Nephrotoxicity of ochratoxin-A in Japanese quail: A clinico-pathological study

Vikram Patial, Rajesh K. Asrani and Rajendra D. Patil

Department of Veterinary Pathology, Dr G C Negi College of Veterinary and Animal Sciences, C.S.K. Himachal Pradesh Agricultural University, Palampur-176 062, Himachal Pradesh, India

Scientist, CSIR-Institute of Himalayan Bioresource and Technology, Palampur-176 062, Himachal Pradesh, India

*Corresponding Author: R D Patil (PhD) E-mail: rdpatil02@gmail.com

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Abstract

The present investigation was designed to study the effect of ochratoxin-A (OTA), a nephrotoxic mycotoxin on the kidneys of Japanese quail. Ninety, day-old quail chicks were divided equally into two groups and fed OTA at a dietary level of 3.0 ppm; and a standard toxin-free feed (control) for 21 days. Dietary inclusion of OTA led to anorexia, diarrhoea, marked depression in body weight and few typical nervous signs in the treated birds. Uric acid values were higher in OTA treated birds as compared to control birds. Serum total protein and albumin levels in OTA treated birds were found to be significantly lower throughout the trial. At necropsy, the kidneys were variably pale and swollen in OTA treated birds. Microscopically, sections of kidneys revealed inflammatory, degenerative, and necrotic changes in ochratoxicated birds. In conclusion, the addition of OTA at 3.0 ppm level in feed produced significant renal damage in Japanese quail.

Keywords: Kidneys, pathology, quail, nephrotoxicity, ochratoxin-A

Introduction

Ochratoxin-A (OTA) was first discovered as a fungal metabolite of Aspergillus ochraceus in 1965 and was recognized as a potent nephrotoxin and nephrocarcinogen shortly thereafter. Being produced by one Penicillium and several Aspergillus species, it has been found in barley, coffee beans, oats, rye, wheat, and other plant products. The kidney is the primary target organ with OTA being a nephrotoxin to all animal species studied to date (Katole et al., 2013). OTA has been shown to inhibit protein synthesis in a wide range of cells (Dirheimer and Creppy, 1991). This effect is discussed to be caused by an inhibition of protein elongation by an interference of OTA with phenylalanine-tRNA synthetase (Marin-Kuan et al., 2006). Many of the toxic effects of OTA are thought to be caused by its interactions with protein biosynthesis (Bennett and Klich, 2003). OTA causes significant losses to the poultry industry due to its effects on growth and health. OTA-contaminated feed on broiler chicks are reported to reduce rates of weight gain (Dwivedi and Burns, 1984a; Stoev et al., 2002a) decre-ased egg production (Haazele et al., 1993), immunosuppression (Dwivedi and Burns, 1984b; Stoev et al., 2002b; Elaroussi et al., 2006) or carcinogenic effects and increased mortality and increased susceptibility to bacterial infections (Kumar et al., 2003). So, the present investigation was designed to study the clinico-pathological effects of OTA in Japanese quail.

Material and Methods

Experimental birds: The present study was conducted using ninety, day-old Japanese quail chicks procured from Central Poultry Development Organization, Chandigarh, India. Prior to arrival of day-old Japanese quails in the Department, the experimental room, cages, trays, waterers, feeder and cage-stands were first washed and room was fumigated with formaldehyde gas, just two days prior to arrival of quail chicks. The animal care and experimental protocol were approved by the Committee for the Purpose of Control
Ochratoxin-A: Ochratoxin-A was produced from the fungus Aspergillus ochraceous (NRRL-3174) on maize and the culture material with known level of ochratoxin A was supplied by Dr. G. E. Rottinghaus, University of Missouri, Columbia, USA on gratis.

Feeding schedule: The quail chicks were maintained on chick mash (Quail mash procured from Department of Animal Nutrition, COVAS, CSK HPKV, Palampur) from day 1 until the end of the experiment. Feed was autoclaved before feeding or mixing with OTA culture material(s) and SBT powder. Pre-boiled drinking water was provided during the experiment. Feed and water were available for ad libitum consumption.

Experimental design: Ninety, day-old Japanese quail chicks were randomly divided into two groups i.e. Control group and OTA treated group at 3.0 ppm in diet, with 45 birds in each of the groups. The dietary treatment was started from day 1 and continued up to 21 days for the study of clinico-pathological changes.

Clinical signs, body weight, mortality pattern and serum biochemistry: Birds from all groups were closely observed daily for the development of clinical signs and mortality pattern. In order to evaluate the effect of OTA on body weight, 9 randomly selected quail from each treatment group were weighed at 0, 7, 14, and 21 day post-feeding (DPF). After weighing, 2 to 3 ml of blood was collected via cardiac puncture from 6 randomly selected birds from each treatment group at 7, 14, and 21 DPF for estimation of total serum proteins, albumin and uric acid. All the biochemical estimations were done using diagnostic kits (Reckon Diagnostics Pvt. Ltd., Baroda, India) in a fully automatic Blood Chemistry Analyzer (RA-50 Auto Chemistry System) according to the manufacturer’s recommendations.

Gross and histopathology: Six randomly selected quail chicks were euthanized from each treatment group at 7, 14, and 21 DPF and examined for gross lesions in both kidneys. Representative renal tissue samples were collected in 10% neutral-buffered formalin for histopathological studies. Fixed tissues were trimmed, embedded in paraffin, sectioned at 3-5 μm, and stained with haematoxylin and eosin stain. The stained tissues were examined for microscopic lesions.

Results and Discussion

Clinical signs, body weight, mortality pattern and serum biochemistry: Feeding diets amended with 3.0 ppm OTA led to anorexia, dullness, diarrhoea, depression in body weight, ruffled feathers (Fig. 1) and incoordinated movements. In OTA treated birds, diarrhoea was more severe from 7 DPF onwards with almost (60-70%) of the birds seen passing watery faeces with an increase in uric acid and mucus content. In addition to this, nearly 5-10% of the birds treated with OTA showed typical nervous signs including pressing of head on the ground, backward motion to gain balance, ataxia and sudden falls onto the ground from day 14 onwards.

A significant reduction in body weight was observed in OTA treated group birds in comparison to control groups at all interval i.e. on 7, 14 and 21 DPF. No mortality was recorded in control group birds throughout the experimental period. However, 51.1% mortality was seen in OTA treated group birds during the experimental trial. Mortality was highest in the first week of the experiment in OTA treated group birds. Serum uric acid values were found to be higher in OTA treated birds however, the values of albumin and total protein were found to be lower in OTA intoxicated birds at all the intervals of the experiment.

Results of the present study showed that feeding diets amended with 3.0 ppm OTA led to anorexia, diarrhoea, and depression in body weight, poor feathering and incoordinated movements. Dwivedi and Burns (1984a) found OTA to be extremely toxic to young growing broiler chicks (2.0 ppm and 4.0 ppm) and the severity of the clinical signs, behavioural changes, growth response and lesions were all dose dependent. Similar clinical signs in chicks fed 5.0 ppm OTA in feed were observed by Stoev et al. (2002a) including weakness and dullness, growth depression and nervous symptoms such as torticollis, lurching, reeling gait, tremor, side-split, sitting posture or layer fashion with tense or flexion of legs. The reduced feed consumption undoubtedly contributed to the depression in body weight observed during the experiment. According to some researchers (Mohiudin et al., 1993), the growth depression in OTA treated chicks may be due to impaired protein synthesis caused by OTA. Huff et al. (1988) reported lower growth rates in chicks fed 4.0 ppm OTA as early as 6 day of age. The presence of diarrhoea may have been an additional factor contributing to the reduction in body weight in all groups fed OTA. Manning and Wyatt (1984), Ramadevi et al. (2000) and Kumar et al. (2003) all reported decreased serum proteins during ochratoxicosis in broilers. The hypoproteinemia might
be due to competitive inhibition of phenylalanyl-t-RNA synthetase with phenylalanine, causes disruption in serum protein levels (Konrad and Roschenthaler, 1977). Stoev et al. (2002b) suggested that a significant increase in serum levels of uric acid indicates that renal function was severely impaired as a result of OTA treatment. Impairment of kidney function may additionally contribute to the decrease of serum total protein by contributing to increased urinary excretion of serum proteins.

**Gross and histopathology:** No gross lesion was observed in the kidneys of quail from the control group when sacrificed at different intervals. In OTA treated group, variably swollen and pale kidneys with the prominent renal tubules were found in comparison to control group birds at all intervals of the study (Fig. 2). Occasionally, the dilated and prominent ureters were also noted in OTA treated birds.

The microscopic changes in control birds showed a normal histological appearance of kidneys (Fig. 3) except for mild to moderate vascular congestion at various intervals. In OTA treated group, the effect was found more pronounced in proximal convulated tubules (PCTs) than the distal convulated tubules (DCTs). Mild to severe vascular congestion was a consistent finding at various intervals. In PCTs, the predominant degenerative changes included epithelial cell swelling due to increase in cytoplasmic granularity (Fig. 4) and varying degree of cytoplasmic vacuolations. At 7 DPF, mild to moderate swelling of tubular epithelial cells was noticed along with a marked increase in eosinophilic granularity in the cytoplasm in OTA treated group birds. Marked variation in the size of nucleus i.e. karyomegaly was also a distinct finding. At places, there was marked sloughing of tubular epithelial cells of PCTs. In addition, there was dramatic increase in the hyaline material in the tubular epithelial cells at 7 and 14 DPF. Focal mild interstitial infiltration with mononuclear cells was also observed at 7 DPF and onwards. At 14 and 21 DPF, the inflammatory changes were more pronounced and individual cell necrosis was also noted in OTA treated group. Some of the nuclei showed karyorrhexis alternating with pyknosis in the tubular epithelial cells. The swelling of glomerular epithelial cells resulting in complete obliteration of Bowman’s space was consistent finding in OTA treated group throughout the experiment. Mesangial cell proliferation in the renal glomeruli was also prominent in comparison to those with the control group birds.

![Fig 1: Photograph of ochratoxin-A treated group bird (left) showing poor weight gain and ruffled feathers as compared with the control group bird (right) at the second week of the experiment.](image-url)
Fig 2: Gross photograph of pale and swollen kidneys from ochratoxin-A treated bird (left) as compared with the normal kidneys from control group bird (right) at 14th day of the experiment.

Fig 3: Photomicrograph of kidney from control group bird showing normal structure of glomeruli (G), PCTs and DCTs at 21 DPF (H&E x132).
Fig 4: Photomicrograph of kidney from ochratoxin-A treated group bird showing severely swollen and degenerated PCT epithelium and the presence of hyaline casts in the tubular lumen (arrow) at 21 DPF. (H&E x132).

The gross lesions observed in the kidneys were mainly paleness and swelling in OTA treated group. Grossly, swollen kidneys observed in the present study were consistent with earlier studies conducted in chickens (Elaroussi et al., 2006, 2008; Dwivedi and Burns, 1984a) and the effect was found to be dose dependent. In a similar study conducted on rats, (Prasanna et al., 2007) observed gross lesions from the 3rd week onwards, and the kidneys were markedly enlarged with occasional petechial haemorrhages in the group treated with 0.5 mg per kg body weight of OTA daily. The tubular enlargement and degenerative changes in the epithelial cells of the kidneys in OTA treated group were probably due to route of elimination of OTA through the kidneys and partly through the liver, and to a direct toxic action of OTA on these cells (Stoev et al., 2000). Similarly, cloudy swelling, granular degenerative changes in the cytoplasm and pyknotic nuclei in the epithelial cells of PCTs were reported when broiler chicks fed diets containing 4.0 and 8.0 ppm OTA for 35 days (Elaroussi et al., 2008).

**Conclusion**

Based on the present findings, it is concluded that the inclusion of OTA at 3.0 ppm dietary level for 21 days produced significant renal damage in Japanese quail. Further studies are needed to check the toxic potential of OTA in combination with other nephrotoxic mycotoxin(s) in quail.

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