Biochemical, Morphological and Molecular Assessments of Flavonoids of *Phoenix dactylifera* L. Following Exposure to Inorganic Mercury on the Liver of Wistar Rats.

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Abstract

Introduction:

Mercury is a highly toxic metal that exerts adverse effects on humans and animals. Date palm (Phoenix dactylifera) is used in folk medicine to treat fever, and inflammation, among others. The present study aimed to evaluate the protective effects of aqueous and ethanol extracts of Phoenix dactylifera on mercury-induced kidney damage in Wistar rats.

Design:

45 male rats (80–125g) were divided into nine groups (I-XI). Group 1 received 1mg/kg of distilled water, group II received mercury chloride (HgCl$_2$) at 5 mg/kg; group III was pretreated with 100 mg/kg of silymarin followed by 5 mg/kg of HgCl$_2$; groups IV and V were pretreated with 500 mg/kg and 1000 mg/kg of aqueous extract of Phoenix dactylifera (AFPD) respectively, followed by 5 mg/kg of HgCl$_2$; groups VI and VII were pretreated with 500 mg/kg and 1000 mg/kg of ethanol extract of Phoenix dactylifera L. (EFPD) respectively, followed by 5 mg/kg of HgCl$_2$ while group VIII and IX received 1000 mg/kg of AFPD and EFPD respectively. After two weeks of oral administration, the animals were sacrificed and the blood and kidney tissue were collected for analyses.

Results

The present study showed that MDA significantly increased in HgCl$_2$ group when compared to control while histological and histochemical changes in the kidney revealed severe degenerative changes. AFPD and EFPD extracts restored serum enzymes, electrolytes and antioxidant enzymes activity with significant increase in SOD activity in the AFPD (high dose) when compared to the HgCl$_2$ treated group. This indicates some level of protection against HgCl$_2$-induced changes in the kidneys of Wistar rats. The protective activity of the AFPD and EFPD may be attributed to the antioxidant properties of the phytochemicals, such as flavonoids, tannins etc.

Conclusion

The aqueous and ethanol extracts of Phoenix dactylifera are potential candidates for the management and treatment of ROS-induced kidney diseases.

Introduction

Mercury is a widespread environmental and industrial pollutant that exerts toxic effects on a variety of vital organs; it induces severe alterations in the tissues$^{1,2}$. Mercury will cause severe disruption to any tissue it comes into contact with in sufficient concentration. The liver and kidney are organs that suffer significant damage caused by inorganic mercury such as mercuric chloride. Inorganic mercury compounds rapidly accumulate in the kidney$^3$, the main target organ for these compounds. Mercury
poisoning can result from inhalation, ingestion, or absorption through the skin and may be highly toxic and corrosive once absorbed into the bloodstream ¹.

Despite massive efforts in search of new drugs that could counteract mercurial toxicity, there is no effective treatment available that can completely abolish its toxic effects. The use of chelating agents assists the body’s ability to eliminate mercury from the tissues ⁴. However, these chelants are of limited use, because of their adverse side effects ⁵. As a result, the need to evaluate plants for their medicinal activities is of paramount importance.

Date palm (*Phoenix dactylifera*) fruits are good source of energy, vitamins such as vitamin A, vitamin B₆ (pyridoxine), vitamin K, and a group of elements like phosphorus, iron, potassium, and a significant amount of calcium ⁶,⁷. Dates are widely used in traditional medicine for the treatment of various disorders e.g., memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders ⁸. It is also used in the treatment of sore throat, to relieve fever, cystitis, gonorrhea, edema, liver and abdominal troubles and to counteract alcohol intoxication ⁹,¹⁰. The World Health Organization (WHO) estimates that up to 80% of the world’s population relies on traditional medicinal systems for some aspect of primary health care ¹¹.

Many pharmacological studies have been conducted on *Phoenix dactylifera* and it has been demonstrated to have antiulcer activity; anticancer activity; anti-diarrhoeal activity; hepatoprotective activity; antimitogenic activity; anti-inflammatory activity; *in vitro* antiviral activity; effect on reproductive system; antihyperlipidemic activity; nephroprotective activity and antioxidant activity ¹². Several researchers have also documented the antioxidant properties of *Phoenix dactylifera* ¹³,¹⁴.

**Materials and Methods**

**Plant Material Collection and Identification**

Dried *Phoenix dactylifera* L. (date palm) fruits were obtained and authenticated in the Herbarium Unit of Department of Biological Sciences, Faculty of Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria where the Voucher Specimen Number, 7130, was obtained.

**Experimental Animals**

A total of forty-five (45) male Wistar rats (80 to 125 g) were obtained from the Department of Pharmacology animal house, Faculty of Pharmaceutical sciences, Ahmadu Bello University Zaria, Kaduna, Nigeria. Animals were housed in the animal house of the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, where they were acclimatized for two weeks prior to the commencement of the experiments. The rats fed on rat chow and water allowed *ad libitum*.

In addition, the rats were administered aqueous or ethanol fruit extract of *P. dactylifera* mercury/silymarin. Rats were weighed at the beginning, during and at the end of the study.
Drugs

• Mercury

Seventy grams of mercuric chloride was obtained and used to induce nephrotoxicity for the experiment. The product was manufactured by British Drug Houses (BDH) chemicals, Poole, England. Batch number-291634U.

• Silymarin

Silymarin was used as the standard antioxidant drug for the experiment. Each tablet is film coated and contains 140 mg of silymarin. The product was manufactured by Micro Labs Limited 92, Sipcot, Hosur-635 126, India. Batch number- SYFH0011.

Animal Feed

Pelletized Vital Feed was obtained and used to feed the animals for the experiment. The product was manufactured by Grand Cereals and Oil Mills Limited (GCOML), Km 17, Zawan Roundabout, Plateau State, Nigeria; RC 54291.

Plant Extraction

Preparation of aqueous and ethanol fruit extracts of *P. dactylifera* were conducted in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna, Nigeria. The maceration methods of Abdul-Wahab *et al.*\(^{15}\) and Agbon *et al.*\(^{16}\) were used for the preparation of the aqueous and ethanol fruit extract of *P. dactylifera* respectively.

**Phoenix dactylifera** Phytochemical Screening

Phytochemical screening of aqueous and ethanol fruit extracts of *P. dactylifera* were done in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna, Nigeria. The method of Trease and Evans\(^ {17}\) for Phytochemical screening was adopted.

**Experimental design**

Forty-five (45) Wistar rats of male sex were divided into common groups (consisting of Groups; I-III) and treatment groups (consisting of Groups IV-IX) having five rats each. Group I served as control and was administered distilled water at 2ml/kg. Nephrotoxicity was induced by HgCl\(_2\) administration. Group II was administered 5 mg/kg of HgCl\(_2\); 12.5% LD\(_{50}\); 40 mg/kg - Sheikh *et al.*\(^ {18}\)) only. Group III was administered 100 mg/kg of Silymarin as reported by Ahmed *et al.*\(^ {19}\), followed by 5 mg/kg of HgCl\(_2\). Groups IV was administered 500mg/kg of aqueous fruit extract of *P. dactylifera* followed by 5 mg/kg of HgCl\(_2\). Group V was administered 1000 mg/kg of aqueous fruit extract of *P. dactylifera* followed by HgCl\(_2\) at 5 mg/kg. Group VI was administered 500 mg/kg of ethanol fruit extract of *P. dactylifera* followed by HgCl\(_2\) at 5 mg/kg. Group VII was administered 1000 mg/kg of ethanol fruit extract of *P. dactylifera* followed by 5
mg/kg of HgCl$_2$. Groups VIII was administered 1000 mg/kg of aqueous fruit extract of *P. dactylifera* only while Group IX was administered 1000 mg/kg of ethanol fruit extract of *P. dactylifera* only. The administrations were orally done and lasted for a period of 2 weeks as shown in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment/ daily</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled water (2 ml/kg)</td>
<td>2 weeks</td>
</tr>
<tr>
<td>II</td>
<td>HgCl$_2$ (5 mg/kg)</td>
<td>2 weeks</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin (100 mg/kg) + HgCl$_2$ (5 mg/kg)</td>
<td>2 weeks</td>
</tr>
<tr>
<td>IV</td>
<td>AFPD (500 mg/kg) + HgCl$_2$ (5 mg/kg)</td>
<td>2 weeks</td>
</tr>
<tr>
<td>V</td>
<td>AFPD (1,000 mg/kg) + HgCl$_2$ (5 mg/kg)</td>
<td>2 weeks</td>
</tr>
<tr>
<td>VI</td>
<td>EFPD (500 mg/kg) + HgCl$_2$ (5 mg/kg)</td>
<td>2 weeks</td>
</tr>
<tr>
<td>VII</td>
<td>EFPD (1000 mg/kg) + HgCl$_2$ (5mg/kg)</td>
<td>2 weeks</td>
</tr>
<tr>
<td>VIII</td>
<td>AFPD (1000 mg/kg) only</td>
<td>2 weeks</td>
</tr>
<tr>
<td>IX</td>
<td>EFPD (1000 mg/kg) only</td>
<td>2 weeks</td>
</tr>
</tbody>
</table>

**Note:** Treatments were administrations orally. Mercuric chloride (HgCl$_2$); aqueous fruit extract of *P. dactylifera* (AFPD), ethanol fruit extract of *P. dactylifera* (EFPD).

### Animal Sacrifice

At the end of the experiment, the rats were humanely sacrificed under chloroform anesthesia. The rats were dissected, blood samples were collected with the use of sample bottles and the kidney was excised, weighed and fixed in 10% formal saline.

### Weight Change

The initial (first day of administration) and final (last day of administration) body weights of animals in all the groups were recorded and analyzed to determine the weight change between the various groups. The organ weight / body weight ratio of animals in all the groups were also recorded and analyzed.

### Biochemical Studies

Biochemical studies were done using the collected blood samples in the Department of Chemical Pathology, Faculty of Medicine, Ahmadu Bello University Teaching Hospital, Shika. Biochemical analysis for kidney electrolytes namely Na$^+$, K$^+$, Cl$^-$, HCO$_3^-$ and Urea; lipid peroxide levels and antioxidant enzyme activity namely malondialdehyde - MDA, superoxide dismutase - SOD, catalase - CAT and glutathione peroxidase - GPx were performed.
Histological and Histochemical Studies

At the end of the experiment, the kidney tissues were fixed in 10% formal saline and processed for routine histological examination, stained with Haematoxylin and Eosin (H&E). Histochemical staining (Periodic Acid Schiff for demonstrating glycogen) was carried out and examined under the light microscope.

Data Analysis

Results obtained were analyzed using Statistical Package for Social Sciences (SPSS version 18.0) and results were expressed as mean ± S.E.M. The presence of significant differences among means of the groups were determined using one way Analysis of variance (ANOVA). Paired sample t-test was employed for the comparisons of means as appropriate. Values were considered significant when $p \leq 0.05$.

Results

Phytochemical Analysis

Phytochemical analysis of aqueous and ethanol fruit extracts of *Phoenix dactylifera* indicating the presence of secondary metabolites is as shown in Table 2.

Weight Change

The initial and final weights of the rats, in all groups (I - IX), were compared. The result showed that weight increase was observed in all treated groups. The result also showed that weight change in all treated groups were significantly lower when compared to the Control, except in Group VII ($p > 0.05$). Weight change in Group VII was significantly higher when compared to Group II ($p < 0.05$) (Table 3).

Table 2: Phytochemical Constituents of the Fruit Extracts of *Phoenix dactylifera*
<table>
<thead>
<tr>
<th>Constituents</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Positive (present)
− = Negative (absent)
Table 3
Weight change and weight comparison of Wistar rats initial day and final day of treatment

<table>
<thead>
<tr>
<th>Group/ Treatment</th>
<th>IW (g)</th>
<th>FW (g)</th>
<th>FW-IW (g)</th>
<th>T</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>125.50 ± 12.34</td>
<td>174.45 ± 39.65</td>
<td>85.28 ± 24.32</td>
<td>1.268</td>
<td>0.425</td>
</tr>
<tr>
<td>II</td>
<td>93.10 ± 3.02</td>
<td>117.07 ± 4.16</td>
<td>41.16 ± 5.14***</td>
<td>2.467</td>
<td>0.090</td>
</tr>
<tr>
<td>III</td>
<td>81.28 ± 5.51</td>
<td>103.45 ± 4.85</td>
<td>40.68 ± 15.17*</td>
<td>2.569</td>
<td>0.083</td>
</tr>
<tr>
<td>IV</td>
<td>102.40 ± 8.78</td>
<td>134.90 ± 9.52</td>
<td>32.50 ± 7.49***</td>
<td>4.339</td>
<td>0.012</td>
</tr>
<tr>
<td>V</td>
<td>97.18 ± 6.98</td>
<td>120.72 ± 5.78</td>
<td>23.54 ± 8.03***</td>
<td>2.933</td>
<td>0.043</td>
</tr>
<tr>
<td>VI</td>
<td>86.48 ± 4.45</td>
<td>114.33 ± 4.11</td>
<td>43.78 ± 13.37**</td>
<td>17.472</td>
<td>0.000</td>
</tr>
<tr>
<td>VII</td>
<td>99.48 ± 2.68</td>
<td>122.40 ± 4.30</td>
<td>71.48 ± 18.68 a</td>
<td>2.145</td>
<td>0.278</td>
</tr>
<tr>
<td>VIII</td>
<td>80.26 ± 3.49</td>
<td>102.10 ± 5.73</td>
<td>21.84 ± 2.36***</td>
<td>9.258</td>
<td>0.001</td>
</tr>
<tr>
<td>IX</td>
<td>95.06 ± 5.34</td>
<td>123.70 ± 4.87</td>
<td>28.64 ± 2.29***</td>
<td>12.518</td>
<td>0.000</td>
</tr>
</tbody>
</table>

n = 5; mean ± SEM; Paired sample t-test; *= p < 0.05, **= p < 0.01; significant difference when compared with the control; a = p < 0.05; significant difference when compared with the MCL group. Gp I = Control (distilled H₂O 0.5 ml/kg); II = HgCl₂ (5 mg/kg); III = Silymarin (100mg/kg); IV and V = Aqueous fruit extract of P.dactylifera (500 and 1,000 mg/kg respectively); VI and VII = Ethanol fruit extract of P.dactylifera (500 and 1,000 mg/kg respectively); aqueous fruit extract of P.dactylifera (1,000 mg/kg); Ethanol fruit extract of P.dactylifera (1,000 mg/kg) FW = Final weight; IW = Initial weight; FW-IW = Weight change.

Biochemical Studies

In the present study, serum kidney electrolytes (urea, Creatinine, sodium ion (Na⁺), chloride ion (Cl⁻), potassium ion (K⁺), and bicarbonate (HCO₃⁻)) levels of experimental animals were analyzed. The results of serum urea showed a non-significant decrease in urea levels in all the treatment groups as compared to the control group except groups II, V and VIII that showed a non-significant increase in urea levels as shown in Table 4.

Creatinine levels revealed non-significant changes in all treated groups, except groups VI and VIII with significantly decreased creatinine levels (p < 0.05) as compared to control. In comparison to the HgCl₂-treated group, there was a non-significant decrease in creatinine levels in all the treated groups (p > 0.05) except group VII with a significant decrease as shown in Table 4.

Na⁺ analysis revealed non-significant changes in all the treatment groups except in group VIII where significant (p < 0.05) decrease was seen when compared to the control group. As compared to the HgCl₂-
treated group, Na\(^+\) analysis also showed significantly decreased levels in groups VI and VIII as shown in Table 4.

When compared to the control group, Cl\(^-\) levels increased significantly in group VII and decreased significantly in group VIII. Similarly, there was a significant \((p < 0.05)\) decrease in Cl\(^-\) level in groups V and VIII when compared to the HgCl\(_2\)-treated group as shown in Table 4.

There were no significant changes in levels of K\(^+\) across all the treated groups except in group IX with a significant decrease as compared to the control. As regards the HgCl\(_2\)-treated group, there were no significant \((p < 0.05)\) changes observed in all treated groups as shown in Table 4.

The results of HCO\(_3^-\) revealed significant \((p > 0.05)\) decrease in HCO\(_3^-\) levels in groups IV, VI and VIII in comparison to the control group. There were no significant changes when compared to the HgCl\(_2\)-treated group as shown in Table 4.

<table>
<thead>
<tr>
<th>Group/Treatment</th>
<th>Urea (mg/dL)</th>
<th>Creatine (mg/dL)</th>
<th>Na(^+) (mmol/L)</th>
<th>Cl(^-) (mmol/L)</th>
<th>K(^+) (mmol/L)</th>
<th>HCO(_3^-) (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.03 ± 0.63</td>
<td>56.50 ± 7.08</td>
<td>138.00 ± 1.73</td>
<td>98.50 ± 1.26</td>
<td>4.68 ± 0.21</td>
<td>19.50 ± 1.89</td>
</tr>
<tr>
<td>II</td>
<td>5.18 ± 0.21</td>
<td>85.75 ± 13.75</td>
<td>140.25 ± 0.85</td>
<td>101.25 ± 1.49</td>
<td>4.23 ± 0.21</td>
<td>21.50 ± 0.96</td>
</tr>
<tr>
<td>III</td>
<td>4.73 ± 0.57</td>
<td>59.25 ± 9.66</td>
<td>141.75 ± 2.02</td>
<td>99.75 ± 0.63</td>
<td>4.48 ± 0.29</td>
<td>19.50 ± 1.50</td>
</tr>
<tr>
<td>IV</td>
<td>4.63 ± 0.47</td>
<td>60.75 ± 15.46</td>
<td>136.50 ± 2.47</td>
<td>98.25 ± 2.10</td>
<td>4.53 ± 0.23</td>
<td>24.50 ± 0.96*</td>
</tr>
<tr>
<td>V</td>
<td>5.20 ± 1.68</td>
<td>74.00 ± 25.39</td>
<td>138.75 ± 2.14</td>
<td>97.50 ± 1.89\textsuperscript{a}</td>
<td>4.60 ± 0.17</td>
<td>22.00 ± 2.45</td>
</tr>
<tr>
<td>VI</td>
<td>4.10 ± 0.00</td>
<td>47.00 ± 0.00\textsuperscript{a}</td>
<td>135.00 ± 0.00\textsuperscript{a}</td>
<td>96.00 ± 0.00</td>
<td>4.20 ± 0.00</td>
<td>12.00 ± 0.00\textsuperscript{**}</td>
</tr>
<tr>
<td>VII</td>
<td>4.63 ± 0.40</td>
<td>56.00 ± 8.46</td>
<td>141.75 ± 3.17</td>
<td>104.25 ± 3.22\textsuperscript{a}</td>
<td>4.75 ± 0.45</td>
<td>18.75 ± 2.56</td>
</tr>
<tr>
<td>VIII</td>
<td>6.70 ± 0.00</td>
<td>41.00 ± 0.00\textsuperscript{**c}</td>
<td>127.00 ± 0.00\textsuperscript{***b}</td>
<td>86.00 ± 0.00\textsuperscript{**c}</td>
<td>4.80 ± 0.00</td>
<td>14.00 ± 0.00\textsuperscript{*}</td>
</tr>
<tr>
<td>IX</td>
<td>3.90 ± 0.23</td>
<td>62.00 ± 12.70</td>
<td>141.50 ± 0.29</td>
<td>100.00 ± 0.58</td>
<td>4.00 ± 0.12\textsuperscript{*}</td>
<td>23.50 ± 0.29</td>
</tr>
</tbody>
</table>

Table 4
Effect of fruit extracts of *P. dactylifera* on kidney electrolytes against mercury-induced kidney toxicity in Wistar rats
Lipid Peroxide Levels and Antioxidant Enzyme Activity

The malondialdehyde (MDA) or thiobarbituric acid-reactive-substances (TBARS) assay was used to estimate lipid peroxidation levels which is a marker of lipid peroxidation, while antioxidant enzymatic activity was estimated by assaying for superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities using the serum of the rats.

Serum MDA levels increased \(p < 0.05\) in the HgCl\(_2\)-treated group when compared to the control group. All other treated groups revealed non-significant changes in MDA as compared to the control. When compared to the HgCl\(_2\)-treated group, MDA levels decreased significantly in all the treated Groups as shown in Fig. 1.

The Serum SOD results showed non-remarkable changes in SOD activity in all the treated groups except in group IV which showed a remarkable increase in SOD activity as compared to the control group. In comparison to the HgCl\(_2\)-treated group, SOD activity increased remarkably in group IV as shown in Fig. 2.

There were no significant changes in serum CAT and GPx activity in all the treated groups when compared to control as well as the HgCl\(_2\)-treated group as shown in Figs. 3 & 4.

Histological and Histochemical Studies of the Kidney

Histological sections of Wistar rats’ kidneys stained with routine (Haematoxylin and Eosin, H&E) stain and histochemical (Periodic Acid Schiff, PAS) stain were examined under the light microscope and revealed the following:

The results of the kidney sections of rats in the control group showed normal histoarchitecture of the kidney tissue as seen by the characteristic appearance of the renal cortex which is comprised of the renal corpuscle (Bowman's capsule, Bowman's space, glomerulus (anastomosing capillaries), blood vessels, proximal convoluted tubule and distal convoluted tubule as shown in Fig. 5, plate A. PAS staining for glycogen revealed positive staining intensity demonstrating the presence of glycogen as shown in Fig. 6, plate A.

The results from the kidney sections treated with HgCl\(_2\) revealed severe histoarchitectural and cytoarchitectural distortion of the kidney tissue. Degenerative changes such as dilatation of Bowman's space, distortion of renal corpuscle, renal tubular necrosis, pyknotic nuclei and cytoplasmic vacuolation as shown in Fig. 5, plate B. Reduced PAS stain intensity was observed as shown in Fig. 6, plate B.

n=5, values are mean ± SEM. \* = \(p<0.05\), ** = \(p<0.01\), *** = \(p<0.001\); significant difference when compared with the control. \(b = p<0.01, c = p<0.001\); significant difference when compared with the MCL group. Gp I=Control (distilled H\(_2\)O 0.5 ml/kg); II = HgCl\(_2\) (5 mg/kg); III = Silymarin (100mg/kg); IV and V= Aqueous fruit extract of P. dactylifera (500 and 1,000 mg/kg respectively); VI and VII= Ethanol fruit extract of P. dactylifera (500 and 1,000 mg/kg); aqueous fruit extract of P. dactylifera (1,000 mg/kg); Ethanol fruit extract of P. dactylifera (1,000 mg/kg)
The histological features of the Silymarin treated followed by the administration of HgCl₂ showed cytoplasmic vacuolation as compared to the control as shown in Fig. 5, plate C. PAS positive stain intensity was similar to the control as shown in Fig. 6, plate C.

AFPD treated group followed by the administration of HgCl₂ showed mild degenerative changes such as, dilatation of Bowman's space as shown in Fig. 5, plates D and E. PAS positive stain intensity was similar to the control as shown in Fig. 6, plates D and E.

EFPD treated group followed by the administration of HgCl₂ showed degenerative changes, such as dilatation of Bowman's space, distortion of renal corpuscle, renal tubular necrosis and cytoplasmic vacuolation, pyknotic nuclei as shown in Fig. 5, plates F and G. Reduced PAS staining intensity revealed glycogen depletion as shown in Fig. 6, plates F and G.

Histological and histochemical examinations of the results of the kidney sections of Wistar rats treated with AFPD and EFPD only revealed mild distortion of the histoarchitecture of the kidney which manifested as dilated Bowman's space as shown in Fig. 5, plates H and I. PAS positive stain intensity was comparable to the Control as shown in Fig. 6, plates H and I.

Discussion

In this study, preliminary phytochemical screening of aqueous and ethanol fruit extracts of *Phoenix dactylifera* was carried out, and their protective effects on mercury-induced kidney toxicity in Wistar rats were assessed using biochemical, histological and histochemical techniques.

Phytochemical screening of the plant (*Phoenix dactylifera*) fruit extracts (aqueous and ethanol), in the present study, revealed the presence of flavonoids, saponins, tannins and alkaloids which have been reported to possess nephroprotective activities in animals and cell culture models.

Exposure to mercury can overwhelm the kidneys making it challenging to perform its biological and physiological functions optimally. According to Wargovich *et al.* and Kumari and Chand, mercury chloride is one of the most toxic forms of mercury because it easily forms organomercuric complexes with proteins in a biological system. Among all heavy metals, mercuric chloride remains the major cause of nephrotoxicity in many parts of the world. The kidney is the first target organ of accumulation and toxicity of inorganic mercury. In fact, in a very short time (1 hour), 50% of an administered dose of inorganic mercury is present in the kidney.

*Phoenix dactylifera* has been reported to possess various pharmacological activities including anti-inflammatory, hepatoprotective, antioxidant and many more. Silymarin is a polyphenolic compound extracted from *Silibum marianum* and *Cynara cardunculus* seeds and fruits. It acts as an antioxidant checkmating free radical induced tissues damage, inhibits lipid peroxidation and alters drug induced
histopathological changes\textsuperscript{30,31}. Silymarin has been used for centuries to treat liver, kidney, spleen and gall bladder disorders\textsuperscript{32}. The antioxidant property of silymarin is well established.

Decreased physical activity exhibited by mercury-treated group could be the result of direct treatment-related toxicity. This is in concordance with reports on drug-related toxicity; altered physical activity manifesting as sluggishness\textsuperscript{33}. Body weight changes serve as a sensitive indication of the general health status of animal\textsuperscript{33}, and are used as an indicator of health status of an organism and adverse effect of drugs and chemicals\textsuperscript{34}. Changes in the body weight of Wistar rats treated with mercury were lower than that of the extracts treated groups. This indicates that the mercury treatment leads to weight loss resulting from loss of appetite, improper assimilation of food and the animals feeling sick while the extracts treated group received some level of protection against mercury treatment as a result of its pharmacological abilities. This is consistent with the works of Jadhav \textit{et al.}\textsuperscript{35,1} and who reported that the body weight gain in the mercury exposed animals lagged behind the controls.

The kidney is responsible for fluid and electrolyte balance in the body, alteration of renal proteins (urea and creatinine) and electrolytes (\(\text{Na}^{2+}\), \(\text{Cl}^{-}\), \(\text{K}^{+}\), and \(\text{HCO}_3^{-}\)) concentrations in serum has been linked to renal injury. Acute or chronic toxicity could lead to changes in electrolyte balance within the body\textsuperscript{36,37}\&\textsuperscript{38}. This study demonstrated increased concentration of \(\text{Na}^{2+}\), \(\text{Cl}^{-}\), and \(\text{HCO}_3^{-}\)in \(\text{HgCl}_2\)-treated group when compared to control. This could be attributed to impaired renal tubular function caused by \(\text{HgCl}_2\) related treatment which could damage the podocytes. This result is consistent with that of Amber \textit{et al.}\textsuperscript{39} that reported alterations in the kidney electrolytes when exposed to inorganic mercury in experimental animal model. Administration of AFPD and EFPD lead to a significant decrease in their concentrations when compared to the \(\text{HgCl}_2\) treated group, an indication that the extracts afforded some level of protection to the kidney against \(\text{HgCl}_2\). In most cases, administration of silymarin modulated the concentrations of these electrolytes towards normal. Similar findings were reported by Kenston \textit{et al.}\textsuperscript{40} and Zhang \textit{et al.}\textsuperscript{41} who observed decrease in serum electrolyte concentrations post \(\text{HgCl}_2\) exposure.

Serum creatinine concentration is one of the traditional screening indices for kidney function and renal structural integrity because creatinine concentration is regulated by glomerular filtration\textsuperscript{18}. In the presence of kidney toxicity, cell membranes are damaged and lose their structural integrity leading to the leakage of urea and creatinine into the blood circulation resulting in the elevation of their serum concentration. Elevated levels of urea and creatinine were observed in \(\text{HgCl}_2\)-treated group in this study, owing to renal damage. Zhang \textit{et al.}\textsuperscript{41} and Goudarzi \textit{et al.}\textsuperscript{42} reported increased urea and creatinine levels to be strongly correlated with kidney injury and oxidative stress. Elevated ROS production may distort the filtration surface area and modify the filtration coefficient; this eventually leads to decrease in glomerular filtration hence, the accumulation of creatinine and urea in the blood serum\textsuperscript{39}.

In this study, the extracts of \textit{Phoenix dactylifera} and silymarin treated groups had lower urea levels when compared to \(\text{HgCl}_2\)-treated group and this could be due to their antioxidant abilities to checkmate free
radicals. This result is in agreement with Chen et al.\textsuperscript{43} and Nasution et al.\textsuperscript{44} on the ameliorative effect of plant extract on cellular damage.

Oral administration of HgCl\textsubscript{2} induced nephrotoxicity as observed by the increase in the levels of oxidative stress biomarkers. Malondialdehyde (MDA) levels significantly increased while the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) decreased as a result of HgCl\textsubscript{2} exposure. This is as a result of the accumulation of toxic metabolites in the system brought about by HgCl\textsubscript{2} induced oxidative stress. Pretreatment with AFPD and EFPD followed by HgCl\textsubscript{2} significantly decreased the level of MDA and restored (increased) the activities of SOD, CAT and GPx towards normal. These findings corroborate the nephroprotective effect extracts of \textit{P. dactylifera} against HgCl\textsubscript{2} induced toxicity. This result agrees with previous reports on the nephroprotective effects of \textit{P. dactylifera} by Saa\textit{f}i et al.\textsuperscript{45}; Ali et al.\textsuperscript{46} and Uzunhisarcikli et al.\textsuperscript{47}.

Light microscopic examination of routine (H & E) and histochemical (PAS) stained histological sections of Wistar rats’ kidney tissues were carried out. In this study, the kidney sections of experimental animals in the control group showed normal histoarchitecture of the kidney cortex (renal corpuscles, proximal convoluted tubule, distal convoluted tubules and blood vessels). It has been shown that kidney is the site of degradation and detoxifications of toxic substances. It is an important organ of excretion and osmoregulation and is directly affected by heavy metal through blood circulation.\textsuperscript{48} The histoarchitecture of the kidney parenchyma from mercury treated group showed severe damages when compared with those from the control. These changes included dilation of Bowman's space, distortion of renal corpuscle, pyknotic nuclei, cytoplasmic vacuolation and necrosis. The present study also revealed cytoplasmic vacuolation and pyknosis in the cells of the proximal convoluted tubule as a result of nephrotoxicity by HgCl\textsubscript{2}. These were in concordance with earlier studies of Oda and El-Ashmawy\textsuperscript{49} and Amber et al.\textsuperscript{18}

Mild histoarchitectural distortion, such as dilation of Bowman's space, and cytoplasmic vacuolation, observed in the groups pretreated with silymarin and extracts (AFPD and EFPD) followed by the administration of HgCl\textsubscript{2} demonstrates their abilities to acts as antioxidants, reducing free radical mediated damage in tissues and inhibiting lipid peroxidation. These results are consistent with reports of Qarawi et al.\textsuperscript{14} and Ben Salah et al.\textsuperscript{20}.

HgCl\textsubscript{2}-treated group showed less reaction to PAS stain owing to glycogen depletion in the podocytes and basement membrane when compared to the control. This agrees with Wang et al.\textsuperscript{50}. A relatively normal PAS staining intensity, similar to the control, was observed in silymarin, AFPD and EFPD treated groups and this is in tandem with Adil et al.\textsuperscript{51}, Kosasih et al.\textsuperscript{52} and Amber et al.\textsuperscript{18} who reported that the nephroprotective potential of plants extracts could be attributed to its phytoconstituents such as polyphenolic compounds.

**Conclusion**
The result of the present study suggested that, the aqueous and ethanol fruit extracts of *Phoenix dactylifera* have some levels of efficiency in ameliorating mercury-induced alterations in the kidney of Wistar rats. The nephroprotective property of the extracts relative to the standard (silymarin), is somewhat similar, and maybe attributed to the antioxidant properties of the constituent phytochemicals, such as flavonoids. Also, the EFPD seems to have more protective effects when compared to the AFPD. This may be due to the quantity and quality of phytochemicals found in the EFPD owing to the preparation process. Thus, these extracts are potential candidates for use in the management and treatment of ROS-induced liver and kidney diseases.

**Declarations**

Ethical Approval was obtained from the Ahmadu Bello University Ethics Committee on the use of Animals for Research and the number ABUCAUC/2023/067 was allocated to this research work.

**Competing of interest**

There was no competing of interest on financial and non-financial grounds.

**Conflict of interest**

There was no conflict of interest between the authors.

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**Authors contributions**

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Figures

Figure 1

Effect of fruit extracts of *P. dactylifera* on blood serum Malondialdehyde (MDA) concentration of Wistar rats
n=5, values are mean ± SEM. ** = p < 0.01; significant difference when compared with the control; a= p < 0.05, b= p < 0.01, c= p < 0.001; significant difference when compared with the MCL group. Gp I=Control (distilled H\(_2\)O 0.5 ml/kg); II= HgCl\(_2\) (5 mg/kg); III= Silymarin (100mg/kg); IV and V= Aqueous fruit extract of \(P.dactylifera\) (500 and 1,000 mg/kg respectively); VI and VII= Ethanol fruit extract of \(P.dactylifera\) (500 and 1,000 mg/kg respectively); aqueous fruit extract of \(P.dactylifera\) (1,000 mg/kg); Ethanol fruit extract of \(P.dactylifera\) (1,000 mg/kg)

Figure 2

Effect of fruit extracts of \(P. dactylifera\) on blood serum Superoxide dismutase (SOD) activity of Wistar rats

n=5, values are mean ± SEM. * = p < 0.05; significant difference when compared with the control; a= p < 0.05; significant difference when compared with the MCL group. Gp I=Control (distilled H\(_2\)O 0.5 ml/kg); II= HgCl\(_2\) (5 mg/kg); III= Silymarin (100mg/kg); IV and V= Aqueous fruit extract of \(P.dactylifera\) (500 and 1,000 mg/kg respectively); VI and VII= Ethanol fruit extract of \(P.dactylifera\) (500 and 1,000 mg/kg respectively); aqueous fruit extract of \(P.dactylifera\) (1,000 mg/kg); Ethanol fruit extract of \(P.dactylifera\) (1,000 mg/kg)
Figure 3

Effect of fruit extracts of *P. dactylifera* on blood serum Catalase (CAT) activity of Wistar rats

n=5, values are mean ± SEM. *p*>0.05; when compared with the control. **Gp I**=Control (distilled H$_2$O 0.5 ml/kg); **II**= HgCl$_2$ (5 mg/kg); **III**= Silymarin (100mg/kg); **IV and V**= Aqueous fruit extract of *P.dactylifera* (500 and 1,000 mg/kg respectively); **VI and VII**= Ethanol fruit extract of *P.dactylifera* (500 and 1,000 mg/kg respectively); **aqueous fruit extract of P.dactylifera** (1,000 mg/kg); **Ethanol fruit extract of P.dactylifera** (1,000 mg/kg)
Figure 4

Effect of fruit extracts of *P. dactylifera* on blood serum Glutathione peroxidase (GPx) activity of Wistar rats

n=5, values are mean ± SEM. *p* >0.05; when compared with the control. **Gp I**=Control (distilled H₂O 0.5 ml/kg); **II**= HgCl₂ (5 mg/kg); **III**= Silymarin (100mg/kg); **IV and V**= Aqueous fruit extract of *P.dactylifera* (500 and 1,000 mg/kg respectively); **VI and VII**= Ethanol fruit extract of *P.dactylifera* (500 and 1,000 mg/kg respectively); **aqueous fruit extract of P.dactylifera** (1,000 mg/kg); **Ethanol fruit extract of P.dactylifera** (1,000 mg/kg)
Figure 5

The kidney section of Wistar rat (H & E x400)

Plate A: Photomicrograph of the transverse section of Kidney of Wistar rats administered 2 ml/kg H₂O (control group) showing Glomerulus (G); Bowman's capsule (B); Bowman's space (S) and renal tubular cell (C).

Plate B: Photomicrograph of the transverse section of kidney of Wistar rats administered HgCl₂ demonstrating histoarchitectural distortion. Dilated Bowman's space (DS); Distorted glomerulus (D); Pyknotic nucleus (P); Cytoplasmic vacuolation (V) and Necrosis (N).

Plate C: Photomicrograph of the transverse section of kidney of Wistar rats administered 100 mg silymarin and HgCl₂ demonstrating cytoplasmic vacuolation (V).
Plate D: Photomicrograph of the transverse section of kidney of Wistar rats administered 500 mg AFPD and HgCl$_2$ demonstrating normal histoarchitecture. Renal tubular cell (C); Glomerulus (G); Bowman's space (S).

Plate E: Photomicrograph of the transverse section of kidney of Wistar rats administered 1000 mg/kg AFPD and HgCl$_2$ demonstrating dilated Bowman’s space (DS); Glomerulus (G).

Plate F: Photomicrograph of the transverse section of kidney of Wistar rats administered 500 mg/kg EFPD and HgCl$_2$ demonstrating dilated Bowman’s space (DS); Glomerulus (G).

Plate G: Photomicrograph of the transverse section of kidney of Wistar rats administered 1000 mg/kg EFPD and HgCl$_2$ demonstrating dilated Bowman's space (DS); Glomerulus (G).

Plate H: Photomicrograph of the transverse section of kidney of Wistar rats administered 1000 mg/kg AFPD only demonstrating dilated Bowman's space (DS); Glomerulus (G).

Plate I: Photomicrograph of the transverse section of kidney of Wistar rats administered 1000 mg/kg EFPD only demonstrating dilated Bowman's space (DS); Glomerulus (G).
Figure 6

The kidney section of Wistar rat (PAS x400)

Plate A: Photomicrograph of the transverse section of Kidney of Wistar rats administered 2 ml/kg H₂O (control group) demonstrating PAS positive stain intensity. Glomerulus (G); Bowman's space (S) and renal tubular cell (C). PAS; (Mag x400).

Plate B: Photomicrograph of the transverse section of kidney of Wistar rats administered HgCl₂ demonstrating reduced PAS positive stain intensity (RSI) due to depletion of glycogen. PAS; (Mag x400).

Plate C: Photomicrograph of the transverse section of kidney of Wistar rats administered 100 mg silymarin and HgCl₂ demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G).
PAS; (Mag x400).

Plate D: Photomicrograph of the transverse section of kidney of Wistar rats administered 500 mg AFPD and HgCl$_2$ demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G). PAS; (Mag x400).

Plate E: Photomicrograph of the transverse section of kidney of Wistar rats administered 1000 mg/kg AFPD and HgCl$_2$ demonstrating PAS positive stain intensity. Renal tubular cell (C); Bowman's space (S). PAS; (Mag x400).

Plate F: Photomicrograph of the transverse section of kidney of Wistar rats administered 500 mg/kg EFPD and HgCl$_2$ demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G). PAS stain (Mag x400).

Plate G: Photomicrograph of the transverse section of kidney of Wistar rats administered 1000 mg/kg EFPD only demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G). PAS stain (Mag x400).

Plate H: Photomicrograph of the transverse section of kidney of Wistar rats administered 1000 mg/kg AFPD only demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G). PAS; (Mag x400).

Plate I: Photomicrograph of the transverse section of kidney of Wistar rats administered 1000 mg/kg EFPD only demonstrating PAS positive stain intensity. Renal tubular cell (C); Glomerulus (G). PAS; (Mag x400).