Biosafety of Ajwa Date against Biotoxicity of Ochratoxin (A) on Proximal Renal Tubules of Male Rat

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ABSTRACT

The purpose of this research was to test the biotoxicity of Ochratoxin in weaning male albino rat’s kidney and to investigate the biosafety of Ajwa dates for prevention and protection against pathological Ochratoxin alterations. Animals were gavage administrated and divided into four groups: first group received (sodium bicarbonate), second group received (289 μg OTA kg⁻¹ B.W day⁻¹), third group received (1 mg Ajwa kg⁻¹ B.W day⁻¹) and fourth group received (289 μg OTA kg⁻¹ B.W day⁻¹+1 mg Ajwa kg⁻¹ B.W day⁻¹). Serum (creatinine-urea) levels were measured in each group at the time of tissue collection, some biopsies were fixed in 10% buffered formalin solution for light microscopy processing stained with Haematoxylin and Eosin (H and E.), Periodic Acid-Schiff (PAS) and Masson’s Trichrome (M.T.). Other biopsies were immediately collected into electron microscopy processing. After 28 days, a significant decrease in body weight, kidney weight and relative weight was detected in Ochratoxin-treated group. Also, Serum (creatinine-urea) level were elevated. The normal cyto-architecture of proximal tubules were lost exhibiting damaged brush border, degenerated, binucleated and karyomegalic cells. The most destructed ultra-structure was the mitochondria which severely swollen with disintegrated membranes. In Ajwa Date extract-group the proximal tubules were normal, whereas in Ajwa date extract+Ochratoxin-group the severity of the lesions was significantly reduced. The present results indicate that Ajwa date have protective effects and ameliorated the lesions of Ochratoxin nephrotoxicity which might led to kidney failure.

Key words: Ochratoxin, ajwa date, proximal tubules, light-structure, ultra-structure, biochemical analysis, morphometry

INTRODUCTION

Mycotoxin (s) contamination of various foods was a problem not only in terms of human and animal health but could have serious economic impact causing losses of millions of dollars in terms of production and reproduction. It reduced the nutritional value of food and feeds and was responsible for causing deleterious effects in form of toxicosis in animal and human populations (Kumar et al., 2007).

Ochratoxin A (OTA) was a secondary metabolite, produced by Aspergillus ochraceus (A. alutaceus) (Bayman et al., 2002). After consumption of contaminated food, OTA had been identified in blood, bile and urine of humans and animals (Okutan et al., 2004). Numerous animal
studies, showed development of renal disease due to OTA oxidative stress (Malekinejad et al., 2010) accompanied by proximal tubular atrophy and cortical interstitial fibrosis after exposition to OTA (Aukema et al., 1999), as kidney was the primary target organ for OTA (Bayman and Baker, 2006).

Nutritional science had been expanding the knowledge of how foods influence consumers in relation to specific health parameters, where high fruit and vegetable consumption was associated with a reduced risk of several chronic diseases such as cancer, cardiovascular disease, neurodegenerative disease and inflammation (Shahidi and Naczk, 2004). The compounds though to be responsible for the protective effects of a fruit- and vegetable-rich diet include carotenoids and antioxidant vitamins as in Syzygium aromaticum L. (SA) (Aisha et al., 2011) and dates extracts (Phoenix dactylifera L.) (Hasan et al., 2010), in this regard, attention has been focused on the significance of phenolics such as phenolic acids, flavonoids, and in particular anthocyanins (Shahidi and Naczk, 2004). All Date varieties served as a good source of natural antioxidants and could potentially be considered as a functional food or functional food ingredient (Al-Farsi et al., 2005), where Date fruit extract had strong antioxidant and antimutagenic properties (Vayali, 2002). Selenium, believed to help in preventing cancer and important in immune function, was also found in Dates. Also, Dates contained 23 types of amino acids and at least six vitamins including a small amount of Vitamin C and Vitamins B (1) thiamine, B (2) riboflavin, niacin acid (niacin) and Vitamin A (Al-Shahib and Marshall, 2003).

Since OTA was nephrotoxic, simultaneous occurrence as food contaminants, might lead to more severe renal damage and there was scarce information on sub-cellular changes and there was no study converting the direct nutritional and functional properties of Dates. So, the present investigation was undertaken to assess the proximal tubules alterations in male rats fed OTA and to investigate detailed information about Ajwa Dates which could lead to a better understanding of their use as functional foods and ingredients in nutraceuticals, pharmaceuticals and medicine against pathological effects caused by OTA.

MATERIALS AND METHODS

Materials: Ochratoxin A: Ochratoxin A (Cat. No. 01877) were purchased from Sigma-Aldrich chemical company (USA). It was dissolved in 0.5 M HNaCO$_3$ PH 7.4.

Preparation of date fruit extract: Fruit flesh was extracted according to Saafi et al. (2010).

Experimental animals: The study was conducted at King Abdulaziz University Jeddah, Saudi Arabia from 2008-2010. Twenty eight weaning male albino rats Wistar (Ratus narvegicus) weighing 40±5 g obtained from the Experimental Animal House Center, King Abdulaziz University Jeddah, Saudi Arabia. All animals were given food (rat chow) and water Ad libitum and were maintained at a relative humidity of 65 to 85%, a temperature of 18-20°C. The use of animals was approved by the ethical requirements approved by the Animals Research Ethic Committee of KAU.

Methods: The animals were distributed equally into four groups and were treated for four weeks (5 days/week) by gavage: First group: control animals received sodium bicarbonate buffer: 0.1 M, pH 7.4. Second group: animal’s received (280 µg OTA kg$^{-1}$ B.W day$^{-1}$). Third group: animal’s

received (1 mg Ajwa dates \textsuperscript{-1} kg B.W. day\textsuperscript{-1}). Fourth group: animals received (1 mg Ajwa dates +289 \textmu g OTA kg\textsuperscript{-1} B.W day\textsuperscript{-1}).

Serum (creatinine-urea) levels were measured in each group at the time of tissue collection. Plasma samples were separated by centrifugation and were measured by radio immunoassay (RIA) according to the methods of Chang et al. (1995).

The animals were weighted then autopsied and the left kidneys were removed weighed and relative weights were calculated. Some biopsies were fixed in 10% buffered formalin solution for light microscopy processing stained with Haematoxylin and Eosin (H and E), Periodic Acid-Schiff (PAS) and Masson’s Trichrome (MT) stains (Bancroft and Gamble, 2002). Five micrometer paraffin sections were stained with H and E for morphometry. Twenty tubules from each group were chosen randomly (at 40x) in the cortex of section were evaluated. The area of proximal tubules, proximal lumen and proximal nuclei were measured under a light microscope (Olympus C×31) using a micrometric ocular. Other biopsies were immediately collected into electron microscopy processing.

Statistical analysis: Body and kidney weight, tubular and nuclei area, creatinine and urea levels were analyzed by using the program SPSS version 15 where one way ANOVA (Mould, 1989) was used to assess the significance of changes between control and treated mice. The data were expressed as Mean±SE.

RESULTS
Regarding general toxicity parameters, in this study, insignificant reduction in both body and kidney weight in the low dose OTA-group were recorded and this led to low relative weight i.e., the OTA-group recorded the minimum value for body weight (163.28±2.71 g), kidney weight (0.03±0.02 g) and relative weight (0.23±0.01) where the control group recorded the maximum value for body weight (173.45±1.10 g), kidney weight (0.51±0.03 g) and relative weight (0.30±0.01). On the other hand, body and kidney weight had a significant decrease in Ajwa date+OTA-group and a significant increase in Ajwa date-group in compare with control-group (Table 1).

With respect to biochemical parameters, creatinine reached the maximum value (35.43±0.43 mmol L\textsuperscript{-1}) in OTA-group and the minimum value in Ajwa date-group (29.71±1.46 mmol L\textsuperscript{-1}) i.e., non significant decrease in creatinine level. In the Ajwa date-group non significant increase in urea level were recorded in compare with control-group i.e., maximum value obtained by Ajwa date-group (6.07±0.04 μmol L\textsuperscript{-1}) and minimum value obtained by Ajwa date+OTA-group (5.57±0.33 μmol L\textsuperscript{-1}). Also, in this study the positive effect of Date appeared

<table>
<thead>
<tr>
<th>Variables</th>
<th>Experimental groups</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Body weight at zero day (g)</td>
<td>35.98±3.57</td>
</tr>
<tr>
<td>Body weight at fourth week (g)</td>
<td>173.45±1.10</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>0.51±0.03</td>
</tr>
<tr>
<td>Relative weight (%)</td>
<td>0.30±0.01</td>
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</tbody>
</table>

Values are as Means±SE. *Significantly different from the control, p<0.05
Table 2: Statistical analysis of serum creatinine and urea concentration in experimental groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>OTA</th>
<th>Ajwa date</th>
<th>Ajwa date + OTA</th>
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</thead>
<tbody>
<tr>
<td>Creatinine (μmol L⁻¹)</td>
<td>0.43±0.02</td>
<td>35.43±0.43</td>
<td>20.71±1.46</td>
<td>33.57±1.84</td>
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<tr>
<td>Urea (mmol L⁻¹)</td>
<td>5.74±0.16</td>
<td>5.83±0.25</td>
<td>6.07±0.04</td>
<td>5.77±0.33</td>
</tr>
</tbody>
</table>

Values are as Means±SE. *Significantly different from the control, p<0.05

Table 3: Histological measurements of proximal tubules

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>OTA</th>
<th>Ajwa date</th>
<th>Ajwa date + OTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of proximal tubules (μm²)</td>
<td>1090.96±56.31</td>
<td>1188.44±28.84</td>
<td>1098.33±48.92</td>
<td>1045.09±34.21</td>
</tr>
<tr>
<td>Area of proximal tubules lumen (μm³)</td>
<td>104.78±5.75</td>
<td>139.88±10.00*</td>
<td>107.91±9.18</td>
<td>99.08±6.07</td>
</tr>
<tr>
<td>Nuclei area of proximal tubule (μm²)</td>
<td>25.94±0.51</td>
<td>42.44±3.48*</td>
<td>29.74±0.61</td>
<td>34.57±3.08*</td>
</tr>
</tbody>
</table>

Values are as Means±SE. *Significantly different from the control, p<0.05

where low level of both creatinine and urea were recorded in Ajwa date+OTA-group in compare with OTA-group (Table 2).

The proximal tubule appeared to be major site of renal injury and this was illustrated in Table 3 where non significant increase in the area of both proximal tubules area (1188.44±28.84 μm²), proximal tubules lumen (139.88±10.00 μm³) and proximal tubules nuclei (42.44±3.48 μm³), were detected in OTA-group which recorded the maximum values. In the other hand, non significant decrease was noticed in the area of both proximal tubules area (1045.09±34.21 μm²), proximal tubules lumen (99.08±6.07 μm³) in OTA+date-group which detected the minimum value while the control group showed the minimum value for proximal tubules nuclei (25.94±0.5 μm²). Serious damage to kidney occurred where the nephrotoxic actions of OTA which led to necrosis of renal tubular cells. The present results showed cloudy swelling of proximal tubules in addition to tubular eosinophilic intraluminal proteinaceous casts (Fig. 1a), atrophied dense tubules separated from basement membrane and congestion in interstitial tissue (Fig. 1b). Also, focal loss of brush border and karyolysis (Fig. 1c). In addition to these findings, karyomegalic nuclei in tubular epithelial cells were observed (Fig. 1d) and as a result of megalocytosis the tubules appeared with no lumen (Fig. 1e). Also, marked interstitial fibrosis was detected (Fig. 1f).

When the kidney specimens obtained from the Date-group and examined with light microscope, normal histological structure of the proximal tubules were observed. As shown in Fig. 2a, tubule with normal brush border and central round nuclei. The brush border was contact and the lumen took the stare-shape (Fig. 2b) and no congestion noticed in interstitial tissue (Fig. 2c).

In the present study, a significant decrease in the severity of histopathological and morphometric changes induced by OTA was observed in animals treated with OTA+Date when compared with the OTA-group. From Fig. 3 it was clear that in some area few proximal tubule still affected which have lytic nucleus but its brush border appeared normal (Fig. 3a) but most of them nearly normal with eccentric nuclei (Fig. 3b), normal abundant brush border, distinct nuclei and lysosome-like vesicles (Fig. 3c).
Fig. 1: Light photograph for section of OTA-group proximal tubules showing: (a) Eosinophilic casts within tubules lumen (arrows). (b) Atrophied dense tubules (PT) separated from basement membrane and congestion in interstitial tissue (⁎) (H and E x100). (c) Tubule with brush border loss (head arrows) and karyolysis (arrows). (d)Megacytosis of tubular cells (arrow) (P.A.S. x1000). (e) Tubule with megacytosis (arrow), no lumen (T.B. x1000). (f) Fibrosis in interstitial tissue (M.T. x100)

Fig. 2: Light photograph for section of Date-group proximal tubules showing: (a) Tubule with normal Brush Border (BB) and central nuclei (N). (b) Tubule have cells with contact Brush Border (BB), round nucleus (N) and stare lumen(P.A.S. x1000). (c) Contact Brush Border (BB) and blood vessel (arrow) in interstitial tissue (T.B. x1000)

The electron microscopic observations of the OTA-group (Fig. 4) showed tubular changes such as shortening and loss of basal infolding, rounded mitochondria with disordered cristae, focal loss of brush border and invagination of the nuclear envelope, the increased number of lysosomes, deformed Golgi zone and nucleus heterochromatin condensation in dense cytoplasm (Fig. 4a), the mitochondrial swelling with lysis of cristae and presence of secondary lysosome (Fig. 4b), apoptotic cell nucleus have peripheral heterochromatin condensation and invagination of nuclear envelope (Fig. 4c). Also and double short basal infolding membrane with bizarre mitochondria were noticed (Fig. 4d).

Ultra-examination of Date-group revealed normal cyto-structure of the proximal tubules where they appeared with contact brush border, small lysosome, active nucleus, large elongated organized mitochondria inbetween extended basal infolding (Fig. 5a) and well developed pinocytic vesicles under the arranged microvilli (Fig. 5b). Also, the nucleus with peripheral heterochromatin
Fig. 3: Light photograph for section of Date+OTA-group proximal tubules showing: (a) Still affected tubule with normal Brush Border (BB) and lytic nucleus (arrow). (b) Nearly normal tubules with cells have eccentric nuclei (N) (PAS. x1000). (c) Tubule with normal Brush Border (BB), distinct nuclei and lysosome-like vesicles (arrow) (T.B. x1000)

Fig. 4: Transmission electron micrograph of ultrathin OTA sections showing: (a) proximal convoluted tubule cell with focal loss Brush Border (BB), numerous apical vesicles (V), numerous lysosome (L) deformed Golgi zone (G), nucleus (N) with marginal heterochromatin in dense cytoplasm. (b) details swollen round mitochondria (M) with indistinct cristae, vesicles (V) and secondary lysosome (L). (c) detail apoptic cell with nucleus have invaginated nuclear envelope and heterochromatin condensation. (d) detail double short basal infolding membrane (arrows) with bizarre mitochondria (M)
Fig. 5: Transmission electron micrograph of ultrathin of Ajwa date-sections showing: (a) part of tubule cell reveals normal Brush Border (BB), small lysosome (L), nucleus (N) and large elongated mitochondria (M) inbetween extended basal infolding (arrows). (b) details intact Brush Border (BB) and well developed pinocytic vesicles (V). (c) details part of nucleus (N) with peripheral heterochromatin, lysosome (L), homogenous mitochondria (M) with normal cristae, double membrane basal infolding (arrow) and free ribosome (R)

surrounded with homogenated cytoplasm contained free ribosome, lysosome, homogenous mitochondria with normal cristae and at the base double membrane basal infolding were seen (Fig. 5c).

The consumption of Date fruit extract restored the tubule damage induced by OTA, as revealed by improvement of ultrastructure changes (Fig. 6) where tubular cell with organized brush border, euchromatic nucleus with nucleolus surrounded by intact nuclear envelope and mitochondria with distinct membrane (Fig. 6a) were observed. The unpacked microvilli and free ribosome illustrated at Fig. 6b, the nucleus surrounded by intact nuclear envelope and mitochondria with distinct membrane were illustrated in Fig. 6c.
DISCUSSION

The study of toxic effects of OTA under low dose exposure regimen was extremely important in order to have more data for human risk assessment (Alvarez et al., 2004). Regarding general toxicity parameters, we observed insignificant reduction in both expected body and kidney weight increase in the low dose OTA-group as recorded before (Alvarez et al., 2004) and this led to low relative weight as agree with Maaroufi et al. (1999) and with Olasore and Samuel (2010) in case of chronic protein-energy malnutrition. In the other hand, body and kidney weight had a significant decrease in Ajwa date+OTA-group and a significant increase in Ajwa date-group in compare with control-group, this might be due to high carbohydrate amount of Date (Al-Shahib and Marshall, 2003).
With respect to biochemical parameters, blood urea and creatinine content were increased in OTA-group but these differences were not statistically significant as recorded before (Stoev et al., 2001; Alvarez et al., 2004). The increase in these substances pointed to pathological alterations in the kidney (Turner et al., 2002), especially degeneration of proximal tubules (Stoev et al., 2001). In the Ajwa date-group non significant decrease in creatinine level with non significant increase in urea level were recorded in compare with control-group and this increase in urea level might be due to high protein content in date which led to increase urea level in blood (Al-Wahyp, 2000).

In animal models, the S3 segment of proximal tubule appears to be major site of renal injury (Sueishi et al., 2002) and this was illustrated in this study where non significant increase in the area of both proximal tubules, proximal tubules lumen and proximal tubules nuclei were detected in OTA-group. In the other hand, non significant decrease was noticed in the area of both proximal tubules, proximal tubules lumen in OTA+Date-group, as agreed with Tarladacalisir et al. (2008) study.

OTA was a widespread mycotoxin which when intaken via diet passed into the blood and accumulated in organs, such as kidneys; potentially imposing serious damage to these organs (Petzinger and Ziegler, 2000) and this might explain the nephrotoxic actions of OTA which led to necrosis of renal tubular cells.

The present results were compatible with Munro et al. (1973), as OTA exhibited cloudy swelling of proximal tubules in addition to tubular eosinophilic intraluminal proteinaceous casts and congestion as indicated before (El-Arab et al., 2006). In addition to these findings karyomegalic nuclei were observed in tubular epithelial cells where karyomegalic of tubular epithelial cells as an early stage marker of the nephrotoxicity by OTA (Arbilla et al., 2008; Adler et al., 2009). Also, marked interstitial fibrosis was detected as indicated by Milicevic et al. (2008).

When the kidney specimens from the Date- group examined with light microscope, normal histological structure of the proximal tubules was indicated in agree with (Junqueira and Carneiro, 2003).

In the present study, a significant decrease in the severity of histopathological and morphometric changes induced by OTA was observed in animals treated with OTA+Date when compared with the OTA-group where Date served as good source of natural antioxidants and could potentially be considered as a functional food ingredient (Al-Parsi et al., 2005) and previously Vayalil (2002) showed that date extracts possess significant antioxidant action in vitro.

The electron microscopic observations of the OTA-group were in agreement with those in the literatures: tubular changes such as shortening and loss of basal infolding, rounded mitochondria with disordered cristae, focal loss of brush border and invagination of the nuclear envelope (Tarladacalisir et al., 2008). The increased number of lysosomes, a result of the attempt to digest toxic substances, was considered a general manifestation of injury. The sequestration of damaged organelles in lysosomes was a mechanism of cellular repair and follows all types of sublethal injury (Cheville, 1994). The mitochondrial swelling and lysis of cristae might reflect the disturbances in oxy-reduction processes taking place in the organelle (Thevenod, 2003) as, the mitochondria were the source of Reactive Oxygen Species (ROS) and a target of excessive ROS generation (Pulido and Parrish, 2003). Excess ROS increased the mitochondrial membrane permeability and damaged the respiratory chain resulting in increased ROS production (Chen et al., 2001). The disruption in the mitochondrial membranes caused the release of cytochrome c from mitochondria which initiated events leading to apoptosis as, ROS were thought to play a role in TNFR and Fas
receptor-mediated apoptosis (Krammer, 1999). Also, changes in the nucleus, such as heterochromatin condensation and margination which was found in the present study, indicate that this organelle was affected in a major way from OTA exposure suggested progressive inactivation of the nuclear component, probably due to inhibition of DNA repair and DNA methylation (Waisberg et al., 2003). The reaction of OTA with proximal tubule cell basolateral membrane might led to loss of basolateral invagination like isplatin C action (Tarladadalis et al., 2008).

Ultra-examination of Date-group revealed normal cyto-structure of the proximal tubules as indicated by Junqueira and Carneiro (2003). In the other hand, the consumption of Date fruit extract restored the tubule damage induced by OTA, as revealed by improvement of ultrastructure changes where in vivo date palm fruit may be useful for the prevention of oxidative stress (Saafi et al., 2010) as the date flesh demonstrated a strong free radical scavenging activity (Chaara et al., 2007). So, The antioxidative properties of Date in vitro (Rock et al., 2009) might exerted a protective effect on OTA-induced damages.

CONCLUSION

Chronic administration of low dose of OTA caused morphological and functional changes in proximal tubules and administration of date-extract would be effective in protecting against OTA-induced tubule’s tissue damage. It was possible that the toxic effect of OTA was some how minimized by a compensatory mechanism involving Date via the induction of antioxidant enzyme activity following administration of Date.

RECOMMENDATIONS

These results highlight the need to reduce OTA exposure where it’s accumulative effect cause extensive damage to the proximal tubules. Additionally, pay particular attention to our diet to be rich in antioxidants such as date where the chronic administration of Date may be of therapeutic benefit on OTA nephrotoxicity.

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REFERENCES


