wasting along with glycemic control<sup>41</sup>.

## CONCLUSION

The results of diabetic study indicated that test ethanolic extracts of *Rhynchosia beddomei* (EERB) and *Glycosmis pentaphylla* (EEGP) have a beneficial effect on normalizing glucose level and lipid profile in alloxan-induced diabetic rats. This suggests the efficacy of *Rhynchosia beddomei* and *Glycosmis pentaphylla* in the maintenance of glucose homeostasis and can be used as a therapeutic agent in the management of diabetes mellitus.

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