PREVENTIVE EFFECT OF MOMORDICA COCHINCHINENSIS (LOUR). SPRENG ON HIGH FAT DIET AND ALONG WITH LOW DOSE ALLOXAN INDUCED DIABETIC NEPHROPATHY AND INSULIN RESISTANCE IN RATS

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ABSTRACT
In the present study, we evaluated the preventive effect of methanolic extract of Momordica cochinchinensis (MEMC) fruit on renal function in alloxan-induced diabetic rats. In addition, we examined (MEMC) extract could prevent diabetic nephropathy and insulin sensitisation action. Initially, we kept rats for acclimatisation along with high fat diet for 20 days and then, Diabetes was induced in Wistar albino rats with a single intraperitoneal injection of alloxan (120 mg/kg). Two days after alloxan injection diabetic rats were selected and divided into groups, MEMC preparations (200 mg/kg per day), (400 mg/kg per day) were given orally for 28 days to diabetic rats. Survival analysis as well as biochemical parameters were measured. Administration of MCC extract to diabetic rats resulted in a significant decrease of Proteinuria and serum parameters (creatinine, uric acid, urea, triglycerides, cholesterol, LDL) in Standard, Test1 and Test2 when compared to Toxic group (only alloxan 120 mg/kg and high fat diet) treated group. Insulin resistance action was measured by glucose tolerance test and there is remarkable decreased blood glucose levels of Standard, Test1 and Test2 compared to Toxic group at different time intervals. MCC treatment improve the impairment of fatty acid metabolism in diabetes. From the results we can conclude that MCC preparations are able to attenuate diabetic renal damage, probably by its anti-oxidative action and its anti diabetic activity. The protective role on diabetic nephropathy and insulin resistance by MCC against the high fat diet and along with Alloxan induced damages in diabetic rats gives a hope that they may have similar protective action in humans.

Keywords
Alloxan, Diabetes, Rats, Momordica cochinchinensis, Kidney.

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Introduction:

Diabetes mellitus is a group of metabolic diseases identified by hyperglycemia resulting from inadequate insulin secretion and/or its action\(^{11}\)\(^{2}\). DN is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli due to longstanding diabetes mellitus\(^{3}\). DN was the main cause for end stage renal failure\(^{2}\). DN can be identified by microalbuminurea, hyper permeability to proteins, which leads to proteinuria and causes renal failure. Long lasting diabetes causes accumulation of advanced glycation end products(AGEs) in kidneys of diabetic patients\(^{14}\). The structural changes of diabetes affected glomeruli show thickening of the glomerular basement membrane, expansion of the mesangium and glomerular hypertrophy.

Insulin resistance is a physiological condition in which cells failed to respond to the normal actions of the insulin\(^{5}\). It is mainly caused due to high fat diet intake compared to large amount of starch\(^{6}\)\(^{7}\)\(^{8}\). ad libitum high fat diet has the tendency to result in caloric intake that’s far in excess of animals energy needs, resulting in rapid weight gain which is similar in human beings\(^{8}\).

The mechanism of hyperglycemia causes free radical generation and thus causes the formation of reactive oxygen species along with increased production of hydrogen peroxide by murine mesangial cells and lipid peroxidation of glomeruli and glomerular mesangial cells\(^{9}\)\(^{10}\). Diabetes promotes glycosylation of circulating and cellular protein and initiate auto oxidative reactions and thus results in the accumulation of AGEs. These formed AGEs can promote tissue damage by free radicals. Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity by changing the activity of membrane bound enzymes and receptors\(^{11}\)\(^{12}\). Thus formed lipid radicals and lipid peroxides are harmful to cells in the body and associated with atherosclerosis and damage to brain, kidney, liver and other organs. Diabetes also causes oxidation of DNA bases and sugar phosphate binding sites thus results in DNA damage\(^{13}\). Hyperglycemia and Increased AGEs, Free radical and Cytokine’s play important role in pathogenesis of diabetic nephropathy.

The purpose of the study is to improve herbal medications for diabetes and its complications and to expose the plants having medicinal values which are using in our day to day life.

Momordica cochinchenensis (MCC) fruits belonging to the family Cucurbitaceae containing licopene, vitamin E, flavanoids(Rutin, myricetin, luteolin, quercetin, Apigenin, Kaempferol)\(^{14}\), diterpene columbin, chondrillasterol, momordica saponins, momordins and pentacyclic triterpenoid esters\(^{15}\). MCC posses reported biological activities like Immunomodulatory\(^{16}\) and anti-inflammatory effect by chymotrypsin inhibitor\(^{16}\), antimicrobial activity\(^{17}\), antioxidant\(^{14}\), DNA protective\(^{18}\), tumor growth and angiogenesis\(^{19}\). Flavanoids present in MCC are phenolic compounds with strong antioxidant properties, free radical scavenging activity\(^{20}\). Licopene is β-carotenoide which is having a potent antioxidant and free radical scavenging activity. It has been reported that Momordica cochinchenensis is having antidiabetic activity in Streptozotocin induced diabetes model\(^{21}\). It has been reported that flavanoids Quercetin and Rutin posses insulin sensitising activity.

In the present study we have investigated the preventive effect of methanolic extract of MCC fruits on high fat diet and along with low dose alloxan induced DN and insulin resistance.

Materials and Methods:

Plant Material:

*Momordica cochinchenensis* (Lour).spreng. (Cucurbitaceae) fruits were collected identified and authenticated from Botanist Dr.K.Madhava Chetty, Department of Botany, sri venkateswara university, Tirupati-517502, Andhra Pradesh, India.

Composition and Preparation of High Fat Diet:

Composition of high fat diet:

<table>
<thead>
<tr>
<th>S.NO</th>
<th>INGREDIENTS</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard laboratory chow</td>
<td>70%</td>
</tr>
<tr>
<td>2</td>
<td>carbohydrates</td>
<td>15%</td>
</tr>
<tr>
<td>3</td>
<td>Olive oil</td>
<td>10%</td>
</tr>
<tr>
<td>4</td>
<td>Egg yolk</td>
<td>5%</td>
</tr>
</tbody>
</table>
Preparation:
- Standard laboratory chow was powdered and all the remaining ingredient was taken and mixed to for a uniform mass.
- Then it was filled into a syringe previously front cutted, then pressed to form pellets identical to standard pellet and then dried.

Preparation of extract:
Fresh fruits of MCC were collected and shed dried and powdered. 600gm of powder was extracted using Methanol by using soxhelt apparatus for 24hrs. Extract was kept for drying at room temperature. The yield of extract was found to be 8.3%(w/w).

Phytochemical Screening:
Preliminary phytochemical screening showed the presence of flavonoids, glycosides, Carbohydrates, Amino acids, oils and fats[14].

Animals:
Male Wistar Albino rats of 160-210gm were taken and housed at (25±2°c) in polyethylene cages in animal house. Rats were kept for acclimatisation with high fat diet and standard laboratory conditions throughout the experiment. All the animals used in the experiment was approved by institutional animal ethical committee smt. Sarojini Ramulamma college of pharmacy with number 51/01/C/CPCSEA/2013/03.

Drugs and Chemicals:
Alloxan, Metformin, Atorvastatin and all other chemical were provided by smt. Sarojini Ramulamma college of pharmacy.
Biochemical reagent were provided by sicra labs kukatpally, IDA, Hyderabad.

Acute Toxicity Studies:
Animals were kept for overnight fasting before extract treatment. Initially a single dose of 2000mg/kg/body weight of MCC extract given orally and animals were observed for every 30min individually for 24hrs and daily upto 14days, for morphological and behavioural changes[22].

Induction of Diabetes:
Diabetes was induced after keeping animals for overnight fasting then by administration of single dose of 120mg/kg/body weight of Alloxan intraperitoneally, hyperglycemic state was confirmed after 48hrs injection of Alloxan, animals with higher than 150mg/dl Blood glucose were included in study.

Treatment of Animals:
Animals with higher than 150mg/dl blood glucose level were selected and grouped in five groups and six animals in each and Group I was named as Normal control administered with 0.9% Nacl normal saline 10ml/kg. Group II were not treated with drug or extract named as diabetic control and provided with high fat diet. Group III were treated with 500mg/kg Metformin, 20mg/kg Atorvastatin and high fat diet were given. Group IV were treated with 200mg/kg MCC extract and high fat diet were given. Group V were treated with 400mg/kg MCC extract and high fat diet were given.
All the above treatment of drugs and extract and high fat diet were for 28 days.

Biochemical Estimation:
Animals were kept for overnight fasting on 28th day and kept in metabolic cages for urine collection and on 29th day fasted animals were anesthetised with diethyl ether and blood was drawn through retro orbital puncture and following parameters were analysed. serum glucose, serum creatinine, serum uric acid, serum BUN, serum triglyceride, serum cholesterol, proteinuria were assayed by using Robonik Diagnostics kits Robonik India, Navi Mumbai India.

Statistical Analysis:
Statistical analysed by using prism Graph pad 5.0. All the results expressed in Mean ± Standard Deviation(S.D). statistically analysed using one way analysis of variance followed by Dunnet's test. Statistical significance was expressed in P<0.05, P<0.01, P<0.001.

Effect of Methanolic extract of MCC 200mg/kg, 400mg/kg on serum glucose, serum uric acid, serum creatinine, serum, Blood urea nitrates(BUN) of high fat diet along with low dose of alloxan induced diabetic nephropathy.
Table 2

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Group &amp; Drug Treatment</th>
<th>Alloxan induced diabetic nephropathy (mg/dl)</th>
<th>Alloxan induced diabetic nephropathy (mg/dl)</th>
<th>Alloxan induced diabetic nephropathy (mg/dl)</th>
<th>Alloxan induced diabetic nephropathy (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glucose Mean±SD</td>
<td>Glucose Mean±SD</td>
<td>Glucose Mean±SD</td>
<td>Glucose Mean±SD</td>
</tr>
<tr>
<td>I</td>
<td>Normal Control 0.9% Nacl for 28days</td>
<td>113.3±13.66***</td>
<td>3.843±1.195**</td>
<td>0.9310±0.3902**</td>
<td>14.08±0.7408***</td>
</tr>
<tr>
<td>II</td>
<td>Toxic Control 0.9% Nacl for 28days</td>
<td>249.5±51.78</td>
<td>13.39±3.865</td>
<td>3.713±0.4145</td>
<td>26.35±1.763</td>
</tr>
<tr>
<td>III</td>
<td>Standard Metformin (500mg/kg)</td>
<td>117.5±14.04***</td>
<td>4.384±0.9569**</td>
<td>1.409±0.3249***</td>
<td>18.50±0.4459**</td>
</tr>
<tr>
<td></td>
<td>Atorvastatin(20mg/kg) for 28 days.</td>
<td>Test-I</td>
<td>120.2±9.806**</td>
<td>7.691±0.7129**</td>
<td>2.071±0.2751†</td>
</tr>
<tr>
<td>IV</td>
<td>MEMC(200mg/kg) for 28 days.</td>
<td>Test-II</td>
<td>116.7±8.733**</td>
<td>4.421±1.404*</td>
<td>1.362±0.2023***</td>
</tr>
<tr>
<td>V</td>
<td>MEMC(400mg/kg) for 28 days.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation (mean of six determination). N=6. *P value < 0.01, **P < 0.001, ***P < 0.0001 vs. Diabetic control.

Effect of Methanolic extract of MCC 200mg/kg, 400mg/kg treated groups showed significant decreased levels of serum glucose, serum uric acid, serum creatinine, serum, Blood urea nitrates (BUN) dose dependently and where as increased in Toxic control group when compared with normal control group. Mean and SD values are given in table 2.

Effect of Methanolic extract of MCC 200mg/kg, 400mg/kg on serum Triglyceride, serum cholesterol, proteinuria of high fat diet along with low dose of alloxan induced diabetic nephropathy.

Table 3

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Group &amp; Drug Treatment</th>
<th>Alloxan induced diabetic nephropathy (mg/dl)</th>
<th>Triglycerides Mean±SD</th>
<th>Triglycerides Mean±SD</th>
<th>Triglycerides Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control 0.9% Nacl for 28days</td>
<td>125.4±8.417***</td>
<td>66.11±6.509**</td>
<td>8.167±0.4803*</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Toxic Control 0.9% Nacl for 28days.</td>
<td>331.1±15.18</td>
<td>273.6±7.255</td>
<td>22.75±2.037</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Standard Metformin (500mg/kg)</td>
<td>144.0±4.014***</td>
<td>66.00±5.810***</td>
<td>11.80±2.146**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atorvastatin(20mg/kg) for 28 days.</td>
<td>Test-I</td>
<td>189.9±3.485*</td>
<td>57.30±2.640**</td>
<td>14.83±1.111***</td>
</tr>
<tr>
<td>IV</td>
<td>MEMC(200mg/kg) for 28 days.</td>
<td>Test-II</td>
<td>172.7±5.066***</td>
<td>47.33±4.091**</td>
<td>8.583±0.7195*</td>
</tr>
<tr>
<td>V</td>
<td>MEMC(400mg/kg) for 28 days.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean ± standard deviation (mean of six determination). N=6. *P value < 0.01, **P < 0.001, ***P < 0.0001 vs.
Diabetic control.
Effect of Methanolic extract of MCC 200mg/kg, 400mg/kg treated groups showed significant decreased levels of serum Triglyceride, serum cholesterol, proteinuria dose dependently and whereas increased in Toxic control group when compared with normal control group. Mean and SD values are given in table 3.

Glucose tolerance test (GTT) of different groups. values are given as mean of six determinations.
Serum glucose levels of different group animals with different time intervals.

Table 4

<table>
<thead>
<tr>
<th>Time</th>
<th>Normal control</th>
<th>Toxic control</th>
<th>Standard 200mg/kg(MCC)</th>
<th>Test 1 400mg/kg(MCC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>102</td>
<td>309</td>
<td>118</td>
<td>148</td>
</tr>
<tr>
<td>30 min</td>
<td>122</td>
<td>343</td>
<td>138</td>
<td>194</td>
</tr>
<tr>
<td>60 min</td>
<td>142</td>
<td>372</td>
<td>147</td>
<td>208</td>
</tr>
<tr>
<td>90 min</td>
<td>115</td>
<td>334</td>
<td>122</td>
<td>164</td>
</tr>
<tr>
<td>120 min</td>
<td>100</td>
<td>316</td>
<td>123</td>
<td>154</td>
</tr>
</tbody>
</table>

value are given mean(mean of three animals).

Effect of methanolic extract of momordica cochinichinensis on GTT of high fat diet and along with low dose of alloxan induced diabetic nephropathy.

Graph 1

DISCUSSION
Diabetic nephropathy is a progressive kidney disease, which is caused by long term vulnerable diabetes. Alloxan is well documented to have cytotoxic effect in the pancreas which is mediated by the generation of reactive oxygen species and free radicals, diabetic state is associated with mesangial expansion, deposition of extracellular proteins, thickening of the glomerular basement membrane, hyaline casts and renal tubular expansion. Administration of alloxan led to an increase in blood glucose, cholesterol, triglycerides, BUN, uric acid, creatinine and proteinuria. Treatment with different pharmacological interventions is well documented to have advantageous effect on DN. Extracts of *Phaleria macrocarpa* have been reported to protect against DN\(^{[23]}\). Croatian propolis has been counted to protect against alloxan induced DN in a 7 day treatment schedule\(^{[24]}\). Volatile oil of *Cinnamomum zeylanicum* protected the diabetic rats against DN in a two week study\(^{[25]}\). We obtained similar results in the present study. Administration of methanolic extract of momordica cochinichinensis at doses of 200 and 400mg kg\(^{-1}\) and standard drugs metformin and atorvastatin was found to decrease the serum glucose, triglycerides, cholesterol, blood urea nitrogen (BUN), creatinine, uric acid and proteinuria(Table 2,3) significantly.
Insulin resistance is one of the most serious effect related to diabetes which is majorly caused by diet and use of sedentary life style and use of protease inhibitors and diseases like hepatitis C. In this study we used high fat diet to produce insulin resistance, olive oil as a major fat source and previous studies has proved that use of olive oil as a fat source in high fat diet showed decreased levels of adiponectin which is a anti insulin resistance protein when compared with that of lard, coconut oil, fish oil, standard rat Chow.[26]

In this study we examined anti insulin resistance activity of MEMC (Table 4). values of blood glucose levels are given in mean of three animals and graph plotted with value of all the different groups(Graph 1). There was significant decrease in blood glucose levels of Standard, Test1 and Test2 groups when compared to Toxic control group at different time intervals this shows improved insulin activity. This is may be due to the presence of quercetin, Rutin and luteolin its has been proved that these three flavonoids have positive effect on insulin mediated glucose uptake and insulin sensitizing activity.

The phytochemicals like flavanoids, glycosides, phenolic compounds were already reported for their nephroprotective, antidiabetic, antioxidant, hypolipidemic and insulin sensitizing activity.

Hence these chemical constituents can be accounted for prevention of diabetic nephropathy and insulin sensitizing activity.[27] Further studies are recommended to evaluate the mechanism involved in preventing diabetic nephropathy and insulin resistance of Momordica cochinchinensis(Lour.) spreng fruits.

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