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Influence of Selenium on Hepatic Antioxidant Capacity in Ducklings Intoxicated with Afatoxin B_1

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Abstract: To investigate the influence of selenium on hepatic antioxidant capacity in ducklings administrated with Afatoxin B_1 (AFB_1), ninety 7 days old ducklings were randomly divided into three groups (groups I-III). Group I was used as a blank control. Group II was administered with AFB_1 (0.1 mg kg^{-1} body weight). Group III was administered with AFB_1 (0.1 mg kg^{-1} body weight) plus selenium (sodium selenite, 1 mg kg^{-1} body weight). All treatments were given once daily for 21 days. The results showed that the activity of hepatic Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-Px) and Glutathione Reductase (GR) in group II ducklings significantly decreased when compared with group I ($p<0.01$). Furthermore, the content of hepatic Malondialdehyde (MDA) significantly increased ($p<0.01$). However, the activities of hepatic SOD, CAT, GSH-Px and GR in group III ducklings significantly increased when compared with group II ($p<0.05$). In addition, the content of hepatic MDA significantly decreased ($p<0.01$). These results revealed that AFB_1 significantly induced hepatic antioxidant function dysfunction. However, selenium could significantly alleviat the negative effect induced by AFB_1.

Key words: Selenium, hepatic antioxidant capacity, afatoxin B_1, activity, ducklings, dysfunction

INTRODUCTION

Afatoxin B_1 is produced by toxigenic fungi belonging to the genus *Aspergillus* and has a long history of association with illness in domesticated animals and in humans (Wogan, 1999; Bondy and Pestka, 2000). The liver is especially sensitive to AFB_1.

In a number of studies, the AFB_1 is firstly a hepatotoxin causing an excessive build-up of hepatic lipids with enlargement of the liver, proliferation of the biliary ducts (Adav and Godinwar, 1997) and hepatocellular carcinoma (Hamilton, 1978). Recently, the direct evidence of the involvement of free radicals in AFB_1 toxicity was demonstrated by Towner *et al*. (2003) who identified free radicals *in vivo* in rat bile following AFB_1 administration.

It was reported that AFB_1-mediated hepatic lipid peroxidation were reduced by the pretreatment of rats with antioxidants, selenium and vitamin E (Shen *et al*., 1996). The aim of the present study was designed to investigate the effect of selenium on hepatic antioxidant capacity in ducklings intoxicated with AFB_1.

MATERIALS AND METHODS

Drugs and chemicals: Afatoxin B_1 and dimethyl sulfoxide were purchased from Sigma. Detection kit which includes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and malondialdehyde were purchased from Nanjing Jiancheng Bioengineering Institute. All other chemicals were of analytical grade.

Animals and treatments: All experimental protocols were approved by the Ethics Committee of South China Agricultural University. This study was carried out on 90 Guangdong white ducklings weighing 180-200 g body weight. About 7 days old ducklings were obtained from the Research Center of Experimental Animals at South China Agricultural University.

The animals were randomly divided into three equal groups containing 30 ducklings with an equal number of male and female ducklings. Group I was used as control and intragastrically administered with Dimethyl Sulfoxide (DMSO). Group II was intragastrically administered with AFB_1 (0.1 mg kg^{-1} body weight). Group III was intragastrically administered with AFB_1 (0.1 mg kg^{-1}

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body weight) plus selenium (sodium selenite, 1 mg kg^{-1} body weight). These treatments were administrated once daily for a period of 21 days under the same condition. AFB_1 was diluted with DMSO for experimental ducklings. All ducklings had free access to water and food at room temperature during the study.

Liver sample preparation: On 7th, 14th and 21st days after treatment in every experimental group, five ducklings were randomly taken out to obtain, respectively about 1 g of liver and prepare liver homogenate in physiological saline. The supernatant fluid was taken out to detect after liver homogenate was centrifuged at 1,000×g for 5 min. All the steps are strictly operated on ice to guarantee the isolation of high-quality liver homogenate supernatant fluid. The protein concentration of the final liver homogenate supernatant fluid was determined with the Bradford's assay using bovine serum albumin as a protein standard (Bradford and Dodd, 1977).

Assessment of hepatic antioxidant capacity: The activities of hepatic superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase were detected according to instruction in the detection kit. Similarly, the content of hepatic malondialdehyde was detected. The activities of hepatic superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase are expressed as international units/mg hepatic protein. The content of malondialdehyde is expressed as nmol/mg hepatic protein.

Statistical analysis: The statistical significance of differences between groups in these studies was determined using a one-way analysis of variance and the results were presented as the mean±SE. The significance level was $p<0.05$.

RESULTS AND DISCUSSION

As shown in Table 1, the activity of hepatic Superoxide Dismutase (SOD) was significantly affected after ducklings were intragastrically administered with AFB_1 for 7, 14 and 21 days. The activity of SOD decreased by 17.19, 43.73 and 43.27%, respectively ($p<0.01$). However, in group III ducklings intragastrically administered with AFB_1 plus selenium, the activity of SOD increased by 13.46, 60.86 and 62.85%, respectively, compared with group II ($p<0.01$).

As shown in Table 2, the activity of hepatic Catalase (CAT) was significantly affected after ducklings were intragastrically administered with AFB_1 for 7, 14 and 21 days. The activity of CAT decreased by 31.91, 37.29 and 55.43%, respectively ($p<0.01$). However, in group III ducklings intragastrically administered with AFB_1 plus selenium, the activity of CAT increased by 36.88, 39.36 and 84.77%, respectively compared with group II ($p<0.01$).

As shown in Table 3, the activity of hepatic Glutathione Peroxidase (GSH-Px) was significantly affected after ducklings were intragastrically administered with AFB_1 for 7, 14 and 21 days. The activity of GSH-Px decreased by 32.42, 34.71 and 39.88%, respectively ($p<0.01$). However, in group III ducklings intragastrically administered with AFB_1 plus selenium, the activity of GSH-Px increased by 27.92, 46.14 and 53.98%, respectively compared with group II ($p<0.05$).

As shown in Table 4, the activity of hepatic Glutathione Reductase (GR) was significantly affected after ducklings were intragastrically administered with AFB_1 for 7, 14 and 21 days.The activity of GR decreased by 27.49, 33.09 and 38.67%, respectively ($p<0.01$).

Table 1: The activity of hepatic Superoxide Dismutase (SOD) for 7, 14 and 21 days after ducklings administrated with AFB_1

	Three different times after treatment (days)		
Groups	7	14	21
I	282.40±21.26	267.53±19.30	229.31±18.98
II	232.16±16.42^{a}	150.54±12.86^{aa}	130.08±9.710^{aa}
III	263.42±19.58^{b}	242.16±17.69^{bb}	211.83±11.42^{bb}

$^{a}p<0.05$, $^{aa}p<0.01$, significantly different, group II compared with group I. $^{b}p<0.05$, $^{bb}p<0.01$, significantly different, group III compared with group II. (the same following)

Table 2: The activity of hepatic Catalase (CAT) for 7, 14 and 21 days after ducklings administrated with AFB_1

	Three different times after treatment (days)		
Groups	7	14	21
I	26.64±2.32	24.27±2.22	22.10±2.240
II	18.14±1.20^{aa}	15.22±1.33^{aa}	9.85±0.890^{aa}
III	24.83±2.30^{bb}	21.21±2.09^{bb}	18.20±1.670^{bb}

Table 3: The activity of hepatic Glutathione Peroxidase (GSH-Px) for 7, 14 and 21 days after ducklings administrated with AFB_1

	Three different times after treatment (days)		
Groups	7	14	21
I	466.22±32.70	409.22±36.48	347.79±30.36
II	315.08±28.01^{aa}	267.17±21.28^{aa}	209.09±18.92^{aa}
III	403.06±40.24^{bb}	390.43±27.79^{bb}	321.95±22.08^{bb}

Table 4: The activity of hepatic Glutathione Reductase (GR) for 7, 14 and 21 days after ducklings administrated with AFB_1

	Three different times after treatment (days)		
Groups	7	14	21
I	22.95±2.25	20.94±1.76	18.00±1.84
II	16.64±1.33^{aa}	14.01±1.17^{aa}	11.04±1.26^{aa}
III	19.53±2.20^{b}	17.95±1.47^{bb}	15.83±1.76^{bb}

Table 5: The content of hepatic Malondialdehyde (MDA) for 7, 14 and 21 days after ducklings administrated with AFB_1

	Three different times after treatment (days)		
Groups	7	14	21
I	0.65±0.08	0.75±0.06	0.81±0.06
II	$1.04±0.04^{aa}$	$1.38±0.09^{aa}$	$1.66±0.06^{aa}$
III	$0.75±0.03^{bb}$	$0.89±0.06^{bb}$	$1.02±0.05^{bb}$

However, in group III ducklings intragastrically administered with AFB_1 plus selenium, the activity of GR increased by 17.37, 28.12 and 43.39%, respectively compared with group II ($p<0.05$).

As shown in Table 5, the content of hepatic Malondialdehyde (MDA) was significantly affected after ducklings were intragastrically administered with AFB_1 for 7, 14 and 21 days. The content of MDA increased by 60.00, 84.00 and 104.94%, respectively ($p<0.01$). However, in group III ducklings intragastrically administered with AFB_1 plus selenium, the content of MDA decreased by 27.88, 35.51 and 38.55%, respectively compared with group II ($p<0.01$).

It is known that AFB_1 may induce the production of free radicals and/or the reduction of antioxidant defenses (Shen *et al*., 1996; Leal *et al*., 1999). Reactive Oxygen Metabolites (ROM) include intracellular thiols (SH), MDA. The MDA production is recognized as an important factor in determining alteration of membrane fluidity (Chen and Yu, 1994; Ferrante *et al*., 2002) and increase of membrane fragility accompanying final cell death (Halliwell and Chirico, 1993). A few researchers (Abado-Becognee *et al*., 1998; Yin *et al*., 1998; Abel and Gelderblom, 1998) reported that some mycotoxins can cause cell membrane damage through the increase of lipid peroxidation. In continuation of these studies, we investigated the effect of AFB_1 on ducklings' hepatic antioxidant capacity in this research. For 7, 14 and 21 days after ducklings administrated with AFB_1, respectively it showed that the activities of hepatic SOD, CAT, GSH-Px and GR in group II ducklings (administered with AFB_1) significantly decreased when compared with group I ($p<0.01$). Furthermore, the content of hepatic MDA in group II significantly increased ($p<0.01$).

Although, the mechanism underlying the hepatotoxicity of aflatoxins is not fully understood, several reports suggest that toxicity may ensue through the generation of intracellular Reactive Oxygen Species (ROS) like superoxide anion, hydroxyl radical and hydrogen peroxide during the metabolic processing of AFB_1 by cytochrome P450 in the liver (Sohn *et al*., 2003). These species may attack soluble cell compounds as well as membranes, eventually leading to the impairment of cell functioning and cytolysis (Blaszczyk *et al*., 2010; Berg *et al*., 2004). But peroxidative damages induced in the cell are encountered by elaborate defense mechanisms including enzymic and nonenzymic antioxidants. AFB_1 mediated hepatic lipid peroxidation were reduced by the pretreatment of rats with antioxidants, selenium and Vitamin E. Defense mechanisms are reinforced by increasing dietary intake of antioxidants and micronutrients such as vitamins and Selenium (Se). The importance of Se is characterized by its role as a constituent of several key antioxidants as well as the unique redox characteristics of selenocysteine and its use in antioxidant enzymes such as thioredoxin reductase. The reduction of reactive oxygen metabolites by glutathione peroxidases helps to maintain membrane integrity (Brenneisen *et al*., 2005). In this study, we assessed the effect of selenium on hepatic antioxidant function in ducklings administrated with AFB_1. For 7, 14 and 21 days after ducklings administrated with AFB_1, respectively it showed that the activities of hepatic SOD, CAT, GSH-Px and GR in group III ducklings (administered with AFB_1 plus sodium selenite) significantly increased when compared with group II ($p<0.05$). In addition, the content of hepatic MDA in group III significantly decreased ($p<0.01$).

CONCLUSION

AFB_1 significantly induced hepatic antioxidant function dysfunction. The result showed that the activity of hepatic Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-Px) and Glutathione Reductase (GR) significantly decreased when compared. Furthermore, the content of hepatic Malondialdehyde (MDA) significantly increased. However, selenium could significantly alleviate the negative effect induced by AFB_1.

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