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# ANTIDIABETIC EFFECT OF *n*-HEXANE FRACTION OF HYDRO-METHANOLIC EXTRACT OF *TAMARINDUS INDICA* LINN. SEED IN STREPTOZOTOCIN-INDUCED DIABETIC RAT: A CORRELATIVE APPROACH WITH *IN VIVO* AND *IN VITRO* ANTIOXIDANT ACTIVITIES

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**Keywords:**

Herbal drug, Diabetes, Antioxidant, Insulin, C-peptide, Pancreas

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**ABSTRACT:** The present study was carried out to evaluate antidiabetic as well as *in-vivo* and *in-vitro* antioxidant activities of n-hexane fraction of *Tamarindus indica* Linn. seed (*T. indica*) in streptozotocin (STZ) induced diabetic rat. Oral administration of n-hexane fraction at the dose of 100 mg/kg body weight for 28 days prevented significantly the STZ-induced hyperglycemia. The plasma insulin and C-peptide levels as well as activities of antioxidant enzymes such as catalase (CAT), peroxidase (Px) and superoxide dismutase (SOD) in the hepatic tissue were found to be decreased in diabetic animals which were corrected after the treatment of *n*-hexane fraction of hydro-methanolic extract of *T. indica*. Oral glucose tolerance test (OGTT) reveals that the fraction at above mentioned dose showed a significant decrease of blood glucose level in normal and diabetic rat. Histopathology of pancreas was performed after n-hexane fraction treatment to diabetic rat and the results were compared with the control as well as diabetic groups. To evaluate the free radical scavenging activities of the n-hexane fraction following *in-vitro* study model with ABTS [2, 2´-azino-bis(3-ethyl benzothiazoline-6-sulphonic acid)] and DPPH [1, 1-diphenyl-2-picrylhydrazyl] were carried out along with determination of $IC_{50}$ values 0.027±0.003 and 0.021±0.002 mg/ml respectively in respect to standard antioxidant such as butylated hydroxytoluene (BHT). Phytochemical studies reveal the presence of flavonoids, alkaloids, terpenoids and steroids in said fraction which is responsible for the possible antidiabetic and antioxidative actions. Acute toxicity study in rats did not show any signs of toxicity upto the dose of 3000 mg/kg body weight in rats.

**INTRODUCTION:** Diabetes mellitus is a chronic metabolic disorder of endocrine system with life threatening complications. This is characterized by hyperglycemia resulting from defect in insulin secretion or insulin action or both. Diabetes mellitus eventually leads to damage of the vital organs of the body.

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It can be classified into two major categories: type 1 and type 2 diabetes mellitus. Among diabetic patients 85 - 95% suffers from type 2 diabetes [1]. The prevalence of diabetes has been rising globally in developed and developing countries. There is an estimate that about 143 million people in the world

are suffering from diabetes and this number will probably double by 2030 [2]. The percentage of people affected by diabetes mellitus was rapidly rising in India. At present more than 40 million people are affected in India alone which represents nearly 25% of total diabetes population worldwide.

Oxidative stress in diabetes is caused by hyperglycemia inducing increased free radical formation *via* interruption of the electron transport chain and glucose auto-oxidation. It also occurs during advanced glycation end products formation [3, 4]. During diabetes or insulin resistance, failure of insulin-stimulated glucose uptake by muscle causes glucose concentrations in blood to remain high. Consequently, glucose uptake by insulin-independent tissues increases. Increased glucose flux both enhances oxidant production and impairs antioxidant defenses by multiple interacting non-enzymatic, enzymatic and mitochondrial pathways [5, 6]. In diabetes, an altered oxidative metabolism is a consequence either of the chronic exposure to hyperglycaemia or of the absolute or relative insulin deficit; insulin regulates several reactions involved in oxido-reductive metabolism [7]. The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity, and treatment [8]. The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world. Medicinal plants are continued to be a powerful source for new drugs, now contributing about 90% of the newly discovered pharmaceuticals [9].

*T. indica* was used as a traditional medicine for the management of diabetes mellitus [10]. *T. indica* is a large and tall tree which belongs to the family of 'Caesalpiniaceae' and it is extremely found all over India. There fruits are also found mainly in summer season and seed coat is brownish black in colour though the kernel is white in colour. Pharmacological studies of the plant revealed that *T. indica* possess anti-snake venom, antibacterial, antifungal, anti-inflammatory, antimalarial, anti-oxidant and hepatoregenerative activities [11 - 14]. In our previous work aqueous extract of seed of *T. indica* was also studied as an antidiabetic agent [15].

## MATERIALS AND METHODS:

**Chemicals:** 1, 1-diphenyl – 2 - picrylhydrazyl (DPPH), 2, 2´-azino-bis(3-ethyl benzothiazoline-6-sulphonic acid) - (ABTS) and streptozotocin was purchased from Sigma Chemical Co. (St Loius, MO, USA). Butylated hydroxytoluene (BHT) was purchased from LOBA CHEMIE Pvt. Ltd., (Mumbai, India).

All other chemicals used here were of analytical grade obtained from E. Merck (Mumbai, India).

**Plant Materials:** Seeds of *Tamarindus indica* Linn. were collected from Badhutola, Paschim Medinipur district, West Bengal, India, in the month of May - June and the materials were identified by taxonomist of Central National Herbarium (CAL), Botanical Survey of India (B.S.I), Shibpur, Howrah. The voucher specimen was deposited in the Central National Herbarium (CAL), B.S.I, Shibpur, Howrah and voucher specimen number, HPCH No-1.

**Preparation of Hydro-Methanolic Extract of *T. indica*:** Pulverized seeds (5000 g) of *T. indica* were taken into 20 L percolator and maceration was carried out with 10L hydro-methanolic solution ($H_2O$: MeOH:: 40: 60) at 25 °C to avoid any degradation or deactivation of the active compound (s). The slurry was stirred intermittently for 1 hr and left for overnight. The extract was collected on the second day after 24 hr of extraction process and then freshly prepared 5L hydro-methanolic solution was added to the extraction chamber and the slurry was stirred again with glass rod. The same procedure was repeated again on the third day with another 5L solvent mixture and last extract was collected on the fourth day.

The extract was filtered first by cotton filter and then by Whatman filter paper (No.1). The filtrate was evaporated under reduced pressure by Rotavapour (BUCHI–R124; Switzerland) at 40 °C for complete removal of methanol. Finally plain aqueous filtrate (9.5 L free from methanol) was lyophilized on VirTis bench top K lyophilizer. The lyophilized extract (920 g) was collected and put into the amber colored glass containers which were finally stored in the refrigerator under vacuum for subsequent fractionation and experimental studies. The lyophilized extract was a mixture of dark brownish sticky layer and light brownish solid powder (slightly hygroscopic in nature).

**Bioassay Guided Fractionation:** In 5L separating flask, 750 g of lyophilized extract of *T. indica* was dissolved with 2L of hydromethanolic ($H_2O$: MeOH :: 40: 60) solution and solvent fractionation was carried out using solvents (n-Hexane, Chloroform, Ethyl acetate and n-Butanol) with increasing polarity. T.L.C was carried out to monitor the progress in fractionation. All fractionates were collected separately and dried under reduced pressure (20 - 200 mbar) using rotavapour instrument at 40 °C. Finally from 500 g lyophilized extract of *T. indica* 5.8 g n-hexane fraction, 36.4 g chloroform fraction, 71.8 g ethyl acetate fraction and 168.5 g n-butanol fractions were obtained. All the fractions were administered orally through gavage.

**Phytochemical Screening:** The n-hexane fraction was subjected to preliminary screening for various active phytochemical constituents such as flavonoids, alkaloids, saponins, tannins, terpenoids, steroids, glycosides, anthraquinon and amino acids [16].

**Acute Toxicity Studies:** Healthy adult Wister albino rats of either sex, starved overnight were divided into six groups (n = 6) and were orally fed with the n-hexane fraction of *T. indica* in escalating dose levels of 100, 500, 1000, 2000, 3000 mg/kg body weight [17]. The rats were pragmatic continuously for 2 h for behavioural, neurological and autonomic profile and after a period of 24 and 72 h for any lethality of death [18].

**Selection of Animal and Animal Care:** Twenty four matured normoglycemic (having fasting blood glucose level 80 - 90 mg/dl) Wistar strain male albino rats, 4 months of age, weighing about 150 ± 10g were selected for this experiment. Animals were acclimated for a period of 15 days in our laboratory condition prior to the experiment. Rats were housed at an ambient temperature of 25 ± 2°C with 12 h light: 12 h dark cycle. Rats were fed pellet diet and water *ad libitum*. The principle of Laboratory Animal Care and instructions given by our Institutional Ethical Committee were followed throughout the experiment.

**Induction of Diabetes in Rats:** Twenty four hours fasted eighteen rats out of twenty four were subjected to a single intramuscular injection at the dose of 4 mg / 0.1 ml of citrate buffer / 100 gm body weight / rat. After 7 days of STZ injection, diabetic rats (fasting blood glucose level >300 mg/dl <400 mg/dl) were selected for the study.

**Animal Treatment:** Eighteen diabetic rats having said criteria were selected. Six rats were categorized into diabetic control and rest rats were placed in n-hexane fraction and glibenclamide administered diabetic group. Other six normoglycemic rats were considered under control group. Fraction and glibenclamide treatment of *T. indica* seed was started from 7$^{th}$ day of post injection period of STZ and was considered as 1$^{st}$ day of experiment. The treatment was continued for next 28 days.

**Group I (Control group):** Rats of this group received single intramuscular injection of citrate buffer (0.1 ml / 100 g bw) at the time of STZ injection to the other animals for diabetic induction.

**Group II (Diabetic control group):** Diabetic rats of this group were forcefully fed with distilled water at a dose of 0.5 ml of distilled water 100 g bw/day for 28 days by gavage.

**Group III (Diabetic + n-hexane fraction):** Diabetic rats were forcefully fed by gavage of n-hexane fraction of seed of *T. indica* at a dose of 100 mg / 5 ml 2% tween 80 / kg body weight / rat / day from 7$^{th}$ day of streptozotocin injection for next 28 days at fasting state.

**Group IV (Diabetic + glibenclamide):** Diabetic rats of this group were administered forcefully by gavage of glibenclamide at a dose of 0.6 mg / 5 ml water / 100 gm bodyweight/rat/day from 7$^{th}$ day of streptozotocin injection for next 28 days at fasting state.

Fraction and glibenclamide administration to the rats of group III and group IV was performed early in the morning and at fasting state by gavage. Animals of control group (Group I) were subjected to gavage of distilled water like group II for 28 days at the time of n-hexane fraction and glibenclamide treatment to the animals of group III to keep all the animals under the same experimental condition and stress imposition if any due to treatment of fraction and animal handling. Starting from first day of n-hexane treatment to diabetic rats, fasting blood glucose levels (12 h after feed

delivery) in all the groups were measured by single touch glucometer on every 7 days interval. On the 35th day of experiment blood was collected from the tail vein and fasting glucose level was monitored by single touch glucometer.

All the animals were sacrificed at fasting state by light ether anesthesia followed by decapitation after recording the final body weight. Blood was collected from the dorsal aorta by a syringe and the serum was separated by centrifugation at 5000 rpm for 5 min for the estimation of serum insulin and C-peptide. The liver was dissected out and stored at -20 °C for the assessment of the activities of the antoxidant enzymes - catalase (CAT), peroxidase (Px), superoxide dismutase (SOD) quantification of the levels of the products of free radicals like conjugated diene (CD) and thiobarbituric acid reactive substances (TBARS).

**Oral Glucose Tolerance Test (OGTT) in STZ-Induced Diabetic Rats:** The oral glucose tolerance test was performed in overnight fasted normal and diabetic rats. Animals were divided into six groups of 6 animals (n = 6) each. Group I, II and III were orally administered 2% Tween 80, n-hexane fraction (100 mg/kg) and glibenclamide (0.6 mg/kg) respectively. Diabetic groups IV, V and VI were orally administered 2% Tween 80, n-hexane fraction (100 mg/kg) and glibenclamide (0.6 mg/kg) respectively served as control and received 2% Tween 80. Fasting blood glucose levels was conducted initially and then blood glucose level was recorded after 30 min of treatment considered as 0 min. A dose of 5 g/kg of glucose was given orally to all the groups. Blood glucose levels were further recorded upto two hours at regular interval of 30 min each, considered as 30, 60, 90 and 120 min values [19].

**Biochemical Estimations:** Serum insulin level was measured according to Brugi *et al*., 1998 [20] using rat insulin enzyme linked immunosorbent assay (ELISA) kit obtained from Millipore Corporation, Billerica, MA 01821. Serum C-peptide level was measured by the method of Bhat *et al*., 2011 [21] using rat C-peptide (Yanaihara, Japan) ELISA kit. The activities of catalase, peroxidase and superoxide dismutase of the hepatic tissues were measured bio chemically according to Beers and Sizer (1952), [22] Sadasivam and Manikam (1996), [23] and Marklund and Marklund, (1974) [24] respectively. Quantification of lipid peroxidation from concentration of thiobarbituric acid reactive substances (TBARS) and conjugated diene (CD) in liver were performed according to Okhawa *et al*., (1979) [25] and Slater 1984 [26].

The radical scavenging activity of *T. indica* against DPPH was determined spectrophotometrically by the method of Kim *et al*., 2003 [27]. The generation of the ABTS radical cation forms the basis of one of the spectrophotometric methods that has been utilized in measuring the total antioxidant activity of solutions of pure substances according to Raja and Pugalandi 2010 [28]. Histology of the pancreas stained with hematoxylin and eosin (H and E) were observed using a light microscope. Diameters of the pancreatic islets were measured by computerized microphotography using software [29].

**Statistical Analysis:** All the data were evaluated statistically using one-way analysis of variance (ANOVA) followed by multiple comparison two tail 't' test by using the Origin Lab (Ver. 6.0) software. P values of less than 0.05 were considered to indicate statistical significance. Data were presented as mean ± standard deviation.

## RESULTS:

**Preliminary Phytochemical Screening:** Our phytochemical studies indicated that n-hexane fraction of seeds of *T. indica* contains flavonoids, alkaloids, terpenoids and steroids while saponins, glycosides, tannins, protein, anthraquinons and phlobatannins were absent **Table 1**.

**TABLE 1: QUALITATIVE ANALYSIS OF THE PHYTOCHEMICALS OF n - HEXANE FRACTION OF *T. INDICA* SEED**

| S. no. | Phytochemical Constituents | *T. indica* seed |
|---|---|---|
| 1. | Flavonoids | + |
| 2. | Alkaloids | + |
| 3. | Saponins | - |
| 4. | Tannins | - |
| 5. | Terpenoids | + |
| 6. | Steroids | + |
| 7. | Glycosides | - |
| 8. | Anthraquinons | - |
| 9. | Proteins | - |
| 10. | Phlobatannins | - |

**Acute Toxicity Studies:** In performing preliminary tests for pharmacological activity in rats, n-hexane fraction did not produce any significant changes in

the auto-nomic, behavioural or neurological responses upto doses of 3000 mg/kg body weight. Acute toxicity studies revealed the non-toxic nature of the n-hexane fraction of *T. indica* **Table 2**.

**TABLE 2: MEDIAN LETHAL DOSE (MLD) DETERMINATION OF THE n-HEXANE FRACTION OF *T. INDICA* ADMINISTERED ORALLY TO WISTAR RAT**

| 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 | |
| 500 | 6 | 6 | 0 | |
| 1000 | 6 | 6 | 0 | |
| 2000 | 6 | 6 | 0 | |
| 3000 | 6 | 6 | 0 | >3.0 g/kg body weight |

**Blood Glucose Level:** Diabetes induced by STZ resulted in a significant elevation in blood glucose in comparison to the control group. After the administration of n-hexane fraction of seed of *T. indica* or glibenclamide to the diabetic animals for 28 days, a significant recovery of blood glucose level was noted and the level was recovered towards the control group. There was a significant difference in the level of fasting blood glucose between fraction treated group and glibenclamide treated group **Table 3**.

**TABLE 3: EFFECT OF n-HEXANE FRACTION OF SEED OF *T. INDICA* ON FASTING BLOOD GLUCOSE LEVEL IN STZ INDUCED DIABETIC ALBINO RAT**

| Groups | Fasting blood glucose level (mg/dl) | | | | |
|---|---|---|---|---|---|
| | 1st day (The day of STZ injection) | 7th day (The day of fraction treatment) | 14th day | 21st day | 28th day |
| Control | 76.21±3.4[a] | 73.78±3.8[a] | 76.04±4.2[a] | 76.36±3.4[a] | 79.48±4.3[a] |
| Diabetic | 75.24±3.4[a] | 342.68±8.4[b] | 338.83±7.1[b] | 339.00±6.7[b] | 343.53±7.9[b] |
| Diabetic + n-hexane fraction | 77.29 ±4.5[a] | 345.92±7.8[b] | 190.59±4.6[c] | 123.36±4.6[c] | 82.62±4.3[a] |
| Diabetic + glibenclamide | 75.45±3.3[a] | 339.28±7.5[b] | 181.37±4.1[c] | 129.46±4.8[c] | 98.42±3.9[c] |

Data are expressed as Mean ± S.E.M; n = 6. ANOVA followed by multiple comparison two tail 't' test. Values with different superscripts (a, b, c) in each vertical column differ from each other significantly, $p < 0.05$.

**Effect of n-hexane Fraction on Oral Glucose Tolerance Test (OGTT) in STZ - Induced Diabetic Rats:** Demonstrate the effect of n-hexane fraction on blood glucose level of normal and streptozotocin-induced diabetic rats during OGTT studies. After 2 h of glucose administration the significant decrease in blood glucose level was observed with the fraction treatment (100 mg/kg) and glibenclamide treated group when compared to the control. In STZ - induced diabetic rats, the fraction treatment and glibenclamide treated group showed significant decrease in blood glucose level respectively when compared to diabetic control **Table 4**.

**TABLE 4: EFFECT OF n-HEXANE FRACTION OF *T. INDICA* SEEDS ON OGTT IN NORMAL AND STZ INDUCED DIABETIC RATS**

| Groups | Blood glucose level (mg/dl) minutes after administration of drugs | | | | |
|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 |
| Control | 86.54 ±2.52[a] | 183.62 ±2.57[a] | 168.84 ± 2.72[a] | 155.70±1.40[a] | 116.35±2.53[a] |
| Control + n-hexane fraction | 88.47±2.79[a] | 178.57±3.53[a] | 158.62 ± 2.28[a] | 118.73±2.64[b] | 97.00±1.95[b] |
| Control + Glibenclamide | 86.64 ± 2.77[a] | 172.2 ± 2.19[a] | 141.00 ± 1.63[b] | 121.20±2.81[b] | 105.33 ± 2.21[b] |
| Diabetic control | 261.70±3.40[b] | 391.80 ± 7.43[b] | 452.42 ± 4.31[c] | 448.43±2.52[c] | 455.54 ±5.43[c] |
| Diabetic + n-hexane fraction | 248.62 ±3.64[b] | 272.62 ±5.17[c] | 310.30 ± 9.38[d] | 365.62±4.75[d] | 405.52 ± 4.52[d] |
| Diabetic + glibenclamide | 262.48 ±5.74[b] | 238.74 ± 4.35[d] | 357.38 ±4.52[e] | 418.54 ±4.69[e] | 413.86±6.19[d] |

Data are expressed as Mean ± S.E.M; n = 6. ANOVA followed by multiple comparison two tail 't' test. Values with different superscripts (a, b, c,d,e) in each vertical column differ from each other significantly, $p < 0.05$.

**Serum Insulin and C-Peptide Level:** A significant decrease was noted in serum insulin and C-peptide level in the diabetic control rats. Administration of n-hexane fraction or glibenclamide to diabetic rats for 28 days resulted a significant increase in serum insulin and C-peptide level in respect to the diabetic control rats. Insignificant difference was noted in both parameters between n-hexane fraction treated group and glibenclamide treated group **Fig. 1** and **2**.

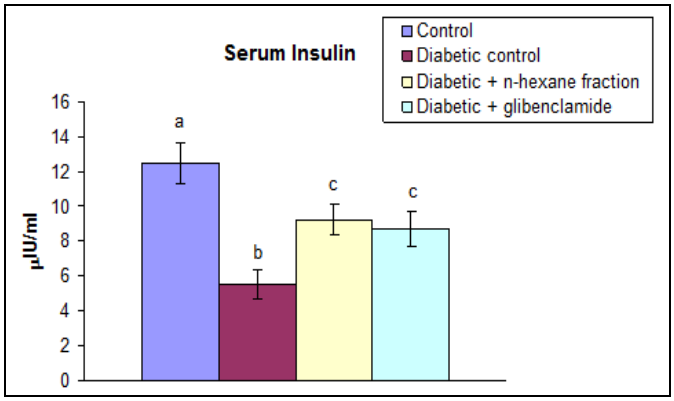


**FIG. 1: RESETTLEMENT OF SERUM INSULIN LEVEL AFTER ADMINISTRATION OF n-HEXANE FRACTION FROM HYDRO - METHANOLIC EXTRACT OF *T. INDICA* SEED IN STZ-INDUCED DIABETIC MALE ALBINO RAT**

Bar represents Mean ± 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, $p < 0.05$.

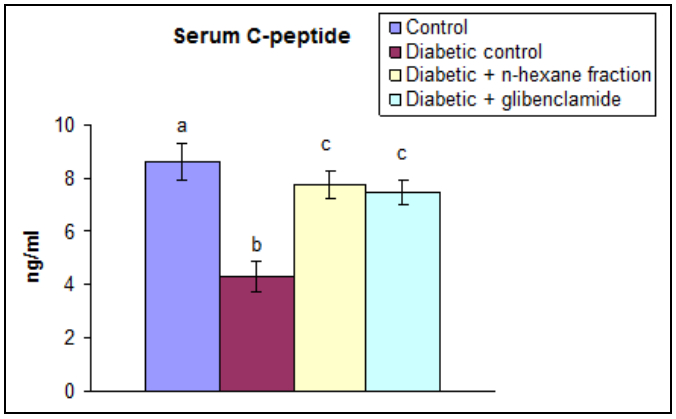


**FIG. 2: RESETTLEMENT OF SERUM C-PEPTIDE LEVEL AFTER ADMINISTRATION OF n-HEXANE FRACTION FROM HYDRO - METHANOLIC EXTRACT OF *T. INDICA* SEED IN STZ-INDUCED DIABETIC MALE ALBINO RAT**

Bar represents Mean ± 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, $p < 0.05$.

### *In vivo* Antioxidant Activities:

**Activities of CAT, Px and SOD:** Activities of CAT, Px and SOD in liver were decreased significantly in diabetic group in respect to control group. After the administration of n-hexane fraction of seed of *T. indica* or glibenclamide to STZ-treated diabetic rat, the activities of the above enzyme were restored towards the control level. Activities of above said enzymes differ significantly between n-hexane fraction of said plant part treated group and glibenclamide treated group **Table 5**.

**Levels of CD and TBARS:** Levels of CD and TBARS in liver were increased significantly in the diabetic group when compared to the control group. Significant recovery was noted in the levels of the above parameters after administration of the said plant part fraction or glibenclamide to diabetic rat. The levels of these parameters were insignificantly differ between fraction treated group and glibenclamide treated group **Table 5**.

### *In vitro* Antioxidant Activities:

**ABTS Radical Scavenging Activity:** *T. indica* was fast and effective scavenger of ABTS radicals as shown in **Fig. 3**. A comparable scavenging activity of this plant part was observed with that of BHT. The $IC_{50}$ values of the fraction and BHT were 0.021 ± 0.002, 0.016 ± 0.003 mg/ml respectively. At 0.5 mg/ml, the plant fraction showed higher inhibitory activity in removing ABTS radicals from the reaction system **Fig. 3**.

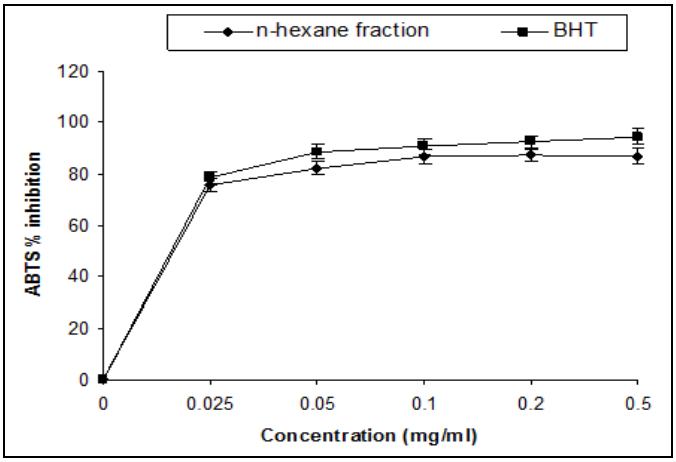


**FIG. 3: TOTAL ANTIOXIDANT ACTIVITY OF n-HEXANE FRACTION OF *T. INDICA* – ABTS RADICAL CATION DECOLOURIZATION ASSAY**

The $IC_{50}$ value of the fraction was 0.021 ± 0.002 mg/ml.

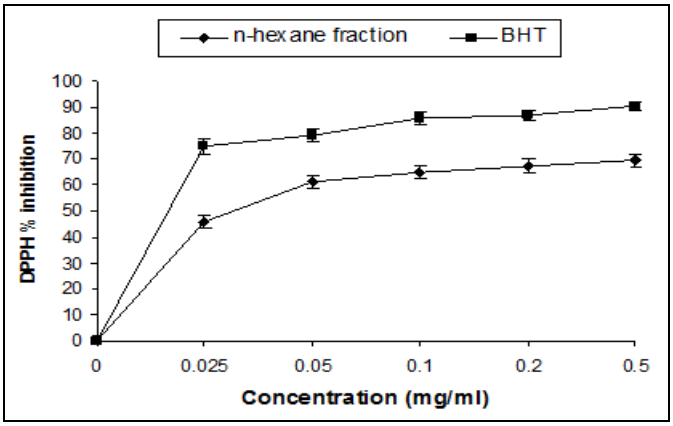


**FIG. 4: INHIBITION OF DPPH RADICAL BY n-HEXANE FRACTION *T. INDICA*, BHT**

The $IC_{50}$ value of the fraction was 0.027± 0.003mg/ml.

**DPPH Radical Scavenging Activity: Fig. 4** shows the dose-response curve of DPPH radical scavenging activity of *T. indica* compared with BHT. It was observed that the fraction, BTH had DPPH scavenging activity with $IC_{50}$ value of 0.027 ± 0.003 and 0.016 ± 0.003mg/ml respectively **Fig. 4**.

**Histological Study:** Diameters of pancreatic islets as well as count of islet cells were significantly decreased in STZ-induced diabetic group in respect to the vehicle control group. The values of these parameters were significantly recovered after the treatment of n-hexane fraction in diabetic rat **Fig. 5**.

**TABLE 5: REMEDIAL EFFECT OF n-HEXANE FRACTION OF SEED OF *T. INDICA* ON THE ACTIVITIES OF HEPATIC ANTIOXIDANT ENZYMES AND LEVELS OF LIPID PEROXIDATION IN STREPTOZOTOCIN-INDUCED DIABETIC ALBINO RAT**

| Groups | Antioxident enzyme activities | | | Lipid peroxidation levels | |
|---|---|---|---|---|---|
| | CAT(mM of $H_2O_2$ consumption/mg of tissue/min) | Px (Unit/mg of tissue) | SOD (unit/mg of tissue) | TBARS (nM/mg of tissue) | CD (nM/mg of tissue) |
| Control | 3.84±0.54[a] | 4.12±0.63[a] | 2.21±0.47[a] | 27.65±1.52[a] | 266.51±7.13[a] |
| Diabetic | 1.54±0.15[b] | 1.87±0.31[b] | 0.57±0.36[b] | 42.56±2.87[b] | 394.74±12.54[b] |
| Diabetic + n-hexane fraction | 3.75±0.57[a] | 3.97±0.74[a] | 2.14±0.52[a] | 30.54±2.23[a] | 278.48±8.25[a] |
| Diabetic + glibenclamide | 3.68±0.67[a] | 3.67±0.68[c] | 1.92±0.63[c] | 32.53±2.35[a] | 286.45±11.76[a] |

Data are expressed as Mean ± S.E.M; n = 6. ANOVA followed by multiple comparison two tail 't' test. Values with different superscripts (a, b, c) each vertical column differ from each other significantly, $p < 0.05$.

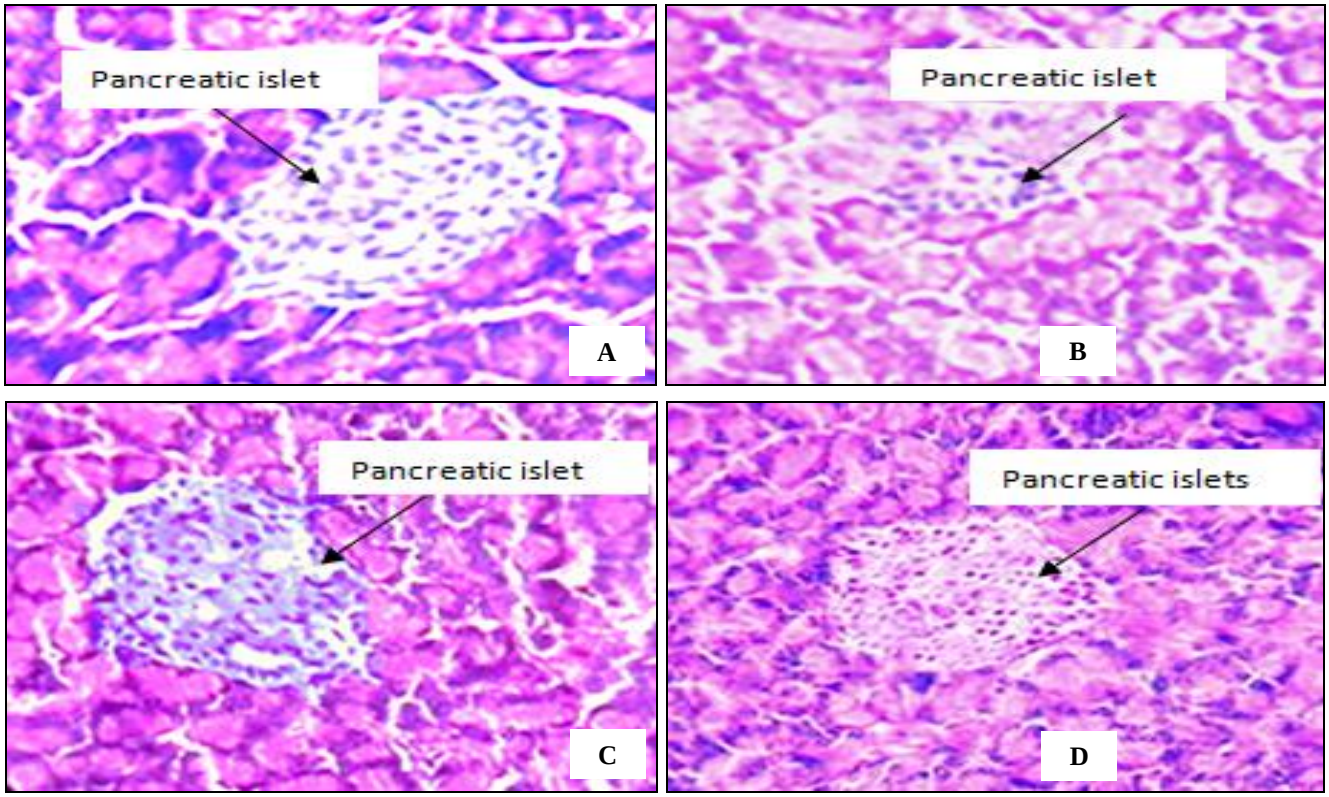


**FIG. 5: PLATE A. REPRESENTATIVE SAMPLE OF PANCREATIC TISSUE OF CONTROL RAT FOCUSING THE NORMAL ISLET DIAMETER. PLATE B. DIMINUTION IN THE DIAMETER OF ISLET IN THE REPRESENTATIVE PANCREATIC TISSUE SAMPLE OF STZ-INDUCED DIABETIC RAT. PLATE C. REPRESENTATIVE PANCREATIC TISSUE SAMPLE SHOWING RECOVERY IN ISLET CELL DIAMETER AFTER N-HEXANE FRACTION OF HYDRO-METHANOLIC 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**

**DISCUSSION:** Streptozotocin induced hyperglycaemia has been described as an useful experimental model to study the activity of antidiabetic agents in our previous work [30 - 33] as well as others [34 - 36]. Streptozotocin selectively destroyed the pancreatic insulin secreting β - cells,

leaving less active cell resulting in a diabetic state [37]. Insulin deficiency is manifested in a number of biochemical and physiological alterations. Insulin estimations and more specifically assessment of C-peptide are generally accepted as an index of β-cell function.

In the present study, we have observed a significant decrease in the levels of insulin and C-peptide in streptozotocin-induced diabetic rats. C-peptide promotes insulin action at low hormone concentration and inhibits it at high hormone levels suggesting a modulatory effect by C-peptide on insulin signaling. After the administration of n-hexane fraction of seed of *T. indica* or glibenclamide a significant recovery of plasma insulin and C-peptide levels was noted.

Antioxidant activity of *T. indica* has been revealed *in-vitro* by free radical scavenging and *in-vivo* by determination of CAT, Px and SOD assays in rats. CAT, Px and SOD were considered biologically essential in the reduction of hydrogen peroxide [38]. Reports have shown that the activities of CAT, Px and SOD were lowered in diabetic rats as well as our previous work [39] and others [40]. However, oral administration of seed of *T. indica* and glibenclamide restored the activities of these enzymatic antioxidants.

This suggests direct or indirect antioxidant nature of n-hexane fraction of seed of *T. indica* and glibenclamide which could be due to the free radical scavenging action of phytochemicals present in the said fraction of *T. indica* and glibenclamide, thereby improving the antioxidant potency in STZ-induced diabetic rats.

We have observed an increase in CD and TBARS levels in liver, a marker of lipid peroxidation in diabetes as well as our previous work and others [32, 41]. The observed increased concentration of lipid peroxides in the liver tissues of diabetic rats may be due to diminution in cytochrome $P_{450}$ and cytochrome b5, this may affect the drug metabolizing activity in chronic diabetes. Increased concentration of lipid peroxide in the liver has been observed in streptozotocin-induced diabetic animals [42]. Oral administration of *T. indica* or glibenclamide decreases TBARS in STZ-induced diabetic rat liver

On the other hand *T. indica* exhibits potent *in-vitro* antioxidant activity in DPPH-radical scavenging assay, ABTS free radical scavenging activity in comparison to the known antioxidants such as BHT. These results showed the ability of said fraction to reduce free radicals which may stop the free radical initiation or consequently inhibits / break free radical chain reaction in the propagation of the oxidation mechanism [43].

The significant antidiabetic activity of n-hexane fraction from hydro-methanolic extract of *T. indica* as shown in **Table 3** may be due to the presence of hypoglycemic flavonoids, alkaloids, terpinoids and steroids. The plant fraction may also contain some active biomolecules that may sensitize the insulin receptor to insulin or stimulates the existing β-cells of islets of Langerhans to release insulin which may finally lead to improvement of area of the pancreatic islets of Langerhans towards the re-establishment of normal blood glucose level [44].

In respect to $LD_{50}$ 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 activities of these enzymes which indicates that it has a promising antidiabetic effect without inducing toxicity [45].

**CONCLUSION:** From the results, it may be concluded that n-hexane fraction of *T. indica* exhibit islet regeneration or protection properties and therefore have a promising anti-diabetic and antioxidative activities in streptozotocin-induced diabetic state that holds the hope of new generation of antidiabetic drugs. 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.

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