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Effects of date fruit extract on paracetamol induced nephrotoxicity in wistar rats
Tijani Idris Ahmad Oseni, Nurah Tijani Oseni, Hauwa Tijani Oseni and Pauline Etuaje Eromon
Effects of date fruit extract on paracetamol induced nephrotoxicity in wistar rats

Tijani Idris Ahmad Oseni¹*, Nurah Tijani Oseni², Hauwa Tijani Oseni³ and Pauline Etuaje Eromon⁴

¹Department of Family Medicine, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria.
²Innovation and Technology Development Department, Nigerian Institute for oil Palm Research (NIFOR), Benin City, Nigeria.
³Department of Biochemistry, University of Benin, Benin City, Nigeria.
⁴Department of Family Medicine, Irrua Specialist Teaching Hospital, Irrua, Nigeria.

Received 3 February, 2017; Accepted 4 April, 2017

This study was aimed to investigate the protective, ameliorative and preventive effects of aqueous Date fruit extract on paracetamol induced nephrotoxicity in wistar rats. A total of 30 male albino wistar rats were used for this study. Rats were randomly divided into five groups containing 6 animals each. Treatments were given daily for fourteen days. The animals in the control group (Group I) did not receive any treatment, while those in group 2 received paracetamol (2 g/kg/day). Group 3 were pretreated with aqueous date fruit extract (400 mg/kg/day) for a week before paracetamol administration. Rats in group 4 received paracetamol (2 g/kg/day) for a week before treatment with aqueous date palm extract (400 mg/kg). Group 5 was administered paracetamol (2 g/kg/day) in concurrent with 400 mg/kg aqueous date fruit extract. Exposure of rats with a nephrotoxic dose of paracetamol disturbed the kidney function tests; blood urea nitrogen (BUN) and serum creatinine (SC) levels increased significantly (P>0.05). The protective use of aqueous date fruit extract on paracetamol-induced nephrotoxicity resulted in a significant improvement in most evaluated parameters.

Key words: Date fruit, paracetamol, wistar rats, kidney.

INTRODUCTION

Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine (Abdelrahman et al., 2012; Iwu et al., 2014). Nephrotoxicity is one of the most common kidney...
problems and occurs when the body is exposed to a drug or toxin that causes damage to the kidneys. Some nephrotoxic compounds are arsenic, cadmium, copper, naturally-occurring plant toxins such as pyrrolizidine alkaloids, mycotoxins and bacterial toxins. Exposure to nephrotoxic compounds may be occupational, environmental or domestic via accidental, homicidal or suicidal ingestion (Lil et al., 2014).

Nephro-protective agents are those compounds which alleviate the kidney injury caused by nephrotoxic compounds. Nephro-protective effects of plant drugs and herbal formulations have been studied against chemicals (alcohol, CCl₄, alcohol, beta galactosamine, thioacetamide) and drugs (paracetamol, nimusalide, antitubercular drugs like isoniazid etc.) induced nephrotoxicity in rats and mice, as they virtually mimic any form of naturally-occurring liver disease (Abdelrahman et al., 2012).

Date fruit (Phoenix dactylifera L) is a good source of rapid energy, due to their high carbohydrate content (70 to 80%) (El-Far et al., 2016). The good nutritional value of dates is also based on the presence of vitamin C (Allaith, 2008). Date fruit provides essential minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, selenium and manganese (Assirey, 2015). The date fruit is listed in folk remedies for the treatment of various infectious diseases and cancer (El-Far et al., 2016).

Accordingly, we hypothesized that date fruit extract may prevent, protect and ameliorate the nephrotoxicity in rats induced by acetaminophen (paracetamol). This study is aimed to investigate the preventive, protective and ameliorating effects of Date fruit extract on paracetamol induced toxicity on kidney function.

**MATERIALS AND METHODS**

The date palm fruit (P. dactylifera) was obtained from Nigerian Institute for oil palm research (NIFOR). Date was sun dried, seeds removed and grounded to fine powder. Fifty grams of the pulverized fruit was soaked in 1 liter of distilled water for 72 h with constant stirring, to avoid fermentation. A 15 g solution of the extract was prepared and stored in a refrigerator. This solution was used as stock crude drug.

**Proximate analysis of date fruit**

Moisture content, total ash and fat content were carried out on the pulverized sample according to AOCS Ca 2c-25, AOCS Ca 11-55, AOCS Am 5-04 (AOCS, 2009). Crude fibre and crude protein were assayed using (Neubert et al., 1940) and (Thiex, 2002), respectively.

**Experimental protocol**

A total of 30 male albino wister rats were used for this study. They were obtained from the anatomy departement of University of Benin, Benin City. The animals were housed in a cage controlled environment at room temperature and 12-h light-dark cycle. Animals were fed meal pellet and fresh water ad libitum for 2 weeks prior to experiments. Rats were randomly divided into five groups containing 6 animals each and all treatments were given daily for 14 days.

Rats in Group 1 served as the control group and were administered fresh water only. Groups 2 received 2 g/kg/day paracetamol orally. Group 3 were administered date extracts (400 mg per kg of body weight) orally for 7 days prior to induction with paracetamol (2 g/kg/day) orally; this served as the preventive group. Rats in group 4 were given extract at 400 mg per kg body weight orally after being induced with oral paracetamol for 7 days, this served as the ameliorating group. Group 5 rats were administered extract orally, delivered concurrently with oral paracetamol administration (protective group).

After two weeks on induction with date extract, animals were anaesthetized and blood samples were withdrawn from retro-orbital sinus by heparinized capillary tubes under light chloroform anaesthesia after 12 to 14 h fasting period. The withdrawn blood was collected in centrifuge tubes for serum separation, allowed to clot for an hour at room temperature and centrifugation done at 3000 rpm for 15 min. Serum were separated and stored at –20°C until used for an estimation of serum urea (BUN), serum creatinine (SC) and Electrolyte (Na, K, Cl and HCO₃⁻).

**Biochemical analysis**

Urea in serum was hydrolyzed to ammonia in the presence of urease. The ammonia was then measured photometrically (550 mm) using Bertholot’s reaction. Levels of serum creatinine were estimated using the Jaffe method as modified by slot (Slot, 1965). Working reagent was mixture of picric acid (55 mM), sodium hydroxide (0.4 M) and sodium carbonate (50 mmol). Working reagent (2 ml) was mixed with 0.1 ml of sample and allowed to stand for 15 min at 20 to 25°C. The absorbance was read at 510 mm.

Chloride ion estimation was carried out using mercurimetric titration method (Schales and Schales, 1941). Bicarbonate ion estimation was done using the Van Slyke titration method. In van Slyke form of titration method, an excess HCl (0.1N) was added to serum. The mixture was then titrated with 0.01N sodium hydroxide solution (Van Slyke, 1919). Sodium and Potassium were assayed using flame photometric method according to (Mosher and Boyle, 1949).

**Statistical analysis**

The statistic used in this research is SPSS version 18.0. Data were analyzed using Shapiro-Wilk normality test and one way analysis of variance (ANOVA) was used for comparison between groups. Data were expressed as mean ± standard deviation (SD) and P<0.05 showed statistically significance.

**RESULTS**

Table 1 depict that, there were significant increase (p<0.05) in the level of urea, creatinine, sodium and phosphate of the control (group 1) compared with
Table 1. Effect of Paracetamol-induced kidney function toxicity on albino Wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Sodium (mmol/l)</th>
<th>Potassium (mmol/l)</th>
<th>Chloride (mmol/l)</th>
<th>Bicarbonate (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.07±0.16</td>
<td>32.5±2.51</td>
<td>136.5±6.12</td>
<td>5.60±0.60</td>
<td>101.33±2.42</td>
<td>19.17±0.98</td>
</tr>
<tr>
<td>2</td>
<td>1.35±0.24</td>
<td>37.1±5.38</td>
<td>141.67±2.66</td>
<td>7.88±0.44</td>
<td>102.67±1.63</td>
<td>19.17±0.98</td>
</tr>
<tr>
<td>3</td>
<td>1.13±0.10</td>
<td>36.83±5.32</td>
<td>140.83±1.83</td>
<td>6.53±0.41</td>
<td>103.66±2.42</td>
<td>19.83±1.60</td>
</tr>
<tr>
<td>4</td>
<td>0.88±0.13</td>
<td>31.4±2.19</td>
<td>141.2±1.64</td>
<td>6.44±0.97</td>
<td>102.0±2.53</td>
<td>19.2±1.64</td>
</tr>
<tr>
<td>5</td>
<td>1.05±0.12</td>
<td>34.67±5.05</td>
<td>135.67±4.18</td>
<td>5.40±0.88</td>
<td>102.0±2.53</td>
<td>20.33±0.52</td>
</tr>
</tbody>
</table>

P value | < 0.05 | < 0.05 | < 0.05 | < 0.05 | > 0.05 | > 0.05 |

*Values are expressed as mean ± SD using one way ANOVA. P value < 0.05 denotes significant differences between means.

Paracetamol altered a number of biomarkers (blood urea nitrogen and serum creatinine levels). These changes occurred as a result of the inactivation of the mitochondrial pathway during acetaminophen-induced cell death.

In the present study, administration of nephrotoxic dose of paracetamol to rats resulted in a significant elevation of serum levels of urea and creatinine in paracetamol toxic group (group 2) when compared to the normal control group. These results are in agreement with that observed by Isik et al. (2006), who noticed an elevation in serum urea and creatinine in rats after 1 g/kg b.w. of paracetamol administration. Karadeniz et al. (2008) and Ajami et al. (2010) explained elevation in the levels of urea and creatinine, by the presence of strong correlation between nephrotoxicity and oxidative stress. The elevated H₂O₂ and O₂⁻ production alters the filtration surface area and modifies the filtration coefficient; both factors could decrease the glomerular filtration leading to accumulation of urea and creatinine in the blood.

There was significant increase in potassium and sodium concentration in paracetamol control group (group 1) when compared with normal group (group 2). This result is in agreement with the work of Yakubu et al. (2006), who reported increased levels of potassium (K⁺) and sodium (Na⁺) in rat treated with artemesine. It is suggested that, increased serum Na⁺ concentration is an indication of alteration in important biochemical parameters, such as an increase production of aldosterone and other mineral corticoids, which will in turn increase the tubular reabsorption of Na⁺ or decrease production of either antidiuretic hormone or tubular sensitivity to the hormone (Anyasor et al., 2011).

Potassium ion plays an important role during transmission of nerve impulses along the nerve cells to receptor cells. The hyperkalaemia observed in toxic treated animals suggests a possible adverse effect on the pump, which maintains the homeostasis of K⁺ in extracellular concentration. Bicarbonate and chloride ion showed no significant changed in all the parameters estimated. This finding is not in line with what was observed by Yakubu et al. (2006).

DISCUSSION

Paracetamol toxic overdose is often linked to many metabolic disorders including serum electrolyte, urea and creatinine derangements (Palani et al., 2009). Ghosh et al. (2010) observed that, exposure of rats with a nephrotoxic dose of paracetamol control (group 2).

However, there were no significant changes (p>0.05) in the levels of bicarbonate and chloride of control and Paracetamol control group. There was no significant difference (p>0.05) in all the kidney function parameters between Paracetamol control (Group 2) and Preventive (Group 3). There was significant decrease (P<0.05) in creatinine and urea of the Paracetamol control (Group 2) and the ameliorative (Group 4).

However there are no significant changes in other parameter. There were significant decrease (p<0.05) in the level of serum Urea, creatinine, Sodium and Potassium of the paracetamol control (Group 2) and the Protective (Group 5).
This may be due to difference in duration of experiment and drug dosage used.

In this study, the preventive treatment of rats with aqueous *p. dactylifera* showed a significant decrease on the paracetamol induced kidney toxicity. The protective group (group 4) rats treated with aqueous *p. dactylifera* showed significant (P<0.05) reduction values of serum creatinine, Urea, Sodium and Potassium as compared to paracetamol toxic (group 2). Previous studies reported the significant decrease in serum creatinine, urea and electrolytes on Paracetamol induced nephrotoxic rats when treated with garlic oil (Gulnaz et al., 2010). This finding supports the findings of present study.

Also, ameliorative treatment of rats with aqueous extract of *p. dactyleria* showed a significant reduction in serum urea and creatinine after honey treatment as it corrects the influence of hemorrhage and food restriction on renal functions.

**Conclusion**

Rats with Paracetamol induced nephrotoxicity, when treated with aqueous date palm extract (protective, ameliorative, preventive) had better renal function parameters compared to the control. It can therefore be concluded from the study that, date fruit has protective effect on kidney function.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**RECOMMENDATION**

Further study should be carried out to investigate the mechanism in which *p. dactylifera* improved kidney function. The active ingredient in it that has the nephron-protective action should be determined. This will help in developing pharmacological agents that will be active against renal diseases. Consumption of dates should be encouraged for better health.

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