EVALUATION OF ANTIDIABETIC POTENTIAL OF MADHURAKSHAK; A POLYHERBAL FORMULATION AGAINST STREPTOZOTOCIN INDUCED DIABETES MELLITUS

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ABSTRACT

Several traditional treatment strategies have been recommended in the alternative system of medicine for the treatment of diabetes mellitus. Present investigation was undertaken to evaluate and examine the antidiabetic potential of Madhurakshak (Mdr), a polyherbal formulation containing extracts from twelve different herbs viz., Pterocarpus marsupium, Cinnamomum tamala, Eugenia jambolana, Gymnema sylvestre, Piper nigrum, Azadirachta indica, Trigonella foenugraecum, Momordica charantia, Phyllanthus emblica, Terminalia chebula, Terminalia belerica and shudh shilajit in streptozotocin (STZ 40mg/kg i.p. single dose) induced type I diabetic rats. Effects of Madhurakshak Powder (600 mg/kg, oral), Mdr concentrate (300mg/kg, oral) and standard herbal drug Diabecon (250 mg/kg, oral) were evaluated on chronic (28 days) administration in STZ induced diabetic rats. This evaluation was based on various parameters studied which included blood glucose, Liver parameters (creatinine, urea, AST, ALT and ALP), Serum markers (serum triglyceride, cholesterol, LDL, bilirubin, HDL& Total Protein) and histopathological studies. Our results concluded that Mdr Powder and Mdr concentrate were both able to depict their antidiabetic potential and were able to bring back the deranged level of liver and serum markers to near normal. This was backed by histopathological studies which revealed the possible beta cell protection potency of this polyherbal formulation.

Keywords: Madhurakshak, Antidiabetic, Polyherbal Formulation, Streptozotocin.
INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder of deranged carbohydrate, fat and protein affecting about 175 million people all over the globe. Diabetes mellitus is not a single disorder but it is a group of metabolic disorder characterized by chronic hyperglycaemia results in defects in insulin secretion, insulin action or both\(^1\). Increased thirst, increased urinary output, ketonuria and ketonemia are the common symptoms of diabetes mellitus which occur due to abnormalities in carbohydrate, fat and protein metabolism\(^2\). When ketones body present in blood and urine it is called ketoacidosis hence proper treatment should be taken immediately, else it can lead to other diabetic complications\(^3\).

Diabetes mellitus has cause significant morbidity and mortality due to microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications\(^4\). Diabetes is mainly attributed to the rapid rise in unhealthy life style, urbanization and aging\(^5\). Hyperglycemia which is the major symptom of Diabetes mellitus produce generates reactive oxygen species (ROS) which cause lipid peroxidation and membrane damage. ROS plays important role in the development of secondary complications in diabetes mellitus such as cataract, neuropathy and nephropathy\(^6\). Antioxidant protects β-cells from oxidation by inhibiting the lipid peroxidation chain reaction thus they play important role in diabetes. Plants containing natural antioxidants such as such as tannins, flavonoids, vitamin E and vitamin C can preserve β-cell function and prevent diabetes induced ROS formation\(^7\). Polyphenols which are classified into many groups such as tannins, flavonoids and stilbenes have been known as health-beneficial properties, which includes free radical scavenging activity, inhibition of hydrolytic and oxidative enzymes, anti-inflammatory and anti-diabetogenic potentiality\(^8\). Aldose reductase as a key enzyme, catalyse the reduction of glucose to sorbitol and is associated with the chronic complications of diabetes such as peripheral neuropathy and retinopathy. Use of aldose reductase inhibitors and α-glucosidase inhibitors has been reported for the treatment of diabetic complications\(^9\).

Although the management strategy for diabetes mellitus includes insulin and synthetic drugs, their continuousadministration may lead to undesirable adverse effects\(^10\), because of which there is an enormous growing interest in discovering traditional medicinal herbs as safer alternative with significant antioxidant activity\(^11\).

Madhurakshak (Mdr), a polyherbal formulation containing powders from twelve different herbs viz., *Pterocarpus marsupium*, *Cinnamomum tamala*, *Eugenia jambolana*, *Gymnema*
sylvestre, Piper nigrum, Azadiracheta indiaca, Trigonella foenum-graecum, Momordica charantia, Phyllanthus emblica, Terminalia chebula, Terminalia bellerica and shudh shilajit have been evaluated for antidiabetic activity as individually each plant have attracted great attention in East Asian countries\textsuperscript{12}.

However, there is still no direct modern report on the antidiabetic activities of Mdr. Therefore, this present study was carried out to investigate the antidiabetic potential of this polyherbal formulation, Mdr and its possible mechanism(s) using in vivo streptozotocin model, with a view to ascertain the use of this formulation in controlling diabetes with least adverse effects.

**MATERIAL AND METHOD**

**Material**

Normal saline, streptozotocin (STZ)(Sigma st louis, USA), all reagents used were of analytical grade. Glucose test kit (span diagnostic , gujrat, India) Triglyceride and cholesterol test kit (Bayer Diagnostics India), rat insulin elisa kit (Mercodia AB, uppsala, sweden), Madhurakshak Powder and concentrate were a generous gift from Dabur Research Foundation, India.

**Preparation of Test Formulations**

**Mdr Powder:** 1.2g of Md. Pwd was weighed and triturated in 0.5% of CMC to dissolve the powder and then transferred to a 50ml calibrated falcon tube. The volume was make up to 30ml for adjusting the final concentration to 600 mg/kg.

**Mdr Concentrate:** 600mg of the Md. Conc. was weighed in a falcon tube and triturated in 0.5% of CMC and kept on a shaker for few minutes to dissolve the concentrate. Finally the volume was make up to 30ml for achieving the final concentration of 300mg/kg.

**Animals**

Female albino wistar rats (180-230 gms) , housed in a light (12h light – 12 h dark) & temperature controlled room with standard pelleted diet (amrut rat food, India) & water ad libitum. The study was approved by the animal ethical committee vide no. AIP/IAEC/2012/12/1.
Experimental design

Induction of diabetes

50 normoglycemic and well acclimated albino wistar rats were selected for this experiment. Diabetes was induced by a single dose of STZ (40 mg/kg body weight via intraperitonial route. Induction of diabetes was confirmed by measuring fasting blood sugar level after 72 hours of STZ injection. Only rats with fasting blood glucose over 250 mg/dl were considered to be diabetic.

A total of 30 rats (n=6) were used in the further experiments. The rats were divided into five groups after the induction of diabetes with STZ. The experimental period was four weeks. Group I (normal control rats) received saline; group II rats served as diabetic control rats. Group III diabetic rats were treated with standard herbal antidiabetic drug diabecon (250mg/kg); group IV diabetic rats received MdrConcentrate (300mg/kg) and group V diabetic rats were administered Powder Mdrpowder (600mg/kg).

At the end of four weeks, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the estimation of blood glucose, liver parameters (creatinine, urea, AST, ALT and ALP) and serum lipid profile (serum triglyceride, cholesterol, LDL, bilirubin, HDL & Total Protein). Pancreas and kidneys were immediately dissected out and washed in ice-cold saline to remove the blood.

Histopathology of pancreas

Pancreas from animals was removed after sacrificing the animal and was washed in ice cold saline. Major portion of the pancreatic tissue was fixed in 10% formalin solution for further histological studies. After fixing the tissue solid section were cut and stained with eosin.

Statistical Analysis

All data were expressed as means ± S.E.M. The statistical significance among multiple groups was assessed by one-way ANOVA using graph pad prism 5 software. P value < 0.01 was considered to be statistically significant.

RESULTS

The antidiabetic effect of diabecon, Mdr concentrate and Mdrpowder on blood sugar levels of normal and diabetic rats is shown is Table 1.
Table 1 | Effects on blood glucose level in STZ induced diabetic rats. (Blood glucose level mmol/L)

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 h</th>
<th>24 h</th>
<th>7 Day</th>
<th>14 Day</th>
<th>21 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>3.14 ± 0.78</td>
<td>2.93 ± 0.13**</td>
<td>3.82 ± 0.48**</td>
<td>3.61 ± 1.04**</td>
<td>2.74 ± 0.39**</td>
</tr>
<tr>
<td>DC</td>
<td>3.21 ± 0.29</td>
<td>12.24 ± 0.22</td>
<td>13.08 ± 1.12</td>
<td>14.27 ± 0.87</td>
<td>15.28 ± 1.36</td>
</tr>
<tr>
<td>D + D</td>
<td>3.65 ± 0.89</td>
<td>14.52 ± 1.84</td>
<td>7.36 ± 1.59*</td>
<td>4.24 ± 0.51**</td>
<td>3.98 ± 1.03**</td>
</tr>
<tr>
<td>D + MC</td>
<td>2.92 ± 0.95</td>
<td>15.07 ± 0.38*</td>
<td>5.89 ± 0.93**</td>
<td>4.02 ± 0.48**</td>
<td>4.73 ± 1.92**</td>
</tr>
<tr>
<td>D + MP</td>
<td>3.14 ± 0.78</td>
<td>15.84 ± 1.52*</td>
<td>6.07 ± 1.69*</td>
<td>5.31 ± 0.92*</td>
<td>4.87 ± 0.67**</td>
</tr>
</tbody>
</table>

NC: Normal control; DC: Diabetic control; D + D: Diabetes + Diabecon; D + MC: Diabetes + Madurakshak Concentrate; D + MP: Diabetes + Madhurakshak Powder. Data were shown as the means ± S.E.M., n=6, *P < 0.05, **P < 0.01 compared to diabetic group.

The results clearly indicated that the diabecon treatment (250mg/kg), Mdr concentrate (300 mg/kg) & Mdrpowder (600 mg/kg) showed a significant antidiabetic activity. The administration of STZ (40 mg/kg) led to about 4 fold elevation of blood glucose levels which was significantly reduced in diabecon, Mdr concentrate and Mdrpowder, however Mdr concentrate showed no significant difference in their antidiabetic activity.

Table 2 | Effect on serum biochemical parameters in STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine</th>
<th>Urea</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.41 ± 0.02*</td>
<td>23.43 ± 0.42</td>
<td>41.11± 0.99**</td>
<td>48.41 ± 0.02</td>
<td>46.03 ± 0.39**</td>
</tr>
<tr>
<td>DC</td>
<td>0.94 ± 0.01</td>
<td>35.72 ± 0.36</td>
<td>126.92± 1.91</td>
<td>157.84 ± 1.29</td>
<td>129.92 ± 1.02</td>
</tr>
<tr>
<td>D + D</td>
<td>0.62 ± 0.02*</td>
<td>14.52 ± 1.84**</td>
<td>68.72 ± 2.91**</td>
<td>81.71 ± 0.82**</td>
<td>59.21 ± 2.64**</td>
</tr>
<tr>
<td>D + MC</td>
<td>0.68 ± 0.04*</td>
<td>15.92 ± 0.28*</td>
<td>77.38 ± 1.02*</td>
<td>98.48 ± 0.63*</td>
<td>101.03 ± 1.93</td>
</tr>
<tr>
<td>D + MP</td>
<td>0.65 ± 0.02*</td>
<td>15.33 ± 0.52*</td>
<td>75.61 ± 2.51*</td>
<td>96.69 ± 2.72*</td>
<td>98.85 ± 1.38*</td>
</tr>
</tbody>
</table>

NC: Normal control; DC: Diabetic control; D + D: Diabetes + Diabecon; D + MC: Diabetes + Madurakshak Concentrate; D + MP: Diabetes + Madhurakshak Powder. Data were shown as the means ± S.E.M., n=6, *P < 0.05, **P < 0.01 compared to diabetic group.
Table 2 depicts the values of creatinine, Urea, AST, ALT and ALP levels of both control and experimental groups. In diabetic control group animals the activities of blood creatinine, Urea, AST, ALT and ALP were significantly increased (P<0.01), which was brought back to near normal in the treated groups compared to diabetic control.

Table 3 shows the effect of test drugs on serum lipid profiles of control and experimental groups. The hyperlipidemic parameters like serum triglyceride, cholesterol, LDL, bilirubin were increased but HDL cholesterol and total protein deceased in diabetic groups in comparison to the normal control group rats. However all these deranged parameters were brought back to near normal in case of treated rats.

Table 3 Effect of polyherbal formulation on lipid profile of STZ induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>HDL</th>
<th>LDL</th>
<th>Bilirubin</th>
<th>Protein</th>
<th>Cholesterol</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>44.58 ± 1.08**</td>
<td>55.75 ± 0.47**</td>
<td>1.38 ± 0.09*</td>
<td>8.24 ± 0.52**</td>
<td>86.03 ± 0.33**</td>
<td>89.03 ± 0.39**</td>
</tr>
<tr>
<td>DC</td>
<td>18.57 ± 0.41</td>
<td>182.05 ± 1.52</td>
<td>4.02 ± 0.05</td>
<td>3.91 ± 0.31</td>
<td>129.38 ± 0.53</td>
<td>134.57 ± 0.49</td>
</tr>
<tr>
<td>D + D</td>
<td>23.14 ± 0.19</td>
<td>54.22 ± 0.04**</td>
<td>1.92 ± 0.04**</td>
<td>6.31 ± 0.46**</td>
<td>89.89 ± 0.92**</td>
<td>91.85 ± 0.24**</td>
</tr>
<tr>
<td>D + MC</td>
<td>29.62 ± 1.09*</td>
<td>61.53 ± 1.18**</td>
<td>2.08 ± 0.02*</td>
<td>5.48 ± 0.78*</td>
<td>91.42 ± 0.37**</td>
<td>94.67 ± 0.23*</td>
</tr>
<tr>
<td>D + MP</td>
<td>28.68 ± 0.84*</td>
<td>63.39 ± 1.21**</td>
<td>2.01 ± 0.03*</td>
<td>5.09 ± 0.95</td>
<td>93.05 ± 0.83*</td>
<td>96.65 ± 0.47*</td>
</tr>
</tbody>
</table>

NC: Normal control; DC: Diabetic control; D + D: Diabetes + Diabecon; D + MC: Diabetes + Madurakshak Concentrate; D + MP: Diabetes + Madurakshak Powder. Data were shown as the means ± S.E.M., n=6, *P < 0.05, ** P < 0.01 compared to diabetic group.

Figure 1 illustrates an islet of Langerhans in the control group. The islet depicts a large number of β cells distributed throughout the islet. In the diabetic group, a decrease in the number of β cells of the islets of Langerhans was observed in comparison to the control group (Figure 2). Few functional β cells were observed and α cells were more prominent. Figures 3, 4 & 5 shows the islets of Langerhans from the diabetic groups treated with Diabecon, Mdr Concentrate and Mdr Powder at concentrations of 250 mg/kg & 600 mg/kg body weight, respectively.
Figure 1 Pancreatic section photomicrograph from the control group rats showing the exocrine region and islets of Langerhans, with scattered β cells and red blood cells.

Figure 2 Pancreatic section photomicrograph of STZ-induced diabetic pancreatic section showing the exocrine region and islets of Langerhans with damaged β cells due to necrosis and a decreased number of β cells.

Figure 3: Pancreatic section photomicrograph of diabetic islet treated with Diabecon 250 mg/kg powder showing evenly distributed β cells and an increased number of β cells.
The damaged β cells seen after the initial induction of diabetes were no longer observed after treatment with Diabecon and Mdr. The recovery of necrotic β cells was especially more pronounced after treatment with 600 mg/kg of Mdr Concentrate when compared to Mdr Powder.

DISCUSSION

The current study was designed to evaluate the antidiabetic potential of Mdr Concentrate and Mdr Powder (a polyherbal formulation) against STZ induced diabetes in albino wistar rats in vivo. The blood glucose levels, liver parameters (Creatinine, Urea, AST, ALT and ALP), serum lipid profile (serum triglyceride, cholesterol, LDL, bilirubin, HDL and total protein) and pancreatic histopathology was performed to assess the antidiabetic potential of this polyherbal formulation in comparison to diabecon as standard herbal antidiabetic agent.
Streptozotocin induced rats exhibited decrease in body weight, polyphagia and polydipsia associated with decrease in endogenous insulin and hyperglycemia. Chronic treatment with Mdr to diabetic rats not only decreased food and water consumption and improved loss of body weight but also depicted decrease in serum glucose and increase in serum insulin levels in diabetic rats. These effects may be attributing to either inhibition of increase in insulin output, inhibition of intestinal absorption of glucose and increase in glucose metabolism because Mdr; a polyherbal drug contains various medicinal plants, each having different mechanisms of action of antidiabetic activity.

Gymnema sylvestre\textsuperscript{15}, Pterocarpus marsupium\textsuperscript{16}, Momordica charantia\textsuperscript{17}, Azadirachta indica\textsuperscript{18}, have been reported to produce antihyperglycemic effect by increase in insulin secretion. While Cinnamomum tamala\textsuperscript{19}, Eugenia jambolana\textsuperscript{20}, Phyllanthus emblica\textsuperscript{21}, have been reported to produce antidiabetic activity by increasing insulin sensitivity. Terminalia chebula and Terminalia belerica have been reported to potentiate the action of insulin\textsuperscript{22}. Shilajeet showed to regenerate the cells in pancreas that secrete insulin\textsuperscript{23}. Many of the plants have been reported to contain substances like glycosides, alkaloids, tannins, and flavonoids etc., which have been proved to be antidiabetic by different mechanism of action\textsuperscript{24}.

Chronic treatment with Mdr showed no increase in serum cholesterol and triglycerides levels. It showed that apart from carbohydrate metabolism it played important role in lipid metabolism. The possible mechanism for decreased lipid levels could be either insulin releasing or insulin sensitizing activity, because insulin proved to inhibit the activity of hormone sensitive lipases in adipose tissue and suppress the release of lipids.

Animals from all the groups did not show any clinical toxicity signs during cage side observation throughout experimental period. Mortality was not observed in any of the group. The feed consumption in all the groups including diabetic control showed almost similar pattern throughout the study.

At prolonged period of diabetes, the standard herbal drug Diabecon at dose i.e., was found to be effective in lowering Serum glucose level. Both the test items showed results equivalent to the standard herbal formulation. All the formulations were found to be effective at early stage of diabetes. In lateral phase of this study all these formulations was found to be less active. This may be due to occurrence of diabetic complications at prolonged time period. These complications may result due to uncontrolled diabetes, which may be unable to recover by
individual treatment of Diabecon. The % anti-diabetic activity of Mdrpowder was found to be equivalent to the standard herbal drug Diabecon. So, it was difficult to interpret that Mdr in which form (powder or Concentrate) works better.

Serum insulin levels were significantly decreased in Diabetic control rats. After 24 day of test and standard drug supplementation to the Wistar rats, there was a significant elevation in serum insulin level in respect to Diabetic control group though the level of this hormone was significantly low than the normal levels. The serum insulin content on day 24 after STZ injection was significantly higher in 24 days formulations treated group when compared to the control group.

It showed that these herbals formulation was effective in controlling the elevation in Serum cholesterol levels and Serum Triglyceride levels which had been seen in STZ induced Diabetes mellitus. Diabecon was found to be most effective in controlling hypercholestremia. After administration of Mdrpowder and concentrate, the lowered serum TC, TG, & LDL levels were restoredback to near normal along with raised HDL/LDL ratio in diabetic rats which were also noted.

Evaluation of liver parameters showed that the activities of AST, ALT in serum of Diabetic control rats were markedly elevated which might be attributed as a result of damage or toxicity to the liver from where these enzymes may leak from the hepatocytes into the circulation thereby elevating their levels. This directly correlates to the hepatocellular damage in case of diabetic rats.

The Pancreas weight on day 24 after STZ injection was significantly higher in 24 days formulations treated group when compared to the control group (Not shown). This indicates that these formulations could protect the pancreatic β-cell destruction against STZ-toxicity. Maximum pancreatic weight gain shown by the Diabecon. The histopathological evaluation undertaken on the islets of Langerhans depicted the recovery of damaged cells and an improvement in overall histopatholgical pattern after treatment with Mdr, when compared to diabetic control group.

Diabetic rats showed a reduced no of islet cells which were restored to near normal upon treatment with diabecon and Mdr concentrate and Mdrpowder. This study further supports the possibility of regeneration as a means for regenerating new beta cells, even in very
severely damaged pancreas as in the case of diabetes control rats or individuals with significant reduced number of beta cells.

CONCLUSION
On the basis of the present results, it could be concluded that Mdrpowder and Concentrate, a polyherbal formulation exerts a significant antihyperglycemic and antihyperlipidemic effect. This could be attributed due to different types of active principles from various plants, which may have different mechanisms of action. One potent mechanism attributes to the protection of pancreatic $\beta$-cells and liver tissues by ameliorating oxidative stress followed by other mechanism which is also associated with amelioration of carbohydrate metabolism by augmenting insulin signalling and regulating rate-limiting enzymes. Both the Mdrpowder and Concentrate have an equivalent effect on lowering serum glucose levels. However, it cannot be concluded that combination of different plants may have synergistic or additive effect. Although, further studies remains to be conducted to investigate this hypothesis this herbal formulation can be considered as safe supplementary therapy for a long term and effective management of diabetic patients.

ACKNOWLEDGMENTS
The authors are thankful to the management of Amity Institute of Pharmacy and Dabur research Foundation for their cooperation during the research work.

REFERENCES


