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HYPOGLYCEMIC AND ANTIOXIDANT POTENCY OF SEED EXTRACT OF *Tamarindus indica* LINN ON STREPTOZOTOCIN INDUCED DIABETES MELLITUS IN MALE RAT: A DOSE DEPENDENT STUDY.

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ABSTRACT

The aim of this study is to investigate dose dependent antidiabetic and antioxidant effects of aqueous methanol extract of seed of *Tamarindus indica* Linn on fasting and postprandial blood glucose level, liver and muscle glycogen content, haemoglobin level, glycosylated haemoglobin level, liver hexokinase activity, serum insulin level and on the antioxidant enzymes such as catalase, peroxidase, glutathione-S-transferase, superoxide dismutase along with lipid peroxidation such as thiobarbituric acid reactive substances and conjugated dienes in streptozotocin induced diabetic rats. Diabetic rats show a significant diminution in the liver hexokinase activity, serum insulin level, haemoglobin level and antioxidant enzymes activities in liver, kidney and skeletal muscle as well as elevation of fasting, postprandial blood glucose level, glycosylated haemoglobin level and lipid peroxidation. Supplementation of aqueous methanol (1:1) extract of seed of *Tamarindus indica* at the dose of 40 mg or 80 mg or 120 mg/100 g body weight/ day by gavage for 14 days to diabetic rats resulted a significant protection of the above mentioned parameters towards the control level at 40 mg dose but complete recovery was noted at 80 mg or 120 mg doses. The applied doses of this extract have no metabolic toxicity which was confirmed here by the assessment of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities. The antidiabetic activity of this extract was confirmed after a comparison with standard oral hypoglycemic drug i.e. glibenclamide. That major nutraceuticals presents in aqueous methanol extract are flavonoids and phenolic compounds.

KEYWORDS: ***Tamarindus indica*, diabetes, antioxidant enzymes, lipid peroxidation, glibenclamide.**

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INTRODUCTION

Diabetes mellitus, a chronic, non-communicable metabolic disorder characterized by hyperglycemia resulting from malfunction in insulin secretion and/ or insulin action caused by impaired regulation of carbohydrate, protein and lipid metabolism[1]. The number of the diabetic population around the globe continuously increasing with a current estimate of 371 million cases in 2012 and it is expected to reach 552 million by 2030[2]. It is also estimated that 5% of all deaths in the world are caused by diabetes and the number is rapidly increasing. Oxidative stress, an excessive production of reactive oxygen species (ROS) above the body's antioxidant capacity, has been implicated in the development of many pathophysiological conditions including diabetes, hypertension, cancer and the process of aging[3-4]. Various studies have shown that diabetes mellitus is associated with oxidative stress, leading to an increased production of ROS, including superoxide radical, hydrogen peroxide and hydroxyl radical or reduction of antioxidant defense system[5-6]. Currently, oxidative stress is suggested as one of the mechanism underlying diabetes and diabetic complications[7]. Oxidative stress induced by chronic hyperglycemia has been associated with dysfunction and apoptosis of several cell types, including pancreatic β cells[8]. Glucose itself and hyperglycemia increase protein glycosylation which are important source of free radicals[9]. Elevated glucose causes significant non-enzymatic glycosylation of proteins in diabetes[10]. Cell utilizes oxygen in the making of energy, but that process produces free radical, toxic byproducts, that damage DNA and protein. The formation of ROS is prevented by an antioxidant system that include non-enzymatic antioxidants (ascorbic acid, selenium, glutathione, β carotene, lipoic acid and tocopherol), enzyme regenerating the reduced forms of antioxidants, and ROS scavenging enzymes such as catalase, glutathione peroxidase and superoxide dismutase[11-12]. Recent decades have shown a resurgent interest in traditional plant medicine for hypoglycemic and antioxidant properties[13-14]. In practice, it is being increasingly recognized to be an alternative approach to modern medicine. The World Health Organization (WHO) has also recommended that this practice should be encouraged especially in countries where access to conventional treatment of diabetes mellitus is not adequate[15]. The first scientific study on *Tamarindus indica* about its antidiabetic activity was conducted by us and the result were highly encouraging where we reported that the aqueous extract of the seed of *T. indica* has an antidiabetic potency by correcting the carbohydrate metabolic enzymes and fasting blood glucose level[16]. Moreover, we have also reported that hyperlipidemia which develops in STZ-induced diabetic states is also recovered significantly after the aqueous extract supplementation[17]. *T. indica* Linn is an indigenous tropical tree type of plant, upto 20-25 ft tall, grown abundantly in all over India, Bangladesh, Pakistan and Burma, belonging to Caesalpiniaceae family and is attributed to have many traditional medicinal properties. The fruits and seed extract have been reported earlier for antibacterial and antifungal activities[18-19], it's fruits is a traditional meal on bioavalibility of aspirin tablets[20]. *T. indica* seed have rich of polysaccharides that have lack of carcinogenic potential [21]. Their fruits are also found mainly in summer season and its seed coat is brown black in colour though the kernel is white in colour. The objective of the present study was to investigate the possible antihyperglycemic and antioxidant potency of seed of *T. indica* Linn in STZ-induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin was purchased from (Sigma Chemical Company, St. Louis, MO, USA). All other chemicals and solvent used in this experiment were of analytical grade. Insulin enzyme linked immunosorbant assay (ELISA) kit purchased from Boehringer Mannheim Diagnostic, Mannheim (Germany).

Plant Material

Plant materials used in this study considered of the seed of *T. indica* 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 was kept in the Central National Herbarium (CAL), BSI, Shibpur, Howrah (voucher specimen number, HPCH No-1).

Preparation of aqueous methanol mixture extract of seed of T. indica

Aqueous methanol (1:1) mixture extract of seed of *T. indica* was prepared according to the method of National Institute of Health and Family Welfare (NIHFW), New Delhi[22]. 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 dissolved in 500 ml of aqueous 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 aqueous methanol extract was obtained. The suspension was then filtered by coarse sieve filter paper and then evaporated to dryness under reduced pressure at low temperature (40^0C - 42^0C) in Buchi rotavapor R-200. A deep brown material was obtained (4 g/100 g of the dried seeds powder). It was stored at (0-4)^{0}C until used. When needed, the residual extract was suspended in olive oil and used in the study.

Selection of animals and care

Fifty matured Wistar strain male albino normoglycaemic rats (*Rattus norvegicus*) having fasting blood glucose level 74±5 mg/dl, 3 months of age and weighing 135±10 g were selected in this experiment. The animals 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). The study was approved by our 'Institutional Ethical Committee'.

Toxicity studies

Acute toxicity related to the determination of LD_{50} value was performed with different concentration of the extract. Overnight fasted rats were divided into four groups (n=6). Three of these groups of animals were orally administered with the seed extract at the dose of 40, 80 and 120 mg/100 g of body weight and one group was used as a control. The animals were observed continuously for the initial 2 h, intermittently for the next 6 h of the study for the following symptoms[23].

i. Behavioural profile: alertness, restlessness, irritability and fearfulness.
ii. Neurological profile: spontaneous activity, reactivity, touch response, tremors.
iii. Autonomic profile: defecation and urination.

The numbers of deaths, if any were recorded between 24 h and 72 h.

Induction of experimental diabetes

Diabetes was made in overnight fasted forty four 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. Food was provided to them 2 h after injection. 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 schedule

Experimental design

Six rats were considered in control group. Thirty diabetic rats were divided into five equal groups and each group consisted to six rats and schedule is as follows:

Group I: Control group

Six rats 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 extracts and glibenclamide treatment to group III, IV, V, VI 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: Aqueous methanol (1:1) extract administered (40 mg dose) group

Six diabetic animals were subjected to oral administration of aqueous methanol extract of seed of *T. indica* at the dose of 40 mg/0.5 ml olive oil/100 g body weight/day/rat for 14 days.

Group IV: Aqueous methanol (1:1) extract treated (80 mg dose) group

Another six diabetic animals were subjected to oral administration of aqueous methanol 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 V: Aqueous methanol (1:1) extract treated (120 mg dose) group

Diabetic rats were forcefully fed oral intubation of aqueous methanol extract of *T. indica* at the dose of 120 mg/0.5 ml olive oil/100 g body weight/day/rat for 14 days.

Group VI : Glibenclamide co-treated (60 μg dose) group

Diabetic rats were given 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 administered at 8 A.M. and food was given at 10 A.M. of each day. On 15th day of experiment final body weight of all the animals were recorded and they were sacrificed under light ether anesthesia. 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, antioxidant enzymes, product of free radicals and transaminases activities. Blood was collected from the dorsal aorta by a syringe. A portion of the blood was used for quantification of haemoglobin and glycated haemoglobin and another part was used for separation of serum by centrifugation at 4000 rpm for 10 minutes for the estimation of serum insulin assay.

Analytical procedures

Estimation of blood glucose was carried out by using single touch glucometer (Life Scan, Johnson and Johnson, USA)[25]. An activity of hexokinase in liver was assayed by the method Chou and Wilson[26]. The liver and skeletal muscle glycogen content was quantified by the method of Sadasivam and Manickam[27]. Serum insulin level was assayed by using ELISA technique[28]. Biochemical estimation of antioxidant enzyme activities such as catalase (CAT) and peroxidase (Px) was measured according to method of Beers and Sizer[29] and Sadasivam and Manickam[30]. Superoxide dismutase (SOD) and glutathione-S-transferase activities in liver, kidney and skeletal muscle was estimated according to standard protocol[31-32]. An estimation of lipid peroxidation was performed from a concentration of thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CD) according to - Ohkawa et al[33] and Slater[34] respectively. Haemoglobin level was measured according to standard method[35]. HbA1c was estimated by the method of Eross et al[36]. Activities of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase in lever and kidney were assessed according to Henry et al[37]. Flavonoids and total phenolic derivatives of *T. indica* seed extract were determined according to method of Costa[38], Singleton and Rossi[39] respectively.

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[40]. Difference were considered significantly when $P<0.05$.

RESULTS

Toxicity studies

There was no death or toxic reaction observed at any of the concentrations of the extract used in the study. Acute toxicity studies revealed the non-toxic nature of the aqueous methanol extract of seed of *T. indica* at a dose of up to 120 mg/100 g body weight. From this result, it may be stated that the applied dose 80 mg/0.5 ml olive oil/100 g body weight/rat) throughout the experiment is biologically safe.

Fasting and postprandial blood glucose level

Diabetes induced by STZ resulted significant elevation in fasting and postprandial blood glucose levels in comparison to control group (Table 1). Co-administration of aqueous methanol mixture extract of seed of *T. indica* at the dose of 80 mg or 120 mg/0.5 ml olive oil/100 g body weight/day/rat for 14 days to the diabetic animals resulted a significant diminution in fasting and postprandial blood glucose levels when compared to STZ-induced diabetic group and the result was resettled to the control group (Table 1). On the other hand, a significant diminution in fasting and postprandial blood glucose level was noted after co-treatment of glibenclamide at the dose of 60 μg/0.5 ml deionized water/100 g body weight/day/rat for 14 days in respect to STZ-treated diabetic animals, which completely recovered to the control level (Table 1), though after co-administration of aforesaid drug in the above mentioned dose and duration, such parameters were insignificantly differed in respect to the doses of 80 mg/day and 120 mg/day of extract co-administered groups (Table 1). After co-administration of this extract at the concentration of 40 mg/0.5 ml olive oil/100 g body weight/day/rat for 14 days to the diabetic animals, a significant decrease in these parameters were observed in respect to diabetic group but the values were not attained to the level of the control group (Table 1).

Glycogen content in liver and skeletal muscle

In this study, it was noted that glycogen contents in liver and skeletal muscle were significantly decreased in diabetic group in respect to the control group (Table 2). But after co-administration of aqueous methanol extract of seed of *T. indica* at the dose of 40 mg for 14 days to the diabetic rats resulted a significant elevation in this parameter in respect to diabetic group of animals and the said parameter in liver and skeletal muscle was partially protected towards the control group (Table 2). On the other hand, the above mentioned parameter of the aforesaid tissues were significantly elevated after co-administration of said extract at the dose of 80 mg or 120 mg/ dose to the diabetic animals when compared to the diabetic group of animals and the values were insignificantly differed from the control group (Table 2**).** Co-administration of glibenclamide to diabetic animals resulted significant elevation in this parameter in above tissues and the result was completely corrected to the control group (Table 2). Whereas, a comparison among 80 mg/day or 120 mg/day dose of extract co-administration and glibenclamide co-treatment group, resulted insignificant difference in glycogen level in above mentioned tissues (Table 2).

Hexokinase activity in liver

Activity of hexokinase in liver was diminished in diabetic group in comparison to the control group (Table 3), but after co-administration of aqueous methanol extract of seed of *T. indica* at the dose of 80 mg or 120 mg for 14 days to the diabetic animals, resulted a significant elevation in the above hepatic enzyme in respect to diabetic group, which was corrected completely when compared to the control group (Table 3). Above mentioned parameter was elevated significantly in comparison to STZ-treated diabetic group after co-administration of glibenclamide to the diabetic animals and the value was resettled to the control group (Table 3). In contrast, aforesaid enzyme in liver was insignificantly differed among the results of 80 mg, 120 mg doses of *T. indica* and glibenclamide co-administered groups (Table 3**).** Whereas, co-administration of the above extract at the dose of 40 mg resulted a significant elevation in hexokinase in above mentioned tissue sample and the value was partially reestablished towards the control group (Table 3).

Serum insulin level

Serum insulin level was decreased significantly in diabetic group when compared to the control group (Table 4). Co-administration of aqueous methanol extract of seed of *T. indica* at the dose of 80 mg or 120 mg for 14 days to the diabetic rats resulted a significant increase in the said parameter when compared to the diabetic group and side by side, the insulin level in serum was found to resettle to the control group (Table 4). On the other hand, a significant elevation in the aforesaid parameter was noted after co-administration of above extract at the dose of 40 mg for the said duration to the diabetic animals when compared to diabetic group and the result of which regained towards the control group (Table 4). Co-administration of glibenclamide to the diabetic rats resulted a significant elevation in serum insulin level in comparison to diabetic group and the level of the specified hormone was completely corrected when compared to the control group (Table 4). Insignificant difference in serum insulin level was noted among 80 mg or 120 mg dose co-administered group and glibenclamide co-treated group (Table 4).

Activities of superoxide dismutase and glutathione-S-transferase in liver, kidney and skeletal muscle

Superoxide dismutase and glutathione-S-transferase activities in liver, kidney and skeletal muscle were decreased significantly in diabetic rats in compared to the control animals (Table 5). Co-administration of aqueous methanol extract of 40 mg dose for 14 days to the diabetic rats a significant elevation in the activities of aforesaid antioxidant enzymes in above mentioned tissues in compared to diabetic group and these parameters were partially resettled towards the control group (Table 5). On the other hand, after 14 days of co-administration of above mentioned extract at 80 mg or 120 mg to the diabetic animals, a complete protection in these enzymes were recorded in the above mentioned tissues in respect to the control group (Table 5), Co-administration of glibenclamide at the concentration of 60 μg/0.5 ml deionized water/100 g body weight/day/rat for 14 days resulted a significant elevation in the above mentioned enzymes in aforesaid tissues were noted in comparison to STZ-induced diabetic group and these enzyme activities were corrected completely to the control group (Table 5). These parameters were insignificantly differed when the results were compared among 80 mg or 120 mg doses of extract co-administration groups and glibenclamide co-treated group (Table 5).

Catalase and peroxidase activities in liver, kidney and skeletal muscle

Activities of catalase and peroxidase in liver, kidney and skeletal muscle were significantly diminished in diabetic group in comparison to the control group (Figure 1). Co-administration of aqueous methanol extract of 40 mg dose for 14 days to the diabetic rats a significant elevation in the activities of above mentioned antioxidant enzymes in liver, kidney and skeletal muscle in comparison to diabetic group and these parameters were partially resettled towards the control group (Figure 1). In contrast, after 14 days of co-administration of above mentioned extract at the dose of 80 mg or 120 mg to the diabetic animals, a complete recovery in these enzymes were recorded in the above mentioned tissues in respect to the control group (Figure 1). Co-administration of glibenclamide at the concentration of 60 μg/0.5 ml deionized water/100 g body weight/day/rat for 14 days resulted a significant elevation in the above mentioned enzymes in aforesaid tissues samples were noted in comparison to STZ-induced diabetic group and these enzyme activities were corrected completely to the control group (Figure 1). These parameters were insignificantly differed when the results were compared among 80 mg or 120 mg doses of extract co-administration groups and glibenclamide co-administration group (Figure 1).

Quantification of thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CD) in liver, kidney and skeletal muscle

Quantification of TBARS and CD levels were significantly differed in liver, kidney and skeletal muscle between diabetic group and control group (Figure 2). Whereas, after 14 days of co-administration of aqueous

methanol mixture extract at the concentration of 80 mg or 120 mg/0.5 ml olive oil/100 g body weight/day/rat to the diabetic animals resulted a significant diminution in the said parameters in respect to the diabetic group which was resettled to the control group (Figure 2). Co-administration of the said extract at the concentration of 40 mg/0.5 ml olive oil/100 g body weight/day/rat for the same duration of the diabetic animals, the levels of TBARS and CD in respective tissues were decreased significantly when compared to the STZ-treated diabetic group of animals that resettled towards the control group (Figure 2). On the other hand, co-administration of glibenclamide at the dose of 60 μg for 14 days to the diabetic animals resulted complete protection in these parameters in aforesaid tissue samples when compared to diabetic group and the values were recovered to the control group (Figure 2). Beside this, after 80 mg or 120 mg co-administered group and glibenclamide co-treated group, TBARS and CD levels in liver, kidney and skeletal muscle samples were insignificantly differed from each other (Figure 2).

Transaminase activities in liver and kidney

Liver and kidney GOT and GPT activities were elevated significantly in STZ-induced diabetic group in respect to control group (Table 6). On the other hand, co-administration of aqueous methanol mixture of seed extract of *T. indica* at the dose of 40 mg/0.5 ml olive oil/100 g body weight/day/rat for 14 days for the diabetic animals resulted a significant diminution in the said parameters when compared to the diabetic group and these results were resettled towards the control group (Table 6**).** In contrast, after co-treatment of said extract at the dose of 80 mg or 120 mg or glibenclamide 60 μg to the diabetic animals resulted a complete recovery in above mentioned parameters in liver and kidney in respect to the control group (Table 6). Comparison between 80 mg/day extract or 120 mg/day co-administered group and glibenclamide co-treated group resulted an insignificant difference in GOT and GPT activities in liver and kidney (Table 6).

Haemoglobin and glycated haemoglobin levels

The haemoglobin level was decreased significantly along with an increase in glycated haemoglobin (HbA_1C) in diabetic group when compared to the control group (Table 7). Co-administration of aqueous methanol extract of seed of *T. indica* at the dose of 80 mg or 120 mg for 14 days to the diabetic rats resulted a significant increase in the said parameter when compared to the diabetic group and side by side, haemoglobin and glycated haemoglobin levels in blood was found to resettle to the control group (Table 7). On the other hand, a significant elevation in the aforesaid parameter was noted after co-administration of the above extract at the dose of 40 mg for the said duration of the diabetic animals when compared to diabetic group and the result of which regained towards the control group (Table 7). Co-administration of glibenclamide to the diabetic rats resulted in a significant elevation in haemoglobin along with diminution of HbA_1C levels of blood in comparison to diabetic group and the level of these parameters were completely corrected when compared to the control group (Table 7). Insignificant difference of the above mention parameters were noted among 80 mg or 120 mg dose co-administered group and glibenclamide co-treated group (Table 7).

UV spectrophotometric study

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

Table 1

Dose dependent effect of aqueous methanol mixture extract of seed of T. indica on fasting and postprandial blood glucose level in streptozotocin-induced diabetic rats.

Group	Fasting blood glucose level (mg/dl)			Postprandial blood glucose level (mg/dl)
	At the time of grouping	Days of *T. indica* supplement		
		0 day	14 days	
Control	80.7±6.0[a]	82.7±5.7[a]	81.4±5.8[a]	124.8±6.1[a]
Diabetic	82.8±5.7[a]	342.0±7.0[b]	331.9±6.8[b]	362.0±6.6[b]
Diabetic + *T. indica* supplement 40 mg	81.0±5.6[a]	339.7±6.6[b]	191.6±6.3[c]	228.3±6.5[c]
Diabetic + *T. indica* supplement 80 mg	80.7±6.0[a]	341.7±6.9[b]	85.6±6.2[a]	127.3±6.0[a]
Diabetic + *T. indica* supplement 120 mg	80.4±6.2[a]	345.5±6.6[b]	83.4±5.8[a]	126.7±6.5[a]
Diabetic + Glibenclamide 60 µg	81.4±6.1[a]	344.4±6.5[b]	84.6±6.3[a]	127.5±6.4[a]

Data are expressed as Mean ± SEM (n=6) . ANOVA followed by multiple comparison two-tail 't' test. In vertical column mean values with different superscripts ([a,b,c]) differ from each other significantly, P<0.05.

Table 2

Glycogen content in liver and skeletal muscle of streptozotocin-induced diabetic male albino rats treated with aqueous methanol extract of seed of T. indica in comparison with glibenclamide treatment.

Group	Glycogen level (µg of glucose/mg of tissue)	
	Liver	Skeletal muscle
Control	27.9±0.64[a]	26.7±0.63[a]
Diabetic	16.2±0.56[b]	14.4±0.54[b]
Diabetic + *T. indica* supplement 40 mg	22.3±0.58[c]	20.6±0.50[c]
Diabetic + *T. indica* supplement 80 mg	27.3±0.53[a]	26.4±0.55[a]
Diabetic + *T. indica* supplement 120 mg	28.1±0.64[a]	26.9±0.57[a]
Diabetic + Glibenclamide 60 µg	27.6±0.61[a]	26.3±0.58[a]

Data are represents as Mean ±SEM (n=6). ANOVA followed by multiple comparison two-tail "t" test. In vertical column mean values with different superscripts ([a,b,c]) differ from each other significantly, P<0.05.

Table 3

Liver hexokinase activity in streptozotocin-induced diabetic male albino rats treated with aqueous methanol extract of seed of T. indica or glibenclamide: a dose dependent effect.

Group	Hexokinase (Unit/mg of tissue)
Control	3.99±0.13[a]
Diabetic	1.68±0.14[b]
Diabetic + *T. indica* supplement 40 mg	3.00±0.12[c]
Diabetic + *T. indica* supplement 80 mg	3.92±0.14[a]
Diabetic + *T. indica* supplement 120 mg	3.94±0.17[a]
Diabetic + Glibenclamide 60 µg	3.84±0.15[a]

Each value represents Mean ±SEM (n=6). ANOVA followed by multiple comparison two tail 't' test. In vertical column mean values with different superscripts ([a,b,c]) differ from each other significantly, P<0.05.

Table 4

Dose dependent effect of aqueous methanol mixture extract of seed of T. indica and glibenclamide onserum insulin level in streptozotocin-induced diabetic male albino rats, after 14 days of treatment.

Group	Serum insulin level (µU/ml)
Control	14.18±0.21[a]
Diabetic	4.06±0.34[b]
Diabetic + *T. indica* supplement 40 mg	8.56±0.22[c]
Diabetic + *T. indica* supplement 80 mg	14.16±0.25[a]
Diabetic + *T. indica* supplement 120 mg	14.23±0.27[a]
Diabetic + Glibenclamide 60 µg	13.98±0.29[a]

Each value represents Mean ±SEM (n=6). ANOVA followed by multiple comparison two tail 't' test. In vertical column mean values with different superscripts ([a,b,c]) differ from each other significantly, P<0.05.

Table 5

Effect of aqueous methanol extract of seed of T. indica on superoxide dismutase and glutathione-S-transferage activities in liver, kidney and skeletal muscle in streptozotocin-induced diabetic male albino rats.

Group	Superoxide dismutase (Unit /mg of tissue)			Glutathione-S-transferage (Unit /mg of tissue)		
	Liver	Kidney	Skeletal muscle	Liver	Kidney	Skeletal muscle
Control	1.63±0.03[a]	1.72±0.04[a]	1.63±0.03[a]	3.61±0.06[a]	3.43±0.07[a]	3.23±0.05[a]
Diabetic	0.82±0.02[b]	0.92±0.04[b]	0.82±0.03[b]	2.12±0.05[b]	1.99±0.05[b]	1.62±0.07[b]
Diabetic + *T. indica* supplement 40 mg	1.42±0.04[c]	1.37±0.03[c]	1.42±0.02[c]	2.93±0.04[c]	2.82±0.06[c]	2.42±0.06[c]
Diabetic + *T. indica* supplement 80 mg	1.58±0.02[a]	1.68±0.04[a]	1.58±0.03[a]	3.47±0.07[a]	3.38±0.05[a]	3.28±0.06[a]
Diabetic + *T. indica* supplement 120 mg	1.60±0.03[a]	1.76±0.04[a]	1.60±0.03[a]	3.67±0.05[a]	3.49±0.07[a]	3.30±0.05[a]
Diabetic + Glibenclamide 60 µg	1.53±0.04[a]	1.63±0.03[a]	1.53±0.04[a]	3.46±0.04[a]	3.35±0.06[a]	3.16±0.07[a]

Each value represents Mean ±SEM (n=6) , ANOVA followed by multiple comparison two-tail 't' test. In vertical column mean values with different superscripts ([a,b,c]) differ from each other significantly, P<0.05.

Table 6

Effect of aqueous methanol mixture extract of seed of T. indica on liver and kidney GOT and GPT activities in streptozotocin-induced diabetic rats: A dose dependent study.

Group	GOT (Unit/mg of tissue)		GPT (Unit/mg of tissue)	
	Liver	Kidney	Liver	Kidney
Control	15.4±0.55[a]	14.8±0.52[a]	12.6±0.53[a]	11.9±0.56[a]
Diabetic	29.1±0.68[b]	27.1±0.58[b]	26.2±0.62[b]	25.0±0.58[b]
Diabetic + *T. indica* 40 mg	23.5±0.60[c]	21.8±0.54[c]	20.3±0.52[c]	19.9±0.55[c]
Diabetic + *T. indica* 80 mg	15.9±0.55[a]	14.9±0.52[a]	12.8±0.57[a]	12.1±0.52[a]
Diabetic + *T. indica* 120 mg	15.3±0.60[a]	14.7±0.56[a]	12.5±0.58[a]	11.5±0.51[a]
Diabetic + Glibenclamide 60 µg	15.7 ±0.61[a]	14.6±0.58[a]	12.8±0.59[a]	12.0±0.53[a]

Each value represents mean ± SEM; (n=6) . ANOVA followed by multiple two tail-t test. In each vertical column, the mean with different superscripts (a,b,c) differ from each other significantly, P<0.05.

Table 7

Dose dependent effect of aqueous methanol mixture extract of seed of T. indica and glibenclamide on haemoglobin and glycated haemoglobin levels in streptozotocin-induced diabetic male albino rats, after 14 days of treatment.

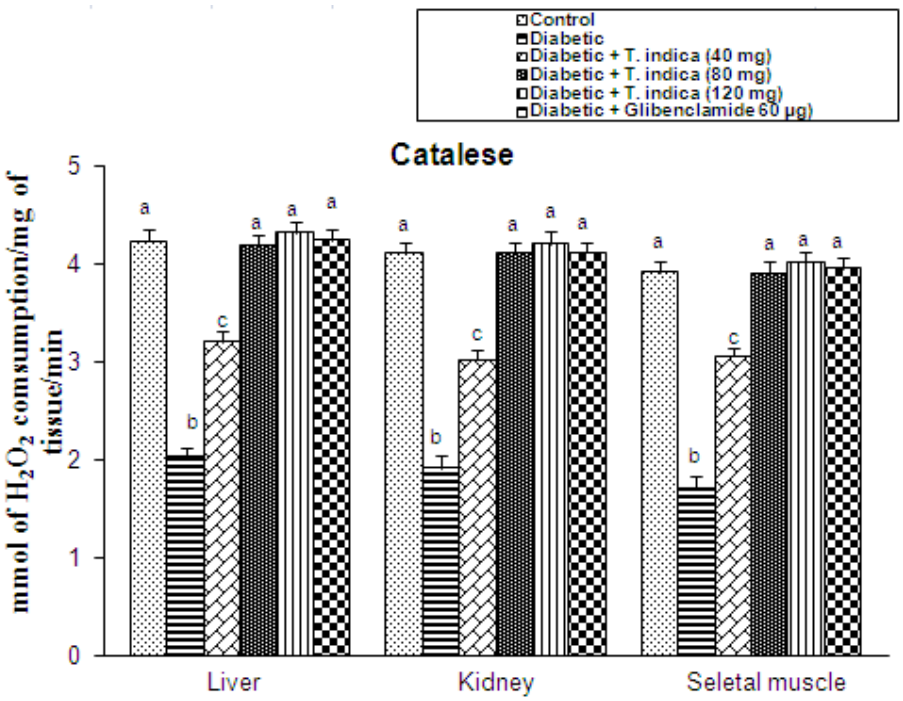
Group	Haemoglobin (g/dl)	Glycated haemoglobin (%)
Control	11.88±0.22[a]	5.90±0.13[a]
Diabetic	7.82±0.31[b]	11.86±0.14[b]
Diabetic + *T. indica* supplement 40 mg	9.56±0.32[c]	8.56±0.12[c]
Diabetic + *T. indica* supplement 80 mg	11.96±0.35[a]	5.96±0.15[a]
Diabetic + *T. indica* supplement 120 mg	11.73±0.28[a]	5.93±0.12[a]
Diabetic + Glibenclamide 60 µg	11.88±0.31[a]	6.08±0.11[a]

Each value represents Mean ±SEM (n=6). ANOVA followed by multiple comparison two tail 't' test. In vertical column mean values with different superscripts ([a,b,c]) differ from each other significantly, P<0.05.

Table 8

UV spectrophotometric results of different biomolecules present in aqueous methanol mixture extract of seed of T. indica.

Name of the extract of seed of *T. indica*	Phenolic compounds (nM/ml)	Flavonoids (µg/ml)
Aqueous methanol mixture extract (1:1)	47.12±3.24	45.49±5.67



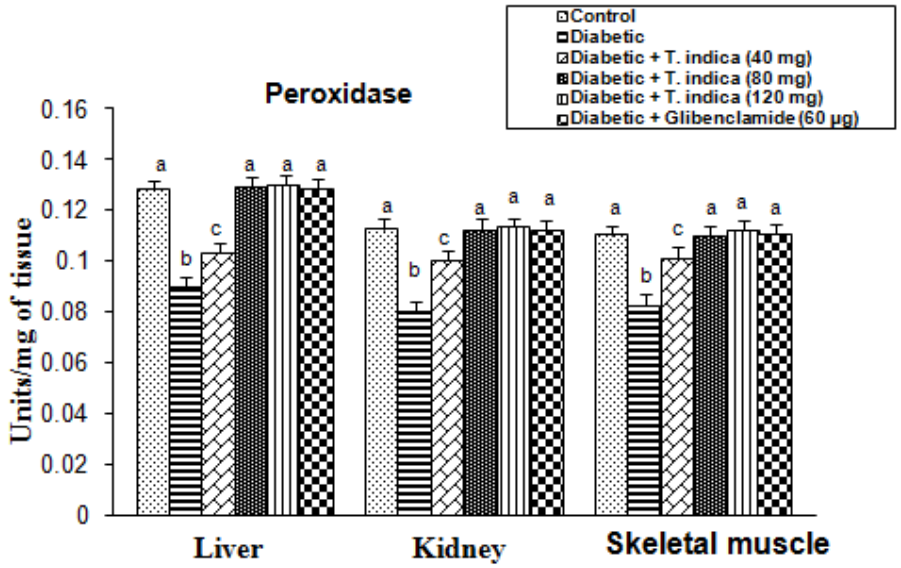
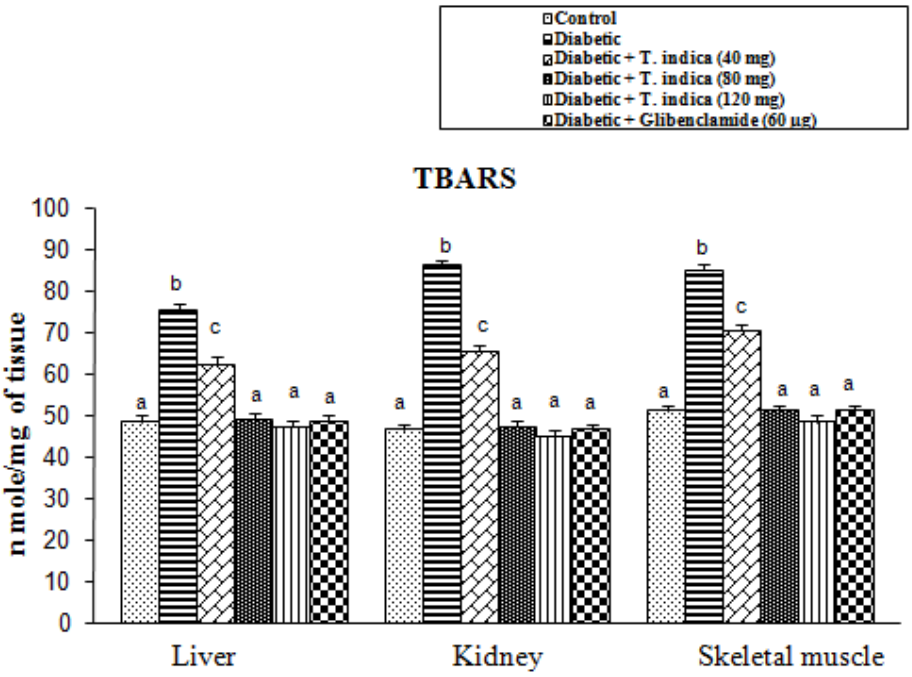


Figure 1. Dose dependent effect of aqueous methanol mixture extract of seed of *T. indica* on liver, kidney and skeletal muscle catalase and peroxidase activities in streptozotocin-induced diabetic male albino rats. Data are expressed as mean ± SEM; n=6. ANOVA followed by multiple two tail t-test. Figure with different superscripts(a,b,c) on each bar differ from each other significantly, P<0.05.



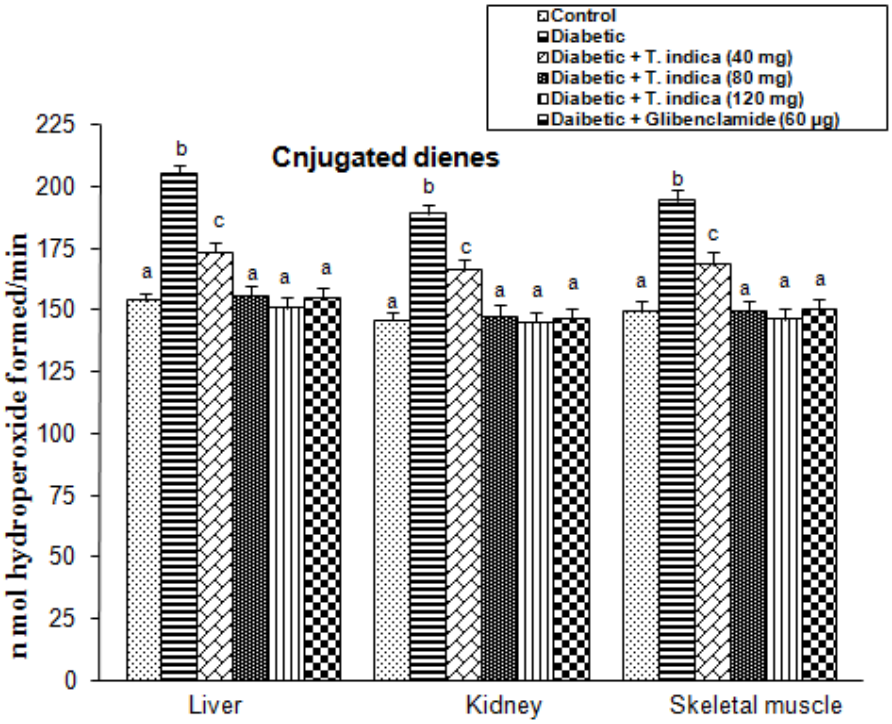


Figure 2

Dose dependent effect of aqueous methanol mixture extract of seed of T. indica on liver, kidney and skeletal muscle TBARS and CD levels in streptozotocin-induced diabetic male albino rats. Data are expressed as mean ± SEM; n=6. ANOVA followed by multiple two tail t-test. Figures with different superscripts (a,b,c) on each bar differ from each other significantly, P<0.05.

DISCUSSION

In the present study *T. indica* seed extract has shown significant antihyhyperglycemic, and potent antioxidant defense mechanism in STZ-induced diabetic rats. The antihyperglycemic effect of *T. indica* is due to its ability to stimulate the secretion of insulin from the surviving pancreatic β-cells. Several authors reported flavonoids, sterols, alkaloids and phenolic compound as bioactive antidiabetic principles[41]. The phytochemical examination of *T. indica* reveals the presence of flavonoids and polyphenolic compounds. We also observed that the status of lipid peroxidation and antioxidant enzymes were restored to the normal level in diabetic rat treated with *T. indica*. Thus we, feel that the *T. indica* prevents the oxidative damage induced by STZ by enhancing the activities of cellular enzymatic antioxidants. From the results of this experiment, it has been indicated that fasting and postprandial blood glucose levels were significantly elevated in STZ-induced diabetic rats. After co-administration of aqueous methanol mixture extract of seed of *T. indica* to the diabetic animals at the dose of 40 mg resulted a partial resettlement in these parameters to the control level but in 80 mg or 120 mg doses as well as 60 µg of glibenclamide, complete resettlement in these parameters was noted. This dose dependent diminution in fasting and postprandial blood glucose levels was confirmed here by the assessment of serum insulin level. In diabetic rats serum insulin level was diminished significantly in respect to control animals because STZ selectively destroy pancreatic β cells that diminish insulin secretion[42] and results high level of fasting and postprandial blood glucose along with low level of serum insulin. A partial resettlement in serum insulin level was noted after co-administration of aqueous methanol mixture extract of seed of *T. indica* to the diabetic animals at the dose of 40 mg though the complete reestablishment of this parameter was noted at the dose of 80 mg or 120 mg as well as glibenclamide

administered group. The possible mechanism by which the aforesaid extract of seed of *T. indica* possesses its hypoglycemic action to the diabetic rats in a dose dependent manner may be through the elevation of insulin secretion from the existing β cells[43]. To establish whether the dose dependent antidiabetogenic effect of the above mentioned extract in the diabetic animals is executed not by modulating glucose absorption from the intestine, but through glucose utilization from extra hepatic tissues.

To support the STZ-induced diabetic state we assessed the activity of the key glycolytic enzymes for the catabolism of glucose i.e., hexokinase. In diabetic state diminution in hexokinase activity in liver was observed. This result corroborates the findings of other investigators[44-45]. This may be due to low level of serum insulin because hexokinase activity is regulated by insulin[46]. Administration of aqueous methanol mixture extract of seed of *T. indica* to the diabetic animals at the dose of 80 mg or 120 mg as well as glibenclamide resulted complete resettlement in the activity of hexokinase enzyme to control level whereas, only partial recovery was noted at lower dose studied here, which focused that a critical amount of extract is required for induction of such corrective effect in STZ-treated diabetic rat In STZ-induced diabetic rats, glycogen contents in liver and skeletal muscles were decreased significantly from its control level. Such types of investigations have also been reported by other investigator[47] and also in conjunction with our previous report[16, 17]. Administration of aqueous methanol mixture extract of seed of *T. indica* at the dose of 80 mg or 120 mg or 60 μg of glibenclamide to the diabetic animals completely protect the liver and muscle glycogen contents, though a partial resettlement of this parameter was noted at 40 mg dose. The glycogen levels in various tissues, especially in skeletal muscle and liver is a direct reflection of insulin activities as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Since STZcauses selective destruction of β-cells of pancreas resulting in a marked decrease in insulin level, it is rational that glycogen levels in tissues were decreased as they depend on insulin for influx of glucose[48]. So, from this experiment, it may be predicted that a critical dose of this extract is required for the correction of diabetes induced glycogen depletion. In diabetes mellitus, hyperglycemia can simply inactive antioxidant enzymes such as CAT, Px, SOD and GST by glycating these proteins and induces oxidative stress which in turn causes lipid peroxidation[49-50]. Decreased antioxidant enzymes levels and enhanced lipid peroxidation have been well documented in STZ- induced diabetes mellitus[51-52]. In the enzymatic antioxidant defense system, SOD is one of the important enzymes and scavenges the superoxide radicals by converting them to H_2O_2 and molecular oxygen. Catalase and peroxidase are the important scavenging enzymes those remove toxic free radicals from the body[53]. STZ-induced experimental model animals exhibited oxidative stress due to persistent and chronic hyperglycemia, thereby, deplete the activity of antioxidant defense system and thus promote de novo free radical generation[54]. Oxidative stress has recently been shown to be responsible, at least in part, for the β-cell dysfunction. Our experimental results reveal that in STZ-induced diabetic rats the key antioxidant enzymes like CAT, Px, SOD and GST activities in liver, kidney and skeletal muscle were decreased significantly in comparison to control animals. After co-administration of glibenclamide or aqueous methanol mixture extract of seed of *T. indica* to the diabetic animals at the dose of 80 mg or 120 mg resulted complete correction in these enzyme activities in its control level. An incomplete resettlement was noted after co-administration of this extract at the dose of 40 mg dose to the control level. Such types of results indicate that STZ produces oxidative stress by means of diminishing the activity of antioxidant enzyme or their levels in the experimental animals that can be protected by this extract at a dose dependent manner that has been further confirmed from the treatment of glibenclamide in this experiment. Oxidative stress imposition in STZ-induced diabetic rats was confirmed here by the assessment of lipid peroxidation by means of quantification of TBARS and CD levels in liver, kidney and skeletal muscle. The levels of these parameters were elevated in the above

tissues in diabetic animals. Administration of this plant extract or glibenclamide to diabetic rats said parameters were completely resettled to the control levels. Hypoinsulinamia in diabetes initiates the β oxidation of fatty acids resulting in lipid peroxidation[55]. Our results reflect that aqueous methanol mixture extract of seed of *T. indica* possibly cause regeneration of β cells and thus increases the insulin level that can correct STZ-induced oxidative stress imposition. The antidiabetic activity of *T. indica* has been further supported here by the measurement of haemoglobin and glycated haemoglobin level as a marker for estimating the degree of protein glycation in diabetes mellitus. In diabetic condition decrease level of haemoglobin and increase level of glycated haemoglobin as reported by others[56]. In diabetes state, the excess glucose present in the blood reacts with haemoglobin to form glycated haemoglobin[57]. This decrease Hb as well as elevated HbA1c level was well regulated by *T. indica* supplementation, this could be due to an improvement in insulin secretion upon *T. indica* treatment. Biochemical assessment of GOT and GPT activities were conducted here to find out whether the extract at the applied doses as well as glibenclamide itself has any toxic effect in liver and kidney, as these parameters are considered as the indicators of toxicity assessment[58]. In STZ-induced diabetic rat, there was a significant elevation in GOT and GPT activities in above tissues which are consistent to previous publications of other investigators and also of our[16 57]. The restoration of GOT and GPT activities of the matched control group, were noted after co-administration of aqueous methanol mixture extract of seed of *T. indica* at the dose of 80 mg or 120 mg or glibenclamide but in low dose (40 mg) partial correction in these parameters was observed in comparison to the control level.

CONCLUSION

In conclusion, it may be stated that the aqueous methanol extract of seed of *T. indica* at the dose of 80 mg is the minimum dose that has the maximum potentiality for correction of above abnormalities in STZ-induced diabetic state. The above mentioned antidiabetic and antioxidative properties of aqueous methanol mixture extract predicted from this experiment has been strengthened from the role of a known hypoglycemic drug as glibenclamide treated side by side with the diabetic animals. It may be stated that the aqueous methanol extract of seed of *T. indica* may provide a new therapeutic avenue against antihyperglycemic and antioxidative capacities along with diabetes related complications.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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