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PATHOLOGICAL, BIOCHEMICAL AND IMMUNOLOGICAL STUDIES ON EXPERIMENTAL OCHRATOXIN A TOXICOSIS IN RABBITS

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INTRODUCTION & AIM

Ochratoxin A (OTA) contamination of feed and food is a common global problem, and spontaneous cases of OTA-induced mycotoxic nephropathy are frequently reported in pigs and poultry in Europe and Africa. Rabbits are highly sensitive to OTA and may develop severe nephrotoxic, hepatotoxic, teratogenic, and immunosuppressive effects. Regardless of the prevalence of OTA in rabbit feed, the pathogenesis and clinicopathological features of ochratoxicosis in this species remain insufficiently studied. The present study aimed to investigate the main pathomorphological, biochemical, and immunological alterations induced by dietary OTA exposure in young New Zealand White rabbits fed a naturally relevant contamination level of 2 ppm for 30 days.

METHOD

The experiment was conducted using 16 New Zealand White rabbits randomly assigned to one control group, receiving standard complete feed free of mycotoxins, and one OTA-treated group, receiving the same diet contaminated with 2 ppm (2 mg/kg) ochratoxin A (OTA) produced from *Aspergillus ochraceus* culture material and verified by HPLC analysis. All animals were housed under standardized conditions, and standard complete feed for growing rabbits and drinking water were available *ad libitum*. All rabbits were immunized against RHDV, and blood samples were collected before immunization and 28 days later to evaluate the humoral immune response. Additional blood samples for biochemical analyses were obtained on day 30 of the experiment. Subsequently, four rabbits (two males and two females) from each group were euthanized, and tissue samples from internal organs were collected for pathomorphological investigations, fixed in 10% neutral buffered formalin, and stained with routine hematoxylin–eosin to characterize OTA-induced lesions.

RESULTS & DISCUSSION

A potent immunosuppressive effect of OTA on the humoral immune response against RHDV was observed. The measurement of the hemagglutination inhibiting antibody titer (HIAT) on day 28 after immunization against Rabbit Hemorrhagic Disease Virus (RHDV) revealed that it was significantly reduced in the group treated with OTA (Table1). The immunosuppressive effect of OTA caused the occurrence of a secondary infection (pasteurellosis), which resulted in the death of two rabbits from the OTA-exposed group. Biochemical investigations revealed decreased levels of glucose and increased levels of creatinine, BUN, triglycerides, ALT, AST, and ALP in rabbits from the OTA-treated group (Table2). Pathomorphological investigations revealed the strongest damage in the liver, kidneys, spleen, and thymus, except in cases of secondary pasteurellosis, where the strongest damage was found in the lung. In the kidneys, the main degenerative changes were found in the epithelium of proximal tubules (A). Liver damage included degeneration in hepatocytes and Kupffer cell activation (B). In the spleen degenerative lesions and cell depletion were seen (C). Lung damage, especially in cases of secondary pasteurellosis, included purulent or croupous pneumonia, accumulation of fibrin, leukocytes and mononuclear cell infiltration (D). The present findings demonstrate that OTA induces pronounced nephrotoxic, hepatotoxic, and immunosuppressive effects in rabbits, as evidenced by severe degenerative lesions in the kidneys and liver, lymphoid depletion in the spleen, significant alterations in biochemical indicators of renal and hepatic function, and a reduced humoral immune response to RHV. These effects are likely associated with OTA accumulation in target organs, impaired protein synthesis, and immune cell depletion, resulting in organ dysfunction and increased susceptibility to secondary infections.

Table 1. Mean values of antibody titer in groups of rabbits (n=8) with or without OTA on day 28 after vaccination against RHDV

Group	0 day	28th day
OTA	0.10±0.021	0.51±0.06
CONTROL	0.10±0.011	1.01±0.16[a]

± SEM (standard error of the mean)
[a] – Samples with an OD value (at a dilution of 1/200) above 0.9 indicate sufficiently developed immune protection against RHDV infection

Table 2. Mean serum values of glucose, creatinine (Creat), urea (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and triglycerides (TG) in groups of rabbits (n=8) with or without OTA .

Group	Glucose mmol/L	Creat mmol/L	BUN µmol/L	AST U/L	ALT U/L	ALP U/L	TG mmol/L
OTA	2.94±0.16[a]	105.06±12.08[a]	9.00±0.33[a]	92.75±7.70[a]	77.50±3.91[a]	583.50±53.02[a]	1.34±0.11[a]
CONTROL	3.92±0.31[b]	72.21±4.48[b]	7.04±0.26[b]	31.37±2.10[b]	53.00±2.68[b]	353.38±24.44[b]	0.99±0.07[b]

± SEM (standard error of the mean)
[a] – significant difference compared to CONTROL group ($p<0.05$)
[b] – significant difference compared to OTA group ($p<0.05$)

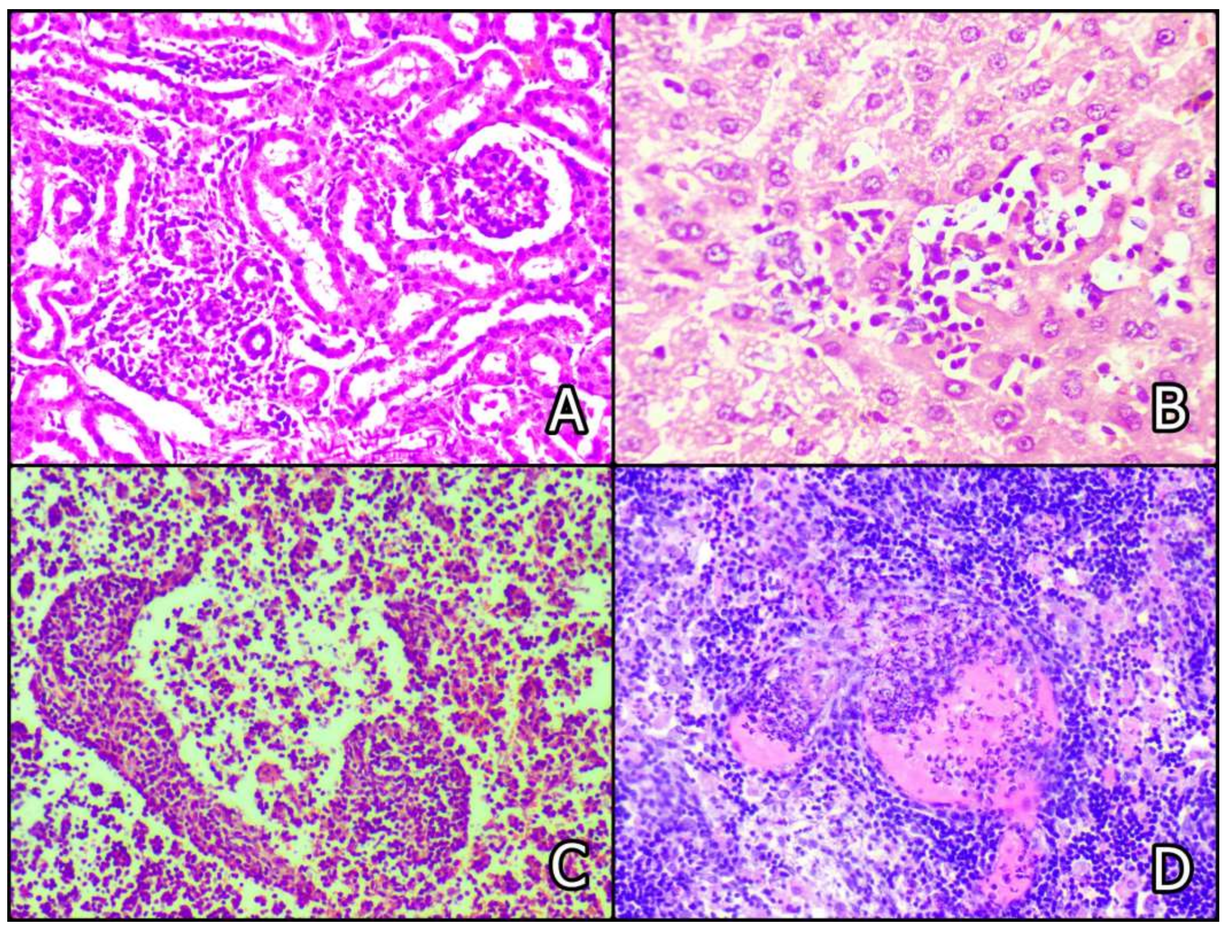

Figure A - Granular degeneration in proximal tubules and mononuclear proliferation in interstitium in kidney of rabbit on day 30 of OTA exposure, H/E.
Figure B - Vacuole degeneration in hepatocytes and activation of Kupffer cells in liver of rabbit on day 30 of OTA exposure, H/E.
Figure C - Reduction of the size of white pulp and degenerative changes or depletion of cells in the lymph follicles in spleen of rabbit on day 30 of OTA exposure, H/E.
Figure D - Purulent or croupous pneumonia, accumulation of fibrin, and leukocytes and mononuclear cell infiltration in lung of rabbit, died from pasteurellosis, H/E.

CONCLUSION

The kidneys and liver were identified as the primary target organs of OTA toxicity in rabbits, exhibiting the most severe pathological alterations. In conclusion, it was found that immunosuppression was the first pronounced toxic effect of OTA that may manifest clinically before pathological and biochemical changes. Humoral immunity was affected to the extent of allowing the development of clinical disease and death of rabbits at only 2 ppm OTA in the ration.

FUTURE WORK / REFERENCES

• Zhivkova K., Stoev S., Petrov V., Ivanov V. – “Susceptibility to Secondary Bacterial Infection in Growing Rabbits Exposed to Ochratoxin A and Protected or Not by Herbal Supplements” *Toxins* 2025, 17(10), 507