Diabetes-induced testicular dysfunction correction by hydromethanolic extract of *Tamarindus indica* Linn. seed in male albino rat

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**Abstract**

**Background:** *Tamarindus indica* Linn. (Caesalpiniaeae) is a well-known traditional plant used by the tribes as well as rural sectors of tropical countries. Locally, it is named as Tantul and recognized as therapeutic uses of different diseases. **Aim:** The present study aimed to evaluate the efficacy of hydromethanolic extract of *T. indica* Linn. seed in streptozotocin (STZ)-induced diabetic testicular dysfunction correction. **Materials and Methods:** STZ-induced diabetic state has been established here by single intramuscular injection of STZ (6 mg/100 g body weight in citrate buffer, pH 4.5) resulted hyperglycemia. STZ-induced experimental diabetes resulted testicular dysfunctions evaluated by sperm count, sperm motility, viability, and seminal vesicle fructose content as well as by the level of serum testosterone. Testicular oxidative stress has been observed by the monitoring of catalase, peroxidase, superoxide dismutase, and glutathione-S-transferase activities. Quantification of thiobarbituric acid reactive substances and conjugated diene in testicular tissue was assessed. General and metabolic toxicity assessment parameters were studied. **Results:** Administration of hydromethanolic extract to STZ-induced diabetic rat at the dose of 80 mg/100 g body weight/day for 28 days resulted a significant protection in fasting blood glucose, serum insulin, and serum testosterone levels (*P* < 0.05) along with the correction of testicular above-mentioned parameters as well as semen analysis parameters toward the control level (*P* < 0.05). This extract has no general toxic effect on the body weight, along with no metabolic toxicity that was reflected here by the assessment of serum glutamate oxaloacetate transaminase and pyruvate transaminase activities. **Conclusion:** These data demonstrated that *T. indica* significantly improved STZ-induced diabetic complication in rat testis. This study suggested that hydromethanolic extract might have a protective role against oxidative stress-induced impairment in testicular functions in diabetic rats.

**Key words:** Antioxidant enzymes, diabetes mellitus, oxidative stress, streptozotocin, *Tamarindus indica*, testicular dysfunction

**INTRODUCTION**

Diabetes mellitus, a state of complex metabolic disorder with multiple etiologies characterized by chronic hyperglycemia, is a major cause of serious micro- and macro-vascular diseases, affecting, therefore, almost every system in the body. Both male and female reproductive impairment has been linked with diabetes mellitus.¹⁻² About 90% of diabetic patients usually suffer from sexual dysfunction that includes decrease in libido, impotence, erectile dysfunction, and infertility.³⁻⁵ Different studies showed that two factors, such as increased oxidative stress and decrease in antioxidant capacity, are playing an important role in the pathogenesis of chronic diabetes mellitus.⁶⁻⁸ Streptozotocin (STZ) is a broad-spectrum antibiotic having cytotoxic activity that induces diabetes

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**Received:** 13-11-2017  
**Revised:** 24-11-2017  
**Accepted:** 29-11-2017
mellitus in experimental models in animals. Mostly, it causes testicular dysfunction and degeneration in animal models. Oxidative stress is one of the major pathophysiological avenues during diabetes mellitus. Chronic and persistent hyperglycemia leads to the formation of advanced glycation end-products (AGEs) which are the products of non-enzymatic reactions between glucose and lipids, proteins, or nucleic acids. AGEs and glucose auto-oxidation might contribute to diabetes-induced sexual dysfunction by generating oxygen free radicals, especially reactive oxygen species (ROS), which induce oxidative cellular damage and quench nitric oxide, terminating in decreased cyclic guanosine monophosphate. Furthermore, ROS are positively correlated with both insulin resistance and the deterioration of cell function in the context of concomitant hyperglycemia, and their cytotoxic effects are always coupled with an increase in lipid peroxidation, alteration of the glutathione redox state, a decrease in the content of individual natural antioxidants, and decreased production of antioxidant enzymes. Fruits and vegetables contain a vast array of antioxidant components, which possess several physiological properties including protection against oxidative stress-mediated diseases. *Tamarindus indica* is one of those most widely used traditional medicine having antidiabetic and antioxidant properties. Diabetes-induced sexual dysfunction is considered as one of the most prevalent diabetic complications where oxidative stress and inflammation are deemed to play crucial role in its pathogenesis. It can be postulated that *T. indica* for its hypoglycemic and antioxidant properties may protect the diabetes-induced testicular dysfunction from the deleterious effects of ROS. The study was designed to investigate the protective ability of *T. indica* on STZ-induced diabetic-related testicular dysfunction in experimental animal.

Persistent hyperglycemia is linked with many complications including male reproductive dysfunctions and infertility. Many herbal medicinal plants have been widely used for the management of the diabetes mellitus in various traditional systems of medicine and in folklore worldwide because as they are a rich source of bioactive phytonutrients, which decrease blood glucose level and/or also act as antioxidant properties, resulting in the amelioration of oxidative stress-induced diabetic complications. The present study describes the ameliorative effects of traditional medicinal plants, especially on male reproductive dysfunctions, in STZ-induced experimental diabetic model animals.

**MATERIALS AND METHODS**

**Chemicals**

STZ was purchased from (Sigma Chemical Company, St. Louis, MO, USA). All other chemicals used in this experimental study were of analytical grade purchased from E. Merck (Mumbai). Insulin enzyme-linked immunosorbent assay (ELISA) kit purchased from Boehringer Mannheim Diagnostic, Mannheim (Germany). Testosterone kit was purchased from IBL, Germany.

**Plant Materials**

In the month of May-June, *T. indica* Linn. seed was collected from Badhutola, Paschim Medinipur District, West Bengal, India, the seeds were identified by taxonomist of Central National Herbarium (CAL), Botanical Survey of India (BSI), Shibpur, Howrah. The voucher specimen number is HPCH No-1 that was kept in the Central National Herbarium (CAL), BSI, Shibpur, Howrah.

**Preparation of Hydromethanolic Extract of *T. indica***

According to the protocol of National Institute of Health and Family Welfare (NIHFW), New Delhi (NIHFW, 2000), hydromethanolic extract of seed of *T. indica* was prepared. Fresh pulverized *T. indica* was dried in an incubator for 2 days at 40°C, crushed and powdered in an electrical grinder. Then, 100 g seed powder of *T. indica* was suspended in 500 ml of hydromethanolic mixture (1:1) and allows it to stay overnight in refrigerator and then extracted for 18 h in a Soxhlet apparatus, and a deep brown hydromethanolic extract was collected. The suspension was then filtered by coarse sieve filter paper. The filtrate was evaporated to dryness under reduced pressure in a Rotavap (BUCHI-R124; Switzerland). A deep brown material was obtained (4 g/100 g of the dried seeds powder). It was stored at 0–8°C until used. When needed, the residual extract was suspended in olive oil and used in this experiment.

**Acute Toxicity Studies**

Acute toxicity study associated with the determination of LD50 value of the *T. indica* seed extract was performed with different concentrations. Overnight fasted animals at the period of 12 h were divided into six groups (*n = 6*). Five groups of animals were administered orally with the single dose of extract at the concentrations of 20, 40, 80, 160, and 320 mg/100 g of body weight. One group was administered distilled water by oral route and considered as control. The animals were observed continuously for the initial 2 h, intermittently for the next 6 h of the study for the following symptoms such as behavioral profiles: Alertness, restlessness, irritability, and fearfulness. Neurological profiles: Spontaneous activity, reactivity, touch response, and tremors. Autonomic profiles: Mentioning defecation and urination. The morbidity, if any was recorded between 24 h and 72 h.

**Selection of Animal and Animal Care**

Wistar strain 24 male albino rats, scientific name *Rattus norvegicus*, 3 months of age with body weight about 125 ± 10 g were selected for the present study. Animals were
housed in plastic cage in light and temperature controlled room and acclimated for 15 days in our laboratory condition before the experiment in an ambient temperature of 25 ± 2°C under 12 h light:12 h dark cycle. Animals were provided with standard pellets rat chow diet and given water ad libitum. All experimental protocol in this study was conducted following the Principle of Laboratory Animal Care and instructions are given by our Institutional Ethical Committee and was followed throughout the experiment having Animal Ethical Committee No.VU/AEC/IV/13 Dated 23.07.2013.

Induction of Diabetes in Rats

Healthy after an overnight fasting 24 rats of which 18 rats were introduced to a single intramuscular injection of STZ at the dose of 6 g/0.5 ml of 0.1 M citrate buffer (pH-4.5)/100 g body weight/rat. From tail vein with the help of single touch glucometer, diabetic state was confirmed by measuring fasting blood glucose (FBG) level after 2 days of STZ injection. Diabetic rats having blood glucose concentrations of 250 mg/dl or higher were considered as diabetic and included in this experimental study. Normoglycemic rats were considered as control and their blood glucose levels were 75 mg/dl.

Animal Treatment

A total of 12 diabetic rats were divided into two groups having six animals in each group. Six rats were considered as diabetic control and rest six diabetic rats were administered with hydromethanolic extract through oral route by gavage. Other six normoglycemic rats were considered under control group, administered to olive oil to overcome stress due to drug administration to oral route. T. indica seed extracts were started from the 7th day of postinjection period of STZ and were considered as the 1st day of experiment because to investigate the stability of diabetic condition. The treatment was continued for next 28 days.

Group I (Control Group)

Rats of this group received single intramuscular injection of citrate buffer (0.5 ml/100 g body weight) at the time of STZ injection to the other animals for diabetic induction and forcefully feed 0.5 ml of olive oil/100 g body weight/day for 28 days.

Group II (Diabetic Control Group)

Diabetic rats of this group were administered forcefully by oral intubation with olive oil at a dose of 0.5 ml/100 g body weight/day for 28 days.

Group III (Diabetic + Hydromethanolic Extract)

Diabetic rats were forcefully fed T. indica seed extract at a dose of 80 mg/0.5 ml of olive oil/100 g body weight/rat/day from the 7th day of STZ injection for next 28 days at fasting state.

In the morning, at fasting state, extract was administered to the rats of Group III. Animals of control group (Group I) were subjected to gavage of olive oil like Group II for 28 days at the time of hydromethanolic extract treatment to the animals of Group III to keep all the animals under the same experimental condition and stress imposition if any due to the treatment of extract and animal handling. Starting from the 1st day of extract treatment to diabetic rats, FBG levels (12 h after feed delivery) in all the groups were measured by single touch glucometer on every 7 day’s 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 10 min for the estimation of serum insulin, testosterone, glutamic oxaloacetic Transaminase (GOT), and glutamic pyruvic transaminase (GPT) activities. Testis, seminal vesicle, and epididymis were dissected out and their relative weights were recorded and stored at −20°C for biochemical analysis of the activities of the antioxidant enzymes such as catalase (CAT), peroxidase (Px), superoxide dismutase (SOD), glutathione-S-transferase (GST), and quantification of the levels of the products of free radicals such as conjugated diene (CD) and thiobarbituric acid reactive substances (TBARS). Cauda epididymis of each animal was stored at 37°C in normal saline and used for epididymal sperm count and sperm viability. Seminal vesicle fructose content was assessed.

Biochemical Estimations

Testing of FBG level

With the help of single touch glucometer, FBG level was measured. Blood was collected from tip of the tail vein of all experimental and control animals in all groups at the initial time of experiment and every 7 days interval throughout the experiment.[19]

Assay of serum insulin by ELISA

By the solid-phase conjugated sandwich ELISA kit for rat, serum insulin level was measured using (EZRMI-13K, Millipore, USA).[20] The optical density of standard and unknown samples was measured against blank using a 480 nm selective filter and a 630 nm differentiating filter. As all samples were assayed at the same time, so no interassay variation was noted.

Serum testosterone level assessment by ELISA technique

Testosterone level in serum was measured using the testosterone kit from IBL, Germany, according to the
standard protocol supplied by that company.\[21\] In this solid-phase conjugated assay, an alkaline phosphatase conjugated hormone was used. Chromogen and stop solution was supplied by the company. The optical density of standard and unknown was measured using the selective filter at 450 nm and differentiating filter at 630 nm. The intra-assay variation was 5.2%. As all the samples were assayed at a time, so there was no interassay variation.

Measurement of Reproductive Profile

**Epididymal sperm count, sperm motility, and sperm viability assessment**

Sperm was collected from equal length of the cauda of the excised epididymis of each rat, and microscopic examination was performed as per standard procedure.\[22\] Under the microscope, motile spermatozoa were counted after placing it on a glass slide and covering it with a cover slip.\[23\] Result was expressed as %, in each field after counting 100 sperms. Viability of the sperm was performed\[24\] using eosin and nigrosin staining. One drop of sperm suspension was mixed with two drops of 1% eosin Y. After 30 s, three drops of 10% nigrosin were added and mixed well. Thick smear was prepared by placing a drop of mixture on a clean glass slide and allowed to air dry. Under phase contrast microscope prepared slide was examined. Dead sperm having pink color was differentiated from unstained live sperm, and their numbers were counted.

**Quantification of seminal vesicular fructose level**

Quantification for fructose content seminal vesicular homogenate was prepared at a tissue concentration of 50 mg/ml. The supernatant was deproteinized by adding 50 µl of zinc sulfate and 50 µl of sodium hydroxide to make a total dilution of seminal plasma 1:16, followed by centrifugation at 2500 rpm/min for 15 min. For fructose measurement, 200 µl of this clear supernatant was used. The optical density of sample and standard was measured against blank at wavelength of 470 nm. By plotting the values in standard curve, the concentration of fructose was obtained and the value was expressed in the unit of µM/ml of seminal plasma.\[25\]

**Antioxidative Enzyme Activity and Lipid Peroxidation Profile Assessment**

**Biochemical estimation of CAT, Px, SOD, and GST activities along with TBARS and CD levels**

The activities of CAT, Px, SOD, and GST of the testis were measured biochemically according to Beers and Sizer (1952),\[26\] Sadasivam and Manikam (1996),\[27\] Marklund and Marklund (1974),\[28\] and Habig et al.,\[29\] respectively.

Estimation of end products of lipid peroxidation such as TBARS and CD in testicular tissue was measured according to Okhawa et al. (1979)\[30\] and Slater, 1984.\[31\]

**Statistical Analysis**

Statistical analysis of all the collected data was carried out using one-way analysis of variance (ANOVA) followed by multiple comparison two-tailed t-test using the Origin Lab (Ver. 6.0) software. Data were expressed as mean ± standard error of the mean. \( P < 0.05 \) was considered to indicate statistically significant \( (P < 0.05) \).

**RESULTS**

**Body Weight and Accessory Sex Organ’s Weight**

At the end of the experiment, the body weight of diabetic rats was significantly decreased \( (P < 0.05) \) when compared with control and extract treated diabetic groups [Table 1]. Administration of hydromethanolic extract of T. indica seed to the diabetic rat for 28 days resulted in a significant recovery of above parameters toward the control group [Table 1]. The relative body weight of testis, seminal vesicles, and epididymis was significantly decreased \( (P < 0.05) \) in diabetic rat when compared with control and supplemented groups [Table 1]. T. indica supplementation to diabetic rats the above-mentioned parameter was recovered toward the control level [Table 1].

**FBG Level**

FBG level >250 mg/dl was noted significantly in untreated diabetic animals when compared with non-diabetic control rats [Table 2]. Treatment of hydromethanolic extract of T. indica seed to the STZ-induced diabetic rat for 28 days resulted in a significant recovery of FBG level toward the control group [Table 2].

**Serum Insulin, Serum Testosterone Levels along with Sperm Count, Sperm Motility, and Sperm Viability**

Serum insulin and serum testosterone levels were decreased significantly in diabetic group in comparison with the control group and extract treated diabetic groups of animals [Table 3]. The administration of the hydromethanolic extract to diabetic animals observed significant protection in the serum insulin and testosterone levels toward the control group [Table 3].

Semen quality assessment reproductive biomarkers such as sperm count, sperm motility, and sperm viability were decreased significantly in STZ-induced diabetic rats in compared to control rats, which furthermore increased significantly in T. indica treated group [Table 3]. Seminal vesicular fructose levels were increased in diabetic group in respect to the control group, which were rectified toward
the control after treatment with hydromethanolic extract of *T. indica* [Table 3].

The Activities of the Antioxidant Enzymes and Products of Free Radicals Levels

Testicular antioxidant enzymes such as CAT, Px, SOD, and GST were decreased significantly in diabetic group in respect to control group. After the treatment with this extract to STZ-induced diabetic rats, the levels of these parameters were restored toward the control levels [Table 4]. Quantification of the levels of the products of free radicals such as TBARS and CD levels in testis was increased significantly in diabetic group when compared with the control group. There was a significant recovery in the levels of the above-mentioned parameters in testicular tissue was noted after administration of hydromethanolic extract of *T. indica* [Table 4].

Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) Activities

Activities of SGOT and SGPT were increased significantly in diabetic group in respect to control group. After coadministration of *T. indica*, a significant recovery was noted in aforesaid parameters toward the control levels [Table 5].

### DISCUSSION

In the present investigation, single intramuscular injection of STZ selectively destroyed pancreatic β-cell of islets of Langerhans that induced diabetes mellitus in experimental animal, which was confirmed by the presence of persistence high blood glucose level. This result agrees with the findings of others.\[32,33\] The persistent high blood glucose level is due to low level of serum insulin observed in this study and also reported by other.\[34\] Our data showed that STZ-induced diabetic rats have severe loss in body weight which is consistent with the findings of other investigators.\[35,36\] This marked reduction in body weight may be breakdown of structural tissue protein in diabetic state. Diabetes causes remarkable increase in skeletal muscle catabolism along with reduction in protein biosynthesis.\[37\] The present study revealed that diabetic state causes significant diminution in relative body weight of testis, epididymis, and seminal vesicle may be on account of degeneration in sex organs as growth of these organs are controlled by key androgenic hormone testosterone,\[38\] which promotes growth and secretary action of the reproductive organs.\[39\]

We observed that diabetic animals showed a remarkable diminution in sperm count, sperm viability, and percentage of live sperm as well as remarkable increase in abnormalities of sperm. These findings were reported by other worker.\[40\]
### Table 3: Amelioration in the epididymal sperm parameters, reproductive biosensors such as serum testosterone, seminal plasma fructose level, and serum insulin level after administration with hydromethanolic extract of *T. indica* in STZ-induced diabetic male albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum insulin (µU/ml)</th>
<th>Serum testosterone (ng/ml)</th>
<th>Sperm count (million/ml of epididymal fluid)</th>
<th>Sperm motility (%)</th>
<th>Sperm viability (%)</th>
<th>Seminal plasma fructose level (µM/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.29±0.10a</td>
<td>11.2±0.16a</td>
<td>38.4±0.61a</td>
<td>89.3±4.6a</td>
<td>90.2±6.1a</td>
<td>3.08±0.12a</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.31±0.02b</td>
<td>5.5±0.13b</td>
<td>16.1±0.41b</td>
<td>60.6±5.2b</td>
<td>59.1±5.2b</td>
<td>4.71±0.13b</td>
</tr>
<tr>
<td>Diabetic+hydromethanolic extract of <em>T. indica</em></td>
<td>1.70±0.06c</td>
<td>8.7±0.15c</td>
<td>25.8±0.52c</td>
<td>77.8±5.9c</td>
<td>78.1±4.9c</td>
<td>3.38±0.16c</td>
</tr>
</tbody>
</table>

Data represent the mean±SEM; *n=6*. ANOVA followed by multiple comparison two-tailed *t*-test. Different superscript letters (a, b, c) indicate significant difference between these groups (*P*<0.05). SEM: Standard error of mean, ANOVA: Analysis of variance, STZ: Streptozotocin, *T. indica*: Tamarindus indica

### Table 4: Effect of oral administration of hydromethanolic extract of *T. indica* after 4 weeks on testicular enzymatic antioxidant and lipid peroxidation in STZ-induced diabetic male albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Antioxidant enzyme activities</th>
<th>Lipid peroxidation levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAT (mM of H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; consumption/mg of tissue/min)</td>
<td>Px (unit/mg of tissue)</td>
</tr>
<tr>
<td>Control</td>
<td>3.91±0.54a</td>
<td>4.12±0.23a</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.62±0.15b</td>
<td>1.87±0.31b</td>
</tr>
<tr>
<td>Diabetic+hydromethanolic extract of <em>T. indica</em></td>
<td>3.15±0.57c</td>
<td>3.08±0.24c</td>
</tr>
</tbody>
</table>

Data represent the mean±SEM; *n=6*. ANOVA followed by multiple comparison two-tailed *t*-test. Values with different superscripts (a, b, c) in each vertical column differ from each other significantly, *P*<0.05. SEM: Standard error of mean, ANOVA: Analysis of variance. STZ: Streptozotocin, *T. indica*: Tamarindus indica, CAT: Catalase, Px: Peroxidase, SOD: Superoxide dismutase, GST: Glutathione-S-transferase, CD: Conjugated diene, TBARS: Thiobarbituric acid reactive substances

### Table 5: Effect of hydromethanolic extract of seed of *T. indica* on biomarkers such as SGOT and SGPT activities in STZ-induced diabetic male albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.2±3.40a</td>
<td>26.3±2.57a</td>
</tr>
<tr>
<td>Diabetic</td>
<td>99.5±4.05b</td>
<td>88.4±2.11b</td>
</tr>
<tr>
<td>Diabetic+hydromethanolic extract of <em>T. indica</em></td>
<td>45.2±3.31c</td>
<td>37.2±3.14c</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM; *n=6*. ANOVA followed by multiple comparison two-tailed *t*-test. Values with different superscripts (a, b, c) in each vertical column differ from each other significantly, *P*<0.05. SEM: Standard error of mean, ANOVA: Analysis of variance, STZ: Streptozotocin, *T. indica*: Tamarindus indica, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase

who confirmed that diabetic rats had pronounced reduction in sperm quantity. Testosterone plays a key role for spermatogenesis and also high testosterone level is essential for the normal physiology of seminiferous tubules.[41] In the present study, plasma testosterone was remarkably decreased in STZ-induced diabetic rats, and also reported by similar study.[42] Ballester *et al.*[43] suggested that the low level of testosterone in diabetic state may be associated with the decrease in Leydig cells or in androgen biosynthesis. Seminal plasma fructose content is the key energy source for sperm viability and motility has been increased in diabetic rats probably due to the diminution of the sperm count that may interfere in fructose utilization due to oxidative stress.[44] Diabetes initiates a state of oxidative damage that was confirmed by an increase in testicular TBARS and CD levels along with a reduction in testicular antioxidant defense enzymes activities such as CAT, Px, SOD, and GST. The oxidative stress caused by diabetes was shown by a sharp diminution in spermatogenesis process.[45] Supplementation of hydromethanolic seed extract of *T. indica* resulted correction of STZ-induced diabetic state through a...
mark recovery in serum insulin toward the control level. On the other hand aforesaid extract also recovered body weight, organosomatic indices, correction of sperm count, sperm viability, and percentage of live sperm. T. indica remarkably ameliorates serum testosterone level by scavenging the ROS and attenuation of testicular enzymatic antioxidant status. The elevated level of seminal vesicular fructose was corrected by hydromethanolic extract supplementation that showed the recovery in fructose synthesis and its utilization by spermatozoa.

The supplemented hydromethanolic extract has no toxicity which has been indicated here from the improvement of markers of general toxicity assessment such as SGOT and SGPT activities seems to be its ability to enhance glucose utilization and reduce metabolic toxicity.

**CONCLUSION**

From the results of the above studies, it can be concluded that the administration of T. indica may be useful in alleviation of diabetes-induced complications, especially male reproductive dysfunction, by virtue of their antidiabetic, antioxidant, and androgenic activities of various bioactive phytochemicals present in this extract. The actual mechanism for such protection is not clear from this work but the work is in progress and the biomolecules responsible for specific function would be expressed from the subsequent work in this line.

**REFERENCES**


Source of Support: Nil. Conflict of Interest: None declared.