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**Original Research Article**

# Comparing the Antidiabetic Effects of Aqueous Extracts of *Stevia rebaudiana* and *Gymnema sylvestre* In Normal and Streptozotocin Induced Experimental Rats

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## Abstract

For management and treatment of diabetes several medicinal plants are used worldwide as well as these are being utilized customarily for the management of diabetes in human. The current study explored Stevia (*Stevia rebaudiana)* and gurmar (*Gymnema Sylvestre*) to determine their hypoglycemic activity compared to the standard drug metformin. Streptozotocin was used to induce diabetes in rats (55 mg/kg) intraperitoneal. For a time period of 60 days stevia (100mg/kg), gurmar (500mg/kg), and their combination (500mg/kg) was administered orally as well as metformin at the rate of 800µg/kg after 2 weeks of STZ injection. Hypoglycemic effects were shown best in combined stevia-gurmar extract than the individual doses of stevia and gurmar. (OGTT) oral glucose tolerance test and measurement of blood glucose levels were assessed. Reduction in body weight by STZ was also found to increase in all rats except stevia treatment. In conclusion, the extracts of stevia and gurmar showed hypoglycemic effect as well as improving body weight effects in diabetic rats. Therefore, it can be suggested that stevia as well as gurmar extracts have a potential in preventing diabetes and can be used as antidiabetics in herbal medicine.

**Keywords**: Anti-diabetic activity, STZ-induce diabetic rats, Stevia, Gurmar, Metformin, Diabetes.



## INTRODUCTION

Rapid increase in diabetes has been identified as epidemic condition across the world by World Health Organization (WHO) (King *et al*., 1991). As per the study published in Lancet, China, India and USA are among the worst affected countries with high number of diabetic people. As per the statistical analysis by the Indian Heart Association, the number of cases has bounced from 11.9 million to 64.5 million in India and is expected to be home to 109 million individuals with diabetes by 2035(Singh U., 2016). Diabetes is defined as a medical condition that occurs either when the pancreas does not produce enough insulin or when the body can't suitably use the insulin it produces. Hyperglycaemia, or raised blood glucose, is a result of uncontrolled diabetes and leads to impairment of different body parts especially nerves and veins. (WHO., 1999). Diabetes of various kinds can increase complexities in various parts of the body. Possible complexities consolidate cardiovascular diseases, stroke, kidney function, leg evacuation, vision adversity etc. In some cases during pregnancy, insufficiently controlled diabetes can be detected and grows the possibility of fatal destruction and diverse complexities (Singh U., 2016). According to Friedman, (2002) diabetes mellitus will cross about 250 million cases throughout the world by 2025.Diabetes alone ranks among the top ten sources of death in western countries (Mitratza, M *et al*., 2020). Concerning its cure, still appropriate medicine is not developed that will help to reduce its risk and can perpetually cure this disease. Insulin was discovered in 1922 and has been proved very successful in the treatment of insulin-dependent diabetes mellitus (IDMM).But it may have some troublesome effects leading to hypoglycemic reactions with adverse effects as it is not administered orally. Continuous administration of insulin causes resistances against insulin which lead to relative insensitivity to different body tissues to the action of insulin (Holst, J.J., *et al* 2017). Contrarily hypoglycemic drugs like glimepiride, metformin, glibenclamide that are consumed orally also have some ill effects on the body etc. From very ancient time period herbal plants are

commonly used in the management and treatment of different disease including diabetes which delivered a wide range of herbal preparations utilized as anti-diabetic agents before the in development of insulin (Kasole, R., *et al* 2019) Usually traditional herbal medicines used in the treatment of diabetes were derived from plants, (Kooti, W., *et al* 2016).More than 12,00 species of plants possessing hypoglycemic activity used as indigenously herbal treatment for diabetes were identified by ethnobotanical studies (Adinortey, M.B., *et al* 2019).Stevia (Stevia Rebaudiana Bertoni) is originated from South American and is herbaceous perennial plant. Rebaudioside and stevioside are the chief compounds present in stevia. Several studies observed that Steviosides has property of stimulating the production of insulin in the beta-cell (in vitro), which rises the secretion of insulin and thereby decreasing blood glucose levels. Assaei, R., *et al* (2016) observed that rats administered with diet of dried Stevia rebaudiana leaves showed antihyperglycaemic influence. Gurmar (Gymnema Sylvestre) also helps in maintaining the blood glucose levels in diabetic patients. The hypoglycemic effect of gurmar leaves has been also studied in normal and less diabetic animals. Gymnema sylvestre consists of anti-sweet component called as Gymnema saponin that consists of 23 hydroxy longispinogenin as the aglycone moiety and near about 36 more antidiabetic compound such as dammarane, dammarane saponins, Gymnemasides etc have been isolated from leaves of Gymnema sylvestre. (Laha.S *et al*, 2019).Still, using the aqueous extract of stevia and gurmar leaves together in treating type-1 diabetes is unclear. Metformin was begun from biguanide group that causes hypoglycemia by (a) releasing insulin from pancreatic β cells, (b) decreasing level of glucagon and (c) enhancing binding of insulin to target tissue or receptor. It is observed that metformin decrese the level of glucose in diabetic rats by a single oral dose of 3 mg/kg in the duration of three hour. (Kulkarni, S.S., *et al* 2016). From the above discussed facts, the present study focuses on comparing the hypoglycemic effects of Stevia (Stevia rebaudiana Bertoni), gurmar (Gymnema Sylvestre) leaves and their combination treatment in the evaluation to Metformin in type-1 diabetic rats.

# MATERIALS AND METHODS

### Animals

The study was carried out on female wistar rats having 5 weeks age with an average weight of 132±3.1g. Collection of rats were done from Institutional Animal Ethics Committee (IAEC) Jamia Hamdard New Delhi, India under the Registration No: 173/GO/RE /S/2000/CPSEA in line with the National research council's guide for the care and use of laboratory animals and animals used were dealt as per the SOP'S (standard operating procedures). All the rats were administered with filtered tap water and food in the form of pellets was provided ad libitum throughout the study.

### Plant materials

Fresh leaves of Stevia and gurmar were obtained from botanical garden, Jamia Hamdard New Delhi, India and metformin was procured from local pharmacy shop. Authentication and identification of both plants were done by a taxonomist from the Deptt. Of Botany, Jamia Hamdard New Delhi, India. Stevia leaves obtained from the garden were dried by oven drying followed by grinding with grinder machine. 1 gram sample of dried stevia leaves were mixed with distilled water (10ml) for 12hours.Similarly aqueous extract of gurmar was collected from 100g of leaves that were grinded by grinder machine and were soaked in 2000 ml of distilled water followed by lyophilization. After mixing with distilled water both the samples of stevia and gurmar were heated at $65^0$ C for 1 hour. Finally herbal preparation of was collected in the powder form by the application of Freeze drying.

### Induction of diabetes

0.1M buffer with pH 4.5 were utilized for dissolving Streptozotocin. As per the body weight 55 mg STZ was injected intraperitoneal in wistar rats. STZ showed rapid changes and signs in the characteristic of wistar rats such as maximum food and water intake, recurrent urination and increase in concentration of blood glucose. STZ injection in rats for one week showed increased concentration of blood glucose that was more than 250 mg/dl and were found with above discussed characteristic signs of diabetes (Rafiq *et al*., 2009). Blood collection for measuring levels of blood glucose at alternate weeks.

### Experimental design

Each group of Wistar rats consists of six rats (6 groups6 rats). These groups were further divided as: Group-1 was served as vehicle without STZ or non-diabetic which received only distilled water and normal feeding food orally. STZ (55 mg/kg, b.w., i.p.) was injected to all other rats except vehicle control for 14 days and these rats were further divided inti five groups (Group 2, 3, 4, 5, 6) .Group-2 (negative control) received (STZ) once a day whereas Group-3 were treated with STZ + aqueous extract of stevia leaves at a dose rate of 100 mg/kg, Group-4 were treated with STZ + aqueous extract of gurmar leaves at a dose rate of 500 mg/kg as well as Group-5 were treated with STZ + combination of stevia and gurmar extracts at a dose rate of 500 mg/kg, and Group-6 served as positive control which was treated by standard of Metformin with dose of 800 µg/kg. Theses doses were given orally once a day.

### Oral glucose tolerance test (OGTT)

OGTT was done after the study was conducted within 60 days. Prior rats were fasted for 12-14 hours

but were allowed free access to water. Collection of blood were done from the tail vain by needle puncture at different time intervals of 30 minutes between four readings after oral glucose was administered orally by gavage 2.0g/kg. (Rafiq *et al*., 2009). The level of glucose was estimated by using glucose-oxidase peroxidase reactive strips and glucometer (Sigma Diagnostics).

## STATISTICAL ANALYSIS

Data was expressed as Mean ± Standard Error of Means (Mean±SE). Student's unpaired t-test was used for statistical analysis. P values below 0.05 were considered statistically significant.

## RESULTS

**Evaluation of anti-hyperglycemic activity in STZ-induced diabetic- rats with aqueous extracts of indigenous medicinal plants**

The level of glucose concentration in blood of vehicle control was observed 89.01±0.10 to 92.18±0.16 mg/dl from beginning to end while as in negative control rats after STZ injection the glucose levels on day 0 was observed 278.01 ±0.03 and on day 60 was 356.02±2.27 mg/dl (P<0.01). The fasting blood glucose levels were significantly decreased by treatment of stevia and gurmar leaves. On the 0 day and 60 day the concentration of blood glucose levels was observed as 282.08±3.24 mg/dl and 230.05±2.35 mg/dl (P<0.05) respectively in rats treated with stevia leaves extract treated. Group 4 showed 276.02±0.34 mg/dl on 0 day and on day 60 was 181.10±0.06 mg/dl (P<0.01). Additionally, Group 5 showed higher hypoglycemic effects in which glucose levels in blood was observed on day 0 was 281.05±0.04 mg/dl and on day 60 was 140.72±3.11 mg/dl (P<0.01). The results calculated from positive control on day 0 was 278.63±3.35 mg/dl and on day 60 was 125.02±2.09 mg/dl (P<0.01) (Figure.1).

**Evaluation of oral glucose tolerance test (OGTT) in STZ-induced diabetic rats**

Glucose levels and its area under curves during OGTT is shown in Figure 2. Marked increase in blood glucose were observed in oral glucose load in STZ induced rats and in area under curve when compared to vehicle control (P<0.01). While as stevia and gurmar leaves extract indicated significantly decreased blood glucose levels and it's AUC during OGTT which is same approach like treatment of metformin in positive control group.

**Determination of body weight in STZ-induced diabetic rats treated with aqueous extracts of indigenous medicinal plants**

Average weight of body in both control as well as STZ groups were determined at 0 day and 60 day of the study and was observed as 137.20±1.24g, 176.92±0.22g and 135.00±2.31g, 122.57±3.26g respectively. While as, group treated with stevia leaves extracts showed 130.15±0.16g, 124.10±3.14g at day 0 and day 60 respectively. Gurmar leaves extract showed 130.80± 0.26g, 129.25±2.08g changes in body weight at day 0 and day 60. Stevia and gurmar extracts combination indicated 132.40±3.08g and 131.40±4.17g body weight at day 0 and day 60 respectively. Rats treated with metformin increase in body weight 128.65±3.13g and 131.85±2.16g from day 0 to day 60 (Figure-3).

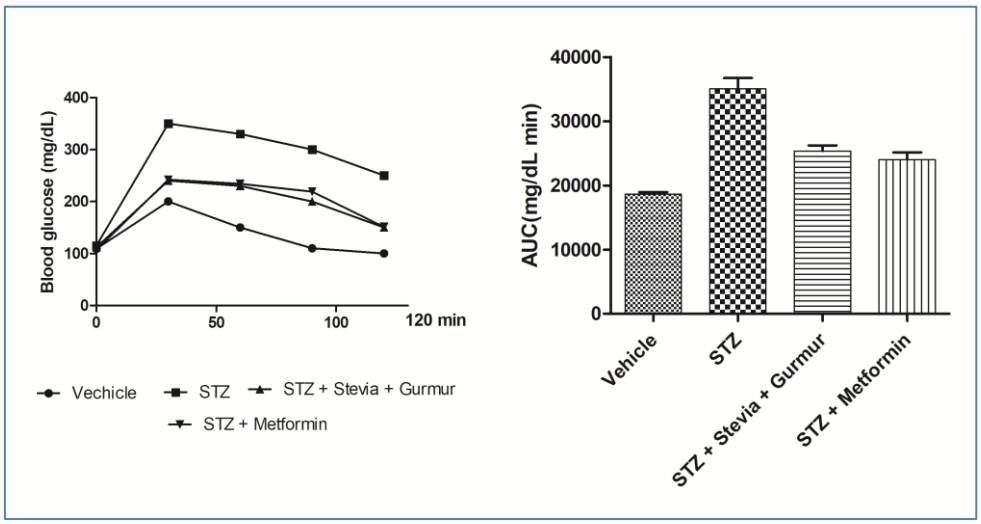


**Fig-1**

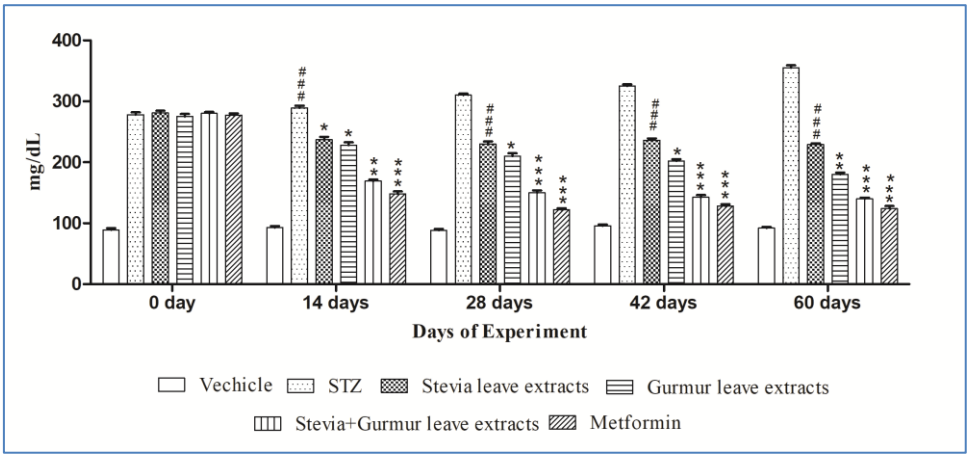


**Fig-2**

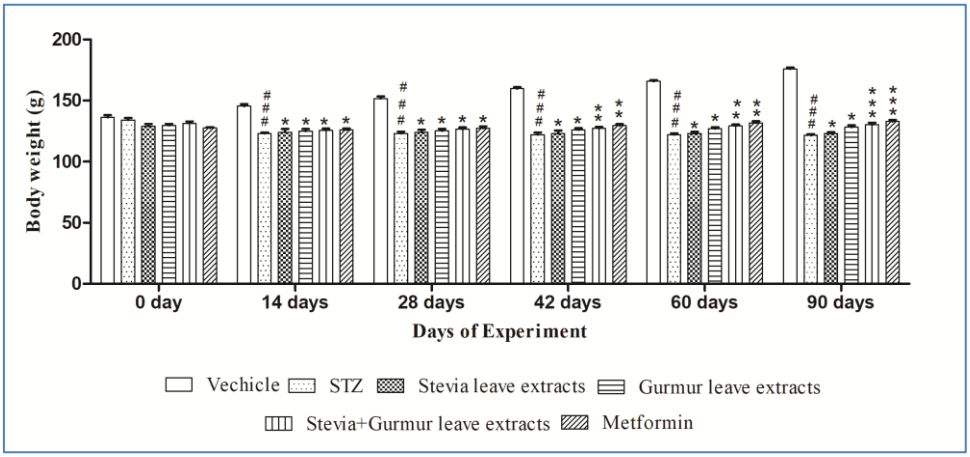


**Fig-3**

## DISSCUSSION

Indian system of medicine and early medicine system of world utilize different types of plants for treating and maintaining the blood sugar levels. As per the modern system of medicine only few plants were evaluated for treatment of diabetes (Mahdi A.A *et al*, 2003).The results of the present revealed that extract of stevia and gurmar leaves and their combination identifies the marked reduction in the levels of blood glucose in vehicle control groups as well as in STZ induced diabetic rats. Diabetes is a chronic disorder that occurs either pancreas does not produce enough insulin or when body is not able to produce required amount of insulin (Faria, A., *et al*, 2017). The most dangerous condition in diabetes is hyperglycemia characterized with reduction in body weight (Vega-López, S., *et al*, 2018. Thus according to (Choudhury, H., *et al*,2018) maintaining glucose level in blood and maintaince of body weight are considered primary and essential parameters for an anti- diabetic agent as well as potential of the herbal extracts should be tested initially based on these two charcteristics. This study proved that STZ injected to rats showed significant (P<0.01) rise in glucose level in blood and drop of body weight. Rafiq *et al*., (2009) showed that one dose of STZ revealed several signs of diabetes. Subsequently metformin revelaed that there is significant reduction in glucose levels (P<0.01).Zhang, S., *et al*. (2017).Several studies observed decrease in glucose level by the treatment of stevia leaves (Chang *et al*., 2005; Raskovi c *et al*., 2004; Jeppesen *et al*., 2003).This experiment also proved that gurmar leaves (Gymnema sylvestre) considerably decreased (P< 0.01) glucose level. These results were same with Laha.S *et al*, (2019) that stated the decrease of glucose levels with gurmar leaves extract. Moreover from this study, gurmar and stevia when used in combination form showed improved hypoglycemic effects as compared to single treatment in STZ-induced rats. Consequently, OGTT was done for assessment of glucose intolerance from the combination of gurmar and stevia treated groups. Reduction in blood glucose intolerances such as metformin treated group were also shown by combination of stevia and gurmar leaves aqueous extract. These results also suggested that combination treatment of stevia and gurmar has both effects of hypoglycemic as well as decreased glucose intolerance effects. Thus we can suggest that these results can provide support in terms of pharmacology with concern to indigenous and ethno therapeutic user of these herbal plants in maintaining and regulating diabetes.

According to our knowledge, no experiment has been done on the combination of stevia aqueous extract and gurmar aqueous extract. Combinatorial treatment with both the extracts (stevia and gurmar) helps in maintaince of diabetes and this treatment was proved more effective than other monotherapies. Additional pharmacological and biochemical research is required to explain the mechanism behind the hypoglycemic effects of stevia and gurmar aqueous extracts.

## CONCLUSIONS

In this study, the aqueous extract of both Stevia (Stevia rebaudiana Bertoni) and gurmar (Gymnema Sylvestre) leaves possessed potential anti diabetic activities in normal glycemic and STZ induced rats. Both the extracts were found to be potent in reducing the glucose levels in diabetic rats. Results seem to approve the alleged antidiabetic activity by different indigenous herbal medicine without any toxic effect. More studies should be carried out in formulating the herbal formulations from the extracts of Stevia rebaudiana Bertoni and Gymnema Sylvestre and determination of their mechanism of action.

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