**Evaluation of hepatoprotective and in-vivo antioxidant activity of *Tamarindus indica* L. seeds extracts in streptozotocin induced diabetic rats**

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**Abstract**

Hydroalcoholic and aqueous extract of *Tamarindus indica* seeds was evaluated for hepatoprotective and antioxidant activity in Streptozotocin induced diabetic rats. Diabetes induced hepatic damage was evaluated by the serum markers such as SGOT, SGPT, ALP, Bilirubin. Further the effect of HAETIS and AETIS on oxidative stress was determined by the markers of oxidative stress: Lipid peroxidation (LPO), Determination of Super Oxide Dismutase (SOD), Determination of Catalase (CAT), glutathione (GSH) were estimated in liver tissue. HAETIS and AETIS at a dose level of 100 mg/kg and 200 mg/kg produce significant (P< 0.05) hepatoprotection by decreasing the activity of serum enzymes, bilirubin and lipid peroxidation, while they significantly increase the level of Glutathione, Super Oxide Dismutase and catalase in a dose dependant manner. Histopathological studies supports the above finding results indicate that hydroalcoholic extracts of *Tamarindus indica* at 200 mg/kg showed more significant hepatic protection as compared to other extracts.

**Keywords:** *Tamarindus indica*, Streptozotocin, Diabetes, hepatoprotective effect, antioxidant

**Introduction**

Diseases are appears to afflicted more to human being in comparison with other animal species. Human being by taking the advantage of plants growing around him. In the past almost all of the medicines used were obtained from plant and plant beings the only chemist for ages [1]. Free radicals are highly reactive and are capable of damaging almost all type of biomolecules. The fact is that free radicals generate free radicals from normal compound which continue as a chain reaction [2]. Ayurveda is a medical system primarily practised in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment [3]. The antioxidants may be defined as a substance that, when present at low concentrations compared to those of the oxidizable substrate, significantly delays, or inhibits, oxidation of that substrate [4].

The plant of *T indica* is a tree-type of plant 12- 18 m. height with spreading branches, which belongs to the Leguminosae, caesalpiniaeae family [5]. It is indigenous to tropical Africa but has become naturalized in North and South America from Florida to Brazil, and is also cultivated in subtropical India, China, Pakistan, Philippines, Spain and Java. In early stage, the fruit shows a reddish-brown color that turns black or black brown, becoming more aromatic and sour after ripening. *T. indica* pulp fruit is used, as a food component and for juices. Its fruit are regarded as a digestive, carminative, laxative, expectorant and blood tonic [6].

*T. indica* was used as a traditional medicine for the management of diabetes mellitus [7]. Different plant parts shows anti diabetic [8], hepatoprotective [9], antioxidant [10], anti inflammatory [11] and antimutagenic [12] activities.

There is increasing evidence indicating that enhanced production of free radicals may be an important contributing factor in the complications seen in diabetes. Species increased in diabetes, especially in uncontrolled diabetes, can lead to the auto-oxidation of glycosylated proteins, activation of the sorbitol pathway, induction of membrane damage, and oxidation of cellular lipids and proteins [13].

Hence, considering the traditional claim, chemical constituents and reported activities of *T indica* seeds extract the present study was planned to screen both aqueous and hydroalcoholic extract of *T indica* seeds for hepatoprotective and antioxidant activity in streptozotocin induced diabetic rat model.

**Materials and methods**

**Plant material**

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Seeds of *T. indica* were collected from local market of Raipur, CG, India. Seeds were washed under running tap water followed by rinsed with distilled water for five minutes. Plant as well as seed of *T. indica* were identified and authenticated by Dr. P. C. Panda, Principal scientist taxonomy division “Regional Plant Resource centre Bhubaneswar, Odisha, India”, a voucher specimen was also deposited (Voucher specimen no: B M -1).

**Preparation of Extracts**

The seeds were dried under shade and then powered with a mechanical grinder to obtain a coarse powder, which was then subjected to successive extraction in a Soxhlet apparatus using petroleum ether (60-80°C), (Water + ethanol, 80:20) and water. Solvent elimination under reduced pressure affords the hydroalcoholic after complete extraction and solvent was removed from the aqueous extract by concentrating at 50°C. The hydroalcoholic extract (9.24% yield) and for aqueous extract (6.43% yields) and Extracts were subjected to preliminary phytochemical screening. Both the extracts were then used for evaluation of hepatoprotective and *in vivo* antioxidant studies [14].

**Drugs and Chemicals**

Glibenclamide was obtained as a gift sample from Bioplus life sciences, Bangalore, India, SGOT, SGPT, ALP, Bilirubin and Total Protein kits were procured from Span Diagnostics, Surat, India. Streptozotocin, Thiobarbituric acid (TBA), 5, 5'- dithiobis-2-nitrobenzoic acid (DTNB) obtained from Himedia laboratories Pvt. Ltd, Mumbai. Reduced Glutathione (GSH) and the rest of the chemicals utilized were of analytical grade and were obtained from Fisher scientific, Mumbai, India.

**Experimental animals**

Studies were carried out using Wistar albino rats of both sex (150-200 g). They were housed in standard cages at room temperature 25±2 C and 50±5% relative humidity, under a light/dark cycle of 10/12 h, for 1 week before the experiment for acclimatization. Animals were provided with standard rodent pellet diet (Amrut, India), and water *ad libitum*. The animals were deprived of food for 24 hours before experimentation, but had free access to drinking water. Experimental protocols were approved by Institutional Ethical Committee which follows guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) The experiment were performed after the approval of Institutional Animal Ethical Committee (IAEC approval no. SBRL/IAEC/2012/11). Normoglycemic animals were selected for this experiment having the fasting blood glucose level 85 ± 5 mg/dl. Instruction given by our institutional ethical committee was followed regarding injection and other treatment of the experiment.

**Acute oral toxicity study and selection of doses**

The acute toxicity study of both the extract HAETIS and ATIS were determined as per the Organization of Economic Co-Operation and Development (OECD) guideline no. 425 (Acute toxic class method). It was observed that the HAETIS and AETIS were not mortal up to 2000 mg/kg dose orally. Hence, 1/10th (200 mg/kg) of this dose was selected for this study [15].

**Streptozotocin-induced diabetes in rats**

In the experiment, a total of 42 rats (6 normal and 36 Streptozotocin induced diabetic rats were used. The animals were divided in to. The animals were assigned into seven groups (I-VII) of six animals each and received the following treatments: Group I: Normal control + distilled water, Group II: Diabetic control (Streptozotocin 55mg/kg) + distilled water, Group III: Diabetic + Glibenclamide (2.5 mg/kg) as standard drug, Group IV: Diabetic + HAETIS(100 mg/kg), Group V: Diabetic + HAETIS (200 mg/kg), Group VI: Diabetic + AETIS (100 mg/kg) And Group VII: Diabetic + AETIS (100 mg/kg) for 14 days . The effects HAETIS and AETIS on Streptozotocin induced diabetic rats were determined weekly once by body weight, blood glucose measurement on overnight fasted rats [16].

**Biochemical studies**

At the end of the experimental period, the animals were fasted overnight and blood was collected for various biochemical estimations. Blood was collected from all animals by puncturing the retro-orbital plexus. The blood sample was allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and subjected for estimation of various biochemical parameters namely SGOT, SGPT, ALP and Bilirubin. After collection of blood samples the rats in different groups were anaesthetized and sacrificed and their livers were excised immediately and washed in ice cold normal saline followed by 0.15 M Tris-HCl (pH 7.4) blotted and dry and weighed . A 10 % w/v of homogenate was prepared in 0.15 M Tris-HCI buffer and processed for lipid Peroxidation.A part of homogenate after precipitating protein with (TCA) for estimation of glutathione. The rest of the homogenate was centrifuged at 1500 rpm for 15 at 14°C. The supernatant thus obtained was used for estimation of SOD and CAT activity.

**Serum hepatospecific markers**

Activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman and Frankel [17]. 0.05 ml of serum with 0.25 ml of substrate (aspartate and -ketoglutarate for SGOT; alanine and - keto glutarate for SGPT, in phosphate buffer pH 7.4) was incubated for an hour in case of SGOT and 30 min. for SGPT. 0.25 ml of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 ml of 0.4N NaOH was added and absorbance was read at 505 nm in **UV-**
Catalase activity was measured based on the ability of the enzyme to breakdown \( \text{H}_2\text{O}_2 \). The method of Maehly et al. was employed in the estimation of CAT activity. The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 1 - 4 C and centrifuged at 5,000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM \( \text{H}_2\text{O}_2 \) and the enzyme extract. The specific activity of catalase is expressed in terms of units/g of liver tissue [22].

Determination of Reduced Glutathione (GSH)

Reduced glutathione (GSH) was determined by the method of Ellman. To 0.1 ml of liver tissue homogenate 2.4 ml of 0.02M EDTA solution was added and kept on ice bath for 10 min. Then 2 ml of distilled water and 0.5 ml of 50 % TCA were added. This mixture was kept on ice for 10-15 min and then centrifuged at 3000 rpm for 15 min. 1 ml of supernatant was taken and 2ml of Tris-Hcl buffer was added. Then 0.05ml of DTNB solution (Ellman’s reagent) was added and vortexed thoroughly. OD was read (within 2-3min after the addition of DTNB) at 412 nm against a reagent blank. Absorbance values were compared with a standard curve generated from known GSH [23].

Histopathology

At the end of treatment, small pieces of tissues of liver were blotted and freed from blood, fixed in 10% neutral buffered formalin for 48h, trimmed and processed for paraffin embedment and 5 mm thickness of tissue sections were stained with haematoxylin and eosin for histo-pathological examination. Histological structures of liver and pancreatic sections were examined using a light microscopy.

Statistical analysis

The experimental results were expressed as the mean ± SEM for six animals in each group. The biochemical parameters were analyzed statistically using one-way analysis of variance (ANOVA), followed by Dunnett’s multiple comparison test. P value of < 0.05 was considered as statistically significant. Statistical analysis was performed using Graph Pad prism 5.0 (San Diego, USA).

Results

The effect of HAETIS and AETIS on serum transaminase, alkaline phosphatase and bilirubin levels in streptozotocin induced diabetic rats are summarized in Table 1. Administration of STZ (55 mg/kg), after 14 days there was significant (p<0.05) elevation in level of hepato- specific serum markers SGOT, SGPT, ALP and bilirubin levels in streptozotocin induced diabetic rats in comparison with normal control group. On administration glibenclamide, at the dose of 2.5 mg/kg (Group III table 1) it was observed that these enzymes were found retrieving towards normalcy and the effect was more significant at (P<0.01). On administration HAETIS and AETIS to (Group IV to VII, Table-1) at the dose of (100 and 200 mg/kg p.o.), HAETS exhibited significant reduction (p<0.05) in plasma level of SGOT, SGPT, ALP. Both the extracts were significantly at (P<0.05) decreased the level of Bilirubin at all dose levels.
Table 1. Hepatoprotective effect of HAETIS and AETIS on normal and diabetic albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (KA unit)</th>
<th>BIL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control (NC)</td>
<td>29.5 ± 1.56</td>
<td>30.66 ± 1.14</td>
<td>77.66 ±2.15</td>
<td>0.61 ± 0.1</td>
</tr>
<tr>
<td>II</td>
<td>Negative control</td>
<td>68 ± 3.99a</td>
<td>61.66 ± 1.94a</td>
<td>158.33 ± 3.73a</td>
<td>2.11 ± 0.12a</td>
</tr>
<tr>
<td>III</td>
<td>Standard (Glibenclamide 2.5 mg/kg)</td>
<td>46.83 ± 1.77c</td>
<td>44.66 ± 1.49c</td>
<td>132.66 ± 2.36c</td>
<td>1.21 ± 0.13c</td>
</tr>
<tr>
<td>IV</td>
<td>Test (HAETIS 100 mg/kg)</td>
<td>59.66 ± 2.51a</td>
<td>53.3 ± 1.14b</td>
<td>143.5 ± 3.66b</td>
<td>1.51 ± 0.12b</td>
</tr>
<tr>
<td>V</td>
<td>Test (HAETIS, 200 mg/kg)</td>
<td>45.33 ± 6.31b</td>
<td>49.83 ± 3.25b</td>
<td>135.16 ± 3.55b</td>
<td>1.35 ± 0.16b</td>
</tr>
<tr>
<td>VI</td>
<td>Test (AETIS, 100 mg/kg)</td>
<td>62 ±0.93a</td>
<td>59.66 ± 1.47b</td>
<td>148.66 ± 3.11b</td>
<td>1.33 ± 0.24b</td>
</tr>
<tr>
<td>VII</td>
<td>Test (AETIS, 200 mg/kg)</td>
<td>63 ±1.67a</td>
<td>56.66 ± 2.33b</td>
<td>142.33 ± 2.95b</td>
<td>1.7 ± 0.03b</td>
</tr>
</tbody>
</table>

All values are expressed as Mean ± SEM (n=6) in each group

aP<0.05 when compared to corresponding value of Normal control
bP<0.05 when compared to corresponding value of Diabetic control
cP<0.01 when compare to corresponding value of Diabetic control

Figure 1. Effects of HAETIS and AETIS on Lipid Peroxidation (LPO)

Values are mean ± SEM, (n = 6); aP < 0.05 (Statistically significant compared with normal control); cP<0.01 (Statistically significant compared with diabetic control); bP < 0.05 (Statistically significant compared with diabetic control).
Figure 2. Effects of HAETIS and AETIS on Superoxide dismutase (SOD)

Values are mean ± SEM, (n = 6); \(^{a}P < 0.05\) (Statistically significant compared with normal control); \(^{c}P < 0.01\) (Statistically significant compared with diabetic control); \(^{b}P < 0.05\) (Statistically significant compared with diabetic control).

Figure 3. Effects of HAETIS and AETIS on Catalase (CAT)

Values are mean ± SEM, (n = 6); \(^{a}P < 0.05\) (Statistically significant compared with normal control); \(^{c}P < 0.01\) (Statistically significant compared with diabetic control); \(^{b}P < 0.05\) (Statistically significant compared with diabetic control).
Figure 4. Effects of HAETIS and AETIS on Glutathione
Values are mean ± SEM, (n = 6); \( ^{a}P < 0.05 \) (Statistically significant compared with normal control); \( ^{b}P < 0.01 \) (Statistically significant compared with diabetic control); \( ^{c}P < 0.05 \) (Statistically significant compared with diabetic control).

Figure 5. Hist. of liver tissues with normal rats showing normal hepatic cells with central vein (CV) and sinusoidal dilation.

Figure 6. Hist. of liver tissue with excess acuolization and granular appearance disappearance of nuclei.
**Figure 7** Hist. of liver tissues of diabetic rat treated with glibenclamide showing less degeneration with normal cells.

**Figure 8** Hist. of liver tissues of diabetic rats treated with HAETIS 100 mg/kg showing less degeneration of cells.

**Figure 9** Hist. of liver tissues of diabetic rats treated with HAETIS 200 mg/kg showing normal hepatocytes with regenerating hepatocytes.

**Figure 10** Hist. of liver tissues of diabetic rats treated with AETIS 100 mg/kg showing mild degree of degeneration of cells.

**Figure 11** Hist. of liver tissues of diabetic rats treated with AETIS 200 mg/kg showing normal hepatocytes with mild degenerations of cells.
Antioxidant activity of the glibenclamide, HAETIS and AETIS in rats liver shows in Figure 1 to 4. There was significant (p<0.05) increase in Liver lipid Peroxidation in (Figure 1) STZ induced diabetic animals as compared to normal control. Administration of glibenclamide (2.5 mg/kg), significantly (P<0.01) decreases lipid peroxidation and HAETIS and AETIS (100 and 200 mg/kg) significantly (P<0.05) decrease the LPO in liver tissue of diabetic rats and the effect was more pronounced in the group of rats treated with HAETIS at the dose of 200mg/kg body weight. A significant (P<0.05) decrease in the activities of glutathione and enzymatic antioxidants (SOD and CAT) were noticed after administration of STZ when compared with the normal control. Upon administration of HAETIS and AETIS (100 -200 mg/kg, p.o.) it was observed that glutathione and enzymatic antioxidants were significantly(P<0.05) reversed near normal(Figure 2-4) in streptozotocin induced diabetic rats and it was more pronounced in HAETIS treated groups at the both the dose levels (100 and 200 mg/kg). 

Histo-pathological studies of rat liver tissue from Group I untreated animals show normal hepatic cells with central vein and sinusoidal dilation (Fig. 5). In STZ induced diabetic group II severe hepatotoxicity was observed with excess vacuolization and granular appearance disappearance of nuclei was observed (Figure 6). Diabetic rats treated with glibenclamide showing less degeneration along with normal cells group III (Figure 7) .In Group IV diabetic animals treated with HAETIS 100 mg/kg observed that less degeneration of hepatic cells (Figure 8). In Group V Diabetic rats treated with HAETIS 200 mg/kg showing normal hepatocytes along with regenerating hepatocytes (Figure 9). In Group VI Diabetic rats treated with AETIS 100 mg/kg showing mild degree of necrosis area of inflammation adjacent to necrotized area degeneration of cells (Figure 10). In Group VII diabetic rats treated with AETIS 200 mg/kg showing normal hepatocytes with mild degenerations of cells (Figure 11).

Discussion

The liver is the important organ which is responsible for metabolic functions and detoxification from the exogenous and endogenous substances like xenobiotics, drugs, microbes and chronic alcoholism. Hepatic injury occurs if challenges to liver become overburden. Liver damage is associated with necrosis of hepatocytes, lipid peroxidation and depletion of tissue GSH levels. In addition to it serum levels of many biochemical parameters like SGOT, SGPT, ALP, TAG, bilirubin levels are also elevated [24]. Hepatoprotective activities of different extracts were monitored by estimating SGOT, SGPT, ALP and bilirubin [25]. High levels of SGOT indicate liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, SGPT is more specific to the liver, and is thus a better parameter for detecting liver damage [26].

On present investigation it was observed that the animals treated with streptozotocin resulted in significant hepatic damage. The study of different serum marker enzymes such as SGOT, SGPT, ALP, BIL have been found to be of great value in assessment of experimental procedure. Upon treatment with HAETIS and AETIS, at the dose of 100 and 200 mg/kg, significantly decreased the elevated serum markers were observed in STZ induced diabetic rats. The normalization of serum markers by HAETIS and AETIS in diabetic rats suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against free radical induced leakage of markers enzyme in to blood circulation.

Serum ALP and bilirubin levels, on the other hand are related to hepatic cell damage. Increase in serum level of ALP is due to increased synthesis in presence of increasing biliary pressure [27]. Effective control of bilirubin level and alkaline phosphatase activity points towards an early improvement in the secretory mechanism of the hepatic cell. In our study we observed that Serum bilirubin was increased significantly in diabetic untreated rats and on treatment with HAETIS serum ALP level was decreased towards normal.

Lipid peroxidation has been postulated to the destructive process of liver injury due streptozotocin induced diabetes. In the present study the elevation in the level of end products of lipid peroxidation in the liver of rat liver treated with streptozotocin was observed. The increased in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. The result of the present study reveals that there was significant increase in lipid peroxidation in diabetic untreated animals, which is responsible for free radical formation and damage of hepatocytes. It is in an agreement with studied by Rauscher FM et al and Kakkar R et al that in diabetes there was increased oxidative stress and disturbances in antioxidant defense mechanism as compared to normal control [28, 22]. The ability of the HAETIS and AETIS to suppress lipid Peroxidation could be due to the anti free radical activities of its phenolic components like Gallic acid and Tocopherol known to act as free radical scavengers, and/or to the increase in the activity of antioxidant enzymes [29]. Treatment with HAETIS and AETIS significantly reverse the damage. Hence it may be postulated mechanism of hepatic protection by HAETIS and AETIS is due to inhibition of free radical formation

The cellular radical scavenging systems include the enzymes such as superoxide dismutase (SOD), which scavenges the superoxide ions by catalyzing its dismutation and catalase (CAT) a haeme enzyme which removes hydrogen peroxide [30]. Therefore, reduction in the activity of these enzymes (SOD, CAT) results in a number of deleterious effects due to the accumulation of superoxide anion radicals and hydrogen peroxide. Elevation in the level of SOD and CAT was noticed upon administration of glibenclamide, HAETIS and AETIS in STZ induced diabetic rats.
Reduced glutathione has an important role in the regulation of cellular redox state and therefore imbalance in reduced glutathione to oxidized glutathione ratio is a putative indicator of cellular oxidative stress. Glutathione peroxidase utilizes reduced glutathione as its co-substrate to scavenge H2O2 formed by the action of SOD. Anuradha CV et al studied that decreases in glutathione content in plasma of diabetic rats are partly being due to its utilization by the tissues to compromise the deleterious effects of lipid peroxidation [31]. Glutathione (GSH), a tri-peptide present in all the cells is an important antioxidant [32]. Decreased glutathione levels in diabetes have been considered to be an indicator of increased oxidative stress [33]. GSH also functions as a free radical scavenger in the repair of radical caused biological damage [34]. Present study indicates that the glutathione level was significantly reduced in streptozotocin induced diabetic rats. After administration of the glibenclamide, HAETIS and AETIS the content of GSH was increased significantly in the liver of diabetic rats. Oxidative stress arises from perturbations in the balance between the production of reactive oxygen species (ROS) and the efficiency of the antioxidant defense mechanisms [35]. Recently, much attention has been focused on the role of oxidative stress and it has been suggested that oxidative stress may constitute the key and common events in the pathogenesis of different diabetic complications and hepatic damage [36]. Histological study findings are in agreement with the degenerative structural changes reported to occur in liver tissue as a result of insulin depletion. In the present study, the treatment with glibenclamide and HAETIS and AETIS decreases degenerative changes in comparison with diabetic untreated groups. Based on the above findings of histo-pathological studies, HAETIS shows an improvement of hepatic cells architecture.

**Conclusion**

Present investigation demonstrates that HAETIS, AETIS exert significant protection against diabetes induced hepatic damage and oxidative stress in rats. Therefore it could be use as a hepatoprotective agent. The protective effects against acute liver damage may be, at least in part, due to the free radical scavenging effect, inhibition of lipid peroxidation, and increased antioxidant enzyme and glutathione activity. Both of the extract exert significant hepatic protection against lipid peroxidation through free radical scavenging activity and enhance antioxidant enzyme and glutathione level in diabetic animals by enhancing the level of antioxidant defense system. Thus, we feel that the *T. indica* prevents the oxidative damage induced by free radicals by restoring antioxidant levels and the activities of cellular enzymatic antioxidants. Since this model simulates many of the features of human liver pathology, we can suggest that natural antioxidants and scavengers in *T. indica* might be an effective as plant hepatoprotective agent in diabetes induces hepatic damage and it may have some therapeutic implications. It was also observed that HAETIS on its 200 mg/kg was the most effective dose among all. It has also been reported that the hydroalcoholic extracts of *T. indica* seeds contain polyphenolic compound which is responsible for its antioxidant activity [37]. *T. indica* seeds extract has a protective effect comparable to glibenclamide against diabetes induce hepatotoxicity and that *T. indica* seeds extracts could have a beneficial effect on hepatocytes if used as a hypoglycemic agent in the treatment of type-II diabetes. The present investigation has also opened avenues for further research especially with reference to the development of potent Phytomedicine for complication of diabetes mellitus from *T. indica* seeds.

**Conflict of interest**

The authors declare that there is no any conflict of interest.

**Acknowledgment**

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**References**


[34] Lu SC. Regulation of hepatic glutathione synthesis: Current concepts and controversies. FASEB J 1999, 16: 1169-1183.

