Evidence of the nephroprotective effect of *Carica papaya* L. leaves in streptozotocin-induced diabetic rats

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Protection against diabetic nephropathy (DN) is one of the main targets in diabetes treatment and present study evaluates the nephroprotective effect of *Carica papaya* L. in streptozotocin (STZ) induced diabetic rats. DN rats were treated with 1.5 and 2.5 gm/dl *C. papaya* leaf extract for 6 weeks to determine its nephroprotective effect with different parameters. Pimagedine (1 ml/mg) served as a reference drug. Compared to diabetic control group, *C. papaya* (1.5 and 2.5 gm/dl) treatment significantly decreased some important parameters including plasma glucose, HbA1-c, urinary AER and albumin/creatinine ratio. Improvement in GFR was also significant by *C. papaya*. However, the decrease in blood urea nitrogen (BUN), plasma creatinine, blood pressure (B.P), total cholesterol and serum albumin levels were significant only in diabetic group treated with 2.5 gm/dl of *C. papaya* leaf extract. Serum triglyceride and urine volume decreased with both low and high doses of *C. papaya*. Histological examination revealed marked improvement in glomerular morphology after *C. papaya* treatment. The study concludes that *C. papaya* leaf extract may exert ameliorative effect on DN.

Keywords: *Carica papaya* L., Diabetic rats, Nephropathy

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Since time immemorial, plant-based therapies have been in use by diverse cultures of the world to treat various ailments. The significance of plants in the treatment of diabetes has long been published and until now more than 270 plant species have been identified as having hypoglycemic activity however, the information regarding their usefulness in the prevention of diabetic complications is limited. Among them lies *C. papaya* L. (*Caricaceae*), popularly known as pawpaw or papaya or papita1,2. The edible portion of the plant is its pleasant fruit which is easily digested and is a source of high nutritional value. It is grown in almost all tropical and many subtropical regions of the world and due to the medicinal properties of different parts of the plant, it is used as therapeutic remedy in the form of infusions3. There are reports that *C. papaya* leaves are useful in subsiding the signs and symptoms of dysentery, worming and asthma4,5. In addition, the leaf extracts of *C. papaya* have long been used for the treatment of cancer and infectious diseases5. The aqueous extract of *papaya* leaf is believed to accelerate wound healing6,7, whereas the methanolic extract possesses vasodilating and antioxidant effects, indicating its usefulness in the prevention and/or delaying of cardiovascular diseases4. In Mexican folk medicine, the plant is used to treat inflammation and diabetes7,8. It also relieves diabetes associated oxidative stress9. The evidence of hypoglycemic and antioxidant properties of *C. papaya* in diabetes mellitus diverted our attention to evaluate the usefulness of the aqueous extract of *C. papaya* leaves in diabetic nephropathy in streptozotocin-induced diabetic rats. Diabetic nephropathy is characterized by hyperfiltration by glomerulus and albuminuria along with the expansion of the glomerular mesangium which is related to the loss of renal function10. It is the major cause of morbidity and mortality and despite treatment with drugs like anti-hypertensives or angiotensin converting enzyme inhibitors (ACEI), nephrotic complications among diabetics are still on the rise in many regions of the world11,12. Persistent hyperglycemia and overproduction of reactive oxygen species (ROS) are the major contributing factors in the development of diabetic nephropathy and this study was designed to determine the possible antinephrotic effects of *C. papaya* in a rat model of diabetic nephropathy.

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Methodology

Animals
A total of 30 male albino rats, 6–8 weeks of age and weighing around 160-200 gm, were purchased from the Animal House of National Institute of Health, Islamabad, Pakistan. The rats were housed and maintained at 25±2 °C temperature under 12-hrs light/12-hrs dark cycle and were fed on standard rat diet and water ad libitum13. Experimental protocol for the present study was approved by Institutional Animal Ethical Committee of Gandhara University, Peshawar.

Induction of diabetes
Diabetes was induced by intraperitonial injection of 60 mg/Kg streptozotocin freshly prepared in distilled water14. Control group received an equivalent amount of normal saline. After a week, rats having fasting plasma glucose values of $\geq 300$ mg/dl were considered diabetic and were included for diabetic nephropathy experiment for a period of six weeks.

Carica papaya leaf extracts processing
Leaves of *C. papaya* were collected during June–September, 2014 from Karachi, Pakistan. The plant was authenticated by Pharmacognosy expert at Gandhara college of Pharmacy Peshawar, before processing and the voucher specimen is kept at University herbarium (Specimen No. 1322). *C. papaya* leaves were first washed in 1% iodine aqueous solution followed by washing with distilled water and then dried. Two leaf portions weighing 10 gm and 15 gm were obtained and homogenized in water followed by filtration through a No. 41 filter paper with 20–25 µm retention (Whatman) to yield 1.5gm/dL and 2.5gm/dL extracts respectively. These doses of *C. papaya* leaves extract selected were based on the reported improvement in glucose tolerance and oxidative stress15. The extracts were administered as drinking water.

Study design
After three weeks of streptozotocin injection, rats began to show signs of nephropathy16,17. The rats were randomized into 5 groups with 6 rats in each group as follows: normal control (NC) and diabetic control (DC) groups received drinking water only; diabetic nephropathy group treated with 1.5 gm/dL *C. papaya* (C.P-1.5 gm/dL); diabetic nephropathy group treated with 2.5 gm/dL *C. papaya* (C.P-2.5 gm/dL) and: diabetic nephropathy group treated with 1 ml/mg pimagedine bicarbonate (PMG-1ml/mg) as a reference drug. The treatment was started on 22nd day of streptozotocin injection and was considered as the 1st day of treatment.

Plasma glucose was determined weekly for 6 weeks. All other parameters were determined at the start (Week 0) and at the end of the experiment (week 6). At the end of the study period, blood was withdrawn by cardiac puncture and collected in tubes to separate plasma. The body weight was measured and 24 hrs urine samples were collected using metabolic cages. Then the rats were anesthetized and sacrificed. Kidneys were removed and weighed and the kidney/body weight ratio was calculated. The kidneys were preserved in 10% (v/v) formalin solution for histopathological changes.

Measurement of biochemical parameters
Various blood and urine parameters including plasma glucose, blood urea nitrogen, total cholesterol, triglycerides, creatinine and serum albumin concentrations were determined on a Boehringer Mannheim / Hitachi 737 automated analyzer (Lewis, UK). HbA1-c was measured using Fast ion-exchange resin separation method. Blood pressure was measured by tail-cuff method18. Albumin excretion rate (AER) and albumin/creatinine ratios in urine were determined according to manufacturer’s specifications19. Glomerular filtration rate (GFR) was calculated using the formula:

$$\text{GFR} = \frac{\text{Urinary creatinine concentration} \times 24 \text{ hrs urine volume}}{\text{plasma creatinine concentration} \times 1440}$$

Differences in mean body weight, kidney weight and water intake/100 gm of body weight were determined at the end of the study period for all the experimental groups.

Histopathological examination
The excised kidneys were cut into about 2 mm thick transverse slices and fixed in 10% formalin. After being embedded in paraffin, several transverse sections were obtained from the kidney and stained with Periodic acid-Schiff (PAS) for histological examination.

Statistical analysis
Results are expressed as mean ±SD. The data was analyzed using repeated measure analysis of variance (ANOVA) followed by Dennett’s test and repeated measure ANOVA followed by Tukey’s test.
Differences with \( p < 0.05 \) were considered statistically significant.

**Results**

All the diabetic groups had elevated levels of biochemical parameters than normal control (NC) group and there were no significant differences between these groups at baseline (Table 1). *C. papaya* exerted ameliorative effect on plasma glucose however, the extent of improvement was different among the groups. There was a dose dependent reduction in C.P-1.5 gm/dL and C.P-2.5 gm/dL groups from baseline to endpoint (17.80±0.70 mmol/L vs. 10.0±0.50 mmol/L, and 17.91±0.60 mmol/L vs. 9.60±0.45 mmol/L, \( p < 0.05 \)). At the endpoint, mean differences in plasma glucose levels of *C. papaya* treated groups and DC group were significant (\( p < 0.05 \)). Comparison between *C. papaya* treated groups revealed low levels of plasma glucose in C.P-2.5 gm/dL group, but statistically non-significant (Fig. 1). *C. papaya* treated groups showed significant reductions in HbA1-c levels compared to DC group at endpoint (10±0.91 vs. 7.55±0.80 and 7.10±0.50, respectively, \( p < 0.05 \)), but the difference between *C. papaya* treated groups was non-significant. Elevated urinary AER in diabetic groups at baseline was decreased by *C. papaya* and the difference was significant when compared to DC group at endpoint (0.895 mg/day vs.0.469 mg/day, and 0.218 mg/day, \( p < 0.05 \), respectively). However, no significant difference was observed between *C. papaya* treated groups (Table 1). Microalbuminuria as indicated by elevated albumin/creatinine ratio was significantly low in *C. papaya* treated groups (\( p < 0.05 \)) compared to DC group. These results were comparable to PMG-1 ml/mg reference group (Fig. 2). When the effect of *C. papaya* on GFR was determined, significant

<table>
<thead>
<tr>
<th>Groups</th>
<th>HbA1-c</th>
<th>AER (mg/day)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>B.P (mmHg)</th>
<th>Serum TC (mg/dl)</th>
<th>Serum Albumin (g/dl)</th>
<th>Serum TG (mg/dl)</th>
<th>Urine volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC Baseline</td>
<td>4.70±0.60</td>
<td>0.071</td>
<td>18.90±1.50</td>
<td>0.15±0.08</td>
<td>100.25±2.40</td>
<td>91.20±5.24</td>
<td>3.42±0.08</td>
<td>87.50±4.15</td>
<td>2.24±0.41</td>
</tr>
<tr>
<td>Endpoint</td>
<td>4.67±0.29</td>
<td>0.070</td>
<td>18.9±1±1.10</td>
<td>0.16±0.05</td>
<td>98.50±4.10</td>
<td>93.40±3.70</td>
<td>3.60±0.06</td>
<td>88.30±5.00</td>
<td>2.65±0.30</td>
</tr>
<tr>
<td>DC Baseline</td>
<td>9.62±0.91</td>
<td>0.873</td>
<td>35.70±1.80</td>
<td>0.71±0.13</td>
<td>118.3±3.50</td>
<td>140.8±6.20</td>
<td>2.39±0.07</td>
<td>173.7±9.20</td>
<td>15.30±1.05</td>
</tr>
<tr>
<td>Endpoint</td>
<td>10±0.91</td>
<td>0.895</td>
<td>37.10±1.21</td>
<td>0.82±0.09</td>
<td>129.50±8.10</td>
<td>144.20±9.10</td>
<td>2.23±0.05</td>
<td>189.3±9.20</td>
<td>15.10±1.00</td>
</tr>
<tr>
<td>C.P-1.5 Baseline</td>
<td>9.70±0.24</td>
<td>0.869</td>
<td>36.22±3.43</td>
<td>0.73±0.41</td>
<td>120±8.70</td>
<td>135.30±6.50</td>
<td>2.33±0.02</td>
<td>177.20±7.30</td>
<td>15.11±1.20</td>
</tr>
<tr>
<td>Endpoint</td>
<td>7.55±0.80</td>
<td>0.469</td>
<td>32.10±2.70</td>
<td>0.70±0.06</td>
<td>118±15.10</td>
<td>132.40±7.10</td>
<td>2.34±0.04</td>
<td>142±7.35</td>
<td>8.75±0.75</td>
</tr>
<tr>
<td>C.P-2.5 Baseline</td>
<td>9.60±0.10</td>
<td>0.865</td>
<td>34.92±2.15</td>
<td>0.74±0.10</td>
<td>121.10±4.90</td>
<td>143.60±8.20</td>
<td>2.30±0.04</td>
<td>178.60±9.50</td>
<td>16.00±1.50</td>
</tr>
<tr>
<td>Endpoint</td>
<td>7.10±0.50</td>
<td>0.218</td>
<td>22.50±1.20</td>
<td>0.21±0.09</td>
<td>106.35±6.70</td>
<td>109.5±4.31</td>
<td>2.95±0.09</td>
<td>98.5±9.00</td>
<td>8.61±0.60</td>
</tr>
<tr>
<td>PMG Baseline</td>
<td>9.49±0.57</td>
<td>0.767</td>
<td>35.45±2.95</td>
<td>0.74±0.25</td>
<td>120.5±3.75</td>
<td>141.85±5.50</td>
<td>2.35±0.06</td>
<td>166.35±12.60</td>
<td>14.350±1.25</td>
</tr>
<tr>
<td>Endpoint</td>
<td>8.10±0.20</td>
<td>0.209</td>
<td>20.15±0.60</td>
<td>0.18±0.18</td>
<td>118.90±17.10</td>
<td>139.20±4.90</td>
<td>3.05±0.02</td>
<td>152±17.50</td>
<td>7.80±0.90</td>
</tr>
</tbody>
</table>

Data are mean ±SD of 6 rats in each group

NC: Normal control group; DC: Diabetic control group; C.P: *C. papaya* treated group; PMG: Pimagedine treated group

\*\( p < 0.05 \) vs. Baseline; \¤\( p < 0.05 \) vs. Diabetic control; \o\( p < 0.05 \) vs. C.P-1.5 gm/dL

![Fig. 1](image1.png) —Effect of aqueous extract of *C. papaya* leaf on plasma glucose overtime.

![Fig. 2](image2.png) —Effect of aqueous extract of *C. papaya* leaf on urinary albumin/creatinine ratio.
improvements were observed in *C. papaya* treated groups compared to DC group at endpoint (0.779 mL/min vs. 1.671 mL/min and 2.160 mL/min, respectively, *p*<0.05). Comparison between *C. papaya* treated groups showed no significant difference at the endpoint (Fig. 3). The levels of blood urea nitrogen (BUN), plasma creatinine, blood pressure (BP), serum total cholesterol (TC) and serum albumin in C.P-2.5 gm/dL group showed significant improvement at endpoint upon comparison with DC group (37.10±1.21 vs. 22.50±1.20 mg/dl; 0.82±0.09 vs. 0.21±0.09 mg/dl; 129.50±8.10 mmHg vs. 106.35±4.31 mg/dl; 2.23±0.05 gm/dl vs. 2.95±0.09 gm/dl, respectively, *p*<0.05). Improvement in these parameters of C.P-2.5 gm/dL group were also significant from baseline (*p*<0.05). Moreover, comparison between *C. papaya* treated groups compared to DC group at endpoint (160.70±5.20 gm vs. 178.80±4.10 gm and 182.50±5.50 gm respectively, *p*<0.05). The ratio of kidney weight to body weight (marker of diabetic nephropathy) significantly decreased in C.P-2.5 gm/dL group compared to DC group (4.61±0.16 vs. 2.90±0.08, *p*<0.05). In addition, *C. papaya* treatment significantly reduced water consumption compared to DC group (894±10.40 mL vs. 551±6.95 mL and 478±8.50 mL, respectively, *p*<0.05). These effects of *C. papaya* were comparable to pimagedine (Table 2).

Periodic-acid Schiff (PSA) stained glomerulus of diabetic control group had expanded glomerular mesangium due to mesangial cell proliferation compared to normal size glomerulus of normal control group. These conditions were reversed and the general morphology of glomerulus was much improved by *C. papaya* as was the case with pimagedine (Fig. 4A-E).

### Discussion

DN is one of the major causes of morbidity and mortality worldwide and is the result of persistent hyperglycemia that causes tissue damage through AGEs formation, PKC activation and stimulation of the aldose reductase pathway. It is characterized by a progressive increase in proteinuria, abnormality in serum creatinine, decline in GFR and hypertension. In the present study, we experimentally induced diabetic nephropathy by injecting STZ in animal models that caused metabolic abnormalities and subsequently kidney damage as evident by elevated

### Table 2—Effect of aqueous extract *C. papaya* leaf on physiological parameters of diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Kidney/ body weight ratio</th>
<th>Water consumption (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC Baseline</td>
<td>230.30±6.50</td>
<td>2.54±0.13</td>
<td>205±4.07</td>
</tr>
<tr>
<td>Endpoint</td>
<td>229.15±5.10</td>
<td>2.50±0.11</td>
<td>211±6.80</td>
</tr>
<tr>
<td>DC Baseline</td>
<td>165.10±8.70</td>
<td>4.02±0.22</td>
<td>710±25.30</td>
</tr>
<tr>
<td>Endpoint</td>
<td>160.70±5.20</td>
<td>4.61±0.16</td>
<td>894±10.40</td>
</tr>
<tr>
<td>CP-1.5 Baseline</td>
<td>166±5.10</td>
<td>3.86±0.40</td>
<td>699.10±21.90</td>
</tr>
<tr>
<td>Endpoint</td>
<td>178.80±4.10</td>
<td>3.44±0.41</td>
<td>551±16.95</td>
</tr>
<tr>
<td>CP-2.5 Baseline</td>
<td>168.90±4.70</td>
<td>4.02±0.42</td>
<td>692±13.50</td>
</tr>
<tr>
<td>Endpoint</td>
<td>182.50±5.50</td>
<td>2.90±0.08</td>
<td>478±18.50</td>
</tr>
<tr>
<td>PMG Baseline</td>
<td>169.50±6.20</td>
<td>3.95±0.20</td>
<td>701±22.90</td>
</tr>
<tr>
<td>Endpoint</td>
<td>185.14±5.75</td>
<td>2.73±0.35</td>
<td>451.35±10.60</td>
</tr>
</tbody>
</table>

Data are mean ±SD of 6 rats in each group

NC: Normal control group; DC: Diabetic control group; C.P: *C. papaya* treated group; PMG: Pimagedine treated group

*p*<0.05 vs. Baseline; *p*<0.05 vs. Diabetic control; *p*<0.05 vs. C.P-1.5 g/dL.
serum creatinine and BUN, microalbuminuria and kidney hypertrophy\textsuperscript{22,23} in order to evaluate the protective effect of \textit{C. papaya} against diabetic nephropathy. Plant based therapies have been in use for the treatment of diabetes and its complications since antiquity\textsuperscript{24} and our study suggest that \textit{C. papaya} leaves extract ameliorated blood glucose level dose dependently along with urinary AER and albumin/creatinine ratio, which is a key measure of renal function. A direct relationship between hyperglycemia and nephropathy has been reported earlier\textsuperscript{25}. We observed no dose response relationship of \textit{C. papaya} leaves extract on plasma creatinine, BUN, urine volume and urine creatinine and only higher doses showed significant improvements in plasma creatinine, urine volume and urine creatinine. These effects may be attributed to the greater hypoglycemic activity of \textit{C. papaya} leaves at a higher dose observed in this study as well as earlier\textsuperscript{15}. We also observed a significant improvement in GFR of diabetic rats treated with \textit{C. papaya} leaves extract. Urine creatinine is an index for evaluating the GFR and there is a gradual decrease in GFR in the presence of DN\textsuperscript{26}. In normal conditions, creatinine is mainly filtered out by kidneys and there is almost no tubular reabsorption that results in a low plasma creatinine level. Hypertension and dyslipidemia are the risk factors both for the initiation and progression of diabetic nephropathy\textsuperscript{27-30} and the observed improvement in blood pressure and blood lipids by \textit{C. papaya} leaves extract particularly at a higher dose may also be the contributing factors in protecting against DN. Moreover, the reported antioxidant activity of \textit{C. papaya} leaves\textsuperscript{31} may inhibit the lipids peroxidation and stabilize the membrane lipids thereby decreasing the oxidative stress. There was significant decrease in body weight of diabetic control group and is the result of persistent hyperglycemia. Treatment with \textit{C. papaya} leaves extract was associated with protection from massive loss of body weight along with improvement in the ratio of kidney weight/body weight and water consumption. The study further shows that marked expansion of glomerular mesangium due to mesangial cell proliferation and excessive accumulation of ECM in diabetic control group was reversed and glomerulus returned to near normal size and configuration in \textit{C. papaya} treated groups with the higher doses showing greater improvements. These findings suggest that \textit{C. papaya} leaves extract at higher dose may exert nephroprotective effects by ameliorating several pharmacological targets in DN and may be a useful therapeutic agent for inhibiting and/or at least retarding the initiation and progression of diabetic nephropathy. Although, we do not have a solid reason, but the non-protective effect of \textit{C. papaya} leaves extract at a lower dose in DN may be the result of its lesser beneficial effects on several biochemical parameters. Moreover, its origin and different methods used in the processing of leaves may also be responsible for variation in activity. Further studies, using a range of \textit{C. papaya} leaves extract (starting from 3 gm/dl) are needed to better evaluate the nephroprotective effects in diabetes mellitus.

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References


