Research Article

Evaluation of hypoglycemic activity of stevia rebaudiana in alloxan induced diabetesin albino rats

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Abstract

Background: The leaves of Stevia rebaudiana have been used in treating diabetes in traditional medicine. Currently Stevia is used as a sweetener but its leaf extract has shown several medicinal properties including hypoglycemic activity. Several animal and human models have demonstrated the hypoglycemic activity of stevia. The present study is designed to compare the hypoglycemic activity of refined stevia extract (90.1 % leaf extract) with standard oral hypoglycemic drug glibenclamide. **Methods:** 36 albino rats with FBS in the range of 80-115 mg/dl were selected for the study. Four groups each containing six rats, were induced diabetes with Alloxan (150mg/kg). They were sub divided into diabetic control (received 0.5 ml normal saline), standard control group (received 5mg/kg glibenclimide), test group-I (received 100 mg/kg Stevia), test group-II (received 200 mg/kg Stevia). Other two groups each containing six rats were non-diabetic, i.e. non diabetic control and non-diabetic test groups and received 0.5 ml normal saline and 100 mg/ kg of Stevia respectively. FBS was recorded on 1, 3, 7, 14, 21and28th day. Data was analysed by using one way ANOVA and Posthoc Tukey's test. **Results:** Refined extract of Stevia showed dose dependent hypoglycemic action in both low dose (100 mg/kg) and high dose group (200 mg/kg). It does not produce hypoglycemia in non-diabetic rats. Hypoglycemic action with high dose Stevia is comparable to that of standard oral hypoglycemic drug glibenclamide. **Conclusion**: Our study demonstrates the hypoglycemic action of stevia in diabetic rats and no hypoglycemic activity in non-diabetic rats, which is an added advantage over conventional antidiabetic drugs which are known to cause hypoglycemia as an adverse effect. This is in accordance with other studies done in animal models. Stevia can be a therapeutic potential to treat type 2 diabetes mellitus.

Keywords: Stevia, diabetes, hypoglycemic action.

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, action or both[1]. Once regarded as a single disease entity, diabetes mellitus is now seen as heterogeneous group of diseases characterized by hyperglycemia resulting from various causes.[2]The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels.[1, 3] According to recent estimates, the prevalence of diabetes in adults was 4% in 2000 and it is projected to be 5.4% by 2005. In India its prevalence is 2.4% in rural

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and 4 to 11.6% in urban dwellers.[2] Various drugs are being used in the treatment of diabetes mellitus but the search for more effective drugs with fewer side effects is on.[4] as an alternative, plant extracts and products are under evaluation. Stevia rebaudiana is a natural non-caloric sweetener and is a native of north eastern Paraguay. Historically the leaves of Stevia plant were used as sweetener and as herbal remedy.[5] Studies done with leaf extract of Stevia have shown hypoglycemic action in both animal and human model.[6, 7] So in this study an effort has been made to investigate the hypoglycemic activity of Stevia rebaudiana and its comparison with currently used oral hypoglycemic agent Glibenclamide.

AIMS AND OBJECTIVES

The aim of the current study is to evaluate the hypoglycemic activity of refined extract of the herb Stevia rebaudiana. The main objectives of this study are To evaluate the hypoglycemic activity of Stevia rebaudiana in alloxan induced diabetes in albino rats. To compare the hypoglycemic activity of Stevia rebaudiana with a currently used oral hypoglycemic Glibenclamide.

METHODOLOGY

Materials

Chemicals: Alloxan monohydrate, Glibenclamide hydrochloride [(Tab. Daonil, 5mg (Aventis Pharma Limited)], Sodium chloride, Refined (leaf extract 90.1% purity) Stevia extract (S.J. Herbals, Bangalore).

Animals: Albino rats

Equipments: Mouth gag, polythene feeding tube, tuberculin syringe and insulin syringe (each lml), Glucometer (Roche diagnostics).

Chemicals

1. **Alloxan monohydrate:** Alloxan monohydrate dissolved in 0.9% sodium chloride solution (normal saline) is used in this study.
2. **Sodium chloride:** It is used to prepare normal saline in distilled water.
3. **Glibenclamide:** Glibenclamide belongs to second generation sulfonylureas. In this study it is taken as the standard drug and results are compared with test drug.
4. **Refined Stevia extract**: used in our study was supplied by S.J.Herbals Ltd, Bangalore

Animals

Animals used are albino rats, of wilstar strain, weighing between 180-220 grams of either sex. The animals were fed with standard laboratory food and water.

Equipments

1. **Mouth gag:** To facilitate the introduction of feeding tube in to the stomach of the rat and administer the drugs.
2. **Feeding tube**: The polythene infant feeding tube was used for oral administration of the drugs.
3. **Tuberculin syringe:** Used for injecting Alloxan in to peritoneal cavity and for ministering the drugs at proper dosage.
4. **Capillary tubes:** for drawing blood from retro orbital veins.
5. **Glucometer:** The glucometer used in this study is the Accu-Chek sensor from Roche diagnostic corporation.

Parts of glucometer

a. Display showing glucometer reading
b. On and off switch
c. Slot to insert test strip and test strips
d. Code strip slot and code strip
e. Check strip

Methodology

Before using the instrument, 2 AA size strips are inserted in to the respective slot and switched on. Then it is calibrated to the respective code by inserting code strip in its respective slot. Now the check strip is inserted in to the slot for test strip until the screen displays "ok" which means the glucometer is properly working. Now a drop of blood is collected and spread on the test strip slot for blood, taking care not to leave any blank space or bubbles. Now the glucometer gives the readings in 30 seconds. Test is repeated with other samples of blood.

Advantages of Glucometer

1. It requires only one drop of blood.
2. It is simple and easy procedure.
3. Results are obtained immediately.
4. It gives a good estimate of glucose levels compared to laboratory methods, (Coefficient of variation ±10%).
5. Test strips can be stored at room temperature.

Methods

The method employed in this study to induce diabetes was chemical method using alloxan monohydrate, given intraperitoneally. Blood glucose estimations were made by using glucometer. Albino rats of Wistar strain with body weight of 180-220 grams were used for the study. Animals were procured from central animal house of department of pharmacology, J.J.M Medical College, Davangere, inbred and grown under suitable laboratory conditions.

Inclusion criteria

- Body weight 180-220 grams.
- Healthy with normal Behaviour.

Exclusion criteria

- Rats weighing more than 220 grams and less than 180 grams.
- Pregnant female and those which have delivered once.

Fasting blood glucose readings were recorded in all rats after an overnight fasting. B1ood samples were obtained from retro bulbar technique, after ether anaesthesia. Blood glucose was estimated by using glucometer. The albino rats with FBS in the range of 80-115mg/dl were selected for the study.

Induction of diabetes

Alloxan monohydrate was used to induce diabetes mellitus. After an overnight fasting, the rats were injected freshly prepared 2% solution of alloxan monohydrate in 0.9% sodium chloride solution (normal saline). The dose injected was 150mg/kg body weight. Following injection, animals were observed for 24-48 hours for evidence of any allergic reaction, behavioural changes, convulsions and hypoglycemic symptoms. No untoward reaction was observed in any animal. Fasting blood glucose was estimated at around 9:30am daily until stable hyperglycemia was established. Rats which developed a stable hyperglycemia with FBS of more than 200mg/dL were selected for the study. They were divided in to six groups as follows.

NDCG: Non diabetic control group **(group-1)**
NDTG: Non diabetic test group **(group-2)**
DCG: Diabetic control group **(group-3)**
SC: Standard control **(group-4)**
TEST-I: Diabetic test group-I (100mg/kg) **(group-5)**
TEST-II: Diabetic test group –II (200mg/kg) **(group-6)**

Non-diabetic control

This group consisted of non-diabetic rats, used as controls for the study. This group received 0.5ml of normal saline daily for 30 days. FBS was recorded on day 1,3,7,14,21 and 28.

Non-diabetic test

This group consisted of non-diabetic rats, used as controls for the study. This group received 100mg/kg body wt of stevia daily for 30 days. FBS was recorded on day 1,3,7,14,21 and 28. The rats in the following four groups were rendered diabetic by injection of *2%* solution of alloxan monohydrate, intraperitoneal in a dose of 150mg/kg body weight. FBS was recorded on day 1,3,7,14,21 and 28.

Diabetic control: received 0.5ml of normal saline daily for 30 days.

Standard control: received glibenclamide in a dose of 0.5mg/kg body weight suspended in normal saline orally, daily for 30 days.

Test group-I: received 100 mg/kg body wt. of refined Stevia extract orally, daily for 30 days. The dose was selected based on previous studies and the effect was observed.

Test group-II: Received 200 mg/kg body wt. of refined Stevia extract orally, daily for 30 days. The dose was selected based on previous studies and the effect was observed.

Observation for side effects

Throughout the experiment, the animals were keenly observed for occurrence of any visible side effects like tremors, loss of body weight, behavioral changes, convulsions, sedation.

STATISTICAL ANALYSIS

Mean, standard deviations were calculated for each group. One way ANOVA was used for multiple group comparisons followed by post hoc Tukey's test for statistical significance between groups. P values less than 0.05 were considered to be significant.

RESULTS

Table 1: Mean (sd) values of blood glucose levels in different group of animals

Groups	DAY 1	DAY 3	DAY 7	DAY 14	DAY 21	DAY 28
NDCG	100.7 ± 10.2	99.7 ± 11.3	95.3 ± 11.8	96.0 ± 11.7	100.3 ± 10.3	101.7± 10.3
NDTG	115.0 ± 11.5	99.8 ± 8.6	104.0 ± 16.6	96.5 ± 11.2	96.7 ± 11.9	100.3 ± 9.4
DCG	401.7 ± 38.0	402.2 ± 39.2	398.0 ± 36.8	395.3 ± 40.4	386.0 ± 34.9	394.7 ± 34.9
SC	403.0 ± 42.3	348.0 ± 47.8	293.7 ± 36.7	260.3 ± 27.7	184.7 ± 22.9	139.2 ± 19.7
TEST-I	420.3 ± 34.8	393.0 ± 33.4	341.8 ± 36.5	361.5 ± 31.4	333.3 ± 20.9	315.7 ± 17.7
TEST-II	477.8 ± 24.4	418.3 ± 38.7	372.3 ± 35.3	310.0 ± 35.7	245.0 ± 25.1	209.0 ± 17.9

NDCG: Non diabetic control group **(group-1)**
NDTG: Non diabetic test group **(group-2)**
DCG: Diabetic control group **(group-3)**
SC: Standard control **(group-4)**
TEST-I: Diabetic test group-I (100mg/kg) **(group-5)**
TEST-II: Diabetic test group –II (200mg/kg) **(group-6)**

Table 2: Comparison of percentage reduction in blood glucose levels between standard and test groups

Groups	DAY 1	DAY 3	DAY 7	DAY 14	DAY 21	DAY 28
SC	4.6%	17.8%	30.6%	38.3%	56.3%	67.1%
TEST-I	4.5%	10.8%	22.4%	17.8%	24.1%	28.1%
TEST-II	1.8%	14.1%	23.6%	36.4%	49.4%	56.9%

Table 2 a: overall mean percentage reduction of blood glucose levels in standard and test group

Groups	Mean Percentage Reduction
SC	35.7%
TEST-I	17.9%
TEST-II	30.36%

Table 3: Statistical analysis of comparison of blood glucose levels between different groups on day 1

Groups	Mean+/-SD	F* Value	Significance
DCG	401.7+/-38.0	6.09	P<0.05 Significant
SC	403.0+/-42.3		
TEST-I	440.3+/-34.8		
TEST-II	477.8+/-24.4		

Table 3 a: Oneway ANOVA Test

Groups compared**	Mean Difference	P value
3and4	1.3	NS
3and5	38.6	NS
3and6	76.1	S
4and5	37.3	NS
4and6	74.8	S
5and6	37.5	S

** Post Hoc Tukey's TestS= Significant P<0.05 NS= Not significant P>0.05

Table 4: Statistical analysis of comparison of blood glucose levels between different groups on day 3

Groups	Mean+/-SD	F* Value	Significance
DCG	402.2+/-39.2	3.17	P<0.05 Significant
SC	348.0+/-47.8		
TEST-I	393.0+/-39.4		
TEST-II	418.3+/-38.5		

* Oneway ANOVA Test

Groups compared**	Mean Difference	P value
3and4	54.2	NS
3and5	09.2	NS
3and6	16.1	NS
4and5	45.0	NS
4and6	70.3	S
5and6	25.3	NS

** Post Hoc Tukey's TestS= Significant P<0.05 NS= Not significant P>0.05

Table 5: Statistical analysis of comparison of blood glucose levels between different groups on day 7

Groups	Mean+/-SD	F* Value	Significance
DCG	398.0+/-36.8	9.12	P<0.05 Significant
SC	293.7+/-36.7		
TEST-I	341.8+/-36.5		
TEST-II	372.3+/-35.5		

* Oneway ANOVA Test

Groups compared**	Mean Difference	P value
3and4	104.3	S
3and5	56.2	NS
3and6	25.7	NS
4and5	48.1	NS
4and6	79.6	S
5and6	30.5	NS

** Post Hoc Tukey's TestS= Significant P<0.05 NS= Not significant P>0.05

Table 6: Statistical analysis of comparison of blood glucose levels between different groups on day 14

Groups	Mean+/-SD	F* Value	Significance
DCG	395.3+/-40.4	18.0	P<0.05 Significant
SC	260.3+/-27.7		
TEST-I	361.5+/-31.4		
TEST-II	310.0+/-35.7		

* Oneway ANOVA Test

Groups compared**	Mean Difference	P value
3and4	135.0	S
3and5	33.8	NS
3and6	85.3	S
4and5	101.2	S
4and6	49.7	NS
5and6	51.5	S

**Post Hoc Tukey's TestS= Significant P<0.05NS= Not significant P>0.05

Table 7: Statistical analysis of comparison of blood glucose levels between different groups on day 21

Groups	Mean+/-SD	F* Value	Significance
DCG	386.0+/-34.9	68.9	P<0.05 Significant
SC	184.7+/-22.9		
TEST-I	333.3+/-20.9		
TEST-II	245.0+/-25.1		

*Oneway ANOVA Test

Groups compared**	Mean Difference	P value
3and4	201.3	S
3and5	52.7	S
3and6	141.0	S
4and5	148.6	S
4and6	60.3	S
5and6	88.3	S

** Post Hoc Tukey's TestS= Significant P<0.05 NS=Notsignificant P>0.05

Table 8: Statistical analysis of comparison of blood glucose levels between different groups on day 28

Groups	Mean+/-SD	F* Value	Significance
DCG	394.7+/-34.9	136.8	P<0.05 Significant
SC	139.2+/-19.7		
TEST-I	315.7+/-17.7		
TEST-II	209.0+/-17.9		

* Oneway ANOVA Test

Groups compared**	Mean Difference	P value
3and4	255.5	S
3and5	79.0	S
3and6	185.7	S
4and5	176.5	S
4and6	69.8	S
5and6	106.7	S

** Post Hoc Tukey's TestS= Significant P<0.05 NS= Not significant P>0.05

ANALYSIS OF RESULTS

The results have been statistically analyzed for significance by using one way analysis of variance (ANOVA) for multiple group comparisons followed by Post Hoc Tukey's Test.

'F' value was calculated using the formula

$$F = \frac{\text{Variance between groups}}{\text{Variance within groups}}$$

In this study, the FBS of animals selected for the study was in the range of 80-115 mg/dl the mean BGL before induction of diabetes in all six groups varied from 94.2-

103.0mg/dl. Both non-diabetic control group and non-diabetic test group did not vary in there BGL throughout the study period. For analysis of results the following groups are considered

- Diabetic control group
- Standard control group
- Test group-I
- Testgroup-II

Table 1 shows variation in blood glucose levels from day 1 to day 28 in each group. Mean BGL in non-diabetic control and non-diabetic test varied between 95.3 to 115.0 mg/dl throughout the study period. In diabetic control group the BGL ranged from 401mg/dl on day 1 to 394.7 mg/dl on day 28. In standard control group there was significant reduction of BGL from day 1(403.0mg/dl) today 28 (139.2 mg/dl) compared to the diabetic control group. In test group-1which received low dose Stevia of 100mg/kg, the BGL decreased appreciably from day 1 to day 7, followed by decrease in BGL on day 21 and day 28 to a minimum of 315.2mg/dl. In test group -II which received high dose Stevia of 200mg/kg, there was no significant reduction of BGL on day 1, but BGL decreased gradually from day 3 to day 28 to a minimum of 209.0 mg/dl. Table.2a shows % reduction in BGL in different groups on day 1, 3, 7,14,21,28. Standard group shows more reduction in BGL (67.1%) compared to test groups (test 1-28.1%, test II-56.9%) at the end of the study. Table.2b shows that the mean % reduction of test II (30.36%) is comparable to that of standard group (35.7%). Table.3-8 give statistical analysis of the results on day 1, 3, 7,14,21,28 by using one way ANOVA followed by post hoc turkeys test. The p value of <. 05 was considered significant. On day 1, there was significant difference between test group II compared to DCG, SC, TEST I. On day 3, there was no statistically significant difference between any groups except for the comparison of standard control group with test II group. On day 7, there was statistically significant difference for the comparison of SC with DCG and TEST II and no significant difference exists between rest of the groups. On day 14, except for the comparison DCG with TEST I and SC with TEST II there was significant difference between all other groups. On day 21 and 28, the difference is maintained in all groups. On day 21 there was better glycemic control in the standard group compared to both the test groups, whereas on day 28 the glycemic control was better with standard group but test II shows comparable glycemic control than test I.

DISCUSSION

In this study the hypoglycemic action of Stevia rebaudiana in the form of refined leaf (90.1% purity) extract has been evaluated and its efficacy is compared with that of standard oral hypoglycemic drug glibenclamide. In our study, low dose Stevia (100mg/kg) decreased blood glucose level (BGL) from 420.3 mg/dl on day 1 to 315.7 mg/dl on day 28 and high dose(200mg/kg) stevia decreased BGL from 477.8mg/dl to 209.0 mg/dl on day 28 (Table.1).The results show that refined extract of Stevia has definitive hypoglycemic activity. The present study is in accordance with the previous studies done by Jeppesan et. al (2002) and Raskovic et.al (2000) who reported the hypoglycemic action of Stevia.[8,9] The percentage reduction in BGL during the study period is 17.9% for low dose Stevia (100mg/kg) and 30.3% for high dose Stevia (200mg/kg) (Table.2A). This shows the dose dependent activity of Stevia. Study done by Jeppesan et. al (2003) has shown antihyperglycemic activity, insulinotropic and glucoganostatic activity of Stevia which suggests that Stevia can be used for treating both type-II DM and metabolic syndrome.[10] In our study Stevia did not produce hypoglycemia in non-diabetic test group (BGL-115 mg/dl on day 1 to 100.3 mg/dl on day 28(Table.1), which suggests that it might have antihyperglycemic activity and no hypoglycemic activity in normal rats. Studies show that Stevia induces insulin secretion only in the presence of high plasma glucose level which supports our above observation.[6] This can be a huge advantage in the therapy of diabetes mellitus, since one of the important adverse effect of using conventional anti diabetic drugs is hypoglycemia. In the present study the standard control drug glibenclamide has shown maximum hypoglycemic activity (35.75% reduction in BGL) and high dose Stevia (200mg/dl) by producing 30.3% reduction showed comparable hypoglycemic activity to that of glibenclamide (Table.2A). The active principles of refined extract of Stevia are stevioside and steviol. These might be responsible for hypoglycemic activity of Stevia.[8]

Mechanism of action

Stevia produces hypoglycemic activity which could be attributed to its several facets of action which are responsible for reduction in BGL. Stevia has shown to increase insulin secretion from β cells which is known as 'first phase insulin response' or acute insulinotropic effect. It also concomitantly suppresses glucagons levels. These two effects i.e.insulinotropic and glucogonostatic effects are mainly responsible for its hypoglycemic activity.[8] The mechanism of action also includes decrease in gluconeogenesis by decreasing PEPCK (phosphoenol pyruvate carboxy kinase) and induction of glycolysis which contribute to the hypoglycemic activity of Stevia.[11] In addition to this Stevia is also shown to increase whole body insulin sensitivity and improved insulin action on skeletal muscle glucose transport in experimental rats. So

Stevia also reduces insulin resistance which is an added advantage.[12] So in conclusion our study shows antihyperglycemic activity of Stevia and no hypoglycemic activity in normal rats which is an advantage over conventional antidiabetic drugs. The efficacy of high dose Stevia (200mg/kg) is comparable to that of standard control glibenclamide.

Limitations of the study

The study has several limitations. The study has been carried out only in one species of animals viz "rats" and needs to be extended to other animals as well. In Our study we have used 100mg/kg as low dose Stevia and 200mg/kg as high dose Stevia. Further studies need to be done to fix proper dosage. Only the fasting blood glucose was estimated in this study, which does not give a clear picture about the effect of Stevia on post prandial glucose, glucose tolerance and other parameters of diabetes mellitus which needs to be studied. Further elaborate studies are needed to evaluate insulin levels, c-peptide levels and serum lipid levels. Acute and chronic toxicity testing need to be undertaken.

CONCLUSION

At the end of the study it can be concluded that:

- Refined Stevia extract has hypoglycemic effect in diabetic rats and it does not have hypoglycemic action in normal rats.
- The hypoglycemic activity is comparable to that of glibenclamide in diabetic rats.

Thus refined Stevia extract could be used as an oral hypoglycemic agent in diabetes. However further extensive studies need to be done to confirm this activity in animal models as well as human trials.

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