

# Protective effect of aqueous extract of seed of *Psoralea corylifolia* (Somraji) and seed of *Trigonella foenum-graecum* L. (Methi) in streptozotocin-induced diabetic rat: A comparative evaluation

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## ABSTRACT

**Background:** *Psoralea corylifolia* (Somraji) and *Trigonella foenum-graecum* L. (Methi), important medicinal plants widely used in India as folk medicine. Local people of West Bengal traditionally used the seeds of these plants to cure diabetes. **Objective:** Present study was designed to investigate the antidiabetic efficacy of aqueous extract of seeds of these plants in separate or in composite manner in streptozotocin (STZ)-induced diabetic rat. **Materials and Methods:** Diabetes was induced by intramuscular injection of STZ at the dose of 40 mg/ml of citrate buffer/kg body weight. Fasting blood glucose (FBG), glycolated hemoglobin (HbA<sub>1c</sub>) and activities of hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase of liver in experimental animals were assessed. Hyperlipidemic state developed in the experimental diabetic rat was assessed by measuring the levels of total cholesterol, triglyceride, and lipoproteins in serum. **Results:** There was significant increased in the levels of FBG, HbA<sub>1c</sub> and lipid profiles along with diminution ( $P < 0.001$ ) in the activities of hepatic hexokinase, glucose-6-phosphate dehydrogenase and elevation in glucose-6-phosphatase in diabetic control animals in respect to the untreated control. Significant recovery ( $P < 0.05$ ) in the activities of above mentioned enzymes along with the correction in the levels of FBG, HbA<sub>1c</sub> and serum lipid profiles were noted towards the control level after the treatment of composite extract (i.e. 100 mg of Somraji: 100 mg of Methi, total 200 mg/kg body weight) than the individual extract (i.e. 200 mg of Somraji or 200 mg of Methi, per kg body weight) treatment. **Conclusion:** Results suggest that composite extract of above plant parts has more potent antidiabetic efficacy than the individual extract.

**Key words:** Carbohydrate metabolic enzymes, glycogen, lipid profiles, streptozotocin

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## INTRODUCTION

Diabetes mellitus is a group of metabolic diseases 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 eyes, kidneys, nerves, heart and blood vessels. The number of people affected with diabetes worldwide is projected to

be 366 million by year 2030.<sup>[1]</sup> In diabetic rats, increased lipid peroxidation was also associated with hyperlipidemia. Liver and insulin dependent tissue that play a vital role in glucose and lipid homeostasis, and these are severely affected during diabetes.<sup>[2]</sup> Liver and kidney participates 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 lipids occurs. In modern medicine, no satisfactory effective therapy is still available to cure the diabetes mellitus. Though, insulin therapy is also used for the management of diabetes mellitus but there are several drawbacks like insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment.<sup>[3]</sup> Insulin also influence lipid metabolism including stimulation of synthesis

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of fatty acid as well as also develop atherosclerosis.<sup>[4]</sup> Atherosclerosis is accompanied by the production of free radicals by endothelial and vascular smooth muscles. Many traditional plants are used for the treatment of diabetes through out the world. Plant drugs and herbal formulations are frequently considered to be less toxic and free from side effects than synthetic one.<sup>[5,6]</sup> Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional medicine are important.<sup>[7]</sup> The attributed antihyperglycemic effects of these plants are due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit in the intestinal absorption of glucose.<sup>[8]</sup> Hence, treatments with herbal drugs have an effect on protecting  $\beta$ -cells and maintain the blood glucose levels. In general, there is very little biological knowledge on the specific mode of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc., that are frequently implicated as having antidiabetic effects.<sup>[9]</sup>

The seeds of *Psoralea corylifolia* (Somraji) are used as a folk medicine in India and China, and a variety of biological effects of extract or constituents are used for the inhibition in mitochondrial lipid peroxidation as well as for the management of hyperglycemic effect.<sup>[10]</sup> It belongs to the Fabaceae family.

The seeds of *Trigonella foenum-graecum* L. (Methi), under family Fabaceae are found all over India. Several human intervention trials demonstrated that the antidiabetic effects of fenugreek seeds (Methi) ameliorate most of the metabolic symptoms associated with type-1 and type-2 diabetes in both humans and relevant animal's models.<sup>[11]</sup>

## MATERIALS AND METHODS

### 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. Metformin was obtained from Aventis Pharmacy Ltd., Goa, India. Biochemical kits were purchased from Span Diagnostic Ltd. Surat, India. Blood glucose levels were measured using a one touch electronic glucometer of Ascensia Entrust, Bayer Diagnostics Ltd., Borada, India.

### Plant material

The seeds of *P. corylifolia* and *T. foenum-graecum* L. have been provided by Pharmaceutical Division of Southern Health Improvement Samity (SHIS), 24-Parganas (S), West Bengal,

India. The plant materials were identified by taxonomist of Botany Department, Vidyasagar University, Medinipur. The voucher specimens were deposited in the Department of Botany, Vidyasagar University and the voucher specimen numbers were recorded as BMLSM/PC/9 (Somraji) and BMLSM/TFL/16 (Methi).

### Preparation of plant extract

Fresh seeds of *P. corylifolia* and *T. foenum-graecum* L. were dried in an incubator for 2 days at 40 °C, crushed separately in an electric grinder and then pulverized. Out of this powder, 50 g was subjected to the extraction separately in 300 ml of distilled water and kept in incubator at 37 °C for 36 h. The slurry was stirred intermittently for 2 h and left overnight. The mixture was then filtered and filtrate was dried by low pressure. About 14.52 g of dark brown colour (*P. corylifolia*) and 19.70 g of yellowish (*T. foenum-graecum* L.) residue were collected. The residue was suspended in water in separately or in composite manner at a fixed dose and used for treatment.

### Selection of animals and animal care

The study was conducted on forty matured Wistar strain male albino rats; 3 months of age weighing about  $130 \pm 5$  g. Animals were acclimated for a period of fifteen days in our laboratory conditions prior to the experiment. Rats were housed in tarsons cages, at an ambient temperature of  $25 \pm 2$  °C with 12 h light: 12 h dark cycle. Rats have free access to standard food and water *ad libitum*. The principle of laboratory animal care was followed throughout the duration of experiment and instructions given by our Institutional Animal Ethical Committee (Ref. No. VU/IAEC/BMLSM/18/12) were considered regarding injection and relevant treatment of the experimental animals.

### Induction of diabetes mellitus

Forty five rats were subjected to streptozotocin induced diabetes by our standard method as mentioned earlier.<sup>[12]</sup> In brief, twenty four hour fasting rats were subjected to a single intramuscular injection of STZ at the dose of 40 mg/ml of citrate buffer (pH 4.5)/kg body weight that produce type I diabetes after 24 h of injection. Diabetic state was monitored for its stability for next seven days. Out of forty five rats, thirty rats with stable diabetes having fasting blood glucose level more than 250 mg/dl were selected as diabetic rat in this experiment.

### Experimental design

Thirty six rats were divided into six groups as follows:

Group I (Untreated Control): Rats of this group received single intramuscular injection of citrate buffer at the level of 1 ml/kg body weight at the time of STZ-injection to other rats.

Group II (Diabetic Control): Rats of this group were made diabetic by single intramuscular injection of streptozotocin at the dose of 40 mg/ml citrate buffer/kg body weight.

Group III (Diabetic + *P. corylifolia*): Diabetic rats of this group were subjected for the treatment with aqueous extract of seed of *P. corylifolia* through oral route at the dose of 200 mg/5 ml distilled water/kg body weight/day for 21 days at fasting state.

Group IV (Diabetic + *T. foenum-graecum* L.): Streptozotocin-induced diabetic rats were subjected for the treatment with aqueous extract of seed of *T. foenum-graecum* L. through oral route at the dose of 200 mg/5 ml distilled water/kg body weight/day at fasting state for 21 days.

Group V (Diabetic + *P. corylifolia* and *T. foenum-graecum* L.): Aqueous extract of seed of *P. corylifolia* and seed of *T. foenum-graecum* L. in composite manner was allow for the treatment of the diabetic rats through oral route at the dose of 200 mg (1:1)/5 ml distilled water/kg body weight/day for 21 days at fasting state.

Group VI (Diabetic + Metformin): Aqueous solution of metformin was provided to the diabetic rats through oral route for 21 days at the dose of 20 mg/5 ml distilled water/kg body weight/day at fasting state.

Extract or metformin treatment was started from 7<sup>th</sup> day after STZ injection and it was continued up to 28<sup>th</sup> day and so the duration of treatment was for 21 days. The time of extract or metformin treatment by forceful gavage was at fasting state at 8 A.M. to eliminate the food-drug interaction if any.<sup>[13]</sup> Animals of control (group I) and diabetic groups (group II) were subjected to forceful feeding of 5 ml of distilled water/kg body weight/day for 21 days at the time of extract treatment to the animals of group III, IV, V and VI to keep all the animals in same experimental condition from the angle of stress due to forceful gavage and animal handling.

Starting from 1<sup>st</sup> day of extract treatment to diabetic rats, fasting blood glucose levels in all the groups were measured by single touch glucometer in every 7<sup>th</sup> day. On 22<sup>nd</sup> day of extract treatment, all the animals were sacrificed by light ether anaesthesia followed by decapitation after recording the final body weight. Blood was collected from dorsal aorta by a syringe and the serum was separated from part of the collected blood by centrifugation at 3000 g for 5 min for the estimation of serum insulin, lipid profiles and toxicity study. Remaining blood was also used for the quantification of glycated hemoglobin (HbA<sub>1c</sub>). The liver and skeletal muscle (gastrocnemius) were dissected out and stored at -20°C. Activities of key carbohydrate metabolic enzymes

like hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase in hepatic tissue were measured. Glycogen content was also determined biochemically in liver and skeletal muscle.

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

At the time of grouping of the animals, FBG levels were measured. Every 7 days interval (8<sup>th</sup> day, 15<sup>th</sup> day, 22<sup>nd</sup> day and 29<sup>th</sup> day) FBG levels were further recorded from all the animals of all groups. Blood was collected from the tip of tail vein or by orbital puncture alternatively and FBG levels were measured by single touch glucometer.

### Glycated hemoglobin (HbA<sub>1c</sub>) level

Glycated hemoglobin (HbA<sub>1c</sub>) level was measured according to the standard protocol.<sup>[14]</sup> For this purpose, 4 ml of blood was collected in EDTA containing bulb and plasma was separated. The packed cell was washed by normal saline for six times. The hemolysate was prepared by adding 1/4<sup>th</sup> part of distilled water and 1/4<sup>th</sup> part of carbon tetrachloride to the packed cell and centrifuge at 3000 r. p. m for 20 min. Hemoglobin concentration was measured from the hemolysate by cyanmethemoglobin method. Hemoglobin concentration was adjusted to 10 mg/dl using normal saline. Hemolysate was taken at the levels of 2 ml of 10 mg/dl hemoglobin containing and 1.0 ml of 0.3 (N) oxalic acid was added to it and mix. The mixture was kept in boiling water bath for one hour then cool to room temperature and 1ml of 40% TCA was added to it. The content was mixed and centrifuged at 3000 r. p. m. Supernatant at the volume of 2 ml was collected and 0.5 ml of 0.7% thiobarbituric acid was added to it and kept at 37°C for 40 min. Reading was taken against blank consisting of 2 ml distilled water and 0.5 ml thiobarbituric acid at 443 nm. Glycosylated hemoglobin was expressed as GHb%.

### Biochemical assay of glycogen content

Glycogen levels in liver and skeletal muscle were measured biochemically.<sup>[15]</sup> Tissue samples were homogenized in 80% ethanol, and extract was collected by centrifugation using anthrone reagent, and quantity of glycogen was measured in relation to standards, which was expressed in µg of glucose/mg of tissue.

### Biochemical assay of hexokinase

Hexokinase activity in hepatic tissue was determined spectrophotometrically using assay mixture.<sup>[16]</sup> The assay mixture contained 3.7 mM glucose, 7.5 mM MgCl<sub>2</sub>, 11mM thioglycerol and 45 mM HEPES buffer. 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 spectrophotometer cuvette 0.9 ml of assay mixture and 0.22 M 0.03 ml ATP were taken and mixed well. After that 0.1 ml of tissue supernatant was added into the cuvette

and then absorbance was noted at 340 nm. One unit of hexokinase was expressed as  $\mu\text{g}/\text{mg}$  of tissue.

#### Biochemical assay of glucose-6-phosphate dehydrogenase

The hepatic glucose-6-phosphate dehydrogenase activity was measured spectrophotometrically. One unit of enzyme activity is defined as that quantity which catalyses the reduction of 1  $\mu\text{M}$  of NADP per min. Activity of this enzyme was recorded by using glucose-6-phosphate as a substrate and absorbance was measured at 340 nm.<sup>[17]</sup>

#### Biochemical assay of glucose-6-phosphatase activity

The hepatic glucose-6-phosphatase activity was measured according to standard protocol.<sup>[18]</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, 0.1ml 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 % trichloro acetic acid (TCA) followed by chilling in ice and centrifuged at  $3000 \times g$  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.

#### Serum insulin level

Serum insulin was measured by enzyme linked immunosorbant assay (ELISA) using the kit (Boehringer Mannheim Diagnostic, Mannheim, Germany).<sup>[19]</sup> 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  $\mu\text{IU}/\text{ml}$ .

#### Serum total cholesterol (TC)

Serum TC was quantified by the addition of enzyme present in reagent kit followed by optical density recording in spectrophotometer.<sup>[20]</sup> The absorbance of red quinoneimine complex was determined at 505 nm. The value of total cholesterol present in serum was expressed in mg/dl.

#### Serum triglyceride (TG)

Serum TG was determined by using kit. The absorbance was noted at 520 nm. The value was expressed in the unit of mg/dl.<sup>[21]</sup>

#### Serum lipoprotein cholesterol

Serum low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) were measured biochemically.<sup>[22]</sup> Other lipoprotein i.e. high density lipoprotein cholesterol (HDLc) level was measured by enzymatic method and using the reagents supplied in kit (Span Diagnostic Ltd. Surat; India).<sup>[23]</sup>

#### Biochemical assay of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT)

The activities of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) in serum were measured by specific kits supplied by Span Diagnostic Ltd. Surat; India. The activities of these enzymes were expressed as IU/L of serum.<sup>[24]</sup>

#### Statistical analysis

Analysis of Variance (ANOVA) followed by multiple comparison 'two-tail t test' was used for statistical analysis of collected data.<sup>[25]</sup> Differences were considered significant at the levels of  $P < 0.001$ ,  $P < 0.01$  and  $P < 0.05$ . All the values were indicated in the tables and figures as Mean  $\pm$  SEM.

## RESULTS

#### Body weight

Body weight was decreased significantly ( $P < 0.001$ ) in streptozotocin-induced diabetic rat in respect to the control animals. Treatment of aqueous extract of seed of *P. corylifolia* or seed of *T. foenum-graecum* L. in separate manner to diabetic rat resulted a partial recovery of body weight. Level of this parameter was significantly recovered in composite (1:1) extract treated diabetic group when the comparison was made with the individual extract treated diabetic group. An insignificant difference was noted in the level of this parameter in between metformin treated and composite extract treated diabetic groups [Table 1].

#### Fasting blood glucose (FBG) level

Significant elevation ( $P < 0.001$ ) in the FBG level of STZ-induced diabetic group when the values were compared with the untreated control group. These parameters

**Table 1: Remedial effect of aqueous extract of seed of *P. corylifolia* and seed of *T. foenum-graecum* L. in separate or in composite manner on body weight in streptozotocin-induced diabetic male albino rat**

| Groups    | Body weight (gm)  |                                |
|-----------|-------------------|--------------------------------|
|           | Initial           | Final                          |
| Group I   | 133.55 $\pm$ 4.22 | 146.02 $\pm$ 4.37              |
| Group II  | 131.16 $\pm$ 4.47 | 103.43 $\pm$ 3.72 <sup>a</sup> |
| Group III | 130.31 $\pm$ 3.44 | 136.71 $\pm$ 4.45 <sup>b</sup> |
| Group IV  | 131.46 $\pm$ 3.96 | 137.28 $\pm$ 4.7 <sup>b</sup>  |
| Group V   | 130.41 $\pm$ 4.08 | 143.04 $\pm$ 3.98              |
| Group VI  | 132.56 $\pm$ 3.95 | 144.87 $\pm$ 4.14              |

Data are expressed as Mean $\pm$ SEM, n=6. ANOVA followed by multiple comparisons two tail 't' test where superscript a-indicates  $P < 0.001$  and b-indicates  $P < 0.01$  compare with untreated control group (Group I). Values without any superscript have no significant variation

**Table 2: Effect of separate or composite aqueous extract of seed of *P. corylifolia* and seed of *T. foenum-graecu* L. on fasting blood glucose (FBG) level in streptozotocin-induced diabetic male albino rat**

| Groups    | 0 day (Day of STZ injection) | Fasting blood glucose level (mg/dl) |                         |                         |                         |                         |
|-----------|------------------------------|-------------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|           |                              | 1 <sup>st</sup> day                 | 8 <sup>th</sup> day     | 15 <sup>th</sup> day    | 22 <sup>nd</sup> day    | 29 <sup>th</sup> day    |
| Group I   | 73.5±4.1                     | 74.61±4.6                           | 76.66±4.6               | 75.51±4.6               | 76.27±4.5               | 75.09±4.7               |
| Group II  | 75.1±4.02                    | 297.2±5.2 <sup>a</sup>              | 318.12±4.4 <sup>a</sup> | 344.14±5.2 <sup>a</sup> | 367.04±4.8 <sup>a</sup> | 389.71±5.8 <sup>a</sup> |
| Group III | 74.53±4.9                    | 326.33±4.6 <sup>a</sup>             | 258.31±4.8 <sup>a</sup> | 198.02±4.7 <sup>a</sup> | 179.28±4.4 <sup>a</sup> | 132.12±3.2 <sup>a</sup> |
| Group IV  | 72.43±3.2                    | 319.84±3.4 <sup>a</sup>             | 261.32±3.6 <sup>a</sup> | 192.2±3.9 <sup>a</sup>  | 180.46±4.1 <sup>a</sup> | 133.5±3.74 <sup>a</sup> |
| Group V   | 73.61±5.1                    | 298.82±4.5 <sup>a</sup>             | 233.36±4.8 <sup>a</sup> | 188.67±4.2 <sup>a</sup> | 106.81±5.3 <sup>a</sup> | 87.5±3.1 <sup>b</sup>   |
| Group VI  | 76.21±3.7                    | 297.98±4.2 <sup>a</sup>             | 187.23±3.8 <sup>a</sup> | 113.98±4.1 <sup>a</sup> | 89.37±3.7 <sup>b</sup>  | 77.31±3.26              |

Data are expressed as Mean±SEM, n=6. ANOVA followed by multiple comparisons two tail 't' test where superscript a-indicates  $P < 0.001$ , and b-indicates  $P < 0.01$  compare with untreated control group (Group I). Values without any superscript have no significant variation

**Table 3: Corrective role of separate or composite aqueous extract of seed of *P. corylifolia* and seed of *T. foenum-graecu* L. on hemoglobin and glyclated hemoglobin levels in STZ-induced diabetic male albino rat**

| Groups    | Hemoglobin (gm/dl)      | Glyclated hemoglobin (GHb %) | Serum insulin ( $\mu$ U/ml) |
|-----------|-------------------------|------------------------------|-----------------------------|
| Group I   | 12.03±2.01              | 2.09±0.88                    | 22.47±1.23                  |
| Group II  | 4.60±0.98 <sup>a</sup>  | 6.23±1.06 <sup>a</sup>       | 8.72±0.59 <sup>a</sup>      |
| Group III | 8.12±1.13 <sup>b</sup>  | 4.21±0.9 <sup>b</sup>        | 14.45±0.71 <sup>b</sup>     |
| Group IV  | 8.94±1.48 <sup>b</sup>  | 4.18±0.79 <sup>b</sup>       | 15.11±0.76 <sup>b</sup>     |
| Group V   | 10.26±1.74 <sup>c</sup> | 3.02±0.83 <sup>c</sup>       | 19.88±0.94 <sup>c</sup>     |
| Group VI  | 11.95±1.97              | 2.11±0.81                    | 20.21±1.06 <sup>c</sup>     |

Data are expressed as Mean±SEM, n=6. ANOVA followed by multiple comparisons two tail 't' test where superscript a-indicates  $P < 0.001$ , b-indicates  $P < 0.01$  and c-indicates  $P < 0.05$  compare with untreated control group (Group I). Values without any superscript have no significant variation

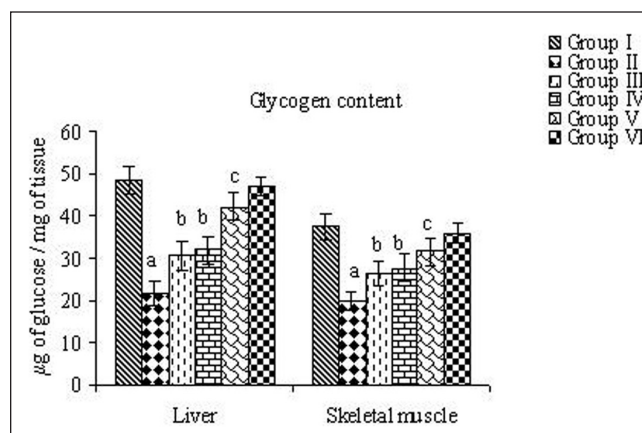
were partially recovered towards the control level after treatment of this extract in separate way. But significant recovery ( $P < 0.05$ ) was noted after treatment of this extract in composite manner (1:1) at the dose of 200 mg/kg body weight to diabetic group than the individual extract treated diabetic groups at the same dose. Metformin treatment (20 mg/kg body weight) to diabetic group resulted resettlement of FBG level to the control level [Table 2].

### Hemoglobin and glyclated hemoglobin (HbA<sub>1c</sub>) levels

Hemoglobin level was decreased significantly along with the elevation in glyclated hemoglobin (HbA<sub>1c</sub>) level in STZ-induced diabetic group in respect to the untreated control group. Significant recovery ( $P < 0.05$ ) was noted after treatment of this extract in composite manner (1:1) than the individual extract treated groups. Insignificant variation was noted in the level of these parameters in metformin treated diabetic group in respect to untreated control group [Table 3].

### Serum insulin level

Serum insulin level was decreased significantly in STZ-induced diabetic control animals in respect to the untreated



**Figure 1:** Corrective role of separate or composite aqueous extract of seed of *P. corylifolia* and seed of *T. foenum-graecu* L. on glycogen contents in liver and skeletal muscle in STZ-induced diabetic male albino rat. Data are expressed as mean ± SEM, n=6. ANOVA followed by multiple comparison two tail 't' test where superscript, a-indicates  $P < 0.001$ , b-indicates  $P < 0.01$  and c-indicates  $P < 0.05$  compare with untreated control group (Group I). Bar without superscript does not differ from one another significantly

control animals. Treatment of these plants extract in separate manner at the above mentioned dose resulted partial recovery of serum insulin level. But in composite (1:1) extract or metformin treated diabetic group, the level of said biosensor was significantly ( $P < 0.05$ ) recovered towards the control level than the individual extract treated groups [Table 3].

### Glycogen levels in liver and skeletal muscle

Significant diminution ( $P < 0.001$ ) in the levels of glycogen in liver and skeletal muscle were noted in diabetic group in respect to the untreated control group. Treatment of these plants extract in separate manner at the above mentioned dose resulted a partial recovery of glycogen content of above mentioned biological tissues. But in composite (1:1) extract treated group, the levels of glycogen in said tissue samples were significantly recovered ( $P < 0.05$ ) towards the control level and the levels of recovery were significantly more than the individual extract treated groups. The levels of this parameter were resettled to the control level in the metformin treated diabetic group [Figure 1].

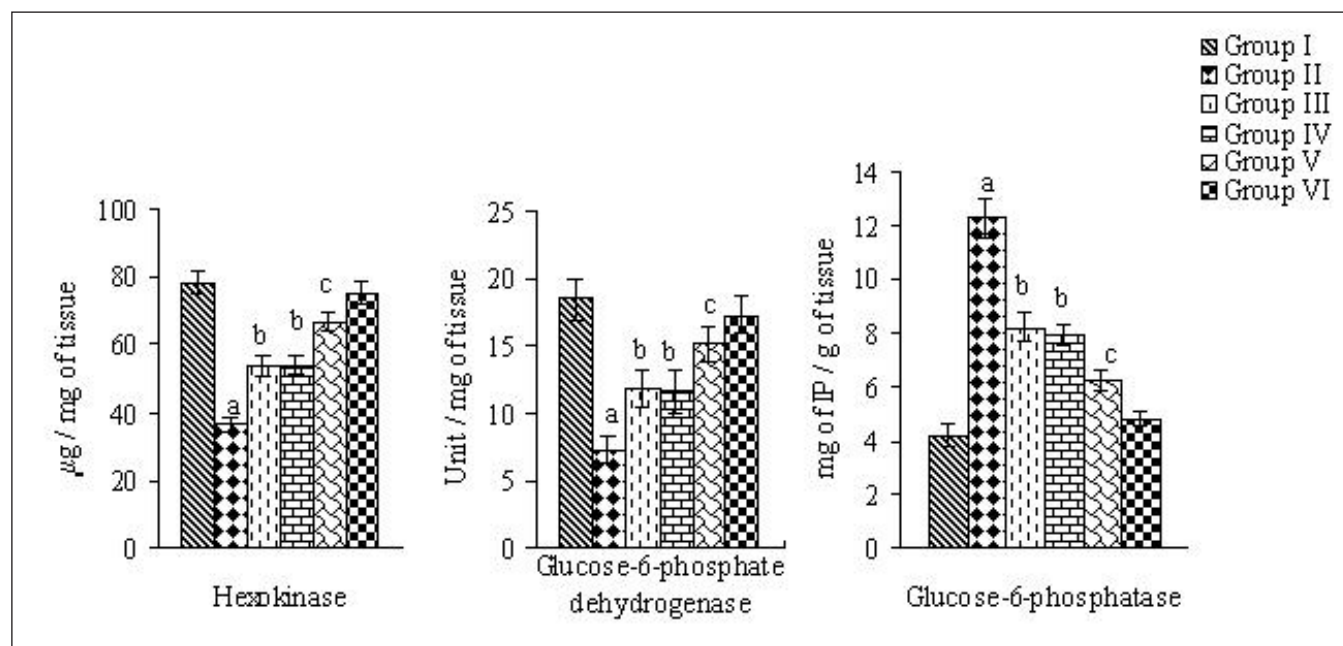
### Carbohydrate metabolic enzymes activities in hepatic tissue

Hepatic hexokinase and glucose-6-phosphate dehydrogenase activities were decreased significantly ( $P < 0.001$ ) along with the increase in the activities of glucose-6-phosphatase in STZ-induced diabetic rat in respect to the untreated control group. Activities of these enzymes were significantly ( $P < 0.05$ ) recovered towards the control level after treatment of this extract in composite manner (1:1) to diabetic rats and the levels of recovery were significant in composite extract treated group than the individual extract treated diabetic groups. An insignificant difference was noted in the activities of these enzymes in between metformin treated diabetic and untreated control groups [Figure 2].

### Serum lipid profiles

Serum total cholesterol (TC) and triglyceride (TG) levels were significantly elevated ( $P < 0.001$ ) in STZ-induced diabetic group in respect to the untreated control group. Treatment of these extract in separate manner at the above mentioned dose to diabetic animals resulted a partial recovery in the levels of these parameters towards the control level. Levels of TC and TG were significantly ( $P < 0.05$ ) recovered towards the control level in composite (1:1) extract treated diabetic group than the individual extract treated diabetic groups [Table 4].

Lipidemic parameters like serum LDLc and VLDLc levels were elevated significantly in diabetic group in comparison with the untreated control group. Treatment of these extract in composite manner (1:1) to diabetic animals



**Figure 2:** Protection in the activities of hepatic hexokinase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase in STZ-induced diabetic male albino rat after treatment of aqueous extract of seed of *P. corylifolia* and seed of *T. foenum-graecu* L. in separate or in composite manner. Data are expressed as mean  $\pm$  SEM, n=6. ANOVA followed by multiple comparison two tail 't' test where superscript, a-indicates  $P < 0.001$ , b-indicates  $P < 0.01$  and c-indicates  $P < 0.05$  compare with untreated control group (Group I). Bar without superscript does not differ from one another significantly

**Table 4: Remedial effect of separate or composite aqueous extract of seed of *P. corylifolia* and seed of *T. foenum-graecu* L. on serum lipid profiles in streptozotocin-induced diabetic male albino rats**

| Groups    | Serum lipid profiles (mg/dl) |                             |                            |                             |                            |
|-----------|------------------------------|-----------------------------|----------------------------|-----------------------------|----------------------------|
|           | TC                           | TG                          | HDL <sub>c</sub>           | LDL <sub>c</sub>            | VLDL <sub>c</sub>          |
| Group I   | 106 $\pm$ 3.08               | 88 $\pm$ 2.97               | 36 $\pm$ 2.16              | 53 $\pm$ 2.17               | 17 $\pm$ 1.13              |
| Group II  | 164 $\pm$ 5.61 <sup>a</sup>  | 139 $\pm$ 3.74 <sup>a</sup> | 19 $\pm$ 1.35 <sup>a</sup> | 118 $\pm$ 4.08 <sup>a</sup> | 27 $\pm$ 2.02 <sup>a</sup> |
| Group III | 139 $\pm$ 5.14 <sup>a</sup>  | 121 $\pm$ 4.03 <sup>a</sup> | 25 $\pm$ 1.68 <sup>a</sup> | 90 $\pm$ 3.98 <sup>a</sup>  | 24 $\pm$ 1.57 <sup>a</sup> |
| Group IV  | 135 $\pm$ 4.32 <sup>a</sup>  | 119 $\pm$ 3.34 <sup>a</sup> | 24 $\pm$ 1.51 <sup>a</sup> | 88 $\pm$ 3.16 <sup>a</sup>  | 23 $\pm$ 1.41 <sup>a</sup> |
| Group V   | 117 $\pm$ 3.78 <sup>b</sup>  | 96 $\pm$ 2.48 <sup>b</sup>  | 33 $\pm$ 2.05 <sup>c</sup> | 62 $\pm$ 3.09 <sup>b</sup>  | 19 $\pm$ 1.37 <sup>c</sup> |
| Group VI  | 110 $\pm$ 3.07 <sup>c</sup>  | 93 $\pm$ 2.13 <sup>c</sup>  | 35 $\pm$ 2.11              | 57 $\pm$ 2.28 <sup>c</sup>  | 18 $\pm$ 1.16              |

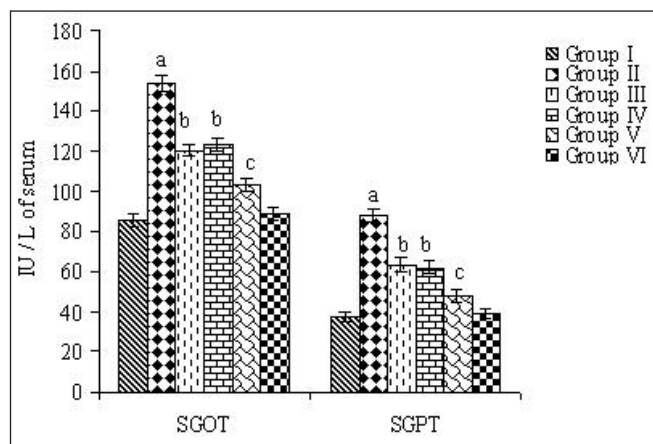
All the values are expressed as Mean $\pm$ SEM, n=6. ANOVA followed by multiple comparison student two tail 't' test where superscript a-indicates  $P < 0.001$ , b-indicates  $P < 0.01$  and c-indicates  $P < 0.05$  compare with untreated control group (Group I). Values without any superscript have no significant variation

resulted a significant recovery in serum LDLc and VLDLc levels towards the control level. Insignificant variation was noted in the level of VLDLc in between metformin treated diabetic and untreated control groups [Table 4].

Serum HDLc level were decreased in STZ-induced diabetic group in respect to the untreated control group. Treatment of the composite (1:1) extract at the above mentioned dose to diabetic rats resulted a significant ( $P < 0.05$ ) recovery of this parameter towards the control level. The composite extract treatment resulted significant recovery in the levels of these parameters than the individual extract treated diabetic groups. An insignificant difference was noted in the level of HDLc in between metformin treated and composite extract treated diabetic groups [Table 4].

### Activities of serum GOT and GPT

Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities in serum were increased significantly ( $P < 0.001$ ) in STZ-induced diabetic group in respect to the untreated control group. Above mentioned parameters were partially recovered towards the control level after treatment of this extract in individual manner. Treatment of this extract in composite manner (1:1) to diabetic animals at the above mentioned dose resulted a significant ( $P < 0.05$ ) recovery in serum GOT and GPT activities towards the control level and the levels of recovery in the composite extract treated group was significantly more than the individual extract treated groups. There was no significant difference in the activities of these enzymes in between metformin treated diabetic and untreated control groups [Figure 3].



**Figure 3:** Remedial effect of separate or composite aqueous extract of seed of *P. corylifolia* and seed of *T. foenum-graecum* L. on serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in STZ-induced diabetic male albino rat. Data are expressed as mean  $\pm$  SEM, n=6. ANOVA followed by multiple comparison two tail 't' test where superscript, a-indicates  $P < 0.001$ , b-indicates  $P < 0.01$  and c-indicates  $P < 0.05$  compare with untreated control group (Group I). Bar without superscript does not differ from one another significantly

## DISCUSSION

Present study was conducted to find out the mechanism of antihyperglycemic and antihyperlipidemic effects of the aqueous extract of seed of *P. corylifolia* and seed of *T. foenum-graecum* L. in separate or in composite (1:1) manner in STZ-induced diabetic rat. Streptozotocin (STZ)-induced diabetic rat is one of the animal models of type-I diabetes mellitus as used by us in our previous studies.<sup>[26]</sup> In this type, diabetes arise from destruction of  $\beta$  cells of islets of pancreas, causing degranulation or reduction of insulin secretion but not total absence of insulin in blood.<sup>[27]</sup>

Here, study was adopted in two dimensions for the management of STZ-induced diabetes by these plant extracts. In one dimension, the fasting blood glucose (FBG), glycated hemoglobin levels and glycogen contents in liver and skeletal muscle as well as activities of carbohydrate metabolic enzymes were measured. In other dimension, antihyperlipidemic potency of these plant extracts was studied as there is a close correlation between hyperglycemia and hyperlipidemia.<sup>[28]</sup> The specific dose of the extract used and duration of the treatment adopted here were selected by pilot study where good promising results were noted without any metabolic toxicity induction.

In the present study, after 24 h of STZ injection, elevation in the fasting blood glucose (FBG) and HbA<sub>1c</sub> levels which were noted in consistent with other reports and this may be due to low plasma level of insulin as reported in the present work.<sup>[29,30]</sup> Treatment of the extract in composite manner (1:1) to STZ-induced diabetic rat resulted a more protective effect on the above mentioned parameters than the treatment of individual plant extract. The recovery in the level of HbA<sub>1c</sub> in the composite extract treated group may be due to high glucose utilization in the peripheral tissues.<sup>[31]</sup> This could be due to the elevation in serum insulin where the possibility of  $\beta$ -cells regeneration by the extract may occurred or increased pancreatic insulin from existing  $\beta$  cells of islets as proposed by others workers as well as our previous report.<sup>[32,33]</sup>

Activities of important carbohydrate metabolic enzymes i.e. hexokinase, glucose-6-phosphatase and glucose-6-phosphate dehydrogenase which are insulin dependent were altered in STZ-induced diabetic rat as reported here also supported by other workers as well as by our previous report.<sup>[34,35]</sup> Treatment of this extract in composite manner (1:1) resulted a significant recovery in the activities of these enzymes in hepatic tissue that may be attributed another possible way of its antidiabetogenic activity which was also investigated by our previous work using other plant.<sup>[36]</sup> The levels of glycogen in liver and skeletal muscle were recovered significantly towards the control level after

treatment of these extract in composite manner (1:1). This type of protection may be due to elevation in the plasma insulin level that stimulates the activity of glycogen synthetase which is supported by our previous publication where we used other plant extracts.<sup>[37]</sup>

Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Hyperlipidemia, which has been noted in STZ-induced diabetic rats, is in agreement with the report of others.<sup>[38]</sup> The hypercholesterolemia and hypertriglyceridemia along with elevation in serum atherogenic lipoprotein levels like LDLc, VLDLc and reduction in the serum levels of main anti-atherogenic lipoprotein i.e. HDLc in STZ-induced diabetic rat are supported by our previous report.<sup>[39]</sup> The impairment in lipid metabolism in diabetes may be due to low level serum insulin as hypoinsulinic state increases the mobilization of free fatty acid from peripheral fat depot.<sup>[40]</sup> Since, insulin inhibits the hormone sensitive lipase; this hyperlipidemic state in diabetes may be regulated as a consequence of the uninhibited action of lipolytic enzymes on fat depot. Treatment of these aqueous extract in composite (1:1) manner resulted in much better correction of hyperlipidemia when compared to the treatment of individual plant extract and this may be due to recovery in the level of serum insulin. The composite extract has no general toxicity induction as body weight remain in positive direction in composite extract (1:1) treated diabetic group like untreated control group. This has been supported here by the assessment of serum GOT and GPT activities as these are considered as good indicators in this concern.<sup>[39]</sup>

## CONCLUSION

It has been noted that the composite extract of seed of *P. corylifolia* and seed of *T. foenum-graecum* L. have promising antidiabetic activity by the corrections of insulin sensitive carbohydrate metabolic enzymes activities as wells as serum lipid profiles. It may be claimed that the active ingredient(s) present in this extract can able to sensitize the  $\beta$ -cells and/or regenerate the  $\beta$ -cells that elevate serum insulin level and thereby regulate carbohydrate metabolic enzymes activities. The actual way of recovery may be explained by the continuation of work in this line in near future.

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