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SHORT COMMUNICATION

Effectiveness of Xiang-Qi-Tang against Avian Pathogenic *Escherichia coli*

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ABSTRACT

Xiang-Qi-Tang (XQT) is a Chinese medicine containing *Rhizoma Cyperi* (40 g), *Andrographis paniculata* (30 g) and *Astragalus membranaceus* (30 g). The purpose of this study is to investigate the therapeutic effects of XQT on Avian Pathogenic *Escherichia coli* (APEC)-infected chickens. The chickens were pretreated with XQT 12 h before being inoculated with 10^8 colony forming unit (CFU) of APEC by subcutaneous injection, and then the mortality and the indexes of health status of chicken in each group were detected. The results showed that high dosage and middle dosage of XQT could significantly decrease the mortality of the chickens challenged with APEC. We further found that XQT improved the infected chickens' health status through improving the water consumption, feed intake, bodyweight gain, routine blood parameters and decreasing the incidences of pericarditis and perihepatitis. The results of present study suggest that XQT can effectively treat chicken colibacillosis as a potential agent.

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INTRODUCTION

Chicken colibacillosis is caused by avian pathogenic *Escherichia coli*, occurring in different types of chicken. The infection results in severe economic losses in the global poultry industry due to reducing poultry meat and egg production (Ron, 2006). Antibacterial agents and inactivated or subunit vaccines are applied to cure or prevent this disease at present. However, because of the mutation of APEC and its complicated pathogenic mechanisms, the vaccines and antibacterial agents are often invalid (Gyles, 2008). Furthermore, antimicrobial agents are continuously or unreasonably fed to chickens, which cause serious drug residues in meat or egg and affect human health (van den Bogaard *et al*., 2001). Now, food safety becomes an increasing public health concern all over the world. People urgently want to find some safe and effective agents to solve the above knotty issues.

The traditional herbal medicine has lots of advantages in curative effect, security, and controlling drug resistance and residue (Kogure *et al*., 2008; Kim *et al*., 2012; Ou *et al*., 2013), so it is more important for prevention and treatment of livestock infectious disease. In our previous study, we had screened a Chinese medical formula named Xiang-Qi-Tang (XQT) for curing chicken colibacillosis through a series of experiments in vitro and in vivo. XQT is composed of *Rhizoma Cyperi*, *Andrographis paniculata* and *Astragalus membranaceus* with an optimal ratio. The current study was planned to further observe its therapeutic effect on APEC-infected chickens.

MATERIALS AND METHODS

A total of 180, day-old white leghorn specific pathogen free (SPF) chicks, were obtained from the Experimental Animal Center of Jilin University, randomly divided into six groups and treated as shown in Table 1. *Escherichia coli* (*E. coli*) O78 (CVCC1418) was purchased from the China Veterinary Culture Collection Center and prepared for APEC suspension to a concentration of 5×10^8 colony forming unit (CFU)/ml. 0.2 ml APEC suspension (containing 10^8 CFU) challenged with chickens in each group except the control group through subcutaneous injection. XQT were prepared as our previous study (He *et al*., 2011). The final concentration of XQT solution was 1 g/ml, which was diluted to serial concentrations to be administered in XQT-treated group 12 h before inoculation with APEC.

On days 1, 2, 3, 4, 5 and 6 after challenge with APEC, water consumption, feed intake, body weight and death in each group were monitored.

After the end of the experiment, body weight (BW) gain and mortality in each group were calculated. Five surviving birds were randomly selected for blood collection through jugular vein for the determination of complete blood count (CBC). Then, all of the chickens in each group were killed and necropsied and the incidence of pericarditis and perihepatitis were detected.

The data are expressed as mean ± standard deviation. Differences in the mortality, BW gain and the incidence of pericarditis and perihepatitis of groups were assessed by Fisher's exact test. Other statistical evaluations were performed using one-way ANOVA (Dunnett's *t*-test) and Student's *t*-test. Statistical difference was accepted at P<0.05.

RESULTS AND DISCUSSION

All of the chickens challenged with APEC appeared the clinical symptoms of chicken colibacillosis about 4 h after inoculation, including depression, ruffled feathers, no reaction to acoustics, lesser balance, closed eyes, decreased water consumption and feed intake. Results of the monitoring of water consumption and feed intake were shown in Fig. 1 and 2. After 1 day of inoculation, the water consumption and feed intake in each drug-treated or APEC-treated group were significantly decrease, compared with the control group (P<0.01). These actions may result largely from the proliferation of APEC in vivo. Because it is reported that APEC could induce a large release of cytokines in brain such as nitricoxide (NO) and interleukin (IL) -1 which were responsible for the repression of the drinking and feeding behavior (Becskei *et al*., 2008). After 2 days, the water consumption and feed intake in XQT high dosage group, middle dosage group and ofloxacin group began to restore to normal levels, which were higher than APEC group (P<0.01). In our previous study, XQT was demonstrated to decrease inflammatory mediator levels in chicken colibacillosis such as TNF-α and IL-1 (He *et al*., 2011). According to these results, it is possible that recovering drinking and feeding behavior of XQT may partly be due to its anti-inflammatory activity.

After being injected with APEC, the death peak of infected chickens was 24 to 48 h. The data in table 2 showed that, after the end of the experiment, the mortality in XQT high dosage group and middle dosage group were significantly (P<0.05, or P<0.01) decreased as compared to APEC group. These results suggest that XQT may prevent the proliferation of APEC. This effect was attributed to the antibacterial activity of *Andrographis paniculata* in XQT, which can directly kill APEC (Mishra *et al*., 2009). After the APEC proliferation being prevented, the health status of the infected chickens could be improved, which was indicated by the growth values. So, the BW gain in each group in our study was detected. The increase of BW gain were observed in XQT high dosage group and middle dosage group as compared to APEC group (P<0.05, or P<0.01). This effect was similar to those of antibiotics such as ofloxacin.

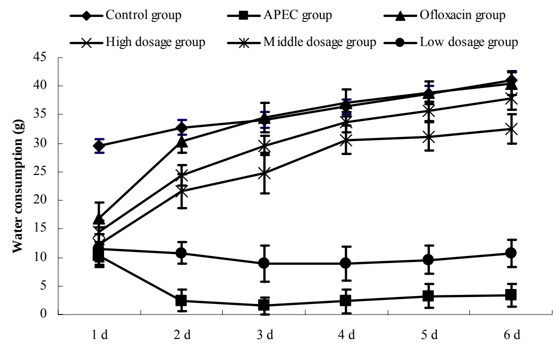

Fig. 1: Effects of XQT on water consumption in each group

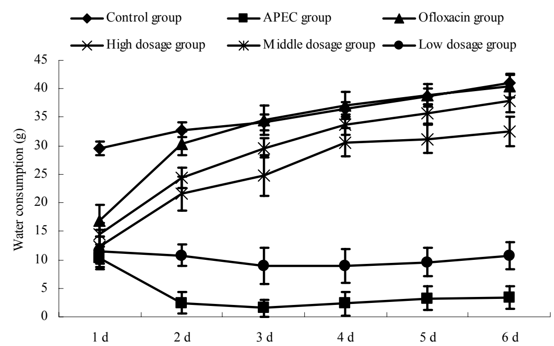

Fig. 2: Effects of XQT on food intake in each group

Table 1: Grouping and carried out treatments

No.	Group	Treatment
1	Control group	No infection, no drug
2	APEC group	Infection, no drug
3	Ofloxacin group	Infection, 10 mg/kg ofloxacin by gavage
4	High dosage group	Infection, 1.2 g/kg XQT by gavage
5	Middle dosage group	Infection, 0.6 g/kg XQT by gavage
6	Low dosage group	Infection, 0.3 g/kg XQT by gavage

In each group, there were 30 chicks.

Table 2: Effects of XQT on mortality and BW gain in each group

Group	No. of Death	Mortality (%)	BW gain (g)	Relative growth rate (%)
Control group	0	0**	42.1	53.5**
APEC group	15	50	8.1	9.7
Ofloxacin group	0	0**	41.6	52.6**
High dosage group	4	13.3**	41.8	52.9**
Middle dosage group	6	20*	22	26.8*
Low dosage group	12	40	4.9	6.5

Significant differences by Fisher's exact test; * P<0.05 vs APCE group, **P<0.01 vs APEC group. Relative growth rate = (the average BW at the end of experiment - the average BW at the beginning of experiment)/ the average BW at the beginning of experiment. In each group, there were 30 chicks.

Table 3 presents the number of red blood cells, white blood cells and thrombocytes. Challenge with APEC could adversely affect these routine blood parameters in chickens. The results showed that red blood cells and thrombocytes in APEC-infected chickens were destroyed by APEC, the number of these two cells were markedly decreased as compared to the uninfected chickens. XQT could ameliorate such adverse effects. The high or middle dosage of XQT significantly increased the number of red blood cells and thrombocytes (P<0.05 or P<0.01). Because the APEC was the direct spoiler of these two cells, we presumed that XQT's protective effect might be due to the anti-APEC activity of its active components. In addition, APEC infection in vivo caused white blood cell

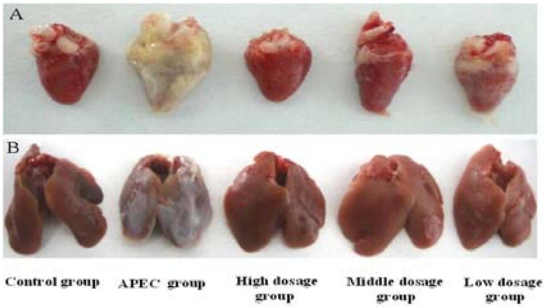

Fig. 3: Pathological change of the heart and liver in each group

Table 3: Effect of XQT on routine blood parameters in each group

Group	Red blood cells ($\times 10^{12}$/L)	White blood cells ($\times 10^{11}$/L)	Thrombocytes ($\times 10^{12}$/L)
Control	28.49±6.23**	0.41±0.42**	2.59±0.17*
APEC	19.16±8.88	1.52±0.1	2.05±0.46
Ofloxacin	27.57±2.64**	0.40±0.06**	2.53±0.27*
High dosage	25.71±3.27**	0.61±0.04**	2.46±0.26*
Middle dosage	21.75±4.48*	0.45±0.16**	2.09±0.35
Low dosage	20.26±2.18	0.76±0.21*	1.76±0.29

Significant differences by Student's *t*-test; * P<0.05 vs APCE group, **P<0.01 vs APEC group

Table 4: Effect of XQT on the incidences of pericarditis and perihepatitis in each group

Group	Pericarditis (%)	Perihepatitis (%)
Control	0	0
APEC	100	93.3
Ofloxacin	0	0
High dosage	66.7**	40**
Middle dosage	73.3**	46.7**
Low dosage	80*	80

Significant differences by Fisher's exact test; * P<0.05 vs APCE group, **P<0.01 vs APEC group

counts to increase. Although the increased white blood cells were beneficial to the clearance of APEC, the additional production of inflammatory response released from white blood cells was harmful to the health of chickens. Therefore, inflammations such as pericarditis and perihepatitis were appeared in APEC-infected chickens (Fig. 3). Necropsy results displayed that the incidences of pericarditis and perihepatitis of the chickens challenged with APEC respectively were 100% and 93.3% (Table 4). However, in the present study, XQT could inhibit the increase of white blood cell counts (P<0.05 or P<0.01) so that it might decrease the inflammatory mediators (Table 3). The incidences of pericarditis and perihepatitis of the chickens treated with XQT were significant lower than those of APEC-infected chickens (Fig. 3 and Table 4).

White blood cells and thrombocytes play important role respectively in inflammation and coagulation. The effect of XQT on these cells suggested that it had potential anti-inflammatory or anticoagulant activity. The components of XQT had been reported having these biological activities in lots of literatures. For example, Lu *et al.* (2012) reported that andrographolide, an active component of *Andrographis paniculata*, had a high therapeutic potential to treat thromboembolic disorders and inflammatory diseases through suppressing nuclear factor-κB (NF-κB) signaling pathway. *Astragalus membranaceus* also possesses a strong anti-inflammatory activity as a NF-κB inhibitor (Yang *et al*., 2013). On the other hand, *Astragalus membranaceus* can treat thromboembolic diseases by up-regulating the PC pathway (Gao *et al*., 2011). *Rhizoma Cyperi* is also extensively used to cure animal infectious diseases in China. Therefore, XQT exhibiting anti-inflammatory and anticoagulant properties may be one of the mechanisms of its treatment of chicken colibacillosis.

Conclusion: XQT could decrease the mortality of the chickens challenged with APEC, and benefit the infected chickens' health status through improving the water consumption, feed intake, BW gain, routine blood parameters and decreasing the incidences of pericarditis and perihepatitis. These results suggest that XQT can be a candidate agent for prevention of or therapy for avian colibacillosis, even be of help to other infectious diseases in poultry.

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