Synergistic Antidiabetic and Antihyperlipidaemic Activity of Glibenclamide by Integrating Momordica charantia Fruit Juice in Streptozotocin Induced Diabetic Rat

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Abstract
Diabetes mellitus is among the most common disorder in developed and developing countries, and the disease is increasing rapidly in most parts of the world. It has been estimated that up to one-third of patients with diabetes mellitus use some form of complementary and alternative medicine. The aim of the present study was to evaluate synergistic antidiabetic and antihyperlipidaemic effects of Momordica charantia fruit juice along with Glibenclamide (lower dose) on glucose tolerance and lipid profile in streptozotocin induced diabetic rat. The Momordica charantia fruit juice (MCFJ) were prepared and different combination of MCFJ and Glibenclamide was evaluated for antidiabetic activity in experimental animals. The administration of MCFJ along with Glibenclamide significantly decreased the blood glucose levels compared to control diabetic rats. The MCFJ along with Glibenclamide significantly lowered the elevated total cholesterol, triglycerides (TGL), total protein and low density lipoprotein (LDL) level while increased the high density lipoprotein (HDL) indicates the antihyperlipidaemic activity. The findings demonstrated the MCFJ enhanced antidiabetic and antihyperlipidaemic activity of Glibenclamide and produced higher protective effect from diabetes.

1 Introduction
Diabetes mellitus (DM) is a severe medical issue being the third most noteworthy reason for death everywhere throughout the world, and if not treated, it is in charge of numerous complications influencing different organs in the body. The chronic hyperglycemia of diabetes is accompanying with long term damage, dysfunction, and failure of different organs. Internationally, starting at 2010, an expected 285 million individuals had diabetes, with type 2 making up about 90% of the cases. The World Health Organization measures an increase from 171 million out of 2000 to 366 million of every 2030. Roughly 70% of this development is anticipated to occur in the developing world and will progressively influence individuals matured more youthful than 65 years who are still in the productive stages of their life cycle.

Type 2 Diabetes mellitus (T2DM), the most well-known type of diabetes, is portrayed by hindered insulin sensitivity and secretion. Unlike Type 1 Diabetes mellitus (T1DM), people with T2DM hold a assured production of insulin although inadequate to keep the blood glucose inside ordinary range. The significant hazard factors which impact the developing T2DM are obesity and a family history of the disease. In type 2 diabetes, the body can deliver insulin however either this isn’t adequate or the body can't react to its belongings, prompting a development of glucose in the blood. Numerous individuals with type 2 diabetes stay ignorant of their sickness for a long time because symptoms may take a very long time to show up or be perceived. They are often diagnosed only when complications of diabetes have already developed. Several important risk factors of type 2 diabetes include obesity, poor diet, physical inactivity, advancing age, family history of diabetes, ethnicity and high blood glucose during pregnancy affecting the unborn child. The oral antihyperglycemic agents currently utilized in clinical practice have trademark profiles of genuine side effects. This prompts expanding interest for herbal products because of their effectiveness, minimal side effects in clinical experience and low cost. Therefore
search for harmless and more effective agents has continued to be a significant area of dynamic research.

Recently, some restorative plants have been accounted for to be helpful in diabetes worldwide and have been utilized observationally as antidiabetic and antihyperlipidemic cures. In excess of 400 plant species having hypoglycemic activity have been accessible in literature, however, searching for new antidiabetic drugs from natural plants is yet alluring because they contain substances which take alternate and harmless result on diabetes mellitus.

*Momordica charantia* commonly known as bitter melon, bitter gourd or balsam pear is a restorative plant having a place with the family Cucurbitaceae. The plant is a climbing plant with blooms and yellow fruits that present red seeds when are ripe. In Nigeria it is called by its neighborhood names which incorporate Ejin (Yoruba), Daladdasu (Hausa), Okwunuolo (Igbo). It is indigenous to tropical and subtropical areas of the world, for example, India, Asia, South America and Nigeria and broadly utilized as nourishment and medication. The phytochemical prospection of the fruit demonstrated the nearness of various classes of secondary metabolites, as flavonoids, alkaloids, saponins, tannins, steroids, triterpens, cumarines, quinones, organic acids and tannins. It possesses many uses, as antidiabetic, carminative, anthelmintic, antimalarial and antifungal, antiviral, anticancerigenous, contraceptive, immunostimulant and laxative, antioxidant and insecticidal, besides its indication in skin treatments (eczema, acne, mycoses, scabies, hemorrhoid and furuncules). Several scientists had revealed the adequacy of its extracts in the treatment of ailments such as lowering of blood sugar, controlling eye disorders, enhancing eyesight due to the presence of beta-carotene, diarrhea, pyorrhea that is bleeding from the gums, piles, hemorrhoids and respiratory problems. The image of fresh fruits of *Momordica charantia* are illustrated in Fig 1.

**Fig 1; Image of fruits of Momordica charantia**

The Glibenclamide is widely used as antidiabetic agent, but associated with side effects. The side effects of Glibenclamide can be reduce by decreasing its dose. Hence, the present study was planned to administer the *Momordica charantia* juice and Glibenclamide together to STZ induced diabetic rats, and investigate their antidiabetic and antihyperlipidaemic efficacy.

## 2 Material and Methods

### 2.1 Plant material

The fresh fruits of *Momordica charantia* were collected and authenticated by an authority in plant taxonomy.

### 2.2 Juice preparation

*Momordica charantia* fruits (MCF) were washed thoroughly, and fresh juice was prepared on a juicer eliminating seeds. It was centrifuged at 1000 rpm on a tabletop refrigerated centrifuge for 10 min at 4°C. The clear supernatant was considered as 100% *Momordica charantia* fruit juice (MCFJ) which was stored at 4°C for further use.

### 2.3 Animals

The animals were carried for experiment from the authorised animal house of Bhopal Nobles College of Pharmacy, Undaipur (RJ), India. All Wistar almino rats were healthy and 150 gm to 220 gm of body weight. The animals were kept in air conditioning environment and temperature was maintained to 25±2°C with conventional laboratory food and fresh drinking water. The bedding of animals was changed every 3rd day.

### 2.4 Induction of diabetes

Hyperglycemia was induced by injecting single intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg, 15 minutes after i.p. administration of 120 mg/kg of nicotinamide. The animals were kept under observation. After 72 hrs, the animals were tested for glucosuria using Diastex strips. Seven days after the STZ injection, rats with fasting blood glucose levels greater than 125 mg/dL were considered diabetic.

### 2.5 Treatment Protocol

Diabetic animals were randomly assigned into the following groups of six animals each and treated as follows.

- **Group I** served as normal rats, administered drinking water daily for 28 days
- **Group II** had diabetic control rats, administered drinking water daily for 28 days
- **Group III** rats were administered standard drug Glibenclamide (50 mg/kg) daily for 28 days
- **Group IV** rats were administered MCFJ (5 ml/kg body weight) + Glibenclamide (0.5 mg/kg) daily for 28 days
- **Group V** rats were administered MCFJ (10 ml/kg body weight) + Glibenclamide (0.5 mg/kg) daily for 28 days
- **Group VI** rats were administered MCFJ (15 ml/kg body weight) + Glibenclamide (0.5 mg/kg) daily for 28 days
The fasting glucose levels were determined on days 7th, 14th, 21st and 28th after juice administration.

2.6 Estimation of biochemical parameters

The biochemical parameters were determined on day 28 after the animals were sacrificed by cervical dislocation. Total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and total protein were determined by using an auto-analyzer\textsuperscript{11-14}.

2.7 Statistical analysis

The results are expressed as mean ± SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunet’s test. A $P < 0.05$ value was considered as statistically significant.

3 Results and Discussions

3.1 Antidiabetic effect of MCFJ

The existence of high fasting glucose level demonstrated the stimulation of diabetes in rats. The antidiabetic activity of MCFJ along with Glibenclamide on serum glucose levels of normal and Streptozotocin-induced rats are exhibited in table 1. The streptozotocin treated rats indicates significant increase in serum glucose level on 7th, 14th, 21st and 28th day compared to normal group rats. The administration of MCFJ at different doses along with Glibenclamide to rats leads to significant decrease in blood glucose level. The findings exhibited that MCFJ (15 ml/kg) along with Glibenclamide (0.5 mg/kg) produces more potent antidiabetic activity compared to other groups. The glucose level of group IV animals were nearer to standard group. This indicates that the administration of MCFJ and Glibenclamide produces synergistic effect and at lower dose of Glibenclamide have potent antidiabetic activity similar to higher dose of Glibenclamide.

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting plasma glucose concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>Normal control</td>
<td>74.8±3.1</td>
</tr>
<tr>
<td>Diabetic control (STZ 50 mg/kg)</td>
<td>159.2±4.8*</td>
</tr>
<tr>
<td>Standard group (Glibenclamide 50 mg/kg)</td>
<td>81.9±2.6*</td>
</tr>
<tr>
<td>MCFJ (5 ml/kg) + Glibenclamide (0.5 mg/kg)</td>
<td>132.1±3.9</td>
</tr>
<tr>
<td>MCFJ (10 ml/kg) + Glibenclamide (0.5 mg/kg)</td>
<td>102.5±4.5*</td>
</tr>
<tr>
<td>MCFJ (15 ml/kg) + Glibenclamide (0.5 mg/kg)</td>
<td>82.7±2.7*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Number of animals, n=6); *Significantly different from the normal control at $P<0.05$; a Significantly different from the diabetic control at $P<0.05$

3.2 Antihyperlipidaemic effect of MCFJ

The management of lipid profiles in different groups of rats is exhibited in table 2. The significant increase in TGL, total cholesterol, LDL and total protein while decrease in HDL observed in diabetic control rats compared with normal rats. The MCFJ along with Glibenclamide and pure Glibenclamide treated rats significantly decreased in TGL, total cholesterol, LDL and total protein; and increased HDL compared to diabetic control group rats. The results of study indicate the antihyperlipidaemic activity of MCFJ. The group IV produces extreme antihyperlipidaemic activity compared with other groups.

The findings indicates the fasting serum glucose of diabetic rats was significantly increased as compared to the normal fasting serum glucose levels of diabetic rats. The serum TGL, total cholesterol, LDL and total protein of diabetic group of rats was found to be significantly enhanced throughout the study period as against normal group of rats. This might have occurred in the diabetic rats as a result of lack of insulin which activates the lipase enzymes, hydrolyzing the stored triglycerides and releasing large amount of fatty acids and glycerol into the circulating blood. Consequently, the excess of fatty acids in the plasma may promote the hepatic conversion of fatty acids into phospholipids and cholesterol, the main products of lipid metabolism. At the same time glycogen, cortisol, catecholamine and growth hormones enhance lipolysis.

The animal treated with the MCFJ along with Glibenclamide significantly decreased the glucose level of diabetic rats compared to untreated diabetic rats. This reduction in the serum...
glucose may be due to inhibition of intestinal absorption of glucose, possible regeneration of pancreas or stimulation of the release of endogenous insulin as reported by the previous research workers. The extracts treated group showed significant reduction in the elevated serum cholesterol and serum triglyceride levels of diabetic rats when compared with untreated diabetic rats due to increased excretion of neutral sterol and acidic steroids in feces. 

Table 2: Determination of biochemical parameters after treatment with MCFJ

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>Total Protein (gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>81.7±2.6</td>
<td>76.4±3.8</td>
<td>62.6±2.4</td>
<td>49.8±4.2</td>
<td>6.7±2.9</td>
</tr>
<tr>
<td>Diabetic control (STZ 50 mg /kg)</td>
<td>205.4±4.1*</td>
<td>183.7±1.7*</td>
<td>23.6±3.1*</td>
<td>142.3±2.6*</td>
<td>15.5±4.5*</td>
</tr>
<tr>
<td>Standard group (Glibenclamide 50 mg/kg)</td>
<td>76.8±2.7*</td>
<td>73.8±2.6*</td>
<td>61.9±1.8*</td>
<td>55.7±3.7*</td>
<td>6.2±3.1*</td>
</tr>
<tr>
<td>MCFJ (5 ml/kg)+ Glibenclamide (0.5 mg/kg)</td>
<td>110.1±2.1*</td>
<td>95.0±3.4*</td>
<td>51.7±2.7*</td>
<td>82.6±2.3*</td>
<td>9.1±2.8</td>
</tr>
<tr>
<td>MCFJ (10 ml/kg)+ Glibenclamide (0.5 mg/kg)</td>
<td>91.6±3.4*</td>
<td>82.0±2.7*</td>
<td>59.6±3.6*</td>
<td>71.03±2.9*</td>
<td>7.8±3.6*</td>
</tr>
<tr>
<td>MCFJ (15 ml/kg)+ Glibenclamide (0.5 mg/kg)</td>
<td>80.4±2.8*</td>
<td>73.0±3.5*</td>
<td>66.1±1.7*</td>
<td>56.8±3.6*</td>
<td>6.9±2.9*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Number of animals, n=6); *Significantly different from the normal control at P<0.05; aSignificantly different from the diabetic control at P<0.05

The antidiabetic activity of MCFJ along with Glibenclamide at lower doses were similar to higher doses of Glibenclamide antidiabetic activity. This indicates that the addition of MCFJ increased the potency of Glibenclamide. Further the lower doses of Glibenclamide can produced minimum side effects to body.

4 Conclusion

The animals treated with the MCFJ along with Glibenclamide at lower doses exhibited synergistic antidiabetic activity. The potency of antidiabetic efficacy of Glibenclamide enhanced, and lower doses of Glibenclamide along with MCFJ produces similar antidiabetic activity produced by higher dose of Glibenclamide. In future studies will be conduct to elucidate the proper mechanism of action of antidiabetic activity impart by MCFJ along with Glibenclamide.

5 Conflict of interest

We declare that we have no conflict of interest.

6 Author’s contributions

AP, JSV, AC and PAR performed whole experimental procedures. All authors read and approved the final manuscript.

7 References


