Original Research Article

Antidiabetic activity of Manomani chooranam aqueous extract on female wistar albino rats

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ABSTRACT

Background: The aim of the present study was to evaluate the antidiabetic potential of Manomani chooranam (MMC), an indigenous polyherbal siddha formulation in Streptozotocin (STZ) induced diabetic female Wistar albino rats.

Methods: Aqueous extract of MMC was prepared. Wistar albino rats were divided into six groups (n=6). Group 1 was kept as normal control, Group 2, 3, 4, 5 and 6 were induced diabetes. After induction, the group 2 was kept as diabetes control; Group 3 received the standard drug metformin (100 mg/kg), whereas Groups 4, 5 and 6 were treated with the aqueous extract of MMC at 500 mg/kg, 1000 mg/kg and 1250 mg/kg doses, respectively for the 21 days. Blood sugar was estimated at the end of each week. At the end of the study, rats were sacrificed and the pancreas was analyzed for histopathological changes. Data expressed as mean±standard error of the mean. Statistical analysis was done using one-way ANOVA followed by post hoc Tukey's test. p<0.05 considered statistically significant.

Results: The groups which received aqueous extract of MMC at 500 mg/kg, 1000 mg/kg and 1250 mg/kg showed a significant decrease in the mean blood sugar level when compared to normal level. The groups which received MMC shows significant reduction in blood sugar level in comparison with standard drug metformin 100 mg/kg.

Conclusions: The aqueous extract of MMC was able to decrease the elevated blood sugar levels in dose dependent manner.

Keywords: Diabetes mellitus, Manomani chooranam, Aqueous extract, Streptozotocin

INTRODUCTION

Diabetes mellitus is one of the metabolic syndromes, which is characterized by hyperglycaemia, hyperuricemia, hyperaminoacidemia and it leads to hypoinsulinemia and reduced action of insulin. The global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year of 2025. Most of the antidiabetic drugs such as sulfonylureas, biguanides, α-glucosidase inhibitors, incretin-mimetic etc., have some kind of adverse effects like nausea, vomiting, diarrhoea, abdominal pain, headache, etc. Thus search for a new safe and potent anti-diabetic herbal formulation drug is essential to overcome these problems. Asper world ethno botanical reports nearly 1000 plants could be used to treat diabetes mellitus. In siddha medicine, many single and polyherbal formulations and higher medicine like chooranam, parpam, chendooram and chunam have been practised cure or control diabetes mellitus from time immemorial.

Manomani chooranam (MMC), a polyherbal siddha formulation containing nineteen ingredients of herbal origin, which is used in traditional medicine to treat type
II diabetes, contains both antidiabetic and antioxidant principles. Justicia adatoda, Solanum trilobatum, Ocimum sanctum, Andrographis paniculata, Aristolochia bracteolata, Azadirachta indica, Murraya koenigii, Eclipta prostrata, Evolulusus sinoeides, Terminalia chebula, Phyllanthus emblica, Zingiber officinale, Piper longum, Elettaria cardamomum, Cumintium cymicum, Alangium salvifolium, Ficus religiosa, Ficus racemosa, Withania somnifera. Cassia auriculata are rich in bioflavonoids helps in metabolic disorders and has antipyretic, hepatoprotective, antidiabetic, antiperoxidative, hypolipidemic and microbicidal activity. Phyllanthus amarus, Terminalia belerica, Piper nigrum, Ficus bengalensis also have hypolipidaemic activity. These formulations help treating diabetic patients as a hypoglycemic and antioxidant agent with fewer side effects. But there is no substantial evidence to prove its efficacy in-vivo.

METHODS

Drug and chemicals

Manomani chooranam was procured from Dr. Iyyankanni, Head and Professor, Department of Sirappu Maruthuvam, Maria Siddha Medical College, Thiruvattaru, Kanyakumari (D.I), India. Streptozotocin was purchased from sigma chemicals, Mumbai. Metformin was procured from MGMC Pharmacy store.

Ethical clearance

The study was one after being approved by Institutional Animal Ethics Committee (Ref. No.06/IAEC/MG/2017-1). Adult female Wistar albino rats (weighing 150-200g) were used for acute toxicity and anti-diabetic activity. The animals were purchased from TANUVAS, Chennai. They were kept individually in cages, fed with standard pellet and water; animals were maintained at a temperature of 27±3°C.

Preparation of MMC aqueous extract

As per Siddha Pharmacopeia, MMC was taken by boiling 100 gm of chooranam with 400 ml of water by boiling in slow flame and then filtered. The aqueous extract prepared is stored in an airtight container.

Acute toxicity study

As per OECD 423 guidelines, MMC aqueous extract was administered through oral gavage to female Wistar rats (n=3) in a dose of 2000 mg/kg body weight. The rats were observed for the first 24 hours for any signs and symptoms of toxicity or death and later for 2 weeks. The procedure was repeated with higher doses of MMC 5000mg/kg body weight using 3 female Wistar albino rats

Dose selection

As the limit dose did not exhibit any signs of toxicity, a dose of 2000 mg/kg body weight p.o., was taken as dose for the main study. Metformin at a dose of 100 mg/kg was taken as a standard control.

Induction of diabetes in Wistar albino rats

The overnight fasted Wistar albino rats were injected with intraperitoneal (i.p.). Inj. streptozotocin in 0.1 M cold sodium citrate buffer, at the dose of 35 mg/kg body weight. To counter-act the drug-induced hypoglycemia, the rats were subjected to drink 5% glucose solution overnight. A week time was given for the development of diabetes. The confirmation of hyperglycemia was determined by monitoring blood glucose level more than 200 mg/dl were considered as diabetic induced rats. Then the animals were divided into the respective groups (6 animals in each group).

Six groups with six rats in each (a total of 36 rats) were used. All groups received respective drugs for 3 weeks.

Group I (control) received normal saline P.O (per orally). Group II (negative control) diabetic Induced rats received normal saline without treatment. Group III (positive control) diabetic induced rats received metformin P.O 100 mg/kg body weight once a day for 3 weeks. Group IV, V and VI treatment group (low, moderate and high dose) received 500 mg, 1000 mg, 1250 mg/kg of the chooranam extract respectively once a day P.O (Table 1).

Table 1: Experimental design of MMCAE.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Purpose</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal control</td>
<td>Standard pellet feed with normal saline</td>
</tr>
<tr>
<td>Group II</td>
<td>Negative control</td>
<td>Standard pellet feed with normal saline</td>
</tr>
<tr>
<td>Group III</td>
<td>Positive control</td>
<td>Standard pellet feed with metformin 100mg/kg</td>
</tr>
<tr>
<td>Group IV</td>
<td>Low dose</td>
<td>Standard pellet feed + 500 mg/kg of MMCAE</td>
</tr>
<tr>
<td>Group V</td>
<td>Moderate dose</td>
<td>Standard pellet feed + 1000 mg/kg of MMCAE</td>
</tr>
<tr>
<td>Group VI</td>
<td>High dose</td>
<td>Standard pellet feed + 1250 mg/kg of MMCAE</td>
</tr>
</tbody>
</table>

MMCAE- MMC aqueous extract.
The blood samples were collected from rat tail vein at the time intervals on 0, 7th, 14th and 21st days to estimate the blood glucose level by Tail snipping method. At the end of the 21st days, animals were sacrificed with I.M Injection sodium thiopentone and diffuse pancreas were collected from all 36 animals and subjected to histopathological study. Rat pancreas was collected and fixed in 10% formaldehyde solution for microscopic examination using haematoxylin-eosin stain.

**Statistical analysis**

Collected data were entered in Microsoft excel 2019 and analyzed using JASP 0.8.4.0 version. Results were expressed in mean±standard error of the mean as a table. Statistical analysis was performed using one way ANOVA followed by post hoc Tukey’s test. The p<0.05 was considered statistically significant.

**RESULTS**

**Acute toxicity study of Manomani chooranam aqueous extract (MMCAE) in rats**

Acute toxicity studies confirmed that the MMCAE up to a dose of 5000mg/kg body weight was non-toxic. There was no mortality or any abnormal behavioral changes were found at any of the selected doses the end of the study.

**Table 2: Effect of MMC aqueous extract formulation on fasting blood glucose levels in STZ induced diabetic rats.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood glucose level in mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th day</td>
</tr>
<tr>
<td>Normal control</td>
<td>115±5.76</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>215.8±11.14</td>
</tr>
<tr>
<td>Positive control (metformin)</td>
<td>218.5±5.96</td>
</tr>
<tr>
<td>MMCAE- low dose</td>
<td>223.5±2.88</td>
</tr>
<tr>
<td>MMCAE- moderate dose</td>
<td>220.2±3.76</td>
</tr>
<tr>
<td>MMCAE-high dose</td>
<td>214.7±3.98</td>
</tr>
</tbody>
</table>

MMC values are expressed as mean±SD (n=6). *p<0.05 when compare to positive control group; **p<0.01 when compared to positive control group (one-way ANOVA followed by post hoc Tukey’s test) STZ-streptozotocin.

**Effect of MMC on blood glucose level**

The blood glucose levels were significantly increased after STZ injection, when compared to that of normal control group and the animals were grouped according to the blood glucose levels. The groups which received low dose (500 mg/kg), moderate dose (1000 mg/kg) and high dose (1250 mg/kg) showed significant reduction in blood sugar level. The blood glucose levels were significantly reduced in all treatment groups under study when compared to positive control (Figure 1) and (Table 2).

**Histopathological study of pancreas**

All the sections of normal control (Figure 2A) showed normal tissue architecture with lobules separated by connective tissue septae. The lobules consist of exocrine acinar cells.

The endocrine islets of Langerhans were embedded within the acinar cells. All the sections of disease control (Figure 2B) showed highly reduced islets number. The number of cells in each islets and the size of islets were also reduced.

The tissue sections of positive control metformin (Figure 2C), low dose of MMCAE (Figure 2D), moderate dose of MMCAE (Figure 2E), high dose of MMCAE (Figure 2F) has shown an increase in size of the islets compared to NC group.

There was an increase in size of cells in each group compared to negative control group compared to normal control.

There was no loss of tissue architecture and necrosis in any of the group. There was protection in all three treated groups which was evident by the increase in size of islets of Langerhans and there was no difference in protection of cells in these groups.

**Figure 1: Effect of MMC aqueous extract formulation on fasting blood glucose levels (mg/dl) in STZ induced diabetic rats.**
Figure 2: (A) Normal pancreas control group showing normal; (B) negative control showing reduced no of islets cells; (C) positive control (metformin) 10x- increase in size of islets cells; (D) MMC aqueous extract low dose 10x- increase in size of islets cells; (E) MMC aqueous extract moderate dose 10x- increase in size of islets cells; (F) MMC aqueous extract high dose 10x- increase in size of islets cells.

DISCUSSION

Diabetes has become a major health problem in most of the countries. Combination of herbs has been extensively used from ancient times and had shown potent antidiabetic activities without toxicity. Therefore a polyherbal formulation was prepared. Since STZ has selective pancreatic islet beta cell cytotoxicity it is used to induce type I diabetes in rat model. Streptozotocin enters the β cell causing alkylation of DNA resulting in necrosis.

In the present study, the anti-diabetic activity of MMC aqueous extract was investigated in STZ induced diabetes in rat models. The 3-week study was conducted in the Central Animal house, Mahatma Gandhi Medical College and Research Institute, Puducherry. In our study, the MMCAE at three doses, that is, 500 mg/kg, 1000 mg/kg, and 1250 mg/kg produced a dose-dependent reduction in the sugar levels especially in first week of treatment when compared to the standard drug metformin (100 mg/kg), which is followed by significant reduction in sugar levels in subsequent weeks till our study (3 weeks).

The poly herbal formulation significantly reduced the blood glucose level in streptozotocin-induced-diabetic rats as compared to the diabetic control group. The possible mechanism by which polyherbal formulation brings about its hypoglycaemic action in diabetic rat may be by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form. Lack of insulin leads to inactivation of the glycogen synthase systems. The possible mechanism of lowering blood glucose level by herbal formulation TAB may be by inhibiting the pancreatic enzymes resulting in an increase in pancreatic secretion of insulin or its release from bound form.

CONCLUSION

The results show that Manomani chooranam is safe and is able to control the blood sugar levels. The ability to reduce the blood sugar levels is due to the presence of active ingredients in this poly herbal formulation. Hence it can be a potent anti-diabetic drug for usage, which needs further evaluation.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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