

# Antidiabetic and Antioxidant Activity of *Rhynchosia Beddomei* Baker

N.V.L. Suvarchala Reddy V\*, J.L. Mary, N.M. Raghavendra and C.V.S Subrahmanyam

Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana State, India

## ABSTRACT

**Objective:** To screen the antidiabetic and antioxidant activity of *Rhynchosia beddomei* whole plant by oral glucose tolerance test, streptozotocin (STZ)-induced and dexamethasone induced diabetic rats using oral administration of methanolic extract (MERB) and ethyl acetate extract (EARB).

**Methods:** In oral glucose tolerance test, both MERB and EARB markedly reduced the external glucose load. The extracts were given orally at doses 150 mg/kg b.w. and 300 mg/kg b.w. and were observed after 21 days in STZ induced diabetes and 11 days in dexamethasone induced diabetes. *In vitro* models using  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory assay were also evaluated. *In vitro* antioxidant study of the methanolic extract (MERB) and ethyl acetate extract (EARB) was done by DPPH assay and NBT inhibition assay respectively.

**Results:** From the preliminary phytochemical investigation *Rhynchosia beddomei* whole plant showed the presence of flavonoids, phenolics and steroids. The antioxidant effect of plants are often attributed to the presence of phytochemical constituents mainly phenolics, flavonoids and flavonols. MERB at 300 mg/kg b.w. was found to have significant antioxidant and antidiabetic activity.

**Conclusion:** This study clearly shows that the extracts of *Rhynchosia beddomei* possess effective antioxidant and antidiabetic activity.

**Keywords:** Diabetes mellitus, streptozotocin, dexamethasone, *Rhynchosia beddomei*.

## Address for Correspondence

Gokaraju Rangaraju  
College of Pharmacy,  
Bachupally,  
Hyderabad, Telangana  
State, India.

**E-mail:**  
[suvarchalakiran@gmail.com](mailto:suvarchalakiran@gmail.com)

## INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and changes in carbohydrate, fat and protein metabolism, associated with fixed or relative deficiencies in insulin secretion and or

insulin action. Diabetes mellitus has been known ever since ages and the sweetness of urine has been mentioned in Ayurveda by Sushruta. Plant materials which are being used as traditional medicine for the curing

diabetes are measured as good sources for a new drug or a lead to make a new drug. Now-a day's more than 400 plants are being used in different forms for hypoglycemic effects. Therefore, proper scientific assessments of *R. beddomei* for pharmacological and chemical evaluation are necessary.<sup>1</sup>

Different parts of *Rhynchosia beddomei* plant have been found to possess medicinal properties; it possesses wound healing<sup>2</sup>, in diuretic<sup>3</sup>, anti inflammatory, carcinoma, antioxidant<sup>4</sup> and antidiabetic activity. *R. beddomei* contains the volatile oils was first reported. The main chemical constituents are flavonoid, steroids, phenols, saponins present in large amounts. So in the present investigation whole plant of *Rhynchosia beddomei* was selected and screened for *in-vitro* antioxidant assay and antidiabetic assay,<sup>5</sup> hypoglycemic and antidiabetic activity.<sup>5</sup>

## MATERIALS AND METHODS

### Plant material and preparation of extract

*R. beddomei* whole plant was collected from Seshachalam hills and authenticated by Dr. K. Madhava Chetty, Assistant Professor in Department of Botany, Sri Venkateshwara University, Tirupati, Chittoor district, Andhra Pradesh. The crude plant material was dried under shade and powdered mechanically to coarse powder. The coarsely powdered plant material (500g) was subjected to extraction with methanol and ethyl acetate using simple distillation. The extract was evaporated to semisolid mass and subjected to preliminary phytochemical investigation.

### Animals

Healthy adult Wistar Albino rats of 180-250 g of were selected for the study. The animals were obtained from Gentox laboratories, Hyderabad. The animals were housed according to CPCSEA guidelines

(under standard temperature condition). They were given a pellet diet and water *ad libitum*. The ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) before the experiment.

### Estimation of total phenolic and flavonoid content

Total phenolic content of *Rhynchosia beddomei* was determined by using Folin-Ciocalteu reagent. Total phenolic content of MERB, EARB was determined by Folin-ciocalteu reagent. 1mL of extract solution was taken in the test tube and 0.2mL of Folin Ciocalteu reagent (1: 2 in distilled water) was added to it and after 20 min, 1 mL of sodium carbonate (15%) and 2 mL of purified water was added. Allowed to react for 30 min and absorbance was measured at 765 nm.<sup>6</sup> The concentration of total phenolic component in the extract was determined as microgram of Gallic acid equivalent.

The MERB, EARB extract was diluted with 80% aqueous ethanol (0.9 mL). Aliquots of 0.5 mL of MERB, EARB extract were added to test tube containing 0.1 mL of 10% aluminum nitrate, 4.3 mL of 80% ethanol and 0.1 mL 1M aqueous potassium acetate was added. The reaction tubes were kept aside for 40 min at room temperature. At the end of this time, optical density of each sample was determined at 415 nm using a UV spectrophotometer.<sup>7</sup> Total flavonoids content was calculated by interpolation on a standard curve established against a reference standard, quercetin.

### *In-vitro* antioxidant assays

#### DPPH radical scavenging activity

The hydrogen donating ability of MERB, EARB was examined in the presence of DPPH stable radical. One milliliter of 0.3 mM DPPH was added to 2.5mL of test solution of different concentrations of MERB, EARB and

allowed to react at room temperature. The absorbance was measured at 517nm after an incubation of 30 minutes. Methanol (1.0mL) and MERB, EARB extract solution (2.5mL) was used as blank, DPPH solution (1.0 mL, 0.3mM) and methanol (2.5 mL) served as negative control. Ascorbic acid was used as standard.<sup>8</sup>

#### NBT reduction assay

A reaction mixture of 3mL in each tube was made up with 1.4 mL of 50 mM  $\text{KH}_2\text{PO}_4\text{-KOH}$  pH 7.4 containing 1mM EDTA, 0.5 mL of 100 $\mu\text{M}$  hypoxanthine, 0.5mL of 100 $\mu\text{M}$  NBT. The reaction was started by adding 0.066 units per tube of xanthine oxidase freshly diluted in 100 $\mu\text{L}$  of phosphate buffer and 0.5 mL of MERB, EARB extract in saline. NBT reduction was measured by Spectrophotometric method at 560nm. Ascorbic acid was used as standard. The results were expressed as the percentage inhibition of NBT.<sup>9</sup>

#### *In-vitro* antidiabetic assays

##### *In-vitro* alpha- amylase inhibitory activity

In a test tube containing 0.2 mL of 0.5 M Tris-Hcl buffer (pH 6.9) and 0.01 M calcium chloride (substrate), starch (2 mg) was perched. Then the test tube was boiled for 5 minutes and pre incubated at 37°C for 5 minutes. 1mL of 0.1% of gum acacia was used to dissolve 1 mg of dried MERB, EARB extracts. 0.2 ml of MERB, EARB extracts were added in the tube containing the substrate solution followed by 0.1 mL of porcine pancreatic amylase in Tris-Hcl buffer (2 units/mL) were added. The process was carried out at 37°C for 10 minutes<sup>10</sup>. The reaction was terminated by the addition of 0.5 ml of 50% acetic acid in each tube and the reaction mixture was centrifuged at 3000 rpm for 5 minutes at 4°C. The absorbance was measured at 595 nm using UV spectrophotometer.

##### *In-vitro* alpha- glucosidase inhibitory activity

$\alpha$ -glucosidase inhibitory activity for MERB, EARB was determined by incubating 0.1 mL of an enzyme solution with 0.2 M Tris buffer, pH 8.0 (1.0 mL) containing different concentrations of MERB, EARB extract at 37°C for 60 minutes. The reaction mixture was boiled for two minutes in water bath to stop the reaction.<sup>11,12</sup> The amount of liberated glucose was measured by glucose oxidation method. (Assay condition 3 °C $\pm$ 0.1°C, pH-8.0; O.D at 540 nm). Same protocol was repeated thrice.

##### Effect of extracts of *Rhynchosia beddomei* on oral glucose tolerance test

Wistar Albino rats (healthy) were selected and randomly divided into seven groups (n=6). Group 'I' serves as normal control receiving distilled water, Group 'II', 'III', 'IV', 'V' serves as test groups receiving MERB (150 mg/kg b.w., and 300 mg/kg b. w.) and EARB (150 mg/kg b.w. and 300 mg/kg b. w.), Blood was withdrawn from the retro orbital plexus at 60, 90, 120 and 150 minutes after 1 h of drug administration.<sup>13,14</sup> The glucose level in blood was measured by using GOD-POD method.

##### Effect of *Rhynchosia beddomei* extracts in streptozotocin induced diabetic rats

Diabetes was induced with streptozotocin (60 mg/kg b.w. i.p.) in healthy wistar albino rats. After the injection animals had food and 0.5% glucose solution overnight to avoid from hypoglycemic shock. Diabetes was induced within 48 hours of administration of streptozotocin. The animals were kept for a stabilization period of 14 days and the animals with blood glucose levels above 350 mg/dL were selected for present the study.

Wistar Albino rats, healthy were selected and divided into seven groups (n=6). Group 'I' serves as normal control receiving distilled water, group 'II' serves as diabetic control which also receives distilled water, Group 'III' serves as standard group, Glimeperide (0.5 mg/kg b. w.), Groups 'IV', 'V', 'VI' 'VII' serves as test groups receiving MERB 150 mg/kg b.w., 300 mg/kg b. w. and EARB 150 mg/kg b.w., 300 mg/kg b. w. respectively. The MERB, EARB extracts was administered orally for 21 days, once daily. Blood samples were withdrawn from retro orbital plexus on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of MERB, EARB administration. The glucose level in blood was measured.<sup>15,16</sup>

#### Effect of *Rhynchosia beddomei* extracts in dexamethasone induced diabetic rats

Diabetes was induced with dexamethasone (10 mg/kg b.w. s. c.) in healthy wistar albino rats. After the injection animals had food and 0.5% glucose solution overnight to avert from hypoglycemic shock. Diabetes was induced within 48 hours of administration of dexamethasone.<sup>17</sup> The animals with blood glucose levels above 350 mg/dL were chosen for present the study.

Wistar Albino rats were selected and randomly divided into seven groups (n=6). Group 'I' serves as normal control receiving distilled water, Group 'II' serves as diabetic control which also receives distilled water, Group 'III' serves as standard group receiving Glimiperide (0.1 mg/kg b. w.), Groups 'IV', 'V', 'VI' 'VII' serves as test groups receiving MERB (150 mg/kg b.w., 300 mg/kg b. w.) and EARB (150 mg/kg b.w., 300 mg/kg b. w.) respectively. The extract was administered orally for 11 days, once daily. Blood samples were withdrawn from retro orbital plexus on 1<sup>st</sup> and 11<sup>th</sup> day of MERB, EARB administration. The blood glucose level was measured.

#### Statistical analysis

The Results were expressed as the mean  $\pm$  S.E.M. The significance of the results was calculated using ANOVA and Dunnet's t-test. The results were considered statistically significant when  $p < 0.01$ ,  $p > 0.05$ .

## RESULTS AND DISCUSSION

DPPH radical scavenging activity and NBT reduction assay showed that the plant possess antioxidant activity. IC<sub>50</sub> value for Vit. C was found to be 8.4 ( $\mu\text{g/mL}$ ). The IC<sub>50</sub> value for the MERB and EARB was found to be 7.4 ( $\mu\text{g/mL}$ ) and 12.5 ( $\mu\text{g/mL}$ ) respectively. In NBT reduction assay, the IC<sub>50</sub> value for the MERB was found to be 12.5 ( $\mu\text{g/mL}$ ) and the IC<sub>50</sub> value for the EARB was found to be 16.5 ( $\mu\text{g/mL}$ ). The results were shown in the table no. 1.

The crude methanolic and ethyl acetate extract of *Rhynchosia beddomei* also showed good inhibitory activity for alpha-amylase and alpha-glucosidase with the IC<sub>50</sub> value of 10.5 $\mu\text{g/ml}$ , 16.2 $\mu\text{g/ml}$ , 9.5 $\mu\text{g/ml}$  and 13.5 $\mu\text{g/ml}$  compared to standard acarbose having IC<sub>50</sub> value 9.4 $\mu\text{g/ml}$  as shown in table no. 2.

The extract treated groups shows significant decrease in the blood glucose levels in oral glucose tolerance in rats when compared with normal control significantly ( $p < 0.001$ ). The methanolic extract decreased blood glucose levels (BGL) better when compared to the EARB as shown in table no. 3.

The pharmacological screening of *R. beddomei* showed a reduction in BGL and MERB at 300mg/kg b.w. was found to be potent, when compared with other extracts. The BGL decreased when compared to the standard. In oral glucose tolerance test the extract reduce BGL in dose dependent manner significantly ( $a = p > 0.05$ ) given in table no. 4. In dexamethasone induced diabetes *Rhynchosia beddomei* decreases

BGL, MERB extract at 300 mg/kg b.w. was found to be potent, when compared with 150 mg/kg b.w. of EARB, MERB and 300 mg/kg b.w. EARB. The BGL decreased nearer to the standard given below in table no. 5.

## DISCUSSION

Total phenolics comprise one of the major groups of compounds acting as chief antioxidants or free radical terminators hence it was practical to detect their amount in the herbal preparation. Flavonoids are group of natural compounds and possibly the most important natural phenolics. Total phenolics and flavonoids are responsible for antioxidant properties. Such property is especially distinct for flavonols. It is claimed that phenolic compounds are powerful chain breaking antioxidants.<sup>9</sup> The scavenging ability of phenolic group is due to its hydroxyl group.<sup>19</sup> The antioxidant activity has been reported to be connected with the improvement of reducing power. Herbal preparation exposed synergistic effects in DPPH scavenging and reducing power.

It is thought that flavonoids may inhibit superoxide producing enzyme xanthine oxidase which is used in the NBT assay by binding to it. Free radical-scavenging activity, possessed by the flavonoids in these plant extracts, is dependent on the concentration and type of radical-scavenging phenolics there in each extract.

In our study, induction of type 2 diabetes showed significant increased blood glucose level and reduced body weight and insulin level compared to control rats which prove the induction of diabetes, and it may be due to partial necrosis of pancreatic  $\beta$ -cell.<sup>12</sup> Also, body weight of diabetic rats was reduced, and it may be due to decrease in amount of insulin. Physiologically, insulin regulates protein synthesis and proteolysis in

skeletal muscle. Oral administration of MERB (150 and 300 mg/kg dose) and glimiperide to the diabetic rats showed significant reduction of blood glucose and increase in body weight and insulin level than diabetic control rats. Hence, MERB mediated above effect possibly due to its protective effect on STZ-mediated  $\beta$ -cell damage in diabetic rats and thereby increases insulin release and inhibits muscle proteolysis which causes improvement in body weight of MERB treated diabetic rats.

Dexamethasone is an effective glucocorticoid used in the healing of inflammation. High exposure to glucocorticoid impairs insulin sensitivity, including insulin resistance and hypertension. The mechanism by which dexamethasone induces peripheral insulin resistance is by inhibiting GLUT-4 translocation, increasing lipase activity.<sup>17</sup> Dexamethasone increases glucose levels leading to hyperglycemia. Therapeutic doses of glucocorticoids induce obesity gene expression in rat adipose tissue within 24 h and are followed by diabetes, development of insulin resistance with enhanced glucose levels. Oral administration of MERB and EARB (150 and 300 mg/kg dose) and Glimiperide (0.1 mg/kg b.w.) to the diabetic rats showed significant reduction of blood glucose and increase in body weight.

## CONCLUSIONS

Preliminary phytochemical investigation showed the presence of bioactive antidiabetic constituents like flavonoids, sterols, alkaloids and phenolics. The MERB and EARB extracts were found to have significant *In vitro* antioxidant activity due to the occurrence of flavonoids and phenolics. Antidiabetic effect of *Rhynchosia beddomei* was found to be effective in STZ induced diabetic rats and dexamethasone induced diabetic rats. Methanolic extract of the *Rhynchosia beddomei* was found to have

strong antidiabetic activity compared to the ethyl acetate extract. Finally the mechanism has yet to be known and further studies are required to ascertain the bioactive components of the plant extract, which can be tested for antidiabetic activity.

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**Table 1.** NBT and DPPH radical scavenging activity of *Rhynchosia beddomei* extracts

Test extract	DPPH radical scavenging activity IC <sub>50</sub> (µg/mL)	NBT inhibition assay IC <sub>50</sub> (µg/mL)
MERB	7.4	12.5
EARB	12.5	16.5
Vit.C (standard)	8.4	8.4

**Table 2.** Alpha amylase and alpha glucosidase inhibitory assay

Test drug	Alpha amylase inhibitory assay IC <sub>50</sub> (µg/mL)	Alpha glucosidase inhibitory assay IC <sub>50</sub> (µg/mL)
MERB	10.5	9.5
EARB	16.2	13.5
Acarbose (standard)	9.4	9.4

**Table 3.** Effect of MERB and EARB on oral glucose tolerance in rats

Groups	Treatment	Blood glucose levels (mg/dL)					
		Fasting	1 h	2 h	3 h	4 h	5 h
I	Control	92±1.95	94±1.28	95±2.57	90±2.79	87±2.06	101±1.64
II	MERB (150 mg/kg b.w.)	97±0.95 <sup>a</sup>	81±1.90 <sup>a</sup>	85±3.61 <sup>b</sup>	71±1.83 <sup>a</sup>	72±3.08 <sup>a</sup>	92±3.54 <sup>a</sup>
III	MERB (300 mg/kg b.w.)	98±3.11 <sup>a</sup>	86±5.11 <sup>a</sup>	63±2.47 <sup>a</sup>	64±3.57 <sup>a</sup>	72±2.74 <sup>a</sup>	74±2.5 <sup>b</sup>
IV	EARB (150 mg/kg b.w.)	95± 3.4 <sup>b</sup>	93± 2.5 <sup>ns</sup>	91± 3.9 <sup>a</sup>	87± 2.4 <sup>a</sup>	92± 4.4 <sup>a</sup>	93±1.35 <sup>b</sup>
V	EARB (300 mg/kg b.w.)	94± 3.32 <sup>b</sup>	64±3.56 <sup>a</sup>	62±2.11 <sup>b</sup>	78±1.77 <sup>b</sup>	84±1.96 <sup>b</sup>	93± 2.8 <sup>ns</sup>

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group and standard group. Significant values are expressed as (a=p<0.001, b=p<0.05,) (\*\*= p<0.01, \*= p<0.05) (ns=non significant).

**Table 4.** Effect of extracts on Streptozotocin induced diabetic rats

Groups	Treatment	Blood glucose levels (mg/dl)			
		1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day
I	Control	82±1.6	83±2.25	89±2.06	80±2.56
II	Diabetic control	393±9.86	372±9.07	396±8.56	410±3.56
III	MERB (150 mg/kg b.w.)	357±5.63 <sup>**</sup> , <sup>a</sup>	323±8.8 <sup>a</sup> , <sup>b</sup>	313±18.2 <sup>ns</sup> , <sup>b</sup>	279±7.44 <sup>**</sup> , <sup>b</sup>
IV	MERB (300 mg/kg b.w.)	406±7.71 <sup>**</sup> , <sup>a</sup>	304±7.65 <sup>**</sup> , <sup>a</sup>	218±4.67 <sup>ns</sup> , <sup>a</sup>	158±7.1 <sup>**</sup> , <sup>a</sup>
V	EARB (150 mg/kg b.w.)	395±6.48 <sup>**</sup> , <sup>ns</sup>	375±6.22 <sup>a</sup> , <sup>b</sup>	363±7.56 <sup>ns</sup> , <sup>a</sup>	353±5.76 <sup>**</sup> , <sup>b</sup>
VI	EARB (300 mg/kg b.w.)	406±4.7 <sup>**</sup> , <sup>b</sup>	371±2.2 <sup>**</sup> , <sup>a</sup>	346±5.6 <sup>ns</sup> , <sup>b</sup>	318±6.3 <sup>**</sup> , <sup>a</sup>
VII	Standard Glimiperide (0.1 mg/kg b.w.)	452±10.56	271±10.44	175±7.23	83±2.23

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group and standard group. Significant values are expressed as (a=p<0.001, b=p<0.05,) (\*\*= p<0.01, \*= p<0.05) (ns=non significant).

**Table 5.** Effect of MERB and EARB on dexamethasone induced diabetic rats

Groups	Treatment	Blood glucose levels (mg/dL)	
		1 <sup>st</sup> day	11 <sup>th</sup> day
I	Control	84±1.6	81±2.2
II	Diabetic control	85±9.86	173±9.07
III	MERB (150 mg/kg b.w.)	91±2.2 <sup>b</sup> , <sup>*</sup>	123±4.7 <sup>*</sup> , <sup>a</sup>
IV	MERB (300 mg/kg b.w.)	89±3.12 <sup>**</sup> , <sup>a</sup>	101±3.12 <sup>**</sup> , <sup>a</sup>
V	EARB (150 mg/kg b.w.)	85±2.20 <sup>ns</sup> , <sup>b</sup>	139 ±2.14 <sup>ns</sup> , <sup>a</sup>
VI	EARB (300 mg/kg b.w.)	93±3.12 <sup>ns</sup> , <sup>b</sup>	127 ±2.14 <sup>ns</sup> , <sup>a</sup>
VII	Glimeperide (0.1 mg/kg b.w.)	86±10.44	87±10.44

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control group and standard group. Significant values are expressed as (a=p<0.001, b=p<0.05,) (\*\*= p<0.01, \*= p<0.05) (ns=non significant).

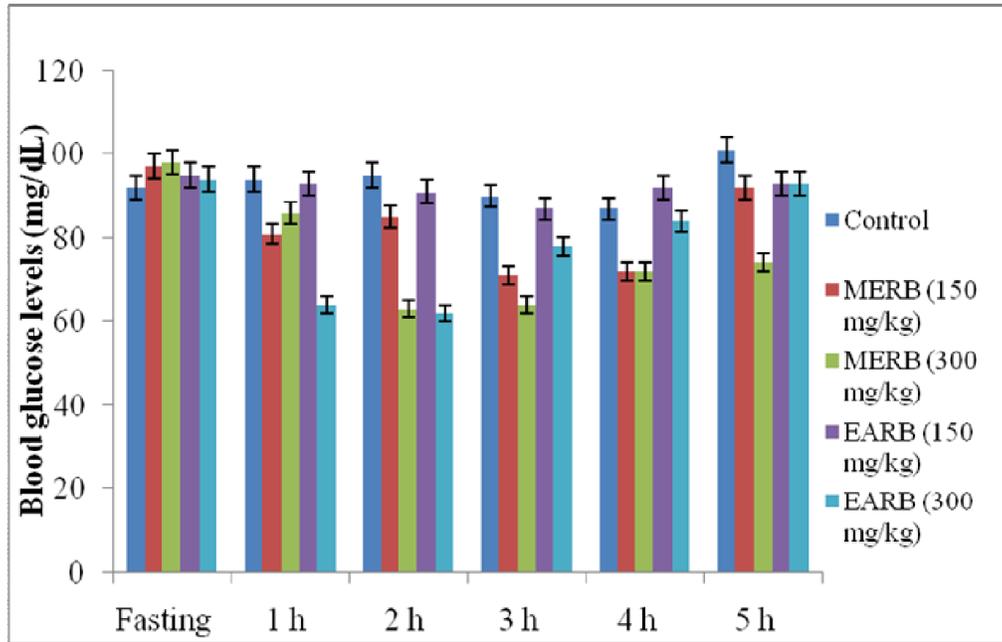


Figure 1. Effect of MERB and EARB extracts on oral glucose tolerance in rats

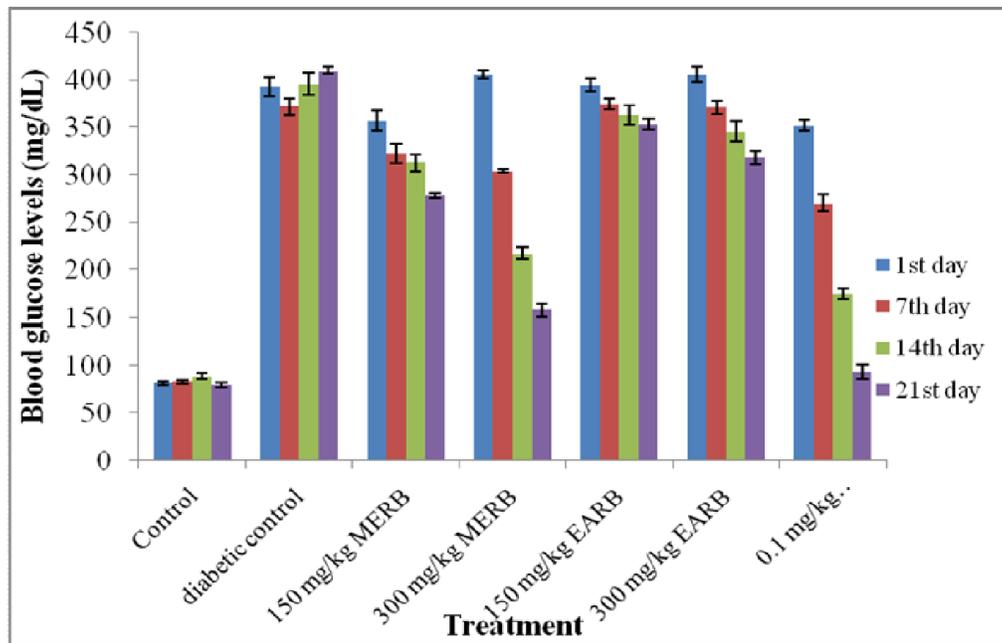


Figure 2. Effect of MERB & EARB on Streptozotocin induced diabetic rats

