EVALUATION OF IN-VIVO ANTIOXIDANT EFFECT OF ETHANOLIC LEAF EXTRACTS OF GREWIA UMBELLIFERA STREPTOZOTOCIN (STZ)-INDUCED DIABETIC RATS

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ABSTRACT
The aim of the present study is to investigate the in vivo antioxidant activities Ethanol Leaf Extract of Grewia Umbellifera (ELEGU) in streptozotocin-induced diabetic rats. Animals were treated with ELEGU for 28 days and then diabetic and oxidative stress was induced with a single dose of streptozotocin 50mg/kg. Treated with 250 mg/kg body wt and 500mg/kg body wt of ELEGU on hepatic and renal thiobarbituric acid reactive substances, hydroperoxides, GSH, SOD, CAT, GPx, GST, and NO enzymes levels was determined. The present study revealed that Grewia Umbellifera has significant in-vivo antioxidant activity and can be used to protect tissue from oxidative stress. The result shows a significant decrease in hepatic and renal thiobarbituric acid substances and Hydroperoxides. The treatment also resulted in a significant increase in liver and kidney GSH, SOD, CAT, GPx, GST and NO when compared with diabetic control groups. Ethanolic Leaf extract of Grewia Umbellifera in the dose of 250mg/kg and 500mg/kg as well as glibenclamide, have improved the GSH, SOD, CAT, GPx, GST levels significantly, which were comparable with diabetic induced rats. Based on this study we conclude that the Grewia Umbellifera possesses in vivo antioxidant- activity and can be employed in protecting tissue from oxidative stress.

KEYWORDS: Grewia Umbellifera, Thiobarbituric acid, Hydroperoxides.
INTRODUCTION
Streptozotocin induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage.\[1\] Diabetic and experimental animal models exhibit oxidative stress due to persistent and chronic hyperglycaemia, which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation.\[2\] Many plants extracts and plant products have been shown to have significant antioxidant activity.\[3\]

There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. The medicinal plants provide a useful source of oral hypoglycemic and antihyperglycemic compounds for the development of new pharmaceuticals as well as a dietary supplement to existing therapies.\[4\]

A number of herbs are traditionally used in different countries during drug or toxin induced in hepatic, renal and cardiac disorders. Grewia umbellifera (Tiliaceae) (GU) is herbaceous medicinal plant that has been distributed in Kanniyakumari district, Tamilnadu, India.\[4\] Extensive phytochemical investigations show that the presence of many chemical constituents including palmitic and linoleic acid such as n-Hexadecanoic acid, 9,12-Octadecatrienoic acid (Z,Z,Z)-, and oleic acid, which are considered significant for Hypocholesterolemic property.\[4\]

Therefore, it seemed worthwhile to assess anti-oxidant potential of Ethanolic extract of Grewia Umbellifera on tissue lipid peroxidise and enzymatic antioxidant in STZ induced diabetic rats.

MATERIAL AND METHODS

Plant material
Grewia Umbellifera’s aerial part plant collected and authenticated by Dr.V.Chelladurai (Research Officer) Botany (C.C.R.A.S) Government of India. Voucher specimen (SIVET C-453/2012-2013) has been retained in the Dept of Biochemistry, S.I.V.E.T College of Arts & Science, Chennai. Materials were cleaned with water and dried in the shade until a constant weight was obtained.

Animals
Studies were carried out using Wistar albino male rats (150–200 g), maintained at animal
house SBST VIT, Vellore, Tamilnadu, India. The animals were housed in polyacrylic cages (38 cm_23 cm_10 cm) and maintained under standard laboratory conditions (temperature 25_20_C) with dark/light cycle (12/12 h). The animals were fed with standard pellet diet and fresh water ad libitum. All the animals were acclimatized to lab conditions for a week before commencement of the experiment. All the procedures described were reviewed and approved by the Animal’s Ethical Committee.

**Extraction**

The whole plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieves and extracted with ethanol (95% v/v) in Soxhlet’s apparatus at 60_C. The solvent was completely removed and obtained dried crude extract which was used for investigation.[5]

**Qualitative Phytochemical Analysis**

Ethanolic extracts of Grewia Umbellifera Leaf were analyzed for the tannins, sterols, lipids, glycosides, terpenoids, phenols, carbohydrates, anthraquinones, resins, reducing sugar, saponins, flavanoids and alkaloids.[4]

**Acute Toxicity Studies:** Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines 423.[6] Administration of stepwise dose of GULE (50 mg/kg - 2000 mg/kg b.w), animals were observed individually at least once during the first 30 minutes and periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for 6 weeks. The dose 2000 mg/kg was found to be safe and no toxicity was observed. One-fifth and one-tenth of upper limit dose were selected as the label for examination of antidiabetic activity.

**Experimental induction of diabetes**

Diabetes was induced in the animals fasted overnight by a single intraperitoneal (ip) injection of freshly prepared solution of STZ (Sigma, USA) 35 mg kg \(^{-1}\) body weight in 0.1M cold citrate buffer pH4.5 (7.8,9) The animals were allowed to drink 5% glucose solution to overcome the drug-induced hyperglycemia. (10, 11) Control rats were injected with citrate buffer (0.1M) alone. Animals were considered diabetic if the blood glucose values were >250 mg dL \(^{-1}\) on the third day after STZ injection. After a fortnight, rats with moderate diabetes having glycosuria (indicated by Benedict’s test for urine) and hyperglycemia with blood glucose range of 200 – 300 mg dL \(^{-1}\) were used for the experiment.
Experimental design
Rats were divided into five groups each have 6 rats as follows after the induction of STZ-induced diabetes. Diabetes was induced in rats two weeks before starting the treatment.

**Group I**: animals were considered as control rats.

**Group II**: animals were treated as diabetic STZ-induced rats.

**Group III**: diabetic-induced animals were fed with 250 mg kg\(^{-1}\) of Ethanolic Leaf extract of GU for 28 days.

**Group IV**: diabetic-induced animals were fed with 500 mg kg\(^{-1}\) of Ethanolic Leaf extract of GU for 28 days.

**Group V**: diabetic rats were given glibenclamide orally (0.6 mg kg\(^{-1}\)) in distilled water daily for 28 days.

**Preparation of liver and kidney homogenate**
Hepatic tissues were homogenized in KCl [10 mM] phosphate buffer (1.15%) with ethylenediamine tetra acetic acid (EDTA; pH 7.4) and centrifuged at 12,000\(\times\)g for 60 min. The supernatant was used for assay of the marker enzymes. The kidneys were removed and dissected free from the surrounding fat and connective tissue. Each kidney was longitudinally sectioned, and renal cortex was separated and kept at \(-80^\circ\text{C}\). Subsequently, renal cortex was homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The renal cortical homogenates were centrifuged at 5000 g for 10 min at 4\(_\circ\text{C}\) and stored for biochemical assays.$^{[12]}$

**Biochemical Assays**
Liver and kidney tissues nitric oxide (NO)$^{[13]}$, Thiobarbituric acid reactive substances (TBARS)$^{[14]}$, Hydroperoxides (HP)$^{[15]}$, Reduced glutathione (GSH)$^{[16]}$, superoxide dismutase (SOD)$^{[17]}$, catalase (CAT)$^{[18]}$, glutathione peroxidise (GPx)$^{[19]}$, glutathione-s-transferase (GST)$^{[20]}$

**Statistical analysis**
Values are presented as mean ± standard deviation for groups of six animals. The results were analyzed by one way analysis of variance (ANOVA) followed by post hoc Dunnett’s multiple comparison test. Differences between means were considered to be statistically significant at (p≤ 0.05).
RESULTS

Table 1 shows the concentration of TBARS and HP in liver and kidney of normal control and experimental groups of rats. The levels of TBARS and HP in diabetic rats were significantly increased, whereas diabetic rats-treated with the Ethanolic Leaf extract of Grewia Umbellifera and glibenclamide restored the altered values to the near normal value.

Table 2 shows the concentration of GSH and NO in liver and kidney of control and experimental groups of rats. The decreased of NO and GSH levels was observed in diabetic rats. Administration of Ethanolic leaf extract of Grewia Umbellifera and glibenclamide tends to bring the NO and GSH levels to near normal.

Table 3 shows the activities of superoxide dismutase (SOD) and catalase (CAT) in the liver snd kidney of normal control and experimental groups of rats. The activity of SOD and CAT in liver was significantly lower in diabetic control rats compared to control group of rats. After administration of Ethanolic Leaf Extract of Grewia Umbellifera as well as glibenclamide in diabetic rats were significant increase in level of SOD&CAT.

Table 4 shows the activities of glutathione-stransferase (GST) and glutathione peroxidise (GPx) in the liver and kidney of normal control and experimental groups of rats. The activity of GST and GPx in liver and kidney was significantly lower in diabetic control rats compared to diabetic induced rats. The GST & GPx levels in liver and kidney were significantly enhanched in Ethanolic Leaf Extract of Grewia Umbellifera treated rats as well as glibenclamide.

Table 1. Effect of thiobarbaturic acid reactive substance (TABARS) and hydrogen peroxides (HP) in liver and kidney of control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LIVER</th>
<th>KIDNEY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBARS (mM/100 g tissue)</td>
<td>HP (mM/100 g tissue)</td>
</tr>
<tr>
<td>Control</td>
<td>9.04 ± 0.69</td>
<td>2.30± 0.16</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>15.76 ± 1.19*</td>
<td>5.35± 0.41</td>
</tr>
<tr>
<td>Diabetic + ELEGU (250mg/kg)</td>
<td>13.10± 0.99*</td>
<td>3.55 ± 0.28*</td>
</tr>
<tr>
<td>Diabetic + ELEGU (500mg/kg)</td>
<td>8.79± 0.69**</td>
<td>2.37 ± 0.18**</td>
</tr>
<tr>
<td>Diabetic + Glibenglamide (0.6 mg/kg,bw)</td>
<td>11.33± 0.84**</td>
<td>2.87± 0.23*</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at *P<0.005&**P<0.001. Diabetic control rats were compared with normal control rats.
Table 2. Effect of reduced glutathione (GSH) and nitric oxide (NO) in liver and kidney of control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LIVER</th>
<th>KIDNEY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO (μmol/g)</td>
<td>GSH (mg/100g tissue)</td>
</tr>
<tr>
<td>Control</td>
<td>22.76±2.91</td>
<td>66.72±6.38</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>12.33±4.70**</td>
<td>37.70±3.65**</td>
</tr>
<tr>
<td>Diabetic + ELEGU (250mg/kg)</td>
<td>18.41±3.57*</td>
<td>60.33±3.39*</td>
</tr>
<tr>
<td>Diabetic + ELEGU (500mg/kg)</td>
<td>20.11±2.57**</td>
<td>69.39±4.59**</td>
</tr>
<tr>
<td>Diabetic + Glibenglamide (0.6 mg/kg bw)</td>
<td>22.12±2.87**</td>
<td>51.78±3.97**</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at *P<0.005 & **P<0.001. Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control.

Table 3. Effect of superoxide dismutase (SOD) and Catalase (CAT) in liver and kidney of control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LIVER</th>
<th>KIDNEY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOD (Units/min/mg of protein)</td>
<td>CAT (μmol/min/mg protein)</td>
</tr>
<tr>
<td>Control</td>
<td>12.22 ± 0.93</td>
<td>85.66 ± 6.52</td>
</tr>
<tr>
<td>STZ induced Diabetic control</td>
<td>6.12 ± 0.47*</td>
<td>40.13 ± 3.06</td>
</tr>
<tr>
<td>Diabetic + ELEGU (250mg/kg)</td>
<td>9.07 ± 0.69</td>
<td>70.04 ± 5.36*</td>
</tr>
<tr>
<td>Diabetic + ELEGU (500mg/kg)</td>
<td>13.03 ± 1.00**</td>
<td>90.05 ± 6.89*</td>
</tr>
<tr>
<td>Diabetic + Glibenglamide (0.6 mg/kg bw)</td>
<td>10.83 ± 0.83*</td>
<td>76.69 ± 5.87*</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at *P<0.005 & **P<0.001. Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control.
Table 4. Effect of glutathione-s-transferase (GST) and glutathione peroxidase (GPx) in liver and kidney of control and experimental groups of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LIVER</th>
<th>KIDNEY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GPx (µg/(min/mg protein))</td>
<td>GST (µmol/(min/mg protein))</td>
</tr>
<tr>
<td>Control</td>
<td>12.17 ± 0.93*</td>
<td>9.00 ± 0.69**</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>3.34 ± 0.25*</td>
<td>3.23 ± 0.25</td>
</tr>
<tr>
<td>Diabetic + ELEGU (250 mg/kg)</td>
<td>7.45 ± 0.57*</td>
<td>6.60 ± 0.51</td>
</tr>
<tr>
<td>Diabetic + ELEGU (500 mg/kg)</td>
<td>12.43 ± 0.97**</td>
<td>9.12 ± 0.70**</td>
</tr>
<tr>
<td>Diabetic + Glibenglamide (0.6 mg/kg.bw)</td>
<td>9.56 ± 0.73*</td>
<td>7.67 ± 0.58*</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of six animals each. Values are statistically significant at *P<0.005 & **P<0.001. Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control.

DISCUSSION

In the present study the Ethanolic leaf extract of Grewia Umbellifera was observed to exhibit antioxidant status in STZ-induced diabetic rats. Moreover the Extract shows enhanced activities of antioxidant enzymes (SOD, CAT, GPx, GSH, GST, and NO) and diminished the amount of lipid peroxides against diabetic rats.

The elevated levels of lipid peroxides and hydroperoxides are unstable, cytotoxic and highly reactive, leading to free radical damage to proteins and DNA.[21] TBARS can react with the free amino group of proteins, phospholipids, and nucleic acids leading to structural modification.[22]

Previous study had reported increased levels of lipid peroxidation in STZ-diabetic rats.[23] However, the oral administration of Grewia Umbellifera to the diabetic group of rats significantly revert back TBARS levels to near normal values (Table 1) which show the anti-lipid peroxidative property of Ethanolic leaf extract of Grewia Umbellifera in experimental diabetes.

Nitric oxide is involved in either the protection against or the production of oxidative stress within various tissues depending on its concentration.[24] The mechanism of inactivation of NO may be of particular relevance for patients with diabetes mellitus.[25]
Here observed that the decreased levels of NO in liver and kidney of STZ diabetic group of rats was increased utilization of NO resulting from oxidative stress. In the current results (Table 2), STZ-diabetic rats treated with Ethanolic leaf extract of Grewia Umbellifera had normal plasma and tissue organs NO levels.

This effect may be because it rapidly reacting with \( O_2 \). The affinity of NO for \( O_2 \) is far greater than the affinity of SOD for \( O_2 \). In fact, NO may compete with SOD for \( O_2 \) - and sparing SOD for other scavenging duties.\(^{26}\) Furthermore, it was reported by Brown and Hu, 2001, that increases bioavailability of antioxidants are expected to decrease superoxide generation by increasing tetra hydrobioperin (BH\(_4\)) a cofactor needed to stimulate endothelial nitricoxide synthase activity. Therefore, it is likely that the antioxidant effect of Ethanolic leaf extract of Grewia Umbellifera contribute to the increased bioavailability of NO.

GSH is a major intracellular non protein sulphydral compound and is accepted as the most important intracellular hydrophilic antioxidant.\(^{27}\) Also, GSH acts as a co-substrate for GPx activity and as a cofactor for many enzymes, stress resistance of many cells is associated with high intracellular levels of GSH. A decreased GSH content may predispose the cells to lower defense against condition of oxidative stress during several degenerative disease conditions including diabetes.\(^{23}\) In the present study (Table 2), the observed elevation in the activities of these antioxidant enzymes in liver and kidney of Grewia Umbellifera diabetic rats compared to the untreated ones reflects the antioxidant potential of Ethanolic leaf extract of Grewia Umbelifera.

SOD, CAT, GPx and GST are enzymes that destroy the peroxides and play a significant role in providing antioxidant defenses to an organism.\(^{28}\) \( \text{GPx}^{29} \), \( \text{CAT}^{30} \) are involved in the elimination of \( H_2O_2 \) and SOD\(^{31} \) acts to dismutate superoxide radicals to \( H_2O_2 \) which is then acted upon by GPx. The function of all three enzymes is interconnected and lowering of their activities result in the accumulation of lipid peroxides and increased oxidative stress in diabetic rats.\(^{32}\)

In the present study Table (3&4) the activities of GPx, SOD and CAT in plasma and different tissue organs extracts of the STZ diabetic rats were significantly lower than their control ones. Impairment of antioxidant machinery may be described by both the damage of antioxidant enzymes caused by protein glycation and consumption by an excess demand.\(^{21}\)
The compromises in enzymatic antioxidant defense system and alterations in their activities have been implicated in the mechanisms of abnormal tissue function observed in diabetes mellitus.[33]

**CONCLUSION**

In the present study showed that the Ethanolic leaf extract of *Grewia Umbellifera* (500 mg/kg) shows significantly increased activity in SOD (Super oxide dimustase), catalase, GPx(Glutathione peroxidase and GST(Glutathione-S-transferase) when compared with 250mg/kg.

**REFERENCES**


