ASSESSMENT OF RENAL FUNCTION IN DIABETIC WISTAR RATS TREATED WITH ETHANOL EXTRACT OF Cucumis sativus FRUIT.

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ABSTRACT

As a fast-growing metabolic disorder diabetes mellitus is one of the leading causes of death worldwide. Nephropathy (damage to kidney leading to renal failure) is a microvascular complication of the disease. The aim of the present study was to assess renal function in diabetic rats treated with ethanol extract of Cucumis sativus fruit. Male Wistar rats (n = 25, mean weight = 215 ± 15 g) were randomly assigned to five groups (5 rats per group): control, diabetic, metformin, 200 mg/kg body weight (bwt) extract and 300 mg/kg bwt extract groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of streptozotocin (STZ) at a dose of 50 mg/kg bwt. The diabetic rats were then treated for 21 days with metformin (50 mg/kg bwt) or the extract at doses of 200 and 300 mg/kg bwt, respectively, leaving the diabetic group untreated. The results showed that induction of diabetes mellitus using STZ significantly increased plasma urease activity, and urea and chloride concentrations, but it reduced the weight of rat kidney and concentrations of sodium, potassium and bicarbonate ions significantly (p < 0.05). However, treatment of the diabetic rats with the extract markedly reduced plasma urease, and urea and chloride ion concentrations, while increasing kidney weight, organ/body weight ratio as well as concentrations of sodium, potassium and bicarbonate ions (p < 0.05). The effect of the extract on potassium and chloride ions was dose-dependent. These results indicate that ethanol extract from the medicinal plant C. sativus fruit can ameliorate kidney dysfunction caused by STZ-induced diabetes mellitus.

Keywords: Electrolytes, Histology, Medicinal plant, Renal toxicity, Urease.

Abbreviations: DM (diabetes mellitus); bwt (body weight); STZ (streptozotocin).
INTRODUCTION
The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure. It accomplishes these homeostatic functions both independently and in conjunction with other organs, particularly those of the endocrine system (Boron and Boulpaep, 2004). Most of the kidney's functions are accomplished by simple mechanisms of filtration, reabsorption, and secretion, which take place in the nephron (Clapp, 2009). Taking place in renal corpuscle, filtration is the process by which cells and large proteins are filtered from the blood to make an ultrafiltrate that eventually becomes urine. The kidney generates 180 L of filtrate per day, while reabsorbing a large percentage, allowing for the generation of only approximately 2 L of urine (Bard et al., 2003; Schrier et al., 1972).

Reabsorption is the transport of molecules from this ultrafiltrate into the blood. During secretion molecules are transported in the opposite direction, from the blood into urine. The kidneys are responsible for elimination of unmodified drugs and metabolites. Alterations in kidney structure and function are frequently found in severe liver disease and once liver function falls below a critical threshold, sodium retention occurs followed by ascites, associated with profound disturbances of splanchnic and systemic hemodynamics which in turn may affect renal function (Cotran et al., 2005). Nephrotoxicity refers to injury to kidneys or impairment of kidney function caused by exposure to xenobiotics (Abu et al., 2021 and 2022a).

Diabetes mellitus (DM) remains a serious health challenge, globally. Strategies currently employed for its treatment are targeted at ameliorating the different metabolic derangement associated with the disease (Abu et al., 2023a, 2023b, 2023c, 2023d and 2023e). Nephropathy (damage to kidney leading to renal failure) is a microvascular complication of the disease (Abu et al., 2022b). Diabetic nephropathy is defined as persistent proteinuria. It can progress to overt nephropathy which is characterized by progressive decline in renal function resulting in end-stage renal disease (Abu et al., 2023f). This study investigated renal function in diabetic rats treated with ethanol extract of C. sativus fruit.

MATERIALS AND METHODS
Chemicals and Kits
Reagents used in this study were of analytical grade. Kidney function tests kits were obtained from Randox Laboratories Limited (UK). All other chemicals were purchased from British Drug House (BDH) (England) and Sigma-Aldrich Ltd. (USA).

Plant Material
Whole fruits of C. sativus were obtained randomly from an open market in Benin City, Nigeria. The plant was identified and authenticated at the University of Benin herbarium domiciled in the Department of Plant Biology and Biotechnology.
Plant Extraction
The fruits were washed and shade-dried to constant weight for 2 weeks at room temperature, and thereafter ground into powder using an electric blender. A portion (500 g) of powdered plant material was steeped in 5 L of absolute ethanol. The resultant extract was filtered through muslin cloth and freeze-dried with a lyophilizer thereafter (Okpiabhele et al., 2018; Abu et al., 2017, 2019 and 2022c).

Experimental Rats
Wistar rats (n = 25) weighing between 200 and 230 g (mean weight = 215 ± 15 g) were kept in metal cages under standard laboratory settings for purpose of acclimatization for 7 days. The animals had unrestricted access to feed (pelletized mash) and potable drinking water. The investigation followed a standard experimental protocol.

Experimental Design
The experimental design used in this study was a completely randomized block design. The rats were assigned to five groups of 5 rats each: control, diabetic, metformin, and 200 mg/kg bwt and 300 mg/kg bwt extract groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of STZ (50 mg/kg bwt). The diabetic rats were subsequently treated for 21 days with metformin (50 mg/kg bwt) or the extract (200 and 300 mg/kg bwt, respectively), leaving the diabetic group untreated.

Blood Sample Collection and Preparation
At the end of day 21 of treatment, the rats were euthanized under mild chloroform anaesthesia after an overnight fast. Blood was drawn via cardiac puncture into heparinized sample bottles and centrifuged at 2000 rpm for 10 min to obtain plasma which was used for biochemical analyses.

Kidney Function Tests
Urea, sodium, potassium, chloride and bicarbonate levels were determined in the plasma. Urease activity was also performed in plasma (Abu et al., 2022a; Baniata et al., 2009; Bheemanet et al., 2013; Feroz et al., 2009).

Statistical Analysis
Data are expressed as mean ± SEM (n = 5). One-Way Analysis of Variance (ANOVA) was performed using SPSS (version 20). Groups were compared using Duncan multiple range test. Statistical significance was assumed at p < 0.05.

RESULTS AND DISCUSSION
Effect of C. sativus Fruit Extract on Weight and Renal Function
Induction of DM using STZ significantly increased plasma urease activity, and urea and chloride concentrations, but it reduced the weight of rat kidney and concentrations of sodium, potassium and bicarbonate ions significantly (p < 0.05). However, treatment of the diabetic rats with the extract markedly reduced plasma urease, and urea and chloride ion concentrations, while increasing kidney weight, organ/body weight ratio as well as concentrations of sodium, potassium
and bicarbonate ions (p < 0.05). The effect of the extract on potassium and chloride ions was dose-dependent. These results are shown in Tables 1 to 3.

Kidney, an organ that metabolizes harmful substances besides liver, is constantly perfused with huge volume of blood carrying different kinds of compounds, thereby making it at high risk of toxicity (Tortora and Derrickson, 2006; Small et al., 2012; Abu et al., 2015). Hyperglycemia due to DM can damage blood vessels in the kidneys as well as nephrons, thus impairing the function of these structures. Many people with DM also develop high blood pressure (hypertension) which in turn can damage the kidneys (Tortora and Derrickson, 2006). High levels of blood urea and creatinine are found in renal dysfunction or muscle injury (Abu et al., 2015). They are considered traditional indices of kidney function. Urea is a by-product of protein catabolism. About 90% of urea produced is excreted through the kidneys (Walmsley et al., 2010).

Creatinine, a waste product of muscle catabolism, is excreted exclusively via the kidneys (Treasure, 2003). Therefore, renal damage reduces the kidney’s capacity to excrete both urea and creatinine, thereby making them to accumulate in the blood. The results of this study showed that STZ caused significant damage to renal corpuscle as exemplified by elevated urea concentration and activity of urease. Levels of specific ions such as sodium (Na⁺), potassium (K⁺), chloride (Cl⁻) and bicarbonate (HCO₃⁻) are used as biomarkers of electrolyte imbalance (Bheeman et al., 2013). Electrolytes promote fluid balance via maintenance of blood volume, fluid absorption and generation of impulses. In pathological conditions, electrolyte imbalance occurs with increased sodium and chloride, and decreased potassium levels (Baniata et al., 2009; Feroz et al., 2009).

Nephrotoxicity is characterized by morphological destruction of intracellular organelles, necrosis, and functional alterations such as depletion of antioxidant defense system and mitochondrial damage (Joy and Nair, 2008). The results of this study are in agreement with those of previous studies (Ebhohon et al., 2019a and 2019b; Omorogbe et al., 2020; Abu et al., 2022; Abu and Ikponmwosa-Ewika, 2022a, 2022b, and 2022c).

Table 1: Comparison of the Effect of C. sativus Fruit Extract on Weight of Rat Kidneys

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight of Kidney (g)</th>
<th>Organ/Body Weight Ratio x 10⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.73 ± 0.05</td>
<td>3.95 ± 0.41</td>
</tr>
<tr>
<td>Diabetic Group (DG)</td>
<td>0.44 ± 0.02</td>
<td>2.71 ± 0.17</td>
</tr>
<tr>
<td>DG + Metformin</td>
<td>0.60 ± 0.30</td>
<td>4.08 ± 0.22</td>
</tr>
<tr>
<td>DG + Extract (200 mg/kg bwt)</td>
<td>0.64 ± 0.24</td>
<td>3.81 ± 0.18</td>
</tr>
<tr>
<td>DG + Extract (300 mg/kg bwt)</td>
<td>0.62 ± 0.14</td>
<td>4.38 ± 0.16</td>
</tr>
</tbody>
</table>

Data are weight and organ/body weight ratio, and are expressed as mean ± SEM (n = 5)

Table 2: Effect of C. sativus Fruit Extract on Kidney Function

<table>
<thead>
<tr>
<th>Group</th>
<th>Urease (U/L)</th>
<th>Urea (mg/dL)</th>
<th>Na⁺ (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.76 ± 0.58</td>
<td>33.78 ± 3.69</td>
<td>69.29 ± 5.49</td>
</tr>
<tr>
<td>Diabetic Group (DG)</td>
<td>17.94 ± 1.50</td>
<td>89.72 ± 5.08</td>
<td>21.02 ± 2.25</td>
</tr>
<tr>
<td>DG + Metformin</td>
<td>8.20 ± 0.51</td>
<td>40.98 ± 3.22</td>
<td>73.41 ± 6.38</td>
</tr>
<tr>
<td>DG + Extract (200 mg/kg bwt)</td>
<td>7.11 ± 0.30</td>
<td>35.55 ± 2.22</td>
<td>68.41 ± 4.12</td>
</tr>
<tr>
<td>DG + Extract (300 mg/kg bwt)</td>
<td>8.58 ± 1.00</td>
<td>42.92 ± 2.03</td>
<td>63.03 ± 4.33</td>
</tr>
</tbody>
</table>

Data are kidney function tests, and are expressed as mean ± SEM (n = 5)
Table 3: Effect of *C. sativus* Fruit Extract on Some Parameters of Kidney Function

<table>
<thead>
<tr>
<th>Group</th>
<th>$K^+$ (mEq/L)</th>
<th>$Cl^-$ (mEq/L)</th>
<th>$HCO_3^-$ (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.17 ± 0.61</td>
<td>51.20 ± 2.54</td>
<td>38.15 ± 2.61</td>
</tr>
<tr>
<td>Diabetic Group (DG)</td>
<td>2.05 ± 0.07</td>
<td>90.52 ± 4.37</td>
<td>22.38 ± 1.93</td>
</tr>
<tr>
<td>DG + Metformin</td>
<td>3.52 ± 0.51</td>
<td>46.62 ± 1.85</td>
<td>31.60 ± 3.37</td>
</tr>
<tr>
<td>DG + Extract (200 mg/kg bwt)</td>
<td>3.29 ± 0.40</td>
<td>61.51 ± 6.28</td>
<td>24.41 ± 2.09</td>
</tr>
<tr>
<td>DG + Extract (300 mg/kg bwt)</td>
<td>3.75 ± 0.28</td>
<td>55.07 ± 5.87</td>
<td>22.90 ± 2.90</td>
</tr>
</tbody>
</table>

Data are kidney function parameters, and are expressed as mean ± SEM (n = 5)

CONCLUSION
The results obtained in this study indicate that ethanol extract of the medicinal plant *C. sativus* fruit can ameliorate kidney dysfunction caused by STZ-induced DM. However, further studies are required to elucidate the underlying mechanism(s).

COMPETING INTERESTS
The authors declare that they have no conflict of interest.

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