ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC EFFECTS OF DIAISHIS, A POLYHERBAL FORMULATION, IN STREPTOZOTOCIN-INDUCED DIABETIC MALE ALBINO RAT: AN APPROACH THROUGH TOXICITY STUDY

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ABSTRACT
To investigate the mode of antidiabetic and antihyperlipidemic activity of Diashis, in streptozotocin (STZ)-induced diabetic male albino rats. Hyperglycemia was induced by single intramuscular injection of STZ at the dose of 4 mg / 0.1 mL of citrate buffer (pH 4.5) / 100 g body weight. For acute toxicity evaluation of Diashis, the formulation was administered orally at the doses ranging from 2.5, 5, 10, 20, 40, 80, 160, 320 mg / 100 g body weight. Significant diminution in the activities of hepatic hexokinase and glucose-6-phosphate dehydrogenase along with elevation in glucose-6-phosphatase were noted in STZ-induced diabetic animals. Levels of fasting blood glucose (FBG), glycated hemoglobin (HbA1c), serum lipid profiles were elevated significantly (P<0.001) along with the diminution in serum insulin level in STZ-induced diabetic animals. Above mentioned parameters were recovered significantly (P<0.01) towards the control level after treatment of Diasih for 30 days at the dose of 5 mg / 0.5 ml distilled water / 100 g body weight / rat / day at fasting state to diabetic animals. In an acute toxicity study, there was no toxic symptom up to the dose level of 320 mg / 100 g body weight. From these results, it may be concluded that Diashis at the dose of 5 mg / 100 g body weight is safe for long term treatment for diabetic protection.
KEYWORDS: Diashis, Insulin, Lipid profile, Streptozotocin.

INTRODUCTION
Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with the long-term damage, dysfunction, and failure of various organs, especially eye, kidney, nerve, heart and blood vessel. The number of people affected with diabetes world wise is projected to be 366 million by the year 2030.\[1\] Several reports indicate that annual incidence rate of diabetes mellitus will increase worldwide in future, especially in India. Presently, there are more than 150 million people with diagnosed diabetes mellitus and another 314 million with impaired glucose tolerance, a pre-diabetic state.\[2\] It has been predicted that approximately 57 million Indians will be affected by diabetes mellitus in 2025.\[2\] Type-1 diabetes mellitus is a complex disease where carbohydrate and fat metabolism are impaired. Significant changes in lipid metabolism are also noted in diabetes. In diabetic rats, increased lipid peroxidation is also associated with hyperlipidemia. Liver and kidney participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. During diabetes, a profound alteration in the concentration and composition of serum lipid occurs. Though, insulin therapy is also used for the management of diabetes mellitus but there are several drawbacks like insulin resistance, anorexia nervosa, brain atrophy, fatty liver along with progressed atherosclerosis.\[3\]

Many traditional plants are used for the management of diabetes throughout the world. Plant drugs or polyherbal formulations are frequently considered to be less toxic and free from side effects than synthetic one.\[4\] Based on the WHO recommendations; hypoglycemic agents of plant origin used in traditional medicine are important. An global attempt has been taken to develop herbal traditional medicine in more effective manner for the treatment of metabolic disorders like diabetes, hypertension, gout etc.\[5\] Recent progress in herbal research focused that polyherbal formulation is the choice of medicine than monoherbal drug. Diashis is an antidiabetogenic polyherbal formulation. Its constituents are including Syzygium cumuni (L.) Skeels., Gymnema sylvestrae R. Br., Holarrhena antidysenterica (Roth) DC., Tinospora cordifolia (Thunb.) Miers., Pongamia pinnata (L.) Pierre., Asphaltum, Psoralea corylifolia (L.) and Momordica charantica Descourt. S. cumuni (L.) Skeels has been shown to have hypoglycemic action noted in our earlier report.\[6\] Gynemic acid from G. sylvestrae R. Br has
proven efficacy in adrenaline and growth hormone induced hyperglycemia.\textsuperscript{[7]} *H. antiquus* (Roth) DC also showed antidiabetic activity reported in our previous findings.\textsuperscript{[8]} *T. cordifolia* (Thunb.) Miers inhibits adrenaline induced hepatic glucose release.\textsuperscript{[9]} *P. pinnata* (L.) Pierre also has antidiabetic and antioxidative activities.\textsuperscript{[10]} *Asphaltum* has pancreateotrophic action and promotes weight gain. *P. corylifolia* (L.) antidiabetic and antioxidative activities.\textsuperscript{[11]} *M. charantica* Descourt potentiates tolbutamide action and promotes peripheral glucose utilization.\textsuperscript{[13]} In our state West Bengal (India), this polyherbal formulation Diashis used in commonly Ayurvedic medicine for the treatment of diabetes. Though, it is used for the recovery of diabetes but the scientific basis of its antidiabetic mode of action was not investigated. Whether this polyherbal formulation has any general toxicity induction is also another field of investigation in this work.

**MATERIALS AND METHODS**

**Preparation of polyherbal formulation Diashis**

Eight medicinal plants used for the preparation of polyherbal formulation Diashis, have been provided by Pharmaceutical Division of Southern Health Improvement Samity (SHIS), 24-Parganas (S), West Bengal, India. These plants were confirmed by the Botany Department in Vidyasagar University. Herbal specimens were preserved in the Departmental Herbarium Museum as SC-Bio-Med-1/9, GS-Bio-Med-2/9, HA- Bio-Med-3/9, TC-Bio-Med-4/9, PP- Bio-Med-5/9, As-Bio-Med-6/9, PC-Bio-Med-7/9, and MC-Bio-Med-8/9.

Desired parts of the plants (Table 1) were dried in an incubator for 24 h at 37\textdegree C, crushed separately in an electrical grinder and the pulverized. Powder forms of the parts of the plants were mixed in fixed ratio as per Table 1 and named as Diashis. Polyherbal formulation of Diashis has been prepared on the basis of an Ayurvedic antidiabetic formulation proposed by some workers.\textsuperscript{[14]}

**Table 1: Composition of ingredients present in polyherbal formulation, Diashis**

<table>
<thead>
<tr>
<th>Botanical name (Family)</th>
<th>Common name</th>
<th>Part used</th>
<th>Ingredients of Diashis (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syzygium cumuni (L.) Skeels. (Myrtaceae)</td>
<td>Jaam</td>
<td>Seed</td>
<td>50</td>
</tr>
<tr>
<td>Gymnema sylvestrae R. Br. (Asclepiadaceae)</td>
<td>Meshasringi</td>
<td>Leaves</td>
<td>75</td>
</tr>
<tr>
<td>Holarrhena antidisenterica (Roth) DC. (Apocynaceae)</td>
<td>Indrayab</td>
<td>Seed</td>
<td>50</td>
</tr>
</tbody>
</table>
Diashis administration

Diashis powder was suspended in distilled water and animals were subjected for the oral treatment of the suspension by gavage method at the dose of 5 mg / 0.5 ml distilled water / 100 g body weight / rat / day at fasting state. This dose was selected from our pilot study using doses starting from 2 mg up to 20 mg / 100 g body weight where the above dose (5 mg / 100 g body weight) was noted as the threshold dose. In traditional medicine, the dose of Diashis given to the human at the dose of 2-3 mg / 100 gm body weight.

Selection of animals

Antidiabetic study was conducted on thirty six matured Wistar strain male albino rats, weighing about (150 ± 10) g. In acute toxicity study, 48 Wistar male albino rats were selected. Animals were acclimated for a period of fifteen days in our laboratory conditions prior to the experiment. Rats were housed in tarsons cages (six rats per cage), at an ambient temperature of (25 ± 2) °C with 12 h light: 12 h dark cycle. Rats have free access to standard food and water ad libitum. The principles of Laboratory Animal Care and instructions given by our Institutional Ethical Committee were followed regarding injection and other treatment of the experiment.[15]

Chemicals

Streptozotocin (STZ) was purchased from Sigma–Aldrich Diagnostic Ltd. USA. D-glucose-6-phosphate (Barium salt), NADP and maleic acid were purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. HEPES buffer and ATP were purchased from Himedia Laboratories Pvt. Ltd. Mumbai, India. Kits for the assessment of lipid profile and the activities of transaminases were purchased from Span Diagnostic Ltd. Surat, India.
Induction of diabetes mellitus

Diabetic model animals were prepared by our standard method as mentioned earlier by intramuscular injection of STZ. Single dose of STZ was injected at the dose of 4 mg / 0.1 ml of citrate buffer (pH 4.5) / 100 g body weight / rat to 24 h fasting rats for the development of type-1 diabetes following the method reported previously.[16] Eighteen rats with stable diabetes having fasting blood glucose level more than 250 mg / dl for seven successive days after STZ injection were selected as moderate diabetic in this experiment.

Experimental design

Wistar male albino rats weighing about (150 ± 10) g were divided into following six equal groups (n=6) for the assessment of antidiabetic and antihyperlipidemic effect of Diashis and the duration of experiment was of 38 days. After confirmation of diabetes (7 days stay after STZ injection), 30 days treatment of Diashis at the said dose (5 mg / 100 g body weight) was conducted which was established as threshold duration of treatment in this concern from duration dependent study in our pilot experiment.

**Group I** (Untreated Control) received 0.5 ml of distilled water / 100 g body weight / rat / day by per oral (p.o).

**Group II** (Diabetic Control) were made diabetic as mentioned before. Six diabetic rats were included here and 0.5 ml distilled water was provided by per oral (p.o) / 100 g body weight / rat / day.

**Group III** (Diabetic + Diashis) diabetic rats were per oral (p.o). treatment of polyherbal formulation i.e. Diashis at a dose of 5 mg / 0.5 ml of distilled water/ 100 g body weight / rat / day.

**Group IV** (Diabetic + Glibenclamide) diabetic rats given aqueous solution of Glibenclamide using forceful gavage at dose of 2 mg / 0.5 ml distilled water / 100 g body weight / rat / day.

**Group V** (Diashis + Normoglycaemic) rats were forcefully feed by gavage of Diashis at a dose of 5 mg / 0.5 ml of distilled water / 100 g body weight / rat / day.

**Group VI** (Glibenclamide + Normoglycaemic) rats were forcefully feed by gavage of Glibenclamide at a dose of 2 mg / 0.5 ml of distilled water / 100 g body weight / rat / day.

Starting from 1st day of Diashis treatment to diabetic rats, fasting blood glucose (FBG) level in all the groups was measured by single touch glucometer in every ten days interval. On the 31st day of experiment (38th day from the day of STZ injection), all the animals were sacrificed by light ether anaesthesia followed by decapitation after recording the final body
weight. Blood was collected from dorsal aorta. Serum was separated by centrifugation of collected blood at 3000 g for 5 min for the estimation of serum lipid profiles, insulin and transaminase activity. Remaining blood was also used for the quantification of glycated hemoglobin (HbA1c). Liver were dissected out and stored at -20 °C for the assessment of activities of key carbohydrate metabolic enzymes i.e. hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase.

**Measurement of fasting blood glucose (FBG) level**

Blood was collected from the tip of the tail vein or by orbital puncture alternatively and FBG levels were measured in every ten days after treatment of Diashis interval by single touch glucometer.

**HbA1c level**

HbA1c level was measured after washing packed cell by normal saline for six times. The hemolysate was prepared from the packed cell. Then 2 ml of 10 mg/dl hemoglobin containing hemolysate was taken and 1.0 ml of 0.3 N oxalic acid was added to it and mixed. The mixture was mixed with 1ml of 40% TCA and incubated for a fixed period at 37 °C. Reading was noted against blank at 443 nm and level of glycated hemoglobin was expressed as GHb%.\(^{[17]}\)

**Biochemical assay of carbohydrate metabolic enzymes activities in hepatic tissue**

Hexokinase activity in hepatic tissue was determined spectrophotometrically using assay mixture of 0.9 ml, 0.03 ml ATP (0.22 M) and 0.1 ml of tissue supernatant in cuvette. The absorbance was noted at 340 nm. One unit of hexokinase was expressed as µg/mg of tissue.\(^{[18]}\) Liver glucose-6-phosphate dehydrogenase activity was measured by using glucose-6-phosphate as a substrate and absorbance was measured at 340 nm. One unit of enzyme activity is the quantity which catalyses the reduction of 1 µM of NADP per min.\(^{[19]}\) The hepatic glucose-6-phosphatase activity was measured by recording the optical density at 340 nm according to standard protocol and slightly after modification by us. Enzyme activity was expressed as mg of inorganic phosphate liberated per gm of tissue.\(^{[20]}\)

**Lipid profile assessment**

Serum total cholesterol (TC) was quantified by the addition of enzyme present in reagent kit followed by optical density recording at 505 nm in spectrophotometer.\(^{[21]}\) Serum triglyceride (TG) was determined by noting the absorbance at 520 nm.\(^{[22]}\) Serum low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) levels were
measured biochemically. High density lipoprotein cholesterol (HDLc) level was measured by using kit.

**Serum insulin level**

Serum insulin was measured by enzyme linked immunosorbant assay (ELISA) using the kit (Bochringer Mannheim Diagnostik, Mannheim, Germany). The intra assay variation was 4.9 %. As the sample were run at a time, so there is no inter assay variation. The insulin level in serum was expressed in µ IU / ml.

**Serum transaminase assessment**

The activities of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) in serum were measured by specific kits. Activities of these enzymes were expressed as IU/L of serum.

**Acute toxicity study of Diashis**

The acute toxicity study (LD$_{50}$ determination) was carried out by the method described by Mythilpriya et al., in which healthy forty eight Wistar albino male rats weighing about 150 ± 10 g were randomly distributed to 8 different groups with six animals in each group. The animals were fasted overnight and the Diashis was administered orally at the dose levels of 2.5, 5, 10, 20, 40, 80, 160, 320 mg / 100 g body weight / day. After the treatment of Diashis, the animals in each group were observed for awareness, interactivity, posture, tremors, salivation, diarrhea, lethargy, sleep, coma and death as well as continuous observation for the first four hours up to 14 days to find out the mortality if any.

**Statistical analysis**

Analysis of Variance (ANOVA) followed by multiple comparison two-tail ‘t’ test was used for statistical analysis of collected data. Differences were considered significant at the levels of P<0.05, P<0.01 and P<0.001. All the values were indicated in the tables and figures by Mean ± SEM.

**RESULTS**

**Body weight**

Body weight was decreased significantly (P<0.001) in STZ-induced diabetic control animals in respect to untreated control group. Treatment of Diashis or Glibenclamide to diabetic animals resulted a significant recovery (P<0.01) of body weight towards the control level.
though there was an insignificant difference (P>0.05) of body weight in between Diashis treated diabetic and Glibenclamide treated diabetic groups. Diashis treatment to normoglycaemic animal resulted a significant elevation in body weight (P<0.05), but Glibenclamide treatment to normoglycaemic animal did not change the body weight significantly in respect to the untreated control group (Table 2).

Glycated hemoglobin (HbA\textsubscript{1c}) level
Glycated hemoglobin (HbA\textsubscript{1c}) level was increased significantly (P<0.001) in STZ-induced diabetic control group in respect to the untreated control group. Treatment of ‘Diashis,’ polyherbal formulation, or Glibenclamide to diabetic group, resulted in a significant recovery (P<0.01) in the level of this parameter towards the control. Insignificant (P>0.05) variation in the level of this parameter was noted in between Glibenclamide, and Diashis treated diabetic groups. Treatment of Diashis or Glibenclamide to normoglycaemic group resulted an insignificant variation in the level of this parameter (P>0.05) in respect to the untreated control group as well as in between the Diashis and Glibenclamide treated normoglycaemic group (Table 2).

Serum insulin level
Serum insulin level was decreased significantly in STZ-induced diabetic control animals in respect to the untreated control animals. Treatment of Diashis, or Glibenclamide to diabetic group, resulted a significant recovery (P<0.01) in the level of this parameter towards the control. Insignificant variation in the level of this parameter was noted in between Glibenclamide, and Diashis treated diabetic groups. Treatment of Diashis or Glibenclamide to normoglycaemic animals resulted an insignificant variation in the level of this parameter in respect to the untreated control group as well as in between the Diashis and Glibenclamide treated normoglycaemic group (Table 2).

Fasting blood glucose (FBG) level
Significant (P<0.001) elevation in fasting blood glucose (FBG) level in STZ-induced diabetic control rat was noted in respect to the untreated control group. Treatment of Diashis to diabetic rats for 21 days resulted a significant (P<0.01) recovery of FBG level towards the control level. Glibenclamide treatment to diabetic rats resulted resettlement of this parameter to the control level. Diashis or Glibenclamide treatment to normoglycaemic animals resulted an insignificantly variation (P>0.05) of FBG levels in respect to the untreated control group (Table 3).
Carbohydrate metabolic enzymes activities in hepatic tissue

Activities of hepatic hexokinase and glucose-6- phosphate dehydrogenase were decreased significantly (P<0.001) in STZ-induced diabetic control rats in respect to the untreated control group. In contrast, the activity of glucose-6-phosphatase was increased significantly (P<0.001) in STZ-induced diabetic group compare to the untreated control group. Treatment of Diashis or Glibenclamide to diabetic animals resulted a significant (P<0.01) protection and the activities of these enzymes were resettled towards the control level. Insignificant variation in the activities of these enzymes was noted in between Diashis or Glibenclamide treated diabetic animals. Treatment of Diashis or Glibenclamide to normoglycaemic group resulted an insignificant (P>0.05) variation in the activities of these enzymes in respect to the untreated control group (Fig. 1).

Serum lipid profiles

Serum TC and TG levels were increased significantly (P<0.001) in STZ- induced diabetic control rats in respect to the untreated control group. Diashis or Glibenclamide treatment to diabetic group resulted a significant recovery (P<0.01) in the levels of serum TC and TG towards the control level where insignificant (P>0.05) variation was noted in between of these two groups. An insignificant (P>0.05) variation in the levels of said biosensors were noted when comparison was made between the Diashis or Glibenclamide treated normoglycaemic group and the untreated control group (Table 4). Serum LDLc and VLDLc levels were increased significantly (P<0.001) in STZ-induced diabetic group in respect to the untreated control group. Treatment of this polyherbal formulation Diashis or Glibenclamide to diabetic rats resulted a significant recovery (P<0.01) in the levels of LDLc and VLDLc towards the control level. There was no significant variation in the levels of these biosensors when the comparison was made between Diashis treated diabetic and Glibenclamide treated diabetic groups. Diashis or Glibenclamide treatment to normoglycaemic rats resulted an insignificant difference in the level of this biosensors (P>0.05) in respect to the untreated control group (Table 4). Serum HDLc level was decreased significantly (P<0.001) in diabetic group when compare to the untreated control group. The level of this parameter was recovered significantly (P<0.01) towards the control level after treatment of Diashis or Glibenclamide to diabetic rats. Serum HDLc was increased significantly in Diashis treated normoglycaemic group in respect to the untreated control group though Glibenclamide treatment has no significant effect on the level of these biosensors in respect to the untreated control group (Table 4).
Activities of serum GOT and GPT
Activities of SGOT and SGPT were increased significantly (P<0.001) in diabetic group compare to the untreated control group. Treatment of Diashis or Glibenclamide to the diabetic animals resulted a significant recovery (P<0.01) in the activities of these two enzymes towards the control. Insignificant variation (P>0.05) was noted in the levels of these parameters when comparison was made between Diashis treated and Glibenclamide treated diabetic groups. Treatment of Diashis or Glibenclamide to normoglycaemic groups resulted an insignificant (P>0.05) variation in the levels of these sensors in respect to the untreated control group (Fig. 2).

Acute toxicity study of Diashis
In the acute toxicity study, Diashis up to the dose level of 320 mg / 100 g of body weight did not exhibit any lethality or toxic symptoms. There was no mortality or morbidity observed in animals through 14-day’s period following single oral administration at all selected dose levels of Diashis. No tremors, salivation, diarrhea, sleep, coma, death or unusual behaviors such as self walking backward, reactivity to handling were all normal. The lethal dose 50 (LD50) value for oral administration of Diashis is larger than 320 mg / 100 g body weight. As 5 mg / 100 g body weight was well tolerated by the animals without any behavioral changes during long term treatment (Table 5).

Table 2: Effect of Diashis, polyherbal formulation, on body weight, glycated hemoglobin and serum insulin levels in streptozotocin-induced diabetic male albino rat.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (gm)</th>
<th>Glycated hemoglobin (GHB %)</th>
<th>Serum insulin (µ IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>Untreated Control</td>
<td>155.72±5.21</td>
<td>157.03±4.42</td>
<td>2.75±0.09</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>156.34±4.74</td>
<td>138.52±3.05b</td>
<td>4.76±0.21a</td>
</tr>
<tr>
<td>Diabetic + Diashis</td>
<td>153.57±4.15</td>
<td>149.01±4.33b</td>
<td>3.06±0.10b</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide</td>
<td>154.61±4.82</td>
<td>152.86±4.12b</td>
<td>2.93±0.12b</td>
</tr>
<tr>
<td>Diashis + Normoglycaemic</td>
<td>153.76±5.06</td>
<td>161.46±4.97c</td>
<td>2.72±0.07</td>
</tr>
<tr>
<td>Glibenclamide +Normoglycaemic</td>
<td>152.81±4.68</td>
<td>159.51±4.45</td>
<td>2.77±0.08</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM, n=6. ANOVA followed by multiple comparison two tail’ t’ test. Values with different superscripts (a, b) differ from each other significantly (a indicates P<0.001, b indicates P<0.01 and c indicates P<0.05).
Table 3: Corrective effect of Diashis on fasting blood glucose (FBG) level in streptozotocin-induced diabetic male albino rat.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting blood glucose level (mg/dl)</th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>1st day</td>
<td>7th day</td>
<td>17th day</td>
<td>27th day</td>
<td>37th day</td>
<td></td>
</tr>
<tr>
<td>Untreated Control</td>
<td>74.2±4.2</td>
<td>73.21±4.5</td>
<td>72.86±4.9</td>
<td>75.51±4.6</td>
<td>73.47±4.5</td>
<td>75.1±4.4</td>
<td></td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>72.21±4.9</td>
<td>299.7±4.3</td>
<td>316.32±4.4</td>
<td>342.54±5.2</td>
<td>368.04±4.9</td>
<td>382.52±5.6</td>
<td>92.02±3.7</td>
</tr>
<tr>
<td>Diabetic + Diashis</td>
<td>73.61±5.1</td>
<td>298.52±4.5</td>
<td>271.31±4.8</td>
<td>191.67±4.2</td>
<td>120.81±5.3</td>
<td>98.7±4.3</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Glibenclamide</td>
<td>75.03±5.2</td>
<td>296.64±5.1</td>
<td>283.3±4.5</td>
<td>172.57±3.9</td>
<td>75.53±5.2</td>
<td>8.9±4.5</td>
<td></td>
</tr>
<tr>
<td>Diashis + Normoglycaemic</td>
<td>73.75±4.96</td>
<td>71.68±4.25</td>
<td>74.35±5.13</td>
<td>73.69±5.08</td>
<td>74.58±4.82</td>
<td>77.34±4.96</td>
<td></td>
</tr>
<tr>
<td>Glibenclamide + Normoglycaemic</td>
<td>74.38±5.03</td>
<td>73.72±5.07</td>
<td>72.77±4.63</td>
<td>75.04±4.98</td>
<td>74.58±4.82</td>
<td>77.34±4.96</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM, n=6. ANOVA followed by multiple comparison two tail ‘t’ test. Values with different superscripts (a, b) differ from each other significantly (a indicates P<0.001 and b indicates P<0.01).

Table 4: Effect of Diashis on serum lipid profiles in streptozotocin-induced diabetic male albino rat.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum lipid profiles (mg/dl)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TC</td>
<td>TG</td>
<td>HDL&lt;sub&gt;c&lt;/sub&gt;</td>
<td>LDL&lt;sub&gt;c&lt;/sub&gt;</td>
<td>VLDL&lt;sub&gt;c&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Untreated Control</td>
<td>73±3.12</td>
<td>88±3.75</td>
<td>25±2.71</td>
<td>30±2.78</td>
<td>17±1.26</td>
<td></td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>121±4.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>159±6.14</td>
<td>19±1.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69±4.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31±2.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Diashis</td>
<td>85±3.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>107±5.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22±2.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41±3.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21±1.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Diabetic + Glibenclamide</td>
<td>83±3.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>109±5.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21±2.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43±3.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21±1.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Diashis + Normoglycaemic</td>
<td>74±3.62</td>
<td>86±4.47</td>
<td>29±2.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28±2.44</td>
<td>17±1.62</td>
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</tr>
<tr>
<td>Glibenclamide + Normoglycaemic</td>
<td>72±3.43</td>
<td>89±4.06</td>
<td>24±2.08</td>
<td>30±2.29</td>
<td>18±1.73</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM, n=6. ANOVA followed by multiple comparison two tail ‘t’ test. Values with different superscripts (a, b) differ from each other significantly (a indicates P<0.001, b indicates P<0.01 and c indicates P<0.05).

TC: total cholesterol; TG: triglyceride; HDL<sub>c</sub>: high density lipoprotein cholesterol; LDL<sub>c</sub>: low density lipoprotein cholesterol; VLDL<sub>c</sub>: very low density lipoprotein cholesterol.

Table 5: Determination of median lethal dose (MLD) (LD<sub>50</sub>) for orally administered Diashis

<table>
<thead>
<tr>
<th>Dose of Diashis (mg / 100 g)</th>
<th>Number of animals used</th>
<th>Number of survival</th>
<th>Number of death</th>
<th>MLD (LD&lt;sub&gt;50&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>&gt; 320 mg / 100 g body weight</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>160</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>320</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a safe dose in human. World Health Organization survey indicated that about 70-80
% of the world’s populations rely on non-conventional medicines, mainly of herbal source, for their primary health care.\textsuperscript{30} Present study was conducted to find out the mechanism of antihyperglycemic and antihyperlipidemic effects of Diashis, a polyherbal formulation, in STZ-induced diabetic rats along with its acute toxicity study. The effects were compared with the standard antidiabetic drug i.e. Glibenclamide. STZ-induced diabetic rat is one of the animal models of type-1 diabetes mellitus as used by us in our previous studies.\textsuperscript{6} In this type, diabetes arises from irreversible destruction of \( \beta \) cells of islets of pancreas, causing degranulation or reduction of insulin secretion but not total absence of insulin in blood.\textsuperscript{31}

In the present study, STZ-induced diabetic animals showed elevation in the levels of FBG and HbA\(_1\)c which may be due to low level of plasma insulin this consistent with the results other as well as our previous study.\textsuperscript{32, 16} Treatment of Diashis (5 mg / 0.5 ml of distilled water/100 gm body weight/rat/day) to STZ-induced diabetic rat for 21 days resulted a significant protective effect on the levels of FBG and HbA\(_1\)c. Recovery in the levels of said biosensors may be due to high glucose utilization in peripheral tissues by increased secretion of insulin.\textsuperscript{33}

Key carbohydrate metabolic enzymes i.e. hexokinase, glucose-6-phosphatase and glucose-6-phosphate dehydrogenase are insulin dependent.\textsuperscript{34} Elevation in the activity of glucose-6-phosphatase in STZ-induced diabetic rat as reported here also supported by other workers, as well as by our previous report.\textsuperscript{35, 12} Treatment of Diashis or Glibenclamide to diabetic animals resulted a significant recovery (P<0.01) in the activity of this enzyme in hepatic tissue that may be another possible way of its antidiabetogenic activity. Similarly the activities of hepatic hexokinase and glucose-6-phosphate dehydrogenase enzymes were recovered towards the control after treatment of Diashis or Glibenclamide to STZ-induced diabetic animals. Insignificant variation (P>0.05) was noted in the activities of the said enzyme, and level of HbA1c after treatment of Diashis or Glibenclamide to the normoglycaemic group in respect to the untreated control group. This result focused that Diashis has no direct inhibitory effect on FBG when insulin level is within the normal range and so, the phyto-ingredients present in Diashis mainly exert their effects through insulin.

Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Insulin is a potent inhibitor of lipolysis. Since it inhibits the activity of the hormone sensitive lipases in adipose tissue and serum and also suppresses the release of free fatty acids, therefore, during diabetes, enhanced activity of this enzyme may increases
the lipolysis process and release more free fatty acids into the circulation. In normal condition, insulin increases the receptor-mediated removal of LDL-cholesterol, and decreased activity of insulin during diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats. Serum TC and TG levels were decreased significantly after treatment of polyherbal formulation Diashis or Glibenclamide to STZ-induced diabetic animals. But treatment of Diashis or Glibenclamide to normoglycaemic animals resulted insignificant variation in the levels of said biosensors in respect to untreated control group. These effects may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis which are under the control of insulin. Treatment of this polyherbal formulation Diashis to STZ-induced diabetic animal also results the significant attenuation in the levels of LDLc and HDLc in serum towards the control level which again strengthen the antihyperlipidemic effect of Diashis. As Diashis has no hypoglycaemic and hypolipidemic effects in normoglycaemic condition so it focus that phyto-ingredients which are present in Diashis can able to exert their effect through recovery of insulin in diabetic state with out exerting any direct effect on such disorders management. This polyherbal formulation has no general toxic effect as body weight remains in positive direction in Diashis treated diabetic groups like untreated control group. This was also supported by the effect of Diashis on body weight gain in normoglycaemic rat. Moreover, insignificant variation in the activities of serum GOT and GPT in Diashis treated normoglycaemic rat as well as recovery of GOT and GPT activities in serum after treatment of Diashis in diabetic rat also suggest its non-toxic effect in general. Animals in all groups did not exhibit any sign of adverse effect up to the dose level of 320 mg / 100 g body weight in acute toxicity study. According to Organization for Economic Cooperation and Development guidelines for acute oral toxicity, a lethal dose 50 (LD$_{50}$) dose of 200 mg / 100 g and above is categorized as unclassified and hence the formulation is found to be safe.

**CONCLUSION**

The present study shows the possible ways of hypoglycemic and hypolipidemic effect of Diashis in diabetic model animals. The active ingredients present here that may recover the diabetes complications by protecting the serum lipid profiles, and or by stimulating the carbohydrate regulatory enzymes activities in target organ. This may be due to the stimulating and regenerating efficacy of Diashis on β-cells in pancreas along with the increased insulin secretion. The actual mechanism is not clear and further preclinical and pharmacological investigations are needed to isolate and identify the active ingredients.
present in the Diashis responsible in this concern. Overall, this study provides valuable data on hypoglycemic and hypolipidemic with toxicity profile of Diashis that should be useful for the planning of future preclinical and clinical studies of the formulation.

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