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EFFECT OF DEOXYNIVALENOL ON THE EXPRESSION AND DISTRIBUTION OF *ERK* AND *JNK* IN LIVER AND KIDNEY OF WEANED RABBITS BY IMMUNOHISTOCHEMISTRY METHOD

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ABSTRACT

Intake of Deoxynivalenol (DON) can activate related signaling pathways and induce toxic reaction. At present, there are few studies focus on the toxic mechanism of DON on rabbits. The purpose of current study was to investigate the effect of DON on expression and distribution of extracellular signal-regulated kinase (ERK) and Jun nuclear kinase (JNK) in liver and kidney of weaned rabbits. 45 weaned rabbits were randomly divided into three groups: Control group, LD group (DON at 0.5 mg/kg b.w.), and HD group (DON at 1.5 mg/kg b.w.), with a total feeding period of 31 days and a pre-feeding period of 7 days. In this study, immunohistochemistry method was used to detect the expression and distribution of positive reactants of ERK and JNK in the liver and kidney of rabbits. The results concluded that intake of DON can change the expression and distribution of ERK and JNK in the liver and kidney, and these effects were dose-dependent.

Key words: Deoxynivalenol, immunohistochemistry, weaned rabbit, liver, kidney

INTRODUCTION

Because of the high detection rate in food and feed worldwide, DON is become a potential pathogenic factor to human and animal species. DON is chemically stable and can seriously threaten human and animal health by contaminating food and feed (Wu *et al*., 2013). Mitogen-activated protein kinases (MAPKs), including ERK and JNK, are one of numerous signaling proteins (Lee *et al*., 2019), which are crucial to proinflammatory gene expression and apoptosis induced by DON (Zhang *et al*., 2020). Activation of ERK plays a pivotal role in inducing cell motility, differentiation and survival, while JNK is closely related to cell cycle and cell differentiation. Currently, the researches on DON-induced toxicity mainly focused on pig, chicken and some cell lines, while the study on rabbits are very limit. With the continuous expansion of rabbit breeding scale and the wide application of full price feed in rabbit industry, the issue concerning contamination of mycotoxins in feed and potential threats to food safety have attracted more and more attention from scientists (Mézes *et al*., 2009). However, there are few studies on toxic effect and mechanism of DON on rabbits, especially for the correlation between MAPK signaling pathway and toxicity mechanism induced by DON. Therefore, the purpose of present study was to investigate the effects of different dose of DON on the expression and distribution of ERK and JNK in liver and kidney of weaned rabbits.

MATERIALS AND METHODS

Animals and experimental design

DON Standards ($C_{15}H_{20}O_6$, purity>98%) purchased from Triplebond Company (Guelph, Canada). The formula of the basic feed was based on that reported by de Blas and Wiseman (1998). The composition of the experimental feeds and nutritional level determined by the normal method are listed in Table 1. 45 healthy weaning Rex Rabbits (35-day) were divorced equally into three groups randomly and 15 rabbits

per group (879 ± 17.62g in mass), namely the control, low-dose (LD), and high-dose (HD) groups. All rabbits were fed a basal diet, and animals in the LD and HD groups were additionally administered DON at 0.5 and 1.5 mg/kg b.w., respectively, via the drinking water. DON supplementation of the drinking water was performed as described by Yang *et al*. (2019). The experiment lasted for 31 days, including 7 days of adaptation and 24 days of experiment. On day 24, 18 rabbits (six per group) were selected randomly and euthanized via injection. The liver and kidney were separated and weighed, then the relative weight of the organ (g/kg) = organ weight (g)/live weight (kg) was calculated. Then the samples of liver and kidney in same part were cutted and fixed using Bouin's solution.

Table 1: The ingredient composition of the basic diets (as feed basis).

Ingredient (%)		Calculated composition	
Maize	14	Dry matter	88.64
Soybean meal	17	Crude protein	20.05
Wheat bran	13	Crude fibre	18.78
Corn germ meal	19	Crude ash	10.45
Rice hulls	10	Crude fat	3.34
Soybean straw powder	7	Calcium	0.72
alfalfa	10	Total Phosphorus	0.55
Malt Sprout	5	Digestible energy（MJ/kg）	10.06
Sweet wormwood	3.5		
Premix material [1]	1.5		
Total	100		

[1]Premix material provided per kg feed: VA, 12,000 IU; VD $_3$, 1,000 IU; VE, 50 mg; VK_3, 1.5 mg; Fe, 60 mg; Zn, 60 mg; Cu, 40 mg; Mn, 9 mg; Se 0.2 mg.

Immunohistochemistry (IHC)

The small intestine samples of rabbits were cutted into the paraffin section about 5-μm thick, which attached to a glass slide containing poly-L-lysine. Citric acid buffer (0.01 M, pH 6.0) was used to thermal unmasking of the antigen after dewaxing and debenzenizing. The sections were then incubated with 3% H_2O_2 to block endogenous peroxidase. For IHC staining, the DAB kit with primary polyclonal rabbit antibodies against ERK and JNK (1:100; BIOSS, Beijing, China) were used. The secondary antibody applied Polink-2 plus immunohistochemical assay kit (ZSBIO, Beijing, China) , and 10% calf serum was used for the seal. The sections were stained with hematoxylin, dehydrated, transparented, follow by sealed with transparent resin. Finally, the immunopositive cells with brownish yellow color could be observed via the microscope technology. Total cross-sectional integrated optical density (IOD)were calculated using Image Pro-Plus 6.0 software (Media Cybernetics, Silver Spring, USA) based on cell staining, which were used to assess the intensity of positive reaction.

RESULTS AND DISCUSSION

Relative organ weight of liver and kidney

The relative weight of liver of rabbits decreased significantly ($p < 0.05$) in LD and HD group compared with the control group ($p < 0.05$). Meanwhile, the relative weight of kidney showed no significant difference ($p > 0.05$) among the groups (Table 1).

Table 2. Effect of DON on the relative organ weight1 of liver and kidney（ Mean ± SD, n=6)

notes: The values with different small letters in the same line differ significantly ($p < 0.05$).

Organ name	Control	LD	HD	p	Root MSE
Liver	35.77±1.12a	29.07±0.57b	30.87±1.45b	0.001	3.136
Kidney	8.00±0.23	7.17±0.26	7.85±0.33	0.101	0.779

Expression and distribution of ERK and JNK

The IOD value of positive reactivity for ERK and JNK in the liver and kidney were higher significantly ($p < 0.05$) compared with the control group after DON was added, especially at higher-dose group (Fig.1). By comparison, the change of ERK in kidney and JNK in liver were more significant.

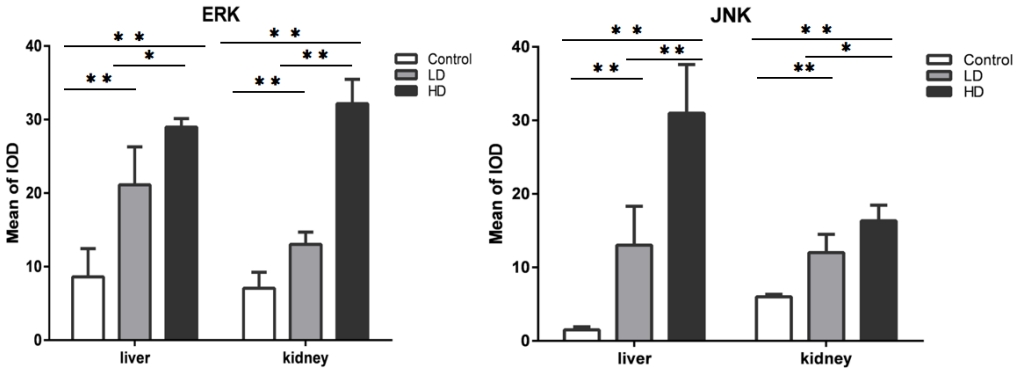

Figure 1. The IOD value of ERK and JNK in samples of liver and kidney examined by IHC method. * and ** indicate the significant difference at $p < 0.05$ and $p < 0.01$, respectively.

The representative photography from IHC staining in liver and kidney are illustrated in Figure 2 and Figure 3 (next page). The results indicated that only a small amount of positive material was detected in the portal area of liver (Fig.2A) and Kidney capsule (Fig.2B) in the control group, however the levels and range of positive reactants distributed in the liver and kidney were increased obviously after DON exposure, especially at the higher dose. in addition, the photographs displayed that few positive reactants of JNK were distributed in the hepatic portal area (Fig.3A) and renal capsule (Fig.3B) in control group, while the levels and range of positive reactants distributed in the liver and kidney were increased obviously after DON addition, especially at the HD group.

CONCLUSIONS

In conclusion the intake of DON can stimulate the expression and distribution of ERK and JNK in the liver and kidney, and these effects were dose-dependent.

ACKNOWLEDGEMENTS

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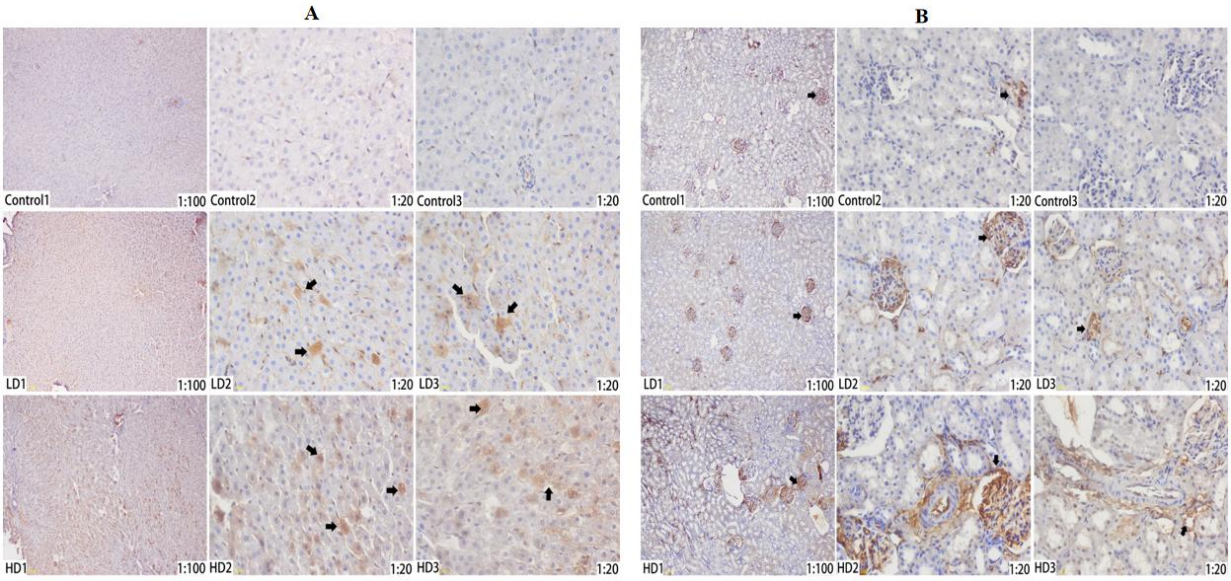

Figure 2. The distribution of ERK in liver (A) and kidney (B) were tested via IHC method. 1:100 and 1:20 represent the magnification of electron microscopy is 10 × and 40 × respectively. The brown positive reactants was emphasized by black arrow.

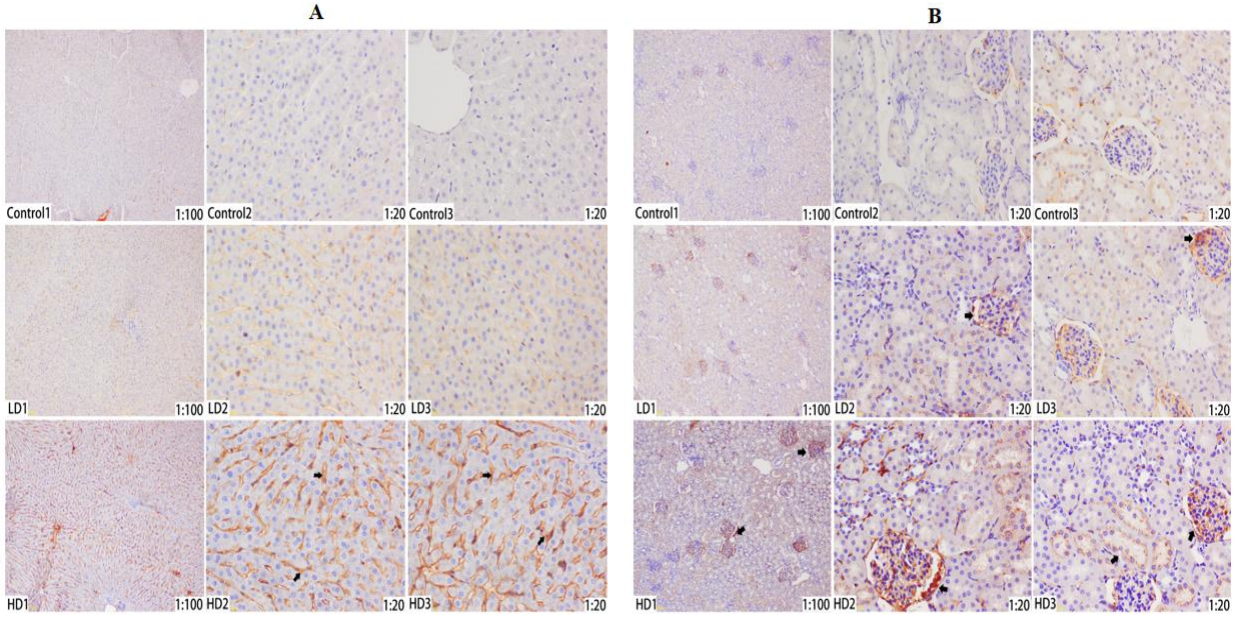

Figure 3. The distribution of JNK in liver (A) and kidney (B) were tested via IHC method. 1:100 and 1:20 represent the magnification of electron microscopy is 10 × and 40 × respectively. The brown positive reactants was emphasized by black arrow.