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Research Article

ANTI - HYPERGLYCEMIC EFFECTS ON *STEVIA REBAUDIANA* ON ALLOXAN INDUCED DIABETIC RATS

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Abstract

Stevia rebaudiana (Asteraceae) ispopular in the name 'Sweet tea' or 'khaa gee because of its pleasant flavor apart from increasing the sweetness of the product. Traditional medicine accounts for its use externally as anti -inflammatory and internally asanemia and as contraceptive. The aim of the study was to investigate anti hyper glycemic activities ofAqueous leaves extract of *Stevia rebaudiana* using alloxan induced diabetic rats. Aqueous leaves extract of *Stevia rebaudiana* was prepared by cold maceration process method. Antihyperglymic activity of the extract was examined by alloxan induced rats with extracts in the concentration of 100 mg/kg body weight (b.w.).The blood was collected from hearts after sacrificing rats. Then the serum was separated by centrifugation at 2500 rpm. The serum was then stored in the refrigerator for 48hrs till the assays were done. The serum was used for the determination of biochemical parameters. From the results it was observed that the blood glucose sugar level reduced. The aqueous leaves extract *Stevia rebaudiana* showed significant protection against diabetes

Keywords: *Stevia Rebaudiana*, blood glucose, Alloxan, Carbohydrate, Albumin

INTRODUCTION

Hyperglycemia or diabetes is one of the most commonly occurring problems around the globe. Technically it is known as Diabetes mellitus. It is the single most important metabolic disorder. Diabetes mellitus is a chronic heterogeneous group of disorders in carbohydrate, protein and lipid metabolism of an absolute or relative deficiency in action of insulin or possibly abnormally high amounts of glucagons growth hormone and other insulin antagonistic substances[1].Diabetes mellitus occurs both in children and adults. It is of different types. Insulin dependent Diabetes Mellitus (IDDM) also known as type 1. Percentage of occurrence of this type of diabetes constitutes less than 5%. If often occurs in childhood and adolescence and requires daily injections of insulin to sustain life. This type of diabetes is characterized by complete lack of insulin secretion due to injury to the β- cells of pancreas[2]**.** Non -insulin dependent diabetes mellitus (NIDDM) also known as type 2. It is a very common form of diabetes and constitutes about 80-90% of diabetes. Unlike IDDM patients who do not secrete enough insulin, problem with NIDDM patients is that they are resistant of action of insulin secreted. Insulin is a protein hormone secreted by β- cells of islets of Langerhans of pancreas. It plays an important role in metabolism causing increased carbohydrate metabolism, glycogenesis, and glycogen storage, fatty acid synthesis, Triglyceride storage and amino acid uptake or protein synthesis[3]**.** Recently the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinedione, sulponylureas D-phenylamine and *α*- glucosidase inhibitors in addition to insulin. Howeverdue to unwanted side effects the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes [4,5].

Hence plants have been suggested as a rich, as yet unexplored source of potentially useful antidiabeticdrugs. Many traditional plants of treatment for diabetes are used throughout the world. Plant drugs and herbal formulations are frequently considered to be less toxic and free from side effects than synthetic one [6].

The anti -hyperglycemic effect of these plants are for their ability to restore the function of pancreatic tissues by increasing insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Medicinal plant has been recently an increasing interest to treat DM. Ethnobotanical information indicates that more than 500 plants are used as traditional remedies for DM treatment [7]. Hence treatment with herbal drugs has an effect on protecting β- cells and smoothing out fluctuation in glucose levels [8].

Stevia rebaudiana (family- asteraceae) is one of them which are herbaceous perennial plant native to subtropical and tropical rainforest areas of south America (Brazil, Venezuela, Colombia and Paraguay)[9]. The leaves are used traditionally in various regions of the world including China, Japan, Korea, Taiwan, Thailand, Malaysia and Paraguay, India and Bangladesh etc. The leaves have been known to contain 100 useful alkaloids among other pharmacologically active compounds. It has been used for the treatment of diabetes and its anti- diabetic effect has been evaluated in diabetic animals in many countries and significant hypoglycemic activities of powdered form of Stevia (*Stevia rebaudiana*) leaves have been reported [10]. *Stevia rebaudiana* is the single sweetener which has antidiabetic activity. To our knowledge no systematic studies have been carried out to find out the effect of aqueous extract of *Stevia rebaudiana*on carbohydrate metabolic enzymes in control and experimental animals. Therefore the present study was an attempt to find the Antidiabetic property in aqueous extract of *Stevia rebaudiana*on the alloxan induced diabetic male albino rat.

MATERIALS AND METHODS:

Plant materials: Fresh leaves of *Stevia rebaudiana*were collected from Salem and Dharmapuri district of Tamilnadu.The plants were authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PADC), Sakthinaga, West Tambaram, Chennai.After thorough washing the leaves were dried completely under mild sun and ground in electric grinder into a coarse powder.

Extraction of the plant material: The dried coarsely powdered leaves of *Stevia rebaudiana*were extracted by cold maceration process. The aqueous extract of *Stevia rebaudiana* was prepared by weighed quantity of powder was placed into the maceration tank and 10 volumes of water was added. This content was macerated for 24 hours, first six with occasional shaking and allowed to stand for remaining hours. After 24 hours of maceration the extract was filtered and solvent evaporated to get dried extract. This dried extract was used for various experimental purposes.

Selection of Experimental Animals: In the present study**,** healthy adult male albino rats were used as experimental animal. A total number of 18 long-Evans male rats were weighing about 120-240gm, age 1 month were selected and housed five in a polypropylene cage. Prior to commencement of the experiment all the rats were acclimatized to the new environmental condition for a period of two weeks. During the experimental period the rats were kept in ventilated animal house at room temperature of 25˚C.

Grouping of experimental rats: 18 long –Evans male rats were randomly assigned into 3 groups, 6 rats in each group.

Group 1 Normal control

Group 2 Diabetic control

Group 3 Diabetic + Aqueous fraction SR (100mg/kg)

Experimental induction of diabetes: Group 1 animals were used for normal control receives only vehicles (normal Saline) Group 2 animals were allowed to fast for 12 hrs and were induced diabetic by injection intraperitoneally a freshly prepared solution of alloxan (60mg/kg) in normal saline after base line glucose estimation was done. The alloxan treated animals were allowed to feed over night to overcome drug induced hyper glycemia. Group 3 animals were treated intraperitoneally with single dose of alloxan and co-administrated with aqueous extract of *stevia rebaudiana* (100mg/kg).

Test of Anti -hyperglycemic effect of plant extracts:

The blood was collected from hearts after sacrificing rats. Then the serum was separated by centrifugation at 2500 rpm. The serum was then stored in the refrigerator for 48 hours. It was used for the analysis of estimation of glucose, estimation of

protein, determination of Albumin and determination of Globulin. The concentrations were absorbance by UV spectrophotometer (Shimidzu UV-1200, Tokyo, Japan).

Estimation of Glucose (King Astoor method)

The main principle of the method was glucose present in the sample. It reacts with copper sulphate in alkaline medium. Copper sulphate is reduced to cuprous oxide. About 3.8 ml of isotonic sodium sulphate –copper sulphate solution and 0.1ml of 10% of sodium tungstate were measured separately and transferred to 0.1ml blood containing centrifuge tube. Then the solution was mixed well and the mixture was centrifuged for 10 minutes and the supernatant was collected. About 1.0 ml supernatant and 1.0 ml of alkaline tartrate solution were measured and transferred in a test tube. Tubes were plugged tightly with cotton wool and the contents were heated in a boiling water bath for 10 minutes. Then it was cooled, 8 ml of phosphomolybdic acid and 3 ml of water was added. About 0.2-1.0ml of working standard solution was taken in a series of test tubes and volume was made upto1ml with distilled water. To this, 3ml of phosphomolybdic and 3ml of distilled water were added. A reagent blank was also prepared. The tubes were kept at room temperature for 10 minutes. Then the solution color was developed and measured at 680 nm colorimetrically.

$$\text{Glucose (mg/dl)} = \frac{Sample\ O.D}{Standard\ O.D} X\ Standard\ conc$$

Estimation of Total Serum protein (Biuret Method):

About 6.0ml of sulphate – sulphite solution was measured and transferred into centrifuge tube and 4.0 ml of serum solution was added and inverted to mix well. From that 2ml of the mixture was pipetted out and 3ml of biuret solution was added. About 0.4-2.0 ml of various concentration of standard solution was pipetted out and transferred into centrifuge tube (S1- S5) and made up to 2 ml with 0.9% sodium chloride. Then 2ml of biuret reagent was added. The colour intensity of the solution was measured at 550nm using green filter.

Determination of Serum Albumin:

About 3ml of ether was added to rest of the serum sulphate – sulphite mixture. The solution was shaked for 40 times twice each second for 20 seconds and centrifuged for 5 minutes. Thin film of globulin layer was formed. From this, 2ml of solution was pipetted out without disturbing the precipitate and 3ml of biuret reagent was added in the test tube. The developed colour intensity was measured at 550nm colorimetrically.

$$\text{Protein, Albumin, Globulin (g/dl)} = \frac{Sample\ O.D}{Standard\ O.D} X\ Standard\ conc$$

Results and Discussion

The effect of the estimation of glucose, determination of protein, Albumin and globulin were investigated in the control and Alloxan induced diabetic male albino rats.

The results were shown in table – 1 and figure-1. From the results it was observed that significant elevation in blood glucose level of alloxan induced diabetic rats and it was compared more than 300% as control. But the treatment of alloxan induced diabetic rats with the stevia extract was show about 40% decreases in blood sugar level when compared with the diabetic rats. The diabetic rats treated with stevia showed about 40% decreasein blood sugar level when compared with the diabetic rats though the blood glucose level was not restored to control level. It could be attributed to the inadequate dosage of stevia extract. These experiment observations reveal that stevia extract has hypoglycemic / antidiabetic effect.Alloxan causes massive reduction in insulin release through β cells destruction of islets of Langerhans[11]. When blood sugar level increases, the condition is called as hyperglycemia. Excess of glucose in the blood causes osmotic disturbance and affects the life of the red blood cells[12].An insulin deficiency exists, glucose utilizations do not take place properly and oxidation isnot complete. The glucose transport across the bio membrane is not facilitated due to the deficiency / absence of insulin [13]. It is evident from group III that *stevia rebaudiana* leaves extract increases the glucose utilization. The active compound is present in the aqueous extract of *Steviarebaudiana*may be used as an effective treatment for diabetes.

Table -1 Determination of Blood glucose

Group of Animals	Mean value ± SD	Group Compared	% Increase (↑) % Decrease (↓)	t-Value
Group-1 Group- II	101.00 ± 6.83 410.25 ± 51.53	Group-I Vs Group-II	306.19 (↑)	P<0.01
Group-1 Group- III	101.00 ± 6.83 247.50 ± 22.45	Group-I Vs Group-III	145.05 (↑)	P<0.01
Group-1I Group- III	410.25 ± 51.53 247.50 ± 22.45	Group-II Vs Group-III	39.67 (↓)	P<0.01

P<0.01 Significant at 1% level

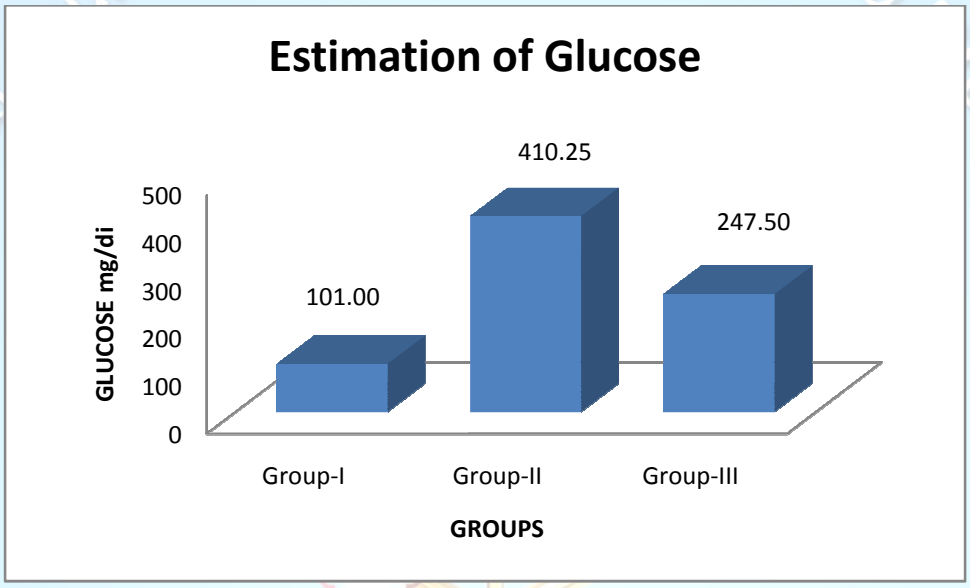

Figure-1 Estimation of Glucose

Serum total protein was moderately decreased in diabetic mellitus rats as compared to normal healthy rats. Total protein level decreased significantly in alloxan induced diabetic rat groups. This may produce due to diminished synthesis of proteins or diminished uptake of amino acids[14]which in turn could be attributed to decrease on absence of insulin in experimental diabetic rats, insulin was more important for the transport of protein synthesis. But the experimental diabetic rats treated with stevia show more than 25% serum total protein level than experimental diabetic rats indicating that the stevia could reverse the inhibition of protein synthesis observed in alloxan induced diabetic rats. Decrease in total proteins is called as hypoproteinemia. Serum protein analysis is regarded as an important procedure for estimating the degree of damage of parenchyma of liver**.** Result from the present studies was demonstrated that during alloxan induced diabetes, there was increased fluidity of membrane and thereby enhanced passive transport of various metabolites. This influence the activity of proteins [15]only moderate changes were exhibited by treated aqueous extract male albino rats. The observed results were shown in table-2 and figure-2.

Table -2 Determination of Total serum Protein

Group of Animals	Mean value ± SD	Group Compared	% Increase (↑) % Decrease (↓)	t-Value
Group-1 Group- II	18.05 ± 3.68 14.00 ± 2.79	Group-I Vs Group-II	22.44(↓)	P<0.05
Group-1 Group- III	18.05 ± 3.68 17.60 ± 2.76	Group-I Vs Group-III	2.49 (↓)	P>0.05
Group-1I Group- III	14.00 ± 2.79 17.60 ± 2.76	Group-II Vs Group-III	25.71 (↑)	P>0.05

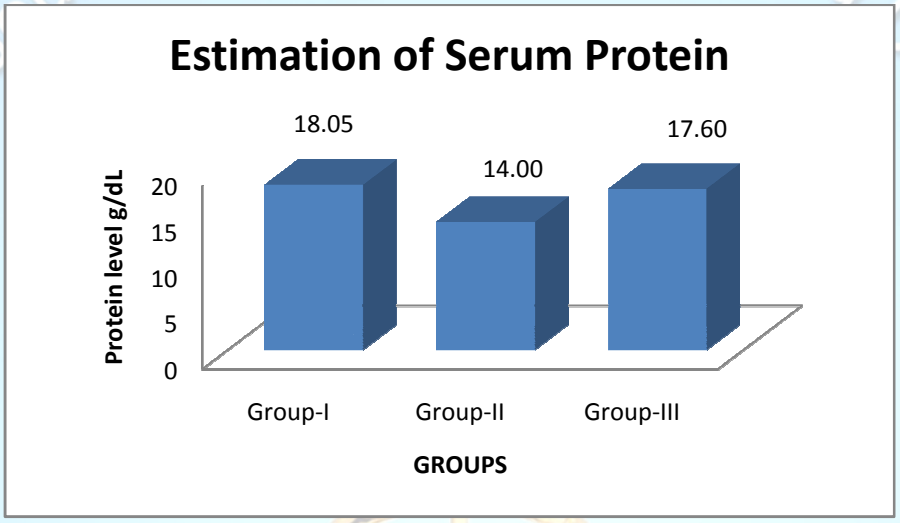

Figure-2 Estimation of Total Serum Protein

Alloxan administrated rats, albumin level in serum was reduced. It could be seen that in group II the level was decreased and the group III the level was moderately elevated. Albumin constitutes more than half of total proteins of plasma. It helps in transport of several substances like thyroxine and drugs. It also plays an importan important role in exchange of water between tissue fluid and blood. In experimental diabetic animal, albumin was decreased by more than 40% as compared to control reported by the literature [16], [17]. In the same case [18] inhibitors of albumin promote activity in the cell-were found, free system in the liver of alloxan induced diabetic rats. Whereas, experimental diabetic rats treated with stevia showed more than 40% increase in the level of serum albumin when compared with the experimental diabetic rats. It showed that stevia aqueous leaves extract could reverse the impairment of albumin synthesis by alloxan as observed in total serum protein.The results of analysis were shown in table -3 and figure -3.

Table -3 Determination of Serum Albumin

Group of Animals	Mean value ± SD	Group Compared	% Increase (↑) % Decrease (↓)	t-Value
Group-1 Group- II	8.50 ± 1.28 5.00 ± 2.79	Group-I Vs Group-II	22.44(↓)	P<0.05
Group-1 Group- III	18.05 ± 3.68 17.60 ± 2.76	Group-I Vs Group-III	2.49 (↓)	P>0.05
Group-1I Group- III	14.00 ± 2.79 17.60 ± 2.76	Group-II Vs Group-III	25.71 (↑)	P>0.05

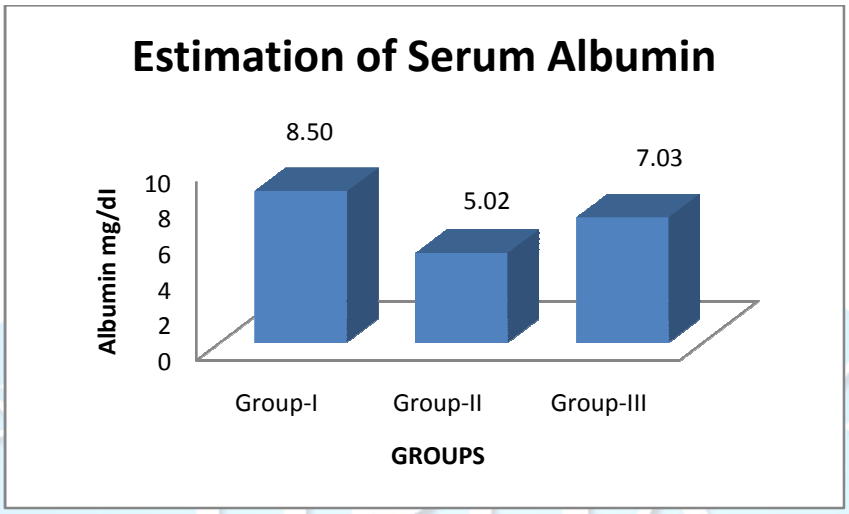

Figure-3 Estimation of Serum Albumin

Table -4 Determination of Serum Globulin

Group of Animals	Mean value ± SD	Group Compared	% Increase (↑) % Decrease (↓)	t-Value
Group-1 Group- II	7.60 ± 0.80 3.48 ± 0.48	Group-I Vs Group-II	54.17(↓)	P<0.01
Group-1 Group- III	7.60 ± 0.80 4.55 ± 0.78	Group-I Vs Group-III	40.13(↓)	P>0.01
Group-1I Group- III	3.48 ± 0.48 4.55 ± 0.78	Group-II Vs Group-III	30.62 (↑)	P<0.05

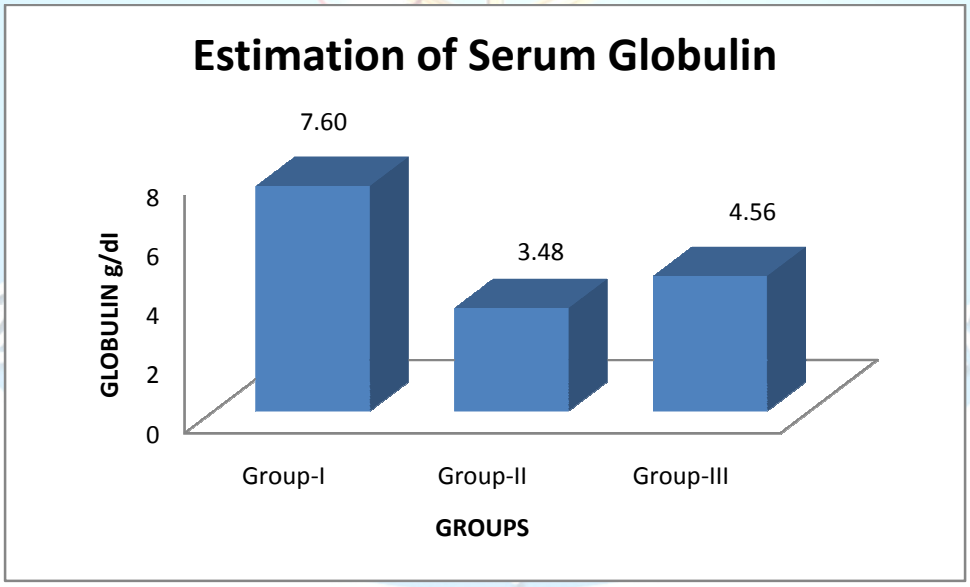

Figure-4 Estimation of Serum Globulin

Serum globulin usually were elevated in drug induced cholestasis or extra hepatic obstruction [19]. Liver was also getting affected in alloxan induced diabetes; globulin synthesis was affected and reduced. The results were shown in table-4 and figure -4.

Serum total protein, albumin and globulin level in alloxan induced diabetic rats were decreased by 50% as compared to control. Whereas, the diabetic rats treated with stevia aqueous leaves extract showed increase in level of serum globulin by more than 30% as compare to diabetic rats, indicating the anabolic effect of stevia extract on protein synthesis.

CONCLUSION:

Diabetes mellitus was presently a burden not only for the individuals affected by the disease but also on the society, particularly the national health systems, life style disorder so it requires a different yardstick for management. The present investigation was aimed to study the effect of alloxan induced diabetes on various biochemical parameters. The results of our study revealed adverse –changes in the blood sugar level test in alloxan induced diabetic rats. It showed that the alloxan was potent diabetogenic chemical used effectively to induce diabetes in the experimental rats. When the alloxan induced diabetic rats were treated with stevia leaves aqueous extract. It was observed that the blood sugar level was reduced. From the findings of the study it could be concluded that *Stevia rebaudiana* plant possess hypoglycemic effect in experimental diabetic rats.

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