Original Article

Hypoglycemic and Hypolipidemic Effect of Seed Hydromethanolic Extract of *Tamarindus indica* L. on Streptozotocin-Induced Diabetes Mellitus in Rat

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ABSTRACT

Objectives: The present available drugs for diabetes management by insulin or oral hypoglycemic agents have one or more adverse effects. Search for new hypoglycemic drugs from herbal plants parts without or a minimum adverse effect is a challenges. Present study was aimed to evaluate the antidiabetic and antihyperlipidemic effect of hydromethanolic extract of seed of *Tamarindus indica* L covering the biochemical parameters for the management of streptozotocin-induced diabetic rat.

Methods: Forty matured Wistar strain male albino rats, fasting blood glucose level 75±5 mg/dl and weighing 135±10 g were selected for this experiment. They were divided into four groups. Diabetes was induced by intramuscular injection of streptozotocin. Fasting blood sugar level was monitored by glucometer. Hydromethanolic extract (1:1) of seed of *Tamarindus indica* was administered at the dose of 80 mg/100 g body weight/day by gavage for 14 days to diabetic rats. Serum was separated and used for total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and also subjected to used for ELISA method for insulin assay. Activities of hepatic and renal biomarker enzymes were measured. The statistical analysis of the results was carried out using ANOVA followed by multiple two tail t test.

Results: Diabetic rats showed elevated blood glucose level and significant diminution in the activities of hexokinase activity in liver. Administration of hydromethanolic extract of *Tamarindus indica* seed to diabetic rats resulted a significant recovery of above mentioned parameters ($P<0.05$) side by side serum insulin level, glycogen content in liver and skeletal muscle, along with diabetic

dyslipidemia was protected in comparison to diabetic rats. Active biomolecule(s) present of this seed extract revealed the presence of flavonoids and total phenolic compounds. There was a significant diminution in the activities of hepatic and renal function biomarkers in diabetic rats treated with seed extract compared to only diabetic rats indicating the non toxic properties of this said extract against liver and kidney damage.
Conclusion: It is concluded that the hydromethanolic extract of *T. indica* seed is capable of managing hyperglycemia, anti-hyperlipidemia and diabetes related complications. Hence this extract may be considered as one of the potential source for the isolation of new oral hypoglycemic drugs.

Keywords: *Tamarindus indica*, Streptozotocin-induced diabetes, Carbohydrate metabolic enzyme, Lipid profile, Glibenclamide.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder of multiple etiologies characterized by high blood glucose level with disturbance of carbohydrate, protein and fat metabolism resulting from defects in insulin secretion, insulin action or insulin insensitivity[1]. Approximately 4% of world populations are affected by diabetes mellitus and by the year 2025 it is expected to increase by 5.4%[2]. The important characteristics features of diabetes mellitus are hyperglycemia, glucosuria, polyuria, polydipsia, polyphagia, ketonemia and kitonurea and glucoma. People with diabetes are at increased risk of vascular complication such as cardiovascular, peripheral vascular, cerebrovascular disease and retinopathy and nephropathy[3,4].

Since ancient times spices and herbal remedies has been used to treat a variety of disorders. Management of diabetes without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use the natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs are having undesirable side effects[5]. The plants with antidiabetic activities provide useful sources for the development of drugs in the treatment of diabetes mellitus. Medicinal plants with hypoglycemic activity were used for many centuries and sometimes as regular constituents of the diet, it is assumed that they do not have any side effects[6]. Aromatic and medicinal plants have played an important role as therapeutic agents for a long time and thus hold immense economic value[7,8]. In the past, herbs were the primary medicinal agents used by people. Knowledge of the attributes and advantageous effects of traditional herbal medicines coupled with basic human nature has led to their increasing use[9-11]. Even today, medicinal plant therapy is extremely used for treatment of many animal and human diseases[12-14]. Phytochemicals isolated from plant source are used for the prevention and treatment of cancer, heart disease, diabetes, high blood pressure etc.[15].

The first scientific study on *Tamarindus indica (T. indica)* of aqueous seed extract for its antidiabetic activity was conducted by us[16] but to our knowledge no detailed investigations had been carried out to shed light on the scientific reports of hydromethanolic seed extract of *T. indica* on

antidiabetic and antihyperlipidemic activities. *T. indica* L. is locally name as Imli in Hindi and Tentul in Bengali, is an indigenous tropical tree type of plant, up to 20-25 ft tall. In summer season fruits are found and its seed coat is black brown in color though the kernel is white in color. It is a dicotyledonous plant, grown abundantly in all over India, Bangladesh, Pakistan, Burma, Brazil, China and tropical Africa belonging to Caesalpiniaceae family and is attributed to have many traditional medicinal properties. The fruits and seed extract have antibacterial and antifungal activities[17,18], its fruits are a traditional meal on bioavailability of aspirin tablets[19]. *T. indica* seed have rich of polysaccharides that have lack of carcinogenic potential[20]. Other parts of the plant present antioxidant[21], antihepatotoxic[22] activities.

In the present study, we explore the role of *T. indica* L in prevention of streptozotocin-induced hyperglycemia and related lipid complications. Hypoglycemic activity of this extract was compared with glibenclamide, an oral antidiabetic drug.

MATERIALS AND METHODS

Plant material

T. indica seeds were collected freshly from Badhutola, Paschim Medinipur district in the month of May-June and the material was authenticated by taxonomist of Central National Herbarium (CAL), Botanical Survey of India (B.S.I.), Shibpur, Howrah, and the voucher specimen (HPCH No-1) was deposited in the Central National Herbarium (CAL), BSI, Shibpur, Howrah.

Chemicals

Streptozotocin was obtained from (Sigma Chemical Company, St Louis, MO, USA). All other chemicals used in this experiment were of analytical grade. Serum Cholesterol and triglyceride kits were purchased from (Reekon Diagnostics Pvt. Ltd. (Boroda, India). Insulin enzyme linked immunosorbant assay (ELISA) kit purchased from Boehringer Mannheim Diagnostic, Mannheim (Germany). The kit for the measurement of GOT and GPT were supplied by Reekon Diagnostics Pvt. Ltd. (Boroda, India).

Preparation of hydromethanolic (1:1) extract of seed of *T. indica*

Hydromethanolic extract of seed of *T. indica* was performed according to the method of National Institute of Health and Family Welfare, New Delhi[23]. Fresh seeds of *T. indica* were dried in an incubator for 2 days at 40^0C, crushed in an electrical grinder to have coarse powdered. Then 100 g seed powders of *T. indica* was suspended in 250 ml of water and 250 ml of methanol mixture (1:1) and allow it to stand overnight in refrigerator and then extracted for 18 h in a soxhlet apparatus and a deep brown hydromethanolic extract was obtained. The suspension was then filtered by coarse sieve filter paper. The filtrate was evaporated to dryness under reduced pressure in a rotary evaporator. A deep brown material was obtained (4 g/100 g of the dried seeds powder). It was stored at $(0\text{-}4)^0$C until used. When needed, the residual extract was suspended in olive oil and used in the study.

Selection of animals and care

Forty matured Wistar strain male albino normoglycaemic rats (*Rattus norvegicus*) having fasting blood glucose level 75±5 mg/dl, 3 months of age and weighing 135±10 g were selected for this experiment. The rats were housed in colony cages (4 rats per cage), at an ambient temperature of 25 ± 2^0 C and humidity (55±10%) with 12 h light/12 h dark cycle. Rats were fed standard rat chow diet and had free access to water *ad libitum*. They were acclimatized to the laboratory conditions for a period of 7 days in the new environment

before carrying out the experimental work. The present study was conducted in accordance with the internationally accepted 'Principles for Laboratory Animal Use and Care' as found in the US guidelines (NIH publication No 85-23). Our Institutional Ethical Committee was approved this study.

Induction of experimental diabetes

Diabetes was induced in overnight fasted thirty two rats by single intramuscular injection of streptozotocin at the dose of 7 mg/0.5 ml normal saline/100 g body weight/rat but had been allowed free access to tap water. After 2 h of injection food was provided to them. Diabetic condition was assessed in STZ treated rats by measuring 12 h fasting blood glucose level after 24 h of STZ injection. Only rats with fasting blood glucose level greater than 250 mg/dl were selected and used for the study. For the stability of diabetes, rats were monitored for blood glucose level for next 7 days and then the experiment was started as per the following protocol.

Experimental design

The rats were divided into the four groups, each with eight animals. Eight rats were incorporated in control group. Twenty four diabetic rats were divided into three equal groups and each group consisted of eight rats and treated as follows –

Group I (Control group)

Eight animals were subjected to oral intubation of olive oil forcefully by gavage method at the volume of 0.5 ml/100 g body weight/day/rat for 14 days through intragastric route.

Group II (Diabetic group)

The rats become diabetic after a single intramuscular injection of streptozotocin (7 mg/0.5 ml normal saline/ 100 g body weight/rat). At the time of seed extract and glibenclamide treatment to group III, IV, and group II (Diabetic) animals were subjected to forceful delivery of olive oil through intragastric route for 14 days at the same time to nullify the effect of stress due to drug delivery and handling of the animals.

Group III [Hydromethanolic (1:1) extracts co-administered (80 mg dose) group]

Eight diabetic animals were subjected to oral delivery of hydromethanolic extract of seed of *T. indica* at the dose of 80 mg/0.5 ml olive oil/100 g body weight/day/rat for 14 days.

Group IV (Glibenclamide co-treated (0.5 mg dose) group)

Diabetic rats were received glibenclaimide at the dose of 60 µg/0.5 ml distilled water/100 g body weight/rat for 14 days through intragastric route[24].

These supplements were ingested at 8 A.M. and food was given at 10 A.M. of each day.

Final body weight of all the animals were recorded on 15th day of experiment. The rats were killed by decapitation after light ether anesthesia and relevant organs like liver, kidney and skeletal muscle were dissected out, washed in ice cold physiological saline, patted dry and stored at -20^{0}C until all the organs of the animals has been collected and was then used for biochemical assay of liver and skeletal muscle glycogen, liver hexokinase, and transaminases activities in liver and kidney. Blood was collected from the dorsal aorta by a syringe and serum was separated by centrifugation at 4000 rpm for 10 minutes for the estimation of serum insulin assay and also used for assessment of lipid metabolic disorders was performed by the measurement of the levels of total cholesterol, triglyceride, high density, and low-density and very low-density lipoprotein cholesterol.

Testing of fasting blood glucose level

After 14 days of all these supplementation, fasting blood glucose level was measured from the animals of all these groups. Fasting blood glucose level was measured using single touch glucometer (Life Scan, Johnson and Johnson, USA)[25] in venous blood collected from tip of the tail vain. Blood glucose levels were expressed in term of mg/dl.

Biochemical assay of glycogen level

Glycogen contents in liver and skeletal muscles were measured according to the standard method[26]. Liver and skeletal tissues were homogenized separately in hot 80% ethanol at the tissue concentration of 100mg/ml and then centrifuged at 8000g for 20 minutes. The residues were collected and allow drying 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 0^0C for 20 min. The collected material was centrifuge at 8000 g for 15 min and supernatant was collected. From supernatant, 0.2 ml was transfer in a graduated test tube and volume was made up to 1 ml by the addition of distilled water. Graded standard were prepared by using 0.1, 0.2, 0.4, 0.6 0.8 and 1.0 ml of working standard solution and 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 µg of glucose/mg of tissue.

Assay of hexokinase in liver

The enzyme activity was determined by the method of Chou and Wilson[27]. Liver tissue was homogenized in ice-cold 0.1 M phosphate puffer, pH-7.4 at the tissue concentration of 50 mg/ml. The homogenate of the tissue was centrifuged at 10,000 g for 20 minutes at 4˚C. The supernatant was collected and used for the assay of enzyme activity. In a spectrophotometric cuvette 0.9 ml of assay mixture, 0.01 ml glucose-6-phosphate dehydrogenase, 0.01 ml NADP and 0.03 ml ATP was added and mixed well. To it 0.6 ml of tissue supernatant was added in to the cuvette and mixed immediately, then read at 340 nm. The subsequent six readings were recorded at 30 sec interval. One unit of hexokinase activity was expressed in units per mg of tissue.

Biochemical assay of serum total cholesterol (TC), triglyceride (TG), LDLc, VLDLc, HDLC

Serum TC was quantified by spectrophotometric method[28] 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**,** **s**erum LDLc and VLDLc were measured according to the protocol[29]. Other lipoprotein cholesterol *i.e.* HDLc was measured by the method of Burstein *et al*[30]. Serum Triglyceride By using kit[31] serum TG was measured noting the absorbance at 546 nm. The value was expressed in the unit of mg/dl.

Biochemical assay of serum insulin level

Serum insulin was measured by enzyme linked imunosorbent assay (ELISA) using the kit[32]. The intra assay variation was 4.9%. As the samples were run at a time, so there was no inter assay variation. The

insulin level in serum was expressed in IU/ml.

Biochemical assay of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities

Separately liver and kidney tissue were homogenized in ice-cold of 0.1M phosphate buffer (pH-7.4) at the tissue concentration of 50 mg/ml. The kit for the measurement of liver and kidney GOT and GPT were supplied by Reekon Diagnostics Pvt. Ltd. (Boroda, India) and activities of these were measured according to the standard protocol[33]. The activities of these enzymes were expressed as unit per milligram of tissue.

UV spectrophotometric determination of active compounds

(a) Total phenolic compound

Total phenolic derivatives were determined according to Folin-Ciocalteau method[34] using a reaction medium containing 50 µl of sample, blank or gallic acid standard respectively, 50 µl of 7% (v/v) acetic acid, 50 µl of Folin-Ciocalteau reagent, 50 µl 35 % (w/v) sodium carbonate and 800 µl of distilled water. After mixing, the reaction was incubated for 90 minutes at room temperature in the dark. Its light absorbance was measured at 725 nm in UV spectrophotometer and the total phenolic contents were expressed as gallic acid equivalent per mg of extract.

(b) Total flavonoids

The total flavonoids were determined in medium containing 200 µl of sample, blank or a quercetin standard, 60 µl of glacial acetic acid, 1 ml of pyridine : water : 12 % ammonium chloride solution (17:80:3), 1.24 ml of DMSO : H_2O (1:1). The reaction product was spectrophotometrically determined at 420 nm[35].

Statistical analysis

One-way ANOVA followed by a multiple two-tail 't' test with Bonferroni modification was used for statistical analysis of the collected data[36] (Sokal and Rohle, 1997). Difference were considered significantly when $P<0.05$.

RESULTS

Body weight

Diabetes induced by STZ resulted significant diminution in body weight in comparison to control. Supplementation of *T. indica* for 14 days to the diabetic animals body weight of all the animals were insignificantly differ from control level (Table 1). On the other hand co-treatment of glibenclamide at the dose of 60 µg/0.5 ml distilled water/100 g body weight/rat for 14 days to the diabetic animals resulted, a significant elevation in body weight, which recovered completely to the control group (Table 1).

Fasting blood glucose level

After 24 h of STZ injection fasting blood glucose level was significantly elevated. Administration of hydromethanolic extract of seed of *T. indica* for 14 days, resulted a significant recovery in fasting blood glucose level and resettled to the control group (Table 2). On the contrary, a significant recovery in fasting blood glucose level was noted after co-treatment of glibenclamide for 14 days in respect to STZ-treated diabetic animals, which recovered towards the control level (Table 2).

Glycogen content in liver and skeletal muscle

In this study, it was stated that quantity of glycogen content in liver and skeletal muscle were decreased significantly in STZ-treated diabetic group when compared with the control group (Table 3). On the other hand, the aforesaid parameter

of the said tissues were significantly increased after administration of *T. indica* or glibenclamide for 14 days to the diabetic animals when compared to the STZ-treated diabetic group of animals and the values showed no significant difference from the control group (Table 3**)**.

Hexokinase activity in live

Hexokinase activity in liver was diminished significantly in STZ-treated diabetic group in comparison to the control group (Table 4). Co-administration of glibenclamide for 14 days to the diabetic rats resulted significantly elevation in aforesaid parameter in comparison to STZ-treated diabetic rats and the values were resettled towards the control group (Table 4). In contrast, above mentioned parameter in liver of STZ-induced diabetic rat was completely recovered to the control group after supplementation of *T. indica* (Table 4**).**

Serum lipid profile

Serum TC and TG levels were significantly elevated in the diabetic group in comparison with the control group. After treatment with the above-mentioned extract to the diabetic animals, serum TC and TG levels were resettled significantly to the control level (Table 5). Other parameters of this lipid profile like serum LDLc and VLDLc levels were elevated and serum HDLc level was decreased in the diabetic group in comparison to the control. The levels of the above-mentioned parameters were recovered significantly to the control group after treatment of *T. indica* seed when compare with the diabetic control group (Table 5). On the other hand, a significant recovery was noted to the diabetic rats after co-treatment of glibenclamide for 14 days in respect to control level (Table 5).

Serum insulin level

Serum insulin level was decreased in STZ-induced diabetic rats in respect to control. Co-treatment of glibenclamide for 14 days to the diabetic animals and the values were resettled to the control group (Table 6). In contrast, aforesaid parameter in serum of STZ-induced diabetic rats was completely recovered to the control group after *T. indica* supplementation (Table 6**)**. There was no significant difference in the level of this parameter between seed extract treated group and the glibenclamide treated group (Table 6).

Transaminase activities in liver and kidney

In STZ-induced diabetic group the activities of GOT and GPT in liver and kidney were elevated significantly in respect to the control group (Table 7). On the contrary, co-administration of hydro-methanolic seed extract of *T. indica* or glibenclamide for 14 days to the diabetic animals resulted a significant diminution in the above mentioned parameters in comparison to the STZ-induced diabetic group and these results were resettled to the control group (Table 7).

UV spectrophotometric study

From UV spectrophotometric study it has been revealed that in hydromethanolic seed of *T. indica* contains specific bioingredient(s) or neutraceuticals i.e. phenolic compounds and flavonoids (Table 8).

DISCUSSION

The present study focuses the antidiabetic, antihyperlipidemic capacities, as well as carbohydrate metabolic disorders management efficacy of the hydro-methanolic extract of seeds of *T. indica* in STZ-induced diabetic male albino rat. In this study, carbohydrate and lipid metabolic disorders in STZ-induced diabetic rat have been established by the study of blood

glucose, hepatic and skeletal muscle glycogen content, cholesterol, triglyceride, and lipoproteins. These results are in the same line of our previous studies[16,37] and of others[38,39]. Glycemic controlling capacity of *T. indica* seed extract in STZ-induced diabetic state has been supported here by the correction of blood glucose, and glycogen content in liver and muscle, important parameters in this study[40]. The significant elevation in the level of glucose in diabetic rats could be due to the destruction of pancreatic β-cells by STZ[41]. The capacity of *T. indica* to decrease the elevated blood glucose to normal level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes[42]. The possible mechanism by which *T. indica* bring about it hypoglycemic action in diabetic rats may be by potentiating the insulin effect of serum by increasing the pancreatic secretion of insulin from the existing β-cells similar to that observed after sulphonylurea administration[43]. As reported earlier[44], in the present study also, hepatic and skeletal muscle glycogen content was reduced significantly in diabetic rats as compared to the control animals. The decreases in both liver and skeletal muscle glycogen content observed in this study may be due to lack of insulin in the diabetic state and this type of result probably due to the inactivation of glycogen synthase system. Supplementation of *T. indica* or glibenclamide prevent this alteration in glycogen level. This prevention of depletion of glycogen in the liver and muscle is possible due to either stimulation of insulin release from β cells[45] or due to insulinomimetic activity of some component of the seed resulting in direct peripheral glucose uptake or due to a combination of the two.

One of the prime enzymes in the glycolytic pathway is hexokinase, which is insulin dependent and plays an important role in the maintenance of glucose homeostasis and all the cells that metabolized glucose by ATP to produce glucose -6-phosphate. The activity of hexokinase in liver was decreased significantly in STZ-induced diabetic rats in respect to control group, resulting in depletion of liver and muscle glycogen which is consistent with other[46]. Administration of hydromethanolic seed extract of *T. indica* at the dos of 80 mg for 14 days to the diabetic rats resulted in a significant increased and resettlement of liver hexokinase to the control level. This increase activity can causes increased glycolysis and increased utilization of glucose by elevating serum insulin level[47,48].

In this study, *T. indica* significantly reduced the TC, TG, LDLc and VLDLc levels with an increase of HDLc in STZ-induced diabetic rats compared to diabetic rats. This may be due to the insulinotropic effect or insulin secretagogue activity of *T. indicia*. This extract treated diabetic rats showed decrease in atherogenic index and increase in percentage of protection against atherogenecity. Decrease in atherogenic index is due to increase in HDLc levels after the treatment. HDLc is known to play an important role in the transport of cholesterol from peripheral cells to the liver by a pathway termed reverse cholesterol transport, and is considered to be a cardio protective lipid. The existence of negative correlation between HDLc and atherosclerosis resulted in improvement of the percentage of protection against atherogenecity in STZ-induced diabetic rats[49].

Under hyperglycemic condition disturbances in carbohydrate, lipid and protein metabolism are likely to affects hepatic and renal functions. Hence our study was also focused to know the protective activity of *T. indica* against hepatic and renal damage caused by diabetes.

In the presence study diabetic rats had showed low body weight as compared to the normal rats. Corrections of body weight of diabetic rats in seed extract treated condition may be due to improving the glycemic control mechanism and insulin secretion from remnant pancreatic β cells in diabetic rats[43]. This study reveals that hepatic and renal transaminases enzyme such as GOT and GPT were used in the evaluation of hepatic and renal damage. GOT and GPT levels are the most useful tests for the detection of hepatic cell damage, because both are present in high concentration in hepatocytes. In diabetic rats an increases in these enzyme activities reflects active liver damage. Increase level of GOT and GPT under insulin deficiency[50] have been related with increased gluconeogenesis and ketogenesis during diabetes. Moreover increased level of these enzymes is reported to be associated with liver dysfunction in diabetes[51].

The antidiabetic and antihyperlipidemic effect of *T. indica* is due to its ability to stimulate the secretion of insulin from the existing pancreatic β cells. Several authors reported flavonoids, sterols, alkaloids and phenolic compounds as bioactive antidiabetic principles. The phytochemical examination of *T. indica* reveals the presence of specific biomolecule (s) or neutraceuticals such as flavonoids and polyphenolic compounds.

CONCLUSION

In conclusion, it may be stated that the hydromethanolic extract of *T. indica* seed may provide a new therapeutic avenue against antidiabetic and antihyperlipidemic capacities along with diabetes related complications.

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Table 1. Effect of hydromethanolic extract of seed of *T. indica* on body weight in streptozotocin-induced diabetic male albino rats

Group	Body weight (g)
Control	138.9±9.7[a]
Diabetic	109.7±10.2[b]
Diabetic + *T. indica* supplement	137.5±9.4[a]
Diabetic + Glibenclamide	136.6±7.8[a]

Data are expressed as Mean ±SEM (n=8). ANOVA followed by multiple comparison two tail 't' test. Values with different superscripts ([a,b]) in each vertical column differ from each other significantly, P<0.05.

Table 2. Effect of hydromethanolic extract of seed of *T. indica* for 14 days treatment on blood glucose level in streptozotocin-induced diabetic male albino rats

Group	Fasting blood glucose level (mg/dl)		
	At the time of grouping	Days of *T. indica* supplement	
		0 day	14 days
Control	78.7±8.2[a]	82.0±9.6[a]	80.9±7.6[a]
Diabetic	79.1±8.7[a]	329.5±8.3[b]	325.4±10.4[b]
Diabetic + *T. indica* supplement	80.6±8.2[a]	324.6±10.9[b]	88.4±7.6[a]
Diabetic + Glibenclamide	79.8±7.3[a]	320.7±6.5[b]	99.6±6.6[c]

Data are expressed as Mean ±SEM (n=8), ANOVA followed by multiple comparison two tail 't' test. In each vertical column mean values with different superscripts ([a,b,c]) differ from each other significantly, P<0.05.

Table 3. Quantification of glycogen content in liver and skeletal muscle of streptozotocin-induced diabetic male albino rats treated with hydromethanolic extract of seed of *T. indica*

Group	Glycogen level (µg of glucose/mg of tissue)	
	Liver	Skeletal muscle
Control	25.9±0.52[a]	25.7±0.58[a]
Diabetic	13.9±0.51[b]	14.1±0.51[b]
Diabetic + *T. indica* supplement	25.7±0.49[a]	26.4±0.52[a]
Diabetic + Glibenclamide	26.1±0.52[a]	25.1±0.58[a]

Data are expressed as Mean ±SEM (n=8). ANOVA followed by multiple comparison two tail 't' test. Values with different superscripts ([a,b]) in each vertical column differ from each other significantly, P<0.05.

Table 4. Hexokinase activity of hepatic tissue in streptozotocin-induced diabetic male albino rats supplemented with hydromethanolic extract of seed of *T. indica*

Group	Hexokinase (Unit/mg of tissue)
Control	3.56±0.12[a]
Diabetic	1.51±0.10[b]
Diabetic + *T. indica* supplement	3.52±0.13[c]
Diabetic + Glibenclamide	3.48±0.14[a]

Each value represents Mean ±SEM (n=8). ANOVA followed by multiple comparison two tail 't' test. In each vertical column mean values with different superscripts (a,b) differ from each other significantly, P<0.05.

Table 5. Effect of hydromethanolic extract of seed of *T. indica* on serum total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) in streptozotocin-induced diabetic male albino rats

Group	Serum lipids (mg/dl)				
	TC	TG	HDLc	LDLc	VLDLc
Control	74.5±4.2[a]	80.6±5.1[a]	63.6±4.2[a]	73.6±4.8[a]	37.56±4.2[a]
Diabetic	125.5±5.2[b]	220.1±6.1[b]	39.1±5.3[b]	116.1±5.0[b]	60.4±4.9[b]
Diabetic + *T. indica* supplement	75.5±5.4[a]	78.2±4.6[a]	60.9±5.3[a]	75.5±5.1[a]	39.5±4.2[a]
Diabetic + Glibenclamide	73.8±4.4[a]	84.8±5.4[a]	62.8±5.1[a]	77.8±4.1[a]	41.4±4.4[a]

Data are expressed as Mean ±SEM (n=8). ANOVA followed by multiple comparison two tail 't' test. Values with different superscripts (a,b) in each vertical column differ from each other significantly, P<0.05.

Table 6. Serum insulin level in streptozotocin-induced diabetic male albino rats supplemented with hydromethanolic extract of seed of *T. indica*, after 14 days of treatment

Group	Serum insulin level (µU/ml)
Control	13.92±0.22[a]
Diabetic	4.31±0.13[b]
Diabetic + *T. indica* supplement	13.78±0.26[a]
Diabetic + Glibenclamide	13.88±0.27[a]

Each value represents Mean ±SEM (n=8). ANOVA followed by multiple comparison two tail 't' test. In vertical column mean values with different superscripts ([a,b]) differ from each other significantly, P<0.05.

Table 7. Activities of GOT and GPT in liver and kidney of streptozotocin-induced diabetic male albino rats administered with hydromethanolic extract of seed of *T. indica*, and glibenclamide

Group	GOT (Unit/mg of tissue)		GPT (Unit/mg of tissue)	
	Liver	Kidney	Liver	Kidney
Control	16.1±0.56[a]	15.6±0.52[a]	13.6±0.51[a]	12.5±0.57[a]
Diabetic	29.4±0.64[b]	26.9±0.60[b]	25.8±0.59[b]	25.5±0.54[b]
Diabetic + *T. indica* supplement	17.0±0.61[a]	16.2±0.55[a]	14.5±0.56[a]	12.9±0.53[a]
Diabetic + Glibenclamide	16.8 ±0.64[a]	16.8±0.56[a]	12.6±0.56[a]	13.7±0.58[a]

Data are represents as mean ± SEM; n=8. ANOVA followed by multiple comparison two tail "t" test. In each vertical column, values with different superscripts (a,b) differ from each other significantly, $P<0.05$.

Table 8. UV spectrophotometric results of active nutraceuticals present in hydromethanolic mixture extract of seed of *T. indica*

Seed of *T. indica*	Phenolic compounds (nM/ml)	Flavonoids (µg/ml)
Hydromethanolic extract (1:1)	46.01±2.95	44.61±5.01