Journal of Animal and Veterinary Advances 11 (8): 1162-1165, 2012
ISSN: 1680-5593

Influence of Longdan Xiegan Decoction on Body Weights and Immune Organ Indexes in Ducklings Intoxicated with Aflatoxin B_1

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Abstract: To explore the influence of Longdan Xiegan Decoction (LXD) on body weights and the immune organ indexes in ducklings administrated with Aflatoxin 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 LXD (as 2% of feed). All treatments were given once daily for 21 days. It showed that the ducklings' bursa of fabricius and thymus indexes, body weights in group II significantly decreased when compared with group I ($p<0.01$). Furthermore, the spleen indexes significantly decreased ($p<0.01$). However, the ducklings' bursa of fabricius and thymus indexes, body weights in group III ducklings significantly increased when compared with group II ($p<0.01$). In addition, the spleen indexes significantly decreased ($p<0.01$). These results revealed that AFB_1 significantly affect ducklings' growth and immune organs development. However, LXD significantly ameliorated the negative effects induced by AFB_1.

Key words: Longdan Xiegan Decoction, body weights, immune organ indexes, aflatoxin B_1, growth, thymus

INTRODUCTION

Aflatoxins, a group of well-known toxic metabolites are produced primarily by certain strains of fungi *Aspergillus flavus* and *Aspergillus parasiticus* under favorable temperature and humidity. In subtropical and tropical areas, the contamination of aflatoxins in food crops is common especially for the crops containing high starch and lipid content (Ostrowski-Meissner *et al*., 1995; Bokhari, 2002; Aba-Alkhail *et al*., 2004). It was reported that afatoxins could be detected in 96.1% of the 334 tested commercial feeds and raw materials collected from Asia (Chen and Rawlings, 2008). Among them Aflatoxin B_1 (AFB_1) is the most prevalent and toxic for human, land animals and aquatic organisms, mostly by its strong carcinogenic, mutagenic and teratogenic effects (Santacroce *et al*., 2008; Han *et al*., 2008). Aflatoxicosis in birds is highly relevant as a problem affecting the broiler chicken industry due to its socioeconomic impact on mortality, feed conversion index, production costs and the negative effects on public health related to the human consumption of exposed animals.

Pretreatment of animals with substances tested before hepatotoxin administration, e.g., carbon tetrachloride, aflatoxin B_1 is a common experimental model for investigation potential hepatoprotective activity (Rafatullah *et al*., 2008; Chavda *et al*., 2010; Bigoniya *et al*., 2010; Bitiren *et al*., 2010). In the studies, researchers have used this model to test whether Longdan Xiegan Decoction (one of the most famous Traditional Chinese Medicine prescriptions) could mitigate toxicity of aflatoxin B_1 with respect to growth performance and the relative weights of the immune organs (spleen, thymus and bursa of fabricius) in ducklings intoxicated with aflatoxin B_1.

MATERIALS AND METHODS

Longdan xiegan decoction preparation: In this research, Longdan Xiegan Decoction (LXD), a famous traditional Chinese medicine prescriptions was comprised of 10 medicinal materials including Radix Gentianae, Radix Scutellariae, Fructus gardenia, Rhizoma alismatis, Caulis Aristolochiae Manshuriensis, Semen plantaginis, Radix Angelica Sinensis, Radix rehmanniae, Radix bupleuri and Radix Glycyrrhizae. In a certain ratio, the ten ingredient herbs were crushed fully and used to feed ducklings (as 2% of feed).

Animals and treatments: All experimental protocols were approved by the Ethics Committee of South China Agricultural University. This study was carried out on 90

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Guangdong white ducklings weighing 180-200 g body weight. About 7 days old ducklings were obtained from 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} body weight) plus LXD (as 2% of feed). 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.

Determinations of body weights and of the immune organ indexes: On 7th, 14th and 21st day after treatment, five ducklings from every experimental group were randomly taken out to be weighed. Afterwards, the ducklings' immune organ (spleen, thymus and bursa of fabricius were taken out and weighed. The duckling's body weights are expressed in grams. The immune organ indexes are expressed in the ducklings' immune organs (spleen, thymus and bursa of fabricius) relative weight (g kg^{-1}). The immune organ relative weight equal to the immune organ weight/the duckling's body weight.

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 body weight was significantly affected after ducklings were intragastrically administered with AFB_1 for 7th, 14th and 21st days. The body weight in group I ducklings decreased by 35.38, 52.38 and 51.92%, respectively compared with group II ($p<0.01$). However, the body weight increased in group III ducklings by 11.38, 17.89 and 24.41%, respectively compared with group II ($p<0.01$).

As shown in Table 2, the spleen index was significantly affected after ducklings were intragastrically administered with AFB_1 for 7th, 14th and 21st days. The spleen index in group I ducklings increased by 30.67, 41.85 and 49.33%, respectively compared with group II ($p<0.01$). However, the spleen indexes in group III ducklings decreased by 10.54, 17.62 and 18.30%, respectively compared with group II ($p<0.01$).

Table 1: Body weights for 7th, 14th and 21st days after the ducklings exposed to AFB_1

	On 7th, 14th and 21st day after the administration of AFB_1		
Groups	7	14	21
I	475.83±37.00	929.17±75.87	1460.00±128.73
II	307.50±26.42^{aa}	442.50±33.16^{aa}	702.00±58.280^{aa}
III	342.50±24.50^{b}	521.67±46.87^{b}	873.33±61.670bb

Table 2: The spleen indexes for 7th, 14th and 21st days after the ducklings exposed to AFB_1

	On 7th, 14th and 21st day after the administration of AFB_1		
Groups	7	14	21
I	2.25±0.20	1.84±0.14	1.50±0.12
II	2.94±0.27^{aa}	2.61±0.19^{aa}	2.24±0.22^{aa}
III	2.63±0.25^{b}	2.15±0.18^{bb}	1.83±0.21^{bb}

Table 3: The thymus indexes for 7th, 14th and 21st days after the ducklings exposed to AFB_1

	On 7th, 14th and 21st day after the administration of AFB_1		
Groups	7	14	21
I	8.22±0.70	10.22±0.88	9.79±0.76
II	5.08±0.41^{aa}	7.17±0.68^{aa}	6.09±0.62^{aa}
III	6.26±0.54^{bb}	8.63±0.59^{bb}	7.85±0.68^{bb}

Table 4: The bursa of fabricius indexes for 7th, 14th and 21st days after the ducklings exposed to AFB_1

	On 7th, 14th and 21st day after the administration of AFB_1		
Groups	7	14	21
I	2.55±0.25	2.24±0.16	1.78±0.14
II	1.74±0.10^{aa}	1.51±0.13^{aa}	1.24±0.16^{aa}
III	2.03±0.24^{b}	1.85±0.15^{b}	1.54±0.18^{b}

$^{a}p<0.05$, $^{aa}p<0.01$, 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)

As shown in Table 3, the thymus index was significantly affected after ducklings were intragastrically administered with AFB_1 for 7th, 14th and 21st days. The thymus index in group I ducklings decreased by 38.20, 29.84 and 37.80%, respectively compared with group II ($p<0.01$). However, the thymus index in group III ducklings increased by 23.23, 20.36 and 28.89%, respectively compared with group II ($p<0.01$).

As shown in Table 4, the bursa of fabricius index was significantly affected after ducklings were intragastrically administered with AFB_1 for 7th, 14th and 21st days. The bursa of fabricius index in group I ducklings decreased by 31.76, 32.59 and 30.34%, respectively compared with group II ($p<0.01$). However, the bursa of fabricius index in group III ducklings increased by 16.67, 22.52 and 24.19%, respectively compared with group II ($p<0.01$).

It was well known the AFB_1 and its metabolites can accumulate in animal tissues after AFB_1 exposure. Aflatoxin contamination has been a potential threat to the health of humans and animals (Agag, 2004; Meissonnier *et al*., 2007). In addition, the greatest economic impacts include reduced productivity,

suppressed immune function, pathological effects on organs and tissues (Anonymous, 1989). It was reported that the inclusion of AFB_1 200 ppb in the diet significantly reduced the growth of mule ducklings, significantly affect immune organ development (Cheng *et al*., 2001). Compared to the controls, the relative weights of the livers and kidneys increased significantly in the broilers exposed to aflatoxins (Raju and Devegowda, 2000).

In this study for 7th, 14th and 21st days after ducklings administrated with AFB_1, respectively it showed that the ducklings' bursa of fabricius indexes, thymus indexes and body weights in group II significantly decreased, compared with group I ($p<0.01$). Furthermore, the spleen indexes significantly increased ($p<0.01$). In all, AFB_1 reduced the growth of the ducklings caused tymus and bursa of fabricius atrophy and induced swelling of the spleen.

Traditional Chinese Medicines (TCMs) have been practiced in China and other eastern countries over long period of time. Usually, TCMs are administrated in the form of formulated preparations consisting of several even >10 ingredient herbs in a certain ratio which may provide polyvalent biological actions. Longdan Xiegan Decoction (LXD), a traditional Chinese formulation has been widely practiced to treat jaundice, conjunctival congestion, earache, scrotum and extremitas inferior eczema, etc. in Chinese medicine for thousands of years (Chinese Pharmacopoeia Commission, 2005).

It has been suggested that for some herbs it is the natural antioxidants they contain conferred their biological activities (Loliger, 1991; Zhu *et al*., 2004). Most of the antioxidant potentials in herbs and spices are due to the redox properties of phenolic compounds that allow them to act as reducing agents, hydrogen donators and free radicals quenchers (Shahidi *et al*., 1992).

Longdan Xiegan Decoction is one of the most famous traditional Chinese medicine prescriptions which is a combinatorial formula including ten drugs. It has been used to treat damp-heat in the liver, gall bladder, congested eyes or as an important drug for acute/chronic cholecystitis (Meng, 2006; Leng, 2006). The prescription consists of 10 medicinal materials including Radix Gentianae, Radix Scutellariae, Fructus gardenia, Rhizoma alismatis, Caulis Aristolochiae Manshuriensis, Semen plantaginis, Radix Angelica Sinensis, Radix rehmanniae, Radix bupleuri and Radix Glycyrrhizae.

Although, LXD preparation as a whole has not been elucidated for its bioactive constituents, some of the compounds contained in the ingredient herbs have been tested and have shown pharmacological properties. For instance, gentiopicroside isolated from Radix Gentianae root indicated antibacterial and free radical scavenging activities (Kumarasamy *et al*., 2003). The flavonoids baicalin and baicalein from Radix Scutellariae root showed multi functional efficacies including anti-bacterial, antivirus, anti-inflammation and liver protective activities (Pan and Feng, 1994). In the study, researchers assessed the effect of LXD on body weight and immune organs indexes in ducklings administrated with AFB_1. It showed that the bursa of fabricius indexes, thymus indexes and body weights in group III ducklings (administrated with AFB_1 plus LXD), significantly increased when compared with group II ($p<0.01$). In addition, the spleen indexes in group III ducklings significantly decreased, compared with group II ($p<0.01$).

CONCLUSION

In this research, these results showed that the ducklings' bursa of fabricius and thymus indexes, body weights in group II significantly decreased, compared with group I. In addition, the spleen indexes significantly decreased. However, the ducklings' bursa of fabricius and thymus indexes, body weights in group III ducklings significantly increased, compared with group II. Besides, the spleen indexes significantly decreased. So it was concluded that Longdan Xiegan Decoction could significantly ameliorate AFB_1 negative effect on ducklings' growth performance and the immune organs' development.

ACKNOWLEDGEMENTS

This research was supported by the Guangdong Province Science Foundation of China (Grant No.: 9151008002000003), the National Natural Science Foundation of China (Grant No. 38071900), the National Twelve-Five Technological Supported Plan of China (No. 2011BAD34B01), the Guangdong province and Ministry of education Cooperation project by Production, Study and research of China (Grant No. 2008B090500250). The S.N. Guo, S.Q. Liao and R.S. Su contributed equally to this research.

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