In vivo Antidiabetic Potential of *Momordica charantia* and *Trigonella foenum graecum* Seed Extracts in Nicotinamide- Streptozotocin Administered Diabetes Induced Rats

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Abstract

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. Antidiabetic potential of *Momordica charantia* seed (MCS) and *Trigonella foenum graecum* seed (TGS) extracts were evaluated in Streptozotocin – Nicotinamide (STZ-NIC) administered diabetes induced rats. In OGTT (Oral Glucose tolerance Test), MCS, TGS and glibenclamide treated rats significantly prevented a rise of the blood glucose level compared to the control group. Diabetes Mellitus was induced by a single intraperitoneal injection of STZ-NIC and rats with blood glucose concentration more than 250mg/dl were used for further study. The rats were divided into seven groups and treated with MCS, TGS and glibenclamide for of 21 days. Rats treated with MCS 400 mg/kg b.w and TGS 400 mg/kg b.w. showed significant decrease in the levels of blood glucose monitored at weekly intervals. Rats treated with plant extracts and glibenclamide showed a significant increase ($p<0.05$) in the total protein content and in liver glycogen.

Key words: Diabetes mellitus, *Momordica charantia*, *Trigonella foenum graecum.*

Introduction

Diabetes is the collection of metabolic illnesses in which increased blood sugar levels persist for a prolonged period due to a malfunction in insulin production that affects the metabolism of various nutrients such as proteins, lipids, and carbohydrates [1]. Research is now being done on medications that can continually control blood sugar levels. Diabetes is primarily managed with oral hypoglycaemic medications and insulin injections 2. These medications have some adverse effects such as severe hypoglycaemia, weight gain, gastrointestinal discomfort, and nausea [3]. Medicinal plants and herbs are excellent sources of alternative and complementary medicine and they have a significant function in disease treatment [4]. Long-term human use of plant extracts provides reliable evidence for diabetes treatment in traditional medicine system. The plant extract contains a variety of phytochemicals that contain many primary and secondary metabolites that can enhance the efficacy of plant-related drugs in treating disease [5]. Bitter gourd (Bitter melon) or *Momordica charantia* Linn. (Cucurbitaceae) (MC) is a tropical and subtropical vine [6]. It is used in the Ayurvedic system of medicine for treating various diseases including diabetes mellitus [7]. This plant has been extensively studied for its various pharmacological properties. The hypoglycaemic

activities of M. charantia extract have been stressed by number of investigators in both normal and diabetic rats. It also improves glucose tolerance [8]. Due to remarkable hypoglycemic properties, *M. charantia* has great potential as an dietary ingredient and in medical foods for diabetic and prediabetic patients, as well as for the regulation of body weight and lipid metabolism [9].*Trigonella foenum-graecum* (also known as fenugreek, locally as methi) possesses diverse biological activities and pharmacological functions. *T. foenum-graecum* seeds have been used as traditional medicines not only in diabetes but also in high cholesterol, inflammation and gastrointestinal ailments [10].*Trigonella foenum-graecum* seeds have also previously been shown to have hypoglycemic and hypocholesterolemic effects on type 1 and type 2 diabetes mellitus patients and experimental diabetic animals [11].

Hence, with this background information, the present study has been designed to explore the potential and efficacy of *Momordica charantia seed* (MCS) and *Trigonella foenum graecum* Seed (TGS) extracts in Nicotinamide- Streptozotocin Administered Diabetes induced rats**.**

Materials and Methods

Plant Collection, Identification and Preparation of Extract

Momordica charantia seeds (MCS) and *Trigonella foenum graecum* seeds (TGS) were dried, finely powdered and stored in airtight containers at room temperature for further use. Five grams of *Momordica charantia* seeds (MCS) and *Trigonella foenum graecum* seeds (TGS) were macerated with 50 ml ethyl acetate for 48 hours filtered and collected the solvent. The solvent was evaporated in water bath shaker to get dry extract and used for further analysis.

Experimental Animals

Adult male albino Wistar rats (6 weeks), weighing 150 to 200 g were used for the present antidiabetic study. The animals were housed in clean polypropylene cages and maintained in a well-ventilated temperature-controlled animal house with a constant 12 h light/dark schedule. The animals were fed with standard rat pelleted diet and clean drinking water was made available *ad libitum*. All animal procedures were performed after approval from the Ethical Committee Clearance No: 53 IAE1012/c/17/CPCSEA-2013 and in accordance with the recommendations for the proper care and use of laboratory animals.

Acute toxicity studies

Acute oral toxicity study of *Momordica charantia* seeds (MCS) extract and *Trigonella foenum graecum* seeds (TGS) extract was studied in healthy rats (n= 3) according to guidelines set by Organisation for Economic Co-operation and Development (OECD). The plant extract was evaluated for the pharmacological potential in normal rats weighing 150 to 200 g. The animals were kept fasting overnight providing water. The animals were treated with MCS and TGS starting dose of 200 mg/kg followed by 500,1000,1500,2000 mg/kg b.w and were evaluated for toxicity. The animals were observed for mortality for 24 hours. Since no mortality was observed in acute toxicity

studies, 1/5th and 1/10th of the highest dose (2000mg/kg b.w) were chosen for performing oral glucose tolerance test (OGTT) in normal rats.

Oral glucose tolerance test

Overnight fasted rats were separated in 6 groups. Animals of all groups were administered with glucose (2g/kg) orally by means of gastric intubation. Animals in group 1 were given normal saline (0.9% w/v NaCl). Group 2 and 3 were treated orally with ethyl acetate extracts of MCS at a dose of 200 and 400 mg/kg and group 4 and 5 were treated with ethyl acetate extracts of TGS at a dose of 200 and 400 mg/kg. Group 6 received standard drug glibenclamide 200 µg /kg b.w. Blood samples were collected by tail nipping of each animal just after oral glucose administration at 0, 60,120 and 180 min for the assay of glucose by Accu-chek glucometer.

Induction of Diabetes Mellitus

The animals were kept overnight fasting and the initial fasting blood glucose was checked from tip of rat tail vein. Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Diabetes Mellitus was induced in overnight fasted rats by a single intraperitoneal injection of 60 mg/kg streptozotocin, 15 min after the i.p administration of 120 mg/kg of nicotinamide. Hyperglycemia was confirmed by the elevated levels of blood glucose determined after 72 hours. The animals with blood glucose concentration more than 250mg/dl were used for further study.

The vehicle (saline), standard drug glibenclamide and plant extracts were administered to the respective group animals for 21 days. Throughout the study period glibenclamide and plant extracts were freshly dispersed in normal saline and distilled water before the administration. The fasting blood glucose level was estimated on 1st, 7th, 14th and 21st day from the tip of rat tail vein.

Evaluation of Antidiabetic Activity

Treatment protocol

The animals were divided into seven groups of six animals each as follows. The experimental period was 21days.The experimental design for evaluation of antidiabetic activities is shown in Table 1.

Table 1

Experimental design for evaluation of antidiabetic activities

Groups	Treatment
Group 1	Control - Only normal saline (0.9% w/v NaCl)
Group 2	STZ-NIC - Diabetic control-Only Streptozotocin 60 mg/kg +Nicotinamide 120mg/kg b.w.
Group 3	MCS-1 - Streptozotocin (60 mg/kg) +Nicotinamide 120mg/kg rats treated with MCS 200 mg/kg b.w.
Group 4	MCS-2- Streptozotocin (60 mg/kg) +Nicotinamide 120mg/kg rats treated with MCS 400 mg/kg b.w.

Group 5	TGS-1- Streptozotocin (60 mg/kg) +Nicotinamide 120mg/kg rats treated with TGS 200 mg/kg.
Group 6	TGS-2- Streptozotocin (60 mg/kg) +Nicotinamide 120mg/kg rats treated with TGS 400 mg/kg b.w.
Group 7	Glib - Streptozotocin (60 mg/kg) Nicotinamide 120mg/kg rats treated with Glibenclamide 200 µg /kg b.w.

Biochemical analysis

Estimation of blood glucose

Blood sample were collected from tip of rat tail vein and glucose levels were estimated using a glucose oxidase-peroxidase reactive strips and Accu-chek glucometer.

Estimation of glycogen [12]

Glycogen is the primary intracellular storage form of glucose and its level in liver is important in the management of Diabetes and hence liver glycogen was estimated using the anthrone reagent

Determination of proteins [13]

In diabetic condition, alterations in hormonal and enzymatic activities can occur. Hence measurement of change in the total protein content would help in the management of Diabetes Mellitus. Total proteins were estimated by the method of Lowry *et al*. (1951)

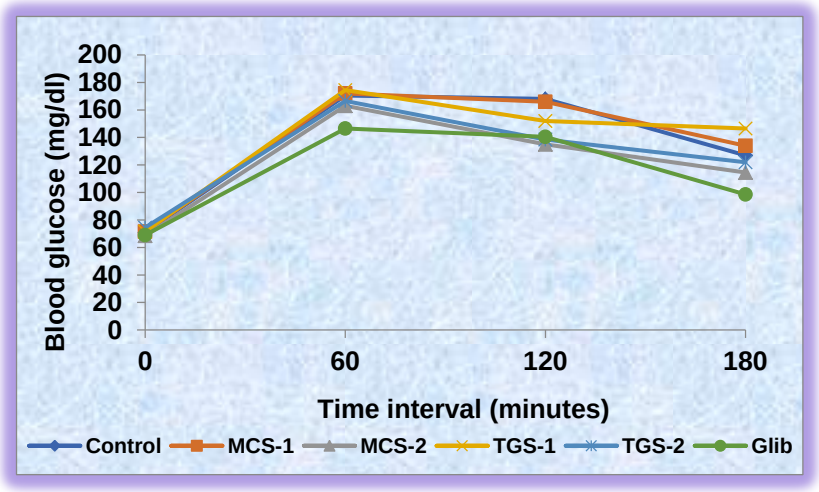
Results & Discussion

Acute toxicity

Acute toxicity study of ethyl acetate extracts of *Momordica charantia* seeds and *Trigonella foenum graecum* seeds given by oral route were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in the normal behavior pattern and no signs and symptoms of toxicity and mortality observed as per Organization for Economic Co-operation and Development (OECD) guidelines. Since no mortality was observed $1/10^{th}$ (200 mg/kg b,w) and $1/5^{th}$ (400 mg/kg b,w) of the highest dose (2000mg/kg b.w) were chosen for further studies.

Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test of *Momordica charantia* seeds extract and *Trigonella foenum graecum* seeds extract was done on fasting normoglycaemic rats and the results are presented in Figure1 OGTT is the only form of glucose tolerance recommended for the diagnosis of Diabetes. The changes in blood glucose concentration, which result from an oral carbohydrate load is theoretically dependent on the rate at which carbohydrate enters the small intestine, the rate of digestion and intestinal absorption of glucose and the rate of insulin-driven metabolism [14]. OGTT determines how quickly glucose is cleared from the blood after a given oral glucose dose. In the present study, a dose-dependent effect was observed. In glucose-fed rats treated with normal saline, there was a significant increase in blood glucose levels after 60 minutes following administration of glucose. The maximum increase in blood glucose was observed 60 minutes after administration of glucose.



Values are expressed as mean (n=6)

MCS -1: 200mg/kg b.w, MCS-2:400mg/kg b.w, TGS -1: 200mg/kg b.w, TGS -2:400mg/kg b.w.

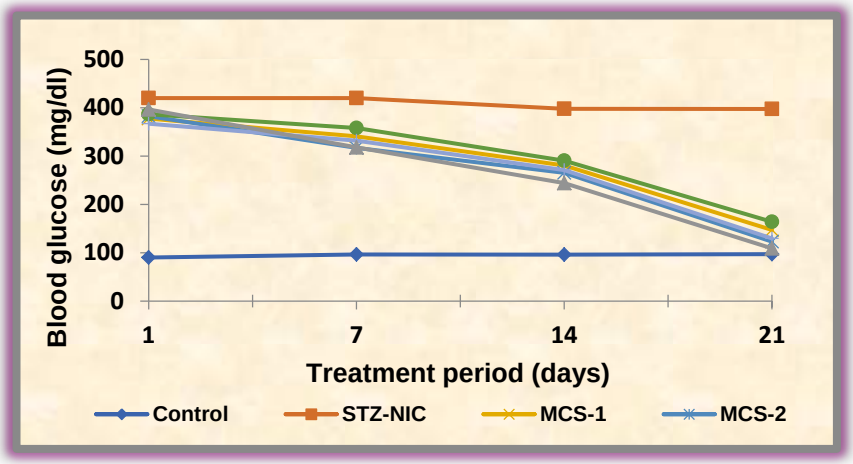
Figure 1

Blood glucose level in normal rats administered with *Momordica charantia* seeds extract and *Trigonella foenum graecum* seeds extract and glibenclamide during OGTT

The ethyl acetate extracts of *Momordica charantia* seeds, *Trigonella foenum graecum* seeds and glibenclamide significantly prevented a rise of the blood glucose level after 60 and 180 minutes compared to the control group. Treatment of the rats with glibenclamide produced a maximum reduction in blood glucose after 60 to 180 min of glucose administration. Higher doses 400mg/kg b.w of both MCS and TGS prevented increase in blood glucose after 60 minutes and it was similar to the action of standard drug glibenclamide. This shows that both the extracts possess glucose lowering effect indicating a better glucose utilization capacity. This could be due to tissue glucose uptake and reduced hepatic glucose output, there by producing an antihyperglycemic effect. Ethyl acetate extracts of *Hypericum perforatum* was administered to fasting normal rats and hypoglycemia was observed after 30 min. The decline in blood sugar level reached its maximum after 2 hours [15].

Blood glucose

The initial blood glucose level among the groups showed no significant differences. But after induction of Diabetes, the blood glucose levels effectively showed hyperglycaemia which was followed with the administration ethyl acetate extracts of *Momordica charantia* and *Trigonella foenum graecum* seeds and standard drug glibenclamide. The fasting blood glucose levels of control, Diabetes induced and treated rats during the experimental period is depicted in Figure 2. After 7 days of treatment, there was a significant decrease in fasting blood glucose levels in MCS, TGS and glibenclamide treated groups respectively. The fasting blood glucose level decreased gradually on 14^{th} and 21^{st} days in MCS, TGS and glibenclamide treated groups as compared to onset of the study.



Values are expressed as mean (n=6)

MCS -1: 200mg/kg b.w, MCS-2:400mg/kg b.w, TGS -1: 200mg/kg b.w, TGS -2:400mg/kg b.w, Glib: 200µg/kg b.w.

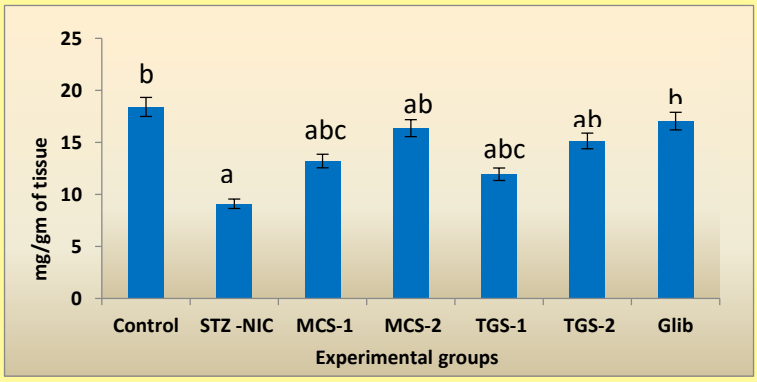
Figure 2

Fasting blood glucose levels in the experimental rats during treatment period

The effect of plant extracts was found to be time dependant up to 21st day of the study. The results indicate that both low (200 mg/kg b.w) and high (400 mg/kg b.w) doses lowered blood glucose levels in diabetic rats. The diabetic rats treated with high dose of 400 mg mg/kg b.w of MCS and TGS showed similar reduction as that of glibenclamide. This difference in reduction of blood glucose observed between groups might be dose dependant. Repeated administration of ethyl acetate and ethanolic extract of *Scindapsus officinalis* fruits (once a day for 21 days) as well as glibenclamide caused significant reduction in the blood glucose level as compared to diabetic control group [16]. *Citrus macroptera* extracts were found to decrease the activity of α-amylase in the digestive canal, improve the metabolism of glucose and increase insulin secretion by stimulating beta cells and also suggested that the bioactive compounds present in the fruit extract might be responsible for multifaceted effects. Among the two doses 200mg/kg bw and 500 mg/kg bw of the extracts used for treatment, as per their observation 500 mg/kg bw of the extract showed greater reduction in blood glucose level which was comparable to glibenclamide as per their observation [17].

Glycogen

The results of liver glycogen in control and experimental rats are shown in Figure 3. The levels of hepatic glycogen were observed to be significantly reduced ($p<0.05$) in STZ-NIC induced diabetic rats. In rats treated with plant extracts and glibenclamide, there was a significant increase ($p<0.05$) in liver glycogen. The increase in glycogen content was found to be dose dependent. The increase in glycogen content of MCS 400 mg/kg bw treated rats was similar to that of glibenclamide treated rats.



Values are mean± SEM (n= 6)
a-p <0.05 compared with control group
b-p <0.05 compared with STZ –NIC group
c-p <0.05 compared with Glib (200µg/kg b.w) treated group
(One way ANOVA followed by Dunnett's multiple Comparison test)
MCS -1: 200mg/kg b.w, MCS-2:400mg/kg b.w, TGS -1: 200mg/kg b.w, TGS -2:400mg/kg b.w.

Figure 3

Glycogen content in the liver of experimental rats

Glycogenesis in muscle and liver is mainly regulated by serum insulin level. The regulation of glycogen metabolism *in vivo* that occurs by the multifunctional enzyme glycogen synthase and glycogen phosphorylase plays a major role in the glycogen metabolism [18]. The reduced glycogen stored in diabetic rats has been attributed to reduced activity of glycogen synthase and increased activity of glycogen phosphorylase [19]. The significant increase in the glycogen content of the treated groups might be due to reactivation of the glycogen synthase enzyme. Hence, improvement of glycogenesis could be another probable way of anti-diabetic action [20].

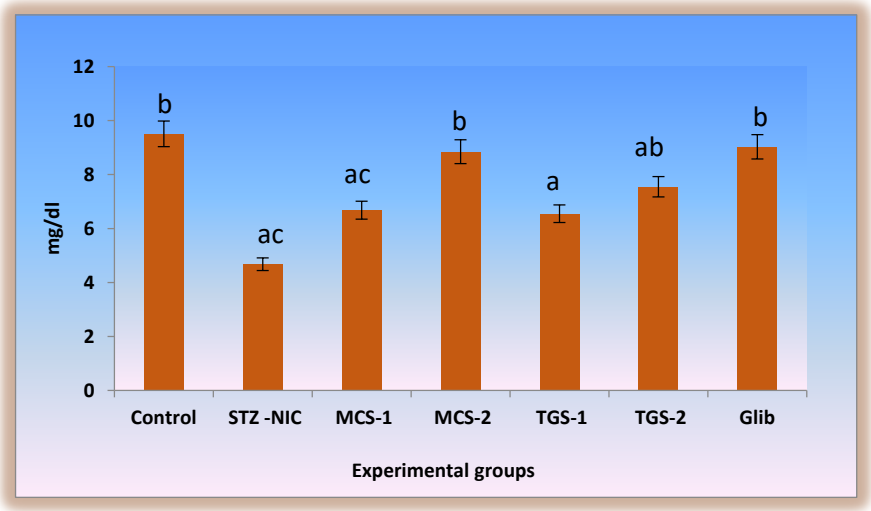
The decrease in hepatic glycogen reported in the present study might be due to low level of serum insulin in diabetic rats, which could have inactivated the glycogen synthesis system. Treatment with MCS and TGS extracts for 21 days in experimental rats was found to result in increased liver glycogen levels. This might highlight one possible way of antidiabetogenic action of the plant extracts.

Serum proteins

The effect of ethyl acetate extracts of *Momordica charantia* and *Trigonella foenum graecum* seeds and standard drug glibenclamide on total serum proteins of the experimental rats was studied at the end of the treatment period and results are depicted in Figure 4.

The serum total protein levels of diabetic control was significantly reduced ($p<0.05$), whereas the rats treated with plant extracts and glibenclamide showed a significant increase ($p<0.05$) in the total protein content. *Ethyl acetate seed extracts of MCS and TGS* treatment were found to increase the content of protein. The increase in serum proteins was observed to be dose dependant. Higher dose

of 400 mg/kg b.w of MCS and TGS showed better results when compared to 200 mg/kg b.w. Restoration of protein content by MCS was more than by TGS extracts.



Values are mean± SEM (n= 6)
a-p <0.05 compared with control group
b-p <0.05 compared with STZ –NIC group
c-p <0.05 compared with Glib (200µg/kg b.w) treated group
(One way ANOVA followed by Dunnett's multiple Comparison test)
MCS -1: 200mg/kg b.w, MCS-2:400mg/kg b.w, TGS -1: 200mg/kg b.w, TGS -2:400 mg/kg b.w.

Figure 4

Total protein levels in the serum of the experimental rats

There is increased protein catabolism with the flow of amino acids into the liver, which feeds gluconeogenesis as a result of insulin deficiency during uncontrolled Diabetes Mellitus [21]. Daily administration of ethyl acetate fraction of *Stereospermum suavelolens* for 14 days caused a significant elevation in serum total protein levels in diabetic rats when compared to diabetic control [22].

The results of the present study showed that the treatment of rats with MCS and TGS ethyl acetate extracts caused a significant increase in serum total protein. This might be attributed to an improvement in glycemic control and insulin secretion that might increase protein synthesis or decrease protein degradation [23]

Summary and Conclusion

In present *in vivo* antidiabetic study *Momordica charantia* and *Trigonella foenum graecum* seed extracts on Nicotinamide- Streptozotocin Administered Diabetes Induced Rats showed considerable decrease in blood glucose levels, increase in liver glycogen levels, and increase in serum protein levels. Hence *Momordica charantia* and *Trigonella foenum graecum* seeds might contribute to management and in natural treatment of diabetes mellitus without any side effects.

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