Effect of Ocimum sanctum leaf on pathology of Escherichia coli infection in broiler chickens

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ABSTRACT

The present study was undertaken to investigate the effect of Ocimum sanctum leaf powder on experimental Escherichia coli infection in broiler chickens. Day-old broiler chicks (160) were divided into 2 groups. The birds of group A were supplemented with dried O. sanctum leaf (OSL) powder in feed @5g/kg and the birds of group B were given feed without OSL supplementation. After 7 days of age, each bird in group A1 and B1 was inoculated with E. coli O78 (@10^7 CFU/0.5 ml) by the intraperitoneal route, whereas groups A2 and B2 were kept as controls. Mortality in group B1 was 35.56% whereas mortality was comparatively low (20%) in OSL supplemented group A1. There was no mortality in the control groups (A2 and B2). Gross lesions produced in group B1 were fibrinous mass on the surface of liver and heart, congestion in visceral organs, peritonitis, reddish intestinal mucosa and atrophy of bursa of Fabricius. The gross lesions in OSL supplemented infected group were of less intensity at different intervals as compared to those observed in non-supplemented infected group. Histopathological lesions observed were fibrinous pericarditis, myocarditis, fibrinous perihepatitis, haemorrhagic enteritis, proventriculitis and depletion of lymphocytes in bursa of Fabricius. In OSL supplemented E. coli infected group lesions were less severe in magnitude and lasted for shorter duration as compared to non-supplemented E. coli infected group, indicating reduction in severity of the disease and early recovery due to OSL supplementation.

Key words: Chickens, Escherichia coli, Gross lesions, Histopathological lesions, Ocimum sanctum leaf supplementation

Indian poultry has emerged as a self-reliant, technology driven industry, with the capability to produce every essential input for successful poultry farming. But a major constraint affecting growth of poultry industry in India is occurrence of disease outbreaks. Acute form of avian colibacillosis, one of the principal causes of morbidity and mortality, is characterized by septicemia resulting in heavy mortality and its subacute form is characterized by pericarditis, airsacculitis and peri-hepatitis (Kabir 2010). E. coli strains are often resistant to cephradine, tetracyclines, chloramphenicol, sulfonamides, ß-lactam antibiotics and antiviral activity in vitro (Geeta et al. 2001, Chauhan et al. 2002), anti-inflammatory (Singh and Jaggi 2003), antioxidant (Reddy et al. 2009, Bharavi et al. 2010), hepatoprotective (Lahon and Das 2011), cardioprotective (Panda and Naik 2009), and immunomodulatory properties (Bharath et al. 2011). O. sanctum leaf supplementation reduces the severity of hydropericardium syndrome in broiler chickens and enhances the cell-mediated response (Batra and Gupta 2006). Similarly, Mamta (2004) observed the immunomodulatory effect of tulsi on Salmonella Galinarum infection in chickens. However, the literature on in vivo studies regarding the effect of this medicinal plant on pathological lesions and immunological response against E. coli infection in poultry is sparse. The present study was conducted to investigate the effect of O. sanctum on pathology of E. coli infection.

MATERIALS AND METHODS

Experimental design: Day-old chicks (160) were divided into group A and B containing 80 birds each. Initial body weights of chicks in group A and B was 35.4±0.51g and 35.6±0.51g, respectively. The birds of group A were fed on feed supplemented with dried Ocimum sanctum leaf powder mixed in feed @5g/kg feed. The birds of group B were given feed without Ocimum sanctum leaf powder supplementation. After 7 days of age, the birds of both the groups were further divided into 2 subgroups (A1 and A2
and B1 and B2) of 45 and 35 birds, respectively. All the chicks of groups A1 and B1 were injected with E. coli O78 @ 10^7 CFU/0.5 ml intraperitoneally. The approval for the protocol of present study including the use of broiler chicks was taken from Institutional Animal Ethics Committee (IAEC) and the experiment was conducted as per its guidelines.

Pathological studies: Randomly selected chicks (5) were sacrificed at day 0, 2, 4, 7, 14, 21 and 28 post infections. Mortality occurring post inoculation was recorded in all groups. Detailed post mortem examination of chickens that died naturally during the course of study or sacrificed at above mentioned intervals was carried out. Organs like heart, liver, spleen, bursa of Fabricius, lungs and intestines were examined for gross lesions. Tissue pieces of these organs particularly at the place of lesions were collected in 10% buffered formalin for histopathological examination. The formalin fixed tissues were processed for paraffin embedding technique. The tissues were properly trimmed, washed in running tap water, dehydrated in graded ethyl alcohol, cleared in cedar wood oil and embedded in paraffin wax (melting point 60–62°C). Sections of 4μ thickness were cut using automatic microtome and stained with haematoxylin and eosin (Luna 1968). Heart blood and liver tissue from the infected groups were also collected in sterilized petri-dish under aseptic conditions for reisolation of the organism.

Lesion score: The colibacillosis specific gross lesion score (GLS) and histopathological lesion score (HLS) in different experimental groups were calculated for different organs/tissues at scale of 0 to 4 as detailed below (Witter 1982): 0, no lesion; 1, mild lesions; 2, moderate lesions; 3, moderately severe lesions; 4, severe lesions. Per cent mean gross and histopathological lesions were calculated as per Witter (1982).

Protective effect: Per cent protective effect due to Ocimum sanctum (tulsi) supplementation in E. coli infected chickens was calculated on the basis of GLS and HLS (Witter 1982).

RESULTS AND DISCUSSION

Mortality: The total mortality due to colibacillosis in non-supplemented E. coli infected group B1 was 35%, which was more as compared to OSL supplemented E. coli infected group and the difference in mortality between both the infected groups was about 15% indicating protective effect of OSL supplementation. There was no mortality in control groups A2 and B2. Decrease in mortality was reported in fowl typhoid and hydropericardium syndrome affected chicks following tulsi leaf supplementation (Mamta 2004, Batra 2004). The reduction in mortality may be attributed to antimicrobial, adaptogenic and anti-inflammatory property of O. sanctum. Furthermore, in vitro studies revealed that alkaloid, steroids and tannins of O. sanctum leaf showed antimicrobial activity against E.coli (Sadul et al. 2012).

Gross pathology: The characteristic gross lesions of colibacillosis observed in the present study were congestion in organs such as liver, heart, lungs, spleen and kidneys, which were observed on 2 DPI. Later on heart and liver were enlarged covered with thick fibrinous layer indicating pericarditis and pericarditis and resulting in adhesions with abdominal wall as well as with other visceral organs (Fig. 1 a). Besides, there was peritonitis, congestion and enlargement of spleen, congestion and consolidation of lungs, hemorrhagic enteritis and atrophy of bursa of Fabricius. Severity of these lesions was highest at 7 DPI. The gross lesions in OSL supplemented infected group were of less intensity at different intervals as compared to those observed in non-supplemented infected group (Fig. 1 b). The reduction in the severity of the lesions due to OSL supplementation might be attributed to antioxidant property of O. sanctum resulting in activation of enzymes such as superoxide dismutase, catalase and glutathione peroxidase (Kim et al. 2010). Furthermore, O. sanctum was also reported to decrease the activities of cardiac marker enzymes (aspartate transaminase, lactate dehydrogenase and creatine phosphokinase) in serum following myocardial injury in rodents (Panda and Naik 2009). These findings support hepatoprotective and cardioprotective properties of tulsi. Protective effect of tulsi on liver and heart was also reported by others (Chattopadhyay et al. 1992, Panda and Naik 2009, Lahon and Das 2011).

Histopathology: Histopathological changes observed in heart due to colibacillosis in the present study were fibrous pericarditis and myocarditis near pericardium characterized by accumulation of fibrin, infiltration of heterophils and lymphocytes in early stage and macrophages in later stage (Fig. 2a). Similarly, liver revealed fibrinous pericarditis and hepatitis along with degenerative changes particularly fatty changes in hepatocytes and dilatation of sinusoids at different intervals of post infection. Pericarditis was also characterized by presence of fibrin and infiltration of heterophils in early stage and macrophages in later stage (Fig. 3a). These histopathological features of fibrinous pericarditis and pericarditis were of lower magnitude in OSL supplemented infected group at different intervals and lasted for shorter duration (Fig. 2b, 3b). Antioxidant activity of O. sanctum and its flavonoids might also contribute for hepatoprotective and cardioprotective potential of O. sanctum (Shetty et al. 2008). O. sanctum extract has also the ability to scavenge highly reactive free radicals (Kelm et al. 2000). The differences in severity of hepatic and cardiac lesions between both the infected groups might be attributed to anti-liperoxidative activity (Bhattacharya et al. 2001) and anti-inflammatory activity (Godhwani et al. 1987). Hepatoprotective and cardioprotective potential of O. sanctum leaf extract were also demonstrated in rats/mice (Chattopadhyay et al. 1992, Sharma et al. 2002).

Histopathological lesions observed in lungs due to colibacillosis in the present study were congestion and hemorrhages in early stage and focal pneumonia lesions at later stage of the infection i.e. 7 DPI onwards (Fig. 4(a)). However, pneumatic lesions in OSL supplemented infected
cells in the mucosa and serosa along with destruction of the glandular epithelium. It appears that there is no report in the literature regarding pathological changes in proventriculus due to colibacillosis. However, inflammatory reaction in serosal layer of different organs particularly in liver and heart was reported as consistent finding of *E. coli* infection (Baliarsingh et al. 1993, Manimaran et al. 2003) and in the present study too.

These histopathological results observed in liver, heart, lungs and intestine suggested that *O. sanctum* leaf supplementation caused considerable reduction in inflammatory reaction due to colibacillosis. However, inflammatory reaction in serosal layer of different organs particularly in liver and heart was reported as consistent finding of *E. coli* infection (Baliarsingh et al. 1993, Manimaran et al. 2003) and in the present study too.

These histopathological results observed in liver, heart, lungs and intestine suggested that *O. sanctum* leaf supplementation caused considerable reduction in inflammatory reaction due to colibacillosis. Compounds isolated from *O. sanctum* such as civsilineol, civsimavatine, isothymonin, apigenin, rosavinic acid and eugenol were reported to possess cyclooxygenase–1 inhibitory activity responsible for anti-inflammatory activity (Kelm et al. 2000). Furthermore, linoleic acid extracted from *O. sanctum* was reported to block both the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism which might be responsible for the anti-inflammatory activity (Singh 1998). Recently, Hemalatha et al. (2011) reported that anti-inflammatory property of *O. sanctum* might be due to suppression of NFkB classicalpathway observed in mice model.

Colibacillosis in the present study caused severe
depletion of lymphocytes in spleen and bursa of Fabricius associated with necrosis of lymphocytes in spleen and infiltration of heterophils and reticulo endothelial cells proliferation in bursa of Fabricius. *E. coli* infected group revealed cyst like structures in the bursal follicles along with inflammatory cells in the interfollicular space (Fig. 5a). However, *E. coli* infected and OSL supplemented group revealed only mild depletion of lymphocytes in the bursal follicles (Fig. 5b). These results revealed that colibacillosis caused immunosuppression and are in accordance with findings of Hegazy *et al.* (2010), who also reported depletion of lymphocytes mainly in bursa of Fabricius in *E. coli* O78 infection in chickens.

On the basis of gross and histopathological lesions in different organs, the lesion scores in both the infected groups were calculated to quantify protective effect of OSL supplementation. Colibacillosis specific gross and histopathological lesion scores in heart, liver, spleen, lungs, bursa of Fabricius and intestine of OSL supplemented infected group were of lower magnitude as compared to non-supplemented infected group (Table 1).

Per cent protective effect due to *O. sanctum* leaf supplementation in *E. coli* infected chicks on the basis of gross lesion scores and histopathological lesion scores was 32.43 and 34.76%, respectively indicating that OSL supplementation in *E. coli* infected group has reduced the severity of pathological lesions of colibacillosis (Table 2).

On the basis of overall results noticed in the present study it may be inferred that supplementation of *O. sanctum* leaf @ 5g/kg feed caused reduction in severity as well as recovery period of *E. coli* infection suggesting its protective effect on limiting the pathology and pathogenesis of *E. coli* infection in broiler chickens.

### REFERENCES


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### Table 1. Overall per cent mean scores in different organs irrespective of post infection period in different experimental groups

<table>
<thead>
<tr>
<th>Lesion score</th>
<th>Groups</th>
<th>Overall per cent mean scores in different organs irrespective of post infection period</th>
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<tr>
<td></td>
<td>Heart</td>
<td>Liver</td>
</tr>
<tr>
<td>HLS Group A1</td>
<td>35.71</td>
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<tr>
<td>Group B1</td>
<td>50.71</td>
<td>40.00</td>
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GLS: Gross lesion score; HLS, histopathological lesion score.

### Table 2. Per cent protective effect due to *Ocimum sanctum* leaf powder supplementation

<table>
<thead>
<tr>
<th>Lesion score</th>
<th>Groups</th>
<th>Overall per cent mean protective effect due to <em>Ocimum sanctum</em> leaf powder supplementation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Heart</td>
<td>Liver</td>
</tr>
<tr>
<td>GLS Group A1</td>
<td>24.28</td>
<td>32.43</td>
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<tr>
<td>Group B1</td>
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<td>HLS Group A1</td>
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<td>34.76</td>
</tr>
<tr>
<td>Group B1</td>
<td>38.69</td>
<td></td>
</tr>
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</table>

GLS, Gross lesion score; HLS, histopathological lesion score.


