Effect of *Adhatoda zeylanica* Ethanol Extract on Attenuated Kidney in Streptozotocin-Induced Diabetic Rats

Prima Swetha D'souza, Rajendra Holla, Gangadhara Swamy

**1**Department of Anatomy, K.S. Hegde Medical Academy, Mangalore, Karnataka, India  
**2**Department of Pharmacology, K.S. Hegde Medical Academy, Mangalore, Karnataka, India  
**3**Department of Anatomy, Subbiah Institute of Medical Sciences and Research Center, Shivamogga, Karnataka, India

Address for correspondence Gangadhara Swamy, MSc, Department of Anatomy, Subbiah Institute of Medical Sciences and Research Center, Shivamogga 577222, Karnataka, India (e-mail: dr.gangadharaswamy@gmail.com).

**Abstract**  
**Objective** The present study was aimed to evaluate the effect of ethanol extract of *Adhatoda zeylanica* (EAZ) leaves on streptozotocin (STZ)-induced diabetes mellitus (DM) and its renal complications in male Wistar albino rats.  
**Materials and Methods** Adult male Wistar albino rats were randomly selected from a colony, divided into four groups, namely, A, B, C, and D, with each having six rats (n = 6) and each weighing between 200 and 250 g. Group A served as control and received only water per oral (p.o.). Group B, C, and D animals received a single dose of STZ at 45 mg/kg body weight (kbw) intraperitoneal administration (i.p.) on day 1 and observed for fasting blood glucose (FBG) to induce DM for next 72 hours. After the DM was induced, group B served as DM control, group C received the standard drug glibenclamide (GL) at 5 mg/kbw p.o. once daily, and group D received EAZ of 500 mg/kbw p.o. once daily for 35 days. After the observation period, the animals were euthanized, serum creatinine and blood urea, antioxidants in the kidney tissue homogenate, and histopathological studies were assessed to know the ameliorative effect of the test drugs.  
**Results** Renal parameters, such as serum creatinine, blood urea, antioxidants activities, in group D were nearer to the control when compared with groups B and C. Histopathological studies revealed that there was minimal renal damage in group D when compared with groups B and C.  
**Conclusion** Administration of ethanol EAZ showed significant ameliorative effects on the FBG, biochemical, oxidative, and histopathological parameters on kidney tissues treated with STZ to induce DM.

**Introduction**  
Diabetes mellitus is a long-standing disorder manifested by hyperglycemia. Etiology of DM is related to defects in insulin secretion and/or insulin action on cell membranes leading to hyperglycemia.  

DM has been implicated in several serious health problems including glomerulonephritis, nephropathy, retinopathy, hepatopathy, and cardiomyopathy, all of which can increase the risk of death.  

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There is a significant increase in DM-positive population worldwide, and calculations based on a study suggest that there will be 210 million people who will be DM positive during 2013 to 2035 (22 years). Unfortunately, DM in the younger age group has been on the rise, and there is an urgent need to combat this disease. Hyperglycemia can lead to high production of reactive oxygen species (ROS) and a simultaneous reduction of the antioxidant defense mechanisms which can cause oxidative stress. It is proposed that DM makes alterations in the activity of antioxidant enzymes such as catalase, glutathione (GSH-Px) peroxidase, and superoxide dismutase (SOD) that leading to imbalance in oxidative status and damage of tissues. Therefore, hyperglycemia-induced oxidative stress due to the disruption of cellular function and cellular damage has a crucial role in the development and progression of diabetic complications.

DM is the leading cause of the end-stage renal disease (ESRD) worldwide, and approximately 40% of ESRD patients were attributed to diabetic nephropathy (DN). The high concentration of blood glucose damages the kidney tissue, leading to altered kidney function in the patients, causing DN and develop an ESRD. The kidney damage in DN is manifested histologically by the thickening of the glomerular basement membrane, mesangial matrix expansion, macrophage infiltration, podocyte loss, and tubular epithelial degeneration. Existing therapy for DM is known to support glycemic control but is believed to do little in regard to the complications to various organs. Besides, these antidiabetic drugs are associated with mild-to-moderate side effects.

Although numerous oral hypoglycemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes. Over the last few decades, the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety.

Evidence has been established that proper glycemic control and replenishment of antioxidant plays a significant beneficial role in slowing the progression of DN; however, reversal of nephropathy to normal condition is not easy once the duration of DM is prolonged.

In view of this, the present study has investigated the effect of a plant extract in management of DM in streptozotocin (STZ)-induced Wistar rats. STZ is frequently used to induce DM in experimental animals through its toxic effects on pancreatic β-cells and as a potential inducer of oxidative stress. Various studies have been done to establish models of DN in rat using STZ.

The medicinal plant used for this study was *Adhatoda zeylanica* which belongs to family fabaceae and subfamily acanthaceae.

It is popularly and frequently used in Ayurveda and Unani medicines and thus, included in the World Health Organization (WHO) manual, entitled The Use of Traditional Medicine in Primary Health Care.

*Adhatoda zeylanica*, also known as the Malabar nut tree, is a part of the acanthaceae plant family. It is a common small evergreen, subherbaceous bush distributed throughout India, especially in the lower Himalayas (up to 1,300 m above sea level), India, Sri Lanka, Burma, and Malaysia. In Ayurveda, in the ancient system of Indian medicine, there is a well-known plant in the indigenous system of medicine, commonly known as vasaca. It grows to a height of 1.5 to 2.0 m with leaves approximately 10- to 15-cm long, 5.0-cm wide, with white or purple flowers, four-seeded fruits. The leaf extract of *A. zeylanica* (EAZ) contains a class of alkaloids, namely, vasicine and vasicinone, and phytochemical, like phenols, tannins, alkaloids, anthraquinones, saponins, flavonoids, amino acids, and it has also been reported earlier that it helps in reducing sugar levels. EAZ has been shown to possess multifarious medicinal properties such as antioxidant, antimicrobial, anti-inflammatory, anti-inflammatory, and hepatoprotective activities.

However, systemic and scientific reports on the investigation of *A. zeylanica* leaves for its effect on renal function are scarce. In our study, EAZ was evaluated for its nephroprotective effect in STZ-induced DM rats.

### Materials and Methods

#### Plant Material

The leaves of *A. zeylanica* were identified, collected, and authenticated by a botanist. The leaves were dried in the shade and powdered in our research laboratory with the help of a pulverizer. The powder was then subjected to extraction using a rotary evaporator. The percentage yield of the extract was (9.2% w/w) from dry leaves.

#### Chemicals

All the chemicals and reagents were purchased from commercial sources and were of analytical grade.

#### Experimental Animals

The animals were procured and housed in the animal house attached to the Department of Pharmacology, K.S. Hegde Medical Academy, Mangalore. Individual rats were housed in separate polypropylene cages with padded husk bedding. All animals were maintained under standard laboratory conditions, with a constant 12-hour light/dark cycle and controlled temperature (25 ± 2°C) with access to drinking water and pellet diet ad libitum.

#### Methodology

##### Acute Oral Toxicity Study

Acute toxicity for EAZ was performed to know the median lethal dose (LD50) in female adult Wistar albino rats and also for the dose fixation for conducting the further study. Each group consists of six rats. For an overnight fasting rats, a single dose of EAZ was administered per oral (p.o.). Groups 1, 2, and 3 had received 175, 550, and 2,000 mg/kg body weight (kbw) of EAZ, respectively. The rats were monitored continuously for first 4 hours, then hourly on day 1, followed by once daily for next 14 days for the body weight, toxicity signs, and mortality.
Induction of Diabetes
To the overnight fasted rats but with free access to water a single dose of STZ intraperitoneal administration (i.p.) at a dose of 45 mg/kbw, dissolved in 0.1 M Citrate buffer (pH = 4.5). Then 5% sucrose was supplemented for 24 hours to prevent the animals from fatal hypoglycemia. After 72 hours of STZ administration, fasting blood glucose (FBG) level was checked using the (Accu-Chek Active, Roche Diabetes Care, Sandhofer Strasse, Mennheim, Germany) from the tail vein. The rats with a FBG more than 300 mg/dL were considered diabetic and included in the study.

Estimation of effective dose of EAZ in STZ-induced DM in male adult Wistar albino rats was done. Adult male Wistar albino rats were randomly selected from a colony divided into five groups with each having six rats per group. DM was induced in them by administration of single dose of STZ at 45 mg/kbw i.p. to induce DM on day 1. After 72 hours, FBG level was checked for analyzing the DM by using glucometer. After the DM phenotype developed, the rats have received the single dose of test drug orally on day 4, and FBG was repeated on the day 5 to know the effectiveness of EAZ in the single dose of test drug orally on day 4, and FBG was repeated on the day 5 to know the effectiveness of EAZ in STZ-induced DM rats. The study chart is mentioned below:
- Group 1: control
- Group 2: STZ-induced DM rats
- Group 3: STZ + EAZ one-fourth of its LD50
- Group 4: STZ + EAZ one-eighth of its LD50
- Group 5: STZ + EAZ one-twelfth of its LD50

Estimation of EAZ Effect on Attenuated Kidney in STZ-Induced DM Rats
Adult male Wistar albino rats were selected randomly from a colony, and they were divided into four groups, namely, A, B, C, and D, with each having six rats per group. DM was induced in them by administration of single dose of STZ at 45 mg/kbw i.p. to induce DM on day 1. After 72 hours, FBG was repeated on the day 5 to know the effectiveness of EAZ in STZ-induced DM rats. The study chart is mentioned below:
- Group 1: control
- Group 2: STZ-induced DM rats
- Group 3: STZ + EAZ one-fourth of its LD50
- Group 4: STZ + EAZ one-eighth of its LD50
- Group 5: STZ + EAZ one-twelfth of its LD50

Preparation of Kidney Homogenate
Kidneys were excised and cleaned with ice cold saline and stored at -20°C in freezer. Tissues were thawed and homogenized in phosphate buffered saline, pH of 7.4, centrifuged at 10,000 rpm for 15 minutes using refrigerated centrifuge and supernatant was stored at -20°C. The supernatant was subjected to determination of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) using the method of Mohun and Cook, SOD assay by the methods of Beauchamp and Fridovich, and GSH-Px by Ellman’s method.

Renal Function Test
Blood urea [urease, Berthelot’s method] and serum creatinine [Jaffe’s method] levels were measured using the respective assay kits (Agapee Diagnostic, Bangalore, Karnataka, India) using a semiautomatic biochemical analyzer.

Histopathological Examination
Kidneys were kept in 10% formalin for 24 hours (postfixation). Kidney tissue paraffin blocks and tissue sections were prepared and stained with hematoxylin and eosin by using standard histological procedures. Tubular dilatation, tubular degeneration, expansion of Bowman’s capsule, interstitial inflammation, and congestion were observed under light microscope.

Ethical Permission
This study was performed at the K.S. Hegde Medical Academy of Mangalore in a CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals)-approved laboratory under registration number 115/1999/CPCSEA, following all ethical practices as laid down in the guidelines for animal care. This study has been approved by the Institutional Animal Ethics Committee (IAEC), KSHEMA/IAEC/31/2011.

Statistical Analysis
Statistical analysis was performed using one-way analysis of variance (ANOVA) by SPSS, Version 22.0 software where ever it is applicable.

Results
Acute Toxicity Study of EAZ in STZ-Induced DM in Male Adult Wistar Albino Rats
No significant changes in the body weight and acute toxicity signs were noticed at the observation period. It is also noticed that no mortality was observed in animal of all the groups, indicating that toxicity is more than 2,000 mg of EAZ in female adult Wistar albino rats.

Estimation of the Effective Dose of EAZ in STZ Induced DM in Male Adult Wistar Albino Rats
The study found that EAZ at dose one-fourth of its LD50 had shown a significant decrease in the FBG levels in the STZ-induced DM in male adult Wistar albino rats (∗Table 1; p < 0.05).

Sample Collection
At the end of the observation period, the animals were deeply anesthetized with ether. All the animals were observed for any gross/macroscopic pathological changes. Whole blood collected by cardiac puncture, and the serum was separated by centrifugation at 2,000 rpm for 20 minutes for analysis.

Kidneys from the representative groups of animals were removed and weight was noted. One side’s kidney tissue was homogenized for the evaluation of the oxidation status and other side’s tissue was stored in the formaldehyde for fixation to further histopathological study.
Effect of EAZ on Attenuated Kidney in STZ-Induced DM Rats

The study showed a significant decrease in the FBG levels in the groups C (80.47%) and D (78.81%) on day 39 in STZ-induced DM rats when compared with group B (►Table 2; \( p < 0.05 \)).

The STZ-induced DM rats when administered with the EAZ for consecutive 35 days have shown the significant increase in the concentration of GSH in the kidney tissues when compared with the groups B and C (►Table 3).

Histological studies in group D have shown minimal damage to kidney tissues’ cytoarchitecture compared with groups B and C (►Fig. 1). But the groups B and C had demonstrated moderate-to-severe degenerative features, like dilated tubules, degenerated tubules, glomerular congestion, interstitial inflammatory infiltration, and atrophy of glomerulus, seen with enlarged glomerular space. The degenerated tubule cells with pyknotic nuclei and vacuolated cytoplasm were observed. Sloughing of epithelium was noticed in tubular lumens.

Table 1 Fasting blood glucose levels in the streptozotocin-induced diabetes mellitus in male adult Wistar albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting blood glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>84.03 ± 14.03</td>
</tr>
<tr>
<td>2</td>
<td>372.52 ± 50.53a</td>
</tr>
<tr>
<td>3</td>
<td>103.43 ± 10.45</td>
</tr>
<tr>
<td>4</td>
<td>180.62 ± 30.63a</td>
</tr>
<tr>
<td>5</td>
<td>270.43 ± 25.72a</td>
</tr>
</tbody>
</table>

Note: Fasting blood glucose levels (mg/dL) was assessed in streptozotocin-induced diabetes mellitus rats \((n = 6)\) on day 1. Values are expressed as mean ± standard deviation. *Indicates significant difference from the control group \((p < 0.05)\).

Table 2 Fasting blood glucose levels assessed in streptozotocin-induced diabetes rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 19</th>
<th>Day 39</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (mg/dL)</td>
<td>82.5 ± 20.5</td>
<td>80.4 ± 13.56</td>
<td>87 ± 16.97</td>
<td>79 ± 15.55</td>
</tr>
<tr>
<td>Diabetic control (mg/dL)</td>
<td>97 ± 9.89</td>
<td>300.16 ± 10.84a</td>
<td>340 ± 48.08a</td>
<td>425.40 ± 49.49a</td>
</tr>
<tr>
<td>Diabetic + glibenclamide (mg/dL)</td>
<td>102.5 ± 7.37</td>
<td>85.5 ± 7.77a</td>
<td>83.22 ± 19.7a</td>
<td></td>
</tr>
<tr>
<td>Diabetic + streptozotocin (mg/dL)</td>
<td>98.10 ± 4.43</td>
<td>167.12 ± 12.21</td>
<td>90.12 ± 38.18a</td>
<td></td>
</tr>
</tbody>
</table>

Note: Fasting blood glucose levels (mg/dL) was assessed in streptozotocin-induced diabetes rats \((n = 6)\). Values are expressed as mean ± standard deviation. *Indicates significantly difference from the control group \((p < 0.05)\).

Table 3 The effect of EAZ leaves on functional enzymes of rat kidney tissues

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/g tissue)</th>
<th>GSH (µmoles/g tissue)</th>
<th>GPT (U/g tissue)</th>
<th>GOT (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (mg/dL)</td>
<td>312.32 ± 10.13</td>
<td>2.636 ± 0.380</td>
<td>0.522 ± 0.19</td>
<td>1.695 ± 0.440</td>
</tr>
<tr>
<td>Diabetic control (mg/dL)</td>
<td>440.33 ± 25.146a</td>
<td>0.240 ± 0.04a</td>
<td>0.937 ± 0.031a</td>
<td>1.99 ± 0.10</td>
</tr>
<tr>
<td>Diabetic + glibenclamide (mg/dL)</td>
<td>327.33 ± 39.79a</td>
<td>1.368 ± 0.477a</td>
<td>0.586 ± 0.09a</td>
<td>1.781 ± 0.159a</td>
</tr>
<tr>
<td>Diabetic + streptozotocin (mg/dL)</td>
<td>320.03 ± 39.82a</td>
<td>1.95 ± 0.31a</td>
<td>0.704 ± 0.068a</td>
<td>1.720 ± 0.06</td>
</tr>
</tbody>
</table>

Abbreviations: EAZ, extract of Adhatoda zeylanica; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; GSH, glutathione; SOD, superoxide dismutase.

Note: The effect of EAZ leaves on functional enzymes of rat kidney tissues \((n = 6)\). Values are expressed as Mean ± standard deviation. *Indicates significant difference from the control group \((p < 0.05)\).

Table 4 The effect of extract of Adhatoda zeylanica (EAZ) leaves on renal functions

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>27.435 ± 6.017</td>
<td>0.76 ± 0.1</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>103.87 ± 2.39a</td>
<td>1.8 ± 0.16</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>61.326 ± 5.39a</td>
<td>1.18 ± 0.04</td>
</tr>
<tr>
<td>Diabetic + streptozotocin</td>
<td>38.58 ± 1.03</td>
<td>0.82 ± 0.6</td>
</tr>
</tbody>
</table>

Note: The effect of EAZ leaves on renal functions \((n = 6)\). Values are expressed as mean ± standard deviation. *Indicates significantly difference from the control group \((p < 0.05)\).
enzymes to the extracellular space. The present study, sig-
ticle membrane changes that allow passage to intracellular
phatase (ALP), and pseudocholinesterase (PChE) activities
vious work. SOD is a free-radical scavenging enzyme that
against oxidative stress and this data are in line with a pre-
an adaption response and could be a protective mechanism
rats were used to induced with DM, and the study period
noticed in DM-induced group. That shows, may be healthy
due to β-cell damage by STZ decreased GSH concentration.
acute hyperglycemic condition and free-radical load formed
GSH is the first-line defense in scavenging free radicals, the
utilization due to oxidative stress induced by STZ. Tissue
protein thiols and disulphides are in equilibrium with the
GSH–GSSG (oxidized gluthathione disulfide) redox pair. Since
GSH is the first-line defense in scavenging free radicals, the
acute hyperglycemic condition and free-radical load formed
due to β-cell damage by STZ decreased GSH concentration.

Induction of diabetes in the present study caused a sig-
nificant increase in antioxidant enzyme SOD activity and a
decrease in GSH concentration in the kidney tissue; this result
is in accordance with a previous study. Normal GSH is needed
to preserve the structural and functional integrity of cells. The
decrease in the level of GSH in tissues represents increase in
the utilization due to oxidative stress induced by STZ. Tissue
protein thiols and disulphides are in equilibrium with the
GSH–GSSG redox pair. Since GSH is the first-line defense in scavenging free radicals, the acute hyperglycemic condition and free-radical load formed due to β-cell damage by STZ decreased GSH concentration.

In our study, an increase in the SOD enzyme level was
noticed in DM-induced group. That shows, may be healthy
rats were used to induced with DM, and the study period
was concise. Overexpression of these antioxidant might be
an adaption response and could be a protective mechanism
against oxidative stress and this data are in line with a pre-
vious work. SOD is a free-radical scavenging enzyme that
has a longer life, and an increase in free radicals induces its
gene expression. So, its activity is higher in STZ control. In
EAZ-treated rats, the possible decrease in free-radical load
has decreased SOD activity.

Evaluation of GOT and GPT enzyme activities in kidney
tissues showed significantly increased activity in STZ-treated
DM rats compared with group A, but the activity of these
enzymes significantly reduced in both the groups C and D.
GOT and GPT are housekeeping enzymes in liver and kidney
tissues involved in protein metabolism. Alterations in the
activity of mentioned enzymes in STZ treated DM rats may
be due to metabolic abnormalities or cellular damage. It has
been reported that the increase in GOT, GPT, alkaline phos-
phatase (ALP), and pseudocholinesterase (PChE) activities
in the kidney tissues of STZ-treated rats is due to the sub-
tle membrane changes that allow passage to intracellular
enzymes to the extracellular space. The present study, sig-
nificantly reduced the enhanced GOT and GPT activities after
the administration of A. zeylanica to the diabetic rats. The
reversal of renal GOT and GPT activity in A. zeylanica–treated
DM rats toward average level is evidence of preventing cellu-
lar and tissue damage under diabetic conditions.

The kidney damage is reflected by an increase in metabolic
wastes such as blood urea and creatinine in the blood. In our
study, the STZ-induced DM rats showed significant increase
in serum creatinine and blood urea. This proved that diabetic
rats have progressed to DN. Increased blood urea and serum
creatinine indicate glomerular damage, leading to a decrease
in these materials' renal excretion. DM seems to increase ROS
production, causing damage to oxygenated mediators, alter-
ging glomerular filtration and increasing the permeability of
the membrane. However, the diabetic rats that were treated
with EAZ showed a significant decrease in serum parameters.
Thus, showed that the EAZ supplementation had ameliorated
kidney damage in diabetic rats, thus attenuating DN.

In accordance with the results obtained in biochemical
analysis, morphological changes were also noticed in the kid-
ney tissues. The histological analysis in DM rats treated with
GL and EAZ showed that a nonsignificant change was noticed
in the histology of kidney tissue ( Fig. 1 ). Thus, the antioxidant
property of the plant extract was demonstrated. Similar find-
ings also saw in many previous histopathological studies. EAZ
was able to render nephroprotective effect in these models
by attenuating oxidative stress. So, is speculated that the nephroprotective effect of EAZ might be due to its
antioxidant properties. The plant extract might contain bio-
active components that have the potential to reverse/pre-
vented the undesirable changes in the kidney associated with
hyperglycemia–induced oxidative stress. Thus, a corrective
measure, even on the histology, of the kidney was noticed.

## Conclusion

Our study demonstrated that the ethanolic EAZ possesses
antidiabetic potential in STZ-induced DM rats when admin-
istered p.o. for 35 days at the dose of 500 mg/kgbw.

The biochemical and histological studies have suggested that
oxidative stress plays a prime role in the pathophysiology of dia-
abetic nephropathy, and the EAZ showed the ability to attenuate
the renal damage in diabetes through its antioxidant action.

## Authors’ Contributions

P.D. designed and worked on the experiment, R.H. col-
lected the review of literature and also planned for the
Effect of *Adhatoda zeylanica* Ethanolic Extract on Diabetic Rats

D’souza et al.

References


