Research Article

Antidiabetic Activity of Ethanolic Extracts of *Rhyncosia beddomei* and *Glycosmis pentaphylla* in Streptozotocin 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 Streptozotocin-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 profile, Liver marker enzymes, liver glycogen, Insulin and Total protein were examined in the study. Streptozotocin (STZ) induced diabetic adult male wistar albino rats were administered ethanolic extract (100, 200 and 400 mg/Kg, p.o.) of *Rhyncosia beddomei* (EERB) and *Glycosmis pentaphylla* (EEGP) or standard drug Glibenclamide 3 mg/kg or Normal diet control (0.9% Saline) or control group (STZ-induced diabetic rats). The blood glucose levels of rats were recorded every week for 28 days in each group. On 29th day of study, after overnight fasting for 12 hours, the rats were sacrificed by cervical decapitation. Blood samples were collected by cardiac puncture and liver, kidney and pancreas were dissected and washed with ice cold normal saline for further investigations. EERB and EEGP showed significant decrease in anti-hyperlipidemic activity as evidenced by significant decrease in serum TC, TG, LDL-C, VLDL-C levels and significant increase in HDL-C level in treated diabetic rats. EERB and EEGP also restored the altered plasma enzymes such as SGOT, SGPT and ALP, total protein, liver glycogen levels to near normal. The effects of EERB and EEGP were proportionate to standard drug glibenclamide. Results of this experimental study showed that EERB and EEGP has anti-diabetic and anti-hyperlipidemic activities. 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, lipid profile, liver marker enzymes, liver glycogen, Insulin and Total protein in STZ-induced diabetic rats. This suggests the efficacy of *Rhyncosia beddomei* and *Glycosmis pentaphylla* in the maintenance of glucosehomeostasis and may be used as a therapeutic agent in the management of diabetes mellitus.

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

INTRODUCTION

Diabetes mellitus (DM) is one of the oldest disease observed in man. 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[1].

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[2, 3].

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[4]. Type1 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[5, 6]. 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[7]. The expected four year survival rate is predicted as 50 %[8].

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[9]. Although the pathogenesis of diabetic cardiomyopathy is multi factorial and complex, metabolic disturbances related to hyper lipidemia, hyper glycemia and insulin resistance, as well as myocardial fibrosis, oxidative stress, and proinflammatory cytokines are proposed to be contributing factors[10].

Streptozotocin [STZ; 2-deoxy-2(3-methyl-3-nitrosoureido)-D glucopyranose] belongs to a family of broad-spectrum antibiotic. Streptozotocin have oncolytic,

oncogenic, and diabetes-producing properties. The diabetes producing activity is due to selective destruction of pancreatic beta cells. For this reason STZ has been universally used as a method for inducing diabetes mellitus in experimental animals and also in the treatment of malignant beta cell tumours and other neoplasms in humans.

A single injection of STZ can produce diabetes. The serum half-life of STZ is about 15 min. The increase in blood glucose levels are achievable within 02 days, and dissolution and phagocytosis of necrotic cells are observed histologically after 03 days. The administration of a single sub-diabetogenic dose produces only mild histologic changes without any evidence of significant hyperglycemia when compared with buffer-injected control animals was observed in rats and mice[11].

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 kinds 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[12-17]. 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.

Rhyncosia 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 consists of flavinoids, alkaloids, glycosides, lignans, tri-terpenoids and noted to be useful as abortifacient, antibacterial, anti diabetic and hepatoprotective. Leaves are also used for wounds, cuts, boils and rheumatic pains by adivasi tribes[18-22].

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[23].

MATERIALS AND METHODS

Animal Selection

Adult male Wistar albino rats weighing 200-250 g, procured from in-house animal breeding facility of SICRA labs and were housed in a clean polypropylene cage with not more than four 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.

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 *Rhyncosia beddomei* were shade dried and crushed into a coarse powder by using grinder. The powdered material was extracted by soxhlet apparatus and ethanol (95%) as solvent for 24 hours. 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.

Acute toxicity

The acute toxicity of the ethanolic extract of *Rhyncosia beddomei* and *Glycosmis pentaphylla* leaves were determined as per the OECD guideline no. 423 (Organization for Economic Cooperation and Development)[24]. It was observed that the test extracts showed no mortality even at a dose of 2000 mg/kg bodyweight. Hence, 100 mg/kg, 200 mg/kg and 400 mg/kg doses were selected for further study.

Oral Glucose tolerance test (OGTT) of EERB and EEGP

Oral glucose tolerance test was performed in normal healthy rats under overnight fasting condition. The rats were divided in to eight groups. Each group contains six

rats (n=6). Group I, administered with normal saline. Group II, administered Glibenclamide 3 mg/kg. Group III, IV & V, administered with EERB 100, 200 & 400 mg/kg. Group VI, VII & VIII, administered with EEGP 100, 200, 400 mg/kg respectively. After 30 minutes of administration of normal saline, glibenclamide and EERB and EEGP 100, 200 & 400 mg/kg extracts, Glucose 2 g/kg was fed to the each rat in each group. Blood was collected from tail vein at 0, 30, 60 and 120 minutes of glucose administration and blood glucose was estimated by commercially available glucose strips of Accu Check.

Induction of diabetes by Streptozotocin

Diabetes mellitus was induced to overnight fasted rats by a single intraperitoneal injection of streptozotocin (40 mg/kg body weight) reconstituted in freshly prepared 0.1 M citrate buffer (1 ml/kg body weight; pH-4.5). Hypoglycemic mortality of STZ was prevented by administering 20% glucose solution. After 04 days, 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.

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: Diabetic Control (STZ- induced Diabetic Rats)

Group- III : STZ- induced Diabetic Rats administered Glibenclamide 3 mg/kg

Group- IV : STZ- induced Diabetic Rats administered EERB (100mg/kg)

Group- V : STZ- induced Diabetic Rats administered EERB (200mg/kg)

Group- VI : STZ- induced Diabetic Rats administered EERB (400mg/kg)

Group- VII : STZ- induced Diabetic Rats administered EEGP (100 mg/kg)

Group- VIII : STZ- induced Diabetic Rats administered EEGP (200 mg/kg)

Group- IX : STZ- induced Diabetic Rats administered EEGP (400 mg/kg)

All the above groups received respective treatments at a single daily dose for a period of 28 days.

Evaluation of Parameters

Effect of EERB and EEGP on Blood glucose levels: The blood glucose of rats of each group were measured before first treatment administration (Day 0) and at 7th, 14th, 21st, 28th Days on the regular treatment administration in fasting condition.

Body weight

The initial and final body weights of the rats were measured.

Estimation of lipid profile

On 29th day of study, after overnight fasting for 12 hours, the rats were sacrificed by cervical decapitation. Blood samples were collected by cardiac puncture and liver, kidney and pancreas were dissected and washed with ice cold normal saline.

Lipid profile includes Total cholesterol (TC), HDL (High Density Lipoproteins), LDL (Low Density Lipoproteins), VLDL (Very Low Density Lipoproteins) and Triglycerides (TG). Lipid profile was estimated by commercially available diagnostic kits. Serum total cholesterol was estimated by CHOD-PAP[25]. Estimation of triglycerides was done by GPO-PAP method[26].

LDL cholesterol was estimated by using Friedwald's (1972)[27] formula as follows:

LDL in mg % = Total cholesterol – HDL-C –Triglycerides/5.

VLDL cholesterol was estimated by using following formula:

VLDL in mg%= Triglycerides/5

Estimation of liver marker enzymes (SGOT, SGPT and ALP):

Estimation of liver marker enzymes serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) and Alkaline Phosphatase (ALP) were estimated by using commercially available diagnostic kits[28,29].

Preparation of tissue homogenates

On 29th day, all the rats in all the groups were sacrificed by cervical dislocation. Liver and kidney were separated and rinsed with ice cold physiological solution. The separated organs were homogenized with Potter Elvehjem homgenizer. 10% liver and kidney homogenates were prepared and stored in -20 degree C for the assay of antioxidant enzymes like superoxide dismutase (SOD), Catalase (CAT), Reduced glutathione (GSH) and lipid peroxidation activity.

Estimation of liver glycogen, Total protein and plasma insulin:

Hepatic glycogen content was estimated by using plummer method[30]. Serum total protein was estimated by using Folin phenol reagent[31]. The insulin was estimated by immuno assay kit[32].

Statistical analysis

The results of the experiments were analysed by using one way analysis of variance (ANOVA) to the mean ± 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

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.

Effect of EERB and EEGP on oral glucose tolerance test

As shown in Figure 1: , Blood glucose levels were reached maximum after administering glucose 2 g/kg by 30 min and then started to decrease from 60 and 120 min. EERB and EEGP showed dose dependent reduction in blood glucose levels. EERB and EEGP 100, 200 & 400 mg/kg and Glibenclamide 3 mg/kg significantly reduced blood glucose levels as compared to normal control.

Effect of EERB and EEGP on Blood glucose levels

Blood glucose levels were measured in all groups on 0, 7, 14, 21 & 28 days of the treatment. Group II (STZ-induced diabetic rats) rats showed significant increase in blood glucose levels as compared to Group I (Normal control) rats. After treatment with EERB and EEGP (Group IV-IX), rats were significantly reduced blood glucose levels as compared to Group II rats. There was a dose dependent reduction in blood glucose levels after administering EERB, EEGP and Glibenclamide for 28 days regularly.

Effect of EERB and EEGP on Body weight

At the end of the study, STZ-induced diabetic rats showed significant reduction in body weight as compared to normal control group. There was a significant increase in body weight was observed in EERB, EEGP and Glibenclamide treated rats as compared to STZ-induced diabetic rats. The increase in body weight was dose dependent of the extract.

Effects of EERB and EEGP on lipid profile

At the end of study, STZ-induced diabetic rats showed statistically significant increase in Total cholesterol (TC), Triglycerides (TG), LDL (Low density lipoproteins), very low density lipoproteins (VLDL) and statistically significant reduction in HDL (High density lipoproteins) as compared to normal control group. There was a statistically significant reduction in TC, TG, LDL & VLDL was observed in EERB, EEGP and Glibenclamide treated rats and statistically significant increase in HDL cholesterol levels as compared to STZ-induced diabetic rats.

Effect of EERB and EEGP on Liver marker enzymes and liver glycogen

At the end of the study, STZ-induced diabetic rats showed statistically significant increase in SGOT, SGPT and ALP and significant decrease in liver glycogen levels as compared to normal control rats. In EERB, EEGP and glibenclamide treated rats, there was a significant reduction in elevated SGOT, SGPT and ALP levels and significant increase in liver glycogen levels as compared to STZ-induced diabetic rats.

Effect of EERB and EEGP on Insulin and Total protein

At the end of study, STZ-induced diabetic rats showed statistically significant reduction insulin and total protein levels as compared to normal control group rats. In EERB, EEGP and Glibenclamide treated rats, there was a statistically significant increase in (towards normal) insulin and total protein levels as compared to STZ-induced diabetic rats.

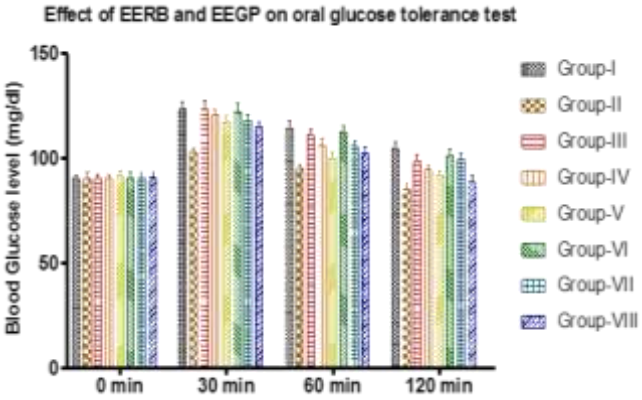

Figure 1: Effect of EERB and EEGP on oral glucose tolerance test:

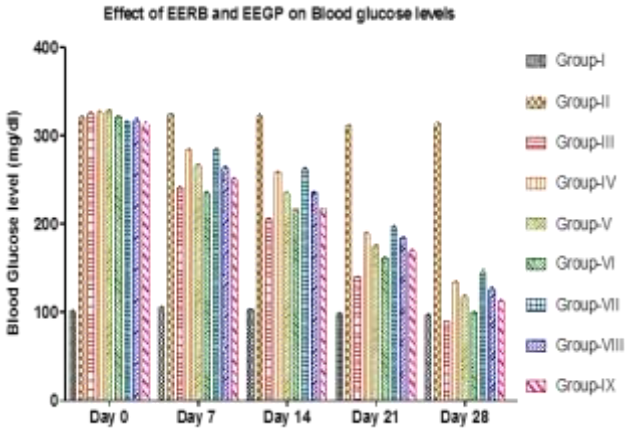

Figure 2: Effect of EERB and EEGP on Blood glucose levels

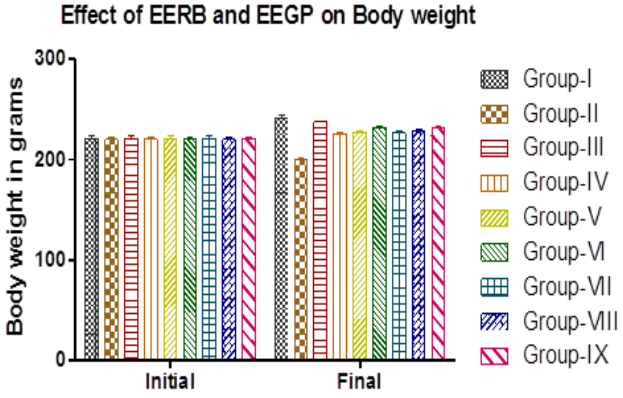

Figure 3: Effect of EERB and EEGP on Body weight

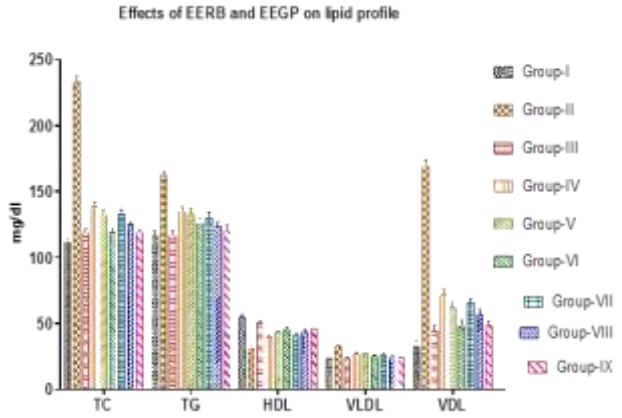

Figure 4: Effects of EERB and EEGP on lipid profile

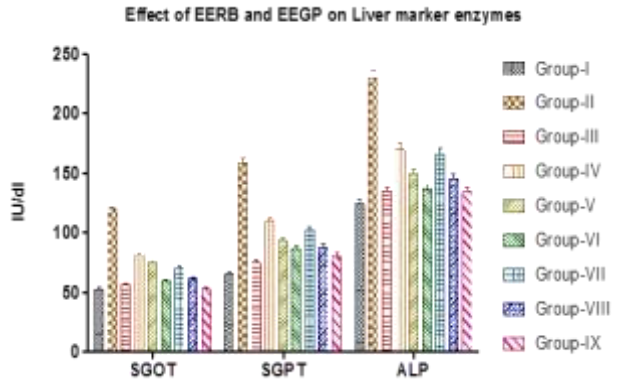

Figure 5: Effect of EERB and EEGP on Liver marker enzymes

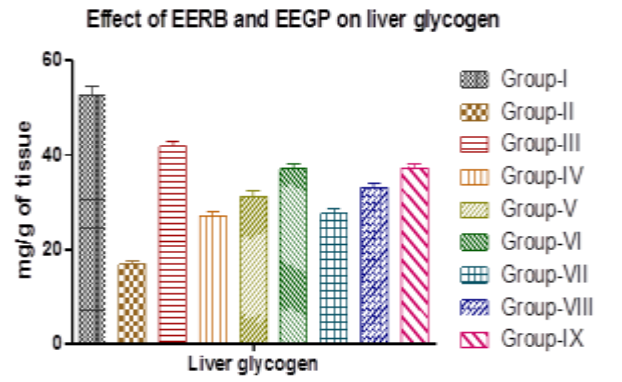

Figure 6: Effect of EERB and EEGP on liver glycogen

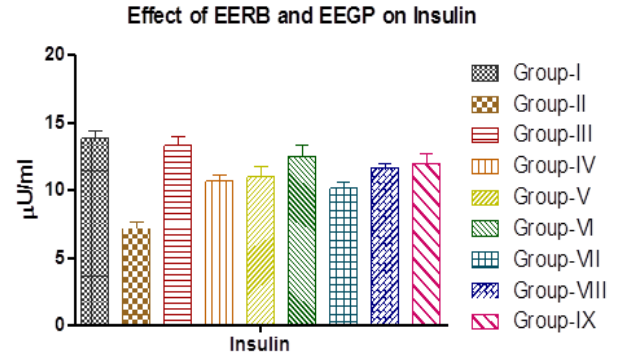

Figure 7: Effect of EERB and EEGP on Insulin

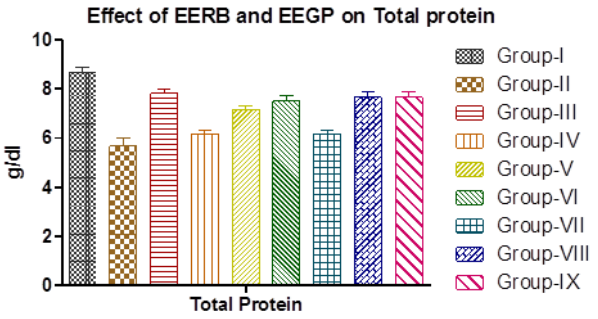

Figure 8: Effect of EERB and EEGP on Total protein

DISCUSSION

In this research study discussed about the antidiabetic efficiency of ethanolic extracts of *Rhyncosia beddomei* and *Glycosmis pentaphylla* in normal rats and STZ-induced diabetic rats. EERB and EEGP showed better glycemic control in oral glucose tolerance test. The efficiency of reducing blood glucose levels may be due to improvement in usage of glucose by peripheral tissues[33]. EERB and EEGP at the tested doses 100, 200 & 400 mg/kg reduced the blood glucose levels in a dose-dependent pattern. The reduced blood glucose levels at high doses of EERB and EEGP were parallel to that of Glibenclamide.

Serum insulin levels were significantly elevated in EERB, EEGP and Glibenclamide treated STZ-induced diabetic rats may be as a result of restoration of pancreatic beta cells which were damaged by STZ[34]. In addition, EERB and EEGP improved glycemic control by potentiating insulin release, regularizing GLUT-2 & 4 translocation and increase in insulin level results in usage of glucose in STZ-induced diabetic rats[35].

The liver is a largest glandular organ with major functions includes regulation of glycogen storage and emulsification of lipids. Liver toxicity is one of the major side effect of STZ. Liver marker enzymes elevates during liver toxicity due to extermination of liver cells. EERB and EEGP treated group rats showed significant reduction in these liver marker enzymes which demonstrating protective action of EERB and EEGP in diabetic condition[36]. Insulin promotes the storage of intra-cellular glycogen. Increase in insulin activity directly promotes synthesis of glycogen in all tissues by stimulating the enzyme glycogen synthase and inhibiting the enzyme glycogen phosphorylase. Liver glycogen levels were greatly reduced during diabetic condition due to insulin deficiency. EERB and EEGP showed elevations in liver glycogen in diabetic rats which is supporting the sufficient levels of insulin availability[37].

The body weight and total protein levels of STZ-induced diabetic rats were significantly reduced as compared to normal control group rats, EERB, EEGP and Glibenclamide treated group rats. This denotes EERB and EEGP were prevented protein degradation and decrease in muscle wasting in diabetic animals[38-40].

Diabetes and insulin resistance are associated with lipid and lipoprotein abnormalities[41]. Insulin insufficiency leads

to increase in free fatty acid mobilization from adipose tissue further results in elevation of total cholesterol (TC), triglycerides (TG), LDL-cholesterol, VLDL-cholesterol and reduction in HDL-cholesterol. EERB and EEGP revised the lipid profile in dose-dependent pattern by reduced TC, TG, LDL & VLDL and elevated HDL cholesterol levels.

CONCLUSION

The results of diabetic study indicated that test extracts of *Rhyncosia beddomei* and *Glycosmis pentaphylla* have a beneficial effect on normalizing glucose level, lipid profile, liver marker enzymes, liver glycogen, Insulin and Total protein in STZ-induced diabetic rats. This suggests the efficacy of *Rhyncosia beddomei* and *Glycosmis pentaphylla* in the maintenance of glucosehomeostasis and may be used as a therapeutic agent in the management of diabetes mellitus.

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