

## Attenuation of Hyperglycemia and Hyperlipidemia in Streptozotocin-Induced Diabetic Rats by Aqueous Extract of Seed of *Tamarindus indica*

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Streptozotocin (STZ)-induced diabetic rats were divided into mild diabetic (MD) and severe diabetic (SD) on the basis of fasting blood glucose (FBG) levels. Diabetes was confirmed here by intravenous glucose tolerance test (GTT), biochemical assay of glycogen content in liver and skeletal muscle, glucose-6-phosphatase activity in liver, and serum insulin levels. Hyperlipidemia developed in these experimental diabetic rats was assessed by quantification of total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc) and triglyceride (TG) in serum. Aqueous extract of seed of *Tamarindus indica* was given to MD and SD rats at the dose of 80 mg and 120 mg/0.5 ml distilled water/100 g body weight/d respectively for 14 d. Significant attenuation of hyperglycemia was indicated by measuring FBG, glycogen level and glucose-6-phosphatase activity along with monitoring of intravenous GTT and serum insulin level. Similarly, correction of hyperlipidemia in diabetic rats after this extract supplementation was confirmed by significant reduction in the levels of above-mentioned hyperlipidemic indicators. Intravenous GTT was performed that highlights the antidiabetic action of this extract is not due to its effect on the intestinal rate of glucose absorption but may be due to modulation of intracellular glucose utilization in target organs. This study focus the efficacy of this extract for the management of experimental diabetes in rat model which may shed some light on the scientific basis of ancient herbal therapy in this line using this seed.

**Key words** streptozotocin-induced diabetic rat; hyperglycemia; hyperlipidemia; *Tamarindus indica*

Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus, an endocrine disorder based disease. In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus.<sup>1)</sup> Though pharmaceutical drugs like sulfonylureas and biguanides are used for the treatment of diabetes but these are either too expensive or have undesirable side effects or contraindications.<sup>2,3)</sup> Insulin therapy affords effective glycemic control, yet its demerits are ineffectiveness through an oral administration, short half life, requirement of constant refrigeration of the drug and in the event of excess dosage results fatal hypoglycemia that limits its uses.

From various reasons, in recent years, traditional and complementary medicine have been an upsurge in its popularity for the treatment of different diseases as herbal drugs are generally out of toxic effect.<sup>4,5)</sup> Though isolated studies screened several plants having folk medicine reputation for antidiabetic potency<sup>6,7)</sup> but there was no scientific publication about the seed of *Tamarindus indica* (*T. indica*) except our publication<sup>8)</sup> where we focused the antidiabetic effect of this seed extract in type-I diabetes or insulin dependent diabetes mellitus (IDDM). There is no report of this seed extract on different types of diabetes mellitus like type-I or IDDM or severe diabetic (SD) where functional  $\beta$  cells are not present or are present in very few in number and type II or non insulin dependent diabetes mellitus (NIDDM) or mild diabetic (MD) where functional  $\beta$  cells are present in a remarkable number. Moreover, no work has been done about the antihyperlipidemic effect of the seed extract of *T. indica* in diabetic state.

*T. indica* LINN is used as traditional medicine for the management of diabetes mellitus.<sup>9)</sup> This plant is tree type, dicotyledonous, found all over India and belonging to Caesalpiniaceae family.

The present aim of this work is to explore the scientific basis of the utility of this aqueous seed extract of *T. indica* for correction of hyperglycemia and hyperlipidemia in diabetes. Moreover, mode of action of this extract for its antidiabetic activity is another part of searching that has been performed here by conducting intravenous glucose tolerance test (GTT), serum insulin assay and by measurement of carbohydrate metabolic enzyme activity that collectively focus the pancreatic and extra pancreatic activity of this extract.

### MATERIAL AND METHODS

**Plant Material** Seeds of *T. indica* were collected from Badhutola, Paschim Medinipur district in the month of May and the material was identified by taxonomist of Central National Herbarium (CAL), Botanical Survey of India (B.S.I.), Shibpur, Howrah. The voucher specimen was deposited in the Central National Herbarium (CAL), BSI, Shibpur, Howrah having the voucher specimen number HPCH No-1.

**Preparation of Aqueous Extract of Seed of *T. indica*** Aqueous extract of seed of *T. indica* was performed according to the method of National Institute of Health and Family Welfare (NIHFW), New Delhi, India.<sup>10)</sup> Fresh seeds of *T. indica* were dried in an incubator for 2 d at 40 °C, crushed in an electrical grinder and then powdered. Out of this powder, 100 g was suspended in 500 ml redistilled water and then extracted for 18 h in a soxhlet apparatus. A deep brown aqueous extract was obtained which was filtered by coarse sieve filter paper. The filtrate was dried at reduced pressure and finally lyophilized. It was stored at (0—4) °C until used. When needed, the residual extract was suspended in distilled water and used in the study.

**Selection of Animals and Animal Care** The study was conducted on forty matured Wistar strain male albino rats

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(Joy Tara Traders, Kolkata, India), 3 months of age weighing about  $138 \pm 5$  g. Animals were acclimated for a period of 15 d in our laboratory conditions prior to the experiment. Rats were housed in colony cages (4 rats per cage), at an ambient temperature of  $25 \pm 2^\circ\text{C}$  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 (NIH, 1985) were followed throughout the duration of experiment and instruction given by our institutional ethical committee was followed regarding injection and other treatment of the experiment. Normoglycemic animals were selected for this experiment having the fasting blood glucose level of  $75 \pm 5$  mg/dl.

**Chemicals** Streptozotocin (STZ) was obtained from Sigma Chemical Company (U.S.A.) and all other chemicals used in this experiment were of analytical grade. Insulin enzyme linked immunosorbent assay (ELISA) kit was purchased from Boehringer Mannheim Diagnostic, Mannheim, (Germany).

**Induction of Diabetes Mellitus** Rats were fasted for 24 h before the induction of diabetes by STZ injection. Experimentally induced MD and SD conditions were developed in a group of normoglycemic male Wistar strain albino rats by single intramuscular injection of STZ at the dose of 4 mg or 7 mg/0.5 ml of physiological saline/100 g body weight/rat respectively. This single dose of STZ produced SD or type-I (having fasting blood glucose level more than 250 mg/dl) and MD or type-II diabetes mellitus (having fasting blood glucose level more than 90 mg/dl but less than 250 mg/dl) after 24 h of STZ injection and this diabetic state was maintained throughout the experimental schedule.

**Experimental Design** Forty rats were divided into five equal groups as follows:

i) Control group: Rats of this group received single intramuscular injection of physiological saline (0.5 ml/100 g body weight/rat).

ii) Mild diabetic group: The rats were made diabetic by a single intramuscular injection of STZ (4 mg/0.5 ml physiological saline/100 g body weight/rat).

iii) Severe diabetic group: The rats were made diabetic by a single intramuscular injection of STZ (7 mg/0.5 ml physiological saline/100 g body weight/rat).

iv) Mild diabetic+*T. indica* supplemented group: The MD rats were forcefully fed aqueous extract of seed of *T. indica* at the dose of 80 mg/0.5 ml distilled water/100 g body weight/d/rat after 24 h of STZ injection for next 14 d at fasting state.

v) Severe diabetic+*T. indica* supplemented group: The SD rats were forcefully fed aqueous extract of seed of *T. indica* at the dose of 120 mg/0.5 ml distilled water/100 g body weight/d/rat after 24 h of STZ injection for 14 d at fasting state.

Animals of control group, MD and SD groups were subjected to forceful feeding of 0.5 ml distilled water/100 g body weight/d for 14 d to keep all the animals at same type of treatment condition in respect to *T. indica* supplemented groups. On starting day of extract supplementation to MD and SD rats, fasting blood glucose (FBG) was monitored of all the animals in each group.

On 15th day of experiment, 4 h before animal sacrifice, intravenous GTT was performed as per standard protocol on all the animals and they were sacrificed under light ether anes-

thesia. The guideline of our institutional ethical committee for this purpose was followed strictly. The rats were sacrificed by decapitation and blood was collected from dorsal aorta and serum was separated by centrifugation at 3000 *g* for 5 min and was kept at  $-20^\circ\text{C}$  for the biochemical assay of total cholesterol (TC), low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc), triglyceride (TG) and for serum insulin assay following ELISA technique.

Relevant organs like liver and skeletal muscle were dissected out and stored at  $-20^\circ\text{C}$  until organs from all the animals have been collected and were then used for biochemical assay of liver and muscle glycogen, and liver glucose-6-phosphatase activity.

**Testing of Fasting Blood Glucose Level (FBG)** At the time of grouping of the animals, FBG level was measured. After 14 d of aqueous extract of seed of *T. indica* supplement, FBG was further determined from the animals of all these groups. Blood was collected from tip of the tail vein and fasting blood glucose level was measured using single touch glucometer.<sup>11)</sup> The results were expressed in term of mg/dl of blood.

**Intravenous Glucose Tolerance Test (GTT)** After overnight fasting, on the day of animal sacrifice, a 0-min blood sample was taken from tip of the tail vein from all the rats in control, MD, SD, MD+*T. indica* and SD+*T. indica* supplemented groups. Glucose solution at the dose of (0.5 g/kg body weight/5 ml physiological saline) administered intravenously through femoral vein very slowly. Blood samples were taken from tail vein at 30th, 60th, 90th and 120th minute after glucose administration<sup>12)</sup> and glucose levels were assessed by single touch glucometer.<sup>11)</sup>

**Biochemical Assay of Glucose-6-phosphatase Activity** The liver glucose-6-phosphatase activity was measured according to the standard protocol.<sup>13)</sup> Tissue was homogenized in ice cold of 0.1 M phosphate buffer saline (pH 7.4) at the tissue concentration of 50 mg/ml. In a calibrated centrifuge tube, 0.1 ml of 0.1 M glucose-6-phosphate solution and 0.3 ml of 0.05 M maleic acid buffer (pH 6.5) were taken and bring to  $37^\circ\text{C}$  in water bath for 15 min. The reaction was stopped with 1 ml of 10% TCA followed by chilling in ice and centrifuged at 3000 *g* for 10 min. The optical density was taken at 340 nm. The enzyme activity was expressed as mg of inorganic phosphate liberated per g of tissue.

**Biochemical Assay of Glycogen Level** Glycogen contents in liver and skeletal muscle were measured according to the standard method.<sup>14)</sup> Liver and skeletal tissues were homogenized separately in hot 80% ethanol at the tissue concentration of 100 mg/ml and then centrifuged at 8000 *g* for 20 min. The residue was collected and allowed to dry over a water bath. To the residue, 5 ml of distilled water and 6 ml of 52% perchloric acid were added. The extraction was done at  $0^\circ\text{C}$  for 20 min. The collected material was centrifuged at 8000 *g* for 15 min and supernatant was collected. From supernatant, 0.2 ml was transferred in a graduated test tube and volume was made up to 1 ml by the addition of distilled water. Graded standards were prepared by using 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard solution and the volume of all these standards were made up to 1 ml by addition of distilled water. In all the test tubes, 4 ml of anthrone reagent was added. The test tubes were allowed to heat in

boiling water bath. Then these were allowed to cool at room temperature and the intensity of green to dark green colour of the solution was recorded at 630 nm. The amount of glycogen was measured from standard curve, prepared with standard glucose solution. The amount of glycogen in tissue sample was expressed in  $\mu\text{g}$  of glucose/mg of tissue.

**Serum Total Cholesterol** Serum TC was quantified by spectrophotometric method<sup>15)</sup> by the addition of enzymes present in reagent kit. The absorbance of red quinoneimine complex was determined at 505 nm. The value of TC present in serum was expressed in mg/dl.

**Serum Lipoprotein Cholesterol** Serum LDLc and VLDLc were measured according to the protocol of Friendwald *et al.*<sup>16)</sup> Other lipoprotein cholesterol *i.e.* HDLc was measured by the method of Burstein *et al.*<sup>17)</sup>

**Serum Triglyceride** By using kit,<sup>18)</sup> serum TG was measured noting the absorbance at 546 nm. The value was expressed in the unit of mg/dl.

**Serum Insulin** Serum insulin was measured by enzyme linked immunosorbent assay (ELISA) using the kit<sup>19)</sup> (Boehringer Mannheim Diagnostic, Mannheim, Germany). The intra assay variation was 4.9%. As the samples were run at a time, so there is no inter assay variation. The level of insulin in serum was expressed in  $\mu\text{IU/ml}$ .

**Statistical Analysis** One way ANOVA followed by a multiple two tail 't' test was used for the analysis of significant differences among the collected data.<sup>20)</sup> Differences were considered significant when  $p < 0.05$ .

## RESULTS

**Fasting Blood Glucose Level** There was significant elevation in FBG after 24 h of streptozotocin injection in respect to control group. Supplementation of aqueous extract of seed of *T. indica* to MD and SD rats for 14 d resulted a significant recovery of FBG level and resettled to the control level (Table 1).

**Intravenous Glucose Tolerance Test** The level of blood glucose in control, MD, SD, MD plus *T. indica*, and SD plus *T. indica* supplemented groups showed a significant change in blood glucose level after intravenous administration of glucose (0.5 g/kg body weight). Rats of both the diabetic groups showed a significant elevation in blood glucose level

Table 1. Effect of Aqueous Extract of Seed of *T. indica* after 14 d Treatment on Blood Glucose Level in STZ-Induced MD and SD Male Albino Rats

Group	Fasting blood glucose level (mg/dl)		
	At the time of grouping	Days of <i>T. indica</i> supplement	
		0 d	14 d
Control	82.0 $\pm$ 9.3 <sup>a</sup>	83.0 $\pm$ 8.5 <sup>a</sup>	84.6 $\pm$ 8.7 <sup>a</sup>
MD	79.4 $\pm$ 8.5 <sup>a</sup>	170 $\pm$ 7.9 <sup>b</sup>	166.4 $\pm$ 7.8 <sup>b</sup>
SD	81.6 $\pm$ 7.9 <sup>a</sup>	302.6 $\pm$ 9.5 <sup>c</sup>	289.4 $\pm$ 10.0 <sup>c</sup>
MD+ <i>T. indica</i> supplement (80 mg)	82.9 $\pm$ 8.1 <sup>a</sup>	176.6 $\pm$ 7.9 <sup>b</sup>	89.4 $\pm$ 7.6 <sup>a</sup>
SD+ <i>T. indica</i> supplement (120 mg)	83.2 $\pm$ 8.5 <sup>a</sup>	316.2 $\pm$ 8.9 <sup>c</sup>	90.4 $\pm$ 7.6 <sup>a</sup>

Each value represents mean $\pm$ S.E.M. ( $n=8$ ), ANOVA followed by multiple two-tail 't' test. In each vertical column, mean with different superscripts (a, b, c) differ from each other significantly,  $p < 0.05$ .

throughout the total measurement period *i.e.* for 120 min, in respect to control (Fig. 1), and at the end of the period, it did not come back to the initial value (0 min level). In both the supplemented groups, blood glucose level decreased significantly and from 60th minutes, this parameter was resettled to the control level (Fig. 1).

**Glycogen Level and Glucose-6-phosphatase Activity** Glycogen levels were significantly decreased in both the MD and SD rats in respect to control (Table 2) but after 14 d of aqueous extract of seed of *T. indica* supplementation to the MD and SD rats, there was a significant elevation in liver and

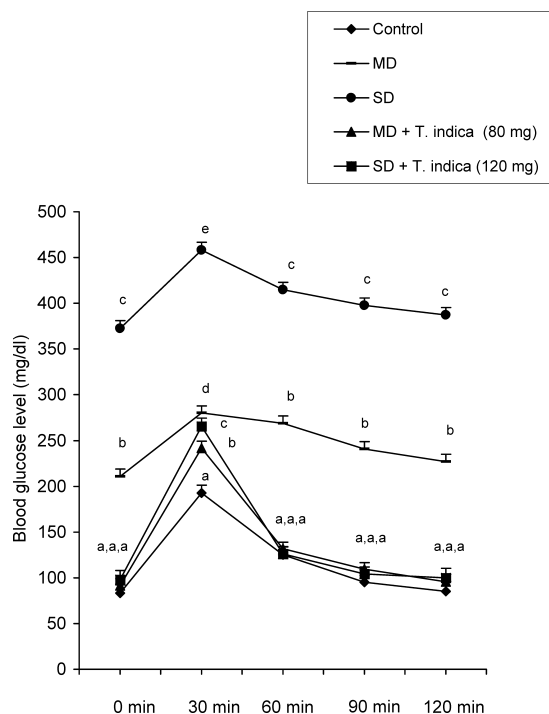


Fig. 1. Effect of Aqueous Extract of Seed of *T. indica* on Intravenous Glucose Tolerance Test in STZ-Induced MD and SD Male Albino Rats

Data are expressed as mean $\pm$ S.E.M.:  $n=8$ . ANOVA followed by multiple two tail 't' test. Lines with different superscripts (a, b, c, d, e) within fixed duration of measurement differ from each other significantly ( $p < 0.05$ ).

Table 2. Effect of Aqueous Extract of Seed of *T. indica* after 14 d Treatment on Glycogen Content in Liver and Skeletal Muscle and Activity of Liver Glucose-6-phosphatase in STZ-Induced MD and SD Male Albino Rats

Group	Glycogen ( $\mu\text{g}$ of glucose/mg of tissue)		Glucose-6-phosphatase activity (mg of IP/g of hepatic tissue)
	Liver	Skeletal muscle	Liver
Control	22.8 $\pm$ 0.8 <sup>a</sup>	22.2 $\pm$ 0.7 <sup>a</sup>	19.2 $\pm$ 1.1 <sup>a</sup>
MD	15.0 $\pm$ 0.7 <sup>b</sup>	13.6 $\pm$ 0.7 <sup>b</sup>	26.3 $\pm$ 1.0 <sup>b</sup>
SD	12.6 $\pm$ 0.8 <sup>c</sup>	11.0 $\pm$ 0.7 <sup>c</sup>	32.2 $\pm$ 1.2 <sup>c</sup>
MD+ <i>T. indica</i> supplement (80 mg)	22.7 $\pm$ 0.8 <sup>a</sup>	22.7 $\pm$ 0.8 <sup>a</sup>	18.9 $\pm$ 1.0 <sup>a</sup>
SD+ <i>T. indica</i> supplement (120 mg)	19.4 $\pm$ 0.7 <sup>d</sup>	18.8 $\pm$ 0.7 <sup>d</sup>	23.1 $\pm$ 1.0 <sup>d</sup>

Each value represents mean $\pm$ S.E.M. ( $n=8$ ), ANOVA followed by multiple two-tail 't' test. In each vertical column, mean with different superscripts (a, b, c, d) differ from each other significantly,  $p < 0.05$ .

skeletal muscle glycogen level in respect to diabetic groups and this parameter was resettled towards the control level (Table 2).

Glucose-6-phosphatase activity was increased significantly in both the MD and SD groups in respect to control (Table 2). After 14 d of aqueous extract of seed of *T. indica* supplementation to the MD and SD groups, there was a significant diminution in this parameter having a tendency to resettle towards the control level (Table 2).

**Serum Lipid Profile** Serum TC and TG levels were significantly elevated in both the diabetic groups in comparison to control (Fig. 2). Supplementation of this extract for 14 d to the MD and SD rats resulted a significant diminution of these parameters and the levels of these parameters were resettled

towards the control level (Fig. 2).

Other hyperlipidemic parameters like serum LDLc and VLDLc, both were elevated in both the diabetic groups in respect to control (Fig. 3). All these parameters were decreased significantly in the extract supplemented groups in respect to the corresponding diabetic groups (Fig. 3), and were resettled towards the control level (Fig. 3).

HDLc, a friendly lipoprotein, was decreased in both the diabetic groups in respect to the control (Fig. 3). After 14 d of aqueous extract of seed of *T. indica* supplementation, there was a significant elevation of this lipoprotein level in serum and was resettled to the control level (Fig. 3).

TC/HDLc and LDLc/HDLc ratios were significantly elevated in both the diabetic groups in respect to the control (Fig. 4) but supplementation of aqueous seed extract of *T. indica* to MD and SD rats for 14 d resulted a significant diminution of these ratios and were resettled towards the control level (Fig. 4).

**Serum Insulin Level** Serum insulin level was significantly decreased in MD and SD rats in respect to control as well as there was also a significant difference of this parame-

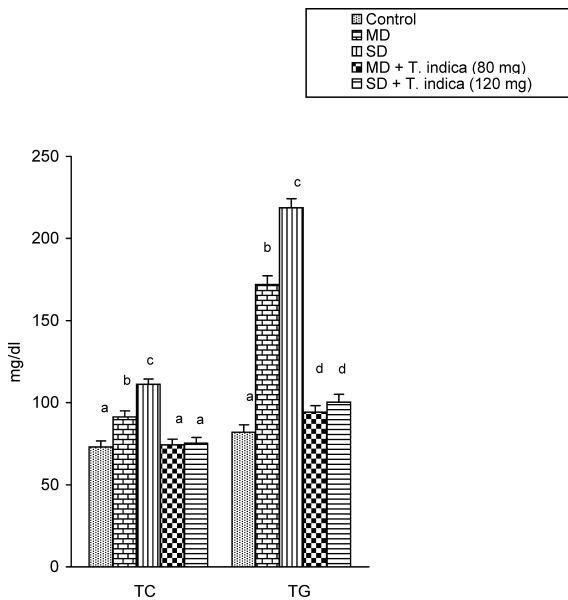


Fig. 2. Effect of Aqueous Extract of Seed of *T. indica* on Serum TC and Serum TG Levels in STZ-Induced MD and SD Male Albino Rats

Data are expressed as mean  $\pm$  S.E.M.:  $n=8$ . ANOVA followed by multiple two tail "t" test. Bars with different superscripts (a, b, c, d) differ from each other significantly ( $p<0.05$ ).

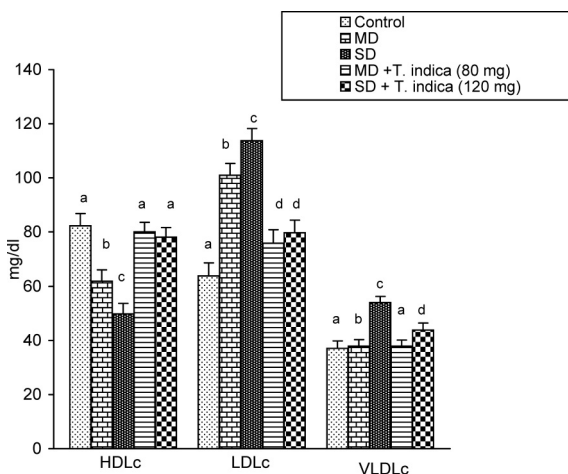


Fig. 3. Effect of Aqueous Extract of Seed of *T. indica* on Serum HDLc, LDLc and VLDLc Levels in STZ-Induced MD and SD Male Albino Rats

Data are expressed as mean  $\pm$  S.E.M.:  $n=8$ . ANOVA followed by multiple two tail "t" test. Bars with different superscripts (a, b, c, d) differ from each other significantly ( $p<0.05$ ).

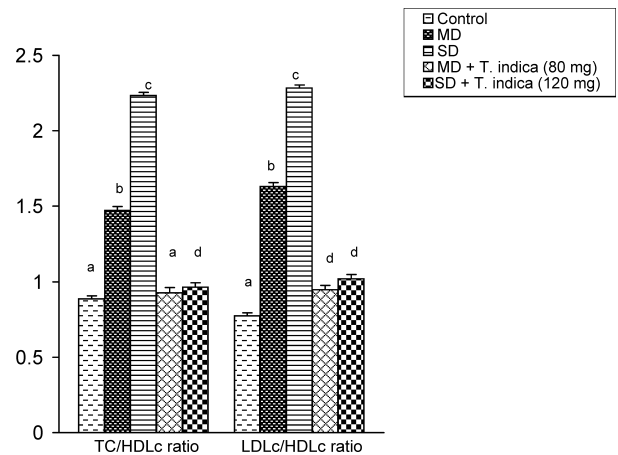


Fig. 4. Effect of Aqueous Extract of Seed of *T. indica* on Serum TC/HDLc and LDLc/HDLc Ratios in STZ-Induced MD and SD Male Albino Rats

Data are expressed as mean  $\pm$  S.E.M.:  $n=8$ . ANOVA followed by multiple two tail "t" test. Bars with different superscripts (a, b, c, d) differ from each other significantly ( $p<0.05$ ).

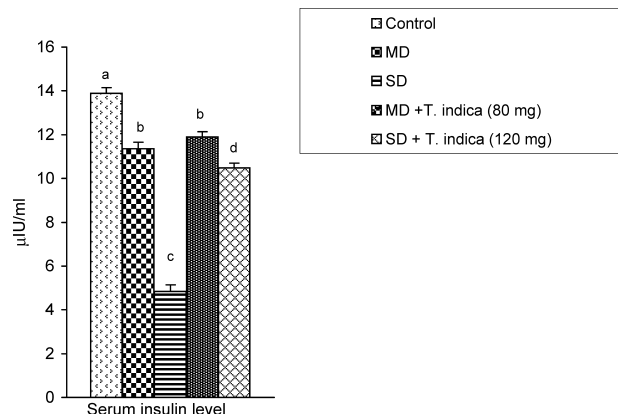


Fig. 5. Effect of Aqueous Extract of Seed of *T. indica* on Serum Insulin Level in STZ-Induced MD and SD Male Albino Rats

Data are expressed as mean  $\pm$  S.E.M.:  $n=8$ . ANOVA followed by multiple two tail "t" test. Bars with different superscripts (a, b, c, d) differ from each other significantly ( $p<0.05$ ).

ter between MD and SD groups (Fig. 5). After 14 d of aqueous extract of seed of *T. indica* supplementation to the SD rats, there was a significant elevation in serum insulin level in respect to SD group though the level of this hormone was significantly low than the control (Fig. 5). In MD rats this extract supplementation resulted an insignificant variation in serum insulin level in respect to only MD group (Fig. 5).

## DISCUSSION

It is generally accepted that SD is of IDDM type and MD is of NIDDM type.<sup>21)</sup> In this present investigation, the main focal points are the efficacy of aqueous extract of seed of *T. indica* for correction of SD or IDDM where  $\beta$  cell degeneration is dramatic in type and of MD or NIDDM where  $\beta$  cells are existed in pancreas which has been indicated here from the serum insulin assay in respect to SD rats. Antihyperglycemic potency of the aqueous extract of seed of *T. indica* in SD and MD rats has been indicated here by the study of FBG level as it is an important basal parameter for monitoring of diabetes and it also supports our previous work.<sup>8)</sup> Furthermore the attenuative effect of this extract on experimental SD and MD has been confirmed here by the study of glucose-6-phosphatase activity in liver and quantification of glycogen in liver and skeletal muscle which are the important indicators of diabetes mellitus.<sup>22)</sup>

Diabetes is also associated with hyperlipidemia.<sup>23)</sup> The serum TC and TG have been decreased significantly in SD and MD rats after the extract supplementation. 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.<sup>21)</sup> This extract supplementation also results the significant attenuation in the level of LDLc and HDLc in serum toward the control level which again strengthen the hypolipidemic effect of this extract. Moreover, LDLc/HDLc and TC/HDLc ratios are markers of dyslipidemia.<sup>24)</sup> Both this ratio have been increased in streptozotocin-induced SD and MD rats, and after the extract supplementation, these ratios resettled towards the control level.

The possible mechanism of antihyperglycemic action of this extract appears to be both pancreatic and extra pancreatic that has been supported here by the serum insulin assay in SD rats. The extra pancreatic effect of this extract has been focused here by the significant recovery of glucose-6-phosphatase activity in liver in MD rats in spite of failure of significant recovery of serum insulin in respect to only MD group. The extra pancreatic effect may be by the sensitization of insulin receptor in target organ or by inhibiting insulinase activity in both liver and kidney. Moreover, it also focuses the antidiabetic effect of this extract in MD rats where serum insulin level is higher than SD but lower than control. So this extract has some antihyperglycemic activity in NIDDM also. From intravenous glucose tolerance test it has been indicated that this extract did not executes the antihyperglycemic effect by the modulation of glucose absorption from the intestine. Another feature of this study is that the extract also corrects the dyslipidemia, which is noted in diabetic condition in sev-

eral cases. This hypolipidemic effect may be due to elevation in HDLc level as this is friendly cholesterol for the prevention of cardiovascular diseases. The actual ingredient(s) present in this extract for such correction is not delineated from this present study. Further investigations are in progress to elucidate the detailed mechanism of hypoglycemic and hypolipidemic effects in IDDM and NIDDM.

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