Antidiabetic activity of *Momordica Sahyadrica* tuber on Streptozotocin induced diabetic rats
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**ABSTRACT**

*Momordica sahyadrica* is a rare endemic species found mainly in the Western Ghats of India. Its medicinal property is not defined but in ancient time it was used as a folk medicine mainly by the tribal communities comprising *M.charantia var.muricata, M.charantia var.charantia, M.dioica* and *M. sahyadrica*. Traditional uses of these species comprising food, medicinal and cosmetic, culinary preparations, vernacular names in local dialects and taboos and religious beliefs relating to their domestication and folk taxonomy. Formerly, material of it was placed under *Momordica dioica*, but it appeared distinct from that species in various morphological and ecological features. Literature review suggests that *Momordica* species seems to have some anti-diabetic activity. Hence, the present study is under taken to explore anti diabetic activity of hydro-alcoholic extract of *Momordica sahyadrica* tuber on streptozotocin induced diabetic rats. Male Wistar albino rats of weighing 150-250 g were procured and diabetes was induced in overnight fasted male albino wistar rats by Streptozotocin (45 mg/kg, i.p) on the 15th day. Blood Glucose level was estimated continuously till 7 days. There was significant increase in the antioxidant levels like SOD and CAT, decrease in MDA, Cholesterol, Triglyceride, blood glucose level compared to the control group. Also there was a significant increase in the Glucose oxidation enzyme Glucose-6-Phosphate Dehydrogenase and decrease in the gluconeogenic enzyme Glucose-6-Phosphatase. Histopathological studies showed that treatment with MS Extract causes increase in β-cells, Langerhans were restored to protection of and pancreatic liver tissue compared to diabetic group.

**KEYWORDS:**

Momordica sahyadrica, diabetic rats, diabetic rats

1.INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. These abnormalities give rise to altered metabolism of lipids, carbohydrates, and amino acids. Chronic hyperglycaemia resulting in impaired function or failure of various organs especially, eyes, kidney, nerves, heart, and blood vessels¹.

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Type 1 diabetes is caused due to a deficiency of insulin secretion from β-pancreatic cells. On the other hand, Type 2 diabetes is closely associated with obesity and is characterized by an initial phase of progressive insulin resistance, with an ensuing reduction in the ability of the pancreatic hormone to promote peripheral glucose disposal and to suppress hepatic glucose output²–³. Sedentary life style, unhealthy dietary habits and genetic predisposition are some of the key factors that have conspired to create the current worldwide epidemic of Type 2 diabetes, an acquired syndrome of elevated blood glucose. Diabetes is the world’s largest endocrine disease with deranged carbohydrate, fats and protein metabolism. As per WHO report, approximately 150 million people are suffering from diabetes mellitus worldwide and this number may well double by the year 2025. The statistical projection suggests that the number of diabetics will rise
from 15 million in the year 1995 to 57 million in 2025, making India apart the country with the highest number of diabetics in the world\textsuperscript{4}.

Despite the great strides that have been made in the understanding and management of diabetes, the disease and disease related complications are increasing unabated\textsuperscript{5}. Inspite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are also used with success to treat this disease\textsuperscript{5}. Many traditional plant used for the treatment of diabetes throughout the world and there is an increasing demand of the natural products with antidiabetic activity\textsuperscript{5}. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes\textsuperscript{8}.

\textit{Momordica sahyadrica} is a rare endemic species found mainly in the Western Ghats of India. Its medicinal property is not defined but in ancient time it was used as a folk medicine mainly by the tribal communities comprising \textit{M.charantia var.muricata}, \textit{M.charantia var.charantia}, \textit{M.dioica} and \textit{M. sahyadrica}. Traditional uses of these species comprising food, medicinal and cosmetic, culinary preparations, vernacular names in local dialects and taboos and religious beliefs relating to their domestication and folk taxonomy. Formerly, material of it was placed under \textit{Momordica dioica}, but it appeared distinct from that species in various morphological and ecological features\textsuperscript{9}. Literature review suggests that \textit{Momordica} species seems to have some anti-diabetic activity\textsuperscript{10, 11, 12, 13}. Hence, the present study is under taken to explore anti diabietic activity of hydro-alcoholic extract of \textit{Momordica sahyadrica} tuber on streptozotocin induced diabetic rats.

\textbf{2.MATERIALS AND METHODS}

\textbf{Plant Materials:} The tuber of \textit{Momordica sahyadrica} was collected and hydro-alcoholic extraction was performed followed by qualitative phytochemical analysis of extract\textsuperscript{9}. All the chemicals and reagents used are of analytical grade and procured from approved vendors. Animal ethical clearance was approved by institutional animal ethics committee with the reference number:IAEC/ ABMRCP/ 2012-13/ 28

\textbf{Acute Toxicity study:} The acute toxicity studies were carried out for hydro alcoholic extract of \textit{Momordica sahyadrica} tuber using up and down procedure according to OECD guidelines no. 425. So based on the concentration of 2000 mg/ kg two doses of one-tenth that is 200 mg/ kg and one-fifth that is 400 mg/ kg were selected to carry out the evaluation of anti diabetic study\textsuperscript{16}.

\textbf{Oral glucose tolerance test:} Prior to an OGT test, rats were fasted for 15 h. Distilled water (control), a reference drug (glibenclamide) and the MS extract of 200 and 400 mg/ kg were orally administered to groups of three rats each. 30 minutes later, glucose (1.25 g/ kg) was orally administrated to each rat. Blood samples were taken from tail veins at 30 (just before the drug administration), 0 (just before glucose administration), 30, 60, 90, 120, 150 and 180 min for the assay of glucose\textsuperscript{17}.

\textbf{Induction of Diabetes:} Diabetes was induced in overnight fasted animals (deprived of food 16 h but was allowed free access of water) by a single intraperitoneal injection of Streptozotocin (STZ) 45 mg/ kg freshly dissolved in 10 mM citrate buffer (pH 4.5). Rats with blood glucose levels greater than 300 mg/ dL was considered to be diabetic\textsuperscript{18}.

\textbf{Pancreatic \textit{β} cell Protective activity:} Treatment with MS extracts 200 and 400 mg/ kg were given twice a day for two weeks after estimation of normal blood glucose level. Diabetes was induced on 15\textsuperscript{th} day of treatment and blood glucose level was estimated regularly from the day of induction till 7 days from 15\textsuperscript{th} day. On the 7\textsuperscript{th} day the animals were decapitated and the organs, liver and pancreas were isolated and stored at -70°C in formalin solution for performing histopathological studies\textsuperscript{19}.

\textbf{Anti-diabetic Activity:} 3 days after STZ induction of diabetes in Male albino wistar rats, the fasting blood-glucose level was measured. The hyperglycemic rats (blood glucose > 300 mg/ dl) were divided on day zero into four groups (each with 8 rats). After the determination of fasting blood glucose level on day zero, Distilled water, MS extract (200 and 400 mg/ kg) and Glibenclamide (5 mg/ kg) was administered orally twice a day to Diabetic control, Treatment and Standard groups respectively for 2 weeks. On the 15\textsuperscript{th} day, after 16 h fasting, the rats were decapitated and blood and liver tissue were isolated under mild thiopentone anesthesia for biochemical estimations. The organs liver and pancreas were also isolated and stored at -70°C for performing histopathological studies\textsuperscript{19}.

\textbf{Experimental study design}

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
\hline
\textbf{GROUPS} & \textbf{GROUPS/DRUGS} & \textbf{DOSE} \\
\hline
Group I & Normal/ Control group (Distilled Water) & - \\
\hline
Group II & Diabetic control group (Streptozotocin ) & 45 mg/ kg \\
\hline
Group III & Standard group (Streptozotocin+ Glibenclamide) & 45 mg/ kg+ 5 mg/ kg \\
\hline
Group IV & Antidiabetic treatment group (Streptozotocin+ MS) & 45 mg/ kg+ 200 mg/ kg \\
\hline
Group V & Antidiabetic treatment group (Streptozotocin+ MS) & 45 mg/ kg+ 400 mg/ kg \\
\hline
Group VI & Antidiabetic Pretreatment Group (MS (200 mg/ kg)+ Streptozotocin) & 200 mg/ kg+ 45 mg/ kg \\
\hline
Group VII & Antidiabetic Pretreatment Group (MS(400 mg/ kg)+Streptozotocin) & 400 mg/ kg+45 mg/ kg \\
\hline
\end{tabular}
\end{table}

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Collection of serum samples: The blood was drawn via carotid artery occlusion of the rats (fasted for 14 h) under mild thiopentone anesthesia. The blood samples were allowed to clot for 30 min at room temperature and then they were centrifuged at 5000 rpm for 20 min. The resulting upper layer was collected in properly labeled, clean and dry micro centrifuge tubes. This serum specimen was used for the estimation of different biochemical parameters.\textsuperscript{19} Fasting blood glucose estimation: Blood glucose level was estimated by Glucometer, Accu Check.

Total Cholesterol: Serum cholesterol was estimated by CHOD/POD method with the help of Clinical Chemistry Analyzer (Metro Lab, 1600 DK-R) by using cholesterol stable reagent (Infinite, Bangalore)\textsuperscript{20}.

Total Triglyceride: Total triglyceride was estimated by GPO/POD method with the help of Clinical Chemistry Analyzer (Metro Lab, 1600 DK-R) by using triglyceride stable reagent (Infinite, Bangalore)\textsuperscript{21}.

Glucose-6-Phosphatase activity: The liver glucose-6-phosphatase activity was measured according to standard protocol (Swanson, 1995). Tissue was homogenized in ice cold of 0.1 M phosphate buffer saline (pH 7.4) at the tissue concentration of 50 mg mL\textsuperscript{-1}. In a calibrated centrifuge tube, 0.1 mL of 0.1 M glucose-6-phosphate solution and 0.3 mL of 0.5 M maleic acid buffer (pH 6.5) were taken and brought to 37 °C in water bath for 15 min. The reaction was stopped with 1 mL of 10% trichloroacetic acid (TCA) followed by chilling in ice and centrifuged at 3000 rpm for 10 min. The optical density was noted at 340 nm. The enzyme activity was expressed as mg of inorganic phosphate liberated per gm of tissue\textsuperscript{22}.

Glucose-6-Phosphate dehydrogenase: The liver glucose-6-phosphate dehydrogenase activity was measured according to standard protocol (Landdon, 1996). Tissue was homogenized in ice-cold 0.1 M phosphate buffer saline (pH-7.5) at the tissue concentration of 50 mg mL\textsuperscript{-1}. In a spectrophotometric cuvette, 0.3 mL of 1 M Tris-Chloride buffer (pH-7.5), 0.3 mL of 2.5X10\textsuperscript{-2} M glucose-6-phosphate, 0.1 mL of NADP and 0.3 mL of 0.2 M MgCl\textsubscript{2} and 0.3 mL ice cold tissue homogenate were taken. The rate of change of absorbance at 340 nm was recorded. One unit of enzyme activity defined as that quantity which catalyses the reduction of 1 μM of NADP per minute\textsuperscript{22}.

Catalase: Liver tissue was homogenized with polytron homogenizer in ice-cold Tris-HCl buffer to produce a 10% v/v homogenate. The homogenate was centrifuged at 15,000 rpm at - 4°C for 10 min. Supernatant 0.1 ml was added to cuvette containing 1.9 ml of 50 mM phosphate buffer. To that mixture, 1.0 ml of freshly prepared 30 mM H\textsubscript{2}O\textsubscript{2} was added and changes in absorbance for 3 min at 240 nm at an interval of 30 sec was measured. A control was prepared using 0.1 ml of distilled water devoid of 0.1 ml of homogenate. One unit of the enzyme activity is defined as enzyme concentration required to inhibit change in absorbance by 50% in one min in the control sample\textsuperscript{23}.

Superoxide dismutase (SOD): The liver tissue was homogenized with polytron homogenizer in ice-cold Tris-HCl buffer to produce a 10% w/v homogenate. The homogenate was centrifuged at 15,000 rpm at - 4°C for 10 min. Supernatant 0.1 ml was added to 1.2 ml of 0.052 M sodium pyrophosphate buffer (pH 8.3) followed by addition of 0.1 ml of 186 μM phenazonium methosulphate, 0.3 ml of 300 μM nitroblue tetrazolium, 0.2 ml of 780 μM NADH. Reaction mixture was incubated for 90 sec at 30°C, and the reaction was stopped by the addition of 1.0 ml of glacial acetic acid. Reaction mixture was stirred vigorously and shaken with 4.0 ml of n-butanol and centrifuged at 4000 rpm for 10 min. The absorbance of organic layer was measured at 560 nm. A control was prepared using 0.1 ml of distilled water devoid of 0.1ml of homogenate. One unit of the enzyme activity is defined as enzyme concentration required to inhibit the absorbance of chromogen production by 50% in control sample under the assay conditions\textsuperscript{24}.

Estimation of MDA in tissue: The liver tissue was homogenized in 0.1 M Tris-HCl buffer (pH 7.4) to produce 10% w/v homogenate. To a sample of 0.2 ml of tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% aqueous solution of TBA were added and mixed thoroughly. The final volume in all the tubes was made up to 5 ml with distilled water. Then the reactants were heated in an oil bath at 95°C for 60 min. Contents were cooled to room temperature with tap water and the resulting chromogen was extracted with 5 ml mixture of n-butanol and pyridine (15:1 v/v) by vigorous shaking. Separation of the organic phase was facilitated by centrifugation at 4000 rpm for 10 min. The absorbance of the solution was measured at the wave length of 532 nm\textsuperscript{25}.

Histopathological studies: After 16 h fasting on the 7\textsuperscript{th} day of induction of diabetes of the Pancreatic β-cell protective Group and 15\textsuperscript{th} day of Anti-diabetic Group rats were sacrificed under anesthesia, pancreas and liver tissues were immediately excised and fixed in 10% solution of formaldehyde and then dehydrated in graduated ethanol (50-100%), cleared in xylene and embedded in paraffin. The pancreatic as well as hepatic sections (4-5μm) were examined with a photomicroscope (40x) after staining with haematoxylin and eosin (H-E) dye. The histopathological studies were carried out\textsuperscript{26}.

Statistical analysis: All the values are expressed as (Mean ± SEM). Total Cholesterol, Total Triglyceride, Glucose-6-Phosphatase, Glucose-6-Phosphate dehydrogenase, Catalase, SOD, MDA in liver tissue were determined by factorial One-way ANOVA. Individual group was compared against diabetic control using Dunnett’s test. Values ranging between P<0.001 – P<0.05 were considered statistically significant. Statistical analysis was performed using GraphPad Prism 5 (version 5.01).
3. RESULTS AND DISCUSSION:

Preliminary phytochemical screening of hydroalcoholic extract of *Momordica sahyadrica* revealed the presence of carbohydrates, protein, glycosides, tannin and flavanoids. The values are expressed as Mean ± S.E.M (n=6). Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett’s test. *p*<0.05 as compared to control group.

### Table 1. Effect of *Momordica sahyadrica* tuber on glucose tolerance in fasted rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood Glucose Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/dl)</td>
<td>Normal</td>
</tr>
<tr>
<td>Normal</td>
<td>Distilled Water</td>
<td>186.6 ±10.85</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Streptozotocin (45 mg/kg)</td>
<td>100.8 ±4.92</td>
</tr>
<tr>
<td>Standard</td>
<td>Glibenclamide (5mg/kg)</td>
<td>110.7 ±3.92</td>
</tr>
<tr>
<td>Treatment (200 mg/kg)</td>
<td>MS (200 mg/kg)</td>
<td>107.67 ±3.6</td>
</tr>
<tr>
<td>Treatment (400 mg/kg)</td>
<td>MS (400 mg/kg)</td>
<td>107.67 ±3.6</td>
</tr>
</tbody>
</table>

### Table 2. Anti-diabetic effect of *Momordica sahyadrica* tuber on blood Glucose levels in Streptozotocin induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Blood Glucose Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/dl)</td>
<td>Normal</td>
</tr>
<tr>
<td>Normal</td>
<td>Distilled Water</td>
<td>186.6 ±7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Streptozotocin (45 mg/kg)</td>
<td>100.8 ±3</td>
</tr>
<tr>
<td>Standard</td>
<td>Glibenclamide (5mg/kg)</td>
<td>110.7 ±1</td>
</tr>
<tr>
<td>Treatment (200 mg/kg)</td>
<td>MS (200 mg/kg)</td>
<td>107.67 ±3.6</td>
</tr>
<tr>
<td>Treatment (400 mg/kg)</td>
<td>MS (400 mg/kg)</td>
<td>107.67 ±3.6</td>
</tr>
</tbody>
</table>

### Table 3. Pancreatic β-cell protective effect of *Momordica sahyadrica* tuber on blood Glucose levels in Streptozotocin induced diabetic rats

The values are expressed as Mean ± S.E.M (n=6). Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett’s test. * ***p*<0.05 as compared to control group. **ns**= non-significant as compared to control group.

### Table 4. Effect of *Momordica sahyadrica* tuber on lipid levels

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The values are expressed as Mean ± S.E.M (n=6). Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett’s test. *", **" and ***" =p<0.05 as compared to control group.

### Table 5: Effect of Momordica sahyadrica tuber on Glucose-6-Phosphatase and Glucose-6-Phosphate Dehydrogenase activity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Glucose synthesis and metabolism pathways</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glucose-6-Phosphatase (mg of IP of Tissue(^{-1}))</td>
<td>Glucose-6-Phosphate Dehydrogenase (units/mg solid)</td>
</tr>
<tr>
<td>Normal</td>
<td>Distilled Water</td>
<td>18.00±1.69</td>
<td>4.41±0.45</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Streptozotocin (45 mg/kg)</td>
<td>337.80±7.54</td>
<td>3.91±0.89</td>
</tr>
<tr>
<td>Standard</td>
<td>Glibenclamide (5mg/kg)</td>
<td>103.10±0.81***</td>
<td>2.53±0.65**</td>
</tr>
<tr>
<td>Treatment (200 mg/kg)</td>
<td>MS (200 mg/kg)</td>
<td>86.34±3.89***</td>
<td>40.13±0.46***</td>
</tr>
<tr>
<td>Treatment (400 mg/kg)</td>
<td>MS (400 mg/kg)</td>
<td>87.80±5.54***</td>
<td>41.16±2.41***</td>
</tr>
</tbody>
</table>

### Table 6: Effect of Momordica sahyadrica tuber on Oxidative Stress levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Oxidative Stress Parameters</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CAT</td>
<td>SOD</td>
</tr>
<tr>
<td>Normal</td>
<td>Distilled Water</td>
<td>0.004970±0.00007375</td>
<td>0.02769±0.004689</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Streptozotocin (45 mg/kg)</td>
<td>0.005136±0.0004284</td>
<td>0.07692±0.01988</td>
</tr>
<tr>
<td>Standard</td>
<td>Glibenclamide (5mg/kg)</td>
<td>0.008104±0.001515</td>
<td>0.1241±0.02779**</td>
</tr>
<tr>
<td>Treatment (200 mg/kg)</td>
<td>MS (200 mg/kg)</td>
<td>0.008504±0.0008023***</td>
<td>0.09836±0.04291***</td>
</tr>
<tr>
<td>Treatment (400 mg/kg)</td>
<td>MS (400 mg/kg)</td>
<td>0.008069±0.0003542***</td>
<td>0.1025±0.002953***</td>
</tr>
</tbody>
</table>

Histogram representing effect of Momordica sahyadrica tuber on glucose tolerance in fasted rats

The values are expressed as Mean ± S.E.M (n=6). Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett’s test. "**" and "***"=p<0.05 as compared to control group.

Histogram representing Anti-Diabetic effect of Momordica sahyadrica tuber on blood Glucose levels in Streptozotocin induced diabetic rats

The values are expressed as Mean ± S.E.M (n=6). Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett’s test. "**" and "***"=p<0.05 as compared to control group.\(^a\)= non-significant as compared to control group.

Histogram representing Pancreatic β cell protective effect of Momordica sahyadrica tuber on blood Glucose levels in Streptozotocin induced diabetic rats

\(^{a}\)= non-significant as compared to control group.
The values are expressed as Mean ± S.E.M (n=6). Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett’s test. ** and *** = p<0.05 as compared to control group. ns = non-significant as compared to control group.

Histogram representing Anti-Diabetic effect of Momordica sahyadrica tuber on Total Cholesterol.

Histogram representing Anti-Diabetic effect of Momordica sahyadrica tuber on Glucose-6-Phosphatase.

Histogram representing Anti-Diabetic effect of Momordica sahyadrica tuber on Catalase.
The values are expressed as Mean ± S.E.M (n=6). Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett’s test. ‘*’ and ‘***’=p<0.05 as compared to control group. ‘ns’= non-significant as compared to control group.

Histogram representing Anti-Diabetic effect of *Momordica sahyadrica* tuber on Superoxide Dismutase.

Histogram representing Anti-Diabetic effect of *Momordica sahyadrica* tuber on Malondialdehyde.

The values are expressed as Mean ± S.E.M (n=6). Data was analyzed by One-Way Analysis of Variance (ANOVA) followed by Dunnett’s test. ‘*’ and ‘***’=p<0.05 as compared to control group. ‘ns’= non-significant as compared to control group.

Histopathology Studies of Pancrease

(a) Normal Group: Beta cells – 75%, Alpha cells – 20%.
(b) Diabetic Control Group: Beta cells – 35%, Alpha cells – 60%.
(c) Standard/Glibenclamide treated Group: Beta cells – 55%, Alpha cells – 40%.
(d) MS Extract(200 mg/ kg) Antidiabetic Group: Beta cells – 45%, Alpha cells – 50%.
(e) MS Extract(400 mg/ kg) Antidiabetic Group: Beta cells – 55%, Alpha cells – 40%.
(f) MS Extract (200 mg/ kg) Pancreatic β-cell protective Group: Beta cells – 60%, Alpha cells – 35%.
(g) MS Extract(400 mg/ kg) Pancreatic β-cell protective Group: Beta cells – 65%, Alpha cells – 30%

Figure 1: Histopathology of Pancreas
Histopathology Studies of Liver

(a) Normal Group.
(b) Diabetic Control Group.
(c) Standard/Glibenclamide treated Group.
(d) MS Extract(200 mg/ kg) Antidiabetic Group.
(e) MS Extract(400 mg/ kg) Antidiabetic Group.
(f) MS Extract (200 mg/ kg) Pancreatic β-cell protective Group.
(g) MS Extract(400 mg/ kg) Pancreatic β-cell protective Group.

Fig. 2 Histopathology of Liver
Management of diabetes is still a challenge to the allopathic medicinal systems since no availability of synthetic agents without more side effects. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is a growing interest in herbal remedies due to the fewer side effects associated with these therapeutic agents. This has lead to an increase in the demand for natural products with antihyperglycemic activity having few side effects. Streptozotocin is toxic to pancreatic β-cells and thus widely used for induction of experimental diabetes mellitus in animals. Streptozotocin enters the β-cell via a glucose transporter (GLUT 2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-riboseylation, a process that is more important for diabetogenicity of streptozotocin than DNA damage itself. Poly ADP-riboseylation leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of streptozotocin action, β-cells undergo the destruction of necrosis.

The Oral Glucose Tolerance study in Table 2 showed plasma glucose levels of the normal rats reached a peak at 30 minutes after the oral administration of glucose and gradually decreased to the pre-prandial level. The hydroalcoholic extract of Momordica sahyadrica tuber at dose of 200 and 400 mg/kg produced plasma glucose levels significantly lower than those of the normal group at 30, 60, 90, 120 and 150 min after the glucose administration. The area under the curve (AUC) during the OGT test was significantly decreased while Glibenclamide (5 mg/kg) produced a significant reduction in the plasma glucose level at 30, 60, 90, 120, 150 and 180 min after oral glucose administration. This indicated the hypoglycaemic activity of the extract.

In the present investigation hydroalcoholic extract of Momordica sahyadrica tuber demonstrated significant anti-diabetic activity. The results from the present study also indicate that Momordica sahyadrica tuber extract can reduce the levels of glucose, cholesterol, triglycerides, MDA levels and increased protein, SOD, Catalase levels confirming the possibility that the major function of the extract are on the protection of vital tissues such as liver and pancreas, thereby reducing the causation of diabetes in the experimental animals.

Studies have shown that in STZ-induced diabetic rats, insulin deficiency is associated with hypercholesterolemia and hypertriglyceridermia. Lipids play an important role in the pathogenesis of diabetes mellitus. Hyperlipidaemia is a recognized consequence of diabetes mellitus demonstrated by the elevated levels of tissue cholesterol, phospholipids and free fatty acids. Diabetes induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose tissue to cells due to the under utilization of glucose. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. On the other hand, glucagon, catecholamine and other hormones enhance lipolysis. The marked hyperlipidaemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease. The level of total serum cholesterol and triglycerides were actually raised in diabetic rats but were lowered significantly with Momordica sahyadrica tuber extract as shown in Table 5. It indicates that the extract of Momordica sahyadrica tuber is more useful in the treatment of diabetes as it has hypolipidemic effect since the diabetes always associated with the hyperlipidaemia. Moreover, its hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis which is usually associated with diabetes. Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease.

Protein levels were significantly decreased in diabetic group as compared to normal rats. Excessive breakdown of body protein in conjunction with either inadequate supply or defective utilization observed in uncontrolled diabetes. Treatment with hydroalcoholic extract of Momordica sahyadrica tuber restored protein level due to the hypoglycaemic status.

A significant loss of body weight was found in diabetic control rats. The loss of weight of diabetic rats as compared to that of the normal control rats could be due to emaciation of skeletal muscle, dehydration and catabolism of fats and proteins. Treatment with hydroalcoholic extract of Momordica sahyadrica tuber improved body weight in diabetic rats. Oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed in diabetes by glucose oxidation, protein glycation, and the subsequent degradation of glycated proteins. High levels of free radicals and the simultaneously declined antioxidant enzyme levels lead to cell damage, inactivation of enzymes and lipid peroxidation. Evidence also indicates that oxidative stress-activated signaling pathways mediate insulin resistance and β-cell dysfunction. These consequences of oxidative stress can promote the development of diabetes complications. Therefore, oxidative stress, antioxidant defense, cellular redox status should be regarded as the central cause in diabetes and its complications. Increased oxidative stress, defined as a persistent imbalance between the production of highly reactive molecular species (chiefly oxygen and nitrogen) and antioxidant defenses, is a widely accepted participant in the development and progression of diabetes and its complications. Diabetes is usually accompanied by increased production of free radicals or impaired
antioxidant defenses. Glucose oxidation is the main source of free radicals. Mechanisms by which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcriptional factors, Advanced Glycated End products (AGEs) and Protein Kinase C. There is a marked increase in the oxidative stress in DM as indicated by elevated concentrations of lipid peroxidation products such as thiobarbituric acid reactive substances (TBARs) in plasma.

Treatment with hydroalcoholic extract of *Momordica sahyadrica* tuber significantly reduced MDA levels and increased SOD and Catalase levels in the liver compared to diabetic control group as shown in Table 7. It indicates that the drug is having antioxidant properties and protects the vital organs.

Glucose-6-phosphatase catalyzes the final step in glucose production by the liver. Overproduction of glucose by the liver is the major cause of fasting hyperglycaemia in both insulin-dependent and non-insulin-dependent diabetes mellitus. 90% of partially pancreatectomized diabetic rats have a 5-fold increase in the messenger RNA and 3-4 fold increase in the protein level of the catalytic subunit of hepatic glucose-6-phosphatase. Prolonged hyperglycaemia may result in overproduction of glucose via increased expression of this messenger RNA.

Treatment with hydroalcoholic extract of *Momordica sahyadrica* tuber significantly reduced the levels of Glucose-6-phosphatase enzyme compared to that of diabetic control (Table 6) indicating the inhibition of hepatic gluconeogenesis in terms of prevention of proteolysis and lipolysis. Glucose-6-phosphatase dehydrogenase is an important regulator of pentose phosphate pathway for the maintenance of normal blood sugar level. This enzyme activity is diminished here in STZ-induced diabetic group as its activity is under insulin. After supplementation of extract there was a significant recovery of the enzyme activity in liver (Table 10). This may be another possible way for antidiabetogenic potency.

The histopathology study on pancreas (Fig. 1) showed presence of pancreatic lobules separated by fibrovascular septa. The pancreatic lobules consist of intact acinar cells and their intralobular ducts. Most of the lobules show light-staining islets of langerhans. In normal group, the center of islet cells consists of Beta-cells (75%), while the periphery comprises of Alpha-cells (20%). In case on diabetic control group, the number of islets appears quantitatively reduced in number compared to normal group. The center of islet cells consists of Beta-cells (35%), while the periphery comprises of Alpha-cells (60%). Inflammatory infiltration were observed between the islet cells.

The number of islets appears quantitatively increased in number in the Standard/ Glibenclamide Group compared to Diabetic Group. The center of islet cells consists of quantitative increase in Beta-cells (55%), while the periphery comprises of Alpha-cells (40%). Inflammatory infiltration were also observed between the islet cells. In MS Extract (200 mg/kg) anti-diabetic group, the number of islets appears quantitatively reduced in number compared to Standard Group, while increased compared to diabetic Group. Some of the beta cells showed degenerative changes. The center of islet cells consists of 45% Beta-cells, while the periphery comprises of 50% Alpha-cells. Inflammatory infiltrations were also observed between the islet cells.

In MS Extract (400 mg/kg) anti-diabetic group, the number of islets appears quantitatively reduced in number compared to Standard Group while increase in Beta-cells (55%) in the center of islet cells and Alpha-cells (40%) in the peripheral region compared to Diabetic Group. In case of MS Extract (200 mg/kg) Pancreatic β-cell Protective Group, the number of islets appears quantitatively increase in number compared to Standard Group. Some of the beta cells showed degenerative changes. The center of islet cells showed quantitative increase in Beta-cells (60%), while the periphery comprises of Alpha-cells (35%). The MS Extract (400 mg/kg) Pancreatic β-cell Protective Group showed the number of islets appears quantitatively increased in number compared to Standard as well as MS Extract (200 mg/kg) Pancreatic β-cell Protective Group. Some of the beta cells showed degenerative changes. The center of islet cells consists of 65% Beta-cells, while the periphery comprises of 30% Alpha-cells. Inflammatory infiltrations were also observed between the islet cells.

The histopathology study on liver parenchyma showed intact architecture (Fig. 2). The perivenular hepatocytes, perportal hepatocytes and midzonal hepatocytes appear unremarkable. The liver parenchyma and sinusoids appear unremarkable. Some of the sinusoids appear congested. While in case of Diabetic Control Group, the liver parenchyma showed partially distorted architecture. The perivenular hepatocytes, perportal hepatocytes and midzonal hepatocytes show abundant clear cytoplasm. Some of the central veins appear dilated and congested. The liver parenchyma shows inflammatory infiltration predominantly lymphocytes. The sinusoids appear unremarkable.

In the Standard/ Glibenclamide Group, liver parenchyma shows intact architecture. The perivenular hepatocytes and midzonal hepatocytes appear unremarkable. The perportal region at focal areas shows mononuclear inflammatory infiltrations. The liver parenchyma and sinusoids appear unremarkable. Section studied from the liver parenchyma in MS Extract (200 mg/kg) Anti-diabetic Group showed partially distorted architecture. The perivenular hepatocytes and midzonal hepatocytes appear unremarkable. The perportal region showed dense inflammatory infiltration. There are focal aggregates of inflammatory cells amidst these hepatocytes. Some of the central veins appear congested. Most of the sinusoids appear dilated. While MS Extract (400 mg/kg) Anti-diabetic Group, the perportal region shows mild inflammatory infiltration. There are scattered inflammatory cells amidst these hepatocytes. The central
veins appear unremarkable. Few of the sinusoids appear dilated. In case of MS Extract (200 mg/kg) Pancreatic β-cell Protective Group, section studied from the liver parenchyma shows intact architecture. The perivenular hepatocytes and midzonal hepatocytes appear unremarkable. The periportal region shows mild inflammatory infiltration. There are focal aggregates of inflammatory cells amidst these hepatocytes. Some of the central veins appear congested. Most of the sinusoids appear dilated.

The MS Extract (400 mg/kg) Pancreatic β-cell Protective Group showed intact architecture of the liver parenchyma. The perivenular hepatocytes, periportal hepatocytes and midzonal hepatocytes appeared unremarkable. Some of the central veins appear congested. Some of the sinusoids appeared dilated and congested.

This study indicates that Streptozotocin induced diabetes cause considerable damage of the pancreatic as well as liver tissue which might be due to DNA alkylation of the pancreatic as well as liver tissues. The Standard, MS Extract 200 and 400 mg/kg Anti-diabetic Group show that treatment on streptozotocin induced diabetic rats causes increase in β-cells compared to non treated diabetic rats indicating further damage of the cells were prevented on treatment. MS Extract Pancreatic β-cell Protective Group both 200 as well as 400 mg/kg showed Langerhans were restored to normalization. This phenomenon could lead to an increase in insulin synthesis and secretion thereby correcting the diabetic state. Similarly, MS Extract 400 mg/kg of Anti-diabetic Group, MS Extract Pancreatic β-cell Protective Group both 200 as well as 400 mg/kg showed prevention of further damage of the hepatocytes with less amount of inflammatory cells indicating its function on the protection of liver tissue.

4. CONCLUSION

The anti-hyperglycemic activity caused by Glibenclamide in streptozotocin induced diabetic rats is an indication of the presence of some beta cells, as Glibenclamide is known to stimulate insulin secretion or may be due to the improvement of carbohydrate metabolic enzymes such as Glucose-6-Phosphate Dehydrogenase which enhances glucose Oxidation or reduction of Gluconeogenesis by reducing the enzyme Glucose-6-Phosphatase or may be due to the hypolipopidaemic activity or improving antioxidant property or due to stimulatory action on secretion or possessing of an insulin like-effect. \textit{m. mauritiana} and \textit{Ziziphus jujuba} mucilages are versatile excipients for novel drug delivery systems and good replacement for synthetic polymers

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CONFLICTS OF INTEREST

The authors do not have any conflict of interest.

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