Evaluation of the Expression of RCC and KIM-1 Biomarkers in Nephrotoxicity of Rabbits Treated with Ochratoxins A

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ABSTRACT

Mycotoxin known as ochratoxin A (OTA) is generated by a number of fungi, including Aspergillus ochraceus, Aspergillus carbonarius, Aspergillus niger, and Penicillium verrucosum. OTA causes kidney tumors and nephrotoxicity in humans and a number of other animal species. This study was conducted to determine the effect of OTA in the kidney of New Zealand White rabbits and the role of RCC and KIM-1 biomarkers in kidney toxicity induced by OTA. Histopathological study showed various pathological effects of rabbit’s kidneys treated with 0.5 mg/kg (body weight intraperitoneally) with OTA in comparison with control animals. RCC and KIM-1 were used as biomarkers for the immunohistochemical study, results showed that there were no expressions in control animals (score =0), while the expressions of RCC and KIM-1 in animals kidney treated with 0.5 mg/kg (body weight intraperitoneally) of OTA were strong (score =3) and moderate (score =2) respectively.

Keywords: Ochratoxin A (OTA), Nephrotoxicity, RCC, KIM-1, Biomarkers

INTRODUCTION

A wide range of filamentous fungi, including species from the genera Aspergillus, Fusarium, Penicillium, and Alternaria that survive in varied climatic conditions on agricultural goods, produce diverse no secondary metabolites, and some of them are well-known mycotoxins [1]. The Food and Agricultural Organization (FAO) of the United Nations has calculated that secondary metabolites of various toxigenic molds are considerably contaminating up to a quarter (25%) of the world's food crops. (FAO 2004 references). OTA is a mycotoxin that is made by a number of fungi, especially Genus Aspergillus which includes; Aspergillus ochraceus, Aspergillus carbonarius, Aspergillus niger, and other fungus includes Penicillium verrucosum[2]. Ochratoxin can be divided into three groups based on its chemical and structural characteristics, Ochratoxin A being the most dangerous and frequently seen metabolite [3]. Numerous studies have demonstrated that OTA ingestion can result in a number of health issues for both human and animal health for example, OTA has been demonstrated to be nephrotoxic, teratogenic, immunotoxic, and carcinogenic to human health when present in foods of plant and animal origin [2]. Exposure of ochratoxins to humans over an extended length of time by any route like contaminated diet, water etc... can cause a number of health issues, including kidney and liver cancer as well as a weakened immune [4]. The International Agency for Research on
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Cancer (IARC) has listed it as a potential human carcinogen [5]. A naturally occurring nephrotoxicant found in mycotoxins called OTA may result in renal failure [6]. The kidneys may become cancerous as a result of long exposure to OTA [7]. A type of kidney cancer called renal cell carcinoma (RCC) develops in the lining of the proximal convoluted tubule, which is a portion of the kidney's incredibly tiny tubes that carry primary urine. RCC accounts for 90–95% of instances of kidney cancer in adults and is the most prevalent kind [8]. According to RCC incidence data, men outnumber women by a ratio of 1.5:1. RCC often develops between the sixth and seventh decade of life [9]. Among the primary organs affected by this xenobiotic-induced toxicity is the kidney. However, it can be challenging to identify renal impairment at an early stage. Transcriptional profiling has recently revealed numerous novel nephrotoxic indicators, including kidney injury molecule-1 (KIM-1) [10]. In healthy kidneys, the mRNA and protein for KIM-1, a type 1 membrane protein containing extracellular immunoglobulin and mucin domains, are generated at incredibly low levels, but after damage, proximal tubule epithelial cells dramatically increase their expression was commonly seen. [11]. KIM-1 is a proximal tubular injury biomarker with varying performance characteristics based on clinical and population circumstances for the early diagnosis of acute kidney injury. [12].

In this study, we tried to find out the impact of this toxin by animal model experimentation based on KIM-1 Biomarker of RCC.

MATERIALS AND METHODS

Analyzing OTA toxic effects
The concentration of OTA has a hazardous impact that is immediate was 0.5 mg/kg body weight intraperitoneally (i.p.) [13].

Experimental Animals
Eight New Zealand White rabbits, both male and female, with body weights ranging from 700 to 1500 g were used to test the toxin effects of OTA. The rabbits were raised in rooms with ideal temperatures between 22 and 25 °C. The animals were fed a locally created diet made of organic food that was adequate for their growth and maintenance.

Experimental Design

Eight rabbits were split into two groups of four each, creating the following:
Group 1 (G1) consists of untreated control animals. Group 2 (G2): Animals received a 0.5 mg/kg body weight intraperitoneal dose of OTA.

Histopathological Study
Following the therapy for 72 hours, all rabbits were sacrificed and then slaughtered to obtain kidneys. Then the separated kidneys were kept in 100 cc of 10% formalin-filled polypropylene jars. Following dehydration in successively stronger alcohols, samples were embedded in paraffin, sectioned off into pieces of 4-5 m thickness, and stained with hematoxylin and eosin (H & E) [14] as follows:
- The parts were heated xylene for 5 to 10 minutes to deparaffinize them. (This process was carried out twice.)
- For dehydration, varying concentrations of alcohol (100%, 90%, and 70%) were employed for 5 minutes each.
- The section was stained for two to three minutes with hematoxylin, then washed for five to ten minutes with distilled water.
- The segment is differentiated for a brief period of time in 1% acidic alcohol (1% HCl in 70% alcohol) until it turns red, typically for 5–15 seconds.
- To eliminate the acid, thoroughly rinse under running tap water for 3-5 minutes.
- Treat this sample with 1% eosin stained for 10 minutes.
- Dehydration was done with graded alcohol (70%, 90%, 100%, and 100%), 5 minutes in each alcohol level.
- By undergoing three adjustments, xylene was able to clean the area (15, 15 and 30 minutes).
- Mounting utilizing a cover slide and a disterne-plastizer xylene (DPX).
- The slides then inspected under an optical microscope for microscopic analysis.

Immunohistochemical Study
Immunohistochemical detection was done by using protocol of Kits of rabbit anti – RCC and anti–KIM-1 antibodies supplied by Neobiotechnology and Mybiosource Companies were used.

Staining Protocol
The epitopes in the sections of the fixed samples were disclosed by dewaxing them, heating them in
a microwave for 10 minutes at 95°C in 10 mmol of citrate acid buffer with a pH of 6, and then letting them cool for 20 minutes. Following that, the slides underwent two 5-minute items of washing in PBS (pH 7.5). Primary antibodies against the RCC and KIM-1 were diluted 1:100 and incubated on tissue slices for 1 hour at room temperature. PBS was used to wash the slides before the secondary antibody, goat anti-rabbit horseradish peroxidase, was applied for an hour at 4°C in a humid room. All slides then were counterstained with Harris hematoxylin, cleared in xylene, and mounted before being washed once more with PBS and treated with the 3,3’ dianinobenzidine substrate for 7 min.2.5.4

Analysis of Immunohistochemistry results

The lack of immunostaining in the indicated positive controls and its presence in obvious brown cytoplasmic staining patterns were regarded good indicators of immunohistochemical signal specificity. The following scale was used to rate the staining level: “0 = (negative), 1 = (weak positive), 2 (moderate positive), 3 (strong positive), 4 (severe positive)” [20].

RESULTS AND DISCUSSION

Histopathological Study

Sections of kidney from control rabbits (G1) showed normal structure appearance of renal tissue with the presence of glomeruli and renal tubules (proximal and distal convoluted tubules) Figure (1). Whereas, the degree of histopathological changes were observed in rabbits treated with 0.5mg/kg body weight (i.p) of OTA (G2) including Glomerular shrinkage and degeneration, with nuclear pyknosis of glomerular cells, Interstitial fibrosis may see, Mild to severe tubular epithelial degeneration or atrophy, loss of cytoplasmic and nuclear detail, and retraction of tubule epithelial cells from basement membranes (Figure. 2, 3, 4) in comparison with control animals.

OTA is a common mycotoxin that builds up in organs including the liver and kidneys, where it may seriously harm these tissues [16]. Numerous domestic and laboratory animals experience morphological and functional alterations in their kidneys after being exposed to modest quantities of this toxin [17]. The kidney, and specifically the tubular epithelial cells, are OTA's primary target [18, 19]. Given that free OTA tubular concentrations are substantially higher than plasma concentrations [19], exposure-related renal tubular proliferative lesions, including focal hyperplasia, atypical hyperplasia, tubular cell adenomas, cystadenomas, and tubular cell carcinomas with metastases. Induced non-neoplastic renal tubular epithelial changes, including cytoplasmic alteration, degeneration, karyomegaly, proliferation, hyperplasia, and cysts. [18, 20]. These findings were in agreement with [21], while [22] reported that rabbits given OTA demonstrated a variety of histological changes, hyperemia in glomerular capillaries, interstitial vasculature, and clearly visible vacuolar tubular epithelial degradation. Other renal tubules also showed cystic dilatation with pressure atrophy on neighboring tubules. Additionally, there are numerous localized mononuclear cell infiltrations that are either periglomerular or interstitial, primarily composed of mononuclear cell infiltrations and lymphocytes with some heterophils. Additionally, cortically or medullary-located isolated regions of coagulative tubular necrosis with condensed eosinophilic cytoplasm and pyknotic nuclei were observed. Additionally, hyaline casts were observed in a few of the tube lumens. According to these studies, OTA may be nephrotoxic and induce kidney histopathologic abnormalities, and oxidative stress may be a key mechanism behind OTA toxicity [23]. Protein synthesis inhibition, DNA damage, cell cycle arrest, and cell death are some of the effects of OTA that cause nephrotoxicity [24]. When OTA builds up in proximal tubule epithelial cells, it causes cellular damage by inducing oxidative stress, DNA damage, apoptosis, and inflammatory response [25].

Immunohistochemical Study

Positive RCC and KIM-1 immunostaining results in rabbit kidneys were seen as brown cytoplasmic staining, whereas negative results did not show any brown stain (Figure. 5, 6, 7). RCC and KIM-1 IHC expression results were described in Table (1). Results in the table (1) showed that there is no expression of RCC and KIM-1 in the kidney of control rabbits (G1) (score = 0), while RCC expression and KIM-1 biomarkers are strong (score = 3) and moderate (score = 2) respectively in rabbits treated with OTA (0.5 mg/kg) (G2) Figures (5, 6, 7).

According to the findings, RCC and KIM-1 are not expressed in control animals, but they are strongly
and moderately expressed in the kidneys of those that received 0.5 mg/kg of body weight OTA as a treatment (i.p.) Renal cell carcinomas (RCC), which originate within the renal cortex, are responsible for 80% to 85% of all primary renal neoplasms [26]. Historically, it has been challenging to determine the origin of renal cells; currently, only immunohistochemistry could do so. As demonstrated by immunohistochemistry and inferred by the renal intraepithelial dysplastic lesion, it is critical to stress that these tumors have renal tubular cell origin and are not urothelial [27].

After prolonged exposure to OTA, renal carcinogenicity was experimentally demonstrated in male mice [28]. OTA is a known cause of kidney cancer in mice; however, it has not been linked to renal cancer in female of pigs that were fed OTA for two years [29].

Kidney injury molecule-1 (KIM-1) is a type-1 transmembrane protein that is formed on the renal tubules' apical membrane after damage, while not being present in most cases and its detection and monitoring are urgently required in both acute and chronic disease scenarios [30]. KIM-1 is a sensitive and specific renal damage biomarker [31]. When HK-2 cells were subjected to OTA, cell viability was reduced and the expression of kidney injury molecule-1 (KIM-1), a kidney damage marker, was raised. OTA-treated mice likewise had a significant increase in KIM-1 [32]. KIM-1 increased in the kidney following OTA therapy in a dose-dependent manner [33]. According to a study by [34], KIM-1 was found in the outer stripe of the outer medulla in both the low- and high-dose groups after rats were given treatments of 70 or 210 g/kg body weight of OTA for 4 or 13 weeks.

The lack of KIM-1 expression in the healthy kidney, its labeled upregulation and insertion through into the proximal tubule's apical membrane, its persistence in the epithelial cell till the cell has recovered fully, the rapid and robust cleavage of the ectodomain, and the ectodomain's stability at room temperature in vivo, all led researchers to believe that the protein might make an excellent biomarker of kidney injury. For prompt diagnosis, severity and outcome prediction, and monitoring of proximal tubule injury in AKI as well as chronic kidney disease, better biomarkers for acute kidney injury (AKI) are urgently required. Currently, KIM-1 and other kidney damage biomarkers are being evaluated in relation to the classification and diagnosis of AKI [35, 36].

**CONCLUSION**

This study was conducted to evaluate the expression of RCC and KIM1 Biomarkers in Nephrotoxicity of Rabbits Treated with Ochratoxins A. Results showed many histopathological changes in rabbit’s kidneys tissues. Immunohistochemical study observed that there was no Expression of biomarkers in control animals while animals treated with OTA showed strongly and moderately expression in their kidneys.

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TABLE 1: Expression of RCC and KIM-1

<table>
<thead>
<tr>
<th>Groups</th>
<th>RCC</th>
<th>KIM-1</th>
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<tbody>
<tr>
<td>G1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G2</td>
<td>3</td>
<td>2</td>
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G1 = Control rabbits without treatment.
G2 = Rabbits treated with 0.5 mg/kg OTA (LP).
FIGURE 5: Cross section of control rabbit’s kidney showing no immune reaction in tissue (Score = 0) (100X).

FIGURE 6: Cross section of rabbit’s kidney at concentration (0.5mg/kg) of OTA showing the intensity of immune reaction in tissue (Score = 3) using RCC Biomarker (400X).

FIGURE 7: Cross section of rabbit’s kidney at concentration (0.5mg/kg) of OTA showing the intensity of immune reaction in tissue (Score = 2) using KIM-1 Biomarker (400X).