ABSTRACT

**Background and Objective:** The present study was undertaken to investigate the effect of Momordica charantia by virtue of its hypoglycemic, antioxidant activity and hypolipidemic activity which would modulate the diabetes gastropathy induced by in high fat diet with streptozotocin induced diabetes. The study also investigates the combined effect of Momordica charantia with oral hypoglycemic agents in diabetes induced gastropathy. **Methods:** The methanolic extract of Momordica charantia was obtained from distillation with soxhlet apparatus. The Sprague-Dawley rats was fed high fat diet (HFD), once a day for 2 weeks followed by i.p. injection of streptozotocin (35 mg/kg) dissolved in 1M/l citrate buffer (pH 4.4) after overnight fasting. Single and multiple dose studies of fenugreek seed extract and standard oral hypoglycemics was performed to assess...
the anti hyperglycaemic activity. The animals were treated for 10 weeks with monotherapy of Glipizide, Quercitine, and Methanolic extract and with the combination of standard oral hypoglycemics & also with combination of polyphenolic extract and the standard drugs. Body weight was measured every week end. Water and feed intake was recorded. The blood samples was collected once in 15 days of the treatment period. Blood glucose (Glu), total cholesterol (TC), HDL-cholesterol (HDL), and triglyceride (TG) levels was measured using standard kits. The following gastropathy models were carried out on the treated groups ie, colonic contractility, gastric emptying time, gastric fundus. Antioxidants status was estimated with the stomach homogenate at the end of the treatment. 

**Results:** The combination therapy of Momordica charantia fruit Methanolic extract with oral hypoglycemic agents (Glipizide) was more effective in glycemic control, regaining of other parameters(body weight, water intake, urine output, cholesterol, triglycerides, HDL) to the normal range as in non diabetic animals. The antioxidants levels i.e. SOD and catalase was increased and TBARS was found to be decreased. The combination of standard drugs showed hypoglycemia at the 8th week. The groups treated with monotherapy of standard drugs was not found to be effective in glycemic control and hence failed in preventing the stomach damage. 

**Interpretation and Conclusion:** The polyphenolic extract of fenugreek seeds were effective in decreasing the gastropathic effect along with glycemic control. The combination of Methanolic extract of Momordica charantia fruit extract and Oral hypoglycemics agents attenuated the progression of Diabetes Gastropathy by improving diabetes induced raised levels of TBARS, and decreased level catalase and superoxide dismutase thereby increased gastric emptying time, improved intestinal motility and enhanced intestinal transit of charcoal meal in diabetic rats. It has also decreased serum triglyceride, total cholesterol levels and increased HDL levels. The strong antioxidant activity of the selected herb improved the response of colonic smooth muscle and may be evaluated as preventive therapy in diabetic induced delay in intestinal motility in patients at risk of developing diabetic autonomic neuropathy. Further studies required on long term studies.

**KEY WORDS:** Diabetic Gastropathy, Colonic Contractility, Gastric Emptying Time, Gastric Fundus, Momordica Charantia Fruit Extract, GLIPIZIDE, Quercitine, Stomach.

**INTRODUCTION**

Diabetes Mellitus (DM) is a complex metabolic disorder primarily characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Changes in
human behavior and lifestyle over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide. Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complication that result in significant morbidity and mortality.\textsuperscript{[1]} It is considered as one of the five leading causes of death in the world. About 150 million or 1.3% people are suffering from diabetes worldwide which is almost five times more than the estimates ten years ago and this may double by the year 2030.\textsuperscript{[2,3]} Clinically, diabetes is divided into two major types (World Health Organization, 1999). Type 1, or insulin dependent diabetes mellitus (IDDM), requires continuous exogenous insulin replacement therapy in addition to diet and activity management to control blood glucose. Type 1 diabetes accounts for 5-10% of all diabetes. The etiology of type 1 diabetes is due to degeneration of pancreatic β-cells. Type 1 diabetes usually develops in young children, and as a result of having diabetes for a longer duration than type 2 diabetes, these patients carry the risk of more serious complications than type 2 diabetes. Type 2 diabetes or non-insulin dependent diabetes (NIDDM) usually occurs in mature individuals, and is extremely common, accounting for 90-95% of all cases of diabetes. This form of diabetes can be managed by diet, exercise and eventually by drugs that lower glycemia by enhancing insulin secretion and/or sensitivity. It is believed that the etiology of type 2 diabetes involves a degree of insulin resistance in part due to a 'faulty' insulin receptor signal transduction. Type 2 diabetes can go undiagnosed for many years, and the number of cases that are being diagnosed is rising rapidly.

**Diabetic Complications**

The diabetic patient is susceptible to a wide variety of chronic complications. There are general 3 kinds of complications, which are macrovascular, microvascular and neurologic. Each may have a different mechanism of development although some factors are common to all. One common denominator in the development of all complications of diabetes is elevated blood glucose levels (Hyperglycemia).

Diabetes is associated with accelerated atherosclerotic macrovascular disease affecting arteries that supply to heart, brain and peripheral vasculature. As a result, patients with diabetes have a much higher risk of myocardial infarction, stroke and limb amputation than non diabetic people. Microvascular and neural complications of diabetes contribute to the retinopathy, nephropathy, gastropathy and neuropathy that are characteristics of diabetes. Most diabetics develop at least some evidence of complications within 16 years of being
diagnosed, which can significantly affect the patient's health and quality of life, and make diabetes one of the most costly and burdensome chronic diseases of our time.\[^5\] The fruits of *Momordica charantia* L (bitter ground, karela) are widely used vegetable in different part of the world. It is reported to possess wide range of pharmacological activities such as hypoglycemic\[^6\], anti-diabetic\[^7\], anti hyperlipidemic\[^8\], and antioxidant activities.\[^9\]

Diabetic animal models exhibit changes in gastrointestinal function that resemble the abnormalities manifested in human disease. Delayed small intestinal transit and megacolon have been demonstrated in streptozotocin treated diabetic rats.\[^10\]-\[^11\] Oxidative stress plays a vital role in contributing to neural and vascular complications.\[^12\] Dietary supplements of antioxidants are required to achieve an increase in antioxidant status which may diminish oxidative stress associated with diabetes mellitus.\[^13\] Oxidative stress is believed to play a role in the development of complications in many tissues.\[^14\] Quercetin has antioxidant-scavenging activity\[^15\]-\[^16\], delays lipid peroxidation of cell membranes\[^13\], and reduces Cu\(^{2+}\)-induced LDL oxidation.\[^17\] Quercetin is reported to chelate copper ions and thus inhibit oxidation of LDL\[^18\] and thus beneficial in preventing lipid peroxidation. *Momordica charantia* are used extensively in diabetic condition, but how far it is authenticated in preventing diabetic complications is questionable. Hence the present study is useful to study its role in prevention of diabetic complications. *Momordica charantia* in combination with oral hypoglycemic agents is not been studied in diabetes induced gastropathy. Hence the present study was undertaken to investigate effect of *Momordica charantia* extract alone and in combination with oral hypoglycemic agents in rats with type 2 diabetes induced gastropathy.

**OBJECTIVES**

The objective of the present study is to evaluate the protective action of *momordica charantia* fruit extract with oral hypoglycemic agents against gastropathy and to delineate its possible adjuvant in diabetic Gastropathy.

**Specific Objectives**

1. To study the role of *Momordica charantia* fruit extract in diabetic gastropathy.
2. To study the role oral hypoglycemics in diabetic gastropathy.
3. To study the role of hypolipidemic activity of *Momordica charantia* fruit extract in diabetic gastropathy.
4. To study the role of antioxidant property of *Momordica charantia* fruit extract in attenuating the diabetic gastropathy.
5. To study the effect of the *Momordica charantia* fruit extract in combination with standard hypoglycemic agents in diabetic gastropathy.

**Methodology**

**Experimental Animals:** Male Sprague dawley Rats weighing 200-300 g were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional ethical committee approved the experimental protocol (KCP/IAEC-27/2008-09). Animal handling was performed according to Good Laboratory Practice (GLP).

**Extraction of Methanolic extract of Momordica charantia:** 100 grams of the finely grounded sample was weighed into a test tube and 1000 ml of methanol was added and the sample was then extracted for 2 hr. in a ultrasonic bath at 65°C. After extraction, the sample was cooled to room temperature and then centrifuged at 1500 rpm for 15 mins.

**Momordica charantia extraction**[146]: An authentic standardize MC extract’s will be obtained from phytotech Extracts Pvt.Ltd., Bangalore, India. The preparation has been standardised and optimised at the commercial plant of Phytotech extract’s Pvt.Ltd., Bangalore, India.

**List of Apparatus and chemical used**

<table>
<thead>
<tr>
<th>Chemicals and Apparatus</th>
<th>Manufacturer</th>
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</thead>
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<td>1. Streptozotocin</td>
<td>Sigma Aldrich</td>
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<td>2. Glipizide</td>
<td>Cadila</td>
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<tr>
<td>3. Acarbose</td>
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<td>4. Analytical balance</td>
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<td>6. Students Physiograph</td>
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<td>7. Calcium chloride</td>
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<tr>
<td>8. Sucrose</td>
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<tr>
<td>9. D- Glucose</td>
<td>Loba Chemicals, Mumbai</td>
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<td>11. Ethanol</td>
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<td>15. Nitro blue tetrazolium(NBT)</td>
<td>Loba Chemicals, Mumbai, India</td>
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<tr>
<td>16. Potassium chloride</td>
<td>Qualigens Fine Chemicals, Mumbai</td>
</tr>
</tbody>
</table>
Experimental models (standardization)

Oral Glucose Tolerance Test\textsuperscript{[147]}: The oral glucose tolerance test was performed in overnight fasted (18h) normal control and diabetic rats. Rats were divided into ten groups and treatment was given as mentioned below. Glucose (2 g/kg. p.o) was fed 30min after the administration of drugs. Blood was withdrawn from the tail vein under light ether anesthesia at 30, 60 and 120 min of glucose administration. Glucose levels were determine using Accuchek active glucose strips by using Accuchek glucometer manufactured by Roche.

The oral glucose tolerance test will be performed on overnight fasted diabetic rats. Rats will be divided into different groups as follows

Group 1: Normal control, rats receive saline/vehicle.
Group 2: Diabetic control
Group 3: \textit{Momordica charantia} fruit extract
Group 4: Glipizide (5 mg/kg)
Group 5: Quercetin
Group 6: Diabetic rats treated with \textit{Momordica charantia}(500mg/kg) fruit extract + glipizide(5 mg/kg).

Preparation of fructose diet\textsuperscript{[148]}

Fructose diet was prepared freshly every day and this consisted of 660g of fructose, 100g of protein 80g of fat,0.04g of zinc carbonate, 5g of vitamin mixture, 5g of mineral mixture and 150g of cellulose all of commercial grade.

Development of high fat diet (HFD) fed / Low dose streptozotocin Treated type 2 diabetic rats\textsuperscript{[148]}: The animals were fed with HFD once a day for two weeks followed by I.P injection of streptozotocin (35mg/kg) dissolved in 0.5M/l citrate buffer ( pH: 4.4) after overnight fasting. STZ injected animals were then given 5% w/v glucose solution for 5-6 hours following the injection to prevent initial drug induced hypoglycaemic mortality. The rats with non fasted plasma level ≥ 300mg/dl were considered diabetic. The blood Sample was collected from tail vein and blood glucose was checked using glucose diagnostic kit (accuchek).
Preparation of citrate buffer pH 4.4: Solution-A was prepared by dissolving 1.92 g of citric acid in 100 ml of distilled water to give 0.1 M citric acid solution. Solution-B was prepared by dissolving 2.94 g of tri-sodium citrate in 100 ml of distilled water to give 0.1 M sodium citrate solution. To 28 ml of solution-A, 22 ml of solution-B is added and diluted to 100 ml with distilled water to give citrate buffer of pH 4.4. The pH of this solution was adjusted to 4.5 with 0.1 M sodium citrate solution.

Single dose study\(^{[149]}\): The animals were segregated into six groups of four rats in each. Group I and II rats were randomly selected from normal rats that received only distilled water and the extract (500 mg/kg, p.o.) respectively. Group III to Group VI animals were selected from the STZ induced rats. Group II animals served as diabetic control. Group III animals received extract (500 mg/kg) and group IV was treated with the Glipizide (5 mg/kg) group V was treated with Quercetin in a similar manner. Glucose will be fed 30 min after the administration of drugs. Blood will be withdrawn from the tail vein under ether inhalation at 30, 60 and 120 min of glucose administration. Glucose levels will be estimated by using Accucheck glucometer. Blood samples were collected from the tip of tail of each rat under mild ether anesthesia at 0 h, 1 h, 2 h and 4 h after the administration of test samples and tested for glucose concentration as above.

Multidose study\(^{[149]}\): For multidose study, administration of test samples was continued for 10 days, once daily through oral route. Blood samples were collected from the tip of tail and the estimation of blood glucose was carried out as above on the 1, 3, 7 and 10 day of the drug administration. Body weights of all the animals were recorded just prior to 10th day for weight.

Experimental Models

Colonic Contractility\(^{[150]}\)

Charcoal meal transit in small intestine

At the end of the treatment period, overnight fasted animals of different groups were administered; po, 2ml/rat with charcoal meal (10% charcoal in 5% gum acacia) and 20 min later the rats were killed by cervical dislocation. The abdomen was opened and the intestine was removed from pyloric junction to ceecal end. Then colon was separated and kept in continuously aerated Tyrode’s solution. The farthest distance traveled by the charcoal meal through the small intestine and total length of the intestine were measured. Gastrointestinal
transit was expressed as the percentage of the distance traveled by the charcoal meal relative to the total length of small intestine

Contractile response of colonic smooth muscle
Immediately after cleansing the colon, 1 cm of distal colon was mounted under a resting tension of 0.5g in an organ bath (40 ml) containing continuously aerated Tyrode’s solution of the following composition (mmol/l): NaCl 118, KCl 4.7, CaCl2 2.5, MgSO4 7H2O 1.2, KH2PO4 1.2, NaHCO3 25, and glucose 11.1. The temperature was maintained at 37°C throughout the experiment and the tissue was allowed to equilibrate for 30 min before exposing to acetylcholine. A primary dose of 100 ng of acetylcholine was tested before starting the actual concentration response curve. The contractile responses were recorded isotonically using a student’s physiograph. At the end of the initial equilibration period dose response curves were obtained for ascending dose of acetylcholine. ED50 values of acetylcholine were calculated from the graph plotted using percent response against log dose. Rats were divided into 6 treatment groups of six animals each.

Group 1: Normal control, rats receive saline/vehicle.
Group 2: Diabetic control
Group 3: Diabetic rats treated with Momordica charantia fruit extract.
Group 4: Diabetic rats treated with glipizide.
Group 5: Diabetic rats treated with Quercetin
Group 6: Diabetic rats treated with Momordica charantia fruit extract + glipizide.(5 mg/kg).

PARAMETERS ANALYZED
Body weight: The body weight of each animal was recorded every week and was tabulated.
Estimation of Blood Glucose\textsuperscript{[148]}: The blood was withdrawn through the tail vein, and the glucose levels was estimated using glucose dioxidoreductase reactive strips and glucometer (Accu-check, Roche diagnostics USA).

Serum Cholesterol Estimation\textsuperscript{[151]}: Serum Cholesterol was estimated by the modified Roeschllau’s method (end point method) with the help of Clinical Chemistry Analyzer using CHOLESTEROL DYNAMIC EXTENDED STABILITY REAGENT (Erba Mannheim GmbH, Germany).
**Principle**

The estimation of cholesterol involves the following enzyme catalyzed reactions as follows

\[
\text{Cholesterol esterase} \\
\text{Cholesterol ester} \rightarrow \text{Cholesterol + Fatty acid}
\]

\[
\text{Cholesterol oxidase} \\
\text{Cholesterol + O}_2 \rightarrow \text{Cholest– 4-en-3-one + H}_2\text{O}_2
\]

\[
\text{Peroxidase} \\
\text{2H}_2\text{O}_2 + 4\text{- Aminoantipyrine + phenol} \rightarrow \text{H}_2\text{O} + \text{Quinoneimine}
\]

The absorbance of quinoneimine so formed is directly proportional to the cholesterol concentration in the sample.

**Procedure:** To 1000 μl of the reagent, 20 μl of standard cholesterol (200 mg/dl) was added and incubated for 10 min at 37°C. This incubated mixture was aspirated and concentration of standard was calibrated to show a value of 200mg/dl. The serum cholesterol was estimated by adding 20 μl of the serum sample to 1000 μl of the reagent, mixed well and incubated at 37°C for 10 min. This incubated mixture was aspirated and absorbance recorded against a reagent blank at 505 nm using Analyzer.

**Calculation**

\[
\text{Cholesterol} = \frac{\text{Absorbance of test}}{(\text{mg/dl})} \times \frac{\text{Absorbance of Std}}{(\text{mg/dl})} \times \text{Concentration of standard (mg/dl)}
\]

**Serum Triglyceride Estimation**\textsuperscript{[152]}: Serum triglyceride was estimated by the Wako method (end point method) with the help of Clinical Chemistry Analyzer (Metro Lab, 1600 DK-R) TRIGLYCERIDES DYNAMIC EXTENDED STABILITY REAGENT (Erba Mannheim GmbH, Germany).

**Principle**

\[
\text{Triglyceride + H}_2\text{O}_2 \rightarrow \text{Glycerol + Free Fatty Acids}
\]

\[
\text{Glycerol + Adenosine Triphosphate} \rightarrow \text{glycerol 3- phosphate + ADP}
\]

\[
\text{Glycerol-3-Phosphate + O}_2 \rightarrow \text{DAP+ H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + 4\text{- Aminoantipyrine + 3,5 DHBS} \rightarrow \text{Quinoneimine + 2H}_2\text{O}
\]
**Procedure:** To 1000 μl of the reagent, 10 μl of standard triglyceride (200 mg/dl) was added and incubated for 10 min at 37°C. This incubated mixture was aspirated and concentration of standard was calibrated to show a value of 200 mg/dl. The fasting serum triglyceride was estimated by adding 10 μl of the serum sample to 1000 μl of the reagent, mixed well and incubated at 37°C for 10 min. This incubated mixture was aspirated and absorbance recorded against a reagent blank at 505 nm using Auto Analyzer.

**Calculation**

\[
\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Std}} \times \text{Concentration of standard (mg/dl)}
\]

**Preparation of stomach Homogenate**[^153]: At the end of each experiment, the stomach was homogenized in 20 ml of cold 0.1 N perchloric acid containing 16.8 mg of disodium EDTA and 50 nmol of isopropyl homocholine as an internal standard, using a homogenizer. The homogenate was centrifuged for 20 min with 10,000 rpm at 48°C.

**Estimation of antioxidant enzymes**

i. **Estimation of total proteins**

The concentrations of proteins in the homogenate was estimated using the method of Lowry et al. Lowry method was based on the formation of colored complex of proteins on addition of Folin Ciocalteau reagent, that can be measured by colorimetric method at 610 nm using Bovine serum albumin as the standard.

**Reagents**

2gm of sodium carbonate was dissolved in 100ml of 0.1N NaOH solution to prepare alkaline reagent. Fresh alkaline mixture was prepared by adding 1ml of 4% aqueous copper sulphate solution to 100ml of alkaline reagent. Phenol reagent (Folin Ciocalteau reagent) was obtained commercially and was diluted with equal volume of distilled water before use and was stored in the refrigerator. Other chemicals required were 0.1% NaOH solution and 90% alcohol.

**Procedure:** 0.9ml of 90% alcohol was added to the 0.1g of stomach homogenate and was centrifuged at 300 rpm for about 10 minutes. The supernatant liquid obtained was discarded and the precipitate was dissolved in 1ml of 0.1% NaOH. 0.05ml of this solution was taken in...
a clean test tube, to which 4ml of freshly prepared alkaline mixture was added and allowed to stand for 10 minutes. To this solution 0.4ml of phenol reagent was added and again allowed to stand for 10 minutes for the reaction to complete. The absorbance was measured at 610 nm using distilled water as blank. 0.1 ml of standard bovine serum was taken in clean test tube and followed the same procedure as above and absorbance was measured. The amount of total protein present was calculated by using the formula

\[
\text{Protein}=\frac{\text{O.D of sample} \times \text{Final Volume} \times \text{Dilution Factor} \times \text{Concentration of std}}{\text{O.D of Standard}}
\]

ii. Estimation of superoxide dismutase\textsuperscript{[154]}

Estimation of SOD is based on the detection of $O_2^-$ be auto-oxidation of hydroxylamine hydrochloride producing nitrite, which is measured colorimetrically at 560 nm. The value of SOD was calculated in term of units defined as the amount of SOD that inhibits the reduction of nitroblue tetrazolium (NBT) by 50 %. NBT is reduced by auto-oxidation of hydroxylamine and nitrite is produced in presence of EDTA, which can be detected colorimetrically. SOD catalyses in the following manner

\[
O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2
\]

**Reagents**

*Sodium carbonate solution:* It is prepared by dissolving 1.06gm of sodium chloride in 100ml of distilled water.

*Nitro Blue Tetrazolium (NBT):* 10 mg of NBT was dissolved in 100 ml of distilled water.

*EDTA solution:* 37 mg of EDTA di-sodium salt was dissolved in 100 ml of distilled water.

*Hydroxylamine hydrochloride:* 16.5 mg of hydroxylamine hydrochloride was dissolved in 100ml of distilled water.

**Procedure:** 100 μl of stomach homogenate in 0.2 M sucrose in 0.25 M phosphate buffer of pH 7.4 was taken in a test tube to which 1ml of sodium carbonate solution, 0.4 ml of NBT and 0.2 ml of EDTA was added and zero minute reading was taken at 560 nm.

The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride to the above test tube. The test tube containing the reaction mixture was incubated at 25°C for 5 minutes during which the NBT gets reduced and was measured at 560 nm.
A parallel control without stomach homogenate was also treated in similar manner as test. One enzymatic unit of SOD is the amount in the form of proteins present in 100 μl of serum required to inhibit the reduction of 24 mM NBT by 50% and is expressed as units /mg of protein.

The concentration of the enzyme is calculated using the formula:

\[
\text{Units/mg} = \frac{1000}{\mu\text{gm of enzyme resulting in } \frac{1}{2} \text{ max. Inhibition}}
\]

### iii. Estimation of Catalase

The rate of decomposition of H\(_2\)O\(_2\) was measured spectrophotometrically at 240 nm. Catalase catalyzes the following reaction:

\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

\[
\text{ROOH} + \text{AH}_2 \rightarrow \text{H}_2\text{O} + \text{ROH} + \text{A}
\]

Catalase was estimated by determining the decomposition of H\(_2\)O\(_2\) at 240 nm in an assay mixture containing phosphate buffer (0.25 M, pH 7).

**Reagents**

*Phosphate buffer (0.25 M, pH 7):* 3.22 gm potassium hydrogen orthophosphate and 7.268 gm of disodium hydrogen orthophosphate was dissolved in distilled water and volume was made up to 1000 ml.

*Hydrogen peroxide:* 0.34 ml of 30% hydrogen peroxide was diluted to 100 ml with distilled water.

**Procedure:** 100 μl of stomach homogenate in 0.15 M KCl buffer was added to test tube containing 1/9 ml of 0.25 M phosphate buffer at pH 7 and absorbance was measured at 240 nm.

To the above reaction mixture 1ml of hydrogen peroxide was added and the sample was allowed to stand for 1 minute and the absorbance was measured at 240 nm using phosphate buffer as blank solution. One international unit of catalase utilized is that amount which catalyzes the decomposition of 1mM hydrogen peroxide per minute at room temperature and is expressed in terms of units/mg of protein.
The catalase was calculated using the following formula

\[ \text{Absorbance}_{240 \text{ nm}} \times 100 \]

\[ \frac{\text{Units/mg}}{43.6 \times \text{mg of enzyme/ml reaction mixture}} \]

**iv. Estimation of lipid peroxidation**\(^{[156,157]}\)

Lipid peroxidation was assessed by the complex formed between malonaldehyde (MDA) and thiobarbituric acid (TBA). Thiobarbituric acid reactive substance (TBARS) in the serum was estimated using the standard protocol. When TBA reacts with MDA, which is a secondary product of lipid peroxidation a reddish pink color was developed which was estimated at 532 nm.

**Reagents**

*5N hydrochloric acid (HCl):* 42.5 ml of Concentrated HCl was added to distilled water and volume made up to 100 ml with distilled water.

*15% Trichloroacetic acid (TCA):* 7.5 gm of TCA was dissolved in distilled water and make up the volume to 50 ml with distilled water.

*0.5 N Sodium hydroxide (NaOH):* 1g of NaOH was dissolved in distilled water and the volume was made up to 25 ml with distilled water.

*0.375% Thiobarbituric acid (TBA):* 0.094 gm of TBA was dissolved in 0.5N NaOH solution and adjusted the volume to 25 ml with 0.5 N NaOH solution.

**Procedure**

To 500 μl of sample stomach homogenate, 300 μl of 5N HCL, 300 μl of 15 % TCA and 300 μl of 0.375% TBA was added and heated at 95°C for 15 minutes. After cooling of mixture, it was centrifuged. The supernatant was collected and its absorbance was measured at 532 nm against reagent blank.

The amount of lipid peroxidation was determined by using the following formula

\[ \varepsilon = 1.56 \times 105 \text{ M-lcm-1} \]

and expressed as nM of TBARS/mg of protein.

**Statistical Analysis**

Data are presented as the mean ± SE from 6 rats per group. Comparison of mean values among the various groups was performed by one way ANOVA. For the single comparison between the groups unpaired Student’s t-test was used. P values less than 0.05 were considered significant.
RESULTS

EXPERIMENTAL MODELS

Effect of Momordica charantia and standard drugs on OGTT in Normal rats
The effect of PEFS and standard drugs Glipizide and Quercetin and their combinations on OGTT were performed on normal rats and the observations are tabulated in the table. The percentage reductions in 2 hours after feeding 2g/kg of glucose are calculated. All the groups showed significant reduction in BGL in 2 hrs when compared to control group.75% of the dose of standard OHA’s was fixed as a low dose for the treatment in.

Effect of Momordica charantia and standard drugs on OGTT in Diabetic rats
The percentage of reduction in blood glucose levels in 2hrs was extremely significant with groups treated with MC alone and in combination with glipizide; and quercetin when compared to diabetic control group. Groups treated with single standard OHA produced significant decrease in blood glucose when compared to combination of the OHA’s which produced hypoglycemia.

Effect of Momordica charantia and standard drugs on body weight
The body weight at different periods of various groups of animals during the study period are given in the table no 9, which shows that the mean body weight of rats at day 0, which was recorded before induction of STZ, and which were recorded during and at the end of the treatment protocol. The mean body weight of normal rats was gained during the study period. The body weights of diabetic control on groups were found to be reduced throughout the treatment period. During the same period of treatment the mean body weight of animals treated with combination of OHA and MC were found to decrease non-significantly in comparison with the diabetic group. Significant loss of weight was seen in groups treated with Standard OHA alone. The MC group and the group treated with G and showed considerable improvement in body weight.

Effect of Momordica charantia and standard drugs on blood glucose levels
The fasting serum glucose levels of different groups of animals during the study period are given in the table. The mean (± SEM) serum glucose level of the Normal control group of rats was 122.5± 0.5 and 125±0.8 mg/dl on 60th day. The values indicate that the serum glucose levels in normal group of rats were maintained throughout the period of study. As shown in the table serum glucose was significantly (<0.01) increased of serum glucose in groups of STZ-induced diabetic rat and no control was observed in diabetic control group.
Groups treated with combination of Standard OHA and MC have shown significant control in serum glucose levels by 60th day when compared to groups treated with standard OHA alone. After 30 days the group treated with MC alone has shown promising control in serum glucose. The groups treated with standard OHA in combination has shown hypoglycemia at the 60th day.

**Effect of Momordica charantia and standard drugs on Total cholesterol and Triglycerides (TC and TG):** The total cholesterol and triglyceride of the DC control of the models was found to be increased when compared to NC. In the models significant decline in TC and TG was observed in the groups treated with G+MC and when compared to DC. Moderate decrease of the TG and TC was seen in Q, MC treated groups in both the models. But the groups treated with G and failed to show the significant effect.

**Effect of Momordica charantia and standard drugs on HDL**
Severe decrease of HDL was observed in the DC group in the models when compared to NC. In the models there was significant improvement in level of HDL in the groups treated with G+MC. Moderate raise in HDL was seen in Q and MC treated groups. And not much significant raise in HDL was observed in groups treated with G and in the models.

**Effect of Antioxidants in serum and stomach homogenate**

*i. Effect of MC in and kidney homogenate in TBARS:* In preventive therapy MC (P<0.001), MC + G (P<0.001) showed significant decrease in the TBARS levels in stomach homogenate when compared to diabetic control(0.42). In serum similarly MC + G and MC showed significant decrease in TBARS when compared to diabetic control groups.

**ii. Effect of MC in stomach homogenate on catalase:** MC +G treated groups significantly (P<0.001) increased stomach homogenate catalase when compared to diabetic control. Other groups also showed significant (P<0.01) increase in the stomach catalase activity when compared to diabetic control. In serum MC+G treated group showed the significant increase in catalase when compared to diabetic control.

**iii. Effect of MC in stomach homogenate on SOD:** MC+G treated groups significantly increased the stomach homogenate SOD levels when compared to diabetic control. Other groups Q and G also significantly (P<0.01) increased the SOD levels in kidney homogenate when compared to diabetic control.
Effect of MC on colonic contractility and charcoal meal transit models

The colonic contractility and gastric fundus contractile response of the DC control of the models was found to be decreased when compared to NC. In the models significant increased in colonic contractility and gastric fundus contractile response was observed in the groups treated with G+MC when compared to DC. Moderate increase of the colonic contractility was seen in MC and Q treated groups in both the models. But the groups treated with G and failed to show the significant effect. The charcoal meal transit in small intestinal decreased in the DC control of the models when compared to NC. In the models significant increased in charcoal meal transit in the groups treated with G+M when compared to DC. Similarly Q and G show less significance than the G+MC.

Out of all the parameters studied above MC, combination of MC + Glipizide(G) showed much significant results than the quercetin.

Table. 1: Effect of Momordica charantia and its combination with Oral Hypoglycemics in OGTT (Mg/dl) (Normal).

<table>
<thead>
<tr>
<th>TIME</th>
<th>0 min</th>
<th>30 min</th>
<th>60min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>84±1.1</td>
<td>120.6±0.8 (↑43.5%)</td>
<td>114.71 ±1.5 (↑36.5%)</td>
<td>86.2± 0.6 (↓2.61%)</td>
</tr>
<tr>
<td>DC</td>
<td>205±2.5</td>
<td>289±2.6 (↑40.9%)</td>
<td>298.3 ±3.0 (↑50.63%)</td>
<td>285.4±2.1 (↑%)</td>
</tr>
<tr>
<td>MC</td>
<td>86.3 ±1.20</td>
<td>108.12± 4.1 (↑25.28%)</td>
<td>104.42±1.9 (↑20.9%)</td>
<td>88.90±0.8 (↑3.0%)</td>
</tr>
<tr>
<td>G</td>
<td>85.6 ±1.20</td>
<td>120.1±3.4 (↑40.1%)</td>
<td>112.33±1.6 (↑31.2%)</td>
<td>80.8 ±2.8 (↑5.7%)</td>
</tr>
<tr>
<td>QE</td>
<td>88.20 ±0.57</td>
<td>116.66± 3.1 (↑32.1%)</td>
<td>102.12±0.6 (↑15.7%)</td>
<td>84.99±0.5 (↑3.75%)</td>
</tr>
<tr>
<td>MC+G</td>
<td>88.66± 0.88</td>
<td>118.9± 2.7 (↑34.1%)</td>
<td>108.42±0.9 (↑22.34%)</td>
<td>78±2.6 (↑10.68%)</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, a P<0.001, bP<0.01, cP<0.05 when compared to normal control group.

Table. 2: Effect of MC and its combination with Oral hypoglycemics in OGTT (mg/dl) (Diabetic).

<table>
<thead>
<tr>
<th>TIME</th>
<th>0min</th>
<th>30min</th>
<th>60min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>89.26 ±1.2</td>
<td>104.1 ±2.0 (↑6%)</td>
<td>112.90± 0.9 (↑15.3%)</td>
<td>90.21± 2.1 (↑2.8%)</td>
</tr>
<tr>
<td>DC</td>
<td>188.06±3.9</td>
<td>249.2± 0.8 (↑17.5%)</td>
<td>298.14 ±0.8 (↑40%)</td>
<td>306.3±0.1 (↑44%)</td>
</tr>
<tr>
<td>MC</td>
<td>179.26 ±0.7</td>
<td>208.9± 3.3 (↑10.4%) b</td>
<td>222 ±1.2 (↑17%)</td>
<td>186.1±1.7 (↑2%) a</td>
</tr>
<tr>
<td>G</td>
<td>184.43± 0.4</td>
<td>236.67± 3.2 (↑17.4%)</td>
<td>257.45 ±4.1 (↑27%) c</td>
<td>212.7±5.0 (↑5.5%) b</td>
</tr>
<tr>
<td>QE</td>
<td>197.26±0.9</td>
<td>229.88±2.4 (↑12.56%) b</td>
<td>243.5±4.3 (↑20%) a</td>
<td>180±2.9 (↓12.4%) a</td>
</tr>
<tr>
<td>MC+G</td>
<td>166.33±1.1</td>
<td>229.3±2.2 (↑10%)</td>
<td>238.76±2.1 (↑19%) a</td>
<td>198.4±0.6 (↑21%) a</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, a P<0.001, b P<0.01, c P<0.05 when compared to diabetic control group.
Table 3: Effect of MC and its combination with Oral Hypoglycemics on Body weight (mg/dl) estimation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th day</th>
<th>15th day</th>
<th>30th day</th>
<th>45th day</th>
<th>60th day</th>
<th>75th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>2252±0.2</td>
<td>230.4±0.2</td>
<td>239.4±1.5</td>
<td>248.4±1.5</td>
<td>262±4.5</td>
<td>268±0.7</td>
</tr>
<tr>
<td>DC</td>
<td>200±0.6</td>
<td>215.1±0.9</td>
<td>196.4±1.7</td>
<td>187.5±1.5</td>
<td>179±4.5</td>
<td>169±0.4</td>
</tr>
<tr>
<td>MC</td>
<td>210±0.4</td>
<td>228.4±0.4</td>
<td>217.7±0.1</td>
<td>202.5±1.5</td>
<td>187.1±1.5</td>
<td>185±0.8</td>
</tr>
<tr>
<td>G</td>
<td>210±0.8</td>
<td>219.4±0.6</td>
<td>208.1±1.6</td>
<td>187.1±1.6</td>
<td>171.1±0.9</td>
<td>169±0.7</td>
</tr>
<tr>
<td>QE</td>
<td>211±0.4</td>
<td>217.4±1.2</td>
<td>202.4±1.4</td>
<td>182.6±0.2</td>
<td>169.1±0.15</td>
<td>170±1.1</td>
</tr>
<tr>
<td>MC+G</td>
<td>218±0.8</td>
<td>227.5±0.5</td>
<td>224.4±0.9</td>
<td>215.2±0.8</td>
<td>209±0.8</td>
<td></td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, a P<0.001, b P<0.01, c P<0.05 when compared to Diabetic control group.

Table 4: Effect of MC and its combination with Oral Hypoglycemics on Blood glucose (mg/dl) estimation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th day</th>
<th>15th day</th>
<th>30th day</th>
<th>45th day</th>
<th>60th day</th>
<th>75th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>124±0.8</td>
<td>123.2±0.12</td>
<td>126±0.24</td>
<td>128±0.5</td>
<td>122±0.5</td>
<td>123±0.8</td>
</tr>
<tr>
<td>DC</td>
<td>265±0.5</td>
<td>273.5±0.2</td>
<td>291.8±0.9</td>
<td>332.8±0.4</td>
<td>395.2±0.5</td>
<td>418±0.4</td>
</tr>
<tr>
<td>MC</td>
<td>241±0.1</td>
<td>248.4±0.8 b</td>
<td>235.8±0.5 b</td>
<td>228.4±0.6 b</td>
<td>219.4±0.5 b</td>
<td>139±0.9 b</td>
</tr>
<tr>
<td>G</td>
<td>245±0.8</td>
<td>252.5±0.5 c</td>
<td>241.2±1.8 c</td>
<td>234.6±0.8 c</td>
<td>225.8±0.6 c</td>
<td>143±0.4 c</td>
</tr>
<tr>
<td>QE</td>
<td>270±0.5</td>
<td>261±0.6 c</td>
<td>248.1±2.3 c</td>
<td>224.7±0.4 c</td>
<td>194.4±0.4 c</td>
<td>143±0.4 c</td>
</tr>
<tr>
<td>MC+G</td>
<td>271±0.8</td>
<td>268.4±0.2 a</td>
<td>247.6±1.5 a</td>
<td>234.5±0.6 a</td>
<td>184.4±0.6 a</td>
<td>144±0.5 a</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, a P<0.001, b P<0.01, c P<0.05 when compared to Diabetic control group.

Table 5: Effect of MC and its combination with Oral Hypoglycemics on HDL (mg/dl) estimation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th day</th>
<th>15th day</th>
<th>30th day</th>
<th>45th day</th>
<th>60th day</th>
<th>75th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>40±0.2</td>
<td>41.56±0.2</td>
<td>42.7±0.2</td>
<td>43.2±0.4</td>
<td>44.8±0.1</td>
<td>45±0.2</td>
</tr>
<tr>
<td>DC</td>
<td>44±0.6</td>
<td>21.4±0.7</td>
<td>25.2±0.7</td>
<td>25.3±0.8</td>
<td>28±0.88</td>
<td>24±0.3</td>
</tr>
<tr>
<td>MC</td>
<td>43.1±0.4</td>
<td>33.1±0.4 b</td>
<td>35.5±0.5 b</td>
<td>37.2±0.4 b</td>
<td>38.4±0.1 b</td>
<td>40±0.1 b</td>
</tr>
<tr>
<td>G</td>
<td>41.1±0.8</td>
<td>31.8±1.04 c</td>
<td>33.6±1.4 c</td>
<td>36.2±0.2 c</td>
<td>37.9±1.2 c</td>
<td>35.3±1.8 c</td>
</tr>
<tr>
<td>QE</td>
<td>42.1±0.4</td>
<td>30.3±0.5 c</td>
<td>34.06±0.8 c</td>
<td>35.5±0.4 c</td>
<td>34.4±1.8 c</td>
<td>37.7±1.2 c</td>
</tr>
<tr>
<td>MC+G</td>
<td>43±0.5</td>
<td>34.4±0.4 a</td>
<td>36.3±0.8 a</td>
<td>39.3±0.9 a</td>
<td>40.3±0.4 a</td>
<td>41.1±2.1 a</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, a P<0.001, b P<0.01, c P<0.05 when compared to Diabetic control group.
Table. 6: Effect of MC and its combination with Oral Hypoglycemics on Triglycerides (mg/dl) estimation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th day</th>
<th>15th day</th>
<th>30th day</th>
<th>45th day</th>
<th>60th day</th>
<th>75th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>94.1±0.8</td>
<td>95.8±0.4</td>
<td>96.6±0.6</td>
<td>98±1.3</td>
<td>96±0.1</td>
<td>96±0.2</td>
</tr>
<tr>
<td>DC</td>
<td>95.3±0.4</td>
<td>153±0.5</td>
<td>164.2±0.4</td>
<td>180±0.3</td>
<td>183±0.7</td>
<td>194.2±0.7</td>
</tr>
<tr>
<td>MC</td>
<td>95.1±0.6</td>
<td>145.3±0.8b</td>
<td>129±0.3b</td>
<td>123±0.34b</td>
<td>108±0.6b</td>
<td>110±0.5b</td>
</tr>
<tr>
<td>G</td>
<td>96.3±0.5</td>
<td>148±2.4c</td>
<td>133±0.3c</td>
<td>130±0.3c</td>
<td>115±0.14c</td>
<td>116±1.4c</td>
</tr>
<tr>
<td>QE</td>
<td>95.4±1.2</td>
<td>146.3±0.7c</td>
<td>133.8±0.6c</td>
<td>132±0.1c</td>
<td>117±0.84c</td>
<td>126±0.8c</td>
</tr>
<tr>
<td>MC+G</td>
<td>98.7±0.8</td>
<td>149.4±1.4c</td>
<td>134±0.45c</td>
<td>133±0.4c</td>
<td>114±0.3c</td>
<td>123.4±0.8c</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, a P<0.001, b P<0.01, c P<0.05 when compared to Diabetic control group.

Table.7. Effect of MC and its combination with Oral Hypoglycemics on Total Cholesterol (mg/dl) estimation

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th day</th>
<th>15th day</th>
<th>30th day</th>
<th>45th day</th>
<th>60th day</th>
<th>75th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>58.2±0.8</td>
<td>58.8±0.4</td>
<td>62.6±0.8</td>
<td>61±0.5</td>
<td>64.8±0.2</td>
<td>64.8±0.12</td>
</tr>
<tr>
<td>DC</td>
<td>60±0.4</td>
<td>110.7±0.6</td>
<td>116.6±0.8</td>
<td>117±2.5</td>
<td>119±0.7</td>
<td>128±0.2</td>
</tr>
<tr>
<td>MC</td>
<td>61.1±0.6</td>
<td>101.6±1.7b</td>
<td>95.6±2.8b</td>
<td>81±0.5b</td>
<td>79±0.7b</td>
<td>82.4±0.8b</td>
</tr>
<tr>
<td>G</td>
<td>62.4±0.5</td>
<td>104.6±1.2c</td>
<td>95.6±2.4c</td>
<td>90.3±1.3c</td>
<td>86±1.4c</td>
<td>95±0.5c</td>
</tr>
<tr>
<td>QE</td>
<td>62.5±1.2</td>
<td>102.7±0.4c</td>
<td>96±1.7c</td>
<td>98±1.6c</td>
<td>92±0.4c</td>
<td>91±0.6c</td>
</tr>
<tr>
<td>MC+G</td>
<td>64.4±0.8</td>
<td>105.6±1.4a</td>
<td>91±3.4a</td>
<td>86±1.6a</td>
<td>82±9.6a</td>
<td>87±0.2a</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, a P<0.001, b P<0.01, c P<0.05 when compared to Diabetic control group.

Table.8. Effect of MC and its combination with Oral Hypoglycemics on anti oxidant estimation in stomach homogenate

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>SOD (U/mg of protein)</th>
<th>CATALASE (U/ug of protein)</th>
<th>TBARS (Mm/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>4.32±0.27</td>
<td>2.83±1.29</td>
<td>4.01±0.02</td>
</tr>
<tr>
<td>DC</td>
<td>1.9±0.4</td>
<td>0.73±0.40</td>
<td>7.56±0.01</td>
</tr>
<tr>
<td>MC</td>
<td>3.6±0.41b</td>
<td>1.1±0.4b</td>
<td>5.60.07b</td>
</tr>
<tr>
<td>G</td>
<td>2.7±0.11c</td>
<td>1.4±0.3c</td>
<td>4.18±0.08c</td>
</tr>
<tr>
<td>QE</td>
<td>2.8±0.11c</td>
<td>1.4±0.11c</td>
<td>5.9±0.14c</td>
</tr>
<tr>
<td>MC+G</td>
<td>5.4±0.9a</td>
<td>3.2±0.14a</td>
<td>5.6±0.13a</td>
</tr>
</tbody>
</table>

All values are mean ± SEM, n=6, a P<0.001, b P<0.01, c P<0.05 when compared to Diabetic control group.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Contractile response of acetylcholine ED₅₀ (µg)</th>
<th>Charcoal meal colon to exogenous transit in small intestine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>1.18 ± 0.03</td>
<td>80.5 ± 3.01</td>
</tr>
<tr>
<td>DC</td>
<td>15.83 ± 2.37</td>
<td>59 ± 4.91</td>
</tr>
<tr>
<td>MC</td>
<td>6.9±0.82b</td>
<td>68±2.56b</td>
</tr>
<tr>
<td>G</td>
<td>6.1 ± 0.73b</td>
<td>73.16 ± 3.31b</td>
</tr>
<tr>
<td>QE</td>
<td>6.5 ± 0.54b</td>
<td>71 ± 0.9b</td>
</tr>
<tr>
<td>MC+QE</td>
<td>13.05±0.82a</td>
<td>67.5±1.23a</td>
</tr>
</tbody>
</table>

P value: *P < 0.001 when compared with control. **P < 0.001 when compared with diabetic control. ***P < 0.01 when compared with diabetic control.

DISCUSSION

Diabetic autonomic neuropathy (DAN) is among the least recognized and understood complications of diabetes despite its significant negative impact on survival and quality of life in people with diabetes.158,159 A subtype of the peripheral polyneuropathies that accompany diabetes, DAN can involve the entire autonomic nervous system (ANS). ANS vasomotor, visceromotor, and sensory fibers innervate every organ. DAN may be either clinically evident or subclinical. It is manifested by dysfunction of one or more organ systems (e.g., cardiovascular, gastrointestinal [GI], genitourinary, sudomotor, or ocular).160

Gastrointestinal tract is a prime target of diabetic autonomic neuropathy. Diabetic animal models exhibit changes in gastrointestinal function that resemble the abnormalities manifested in human disease. Delayed small intestinal transit and megacolon have been demonstrated in streptozotocin treated diabetic rats.161,162 Several different factors have been implicated in this pathogenic process. Hyperglycemic activation of the polyol pathway leading to accumulation of sorbitol and potential changes in the NAD: NADH ratio may cause direct neuronal damage and/or decreased nerve blood flow.163–165 Activation of protein kinase C induces vasoconstriction and reduces neuronal blood flow.165 Delayed gastrointestinal transit may be associated with cardiac autonomic neuropathy, blood glucose concentration, and gastrointestinal symptoms.166

In type 2 diabetic patients, the ingestion of 15 g of powdered (water soaked) Momordica charantia significantly reduced postprandial glucose levels during the glucose tolerance test. The treatment of diabetic rats with combined doses of vanadate and Momordica charantia was most effective in the normalization of plasma glucose levels and correction of altered metabolic parameters.
GLUT4 distribution. It has been shown that when whole seed powder added to the diet favorably affected glycolytic, gluconeogenic, and lipogenic enzymes to restore glucose homeostasis in alloxan-induced diabetic rats. The present study also showed similar results as reported earlier. There was a significant increase glucose tolerance when the extract was subjected to OGTT. The diabetic when treated with the extract (MC) showed a significant fall in the blood glucose level but it was less effective than the combined therapy of TFG and OHA’s. Low dose of OHA’s with MC had produced significant fall in the blood glucose level at the end of the treatment period.

A decrease in small intestinal transit of charcoal meal was observed in diabetic rats when compared to normal rats. This was in agreement with previous reports.\textsuperscript{167} There was a significant increase in the percentage distance traveled by charcoal meal in the small intestine of diabetic rats treated with MC and its combination with oral hypoglycemic agents. Since the delayed gastrointestinal transit in diabetes is associated with hyerglycemia, the therapy which can normalize the blood glucose level may be useful in the treatment diabetic autonomic neuropathy (DAN). MC and its combined therapy with OHA’s may be useful in the treatment of DAN. Increased oxidative stress, with increased free radical production, causes vascular endothelium damage and reduces nitric oxide bioavailability.\textsuperscript{165,166} Alternately, excess nitric oxide production may result in formation of peroxynitrite and damage endothelium and neurons, a process referred to as nitrosative stress.\textsuperscript{167,168,169}

Patients with diabetes mellitus may show gastrointestinal disturbances due to the damage of peripheral cholinergic neurons as a result of oxidative damage induced by the noted increase in the plasma and tissue levels of ROS.\textsuperscript{167} The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress. Alterations in the antioxidant enzyme activities and increased oxidative damage have been demonstrated in different tissues of diabetic animals. In addition to alterations in activities, changes in the mRNA expression of the antioxidant enzymes have also been reported. Diabetes is accompanied by several morphological and functional changes in the small intestinal mucosa. Suppression in apoptosis of intestinal epithelial cells during diabetes has been reported. This can result in a decline in the rate of disposal of oxidatively damaged epithelial cells further aggravating the damage. Since the small intestine is a primary site for the action of dietary antioxidants, their ability to prevent diabetes-induced oxidative damage to the small intestine may be significant in overcoming oxidative stress. The fruits of
Momordica charantia L were found to contains 5-hydroxy tryptamine, alkaloids, alpha spinasterol, ascorbic acid, ascorbigen, ash, beta alanine, beta carotene (5-6 epoxy), charantin, charine, are reported to be present in MC fruit. Extract has proven to protect against the development of diabetic gastropathy by inhibition of lipid peroxidation and restoration of antioxidant enzymes in diabetic rats. Earlier, vitamin E has been reported to produce significant improvement in small intestine transit percentage and this activity has been attributed to the antioxidant activity of vitamin E.[167]

Momordica charantia fruit also lower serum triglycerides, total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C). The present study also showed that there is significant fall in LDL, TG & TC while HDL increased when the diabetic rats were treated with MC and its combination therapy with OHA’s. As stated earlier, the present study also showed a profound inhibition of lipid peroxidation when the rats were treated with MC.

Poor glycemic control has the potential to cause delayed gastrointestinal transit in diabetic patients. Therefore the diabetic rats when treated with MC and in combination with OHA’s had reversed back the gastrointestinal transit towards normalcy due to better glycemic control.

Distal colons from untreated diabetic rats were found to be less sensitive to acetylcholine because the ED50 of acetylcholine required to produce adequate contractility was increased significantly when compared to control rats. These observations are in agreement with previous report. It is reported that there is diminished release or production of neurotransmitter, i.e; acetylcholine and enhanced degradation of the neurotransmitter, or diminished end organ sensitivities to the neurotransmitter itself. The reduced contractile response of colonic smooth muscle to exogenous acetylcholine may be the result of excessive degradation of acetylcholine by tissue acetylcholine esterase, diminished muscarinic receptor sensitivity or density or defective interaction between muscarinic receptor and intracellular contractile process.

Administration of diabetic rats with MC, improved the response of colonic smooth muscle to acetylcholine induced contractions. These effects may be due to antioxidant activity of the herb and correlates with other study, which had shown the effectiveness of vitamin E in improving the response of colonic smooth muscle in STZ induced diabetic rats towards acetylcholine.[167] A large body of evidence indicates that chronic diabetes causes decreased
relaxation responses at different regions of gastrointestinal tract of experimental diabetic rats. Therefore, the study investigated the influence of different treatment groups on contractile responses of carbachol in the gastric fundus study. Chronic diabetes caused hypersensitivity in fundus contractile responses to carbachol compared with the controls. Carbachol induce contractile responses in fundic strips at low contractions when compared with control which indicates hypersensitivity of the muscle. This effect is unusual for which the cause is not known. Hypersensitivity was reversed by combined therapy of MC and OHA when compared to control.

CONCLUSION
Treatment with MC and in combination with OHA’s has improved intestinal motility and enhanced intestinal transit of charcoal meal in diabetic rats. Treatment with OHA’s & MC produced a significant reversal of all the parameters measured, suggesting a role of hyperglycemia, hypolipidemia and oxidative stress involvement in diabetic complication. Further studies are required to study the combined effect of MC and OHA’s on a long term basis.

SUMMARY
The current investigation dealt with the pharmacodynamic interaction of momordica charantia fruit extract with oral hypoglycemic agents in diabetes induced gastropathy. The oral glucose tolerance test was performed on overnight fasted normal and diabetic rats. The treatment protocol was carried out after feeding the rats with high fat diet for fifteen days and later induction of diabetes by STZ. The treatment was given for 10 weeks for all the groups’ ie. OHA monotherapy, combination of OHA, and combination of polyphenolic fenugreek seed extract with OHA. Blood glucose and body weight of the animals was checked every week. Total cholesterol, triglycerides and HDL was estimated once in every 15 days throughout the treatment period. At the end of the treatment period 3 animal models ie, gastric emptying time, Colonic contractility and gastric fundus was used to evaluate the protective action of fenugreek seeds and low dose of oral hypoglycemic agents. Antioxidant status was estimated in the stomach homogenate at the end of treatment period. The present study also showed that there was a significant increase glucose tolerance when the extract was subjected to OGTT. The diabetic when treated with the combination of two standards OHA produced hypoglycemia which is an adverse effect as reported earlier. The extract MC showed a significant fall in the blood glucose level but it was less effective than the combined
therapy of MC and low dose of OHA’s. A decrease in small intestinal transit of charcoal meal was observed in diabetic rats when compared to normal rats. There was a significant increase in the percentage distance traveled by charcoal meal in the small intestine of diabetic rats treated with MC and its combination with oral hypoglycemic agents. Distal colons from untreated diabetic rats were found to be less sensitive to acetylcholine. Administration of diabetic rats with MC, improved the response of colonic smooth muscle to acetylcholine induced contractions. Alterations in the antioxidant enzyme activities and increased oxidative damage have been demonstrated in different tissues of diabetic animals. In addition to alterations in activities, changes in the mRNA expression of the antioxidant enzymes have also been reported. Momordica charantia extract has proven to protect against the development of diabetic gastropathy by inhibition of lipid peroxidation and restoration of antioxidant enzymes in diabetic rats. Earlier, vitamin E has been reported to produce significant improvement in small intestine transit percentage and this activity has been attributed to the antioxidant activity of vitamin E. The present study also showed that there is significant fall in LDL, TG & TC while HDL increased when the diabetic rats were treated with MC and its combination therapy with OHA’s. The diabetic rats when treated with MC and in combination with OHA’s had reversed back the gastrointestinal transit towards normalcy due to better glycemic control. Combined therapy of MC and OHA decreased hypersensitivity of the fundus contractile response of carbachol when compared to diabetic control. In the model of bethanechol, in diabetes Fundus showed decreased contractile response, Pylorus showed increased contractions and no response was observed in antrum when compared to Diabetic control. The symptoms of delayed gastric emptying, inhibition of migrating motor complexes and pyloric contractions has found to be significantly decreased in concomitant administration of MC and OHA.

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