Hypolipidemic effect of *Momordica charantia* seeds extract on streptozotocin-induced diabetic rats

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Abstract

Objective: The aim of the present study was to evaluate the hypolipidemic efficacy of the seeds of two common varieties of *Momordica charantia* extract (MCSEt1 and MCSEt2) in streptozotocin induced diabetic rats. Methods: Streptozotocin (55mg/kg body weight) was used for induction of diabetes in rats. MCSEt1 and MCSEt2 extracts (150 mg/kg body weight) was administered orally for 30 days to experimental rats. The fatty acid composition of liver and kidney were analyzed by gas chromatography. Results: Oral administration of MCSEt1 and MCSEt2 extracts resulted in a significant reduction in the levels of cholesterol, phospholipids, triglycerides and free fatty acids in plasma and tissues of STZ-induced diabetic rats. The altered fatty acid composition in liver and kidney were restored by the treatment. Glibenclamide was used as a reference drug to compare the efficacy of the seeds extract. Though both the varieties exhibited significant lipid lowering properties, the efficacy was pronounced in MCSEt1 compared to MCSEt2 and glibenclamide. Conclusion: The results of this study confirm the hypolipidemic effect of *Momordica charantia*.

Keywords: Diabetes, *Momordica charantia*, Hypolipidemic, Streptozotocin, Glibenclamide.

1. Introduction

Diabetes mellitus is a complex syndrome involving severe insulin dysfunction in conjugation with gross abnormalities in glucose homeostasis and lipid metabolism, which has affecting several millions of population all over the world. The World Health Organization (WHO) estimates India to be home in the largest number of diabetes patients in the world and this trend will continue into the future [1]. The individual with diabetes has a 25-fold increase in the risk of blindness, a 20-fold increase in the risk of renal failure, a 20-fold increase in the risk of amputation as a result of gangrene and a 2 to 6 fold increased risk of coronary heart disease and ischaemic brain damage [2]. In a recent review by Tsuyki and Bungard [3] non-compliance with prescribed hypolipidemic agents was said to have annual associated costs of about $ 100 billion in the US alone. Treatment of hyperlipidemia in diabetes involves improving glycemic control and the use of lipid lowering diets and drugs.

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Drugs are plenty in use for controlling hyperglycemia associated with hyperlipidemia but none claims to have the expected efficacy. On the other hand, it has been known that the dietary ingredients affecting glucose metabolism may also influence lipid metabolism [4].

From the beginning of the last century, evidence of the cholesterol-lowering properties of medicinal plants has been accumulating. The health awareness and fear of the side effects of allopathic medicines are the main causes for diversion to herbal treatment and the demand of herbal plants are increasing day by day in the global perspective. Even as we commence the new century with its exciting prospect of gene therapy, herbal medicine remains as one of the common mode of therapy available to much of world’s population.

According to World Health Organization, still 80% of the world populations rely mainly on plant drugs and WHO has also recommended the elevation of the effectiveness of plants in conditions where we lack safe modern drugs [5]. About 20% of the modern drugs are derived from any of the edible products of common use [6]. There was a gap in proper understanding of medicinal plants for mankind in past because traditional medicines generally lack scientific explanation.

Many plants are credited with anti hyperglycemic activity. Among these herbal resources, *Momordica charantia* is selected for the present study.

*Momordica charantia* (MC) Linn, commonly referred to as bittergourd or karela, belongs to the Curcurbitaceae family. It is a climbing plant, cultivated throughout Southern Asia. Its fruits are very cheap and available throughout the year. Immature fruits are used to prepare different dishes for human consumption, while ripped fruits are considered as not worthy for consumption. There are two varieties of this vegetable based on size and shape. The large variety is long, oblong and pale green in color. The other one is small, little oval and dark green in color. The yield of small variety per plant is much less when compared to large ones; as a result the cost of the small variety is almost thrice when compared to the larger ones.

Both the types are bitter in taste. The pulp is blood red or scarlet after dehiscence. The seeds are dappled, flat, thick notched margin, red aril in morphology and it is white color in raw fruits and become red when they are ripe. Different parts of these plants have been used in the Indian system of medicine for a number of ailments.

Our previous experimental results were highly encouraging as they revealed that blood glucose level was significantly lowered after oral administration of aqueous extract of *Momordica charantia* seeds in glucose load condition and in streptozotocin induced diabetes [7]. Our study also suggests that the seed extracts helps to decrease the toxicity of reactive oxygen species by its antioxidant property [8,9].

In view of the above considerations, the intention of the present study is to evaluate the lipid lowering properties of MC seeds on streptozotocin induced experimental diabetes. The efficacy was compared with a standard hypoglycemic drug, glibenclamide.

2. Materials and methods

2.1 Chemicals

Streptozotocin (STZ) was procured from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals used were of analytical grade.

2.2 Plant material

Fresh fruits of *Momordica charantia* were procured from a vegetative farm of Chengalpattu, India. Authentication of the plant was carried out by Prof. V. Kaviyarasan, Centre for Advanced
2.3 Preparation of seed extracts

The fruits were sliced into two halves and the seeds were selectively collected manually, washed with fresh water and dried in shade at room temperature. The dried seeds were grounded into fine powder by an electrical mill and mesh (mesh number 50). The powdered seeds were kept in airtight containers in a deep freeze maintained at 4°C until the time of further use. The seed extract was prepared by dissolving a known amount of seed powder in distilled water using a magnetic stirrer. It was then filtered and evaporated to dryness under reduced pressure in a rotary evaporator.

An aqueous suspension, which is the form customarily, used in folk medicine, was prepared to facilitate easy handling. The drug solutions were prepared freshly each time and administered intragastrically. The dosage schedule used for the drug was once per day.

2.4 Animals

Male albino rats of Wistar strain weighing around 160-180 gms were purchased from Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Chennai for the present study. They were acclimatized to animal house conditions, fed with commercial pelleted rat chow (Hindustan Lever Ltd., Bangalore) and had free access to water.

The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC number: 01/033/04).

2.5 Induction of diabetes

STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents [10]. After overnight fasting (deprived of food for 16 h had been allowed free access to water), diabetes was induced in rats by intraperitoneal injection of STZ (Sigma, St. Louis, Mo) dissolved in 0.1M cold sodium citrate buffer pH 4.5 at a dose of 55mg/kg body weight [11]. The control rats received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia.

After a week time for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the further experimental work. The treatment was started on the 8th day after STZ-injection and this was considered as 1st day of treatment. The treatment was continued for 30 days.

2.6 Experimental set up

The rats were divided into five groups with minimum of ten rats in each group.

Group I : Normal control rats
Group II: Diabetic control rats
Group III: Diabetic rats treated with MCSEt1 (150mg/kg b.w/day) in aqueous solution orally for 30 days.
Group IV: Diabetic rats treated with MCSEt2 (150mg/kg b.w/day) in aqueous solution orally for 30 days.
Group V: Diabetic rats administered with glibenclamide (600µg/kg b.w/day) in aqueous solution orally for 30 days.

At the end of the experimental period, the fasted rats were then sacrificed by cervical decapitation. Blood was collected with EDTA as anticoagulant and centrifuged at 3000 rpm for 15 min to separate plasma. Liver and kidney were dissected out and immediately washed in ice-cold saline and homogenized in Tris-HCl.
buffer, pH 7.4 (0.1 M) with a Teflon homogenizer. The total lipids were extracted from the tissue homogenate using chloroform and methanol by the method of Folch et al. [12].

2.7 Assay of lipid profile

Cholesterol content in plasma, liver and kidney were estimated by the method of Parekh and Jung [13]; triglycerides were estimated by the method of Foster and Dunn [14] and free fatty acids by the method of Itaya [15]. Total phospholipids content was estimated by the method of Bartlett [16] after digestion with perchloric acid and the phosphate liberated was estimated by the method of Fiske and Subbarow [17]. High-density lipoproteins (HDL) and low-density lipoproteins (LDL) were separated from the plasma according to dual precipitation technique by the method of Burstein and Scholnick [18] and the cholesterol content of the lipoproteins was estimated.

2.8 Fatty acid determination by gas chromatography

Analysis of fatty acid composition in lipid extract was performed in gas chromatography according to the method of Morrison and Smith [19]. Fatty acids were transmethylated and the resulting fatty acid methyl esters were analyzed by Tracer 540-gas chromatograph equipped with flame ionization with a detector temperature of 220°C. Separating column constitutes 2cm long x 2mm internal diameter packed with 10% cilar or chromosorb W, 80/100 mesh.

Fatty acids separated were identified by the comparison of the retention times with those obtained by the separation of a mixture of standard fatty acids. Electronic integrator was used to measure peak areas and data processing. Individual fatty acids were expressed as percentage of total fatty acids in 100 mg of tissues.

2.9 Histopathological observations

A portion of the liver and kidney tissues were fixed in 10% buffered neutral formal saline solution for histological studies. After fixation, tissues were embedded in paraffin; solid sections were cut at 5 µm and stained with haematoxylin and eosin. The sections were examined under light microscope and photomicrographs were taken [20].

2.10 Statistical analysis

All the grouped data were statistically evaluated with SPSS/10 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as Mean±S.D for six animals in each group.

3. Results

Figure 1 show the levels of plasma cholesterol, triglycerides, free fatty acids and phospholipids in control and experimental groups of rats. Hyperlipidemia, a common feature of diabetes, is evidenced by the increased plasma level of cholesterol, triglycerides, free fatty acids (p<0.05) in diabetic rats when compared to normal control rats. Significant restorations of the plasma lipid contents (p<0.05) to near normal levels in diabetic rats were observed after the oral treatment with MCSEt1, MCSEt2 and glibenclamide.

Figure 2 and 3 shows the levels of cholesterol, phospholipids, triglycerides and free fatty acids in liver and kidney of control and experimental groups of rats. A significant increase in the levels of liver and kidney were observed in diabetic rats when compared to normal control group. Treatment with MCSEt1, MCSEt2 and glibenclamide to diabetic rats resulted in a significant decrease (p<0.05) in the levels of tissue lipid content when compared to diabetic control rats.
Table 1.
Fatty acid composition in Liver of control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Percentage of fatty acid 100mg of tissue</th>
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<tbody>
<tr>
<td></td>
<td>Palmitic acid (16:0)</td>
</tr>
<tr>
<td>Control</td>
<td>20.40 ± 1.2</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>29.01 ± 1.9*a</td>
</tr>
<tr>
<td>Diabetic + MCSEt1</td>
<td>23.45 ± 1.5*b</td>
</tr>
<tr>
<td>Diabetic + MCSEt2</td>
<td>24.50 ± 1.4*c</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide</td>
<td>25.50 ± 1.8*d</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D, for six rats per group; Values are statistically significant at * p < 0.05.
Statistical significance was compared with in the groups as follows:
a. Diabetic control rats were compared with normal control rats; b. MCSEt1 treated diabetic rats were compared with diabetic control rats; c. MCSEt2 treated diabetic rats were compared with diabetic control rats; d. Glibenclamide treated diabetic rats were compared with diabetic control rats.

Figure 1.
Levels of plasma cholesterol, phospholipids, triglycerides and free fatty acids in control and experimental groups of rats

Values are given as mean ± S.D, for six rats per group.
Values are statistically significant at * p < 0.05.
Statistical significance was compared with in the groups as follows: a. Diabetic control rats were compared with normal control rats; b. MCSEt1 treated diabetic rats were compared with diabetic control rats; c. MCSEt2 treated diabetic rats were compared with diabetic control rats; d. Glibenclamide treated diabetic rats were compared with diabetic control rats.

Figure 4 represents the cholesterol content of plasma lipoproteins in control and experimental groups of rats. The levels of LDL and VLDL-cholesterol were significantly (p<0.05) higher, whereas the HDL-cholesterol was markedly lower in diabetic rats when compared with normal control rats. In diabetic rats treated with MCSEt1, MCSEt2 and glibenclamide to diabetic rats resulted in significant alterations of the lipoprotein levels to near normalcy.

Table 1 and 2 depicts the changes in the fatty acid composition of total lipids in liver and kidney of control and experimental groups of rats. There was a significant elevation in palmitic acid, stearic acid and oleic acid observed in tissues of diabetic rats. MCSEt1, MCSEt2 and glibenclamide were restored these altered fatty acid composition to near normal levels.

The histopathological examination revealed extensive alterations in liver and kidney of STZ-induced diabetic rats. The liver of control rat (Fig 5a) shows normal architecture. The liver of
diabetic rat (Fig 5b) shows perivenular inflammatory infiltration over the sinusoidal vacoulation of hepatic nuclei.

The pathomorphological changes observed in STZ-induced diabetes become apparently normal after treatment with MC seeds extract and glibenclamide (Fig 5c, d and e). The kidney of control rat (Fig 6a) shows normal glomeruli and tubules. The kidney of diabetic rat (Fig 6b) shows thickening of vesicles, glomeruli show some cellular proliferation with fibrosis. MC seeds extract and glibenclamide (Fig 6c, d and e) treated diabetic rat show glomeruli, which appear normal with mild dilated tubules.

4. Discussion

Streptozotocin, the most potent diabetogenic agent, has been widely used to induce experimental diabetes in rats [21], since it causes alterations similar to those found in diabetic humans [22]. STZ-induced diabetes in rats is frequently used to study the disturbances in lipid metabolism under diabetic conditions [23]. The concentration of serum lipids, total cholesterol, low density and high-density lipoprotein (LDL and HDL), cholesterol and triglycerides is an important index of overall metabolic control in diabetic animal.

Both the World Health Organization Multinational Trial and the Paris Prospective Study have shown that hypertriglyceridemia is a significant predictor of subsequent cardiovascular mortality in persons with diabetes [24]. Diabetes is known to be associated with an increase in the synthesis of cholesterol, which may be due to increased activity of HMG CoA reductase [25].
Hyperlipidemia in diabetes especially Type I is attributed to hyperglycemia resulting from insulin deficiency. Some authors have reported the presence of insulin-like compounds from fruits and seeds of *Momordica charantia*, which is effective on both subcutaneous and oral administration [26]. The strong hypolipidemic effect shown by the glibenclamide is in accordance with the earlier findings [27].

### 4.1 Plasma lipid profile

The abnormal high concentration of plasma lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots in the absence or deficiency of insulin, since insulin inhibits hormone sensitive lipase [28].

On the other hand, glucagons, catecholamines and other hormones enhance lipolysis. The marked hyperlipemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [25].

Our results of increased plasma lipids correlated with the above findings of hyperlipidemia in diabetes. Treatment with MCSEt1 and MCSEt2 enhances glucose metabolism by increasing glycolysis and glycogen synthesis and decreasing gluconeogenesis and glycogenolysis. Thus the extract optimizes glucose utilization and decrease mobilization of fat depots, which decrease hyperlipidemia.

### Table 2.

Fatty acid composition in Kidney of control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Percentage of fatty acid 100mg of tissue</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Palmitic acid (16:0)</td>
</tr>
<tr>
<td>Control</td>
<td>21.78 ± 1.6</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>34.06 ± 3.4*a</td>
</tr>
<tr>
<td>Diabetic + MCSEt1</td>
<td>24.64 ± 2.5*b</td>
</tr>
<tr>
<td>Diabetic + MCSEt2</td>
<td>25.3 ± 2.2*c</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide</td>
<td>26.31 ± 2.5*d</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D, for six rats per group; Values are statistically significant at * p < 0.05; Statistical significance was compared with in the groups as follows: a. Diabetic control rats were compared with normal control rats; b. MCSEt1 treated diabetic rats were compared with diabetic control rats; c. MCSEt2 treated diabetic rats were compared with diabetic control rats; d. Glibenclamide treated diabetic rats were compared with diabetic control rats.
4.2 Lipoproteins
The Framingham study highlights the profound effects of lipoprotein abnormalities on the incidence of coronary artery disease [29] in diabetic subjects. Under normal circumstances, insulin activates enzyme lipoprotein lipase (LPL) and hydrolyses triglycerides. Previously it was thought that unlike cholesterol, triglycerides don’t deposit in the lining of arteries. But new researchers show that triglycerides also start depositing in the arteries, if present excessively. Lipid disorders, most often encountered in diabetic patients, include increased levels of VLDL –c, LDL–c and low level of HDL–c [30]. Increased LDL-cholesterol may arise from glycosylation of the lysyl residues of apoprotein B as well as from decreasing affinity for the LDL receptor and hence decreased metabolism [31].

Fig. 5 (a-e).
Histopathological observations made on the liver tissue of control and experimental groups of rats (HE 100 X).

5a

5b

5c

5d

5e

Fig 5a. Represents the section of liver tissue from normal control rat showing normal architecture.

Fig 5b. Liver of STZ-induced diabetic rat showing inflammatory infiltration filling over the sinusoidal vacuolation of hepatocytic nuclei.

Fig. 5c. MCSEt1, and Fig. 5d. MCSEt2 treated diabetic liver showing near normal architecture.

Fig. 5e. Glibenclamide treated diabetic rat showing more or less normal architecture with mild changes in hepatocytes.
It has been demonstrated in IDDM, that HDL-cholesterol levels correlate with lipoprotein lipase levels [32]. Inhibition of lipoprotein lipase activity due to insulin deficiency may decrease HDL cholesterol levels [33].

Bruan and Severson [34] reported that a deficiency of lipoprotein lipase activity might contribute significantly to the elevation of triglycerides in diabetes [33]. Lopes-Virella et al (1983) reported that treatment of diabetes with insulin resulted in lowered plasma triglyceride levels by controlling lipoprotein lipase levels [35].

An increase in all lipoprotein fractions invariably leads to elevated levels of triacylglycerol and phospholipids. Jain et al [36] suggested that, the levels of glycemic control and elevated levels
of HDL cholesterol and triglycerides in the blood are significantly correlated with the phospholipid levels. Keelan et al [37], have observed a significant increase in phospholipids of diabetics.

The restoration of phospholipids by the seed extracts may be mediated through controlled mobilization and metabolism of plasma triglycerides, by improving the secretion and action of insulin.

4.3 Tissue lipid profile

During diabetes a profound alteration in hepatic lipid composition occurs, which results in an increase in hepatic lipid concentration and in contrast a decrease in lipogenesis. Persistent hyperlipidemia alters lipid metabolism in kidney [38] and leads to initiation and progression of renal injury. Liver is regarded as one of the central metabolic organs in the body, regulating and maintaining homeostasis.

It performs most of the reactions involved in the synthesis and utilization of glucose. Decreased glycolysis, impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver [39]. The balance between glucose production and its utilization in the liver is regulated primarily by insulin.

Similarly, liver plays a key role in fatty acid metabolism in IDDM. During diabetes, the hepatic lipogenesis is decreased and lipolysis increased [40]. Fatty acids and ketone bodies are important source of energy for the kidney. The lipid accumulates in the renal cortex during in the form of triacylglycerols.

Increased levels of hepatic and renal lipid content observed in the present study are consistent with the previous reports [41,42]. MC seeds extract treatment to diabetic rats for 30 days produced a marked reduction in the elevated lipid status in both liver and kidney.

4.4. Tissue fatty acid composition

Diabetes is consistently associated with change in the concentration and composition of lipid. The alterations in tissue fatty acid composition were found in both animals [43] and human beings [44], reflecting a disturbed lipid metabolism. Faas et al. [45], found that there is an alteration in the erythrocyte membrane and plasma fatty acid composition in diabetic patients. Seigneur et al [46] reported that there is a significant alteration in the fatty acid composition of serum and variety of tissues in experimental diabetes.

In the present study, there was an increase in the concentrations of palmitic, oleic and stearic acids and a significant decrease in the concentration of linolenic and arachidonic acids observed in diabetic rats. This observation coincides with the previous report that there is a preferential synthesis of saturated fatty acids in type 1 diabetes [47]. Seigneur et al [46], also reported that, the increased concentration of oleic acid was observed in the membranes of both type 1 and type 2 diabetic patients. Linolenic and arachidonic acids are rich in polyunsaturated fatty acids; they are the major targets for reactive oxygen species damage.

A significant decrease in the levels of polyunsaturated fatty acids (PUFA) in diabetic tissues may be attributed to the diminished activity of 76 desaturase activity. In diabetic rats treated with extract (MCSet1 and MCSet2), restored the fatty acid composition to near normal level and this may be due to the free radical scavenging effect of the seeds extract [8,9].

Shin et al [48], have reported that, insulin therapy restores fatty acid composition in tissues of streptozotocin-induced diabetic rats. The activation of beta cells with MC (Bittergourd) seed treatment were observed in mildly STZ diabetic animals in which some beta cells were
found active and granulation returns to normal giving insulinogenic effect [49].

Thus the administration of MC seeds extract had a beneficial effect on the hyperlipidemia associated with hyperglycemia.

5. Conclusion

It may be concluded from the results of the present investigation that Momordica charantia seeds possess beneficial effect on the hyperlipidemia associated with hyperglycemia. Several authors reported that, antioxidants like saponin, glycosides, phenolic constituents, alkaloids and other compounds have hypolipidemic and hypocholesterolemic effect.

In addition to the antioxidant potential, hypoglycemic effect of the seeds extract may be implicated as the major reason for this observed antihyperlipidemic effect. Because, hyperglycemia is the major culprit for the observed hyperlipidemia in diabetes. The hypolipidemic effect produced by the extracts may be due to the presence of the above active compounds present in it. Further studies to fractionate the active principles to elucidate the exact mechanism of action are, underway.

References


