Cytoprotective effect of *Semecarpus anacardium* against toxicity induced by Streptozotocin in rats

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**Abstract:** Leakage of cellular enzymes into the plasma is a clear indication of cell damage. When liver plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream and their estimation is a quantitative marker for the extent of damage. The cytoprotective effect of *Semecarpus anacardium* was evaluated in rats that were rendered diabetic by administration of streptozotocin at a dose of 50 mg/kg body weight. The activities of the marker enzymes were assayed in the serum, liver and kidney. The indicators of renal damage such as urea, uric acid and creatinine were assayed in addition to the blood profile. The results of the present study reveal that *Semecarpus anacardium* was able to reverse the levels of the marker enzymes, and protect the kidney by reverting back to the normal levels of urea, uric acid, and creatinine. The abnormal blood parameters were also reverted to near normal levels indicating the drug’s cytoprotective effect.

**Keywords:** marker enzymes, *Semecarpus anacardium*, blood profile, cytoprotective effect

**Introduction**

Diabetes mellitus is probably the single most important metabolic disease and is widely recognized as one of the leading causes of death and disability. It affects every cell in the body and the body’s essential biochemical processes, and it is a major public health problem in developed as well as developing countries.¹ Diabetes arises due to a deficiency of insulin secretion from the beta cells of the pancreas or a deficiency of insulin action. The endocrine pancreas is arranged in clusters of secretory cells in the islets of Langerhans. It contains about one million islets that comprise 1%–2% of the total mass of the gland. These islets are separated from the exocrine tissue by a capsule made up of connective tissue fibers and glial like cells.² Four different endocrine cell types are contained in the islets: β cells, which produce insulin and constitute 60%–80% of the endocrine cell mass, glucagon-secreting α cells (10%–20%), somatostatin-producing d-cells (~5%) and pancreatic polypeptide-secreting PP-cells (<1%).³

Streptozotocin (STZ) is a metabolite of the soil organism *Streptomyces achromogenes*. Owing to a structural resemblance to D-glucose it is taken up by the glucose transporter (GLUT2), which is expressed in the pancreas, liver and the kidney of rodents.⁴ Beta cell necrosis can be detected by electron microscopy within a few hours after STZ administration.³ Intracellular action of STZ results in nitric oxide (NO) liberation, alkylation, and fragmentation of DNA, leading to cell death. DNA damage activates poly ADP-ribosylation which leads to the depletion of cellular NAD+, reduc-
tion of ATP, and subsequent inhibition of insulin synthesis and secretion. Six hours after STZ injection, hyperglycemia develops and blood insulin levels decrease.7

The use of medicinal plants for the treatment of diabetes mellitus dates back from the Ebers papyrus of about 1550 BC. A multitude of herbs, spices, and other plant materials have been described for the treatment of diabetes throughout the world.8 Semecarpus anacardium Linn., commonly called marking nut, is found in the outer Himalayas and the hotter parts of India. Its bark is an astringent and the tree exudes a gum which is used in leprous infections and nervous debility. The chloroform extract of the nut possesses antitumor action against L1210, P388, advanced P388 leukemia, B16 melanoma, and glioma 26.9 It also potentiates the action against L1210, P388, advanced P388 leukemia, and glioma 26,9 and various types of cancers.16 It also has hypoglycemic,17 antioxidant,18 anti-inflammatory, and COX inhibitory properties.19 The present study was undertaken to evaluate the cytoprotective effect of Semecarpus anacardium against the toxicity induced by streptozotocin in rats by using biological markers, since they permit the early identification of the adverse effects and the potentiality of the drug used in the treatment of diseases.

Material and methods

The drug Semecarpus anacardium (SA) nut extract contains purified nuts of Semecarpus anacardium and cow’s milk in the ratio as indicated in the Formulary of Siddha Medicine. 200 g of the nut was boiled with 500 mL of milk, which was repeated thrice. The decoction was stored at room temperature.20

Animals

Male albino rats of Wistar strain, 8–10 weeks old, weighing 260 ± 10 g were used in this study. The animals were housed in polypropylene cages under a control environment with 12-hour light/dark cycles and a temperature between 27 and 37°C, and were given a commercial diet with water ad libitum. All experiments involving animals were conducted according to NIH guidelines, after obtaining approval from the Institute’s Ethical Committee (02/075/06).

Experimental design

Rats were divided into five groups of six animals each.

Group 1, control animals: Normal healthy controls received olive oil (0.5 mL) orally by gastric intubation for 21 days daily.

Group 2, diabetes induced: Streptozotocin (50 mg/kg body weight) dissolved in 0.5 mL of 0.1 M citrate buffer pH 4.5.

Group 3, SA treated: Three days after the induction of diabetes, SA (300 mg/kg body weight dissolved in 0.5 mL olive oil) was administered by gastric intubation for 21 days daily.

Group 4, metformin treated: Three days after the induction of diabetes, metformin (500 mg/kg body weight dissolved in 0.5 mL physiological saline) was administered by gastric intubation for 21 days daily.

Group 5, drug control: Animals received SA (300 mg/kg body weight dissolved in 0.5 mL olive oil) orally by gastric intubation for 21 days daily.

Biochemical analysis

After the experimental period, the animals were killed by cervical decapitation. The pancreases were excised immediately and immersed in ice-cold physiological saline. Ten percent homogenate was prepared with fresh tissue in 0.01 M tris-HCl buffer (pH 7.4) and these were used for the assays. Blood was collected, the serum was separated and also used for the analysis.

Hematological indices

Red blood cell count (RBC) and white blood cell count (WBC) were done according to the method of Dacie and Lewis21 in an improved Neubauer chamber. Platelets were counted using the improved Neubauer counting chamber by the method of Dacie and Lewis.21 Packed cell volume (PCV) was estimated by the Wintrobe macro method.23 Erythrocyte sedimentation rate (ESR) was estimated by the
method of Westergren. Hemoglobin content in blood was estimated by the method of Drabkin and Austin. The mean volume of the red cell in terms of cubic microns was calculated from the formula:

\[
\text{M.C.V.} = \frac{\text{Hematocrit (volume of packed red cells)}}{\text{RBC in millions/mm}^3} \times 10
\]

MCH is the average quantity of hemoglobin in each corpuscle.

\[
\text{M.C.H.} = \frac{\text{g Hgb/100 mL.}}{\text{RBC in millions/cu.mm.}} \times 10
\]

M.C.H.C was calculated from the following formula.

\[
\text{M.C.H.C} = \frac{\text{g Hgb/100 mL.}}{\text{Hematocrit}} \times 100
\]

Estimation of the enzymes involved in cellular integrity

Alanine and aspartate aminotransferases were assayed by the method of King; alkaline phosphatase activity (ALP) was assayed by the method of King; 27 gamma-glutamyl transpeptidase (GGT) was assayed by the method of Orlowski and Meister; 28 5’ nucleotidase (5’-NT) was assayed by the method of Luly et al; 29 albumin was estimated by the method of Fine; 30 total bilirubin was estimated by the method of Dangerfield and Finlayson; 31 amylase was estimated by the method of Henry and Chiamori. 32

Estimation of renal function parameters

Urea was estimated by the method of Natelson et al; uric acid was estimated by the method of Caraway; creatinine was estimated by the method of Owen et al. 35

Histopathological observations in the liver of control and experimental animals

All histopathological studies for liver were performed using standard procedures with uniform conditions of fixation and staining of 5-µm sections with hematoxylin and eosin (H&E).

Statistical analysis

The values are expressed as mean ± SD for six rats in each group. Statistically significant differences between the groups were calculated using one-way analysis of variance (ANOVA), followed by Student Newman – Keuls for multiple comparisons using a statistical package for social sciences (SPSS) computer package. Values of \( P < 0.05 \) were considered to be significant.

Results

Effect of SA on hematological indices

Study of hematological status is one of the important ways for the diagnosis of the root cause of diseases. Alterations in blood parameters may be due to changes in cellular integrity, membrane permeability, and metabolism, or even due to exposure to toxic chemicals.

The blood profiles of control and experimental animals are given in Table 1. The increase in WBC count (1.33-fold),

| Table 1 Blood profile of control and experimental animals |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Parameters                      | Group 1 (control) | Group 2 (STZ)  | Group 3 (STZ + SA) | Group 4 (STZ + metformin) | Group 5 (SA)    |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| RBC × (10⁶/mm³)                 | 7.37 ± 0.71     | 5.31 ± 0.59a,b  | 7.05 ± 0.72a,b  | 6.76 ± 0.63a,b,c | 7.41 ± 0.81     |
| Total WBC × (10³/mm³)           | 6.22 ± 0.61     | 8.31 ± 0.91a,b  | 6.91 ± 0.64a,b  | 7.74 ± 0.82a,b,c | 6.18 ± 0.59     |
| Platelet count × (10³/mm³)      | 953.10 ± 98.15  | 1121.83 ± 100.14a,b | 979.52 ± 89.45a,b | 1034.25 ± 115.15a,b,c | 954.59 ± 98.74 |
| PCV (%)                         | 41.6 ± 3.84     | 33.07 ± 2.91a,b | 39.32 ± 3.12a,b | 37.13 ± 3.97a,b,c | 42.13 ± 4.12     |
| Hb (g/dL)                       | 14.23 ± 1.43    | 8.72 ± 0.96a,b  | 13.87 ± 1.04a,b | 11.86 ± 1.12a,b,c | 14.05 ± 1.33     |
| ESR (mm/hr)                     | 9.97 ± 0.10     | 13.80 ± 1.41a,b | 10.05 ± 1.18a,b | 11.67 ± 0.99a,b,c | 10.16 ± 1.32     |
| MCV (fl)                        | 56.44 ± 5.52    | 58.45 ± 5.73a,b | 55.74 ± 5.21a,b | 54.23 ± 5.42a,b,c | 56.89 ± 5.38     |
| MCH (pg)                        | 19.23 ± 1.88    | 16.46 ± 1.72a,b | 19.75 ± 1.86a,b | 17.25 ± 1.68a,b,c | 19.43 ± 1.91     |
| MCHC (%)                        | 34.41 ± 3.51    | 28.11 ± 2.48a,b | 35.18 ± 3.42a,b | 31.74 ± 3.09a,b,c | 33.37 ± 3.16     |

Notes: Values are expressed as mean ± SD for six animals. Comparisons are made between: aGroup 2 vs Group 1; bGroups 3 and 4, vs Group 2; cGroup 4 vs Group 3; dGroup 5 vs Group 1; *statistical significance at \( P < 0.05 \).

Abbreviations: RBC, red blood cell count; WBC, white blood cell count; PCV, packed cell volume; Hb, hemoglobin; ESR, erythrocyte sedimentation rate; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.
platelet count (1.17-fold), ESR (1.38-fold), and MCV by 1.03-fold, and the decrease in RBC by 38.7%, hemoglobin (Hb) by 38.72%, MCH by 14.4%, and MCHC by 53.08%, shows the changes brought about diabetes in Group 2 animals. These changes were reverted back ($P < 0.05$) to near normal levels upon administration of SA and metformin. When Group 4 animals were compared with Group 3 animals significant difference ($P < 0.05$) between the two groups were observed with SA being more effective than metformin. No significant difference was observed when Groups 1 and 5 were compared.

**Effect of SA on marker enzymes in the serum, liver and kidney**

The level of marker enzymes in serum is given in Figure 1. Serum glutamic oxaloacetic transaminase (SGOT) increased by 1.37-fold, serum glutamic pyruvic transaminase (SGPT) by 2.8-fold, ALP by 1.8-fold, $5$'-NT by 2.22-fold and GGT by 1.60-fold. These were restored to near normal levels upon administration of SA and metformin. Metformin-treated animals had higher values of marker enzymes ($P < 0.05$) when compared with SA-treated animals. Figures 2 and 3 shows the activities of ALT, AST, ALP, GGT, and $5$'-NT in the liver and kidney of control and experimental animals. In diabetic untreated animals (Group 2) the activities of ALT, AST, GGT, ACP and $5$'-NT were significantly increased ($P < 0.05$) when compared with Group 1 animals. Administration of SA (Group 3) and metformin (Group 4) to STZ-induced animals decreased the activities of the marker enzymes significantly ($P < 0.05$). No significant changes were observed when Group 1 and Group 5 animals were compared, whereas a significant difference ($P < 0.05$) was observed between Group 3 and 4.

**Effect of SA on parameters in serum and urine**

Tables 2 and 3 shows the levels of albumin, A/G ratio, bilirubin, amylase in serum and urea, creatinine and uric acid in serum and urine of control and experimental animals. The increase in the level of bilirubin was 2.27-fold in Group 2 animals which decreased by 0.46-fold and 0.74-fold in Group 3 and Group 4 animals. The increase in albumin was 1.32-fold in Group 2 animals which decreased by 0.78-fold in Group 3 and 0.89 fold for Group 4. A/G ratio in Group 2 animals decreased significantly ($P < 0.05$), which increased upon drug treatment ($P < 0.05$). Bilirubin and amylase increased by 2.27- and 1.52-fold in Group 2 animals and these levels were significantly decreased ($P < 0.05$) upon the administration of the drugs.

The levels of urea and creatinine in serum increased by 1.93-fold and 2.14-fold, and uric acid decreased by 50% in Group 2 animals. Upon SA treatment, urea and creatinine decreased by 1-fold and 0.5-fold whereas the level of uric acid increased by 96%. In Group 4 animals the values of urea and creatinine decreased by 0.62- and 0.73-fold and uric acid increased by 52%.

The levels of urea, creatinine, uric acid and albumin in urine increased significantly ($P < 0.05$) in Group 2 animals. These were reverted back to near normal levels upon administration of SA and metformin. Significant differences ($P < 0.05$) were found when SA and metformin treated groups were compared. For all the above given parameters there was no significant change when animals in Groups 1 and 5 were compared.

**Figure 1** Levels of marker enzymes in the serum of control and experimental animals.

**Notes:** Values are expressed as mean ± SD for six animals. Comparisons are made between: aGroup 2 vs Group 1; bGroups 3 and 4, vs Group 2; cGroup 4 vs Group 3; dGroup 5 vs Group 1; *statistical significance at $P < 0.05$. 

![Graph A](https://example.com/graphA.png)

![Graph B](https://example.com/graphB.png)
cytoprotective effect of *Semecarpus anacardium*

|                | Group I | Group II | Group III | Group IV | Group V |
|----------------|---------|----------|-----------|----------|---------|
| ALT Units      |         |          |           |          |         |
| AST Units      |         |          |           |          |         |
| ALP Units      |         |          |           |          |         |
| γ-GT Units     |         |          |           |          |         |
| 5' NT Units    |         |          |           |          |         |

Figure 2 Marker enzymes in the liver of control and experimental animals.

Notes: Values are expressed as mean ± SD for six animals. Comparisons are made between: Group 2 vs Group 1; Groups 3 and 4, vs Group 2; Group 4 vs Group 3; Group 5 vs Group 1; *statistical significance at P < 0.05.

Units: AST, µmol of pyruvate liberated/min/mg protein; ALT, µmol of pyruvate liberated/min/mg protein; ALP, µmol of phenol liberated/min/mg protein; ACP, µmoles of phenol liberated/min/mg protein.

Histopathological observations in the liver of control and experimental animals

Figure 4 shows the changes that are observed histologically in the liver of various groups of animals.

Discussion

In diabetes, hematological disorders play a substantial role in the pathogenesis of its complications. Reduced RBC count, Hb and hematocrit, in untreated diabetic rats indicate the progression towards anemia, which may be the result of impaired red blood cell production. The reduction in Hb is due to decreased formation of globulin or circulating failure, which decreases the circulatory blood volume. The decrease in MCH and MCHC values, observed after administration of STZ, is an indication of abnormal hemoglobin synthesis, failure of blood osmoregulation, and plasma osmolarity. These alterations in blood indices imply that the extract could alter the incorporation of haemoglobin into red blood cells as evident from the altered levels in Group 3 animals. Though metformin has the capacity to revert back the altered levels of blood volume in diabetic rats, the effect was not as pronounced as the plant extract. The profile of the WBC count reflects the balance between the rate of granulocyte production and that of WBC. Kozlov et al. reported that diabetes in mice was accompanied by moderate neutrophilic leukocytosis, monocytopenia, depressed monocytopoiesis, prolonged circulation times of
which indicates the loss of membrane integrity. The present increase in serum alkaline phosphatase activity may be due to the presence of polyphenols. The increased gluconeogenesis and ketogenesis may be due to variations in protein metabolism. The change in alanine and aspartate transaminase activities suggests its role in the pathogenesis of the disease. The increased activity of GGT observed in the present study may be attributed to the administration of STZ, which is capable of altering the redox balance of the cell by drastic reduction of GSH in the tissues. The hepatic level of adenosine 3',5'-monophosphate is increased in STZ-treated diabetic rats as a compensatory mechanism to avoid an excessive amount of nucleotides in blood circulation. Determination of serum bilirubin represents an index for the assessment of hepatic function. Increased bilirubin production, enhanced hepatic conjugation, and biliary excretion of the pigment present in diabetic animals may be as a result of decreased uptake, conjugation, or increased bilirubin production. The decreased level of bilirubin in Group 3 animals upon treatment confirms the hepatoprotective effect of the drug in STZ-induced diabetic animals.

### Table 2 Effect of SA on serum parameters of control and experimental animals

| Parameters          | Group 1 (control) | Group 2 (STZ) | Group 3 (STZ + SA) | Group 4 (STZ + metformin) | Group 5 (SA) |
|---------------------|-------------------|---------------|--------------------|---------------------------|--------------|
| Urea (mg/24 hr urine) | 13.04 ± 1.92      | 23.34 ± 2.69** | 16.04 ± 1.55**     | 19.4 ± 1.76**             | 13.5 ± 1.44  |
| Uric acid (mg/24 hr urine) | 3.56 ± 0.34      | 8.44 ± 0.92**  | 4.76 ± 0.42**      | 7.66 ± 0.54**             | 3.58 ± 0.32  |
| Creatinine (mg/24 hr urine) | 8.39 ± 0.79      | 16.13 ± 1.72** | 9.07 ± 0.93**      | 13.75 ± 1.32**            | 8.51 ± 0.94  |
| Albumin (µg/24 hr urine)   | 302.75 ± 29.36    | 973.43 ± 93.36** | 344.64 ± 36.4**   | 623.45 ± 54.8**           | 306.91 ± 32.3 |

**Notes:** Values are expressed as mean ± SD for six animals. Comparisons are made between: aGroup 2 vs Group 1; bGroups 3 and 4, vs Group 2; cGroup 4 vs Group 3; dGroup 5 vs Group 1; *statistical significance at P < 0.05.

### Table 3 Urinary excretion of urea, uric acid, creatinine and albumin in control and experimental animals

| Parameters          | Group 1 (control) | Group 2 (STZ) | Group 3 (STZ + SA) | Group 4 (STZ + metformin) | Group 5 (SA) |
|---------------------|-------------------|---------------|--------------------|---------------------------|--------------|
| Urea (mg/24 hr urine) | 13.04 ± 1.92      | 23.34 ± 2.69** | 16.04 ± 1.55**     | 19.4 ± 1.76**             | 13.5 ± 1.44  |
| Uric acid (mg/24 hr urine) | 3.56 ± 0.34      | 8.44 ± 0.92**  | 4.76 ± 0.42**      | 7.66 ± 0.54**             | 3.58 ± 0.32  |
| Creatinine (mg/24 hr urine) | 8.39 ± 0.79      | 16.13 ± 1.72** | 9.07 ± 0.93**      | 13.75 ± 1.32**            | 8.51 ± 0.94  |
| Albumin (µg/24 hr urine)   | 302.75 ± 29.36    | 973.43 ± 93.36** | 344.64 ± 36.4**   | 623.45 ± 54.8**           | 306.91 ± 32.3 |

**Notes:** Values are expressed as mean ± SD for six animals. Comparisons are made between: aGroup 2 vs Group 1; bGroups 3 and 4, vs Group 2; cGroup 4 vs Group 3; dGroup 5 vs Group 1; *statistical significance at P < 0.05.
Figure 4  Histopathological observations in the liver of control and experimental animals.

(A) Group 1. Control  Control section shows normal histology of liver (100×).
(B) Group 2. STZ induced  This section shows periportal chronic inflammatory infiltrate with focal fatty change (100x).
(C) Group 3. SA-treated  SA-treated animals shows moderate reduction in periportal chronic inflammatory infiltrate when compared to STZ induced (100×).
(D) Group 4. Metformin-treated  Metformin-treated section shows marked reduction of periportal chronic inflammatory infiltrate when compared to STZ induced (100×).
(E) Group 5. SA drug control  The section shows normal histology of liver (100×).

An increased albumin excretion rate leading to microalbuminuria is widely acknowledged as the earliest index of diabetic nephropathy and as a risk factor for the development of overt diabetic renal and macrovascular disease.53 The decreased albumin excretion seen in SA-treated animals proves the renoprotective effect of the drug, which may be due to the downregulation of various autocrine and paracrine factors such as transforming growth factor beta (TGF-β), insulin-like growth factor 1 (IGF-1), type IV collagen and upregulation of renin. In rats, uncontrolled diabetes mellitus increases corticosterone production and urea excretion.54 The decreased levels of serum and urine urea after treatment with
SA is consistent with earlier reports on herbal drugs for the treatment of diabetes. The increased excretion and uric acid synthesis can be related to the energetic deficiency in the cells that are unable to utilize glucose in the state of insulin deficiency. This can lead to excessive dephosphorylation of high-energy purine nucleotides (ATP) and their enhanced catabolism with uric acid as an end product. Increased renal excretion of uric acid in patients with overt diabetes may be due to increased tubular secretion of urate, impaired glomerular filtration rate, or proximal tubule alteration. The metabolic and microvascular complications of diabetes are associated with multiple effects on renal function and creatinine metabolism. Extreme hyperglycemia, osmotic diuresis, and depletion of extracellular fluid volume may cause a decline in glomerular filtration rate (GFR) and an increase in serum creatinine. In diabetes, there is a relationship between glucose homeostasis and renal damage which can be confirmed by the elevation of the serum creatinine. Treatment with SA reversed the alterations caused by STZ and increased glycemia, which could be due to the reduction of hyperfiltration, alteration in GFR by decreasing the hemodynamically mediated renal injury and by improving the glycemic control. The absence of structural and morphological changes of kidney in the drug control Group 5 proves the nontoxic nature of SA.

The release of cellular enzymes reflects a nonspecific alteration in the plasma membrane integrity and/or permeability as a response to STZ administration. The altered activities of the marker enzymes in the serum and tissues of the control and experimental animals may be due to the presence of flavonoids in SA which have membrane-stabilizing properties. Sanz et al have reported that natural flavonoids present in medicinal plants have the ability to decrease serum transaminase activity in animals. The presence of flavonoids may be responsible for these beneficial effects.

Disclosure

The authors report no conflicts of interest.

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