Diabetes mellitus is a major global metabolic disorder of current century. Plants represent a vast source of potentially useful dietary supplements for improving blood glucose control and preventing long-term complications in type 2 diabetes mellitus. This study was undertaken to investigate the microscopic characteristics of Momordica charantia fruits and phytochemical analysis of extracts. Glucose tolerance test, hypoglycemic, antidiabetic effect, serum lipid profile and histopathological studies of pancreas, kidney and liver in normal control rats, diabetic control rats and extract treated rats were also performed in sub-acute studies and it showed 72% reduction in blood glucose level in normal rats. The same dose of 500 mg/kg produced a fall of 30% in blood glucose level within 90 min during glucose tolerance test (GTT) in normoglycemic rats. This dose has closer effect as that of standard drug glibenclamide (10 mg/kg b.w.). In diabetic rats in acute studies it lowers blood glucose level around 48% after 3 h of administration and in sub-acute studies it showed 22% reduction in blood glucose level in normal rats. The same dose of 500 mg/kg produced a fall of 30% in blood glucose level within 90 min during glucose tolerance test (GTT) in normoglycemic rats. This dose has closer effect as that of standard drug glibenclamide (10 mg/kg b.w.). In diabetic rats in acute studies it lowers blood glucose level around 4%/after 5 h of administration and in sub-acute studies it showed 39% reduction in fasting blood glucose level on 7th day. Total cholesterol (TC), low density lipoprotein (LDL), albumin and triglyceride (TG) levels also decreased in severely diabetic rats whereas, cardiac protective, high density lipoprotein (HDL) and protein level in serum were increased. Concurrent histological studies of the pancreas of these animals showed comparable regeneration by aqueous extract and kidney and liver showed normal structure in the rats which were earlier, necrosed by alloxan. These results clearly indicate that aqueous extract of Momordica charantia has high antidiabetic potential along with significant hypoglycemic and antidiabetic effects. Further studies are warranted to fractionate the active principles and development of polyherbal formulations by using other traditional antidiabetic plant extracts and determination of its synergistic effects.

Keywords: Blood glucose, Antihyperglycemic, Glibenclamide, Pancreas, Momordica charantia

INTRODUCTION

Diabetes mellitus is a major global metabolic disorder of current century. This pandemic is characterized by excessive sugar in the blood (hyperglycemia) due to deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. India is the number one danger zone of diabetes in the World. It is estimated that by the year 2030 there will be 79.4 million diabetic patients in India. Meanwhile, China and US rank second and third in the world with 42.3 million and 30.0 million respectively. In the developing countries, it is the younger age groups which are affected whereas in industrialized countries increase in the number of cases of diabetes occurs among elderly people. Over 13% adults in urban India suffer from diabetes whereas 5% in the countryside. The hereditary background, aging, obesity, dietary imprudence, endocrine imbalance, psychic stress, reduction in physical labour and discriminated social structure are the important factors, that have exploded the prevalence of diabetes in India and other affected countries. It is estimated that in year 2000 about 171 million people were affected with diabetes worldwide and this is expected to double by the year 2030. In India more than 35 million people suffering from diabetes. It is likely that these figures are a gross under estimation of the problem; particularly considering the fact that 50% of diabetics in India do not know that they suffer from diabetes. Diabetic patients have a considerable risk for cardiovascular disorder which further compounds the medical and public health challenges. Up to 80% death within this high risk population are due to associated cardiovascular disease. The health care burden of diabetes is enormous, and effective steps to combat the indiscriminate rise in the global incidence and prevalence of diabetes are urgently needed.

Herbal Approaches to Diabetes Therapy.

For thousands of years herbs have been used as a form of medicine to treat many diseases. Only organic living substances can replace cells that have been used up through the body’s metabolism. Herbalists believe that nature provides a bounty of fruits, vegetables, grains, and nuts for food; it therefore stands that nature should also supply remedies for diseases. The African, Indian, and Chinese cultures have played a major part in teachings on how to treat diseases with herbs. In recent years there has been an increased interest in uses of herbal medicines. Our widespread use of packaged, brand-named medicines to help us combat everything from the common cold to heart disease has seldom led us to believe that plants could be involved. In modern Western medicine, herbal treatments are seen as quaint relics of the past and next to useless when it comes to treating serious illnesses; however, one in four of all prescription drugs dispensed contain ingredients derived from plants.

Plant chemicals are employed in three main ways in Western medicine. The first is by incorporating them directly into medicines and medications, the second uses chemical compounds as blueprints or starting points for the manufacture of new or synthetic drugs and medicines, and the third uses plant chemicals as tools to help us understand physiological and pharmacological mechanisms, especially in drug development and testing.

Momordica charantia L. (Family: Cucurbitaceae)

Since antiquity Momordica charantia (MC – Karela) fruit has been an edible vegetable item in Indian food and has been known to exhibit blood sugar lowering potential. Diabetic patients use it in various forms e.g. Juice of MC as home remedy against diabetes mellitus. Common name is karela in Hindi and bittergourd in English. Momordica charantia displays insulin-like properties, remarkably stimulates glycogen storage by the liver and improves peripheral glucose uptake. Charantin, a steroidal saponin isolated have hypoglycemic potential. The health benefits of bitter gourd have been well documented, especially its anti-diabetic properties. It is also used as carminative, emmenagogue, in the treatment of colics, and as antiviral, anthelmintic, antiinflammatory, and antimicrobial remedy 5-6.

Principal Constituents

Mcharantia fruits consists glycosides, saponins, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free acids.
M. Charantia consists the following chemical constituents those are Alkaloids, charantin, choline, cryptoxanthin, curcubitins, curcubitacins, curcurbitanes, cycloartenols, diosgenin, elaostearic acids, eurythiol, galactouronic acids, gentisic acid, goyaglycosides, goysaponins, guanolate cyclase inhibitors, gynogenin, hydroxyprostamines, kauranoids, lanosterol, lauric acid, linoleic acid, linolenic acid, momorcharasides, momorcharins, momordolen, momordicin, momordicins, momordicinins, momordicosides, momordin, momordol, multiflorone, myristic acid, nerolidol, oleic acid, oxalic acid, pentadecane, peptides, aci, petroselinic acid, polypeptides, proteins, ribosome-inactivating proteins, rosmarinic acid, rubixanthin, spinasterol, steroidal glycosides, stigmasta-diols, stigmasterol, taraxerol, trehalose, trypsin inhibitors, uracil, vaccine, v-insulin, verbascoside, v-kine, zeatin, zeatin riboside, zeaxanthin, zeinoxanthin Amino acids - aspartic acid, serine, glutamic acid, thscinne, alanine, g-aminobutyric acid and piperolic acid, acorbigen, s-sitosterol-d-glucoside, citrulline, elasterol, flavochrome, lutein, lycopene, piceolic acid. The fruit pulp has soluble pectin but no free glucoside, citrulline, elasterol, flavochrome, lutein, lycopene, vitamins 7-8.

Both the edible fruit and the leaves are great sources of the B vitamins 9-10.

Clinical Studies
An aqueous extract of M. charantia (100 g reduced to a 100 ml volume dose) given once per day was found to be highly effective in lowering blood sugar levels in Type II diabetics over a period of 7 weeks. In addition to hypoglycemic effects, the subjects showed a significant delay in the appearance of cataracts compared to the control group. Administration of M. charantia has also been shown to improve the outcome of the oral glucose tolerance test in Type II diabetics. In one study, the administration of 100 ml of M. charantia fruit juice improved glucose tolerance in 73% of test subjects following an oral glucose tolerance test. Similar improvements in glucose tolerance were observed following the administration of 50 ml of M. charantia fruit juice.

Materials and Methods
Plant Material
The fresh fruits of Momordica charantia were purchased from the local market. Plant materials were authenticated by Dr. Mrs. P.Y. Bhogaonkar, Ex. Head of the Botany Department, Annavati (Maharashtra, India).

Chemicals
Alloxan (S D Fine-Chem, India), insulin (USV Ltd., India) and glibenclamide (Sun Pharma, India) were used in this study. Other chemicals used were of analytical grade and were obtained from Qualigens, India.

Microscopy
Detailed pharmacognostic study of Momordica charantia fruit is carried out to lay down the standards which could be useful in future experimental studies 10-12.

Preparation of Plant Extracts
Fruits were cut into slices and shade dried ground to a coarse powder and passed through a 80 mesh sieve. The powdered plant (250 g) was defatted with petroleum ether, chloroform, and ethan (90%) successively using Soxhlet apparatus and later extracted using 50% ethanol and water by maceration. All the above extracts were also tested for the identification of phytocannabinoids. The semisolid aqueous extract (6.7%) W/W was suspended in distilled water and employed for anti-diabetic activity 12.

Identification of Phytochemical Constituents
Chemical tests were carried out on the alcoholic extracts for the qualitative determination of phytochemical constituents as described by Harborne (1998), Trease and Evans (1983) and Sofowora (1993).
prick method for the determination of serum glucose levels. The animals were treated for 7 days and were given free access to food and water ad libitum. On the 7th day animals were killed by decapitation and blood was collected from the arterial jugular and serum was separated. Biochemical parameters

Blood glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, total protein and albumin levels in serum were measured spectrophotometrically by methods prescribed by the manufacturer.

Histopathological studies

Animals were sacrificed on 7th and 14th day during prolonged treatment. Pancreas, liver and kidney were removed, washed with cold saline and preserved in 10% formalin in buffered form.

Blocks from tissues were routinely processed and embedded in paraffin. Thin sections were cut using rotary microtome and stained with hematoxilin and eosin for histomorphology evaluation.

Statistical analysis

Results were presented as mean±S.E.M. of six observations. Statistical analysis was made using two-way analysis of variance using the statistical software program, Graph Pad Prism 4.0. Statistical significance was considered at P<0.05.

RESULTS AND DISCUSSION

The phytochemical screening of MC fruit revealed the presence of glycosides, saponins, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free acids.

Microscopically unripe fruit showed the outer rind with prominent interrupted irregular large ridges and tapering outgrowths which are extensions of the pericarp. The epicarp is very thick and it is covered with a thick stratified cuticle. The epidermis consists of a layer of relatively small parenchyma rich in chlorophyll. The epidermis also bears two-celled uniseriate covering trichomes as well as glandular trichomes commonly found on the outgrowth. The sub-epidermal tissues consist of several layers of round to oval parenchyma enclosing chloroplasts and colored matter. The juicy mucilaginous mesocarp consists of large oblong parenchyma almost devoid of chlorophyll but occasionally contain colored matter. These are underlain by a few layers of almost colorless small-sized parenchyma followed by several layers of collenchyma traversed by non-lignified branching vascular strands some of which form loop structures. Very long intricate fibres are densely distributed within the cells.

Fig. 1: Microscopy of Momordica Charantia Fruits
Oral administration of the extracts in doses from 1000 to 5000 mg/kg/day did not produce significant changes in behaviors. In a single dose administration no adverse effects was observed for the crude aqueous fruit extract of *Momordica charantia*, indicating that the extracts are not toxic under the observable condition.

The aqueous fruit extract of *Momordica charantia* shown significant (P<0.001) increase in glucose tolerance. The results are given in Table 1. The blood glucose levels were reduced considerably (30.15%) within 90 minutes of the drug administration. Maximum effect was observed for dose of 500 mg/kg b.w.in glucose loaded. While standard drug glibenclamide (10mg/kg b.w.) showed reduction in the blood sugar level after 30 min. (21.20%) and ayurvedic marketed antidiabetic preparation showed reduction in the blood sugar level after 30 min. (4.34%).

The acute hypoglycemic effect of the aqueous extract of fruits of *Momordica charantia* on the fasting blood sugar levels of diabetic rats is shown in the Tables 2. After a single dose of extract only the dose of 500 mg/kg showed a significant reduction in the blood sugar level after 3 h. Where as standard drug glibenclamide showed reduction in the blood sugar level after 3 h (19.46%) and ayurvedic marketed antidiabetic preparation showed reduction in the blood sugar level after 7 h (12.4%).

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Each value represents mean±S.E, n=6.

Allocan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycaemia. The acute and sub-acute anti-hyperglycemic effect of the extracts on the fasting blood sugar levels of diabetic rats is shown in Table No. 4 and 5. Administration of alloxan (150 mg/kg, i.p.) led to a 1.5-fold elevation of fasting blood glucose levels, which was maintained over a period of 3 weeks. One week of daily treatment of various extract of Momordica charantia led to a dose-dependent fall in blood sugar levels by 12–39%. Vehicle control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 7 days. Alloxan caused body weight reduction, which is reversed by aqueous extracts of Momordica charantia after 7 days of treatment.

### Table 4: Acute antidiabetic studies of aqueous fruit extract of Momordica charantia in alloxan induced diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>75.5±3.4 74.6±2.9 73.3±3.01 74.1±2.7</td>
</tr>
<tr>
<td>II</td>
<td>Control Diabetic</td>
<td>191.1±3.0 192.5±2.5 193.6±2.8 195.0±2.8</td>
</tr>
<tr>
<td>III</td>
<td>M.C(Aq) 250 mg</td>
<td>187.8±2.9 186.5±2.1 184.6±1.6 182.5±1.8 ***</td>
</tr>
<tr>
<td>IV</td>
<td>M.C(Aq) 500 mg</td>
<td>186.6±3.6 184.5±3.2 181.8±3.3 178.3±2.9 ***</td>
</tr>
<tr>
<td>V</td>
<td>Ayurvedic Marketed Antidiabetic Preparation</td>
<td>185.5±10.3 182.8±10.2 179.0±11.1 173.6±9.7 ***</td>
</tr>
<tr>
<td>VI</td>
<td>Std Drug Glibenclamide (0.25 mg/kg)</td>
<td>183.3±2.9 181.5±2.8 173.6±2.4 164.1±2.4 ***</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E, n=6.

### Table 5: Sub-acute antidiabetic studies of aqueous fruit extract of Momordica charantia in alloxan induced diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>85.1±1.1 84.8±0.7 84.8±1.1 85.3±1.2</td>
</tr>
<tr>
<td>II</td>
<td>Control Diabetic</td>
<td>194.3±4.8 205.6±8.5 217.5±12.1 232.1±14.3</td>
</tr>
<tr>
<td>III</td>
<td>M.C(Aq) 250 mg</td>
<td>194.5±5.5 185.0±7.2 176.6±7.7 168.1±6.8 158.5±6.4 ***</td>
</tr>
<tr>
<td>IV</td>
<td>M.C(Aq) 500 mg</td>
<td>191.5±6.9 168.1±3.3 158.8±6.8 139.6±6.8 116.5±9.3 ***</td>
</tr>
<tr>
<td>V</td>
<td>Ayurvedic Marketed Antidiabetic Preparation</td>
<td>196.8±7.7 191.3±7.9 186.8±7.5 182.1±7.5 176.3±7.6 ***</td>
</tr>
<tr>
<td>VI</td>
<td>Std Drug Glibenclamide (0.25 mg/kg)</td>
<td>187.8±7.8 166.6±7.3 149.6±7.9 127.1±6.2 107.1±3.1 ***</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E, n=6.

Serum cholesterol, serum triglycerides, serum albumin, serum LDL levels were decreased significantly by glibenclamide and all the extracts of Momordica charantia due to 7 days of treatment. LDL levels and total proteins were increased by glibenclamide and aqueous extracts. As shown in Table No. 6.

### Table 6: Effect of aqueous extract of Momordica charantia on serum lipid profile in diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Triglyceride(mg/dl)</th>
<th>Total cholesterol(mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>LDL cholesterol (mg/dl)</th>
<th>Total protein (mg/dl)</th>
<th>Albumin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>97.4±2.0</td>
<td>60.5±1.4</td>
<td>54.1±0.9</td>
<td>53.1±1.0</td>
<td>7.6±0.5</td>
<td>4.3±0.5</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>175.7±4.0</td>
<td>125.4±2.5</td>
<td>36.8±2.0</td>
<td>43.1±1.0</td>
<td>7.3±0.4</td>
<td>3.9±1</td>
</tr>
<tr>
<td>III</td>
<td>Std.Drug Glibenclamide</td>
<td>54.9±0.1</td>
<td>63.6±0.8</td>
<td>18.6±0.7</td>
<td>34.2±0.5</td>
<td>5.9±0.1</td>
<td>3.7±0.3</td>
</tr>
<tr>
<td>IV</td>
<td>Ayurvedic Marketed formulation</td>
<td>85.6±2.1</td>
<td>66.8±0.5</td>
<td>18.3±0.4</td>
<td>33.1±1.0</td>
<td>7.3±0.8</td>
<td>4.1±0.3</td>
</tr>
<tr>
<td>V</td>
<td>Aq.MC extract (250mg)</td>
<td>85.6±1.2</td>
<td>66.5±0.9</td>
<td>17.3±0.4</td>
<td>32.5±0.5</td>
<td>7.7±0.2</td>
<td>3.8±0.2</td>
</tr>
<tr>
<td>VI</td>
<td>Aq.MC extract (500mg)</td>
<td>81.6±1.2</td>
<td>53.1±2.1</td>
<td>53.9±2.3</td>
<td>33.1±1.0</td>
<td>8.0±0.9</td>
<td>3.4±0.2</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E, n=6.

Photomicrographs (Fig.3) of pancreas in slide no.(A) showed normal acini, and normal cellular population in the islets of langerhans in pancreas of vehicle-treated rats. In slide no. (B) extensive damage to the islet of langerhans and reduced dimensions of islets. In slide no. (C) and (D) restoration of normal cellular population size of islets with hyperplasia by Momordica charantia extract 500 mg/kg b.w. (C) was also shown. The partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia was shown by aqueous extract.
Photomicrographs of the pancreas in the diabetic rats at 1 week post-treatment (A) Normal control B) Diabetic control C) Photomicrograph of pancreas treated with ayurvedic marketed preparation D) Photomicrograph of pancreas treated with Momordica charantia aqueous extract 500 mg/b.w.

Photomicrographs (Fig.4) of Kidney tissues in the control group showed normal renal corpuscles (Slide A). But some morphological and pathological changes occurred in the kidney tissues of diabetic control albino rats (Slide B). In slide C and D treated with aqueous extracts of Momordica charantia extract 250 and 500 mg/kg b.w., the glomerular region of the kidney, some atrophic changes and haemolysis were seen but cellularity and basement membrane were normal. No inflammatory cells were found. The tubular portion also showed atrophic changes, shedding of epithelium and oedema.

Photomicrographs of the kidney in the diabetic rats after 7 days post-treatment (A) Normal control B) Diabetic control C) Photomicrograph of kidney treated with ayurvedic marketed preparation D) Photomicrograph of kidney treated with Momordica charantia aqueous extract 500 mg/b.w.

Photomicrographs (Fig.5) of liver in normal animal showed normal hepatic cells with well preserved cytoplasm, nucleus, nucleolus and central vein. In diabetic control liver section showed that the lobular architecture was maintained but there was also severe fatty changes, sinusoidal dilation and congestion, mild periportal inflammation, fibrosis, severe feathery degeneration and necrosis. In diabetic rats treated with aqueous Momordica charantia fruit extract 500 mg/kg b.w, liver section maintained lobular architecture and had mild fatty change, mild sinusoidal dilation and congestion, mild periportal inflammation and mild feathery degeneration.

From the above results, it was concluded that the present study seems to support the claims by traditional medicine practitioners about the usefulness of Momordica charantia fruits for the treatment of diabetes.

CONCLUSION

Momordica charantia fruit juice is claimed to be useful in diabetes. Results of anti-diabetic activity of Momordica charantia fruit extracts established the scientific basis for the utility of this plant in the treatment of diabetes. Over the years scientists have verified many of the traditional uses of this bitter plant that continue to be an important natural remedy for various diseases. Concentrated fruit or seed extracts can be found in various herbal preparations (capsules and tablets) that are marketed today. MC preparations are becoming more widely available in the U.S as well as rest of the world and are employed by practitioners of natural health for treatment of diabetes, viral diseases, including flu and psoriasis. Role of MC in diabetes is of paramount importance as this plant serves various purposes in these patients lowers blood sugar, delays complications (nephropathy, neuropathy, gastroparesis and cataract, atheroarosclerosis) and is anti-infective (diabetics are known to be more susceptible to infections). Moreover till now there is no pharmacological agent that can control diabetic complications. Most importantly it is cheap and easily available in tropical countries. However, standardization of MC and its antidiabetic component followed by a controlled clinical trial is needed.

The aqueous extract have shown significant reduction in blood glucose levels in both glucose loaded, normal and alloxan induced diabetic rats. In glucose loaded animals, the drug has reduced the blood glucose to the normal levels. It is possible that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. Repeated administration of Momordica charantia extracts had decreased the blood glucose, total cholesterol and triglycerides significantly. Histopathological examination of pancreas, liver and kidney showed the recovery of damaged tissues when section of treated groups compared with diabetic control.

In conclusion, Momordica charantia aqueous extract showed significant anti-diabetic effect in diabetic rats after oral administration. Long-term administration of 500 mg/kg of aqueous
extract of Momordica charantia showed significant anti-diabetic
effects, decreased post-prandial glycemia but not fasting blood
glucose. Thus the claim made by the traditional Indian systems of
medicine regarding the use of fruit juice of this plant in the
treatment of diabetes stands confirmed.

In future studies the aqueous *Momordica charantia* fruit extract may
be used along with other traditional antidiabetic plant extracts in
development of polyherbal formulations and elucidation of its
synergistic effects.

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