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Hypoglycemic and antioxidant potency of ethyl acetate fraction of hydro-methanolic extract (60:40) of *Tamarindus indica* Linn. seed in streptozotocin-induced diabetic experimental animal

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Abstract---Diabetes mellitus is a chronic metabolic syndrome characterized by hyperglycemia due to defective insulin secretion, insulin action or both. Nowadays massive developments in variety of synthetic modern medicines, alternative and traditional medicinal plants are still widely used for the management of diabetes mellitus. In this context when ethyl acetate fraction at the dose of 100 mg body weight/kg body weight was administered for 28 days, significant diminution in the STZ-induced hyperglycaemia was noticed. Plasma insulin and C-peptide levels as well as activities of antioxidant defense enzymes such as catalase (CAT), peroxidase (Px) and superoxide dismutase (SOD) in the hepatic tissue were found to be decreased in diabetic animals. This was corrected after the treatment of ethyl acetate fraction of hydro-methanolic extract of *T. indica*. Oral glucose tolerance test (OGTT) reveals that the fraction at the above said dose showed a remarkable decrease of blood glucose level in normal and diabetic rat and also increased glucose tolerance. On the other hand, in diabetic animals body weight decreases along with a decrease in total hemoglobin content in blood and increase in HbA1C level. These animals, however, recover towards the control level by means of a fraction treatment. Histopathology of pancreas was performed after ethyl acetate fraction treatment to diabetic rat and the results were compared with the rats having controlled as well as diabetic groups. To evaluate the free radical scavenging activities of ethyl acetate fraction *in-vitro* study model with ABTS [2,2'-azinobis-(3 ethyl enzothiazoline-6-sulfonic acid)] and DPPH [1,1-diphenyl-2-picryl hydrazil] were performed along with measurement of IC₅₀ values 0.027±0.003 and 0.021±0.002 mg/ml respectively in respect of established antioxidant namely butylated hydroxytoluene (BHT). Phytochemical screening showed that constituents like flavonoids, alkaloids, terpenoids and steroids were found in aforesaid fraction

which has potency for the possible antidiabetic and antioxidative properties. Acute toxicity study in rats did not reveal any symptoms of toxicity upto the dose of 3000 mg/kg body weight in rats.

Keywords---*Tamarindus indica*, diabetes, antioxidant, insulin, C-peptide, glycosylated hemoglobin, pancreas.

1 Introduction

Diabetic mellitus is a multifactorial chronic metabolic disorder that has become a concern for people all over the globe. The disease is characterized by polyurea, polyphasia and polydepsia along with hyperglycemia which is caused due to diminution in insulin secretion, insulin action or ineffective production of insulin in the pancreas (American Diabetes Association, 2019). It has become a life-threatening major health problem globally. Diabetes associated complication can cause early mortality. According to the current data supplied by International Diabetic Federation, the worldwide adult population (20-79 years) with diabetes mellitus was about 463 million (9.3% of the world adult population) in the year 2019 and this number was expected to be about 578 million by the year 2030 and upto 700 million by the year 2045.

Uncontrolled continuation of blood sugar level may result in several associated complications like diabetic nephropathy, diabetic retinopathy, hyperlipidemia, atherosclerosis, coronary heart disease, hypertension, cerebrovascular disease, foot damage, etc. These life-threatening complications make diabetes more dangerous than other life style diseases. DM can be categorized into two broad classes i.e., type-1 diabetes mellitus and type-2 diabetes mellitus. Type-1 is caused due to autoimmune destruction of pancreatic β cells which leads to a complete deficiency of insulin secretion. On the contrary, type-2 results form a combination of both insulin resistance and insufficient secretion of insulin (American Diabetes Association, 2017, Wang, & Shah, 2017). In recent days approximately 90% people with low and middle income of different countries are at a high risk of becoming affected from type-2 diabetes (International Diabetes Federation, IDF Diabetes Atlas, International Diabetes Federation, Brussels, Belgium, 9th edition, 2019).

In modern times although a lot of synthetic drugs have been available in the market, but still, total cure cannot be provided by these drugs. Prolonged use of few synthetic drugs may cause many harmful effects, and thus research is still going on to find out drugs which will be budget friendly, non-toxic for the treatment of DM. Emphasis is being given to traditional and herbal treatments as valued resources of medicine globally. These are widely used worldwide indicating that plants are an evolving part of current and efficient technology dependent drugs. Products obtained naturally from plants are the main source for inventing leading candidates and it also plays significant role in the forthcoming drug development programme (Chukwuma et al., 2019; Jugran et al., 2021; Shanak et al., 2022)

According to the World Health Organization (WHO), a total of 21,000 plants are used for medicinal purposes all around the globe. Out of these about 400 plants are suitable for the management of diabetes. In spite the fact of that several herbal drugs are available for treatment of diabetes, only a negligible number of these plants have gone through scientific and medical screening to prove their potency.

Tamarindus Indica Linn is a traditional medicinal plant under the subfamily Caesalpiniaceae of the family Leguminosae (Fabaceae). The plant is locally known as imli or tamarind and is one of the most popular tropical tree types of plant found in India. In summer season mainly the plants bear fruits. The seed coat is brownish black in colour and the kernel is white.

In our earlier publication *T. indica* was used as a traditional and supportive medicine for the management of diabetes induced complications (Maiti et al., 2004). Recent studies have disclosed that this plant possess major role in curing A diet-based strategy against lifestyle maladies, antibacterial, anti-alzheimer potential, anticancer activity along with antioxidant and hepatoprotective activities (Sudjaroen et al., 2005; Pimple et al., 2007; Adeniyi et al., 2017; Arshad et al., 2019; Gomathia et al., 2020; Elmaidomy et al., 2022).

The ultimate goal of this work is to find out recent advances in the field of herbal medicine based on hypoglycemic and antioxidant properties of ethyl acetate fraction of hydro-methanolic extract (60:40) of *Tamarindus indica* Linn. seed in streptozotocin-induced diabetic rat and also to protect complications related with diabetes mellitus.

2 Materials and Methods

Chemicals

All chemicals were of analytical grade used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant Materials

Tamarindus indica Linn. seeds were collected in the month of March and April from Bhadutola, Midnapore, Paschim Medinipur, West Bengal, India, and the materials were authenticated by taxonomist of Botanical Survey of India, Shibpur Howrah. The voucher specimen was kept in the herbarium of Botanical Survey of India and numbered as HPCH NO.1

Preparation of Hydro-methanolic Extract of *T. indica*

T. indica seeds were at first washed with distilled water. The seed were then dried and ground by electrical grinder to a fine powder. Thereafter, 500 gm powder was added into 5L hydro-methanolic solution (H₂O: MeOH :: 60:40) at room temperature in order to prevent any means of inactivation of the bio-active compound(s). This mixture was stirred frequently about for 2 hours and then kept for overnight. On the next day this extract was collected after 24 hours of extraction process and to which freshly prepared 5L hydro: methanolic solution.

The slurry was intermittently stirred once again with glass rod. With the help of cotton filter the extract was filtered at first followed by Whatman filters paper. The filtrate was evaporated taking the help of Rotavapour (BUCHI-R124; Switzerland aqueous filtrate and) under reduced pressure at 37°C for total removal of methanol. Ultimately the filtrate was lyophilized on VirTis bench top K lyophilizer. 100 g of this lyophilized extract was collected and stored in the refrigerator for further experimental studies.

Bioassay Based Fractionation

100 g of lyophilized extract of *T. indica* seed was taken in a separate flask and dissolved with 1L of hydromethanolic (H₂O: MeOH :: 60:40) solution. Solvent fractionation was performed using solvents (Ethyl acetate, n-Hexane and Chloroform) with increasing polarity. The progress in fractionation was monitored with the help of T.L.C. Rotavapour instrument was used to collect the fractionates separately which were then dried under reduced pressure at 38°C. At last 10.4 g ethyl acetate 5 g n-hexane fraction and 20 g chloroform fractions were obtained from 100 g of lyophilized *T. indica* seed. These fractions were given orally by the gavage method.

Phytochemical Screening

The primary screening of ethyl acetate fraction of *T. indica* seed was necessary for obtaining various active phytoconstituents i.e flavonoids, alkaloids, tannins, phenolic compounds terpenoids, steroids, glycosides, proanthrocianine, quirecetin, proanthrocianine and quirecetin anthraquinones (Farnsworth, 1966). The presence of the above mentioned phytochemicals are the main regulators for the antidiabetic and antioxidative properties of the medicinal plants. These phytochemical ingredients have shown a drastic reduction in blood glucose as well as levels of free radicals.

Acute Toxicity Studies

Overnight starved healthy, mature Wistar strain male albino rats were divided into six equal groups (n=6) and were orally fed with the ethyl acetate fraction of *T. indica* by gavage method with elevating dose levels of 50, 100, 200, 400, 800 mg/kg body weight (Ghosh, 1984). For studying the neurological, autonomic and behavioural parameters the rats were kept under continuous observance for every 2 hours and after a period of 48 hours for any lethality or death (Shetty et al., 2007).

Selection of Animal and Care

Thirty-two matured Wistar strain male albino rats of 4 months of age weighing about 140 ± 10g having fasting blood glucose level 80-90 mg/dl were selected for this experiment. Prior to the initiation of the experiment the animals were acclimatized for 15 days in our laboratory condition. They were kept in an animal house maintaining an ambient temperature of 28 ± 2°C with 12 h light:12 h dark cycle. Rats were provided pellet diet along with water *ad libitum*. The experiment

was performed maintaining the Principle of Laboratory Animal Care and instructions given by our Institutional Ethical Committee.

Induction of Diabetes in Rats

Out of thirty-two rats, twelve hour fasted twenty six rats were subjected to an intramuscular injection at a single dose of 4mg/0.1ml of citrate buffer/100 gm body weight/rat. Fasting blood level was monitored after 7 days of STZ injection. Eighteen rats having fasting blood glucose level >300 mg/dl were considered for the study.

Animal Treatment

Out of eighteen selected diabetic rats, six of them were divided into diabetic control and remaining twelve were placed in ethyl acetate and glibenclamide co-administered diabetic group respectively. Normoglycemic six rats were considered as control group. From 7th day of post STZ injection ethyl acetate fraction of *T. indica* and glibenclamide treatment were started. STZ injection was considered as 1st day of experiment and the treatment lasted for next 28 days.

Group I (Control group): At the time of STZ injection to the rats for diabetic induction, on the other hand animals of control group received single intramuscular injection of 0.1ml citrate buffer /100 g body weight.

Group II (Diabetic control group): Distilled water at a dose of 0.5 ml/100g body weight/day was forcefully fed through gavage method for 28 days.

Group III (Diabetic + Ethyl acetate fraction): Ethyl acetate fraction of seed of *T. indica* at a dose of 100 mg/0.5 ml/100 g body weight/rat/day for 28 days at fasting state to the diabetic rats.

Group IV (Diabetic + Glibenclamide): Diabetic rats belonging to this group were administered glibenclamide forcefully by gavage at a dose of 0.6 mg/ 0.5 ml water/100gm body weight at fasting state for next 28 days.

Administration of ethyl acetate fraction and glibenclamide to the rats of group III and group IV was carried out orally in the morning at fasting state. To maintain same experimental condition and also to limit stress if any due to treatment of ethyl acetate and glibenclamide diabetic and control group of animals were forcefully injected 0.5 ml of distilled water for a period of 28 days. Fasting blood glucose level was monitored in all the group beginning from the very first day of ethyl acetate fraction and glibenclamide treatment to the diabetic rats at 12 hours fasting state on every 7 days interval by single touch glucometer. Blood was collected from the tail vein on the 35th day of experiment, total hemoglobin content along with the help of single touch glucometer fasting blood glucose level and OGTT were monitored. After recording the final body weights all the rats were sacrificed at fasting state by light ether anesthesia which was followed by cervical decapitation. For the measurement of C-peptide and serum insulin blood was collected from the dorsal aorta and serum was prepared by centrifugation at 6000 rpm for 10 minutes. The hepatic tissue was dissected and stored at -40 °C for the estimation of key antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (Px), along with quantification of the levels of the products of free radicals i.e conjugated diene (CD) and thiobarbituric acid reactive substance (TBARS).

Oral glucose tolerance test (OGTT)

In overnight fasted normal, diabetic, ethyl acetate and glibenclamide treated rats the oral glucose tolerance test was performed. Fasting blood glucose level was performed initially in all the above-mentioned groups. Then blood glucose level was recorded in group III and IV after 30 minutes of treatment of ethyl acetate and glibenclamide considered as 0 min. To all the groups of animals a dose of 0.5 g/100 g body weight of glucose was administered orally. Blood glucose levels were again monitored for 2 hours at regular interval of 30 minutes each (Satyaprakash et al., 2010).

Biochemical estimations

According to Brugi et al., 1998 serum insulin level was recorded with the help of rat insulin ELISA kit purchased from Millipore Corporation, Billerica, MA. Following the method of Bhat et al., 2011, serum C-peptide level was assessed taking the help of rat C-peptide (Yanaihara, Japan) ELISA kit. Total hemoglobin content in blood (Henry, 1964) and HbA1c (Nayak, 1981) were measure by standard protocol. The antioxidant enzymes namely superoxide dismutase, catalase, peroxidase (Sadasivam and Manikam, 1996) were monitored biochemically in liver tissues according to methods of (Marklund and Marklund, 1974), (Beers and Sizer, 1952). Concentration of thiobarbituric acid reactive substance (TBARS) and conjugated diene (CD) in liver was used for quantifying lipid peroxidation according to protocol of (Okhawa et al., 1979) and (Slater, 1984). According to the method of (Kim, et. al., 2003), the radical scavenging activity of *T. indica* against DPPH was measured spectrophotometrically. An ABTS radical cation form was used to assess the total antioxidant activity of solutions of pure substances according to (Raja and Pugalandi, 2010). Histological study of the pancreas was done using hematoxylin and eosin stain and cytoarchitecture of pancreatic islets were observed using a compound microscope.

Statistical analysis of the collected data

All the data were statistically evaluated with the help of one-way analysis of variance (ANOVA) followed by multiple comparison two tail't' test. The statistical analysis was done based on Origin Lab (Ver. 6.0) software. P values of less than 0.05 were set as statistical significance. Data were represented as mean $\pm$ standard deviation.

3 Results

Preliminary phytochemical screening

From phytochemical screening it was revealed that ethyl acetate fraction of seeds of *T. indica* contains flavonoids, alkaloids, tannins, phenolic compounds terpenoids, steroids, glycosides, proanthrocianine, quirecetin, proanthrocianine and quirecetin anthraquinons (Table 1).

Acute toxicity studies

Pharmacological study in rats revealed that ingestion of ethyl acetate fraction of *T. indica* seed produce insignificant changes in the behavioral, autonomic and neurological responses upto doses of 800 mg/kg body (Table 2).

Body weight, blood glucose level, total hemoglobin content and glycosylated hemoglobin level

Significant elevation in blood glucose and glycosylated hemoglobin levels along with diminution of body weight and total hemoglobin content noted in STZ induced diabetic rats compared to the control group of animals (Table 3 and 4). A significant change in the above-mentioned parameters were found in the diabetic rats after the administration of ethyl acetate fraction of seed of *T. indica* or glibenclamide for a period of 28 days and the level was towards the control group. A comparison was made between fraction treated and glibenclamide treated group and an insignificant difference was noted in aforesaid parameters (Table 3 and 4).

Effect of ethyl acetate fraction on oral glucose tolerance test (OGTT) in STZ-induced diabetic rats

OGTT study of ethyl acetate fraction of *T. indica* seed on normal and streptozotocin-induced diabetic rats resulted marked change in blood glucose level. Decrease in blood glucose level was noticed after 2 h of glucose administration with the ethyl acetate fraction (80 mg/100g) and glibenclamide treated group of normal rats in comparison to control. A significant decrease in blood glucose level was also noticed in STZ-induced diabetic rats treated with ethyl acetate fraction and glibenclamide treated group of animals when compare to diabetic control group. (Table 5).

Serum insulin and c-peptide level

In the diabetic control rats serum insulin and c-peptide level showed marked decrease in comparison to control rats. In diabetic rats supplementation of ethyl acetate fraction or glibenclamide for a period of 28 days revealed a significant increase in serum c-peptide and serum insulin level compared to the diabetic control animals. No significant difference was noted in both parameters in case of ethyl acetate fraction and glibenclamide treated group (Figure 1 and 2).

In vivo antioxidant activities

Activities of SOD, CAT, and Px

The antioxidant enzyme activities like SOD, CAT and Px in hepatic tissue were diminished remarkably in diabetic group compared to control group. Administration of ethyl acetate fraction of seed of *T. indica* or glibenclamide to STZ-induced diabetic rat showed significant protection of antioxidant enzymes towards the control animals (Table 6). The above mentioned enzyme activities insignificantly differ in glibenclamide or ethyl acetate fraction treated group (Table 6).

Levels of lipid peroxidation like TBARS and CD

In hepatic tissue quantification in levels of TBARS and CD were increased significantly in diabetic group in comparison to the control group (Table 6). The levels of the aforesaid parameters showed significant restoration towards the control level as compared between ethyl acetate or glibenclamide treated diabetic rats (Table 6). No significant difference was found in the levels of the above mention parameters between fraction treated ethyl acetate and glibenclamide treated group of animals (Table 6).

In vitro antioxidant activities

ABTS radical scavenging activity

Ethyl acetate fraction of T. indica seed is an effective scavenger of ABTS radicals as depicted in Figure 3. The scavenging activity of this seed extract was compared with BHT. The IC₅₀ values of the Ethyl acetate fraction and BHT was 0.024±0.001 and 0.018±0.002 mg/ml respectively. Maximum inhibitory activity of the seed extract was observed at 0.5 mg/ml in releasing ABTS radicals from the reaction system (Figure 3).

DPPH radical scavenging activity

The dose-dependent curve of DPPH radical scavenging activity of *T. indica in comparison with BHT* is showed in (Figure 4). Scavenging activity of ethyl acetate fraction, DPPH and BTH was noticed with IC₅₀ values of 0.028±0.002 and 0.017±0.002 mg/ml respectively (Figure 4).

3.8. Histological study

In STZ-induced diabetic group of animals diameter of pancreatic islets was significantly decreased in comparison to the control group of animal. Administration of ethyl acetate fraction and gluibenclamide to diabetic rat shows significant recovery of this parameter towards the control (Figure 5).

4 Discussion

Streptozotocin is a broad-spectrum antibiotic, a glucosamine nitrosourea obtained from *Streptomyces achromogenes* that specifically destroy pancreatic β cells through DNA alkylation; it causes hyperglycemia due to low serum insulin level (Lenzen, 2008).

Streptozotocin results hyperglycaemia which has been elicited as an effective experimental model. Our previous publication emphasizes on the efficacy of *T. indica* as an important herbal hypoglycemic agent (Maiti et al., 2004). A number of physiological and biochemical changes take place due to insulin deficiency (Alam et al., 2021). Function of β cells can be monitored by assessments of serum insulin and C-peptide (Suh et al., 2022). In this study streptozotocin induced diabetic animals resulted in a significant diminution in serum insulin as well as C-peptide levels. Insulin action is activated by C-peptide at low hormone concentration and suppressed at high level of hormone resulting a regulatory effect of C-peptide on insulin signaling (Petersen and Shulman, 2018).

Supplementation of ethyl acetate fraction of seed of *T. indica* or glibenclamide resulted in a significant elevation of serum insulin and C-peptide levels.

Streptozotocin-induced diabetic rats showed significant diminution in body weight in respect to control rats as a result of breakdown of tissue protein (Ren et al., 2017). Supplementation of *T. indica* results elevation of body weight in diabetic rats to certain limit that showed improvement of muscle wasting resulted from glycemic control (Gandhi. & Sasikumar, 2012). Elevated non-enzymatic glycosylation is one of the probable mechanisms that is associated with hyperglycaemia and diabetic complications (Chen et al., 2018). In diabetic animals consistent high level of glucose in blood combines with hemoglobin to form HbA1C (Babukumar et al., 2017). In this study, elevated level of HbA1C and diminution in total hemoglobin content were found in diabetic rats in comparison with normal rats, that results poor glycemic control in diabetes. In extract administered diabetic animals significant diminution in the level of HbA1C along with elevation of total haemoglobin level in blood could be the possibility cause for an improvement in the glucose metabolism (Sankaranarayanan, et. al., 2018).

Antioxidant properties of ethyl acetate fraction of seed of *T. indica* has been shown by the assay of *in-vitro* radical scavenging along with *in-vivo* by determination of CAT, Px and SOD enzyme activities in experiment animals. The above-mentioned antioxidant enzymes like SOD, Px and CAT were considered biologically important in diminution of free radical like hydrogen peroxide (Lin, et al., 2018). In diabetic rats antioxidant enzyme activities of SOD, Px and CAT were decreased in our present findings like our previous publication (Maiti et al., 2015) along with observation of other (Chukwuebuka et al., 2021). On the other hand said antioxidant enzyme activities were resettled towards control levels after administration of seed of *T. indica* and glibenclamide. This reveals that ethyl acetate fraction of seed of *T. indica* and glibenclamide have the antioxidant potency via direct or indirect way, which may account for free radical scavenging properties of phytoconstituents present in the aforesaid fraction of *T. indica* and glibenclamide (Martinello et al., 2017).

Streptozotocin-induced diabetic animals had showed an elevated level of TBARS and CD in hepatic tissue that was strengthened by our previous publication as well as by observation of other (Maiti et. Al., 2015; Gutiérrez et al., 2021). Supplementation of ethyl acetate fraction of seed of *T. indica* and glibenclamide reduces the hepatic CD and TBARS level in STZ-induced diabetic animal. On the contrary, ethyl acetate fraction of *T. indica* showed effective *in-vitro* antioxidant properties in DPPH and ABTS radical scavenging assessment when compared with standard antioxidant like BHT. The results revealed the capability to decrease free radicals of aforesaid fraction that might stop the initiation of free radical or minimize free radical chain reaction in account for the propagation of the oxidation process (Bhadoriya, et. al., 2018).

Possible antidiabetic and anti-oxidative properties of ethyl acetate fraction obtained from hydro-methanolic (60:40) extract of *T. indica* might be due to the presence of phenolic compounds, flavonoids, terpenoids, steroid and alkaloids. The active bioactive principles derived from this fraction acts with the help of several antidiabetic and antioxidative mechanisms by inhibiting α -amylase, α -

glycosidase and protein tyrosine phosphatase activities (Hossain et al., 2020, Khalid et al., 2022). The other possible ways include stimulation of existing β -cells of islets of langerhans for releasing insulin, increase hepatic glycogen level, increase glucose oxidation, elevated glucose uptake by peripheral tissues along with diminution of hepatic gluconeogenesis and intestinal glucose absorption. The advantages of herbal hypoglycemic drugs are low cost, has less side effect and easily available for the management of diabetes. In respect to LD₅₀ values and maximum non-fatal doses studies revealed the non-toxic nature of the n-hexane fraction of this plant. There was no lethality or any toxic reactions found at any doses selected until the end of the study period. The plant fraction was shown to normalize the levels of these enzymes, which indicates that it has a promising antidiabetic effect without inducing toxicity. The Further pharmacological and chemical researches are in progress to elucidate in detail the active principles and the real mechanism of action of this plant fraction.

5 Conclusion

From this present work it was concluded that ethyl acetate fraction of hydromethnoic seed extract of *T. indica* has antidiabetic and antioxidatave properties against STZ-induced diabetic rats and the effects was compared to a standard antidiabetic drug i.e glibenclamide.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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Table 1
Qualitative detection of the phytochemicals of ethyl acetate fraction of
hydromethanol extracts of *T. indica* seed

Sl. no.	Phytochemical Constituents	<i>T. indica</i> seed
1.	Alkaloids	+
2.	Flavonoids	+
3.	Saponins	-
4.	Tannins	-
5.	Terpenoids	+
6.	Steroids	+
7.	Glycosides	+
8.	Anthraquinons	+
9.	Phenolic compounds	+
10.	Phlobatannins	-

Table 2
Determination of median lethal dose (MLD) of the ethyl acetate fraction of
hydromethanolic extract (60:40) of *T. indica* administered orally on Wister strain
albino rats.

Dose (mg/kg body weight)	Number of animal used	Number of survived	Number of dead	Median lethal dose (LD ₅₀)
00 (Control)	6	6	0	
100	6	6	0	
200	6	6	0	
400	6	6	0	
600	6	6	0	
800	6	6	0	>800mg/kg body weight

Table 3

Effect of ethyl acetate fraction of seed of *T. indica* on fasting blood glucose level in STZ induced diabetic male albino rat. Data are expressed as Mean $\pm$ S.E.M; n=6. ANOVA followed by multiple comparison two tail't' test. Values with superscripts (a,b,c) in each vertical column differ from each other significantly, $p < 0.05$.

Groups	Fasting blood glucose level (mg/dl)				
	1 st day (The day of STZ injection)	7 th day (The day of Ethyl acetate fraction treatment)	14 th day	21 st day	28 th day
Control	73.2 $\pm$ 3.8 ^a	77.98 $\pm$ 3.7 ^a	78.14 $\pm$ 4.1 ^a	75.96 $\pm$ 3.8 ^a	80.08 $\pm$ 4.7 ^a
Diabetic	76.29 $\pm$ 3.6 ^a	341.09 $\pm$ 7.2 ^b	339.33 $\pm$ 6.7 ^b	329.90 $\pm$ 6.7 ^b	338.13 $\pm$ 7.1 ^b
Diabetic + <i>Ethyl acetate</i> fraction	75.87 $\pm$ 4.2 ^a	337.72 $\pm$ 7.4 ^b	210.76 $\pm$ 5.6 ^c	128.35 $\pm$ 4.4 ^c	87.69 $\pm$ 4.6 ^a
Diabetic + glibenclamide	74.85 $\pm$ 3.8 ^a	338.59 $\pm$ 7.5 ^b	191.43 $\pm$ 4.4 ^c	127.96 $\pm$ 4.7 ^c	99.02 $\pm$ 4.2 ^c

Table 4

Attenuate effect of ethyl acetate fraction of seed of *T. indica* on body weight, total hemoglobin content and glycosylated hemoglobin level in STZ-induced diabetic male albino rat. Data are expressed as Mean $\pm$ S.E.M; n=6. ANOVA followed by multiple comparison two tail't' test. Values with superscripts (a,b,c) in each vertical column differ from each other significantly, $p < 0.05$.

Groups	Body weight (gm)		Hemoglobin gm%	Glycosylated hemoglobin level (GHb%)
	Initial	Final		
Control	142.8 $\pm$ 5.63 ^a	161.12 $\pm$ 6.11 ^a	13.24 $\pm$ 0.57 ^a	5.93 $\pm$ 0.35 ^a
Diabetic	139.4 $\pm$ 6.12 ^a	121.17 $\pm$ 5.46 ^b	8.51 $\pm$ 0.66 ^b	9.15 $\pm$ 0.71 ^b
Diabetic + Ethyl acetate fraction	143.5 $\pm$ 6.16 ^a	159.86 $\pm$ 5.89 ^a	11.14 $\pm$ 0.69 ^c	6.91 $\pm$ 0.53 ^c
Diabetic + glibenclamide	141.28 $\pm$ 6.12 ^a	158.12 $\pm$ 6.01 ^a	11.42 $\pm$ 0.86 ^c	7.03 $\pm$ 215 ^c

Table 5
Beneficial effect of ethyl acetate fraction of *T. indica* seeds on OGTT in normal and STZ- induced diabetic male albino rats. Data are expressed as Mean $\pm$ S.E.M; n=6. ANOVA followed by multiple comparison two tail't' test. Values with superscripts (a,b,c) in each vertical column differ from each other significantly, $p < 0.05$.

Groups	Blood glucose level (mg/dl) minutes after administration of drugs				
	0	30	60	90	120
Control	76.94 $\pm$ 3.84 ^a	188.61 $\pm$ 2.96 ^a	198.84 $\pm$ 2.72 ^a	158.72 $\pm$ 2.46 ^a	88.34 $\pm$ 2.31 ^a
Diabetic	331.70 $\pm$ 4.41 ^b	491.80 $\pm$ 7.43 ^b	442.44 $\pm$ 4.39 ^b	423.47 $\pm$ 2.52 ^b	405.13 $\pm$ 5.02 ^b
Diabetic + Ethyl acetate fraction	328.52 $\pm$ 4.22 ^b	472.62 $\pm$ 5.17 ^c	316.91 $\pm$ 6.18 ^c	275.68 $\pm$ 4.71 ^c	255.42 $\pm$ 4.76 ^c
Diabetic + glibenclamide	332.12 $\pm$ 5.80 ^b	466.74 $\pm$ 8.35 ^d	337.98 $\pm$ 3.57 ^d	278.34 $\pm$ 5.09 ^c	267.51 $\pm$ 5.32 ^c

Table 6
Protective effect of ethyl acetate fraction of seed of *T. indica* on the activities of hepatic antioxidant enzymes and levels of lipid peroxidation in streptozotocin-induced diabetic male albino rat. Data are expressed as Mean $\pm$ S.E.M; n=6. ANOVA followed by multiple comparison two tail't' test. Values with superscripts (a,b,c) in each vertical column differ from each other significantly, $p < 0.05$.

Groups	Antioxidant enzyme activities			Lipid peroxidation levels	
	SOD (Unit/mg of tissue)	CAT(mM of H ₂ O ₂ consumption/mg of tissue/min.)	Px (Unit/mg of tissue)	TBARS (nM/mg of tissue)	CD (nM/mg of tissue)
Control	2.84 $\pm$ 0.43 ^a	3.82 $\pm$ 0.62 ^a	4.24 $\pm$ 0.57 ^a	25.15 $\pm$ 1.38 ^a	266.51 $\pm$ 7.13 ^a
Diabetic	0.84 $\pm$ 0.52 ^b	1.57 $\pm$ 0.39 ^b	1.59 $\pm$ 0.56 ^b	41.55 $\pm$ 2.86 ^b	384.78 $\pm$ 10.94 ^b
Diabetic + n-hexane fraction	2.75 $\pm$ 0.59 ^a	3.77 $\pm$ 0.81 ^a	4.14 $\pm$ 0.62 ^a	28.64 $\pm$ 2.03 ^a	276.12 $\pm$ 9.25 ^a
Diabetic + glibenclamide	2.88 $\pm$ 0.48 ^a	3.97 $\pm$ 0.68 ^a	4.19 $\pm$ 0.66 ^c	27.83 $\pm$ 2.15 ^a	270.12 $\pm$ 8.75 ^a

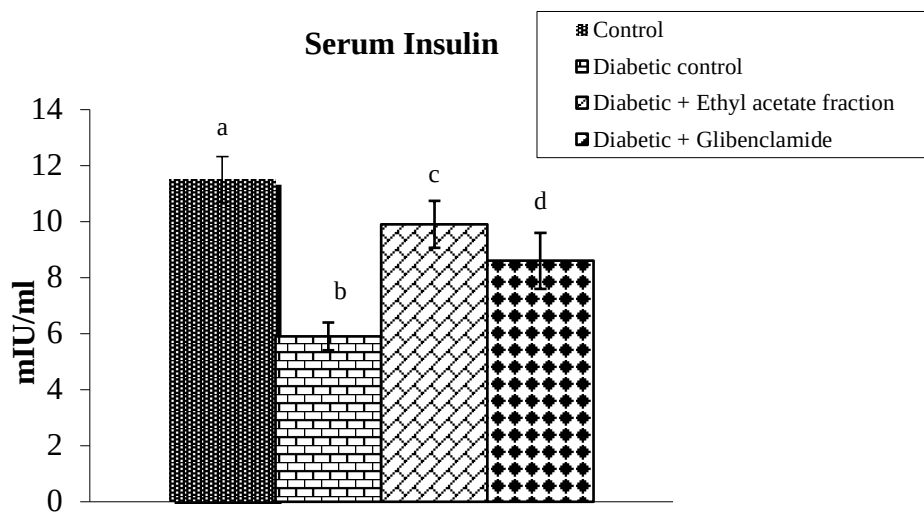


Figure 1. Restoration of serum insulin level after co-administration of ethyl acetate fraction from hydro-methanolic extract of *T. indica* seed in STZ-induced diabetic male albino rat. Bar represents Mean $\pm$ S.E.M; n=6. ANOVA followed by multiple comparison two-tail 't'-test. Bars with different superscripts (a,b,c,d) differ from each other significantly, $p < 0.05$.

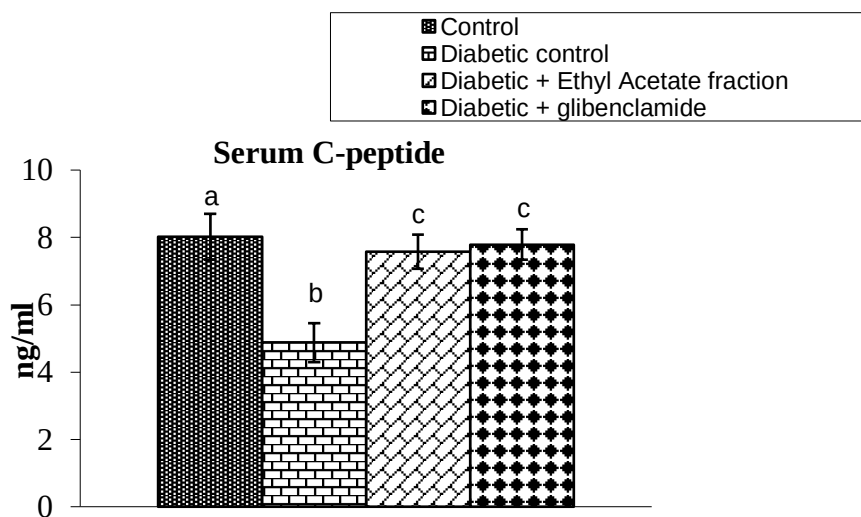


Figure 2. Resettlement of serum c-peptide level after administration of ethyl acetate fraction from hydro-methanolic extract of *T. indica* seed in STZ-induced diabetic male albino rat. Bar represents Mean $\pm$ S.E.M; n=6. ANOVA followed by multiple comparison two-tail 't'-test. Bars with different superscripts (a,b,c) differ from each other significantly, $p < 0.05$.

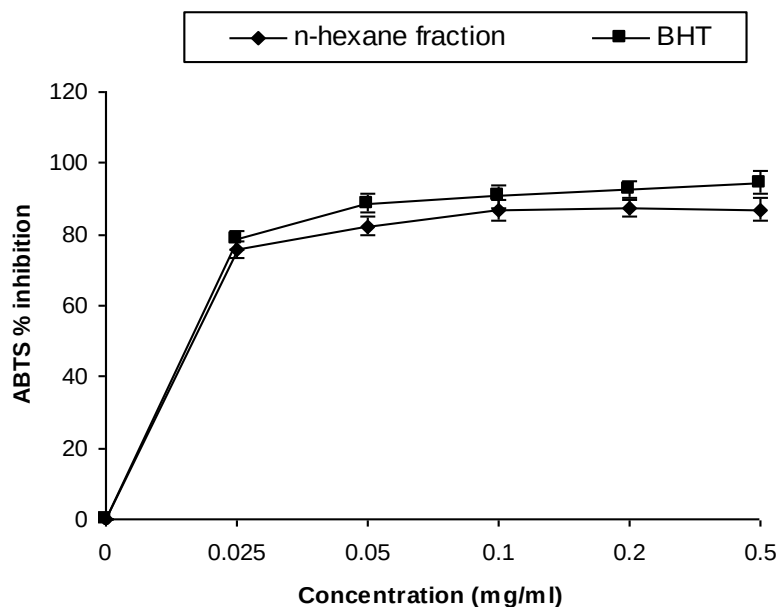


Figure 3. Total antioxidant activity of ethyl acetate fraction of seed of *T. indica* – ABTS radical cation decolourization assay. The IC₅₀ value of the fraction was 0.021± 0.002 mg/ml.

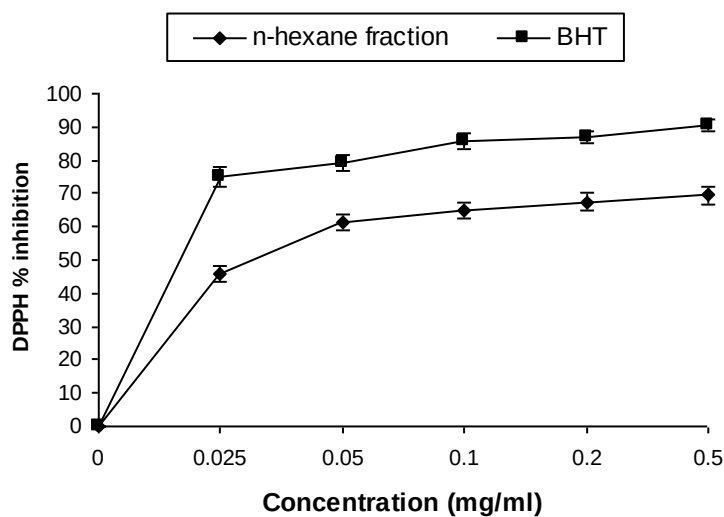


Figure 4. Inhibition of DPPH radical by ethyl acetate fraction of *T. indica* seed extract BHT. The IC₅₀ value of the fraction was 0.027± 0.003mg/ml.

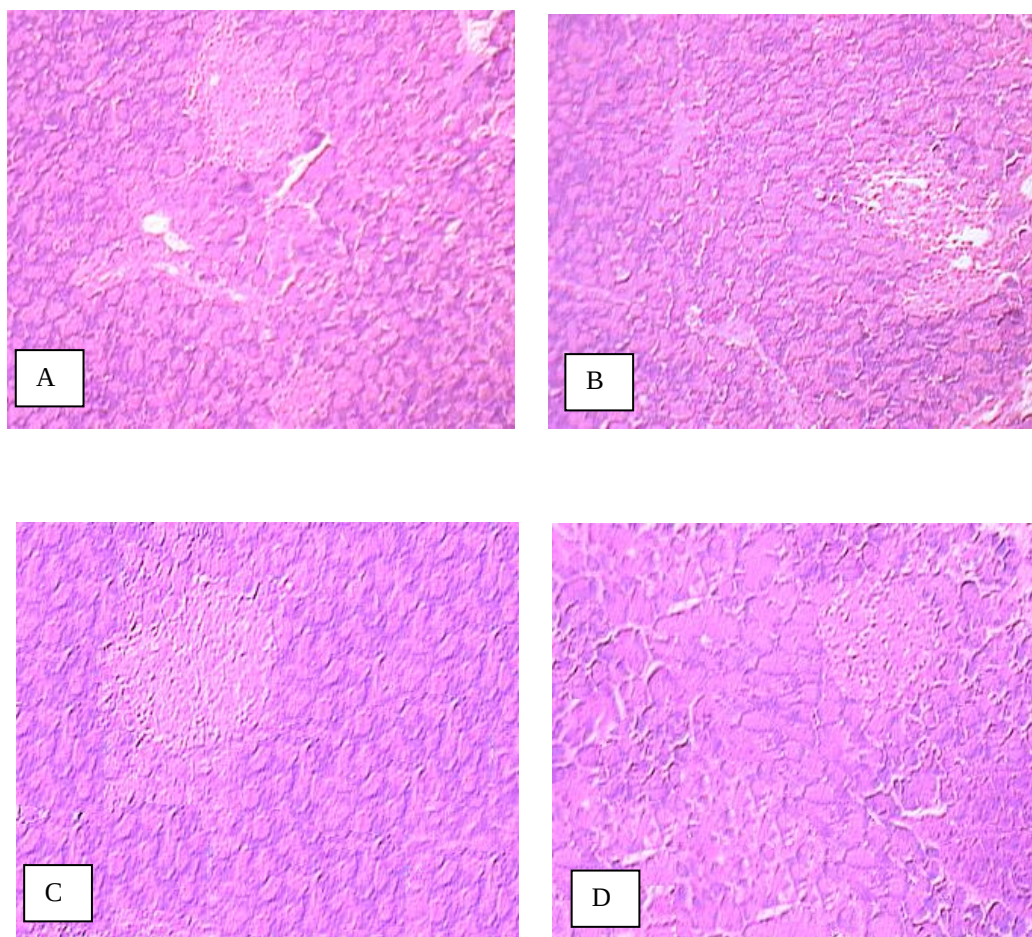


Figure 5: Plate A. Representative sample of pancreatic tissue of control rat focusing the normal islet diameter. Plate B. Diminution in the diameter of islet in the representative pancreatic tissue sample of STZ-induced diabetic rat. Plate C. Representative pancreatic tissue sample showing recovery in islet cell diameter after ethyl acetate fraction of hydro-methanol extract treatment in streptozotocin-induced diabetic rat. Plate D. Representative pancreatic tissue sample showing recovery in islet cell diameter after glibenclamide treatment in STZ-induced diabetic rat.