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Research Article

PROTECTIVE EFFECTS OF *TINOSPORA CORDIFOLIA* ON CLINICAL MANIFESTATIONS OF EXPERIMENTAL COLIBACILLOSIS IN BROILER CHICKEN

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ABSTRACT

The present study was undertaken to investigate the protective effects of *Tinospora cordifolia stem* extract (TCE) against experimental *Escherichia coli* O78 infection in broiler chicken. For this, day-old broiler chicks were fed TCE @ 1 gm/kg feed and infected with *E. coli*, intraperitoneally (group A1) at 7 days of age. In non-supplemented infected group A2, clinical signs of *E. coli* infection started appearing at 24 h post infection that included dullness, depression and drooping of the head and neck. Thereafter, the chicks showed anorexia, listlessness, ruffled feathers closing of eyes, stunted growth, outstretching, drooping of wings, inability to stand, respiratory distress, diarrhoea, soiling of the vent resulting in dehydration. The survived birds in this group appeared almost normal after 18 days post infection. On the other hand, clinical signs of *E. coli* infection in infected and supplemented with TCE group (B2) started appearing at 36 h post infection. Clinical signs were similar to those observed in group A2 but the severity of the signs was of lesser intensity and lasted for a shorter duration. In group A2, mortality occurred within 24 h post infection and continued till 10 DPI and a total of nine chicks (22.5%) died. Mortality in group B2 started from 2 DPI and continued till 8 DPI and six chicks (15%) died in this group. The mortality in infected non-supplemented group was considerably higher as compared to infected and supplemented group. Mean body weight of infected groups A2 and B2 were significantly lower as compared to their corresponding control groups A1 and B1, respectively from 4 DPI onwards. However, body weight in group B2 was comparable to group A1 at 25 DPI. It was concluded that supplementation of *T. cordifolia* stem extract had ameliorating effects on clinical signs, high mortality and reduced growth in broiler chicken experimentally infected with *Escherichia coli* O78.

Key words: body weight, clinical signs, *Escherichia coli*, mortality, *Tinospora cordifolia stem* extract

Escherichia coli infections are quite common in poultry and include disease conditions such as pericarditis, perihepatitis, air sacculitis, peritonitis, salpingitis, panophthalmitis, omphalitis, cellulitis, colisepticemia, coligranuloma and swollen-head syndrome (Saif *et al*., 2003). Colibacillosis is one of the main causes of economic losses in the poultry industry worldwide (Ewers *et al*., 2003). Majority of economic losses result from mortality, decreased egg production, condemnations and costs of vaccination, chemotherapy and eradication programmes. *E. coli* strains are often resistant to antimicrobials such as cephradine, tetracyclines, chloramphenicol (Rahman *et al*., 2004; Hooda, 2009), sulfonamides, β-lactam antibiotics (Li *et al*., 2007; Renu, 2010) and amino-glycosides (Hooda, 2009; Renu, 2010). Resistance to fluoroquinolones was reported within several years of the approval of this class of drugs for use in poultry (Li *et al*., 2007). The medicinal plants have been used in many countries because of natural origin, fewer side effects, no drug resistance, low cost, easy availability, affordability, good antimicrobial nature, reduced diseases associated risks, diversified functions in improving performance, growth rate, feed conversion rate and weight gain in birds (Lewis *et al*., 2003). *Tinospora cordifolia* (*T. cordifolia*) is an herbaceous shrub indigenous to the tropical areas of India and is well known for its medicinal properties. It is one of the most commercially exploited plants in pharmaceuticals. The whole plant possesses diverse health benefits and has been used as traditional medicine against various human ailments since the distant past (Bhattacharyya and Bhattacharyya, 2013). The immunomodulatory, antimicrobial, antioxidant, antineoplastic, hypoglycemic, antipyretic, hepatoprotective, diuretic, anti-stress, antihyperglycemic, antidiabetic and anti tuberculotic properties of this plant have also been reported (Sinha *et al*., 2004).

MATERIALS AND METHODS

After grant of permission from Institutional Animal Ethics Committee to use poultry birds for conducting the experiment, 140, day-old healthy and unvaccinated Cobb broiler chicks were procured from a local hatchery and divided into two groups A and B containing 70 birds each. The chicks were kept under strict hygienic conditions under cage system in well ventilated and well-lit rooms. The birds of group A were fed on basal feed with no

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supplementation. The birds of group B were given feed along with *T. cordifolia* stem extract (TCE) @ 1g/kg feed. At 7 days of age, the birds of both the groups were further divided into two subgroups (Group A into A1 and A2 and Group B into B1 and B2) of 30 and 40 birds, respectively. All the chicks of groups A2 and B2 were injected with *E. coli* O78 @ 10^7 CFU/0.5 ml, intraperitoneally.

Preparation of TCE: *T. cordifolia* stems were collected from medicinal and aromatic plants section, Department of Genetics and Plant Breeding College of Agriculture, CCS Haryana Agricultural University, Hisar. Stems were washed, dried in shade and then powdered. Powdered material was sequentially extracted with water in Soxhlet apparatus for 6 h. The extract thus obtained was reduced to powdered form by evaporation of water from extract by heating it at low temperature till a semisolid powder is obtained. The TCE so prepared was used as supplementation in feed @ 1g/kg feed (Bhardwaj *et al*., 2011) for chicks of group B from 0 day of age till the end of experiment. The infective dose @ 10^7 CFU of O78 serotype of *E. coli*/0.5 ml was prepared for the experiment as *E. coli* inoculum (Kumar *et al.,* 2003).

Parameters Studied: Birds in different groups were closely observed daily for clinical signs and mortality if any. Six randomly selected birds from each group were weighed at 0, 4, 11, 18 and 25 day post infection (DPI). Viable bacterial cell count of *E. coli* in liver of infected groups (A2 and B2) was determined at 4, 11, 18 and 25 days post infection by plate count method (Cruickshank *et al*., 1975).

RESULTS AND DISCUSSION

Clinical Signs: No clinical signs were observed in groups A1 and B1 throughout the experiment. Clinical signs of *E. coli* infection in the group A2 started to appear at 24 h post infection. These included dullness, depression and drooping of the head and neck (Fig. 1). They showed huddling together near the heat source. Thereafter, the chicks showed anorexia, listlessness, ruffled feathers closing of eyes and stunted growth. The birds showed outstretching and drooping of wings and

Fig 1. *E. coli* infected group A2 chicks showing dullness, depression and drooping of the head and neck

Fig 2. *E. coli* infected group A2 chicks showing outstretching and drooping of wings and they were unable to stand

they were not able to stand (Fig. 2). Infected birds exhibited respiratory distress and diarrhoea which was watery or pasty white, in few birds soiling of the vent and resulting in dehydration. These clinical signs were more severe at 11 DPI; the severity reduced in survived birds from 18 DPI. There was almost complete recovery in clinical signs at 20 DPI. Similar clinical signs in natural and experimental colibacillosis have been reported by other workers (Verma and Swamy, 2013; Abd El-Tawab *et al*., 2015; Kumari, 2015; Sharma, 2015). On the other hand, clinical signs of *E. coli* infection in group B2 started appearing at 36 h post infection. Clinical signs were similar to those observed in group A2 but the severity was less and they lasted for a shorter duration. This might be due to improved bacterial clearance from

Table 1
Mortality of broiler chicks in different experimental groups at different intervals

Group	Number of birds died at different days post-infection										Total (%)
	1	2	3	4	5	6	7	8	9	10	
A1	0	0	0	0	0	0	0	0	0	0	0
A2	1	1	1	1	1	2	0	1	0	1	9 (22.5)
B1	0	0	0	0	0	0	0	0	0	0	0
B2	0	1	1	1	0	1	1	1	0	0	6 (15.0)

host, antimicrobial, adaptogenic and immunomodulatory effects of TCE (Desai *et al*., 2007; Upadhyay *et al*., 2011).

Mortality: Mortality pattern in different experimental groups is given in Table 1. No mortality was observed in non-infected groups. In group A2, mortality occurred within 24 h post infection and continued till 10 DPI. Total number of chicks that died in group A2 was 9 (22.5%). On the other hand, mortality in group B2 started from 2 DPI and continued till 8 DPI. Number of birds died at different intervals in this group was 6 (15%). The mortality in A2 group was higher than group B2. These results indicate that the economic losses due to colibacillosis may be reduced to certain extent by supplementation of TCE in feed. The results of present study are in agreement with the findings of Thatte *et al*. (1987) who observed that pre-treatment with *T. cordifolia* imparted protection against mortality induced by intra-abdominal sepsis following coecal ligation in rats. Similarly, reduction in mortality due to *E. coli* infection in mice was reported by Thatte *et al*. (1994). Desai *et al*. (2007) reported 100% protection against mortality in mice due to *E. coli* LPS when pretreated with G1-4A isolated from *T. cordifolia.* Baishya *et al.* (2008) observed significant reduction in mortality due to *Salmonella enteritidis* in broiler chicken when treated with polyherbal preparation containing *T. cordifolia*. This might be due to protective effect of TCE due to its early and fast bacterial clearance from host, antimicrobial, hepatoprotective (Bishavi *et al*., 2002) and immunomodulatory effects (Kolte *et al*., 2007).

Body Weight: It was observed that mean body weights in non-infected groups were higher than the infected groups (Fig. 3). Among non-infected groups, supplemented group (B1) had significantly (P≤0.05) higher body weight at all intervals as compared to group A1 (Fig. 3). It was observed *E. coli* infection in chicks caused significant decrease in body weight gain. Similar results of reduction in weight gain have been reported by other workers due to *E. coli* infection in chicken (Saini, 2004; Kumari, 2015). Huff *et al*. (2008) observed a decrease in body weight and feed efficiency in broilers infected with avian pathogenic *E. coli* infection Mean body weight in group B2 were significantly higher as compared to A2 at all intervals (Fig. 3). Body weight in group B2 was equal to control A1 group at 25 DPI revealing ameliorating effects of supplementation on body weight in *E. coli* infection. These results clearly indicated that feeding of TCE to broiler chicken accelerated growth response of birds. Enhancement of body weight gain due to *T. cordifolia* supplementation in chicks has been reported (Sarag and Khobragade 2003; Bhardwaj *et al*., 2011; Singh *et al*., 2014). The enhanced growth rate of broiler chicks on TCE supplementation may be due to improved feed conversion ratio, improved antioxidant activity and immunomodulatory effect of *T. cordifolia.*

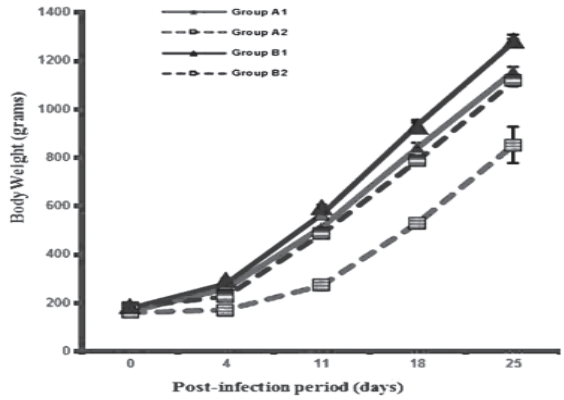

Fig 3. Mean body weights in different experimental groups

Viable Bacterial Cell Count: The bacterial cell count in group A2 was significantly higher than group B2 at all intervals (Table 2). The bacterial clearance in group B2 was faster as revealed by zero bacterial cell count as compared to 0.004±0.001 bacterial count in group A2 at 25 DPI (Table 2). These findings suggested that bacterial load decreased due to supplementation of TCE. Similar observations were recorded by Thatte *et al*. (1992). *T. cordifolia* improved phagocytic and intracellular bactericidal capacities of neutrophils (Thatte *et. al.,* 1994).

It can be concluded that experimental *E. coli* O78 infection in broiler chicken produced high mortality of 22.5%, caused significant reduction in body weight gain and other clinical signs like anorexia, listlessness, ruffled feathers, closing of eyes, outstretching and drooping of wings, respiratory distress and diarrhoea. Supplementation of *T. cordifolia* stem extract (TCE) had ameliorating effects leading reduction in mortality and bacterial load, better growth and clinical signs of lesser severity and duration. Hence, *T. cordifolia* may be used for the reduction of economic losses due to *E. coli* infections which are quite common in poultry.

Table 2
Mean viable bacterial (*E. coli*) cell counts in the liver (per gram) of broiler chicks in different experimental groups at different intervals (Mean±SE)

Group	Days post-infection			
	4	11	18	25
A2	1.40±0.15^{b}	3.44±0.23^{b}	0.13±0.03^{b}	0.004±0.001
B2	1.02±.0.9^{a}	1.88±0.27^{a}	0.06±0.01^{a}	0

Different superscripts in the same column differ significantly (P≤0.05)

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