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ANTIDIABETIC ACTIVITY OF *BAUHINIA RACEMOSA* ROOT EXTRACT ON ALLOXAN INDUCED DIABETIC RAT MODEL

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Abstract: Bauhinia racemosa, also known as 'Aapta' and belonging to the Fabaceae family, is widely utilized in traditional medicine for managing diabetes mellitus. In this study, the Ethanolic extract of Bauhinia racemosa roots (EEBR) underwent phytochemical analysis and was assessed for its potential antidiabetic effects on blood glucose levels in alloxan-induced diabetic rats. Both EEBR (at doses of 200 and 400 mg/kg) and Glibenclamide (administered at 0.5 mg/kg) were orally administered to rats induced with alloxan (100 mg/kg, i.p.). Notably, EEBR at the 400 mg/kg dosage exhibited a significant ($p<0.001$) reduction in blood glucose levels. Preliminary phytochemical analysis identified alkaloids, phenolics, flavonoids, saponins, and tannins as the major constituents in the ethanolic extract. These findings strongly suggest that EEBR, particularly at the 400 mg/kg dose, possesses antihyperglycemic properties in alloxan-induced diabetic rats.

Index Terms - Diabetes mellitus, Bauhinia racemosa, Roots, Alloxan, Glibenclamide, Blood Glucose Level, Alloxan-induced diabetes, Phytochemicals, Glucose tolerance, Ethanolic extract of bauhinia racemosa root, Blood glucose levels.

1. INTRODUCTION

The word "diabetes" was coined by Thomas Willis, who discovered sugar in the urine of diabetics in 1675. "Diabetes" means "polyurea" and "mellitus" means honey.[1] It is a family of metabolic diseases caused by various pathogenic mechanisms, all of which lead to hyperglycaemia.

The world's population is increasing. Both genetic and environmental factors contribute to the disease, including decreased insulin secretion, decreased insulin sensitivity, increased glucose production, and impaired fat and protein metabolism.[2]

While the World Health Organization estimated that approximately 30 million people had diabetes in 1985, this number is estimated to exceed 171 million in 2000.As of 2019, an estimated 463 million people worldwide had diabetes, accounting for 8.8% of the adult population 90% of diabetes cases are type 2 diabetes.[3] large increases will occur in developing countries, especially in people aged between 45 and 64 years.[4]

The major regulator of glucose concentration in the blood is insulin, a hormone synthesized in and secreted by the β cells of the islets of Langerhans in the pancreas (sometimes called beta cells). Hyperglycaemia may be due to a lack of insulin or to an excess of factors that oppose its action.[5]

- Type 1 Diabetes
- Type 2 Diabetes
- Gestational Diabetes

1. **Type 1 diabetes mellitus (T1DM**) is a chronic autoimmune condition characterized by elevated blood glucose levels resulting from insulin deficiency, which occurs due to the destruction of pancreatic islet β-cells. T1DM is among the most common endocrine and metabolic disorders seen in childhood. In the majority of cases (70—90%), the loss of β-cells is attributed to autoimmune activity related to T1DM.[6]
2. **Type 2 diabetes mellitus (T2DM)**, also known as non-insulin dependent diabetes mellitus (NIDDM), result from a reduction in target tissue for the metabolic effect of insulin. Decreased sensitivity to insulin is often referred to as insulin resistance.[4] Type 2 diabetes is characterized by insulin resistance and initially, a relative deficiency in insulin secretion. While plasma insulin levels (both fasting and meal-stimulated) are typically increased, they are insufficient relative to the severity of insulin resistance to maintain normal glucose levels. Over time, there is progressive beta cell dysfunction leading to insulin deficiency. [7]
3. **Gestational diabetes mellitus (GDM)** Women with a history of GDM are also at an increased risk of developing type II diabetes mellitus in the years following their pregnancy, and their children have a higher risk of developing obesity and type II diabetes at an early age. Therefore, it is crucial to carefully monitor and manage GDM to mitigate these risks.[5] GDM affects approximately 15% of pregnancies worldwide and is responsible for approximately 18 million birth per year.[8]

Unfortunately, apart from having number of side effects, none of the oral synthetic hypoglycaemic agents has been successful in maintaining euglycemia and controlling long-term microvascular and macrovascular complications. [5,12,13] Medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and in traditional system. The approximate composition, seed protein composition, minerals amino acid composition and antinutritional factors of *Bauhinia racemosa* were evaluated *Bauhinia racemosa* seed is rich in Ca and Fe. The essential amino acids lysine, tyrosine and phenylalanine are high in content, while sulfur amino acids are low. Section Total free phenols, tannins, levodopa and Phyto hemagglutination activity, And Antimicrobial, Antipyretic, Analgesic, anti-inflammatory activity shows that plant. [9,14,15,16] However, the antihyperglycemic activity of *Bauhinia racemosa* Root has not been reported. Hence, the present study

was under-taken to explore Antidiabetic activity of *Bauhinia racemosa* Root Extracts on alloxan induced diabetic rats.

2. MATERIALS AND METHODS

1. Collection and Authentication of plant

The Root of *Bauhinia racemosa* were collected from regional farm, Akola District, Maharashtra, India. The plants were identified and authenticated by Ms. J.B. Patil, Department of Botany, Vidyabharti Mahavidyalaya, Amravati. The roots were cleaned, dried in shade. The roots were kept in air tight container for further studies.

2. Experimental Animals

The experiment is performed on albino Wistar rats (weighing 150-250 gm), which are obtained from the Animal house of Department of Pharmacology. Vidyabharti college of pharmacy Amravati reg.no. 1504/PO/RE/S/11/CPCSEA. All the Animals are acclimatized to the animal house prior to use. They are kept in cages in animal house with n 12 h light: 12 h dark cycle. Animals are fed on pellets and top water ad libitum. The care and handling of rat were in accordance with the internationally accepted standard guidelines for use of animals (CPCSEA). Permission und approval for animal studies were obtained from the Institutional Animal Ethics Committee (IAEC) of Vidyabharti college of Pharmacy, Amravati. SGBAU) Amravati University.

3. Drugs and Chemicals

a. Inducing Agent:

- Alloxan Monohydrate (100mg/kg) was used to induced diabetes on rats.
- Product number: 00870 (10mg)
- CAS Number: 2244-11-3
  Was purchased from Loba Chemie, PVT, LTD, Mumbai, India.

b. Treatment drug:

- *Bauhinia racemosa* Plant were obtained from Regional Farm.

c. Standard drug:

- Glibenclamide manufactured by Chem Land Ind was purchased from Abmole Bioscience.

d. Others Chemicals & Reagents

Saline, Ethanol, acetone, Citrate buffer. Fehling's Reagent, Benedict's Reagent, etc. The chemicals used and other solutions were of analytical grade. All drugs and reagents were prepared immediate for use.

e. Equipment

Round bottom flask, Soxhlet Extractor and condenser, mesh sieve, glucometer, syringe, test tube, China dish, petri dish, etc.

4. Preparation of Extract:

The Dried roots of Bauhinia racemosa (250 gm) were extracted with ethanol: water (1:1) as solvent by Soxhlet extraction and solvents were evaporated and concentrated on water bath at controlled temperature. The yield of extract was found to be 14.41% w/w.

The percentage yield of the extract was calculated and the extract was then subjected to different phytochemical tests.

5. Preparation of Diabetic Rat:

Male Wistar rats weighing 150-250 g are deprived of food 18 h prior to the experiment but are allowed free access to water are used. Alloxan monohydrate 100mg/kg body weight was dissolved in normal saline (0.9% w/v) and injected intraperitoneally to induce hyperglycemia in experimental rats. The experimental animals were fasted for 18 hours before alloxan injection and the blood glucose level (BGL) was monitored after alloxanization in blood samples collected by tail tipping method using a Glucometer. Treatment was continued for 21 consecutive days, with once-a-day dose. Before the treatment (0 day) and at the end of 1- and 21-day, blood samples were collected from the tip of the tail of each rat under mild ether anaesthesia. The blood glucose level was monitored after 72 h of alloxanization.[10] The blood sample was collected by tail vein and glucose level was estimated using glucometer.[11] The animals which did not developed hyperglycemia i.e. glucose level > 200mg/dl, were rejected and replaced with new animals.[10]

6. Experimental Design

The rats were divided into five groups. Group I (controlled group/untreated normal rats), administered with Saline water, Group II (untreated diabetic rats), Group III (diabetic rats receiving Glibenclamide orally at 0.5mg/kg body weight in saline), Group IV and Group V (receiving200 mg/kg and 400 mg kg of body weight of test extracts respectively). Glibenclamide was used as the standard antidiabetic throughout the experimentation. They were carefully monitored every day. Animals described as fasted were deprived of food for at least 12 hours but allowed to free access for drinking water. Fasting blood glucose measurement was done on day 1, 7th, 14th and 21st of the study. Blood glucose levels were measured by glucometer.

7. Statistical analysis:

The data obtained from the screenings were subjected to statistical analysis following one-way ANOVA followed by Dunnett's Multiple Comparison Test to assess the statistical significance of the results using GraphPad Prism-5 software. *p<0.001 were considered as statistically significant.

3. RESULT

3.1 Pharmacognostical Examination.

% yield = (weight of the extract / weight of powder taken) × 100

table 3.1 percentage yield of roots of *bauhinia racemosa*

Drug	Leaves of *Bauhinia racemosa*
Percentage of yield	14.41 % w/w

table 3.2 physical examination of extract

Extract	**Colour**	**Odour**	**Solubility**
EEBR	Brown	Characteristics	In water

3.2 Phytochemical Testing:

table 3.3 phytochemical test:

SR. NO	CHEMICAL CONSTITUENTS	RESULTS
1	ALKALOIDS	-
2	FLAVONOIDS	+
3	CARBOHYDRATE	-
4	TANNINS	+
5	GLYCOSIDES	+
6	PHENOLS	+
7	SAPONINS	+
8	PROTEIN AND AMINO ACIDS	-
9	PHYTOSTEROLS	

Where, indicates (+) Present, (-) Absent.

3.3 Average Blood Glucose Profile:

In Alloxan treated diabetic rats, values of glucose level were elevated to high level during the study. Chronic treatment with the methanolic extract of *Lycopersicon esculentum* leaves at 200 and 400 mg/kg of body weight significantly ($P < 0.001$) causes decrease in blood glucose on 1, 7, 14th and 21st day as shown in table & figure.

table 3.4 average blood glucose profile

GROUP	TREATMENT	AVERAGE BLOOD GLUCOSE PROFILE			
		DAY 1	DAY 7	DAY 14	DAY 21
I	Control	86.17±0.9458*	84.0 ±1.291*	85.83±1.222*	87.83±0.6009*
II	Alloxan (100mg/kg)	297.8 ± 2.167	301.0±2.352	304.3±2.603	307.7 ±2.390
III	Alloxan(100mg/kg) +Glibenclamide (0.5mg/kg)	249.5 ± 7.805*	182.5±1.893*	139.0±1.693*	102.3±0.8433*

IV	Alloxan(100mg/kg) +EEBR (200 mg/kg)	289.0 ± 3.458	210.0±6.340*	151.0±3.256*	121.5±0.7188*
V	Alloxan(100mg/kg) +EEBR (400 mg/ kg)	288.2± 3.167	193.7±1.745*	143.7±1.308*	111.8±0.8724*

Values Expressed as Mean ± SEM: (n =6)

*p<0.001 compared with Alloxan(induced) group.

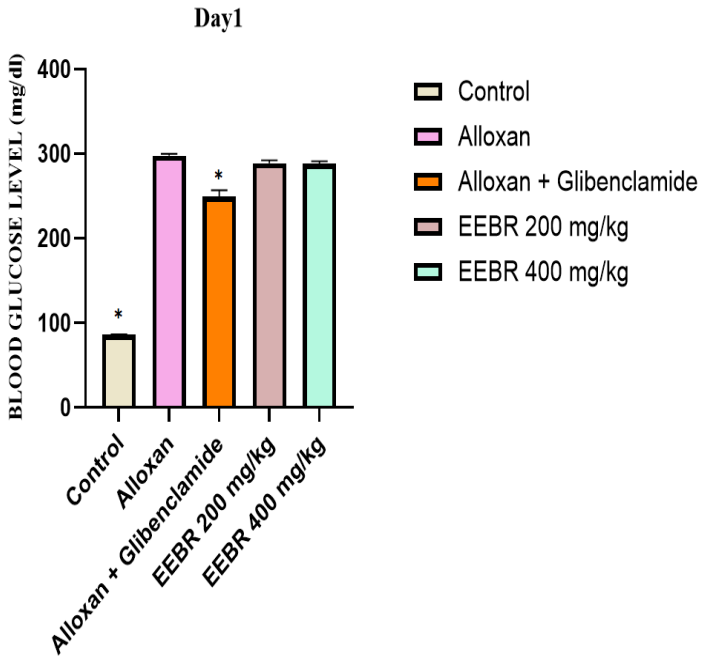

figure 3.1: effect of *bauhinia racemosa* on blood glucose level on 7th day

All Values are expressed as mean ± SEM (n=6). Statistical comparisons between each treatment groups with Alloxan(induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. *P<0.001 when compared to Alloxan(induced) group.

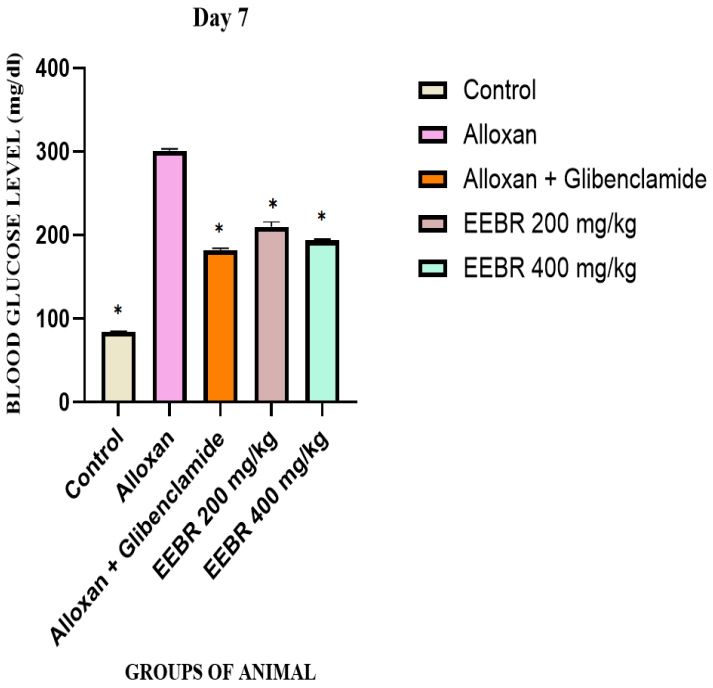

figure 3.2: effect of *bauhinia racemosa* on blood glucose level on 7th day

All Values are expressed as mean ± SEM (n=6). Statistical comparisons between each treatment groups with Alloxan(induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. *P<0.001 when compared to Alloxan(induced) group.

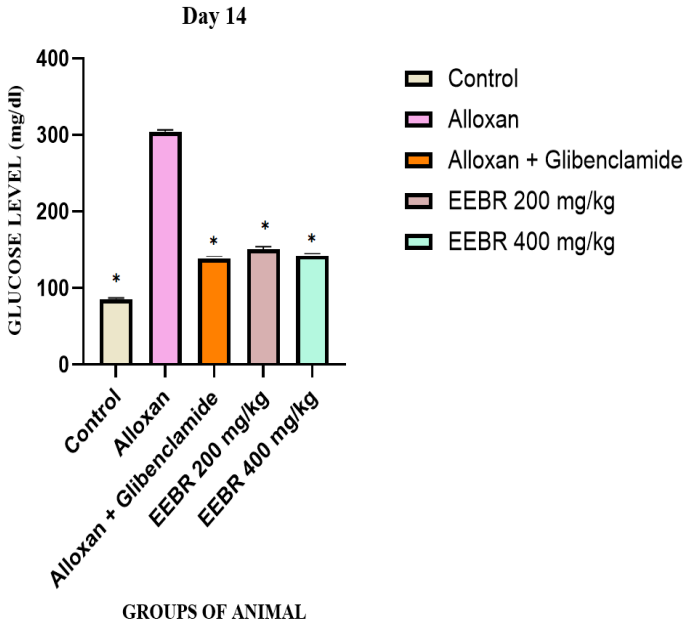

figure3.3: effect of *bauhinia racemosa* on blood glucose level on 14th day

All Values are expressed as mean ± SEM (n=6). Statistical comparisons between each treatment groups with Alloxan(induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. *P<0.001 when compared to Alloxan(induced) group.

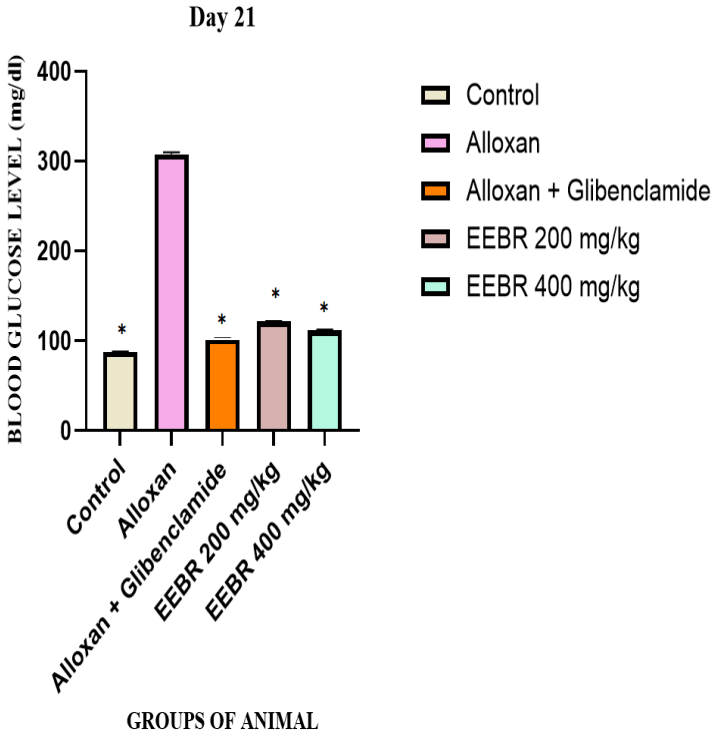

figure 3.4: effect of *bauhinia racemosa* on blood glucose level on 21st day

All Values are expressed as mean ± SEM (n=6). Statistical comparisons between each treatment groups with Alloxan(induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. *P<0.001 when compared to Alloxan(induced) group.

3.4 ORAL GLUCOSE TOLERANCE TEST

ORAL GLUCOSE TOLERANCE TEST OF *BAUHINIA RACEMOSA* ROOT EXTRACTS ON ALLOXAN INDUCED DIABETIC RATS.

table 3.5oral glucose tolarance test

Groups	Treatment	Oral Glucose Tolarance Test		
		Baseline	30 Min	90 Min
I	Control	87.83±0.6009	94.17±1.014*	86.33±1.116
II	Alloxan (100mg/kg)	307.7±2.390	331.8±4.778	367.2±1.249
III	Alloxan(100mg/kg) +Glibenclamide (0.5mg/kg)	102.3±0.8433*	183.3±4.869*	103.3±0.9545*
IV	Alloxan(100mg/kg) +EEBR (200 mg/kg)	121.5±0.7188*	205.3±1.282*	122.0±0.8563*
V	Alloxan(100mg/kg) +EEBR (400 mg/ kg)	111.8±0.8724*	194.2±1.537*	112.8±1.424*

VALUED EXPRESSED AS MEAN ± SEM: (N =6)

*P<0.001 COMPARED WITH ALLOXAN (INDUCED) RATS

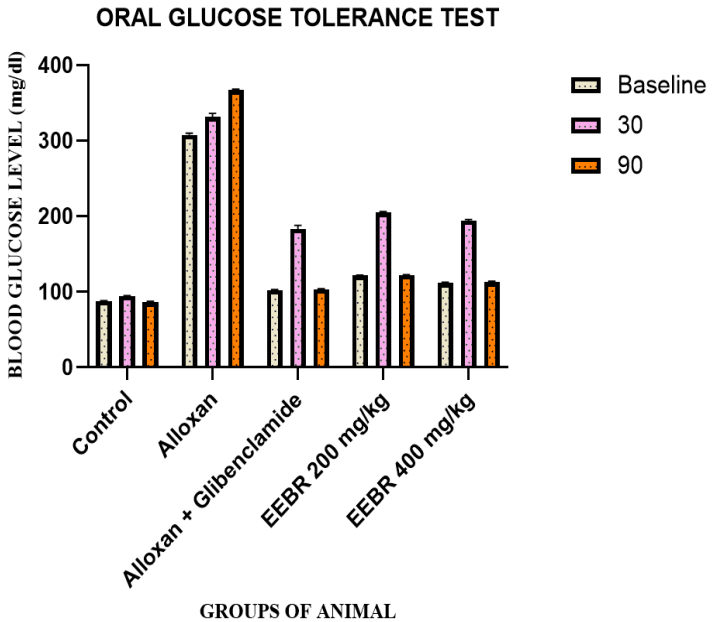

figure 3.5: oral glucose tolarance test

All Values are expressed as mean ± SEM (n=6). Statistical comparisons between each treatment groups with Alloxan(induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. *P<0.001 when compared to Alloxan(induced) group.

3.5 HbA1c (Glycated haemoglobin Test)

table 3.6: hba1c test

Group	Treatment	Before	After
I	Control	4.570±0.01633	4.717±0.1537*
II	Alloxan (100mg/kg)	4.710±0.03812	12.37±0.2519
III	Alloxan(100mg/kg) +Glibenclamide(0.5mg/kg)	4.960±0.01155	5.165±0.08366*
IV	Alloxan (100mg/kg) +EEBR (200 mg/kg)	4.920±0.01826	5.875±0.1260*
V	Alloxan (100mg/kg) +EEBR (400 mg/ kg)	4.990±0.06011	5.533±0.1644*

Valued Expressed as Mean ± SEM: (n =6)

*P<0.001 compared with Alloxan (induced) rats

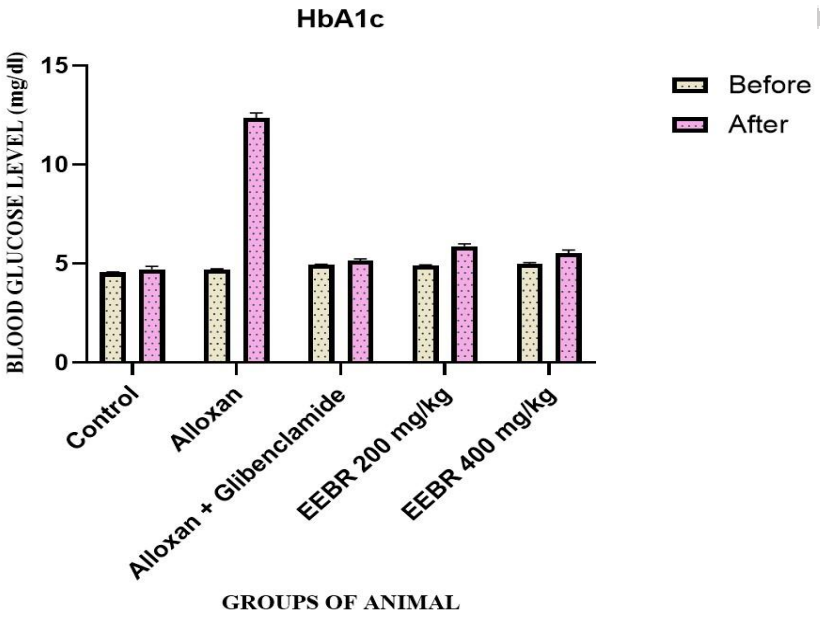

figure 3.6: hba1c test on before and after treatment

All Values are expressed as mean ± SEM (n=6). Statistical comparisons between each control before treatment groups & after Alloxan(induced) rats were carried out by one way ANOVA followed by Dunnet multiple comparison test. *P<0.001 when compared to Alloxan(induced) group

4. DISCUSSION

In this study, the ethanolic extracts of *Bauhinia racemosa* were assessed for their effectiveness in managing alloxan-induced diabetes. The diabetic model utilized in the study was indicative of type 2 diabetes, as the dose of alloxan (100 mg/kg) selectively targets a portion of pancreatic beta cells, resulting in insufficient insulin secretion. Following the induction of diabetes mellitus, continuous treatment with two different doses of ethanolic extracts of *Bauhinia racemosa* (200 and 400 mg/kg) was administered for a duration of 21 days. Glucose levels were monitored using a glucometer on the 1st, 7th, 14th, and 21st days of treatment. Throughout the 21-day treatment period, there was a gradual decrease in glucose levels. Initially, after the induction of diabetes, the rats exhibited glucose levels above 300 mg/dl, which subsequently decreased to approximately 289.0 ± 3.458 mg/dl and 288.2 ± 3.167 mg/dl after administration of the extract at doses of 200 mg/kg and 400 mg/kg, respectively, on the 1st day. By the 7th day of treatment, glucose levels further decreased to 210.0 ± 6.340 mg/dl and 193.7 ± 1.745 mg/dl with doses of 200 mg/kg and 400 mg/kg, respectively. On the 14th day, glucose levels lowered to 151.0 ± 3.256 mg/dl and 143.7 ± 1.308 mg/dl, eventually reaching normal levels by the 21st day, i.e., 121.5 ± 0.7188 mg/dl and 111.8 ± 0.8724 mg/dl with doses of the extract at 200 mg/kg and 400 mg/kg, respectively.

The oral glucose tolerance test (OGTT) was conducted to assess the EEBR Extract's efficacy in managing blood glucose levels in Alloxan-induced diabetic rats. Findings indicated that the positive control group (Alloxan-induced diabetic rats) had notably higher blood glucose levels at 30 minutes and 90 minutes compared to the negative control group (non-diabetic rats). Conversely, the groups administered with the EEBR at doses of 200 mg/kg and 400 mg/kg demonstrated reduced blood glucose levels compared to the positive control group. These results suggest that the EEBR Extract may have the potential to enhance glucose tolerance in diabetic conditions. Blood glucose levels were promptly measured using a Glucometer. The study also evaluated the impact of EEBR on glycosylated haemoglobin (HbA1c) levels within different treatment groups. HbA1c serves as a marker for assessing long-term glycaemic control, with elevated levels being associated with diabetic complications. Results revealed that the positive control group (Alloxan-induced diabetic rats) displayed significantly higher HbA1c levels compared to the negative control group (non-diabetic rats). Conversely, the groups administered with EEBR at doses of 200 mg/kg and 400 mg/kg exhibited lower HbA1c levels in comparison to the positive control group. This suggests that EEBR may contribute to improving long-term glycaemic control in diabetic condition The phytochemical screening of *Bauhinia racemosa* roots extract revealing the presence of tannins, flavonoids, glycosides, phenols, and saponins suggests a rich chemical composition with potential health benefits. These constituents have been associated with various pharmacological effects, including antidiabetic properties The observed antidiabetic effect of the crude ethanolic extract, particularly at a dose of 400mg/kg, further supports the potential therapeutic application of this plant in managing diabetes.

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5. CONCLUSION

In conclusion, the extract derived from *Bauhinia racemosa* roots demonstrated notable antihyperglycemic effects in alloxan-induced diabetic rats. Additionally, the ethanolic extract exhibited enhancements in the regeneration of pancreatic β-cells, indicating its potential therapeutic value in diabetes treatment. Based on the findings, this study indicates that the ethanolic extract of *Bauhinia racemosa* root demonstrated considerable anti-hyperglycaemic activity in alloxan-induced hyperglycaemic rats. This effect is likely attributed to the presence of bioactive compounds such as flavonoids, tannins, glycosides, phenols, and saponins in the extract. The findings of this study demonstrate that *Bauhinia racemosa* root extract restores fasting blood glucose levels to normal in alloxan-induced diabetic rats, indicating hypoglycaemic activity and the chemical compounds responsible for this effect. *Bauhinia racemosa* root Extract has the potential to serve as a natural source of medicinal compounds, containing key therapeutic chemical constituents that play a crucial role in preventing and managing different types of diseases. Efficacy of this extract is appreciably good when compared to standard drug Glibenclamide. Finally, it can be concluded that *Bauhinia racemosa* root extract exhibited significant ($P<0.001$) anti-diabetic activity in alloxan-induced diabetic rats.

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