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SYNERGISTIC ANTIHYPERGLYCEMIC, ANTIHYPERLIPIDEMIC AND ANTIOXIDANT EFFECTS OF MOMORDICA CHARANTIA AND METFORMIN IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus (DM) is one of the complex and multifactorial group of disorders characterized by hyperglycemia. In recent years people suffering from diabetes use natural plants along with synthetic drugs in long term treatment of diabetes. Hence the aim of the present study was to evaluate antihyperglycemic, antihyperlipidemic and antioxidant effects of ethanolic fruit extract of *Momordica Charantia* and standard drug Metformin in combination and alone for treating diabetes. Type I diabetes was induced by streptozotocin (STZ, 65 mg/kg ip single dose) in male albino Wistar rats. The ethanolic fruit extract of *Momordica Charantia* (500 mg/kg) and standard drug metformin (55mg/kg) was administered orally for 28 days. The blood glucose was estimated upto 28 days. A significant decrease in STZ induced serum glucose (306.38±5.13 mg/dl) was observed with combination (76.16±1.65mg/dl) when compare to drug alone (89.48±3.07mg/dl) and fruit extract alone (82.28±0.714mg/dl). The serum triglycerides levels were also reduced drastically with significant increase in the levels of various antioxidant enzymes when administered in combination as compared to individual dose. Hence it can be concluded that combination of Momordica *Charantia* and metformin showed beneficial and synergistic effects in the treatment of type I diabetes.

KEYWORDS: Antihyperglycemic, antihyperlipidemic, antioxidant, *Momordica Charantia*, metformin, streptozotocin.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder that affects the normal level of glucose in the blood that is characterized by rise in the blood glucose (Hyperglycemia) and may be either due to insufficient or ineffective insulin. Diabetes is a chronic disease without permanent cure but, with proper diet management, regular exercise and treatment diabetics can also live a normal and healthy life.[1] Hyperglycemia manifests adverse effects on β-cell insulin secretion and insulin resistance and leads to long-term damage, dysfunction and failure of various organs especially the eyes (Diabetic retinopathy), kidneys (Nephropathy), nerves (Diabetic neuropathy), heart, and blood vessels etc. and creates a huge economic burden related to the management of diabetic complications.[2, 3] Oxidative stress plays an important role in chronic complication of diabetes and is associated with increased lipid peroxidation. [4] Oxidative stress results from imbalance between free radical generation and free radical scavenging systems which results in production of more free radicals or reduced activity of free radical scavenging defense systems or both. [5, 6]

The World Health Organization (WHO) has also recommended the evaluation of the plants for their effectiveness and in conditions where we lack safe modern drugs.[7] The management of diabetes without any side effects is still a challenge to the medical system. There is an increasing demand from patients to use the natural products with antidiabetic activity. [8] Of the many traditionally used plants *Momordica charantia* (Family: Cucurbitaceae Genus: Momordica Species: charantia), commonly known as bitter gourd is used in the management of diabetes. The plant traditionally has been used for its various therapeutic activities such as antibacterial, antioxidant, antispasmodic, antimicrobial, cytotoxic, anti-inflammatory, immunostimulant, hepatoprotective activity and for its free radical scavenging activity. Phytochemical analysis of leaf, fruit and seeds of *Momordica charantia* revealed the presence of phytosterols, phenolics, tannins, flavonoids, glycosides, and saponins which are responsible for antioxidant activity [10, 11] and antihyperglycemic activity.[12] Antihyperglycemic activity of Fractionated Momordica charantia seed extract was also established. [13]

Hence, the present study was under-taken to explore anithyperglycemic, antihyperlipidemic and for free radical scavenging activity of *Momordica charantia* fruit extract on Streptozotocin-induced diabetic rats in combination with metformin and alone.

MATERIALS AND METHODS

Metformin was a gift sample of M/s Hetero drugs Hyderabad. Glucose, Cholesterol, HDL-c Cholesterol, Triglyceride estimation kits were purchased from ERBA diagnostics. Streptozotocin (STZ) all other reagents used were of analytical grade.

Preparation of Ethanolic extract of *Momordica charantia* fruits:

Momordica charantia fruits were purchased locally from vegetable market Hyderabad, Andhra Pradesh.

Identification and collection of *Momordica Charantia*: The collected whole fruits were sliced into small pieces and dried in shadow without exposing to sunlight for 12 days and grinded in mixer to get coarse powder, which is further used for extraction process.

Preparation of *Momordica Charantia* fruit extract(MCFE):

The fruit extract of momordica charantia was prepared by continuous hot percolation method using soxhlet apparatus. The powder was packed in whatmann filter paper and extracted using 70% ethanol for seven days. The final extract was concentrated, dried and stored in refrigerator for further study.

Experimental Animals

Wistar rats weighing 150-200g were used for the present study. The animals were maintained in the animal house for experimental purpose. The animals were maintained under controlled conditions of temperature (23 ± 2 °C), humidity (50 ± 5%) and 12-h light-dark cycles. All the animals were acclimatized for seven days before the study to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (Approved through the Teena Biolabs under Reg.No. **TBLSTPRJ0012013)** according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. We selected male animals for all our studies, since females are shown to be protected from changes in lipid– induced insulin action.

Experimental Protocol

Toxicity Study

An acute oral toxicity study was performed as per OECD guidelines 423. For acute toxic class method Wistar rats weighing 150-200gms were used for the study. Acute toxic class method is a stepwise procedure with use of three animals of a single set per step.

Depending on mortality or morbidity status of the animal's average 2-4 steps may be necessary to allow judgments on the acute toxicity of the substance. Three animals were used for each step. The animal were placed individually and observed for any sign of toxicity, morbidity or mortality during the first 24hrs, with special given attention during the first 4 hrs and daily thereafter for a total of 14 days.

Induction of diabetes in rats

Wistar rats (n=30) were fasted for 48hrs. Diabetes was induced by administering freshly prepared streptozotocin in 0.02M citrate buffer pH 4.5 , intraperitonially at a dose of 65 mg/kg, body weight as single dose. After 72hr of streptozotocin, 18hr fasting blood was collected from those that survived. Sugar estimated by glucose oxidase method. Twenty four diabetic rats with blood glucose level of 200-300 mg/dl were selected and were divided into four groups of six each. Healthy adult Wistar rats (n=6) were included in group 1 to serve as normal animals.

The *Momordia Charantia* fruit extract (500 mg / kg) in 0.5% carboxy methyl cellulose (CMC orally adminstered after 3 days of STZ injection and it was adminstered daily for 28 days. Various physical, biochemical and antioxidant parameters were measured.

Body Weight Determination: Body weight of rats was recorded before and after the study period of 28 days. Change in body weight was calculated and the same was plotted.

Blood sampling

At the end of 28 days the animals were kept for overnight fasting and the blood samples were collected and allowed to clot for 30 minutes at room temperature. The blood samples were centrifuged at 5000 rpm for 20 minutes and serum was separated and stored at -20^{o} C until analysis was done.

Biochemical analysis: Serum samples were analyzed spectrophotometrically for serum glucose, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, SGOT, and

SGPT using respective kits.

Assessment of oxidative stress related markers

The animals were sacrificed after collecting the blood. Liver and kidney were isolated from each animal and weighed. Respective tissues were finely sliced and homogenized in chilled tris buffer for 15 minutes. The homogenates were centrifuged and clear supernatant was collected and used for estimation of various antioxidant parameters like superoxide dismutase (SOD) [14], catalase [15], reduced glutathione (GSH) [16] and thiobarbuturic acid reactive species (TBARS) formation was also determined as per Slater and sawyer. [17]

Statistical Analysis

Results of the experiment were expressed as mean ± SEM. Statistical analysis was carried out by using one way analysis of variance followed by Dunnets p test using Graph pad prism software version 4.0. A value of $p<0.05$, $p<0.01$ and $p<0.001$ were considered significant.

RESULTS

Body Weight Determination: The change in body weight after 28 days treatment was noted for all the study groups as shown in table 1. The body weight was increased by 9.31% in case of control group whereas a significant decrease in the body weight was observed in the diabetic control rats by 19.5% after 28 days. The animals treated with combination the body weight was restored by 10.4%. In diabetic animals treated with metformin alone the body weight was increased by 3.77% and 2.65% in case of MCFE group.

Table 1: Effect of MCFE and Metformin in control and STZ induced diabetic rats on Body weight (gm.)

Group	Initial	Final
Control.	147±2.670	162.16±3.19
Diabetic Control.	146.33±2.703	114.56±3.09
Diabetic + **Metformin (55mg/kg)**	148±2.63	153.83±2.65*
Diabetic + Fruit **extract (500mg/kg)**	146.66±2.45	146.66±3.99*
Diabetic + Metformin (55mg/kg) + Fruit extract (500mg/kg)	142.33±2.703	158.83±2.78*

*=P<0.001= Extremely significant. **=P<0.05= Statistically significant.

Serum glucose levels

The blood glucose levels of normal and experimental animals (initial and final) after oral administration of extract at high dose and with the standard Metformin and also treatment group with combination of extract and Metformin.The results of the extract as well as standard drug treated animals is showed in fig no:1, table 2. The serum glucose was increased to 306.38 mg/dl in case of diabetic control group when compare to normal control group with serum glucose 75.61mg/dl. In case of metformin treated group the glucose level was 89.48mg/dl, 82.28mg/dl in case of MCFE group. But the diabetic group (group5) treated with both the fruit extract and metformin reduced the serum glucose levels to 76.16mg/dl. The results reveal that the combination of extract and metformin produced significant decrease ($P<0.001$) in the blood glucose levels when compared with the diabetic controls in streptozotocin induced hyperglycemic rats.

Table-2 :Effect of MCFE and Metformin in control and STZ induced diabetic rats on biochemical parameters

Group	Serum Glucose levels mg/dl	Serum triglycerides mg/dl	Serum Cholesterol mg/dl	HDL-Cholesterol mg/dl	LDL-Cholesterol mg/dl
Control.	75.61±1.82	81.51±2.90	132.66±1.58	37.16±1.07	34.95±1.93
Diabetic Control.	306.38±5.13	197.81±4.90	287.66±2.72	21.5±1.97	71.60±4.29
Diabetic + Metformin (55mg/kg)	89.48±3.07*	85.83±3.38*	147.33±2.41*	33.5±1.52*	42.07±2.32*
Diabetic + Fruit extract (500mg/kg)	82.28±0.714*	82.23±1.88*	139±2.75*	39.33±1.60**	40.23±4.7*
Diabetic + Metformin (55mg/kg)+ Fruit extract (500mg/kg)	76.16±1.65*	81.4±3.04*	136±1.52*	38.16±1.91**	43.48±2.37*

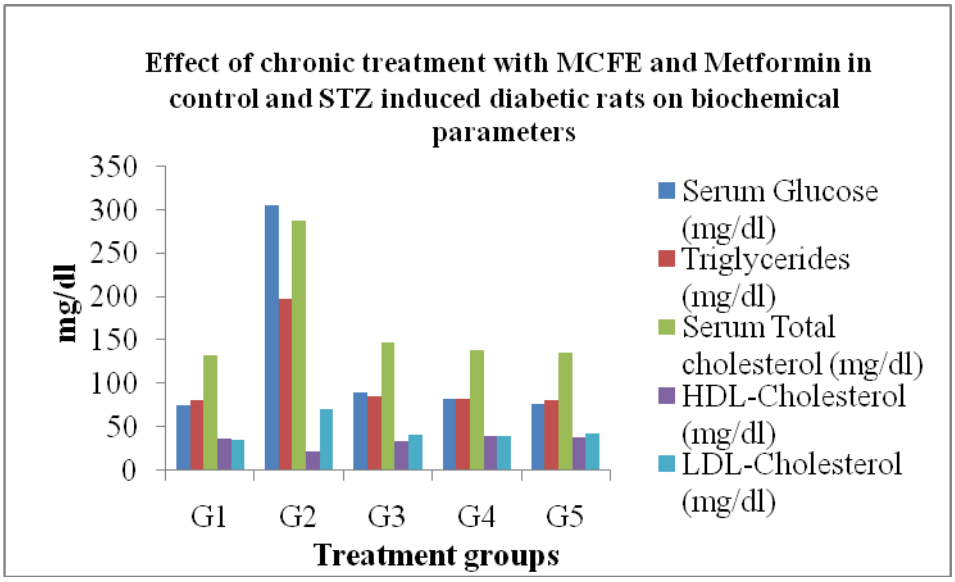

Fig. 1: Effect of chronic treatment with MCFE and Metformin in control and STZ induced diabetic rats on biochemical parameters

Where, G1 = normal control.G2 = Diabetic control. G3 = Diabetic rats treated Metformin (65mg/kg body weight) G4 = Diabetic rats treated with high dose of MCFE extract (500 mg/kg body weight). G5 = Diabetic rats treated with Metformin (65mg/kg body) and high dose of extract (500 mg/kg body weight).

Serum lipid profile and Transaminases

The elevation of biomarker enzymes such as Triglycerides, Total cholesterol, HDL-Cholesterol, LDL-Cholesterol, SGPT, and SGOT was observed in diabetic control rats and alteration in their levels indicates the hepatic damage. The data of the same is given in fig no:1 and table 3. The hepatic damage was restored in hepatocytes and the elevated transaminases, lipoproteins(LDL-C), triglycerides were significantly reduced by combination of *Momordica charantia* fruit extract and Metformin, these combination shows additive effect in decreasing of elevated above biomarker enzymes in diabetic rats induced by streptozotocin. The SGOT and SGPT levels were significantly reduced in the diabetic group treated with combination as compared to the diabetic group. The results demonstrated that combination of *Momordica charantia* fruit extract (500mg/kg) and Metformin exhibited a potent additive hypocholesterolemic effect.

Table 3: Effect of MCFE and Metformin in control and STZ induced diabetic rats on SGOT & SGPT

Group	SGOT (units/dl)	SGPT (units/dl)
Control.	70.33±1.67	84.28±3.15
Diabetic Control.	272.31±4.41	189.58±6.03
Diabetic+Metformin (55mg/kg)	82.93±2.50**	111.75±3.21**
Diabetic+Fruitextract (500mg/kg)	72.73±1.15*	93.66±3.51*
Diabetic+Metformin (55mg/kg) +Fruitextract (500mg/kg)	71.50±1.399*	89.93±2.96*

Each value represents Mean ± S.E.M.(Standard error mean),n=6.

Antioxidant parameters in liver and kidneys

The data of TBARS, SOD, CAT and GSH levels in liver and kidneys are shown in table 4 and 5. The TBARS levels were raised in diabetic control rats which were lowered in the treatment groups with *momordica charantia* fruit extract and the standard Metformin treated groups, the reduction in the TBARS levels is more significant by the combination of *Momordica charantia* fruit extract (500mg/kg) and Metformin to have the protective action from the peroxides.

The SOD, CAT and GSH activity significantly decreased (P<0.001) in diabetic control group of rats, which may be due to inactivation caused by free radicals. However, administration of *Momordica charantia* fruit extract (500 mg/kg), Metformin reversed the progress of the disease. The above observations may clearly suggest that significant increased levels of SOD, CAT and GSH due to combination which has very significant free radical scavenging activity, compared with individual effects, which may exert a beneficial effect against pathological alterations caused by reactive oxygen species.

Table 4: Effect of MCFE and Metformin in control and STZ induced diabetic rats on antioxidant parameters in liver tissue

Group	TBARS	SOD	CAT	GSH
Control.	0.87±0.052	27.26±0.56	0.351±0.023	61.2±2.93
Diabetic Control.	1.86±0.065	13.18±0.63	0.11±0.012	22.48±1.42
Diabetic + Metformin (55mg/kg)	1.126±0.043	17.61±0.33	0.326±0.016	55.05±2.28
Diabetic+Fruit extract (500mg/kg)	0.885±0.027	23.93±3.48	0.403±0.013	67.21±1.32
Diabetic + Metformin (55mg/kg)+ Fruit extract (500mg/kg)	0.961±0.028	23.81±1.55	0.35±0.014	59.53±1.93

Each value represents Mean ± S.E.M.(Standard error mean),n=6.
*=P<0.001=Extremely significant. **=P<0.05=statistically significant.

Table 5: Effect of MCFE and Metformin in control and STZ induced diabetic rats on antioxidant parameters in kidney tissue

Group	TBARS (nM/mg protein)	SOD (units/min/mg of protein)	CAT (units/min/mg of protein)	GSH 9µg/mg of protein
Control.	1.37±0.032	14.36±1.69	0.18±0.011	43.18±1.07
Diabetic Control.	3.17±0.10	9.67±0.52	0.078±0.003	23.07±1.21
Diabetic+Metformin (55mg/kg)	2.02±0.074**	13.51±0.82**	0.136±0.012*	38.2±1.42*
Diabetic+Fruit extract (500mg/kg)	1.33±0.018*	15.73±0.39*	0.205±0.017*	45.36±1.06*
	1.44±0.10*	14.38±0.57*	0.176±0.016*	38.33±1.38*

Each value represents Mean ± S.E.M.(Standard error mean),n=6.

*=P<0.001=Extremely significant. **=P<0.05=statistically significant.

DISCUSSION

The hypoglycemic effect of *Momordica charantia* was compared with metformin in newely diagnosed type-2 diabetes patients. [18] It has been reported that the acetone extract of *Momordica charantia* fruits in alloxan induced diabetic rats results in activation of *ß*-cells and insulinogenic effects may also have brought about hypoglycemic action through stimulation of surviving *ß* cells of islets of Langerhans to release more insulin. [19] This was clearly evidenced from the results that the percentage fall in serum glucose levels. The concentrations of lipids, such as cholesterol, TG, LDL-C and HDL-C, were significantly higher in diabetic rats than in the control group (Table 2). A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. [20] Further, it can be ascertained that diabetic rats treated with metformin, *Momordica charantia* fruits extract shows normalized lipid levels. Thus, the results indicates that combination of metformin and *Momordica charantia* fruits extract shows insulin-like action by virtue of its lipid lowering effect. [21]

The protective role of *Momordica charantia* could be due to the antioxidative effect of flavonoids present in the fruit which in turn acts as strong superoxide radicals and singlet oxygen quenchers. [10, 11]

In the current study, the SOD, CAT and GSH activities were significantly reduced in the liver and kidneys of diabetic rats. These observations emphasize the critical importance of

maintaining the antioxidant potential of the pancreatic ß cell in order to ensure both its survival and insulin secretion capacity during the times of increased oxidative stress. [22] The decreased activities of SOD, CAT and GSH in both liver and kidneys during diabetes mellitus may be due to the production of reactive oxygen free-radical that can themselves reduce the activity of these enzymes. Combined treatment resulted in the significant ($P<0.001$) elevation of the catalase, SOD and GSH levels which protect the cell membrane against oxidative damage of protein in the membrane when compared to their individual treatment, this combination showed synergistic effect in its antioxidant activity.

CONCLUSION

Diabetes Mellitus (DM) is a leading cause of illness and death in most of the developed and in many developing countries. The results from the study clearly highlights the beneficial effects of *Momordica charantia* when given in combination with metformin helps in controlling blood glucose levels, improve the lipid metabolism and prevents diabetic complications from lipid peroxidation and antioxidant systems in experimental streptozotocin induced diabetic rats.

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