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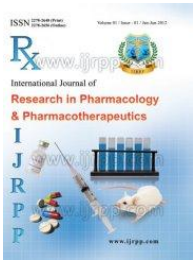

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An anti-diabetic activity of *stevia rebaudiana* with vitamin E

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ABSTRACT

The present research work was carried out to find out the alternative medication for treating the diabetes mellitus and its further complications such as atherosclerosis, pre-eclampsia and male and female infertility, end-stage renal disease, IHD, gangrene of the lower extremities, and blindness in the adults with poor glycemic control. Therefore, rationally selected the combination of leaf extract of Stevia rebaudiana and vitamin E supplement. The phytochemical tests of plant extract of Stevia rebaudiana revealed the presence of Alkaloids, Carbohydrates, Flavonoids, Phenolic compounds, proteins. This study is an attempt to find out an adjuvant medication with hypoglycemic action in combination therapy which has shown beneficial effect in animal models maybe useful in supplementary dosage.

Keywords: Diabetes mellitus, Diabetes mellitus complication, Stevia, vitamin E, Adjuvant medication

INTRODUCTION

Stevia is grown all across the world; it is known as stevia for its sweet leaves. The leaves are the source of diterpene glycosides, stevioside and rebaudioside. Stevioside is non-caloric and is reported to be 30 times sweeter than sugar. In India diabetic patients are increasing day by day and according to world diabetic foundation it has the world's largest diabetes population, followed by China with 43.2 million and it has major concern among health experts and national and international healthcare [1]. A recent scientific trail shows that this herb has many health benefiting. Stevia contains phytochemical compound that helps to cure blood sugar, cholesterol and blood pressure. It is used as natural sweetener and is having low calorie.

Following health benefits has been noticed about Stevia:[1]

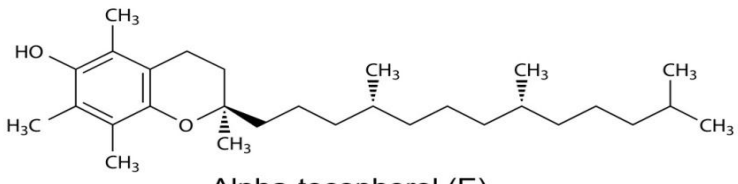
- Stevia is having low calorie as compared to sugar.
- Chlogenic acids reduce conversion of glycogen to glucose and reduces absorption of glucose and reduce blood sugar level. It is used as a flavor enhancer, taste enhancer and anti bacterial effect.
- It is heat stable at high temperature can be cooked with tea and food.

- Stevia can be used widely in Jams, Sauces, Jelly, Confections, Beverages, Pharmaceuticals and in Alcoholic beverages and in Dental products.
- It is used in tea, coffee and dairy products.
- Leaf has stevioside of 10% to 12% on dry basis, even 50 gm of Stevia leaf can replace 1000g of cane sugar.
- The sweetness of stevioside is non-fermenting and it does not display browning while cooking.

World health organization (WHO) has identified diabetes as an epidemic condition [2] and one of the major killer of the decade. Estimation by WHO, there will be about 250 million cases of diabetes mellitus throughout the world by 2025[3]. Though various drugs are available in Indian market for diabetic control like oral hypoglycemic agents and insulin for the treatment of diabetes mellitus, however the therapy with oral hypoglycemic agents is not satisfactory. Several drugs such as biguanides and sulfonylureas are presently used to reduce hyperglycemia in diabetes mellitus. Even these drugs have side effects and management of diabetes without any side effect is big a challenge to the medical community. Along with mainline anti diabetic drugs, several add on like multivitamins and trace minerals are used in the therapy[1].

Stevia rebaudiana

Vitamin E is the term for a group of tocopherols and tocotrienols, of which a-tocopherol has the highest biological activity. Due to the potent antioxidant properties of tocopherols, the impact of α-tocopherol in the prevention of chronic diseases believed to be associated with oxidative stress has often been studied, and beneficial effects have been demonstrated. Vitamin E is a term that encompasses a group of potent, lipid-soluble, chain-breaking antioxidants. Structural analyses have revealed that molecules having vitamin E antioxidant activity include four tocopherols (α, β, g, d) and four tocotrienols (a, b, g, d)[4] One form, a-tocopherol, is the most abundant form in nature In 1922, Evans and Bishop discovered vitamin E—a micronutrient essential for reproduction in rats.[5] The antioxidant activity of vitamin E has persuaded many groups to study its ability to prevent chronic diseases, especially those believed to have an oxidative stress component such as cardiovascular diseases, atherosclerosis, and cancer.



Alpha-tocopherol (E)

Diabetes Mellitus (DM), a state of chronic hyperglycemia, is a major cause of serious micro and macrovascular diseases, affecting, therefore, nearly every system in the body. Growing evidence indicates that oxidative stress is increased in diabetes due to overproduction of reactive oxygen species (ROS) and decreased efficiency of antioxidant defenses, a process that starts very early and worsens over the course of the disease. During the development of diabetes, oxidation of lipids, proteins and DNA increase with time. Mitochondrial DNA mutations have also been reported in diabetic tissues, suggesting oxidative stress-related mitochondrial damage. Diabetes- related oxidative stress may also be the trigger for many alterations on sexual function, which can also include decreased testicular mitochondrial function. Although sexual disorders have been extensively studied in diabetic men, possible changes in the sexual function of diabetic women have only recently received attention. The prevalence of sexual dysfunction in diabetic men approaches 50%, whereas in diabetic women it seems to be slightly lower. Testicular dysfunction, impotence, decreased fertility potential and retrograde ejaculations are conditions that have been described in diabetic males. Diabetes is also the most common cause of erectile dysfunction in men. Poor semen quality has also been reported in diabetic men, including decreased sperm motility and concentration, abnormal morphology and increased seminal plasma abnormalities. In addition, diabetic men may have decreased serum testosterone due to impaired Leydig cell function. Among diabetic women neuropathy, vascular impairment and psychological complaints have been implicated in the pathogenesis of decreased libido, low arousability, decreased vaginal lubrication, orgasmic dysfunction, and dyspareunia. An association between the production of excess radical oxygen species and disturbed embryogenesis in diabetic pregnancies has also been suggested. [6]In fact, maternal diabetes during pregnancy is associated with an increased risk of complications in the offspring, such as altered fetal growth, polyhydramnios, fetal loss and congenital malformations.

The increasing incidence of DM, particularly in affluent Western societies, is contemporaneous with falling fertility and birth rates [7] Significant development in the last 10 years in the study of human infertility has been the discovery that oxidative sperm DNA damage has a critical role in the etiology of poor semen quality and male infertility.

Supplemental vitamin E may improve semen quality and have beneficial and protective effects, especially on sperm motility[8].

Oxidative stress is increased in patients with polycystic ovary syndrome. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. Increased homocysteine levels and decreased antioxidant capacity may contribute to the increased risk of cardiovascular disease in women with PCOS, in addition to known risk factors such as insulin resistance, hypertension, central obesity, and dyslipidemia. So, the treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidative damage. [9]

Antioxidant supplementation has been shown to improve insulin sensitivity and other health threating conditions in women with PCOS [10, 11]

Diabetes has significant impact on fertility of affected population, Infertility is a significant public health problem and diagnosis and treatment are stressful, invasive, and costly. Hence attempt has been made here to combine supplemental therapy of vitamin E with *Stevia rebaudiana* to prove it's hypoglycemic effect as adjuvant over it's proven antioxidant activity.

MATERIALS AND METHODS

Drugs

Fresh leaves of Stevia rebaudiana were collected from botanical garden, Dombivali,Thane District Maharashtra and Authentication is done. The authenticated leaves of Stevia rebaudiana was extracted with methanol in Soxhlet extractor. Dried methanolic extract used further for the study. Further, these extracts subjected to a battery of phytochemical tests to evaluate the constituents. Drug used as standard was Afdiex (Glibenclamide). Vitamin E was the marketed preparation. Alloxan purchased from Thomas Baker.

Animal

The animals used for experiments were healthy adult Swiss-albino mice age 3-4 weeks (25g-30g) were procured from Bharat Serum and Vaccines Pvt. Ltd. Thane, Mumbai. Maintained under controlled

temperature (22±2^0 C) and humidity (55±5%) with 12h light-dark cycle (lights on from 7:00am to 7:00 pm), with food and water available ad libitum.

Experimental design

Animals were divided into different groups of 6 animals each. Group I served as normal control group receives vehicle (distilled water) only. Group II was untreated diabetic mice. Group III, IV and V were diabetic mice administered Stevia leaves extract 300mg/kg[12], vitamin E 20 mg/kg[13], Stevia300mg/kg + Vit E 20mg/kg respectively.

Induction of Diabetes mellitus

In experiment Diabetes mellitus was induced in all groups except control group following overnight fasting (deprived of food for 12 hr allowed free access to water) by single intraperitoneal injection of 150 mg/kg of Alloxan (ALX)[12] dissolved in fresh distilled water. The animals of control group (Group I) were injected with distilled water alone. Alloxan injected animals were given glucose solution for 24 hr following Alloxan injection to prevent initial drug-induced hypoglycemic mortality. After 5days of ALX-injection, animals with fasting blood glucose above 140mg/dl were considered as diabetic and included in the study.Blood samples were collected from tail - vein method to calculate approximately glucose levels were estimated by using glucometer (Accu chek, Roche, Germany) glucose oxidase peroxidase method using strips for all groups.

Statistical Analysis

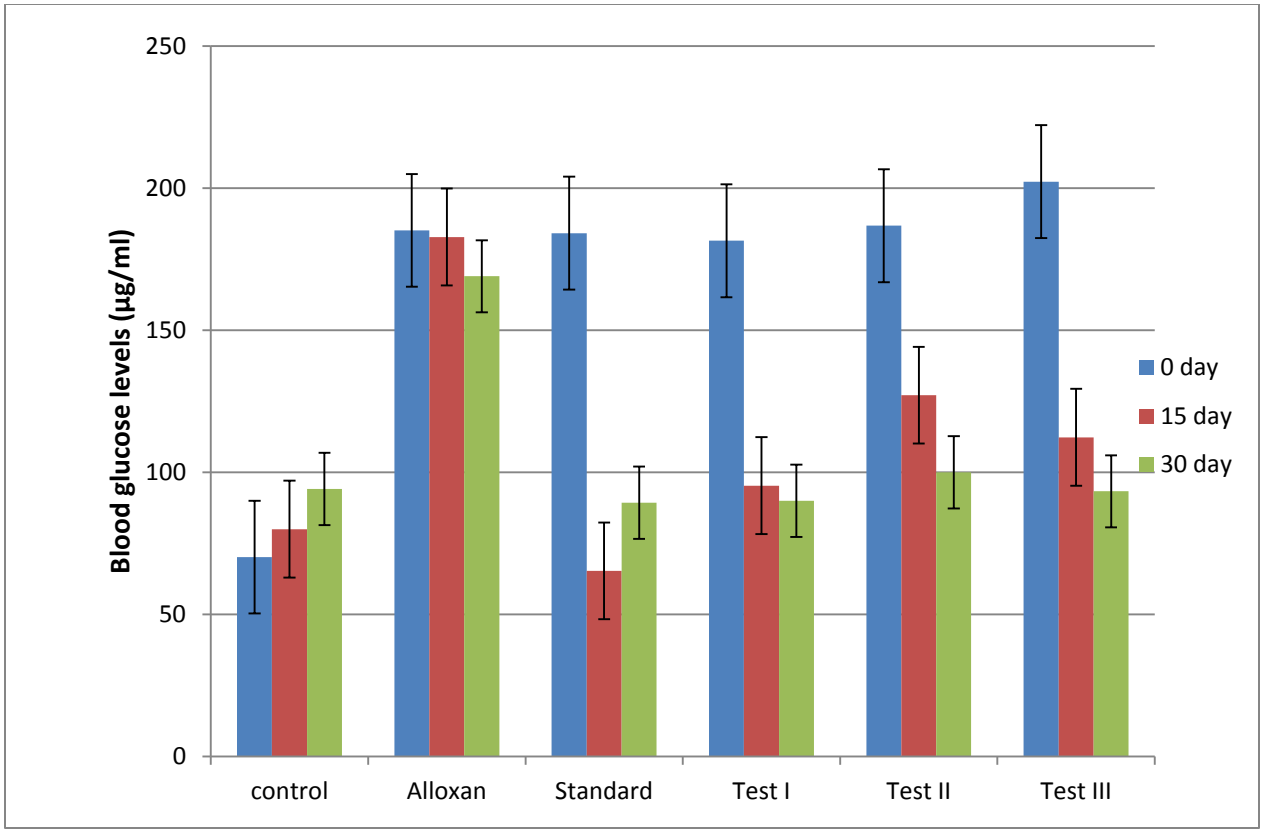
Values are expressed as Mean ± Standard error of mean. Statistical analysis was performed by one-way analysis variance (ANOVA) followed by Tukey test by using GraphPad Prism Version 7, San Diego USA. The results were considered statistically significant if $p< 0.05$.

RESULT

Alloxan induced Anti-diabetic activity

	Dose	**Glucose level**		
		0 day	**15 days**	**30 days**
Control	0.5 ml/25gm Distilled water	70.16±3.79	80±4.02	94.16±7.18*
Diabetic Positive Control	150mg/kg Alloxan monohydrate	185.16±5.16	182.83±6.50	169±5.46*
Standard	5mg/kg Glibenclamide	184.66±6.60	95.33±3.29	89.33±9.22*
Test I	300mg/kg *Stevia rebaudiana*	181.5±7.23	95.33±10.68	90±8.19*
Test II	20mg/kg Vit E	186.83±16.82	127.16±12.65	100±5.55*
Test III	300mg/kg *Stevia +20mg/kg Vit E*	202.33±8.30	112.33±8.39	93.33±6.28*

Each value is given as Mean ± S.D. (n= 6) One way ANOVA followd by tukey test ***p<0.001, **p<0.01, *p<0.05 when comparing to corresponding values of control.



Effect of *Stevia rebaudiana* with vitamin E in Alloxan Induced diabetic mice.

Methanolic extract of Stevia rebaudiana leaves with vitamin E significantly ($p<0.05$) reduced blood glucose levels in Alloxan induced diabetic mice.

DISCUSSION

Stevia leaf extract has been used traditionally in the treatment of diabetes [14, 15]. Their ingestion causes a slight suppression of plasma glucose levels and significantly increased glucose tolerance in normal adult humans [16]. Steviol glycosides have an enhancing effect on insulin secretion by directly acting on β-cells without altering the K+ - ATP channel activity and cAMP level in the islets, thus documenting stevioside and steviol as potent antihyperglycemic agents [17]. Stevioside regulate blood glucose levels by enhancing not only insulin secretion, but also insulin utilization in insulin-deficient rats; which was due to decreased phosphoenolpyruvate carboxykinase (PEPCK) gene expression in rat liver by stevioside's action of slowing down gluconeogenesis suggested by Chen et al.[17]. Overall, *Stevia* possess the ability to increase the insulin effect on cell membranes, increase insulin production, stabilize glucagon secretion and blood sugar levels, and improve glucose tolerance to ingested carbohydrates and lower post-prandial blood sugar levels in both animals and humans. In other words, *Stevia* is shown to provide a comprehensive set of mechanisms that counter the mechanics of type II diabetes and its eventual complications. Thus, sugars can be replaced with steviol glycosides or stevioside of *Stevia* leaf to support healthy glucoregulation. The addition of leaves of *Stevia*, dried or in powder form in supplementary food products of diabetic patients aid in increasing the natural sweetness and also help in rejuvenating the pancreatic gland.[18]

In the present study, administration of AqF of methanolic extract of S.rebaudiana and vit E resulted in significant reduction in fasting blood glucose levels on various days of treatment. The standard hypoglycemic drug, Glibenclamide (5mg/kg) trated diabetic mice also exhibited significant reduction in fasting blood glucose levels. Since, S. rebaudiana and vit E reduced the blood glucose levels potently in the ALX- diabetic mice like glibenclamide, it may have

hypoglycemic principle that are similar in action to glibenclamide.

CONCLUSION

Diabetes mellitus is a multifactorial, multi-systemic endocrine disorder characterized by persistent hyperglycemia resulting from the defects in insulin secretion, action or both. Diabetes mellitus is expected to continue as a major health problem owing to its serious complications,especially end-stage renal disease, IHD, gangrene of the lower extremities, and blindness in the adults with poor glycemic control. Growing evidence indicates that oxidative stress is increased in diabetes due to overproduction of reactive oxygen species (ROS) and decreased efficiency of antioxidant defences. Oxidative stress has been associated with numerous adverse health effects including atherosclerosis, pre-eclampsia and male and female infertility.

The present research work was carried out to find out the alternative medication for treating the diabetes mellitus and its further complications such as atherosclerosis, pre-eclampsia and male and female infertility. Therefore, rationally selected the combination of leaf extract of Stevia rebaudiana and vitamin E supplement. The phytochemical tests of plant extract of Stevia rebaudiana revealed the presence of Alkaloids, Carbohydrates, Flavonoids, Phenolic compounds, proteins. The evaluation of hypoglycemic effect in oral glucose tolerance test and anti-diabetic activity in alloxan induced diabetic mice showed significant result ($p<0.05$).

This study is an attempt to find out an adjuvant medication with hypoglycemic action in combination therapy which has shown beneficial effect in animal models maybe useful in supplementary dosage.

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