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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 peri-carditis, airsacculitis and peri-hepatitis (Kabir 2010). *E. coli* strains are often resistant to cephradine, tetracyclines, chloramphenicol, sulfonamides, ß-lactam antibiotics and amino-glycosides (Bass *et al.* 1999, Rahman *et al.* 2004, Li *et al.* 2007, Hooda 2009, Renu 2010). Resistance to fluoroquinolones is also reported in poultry (Blanco *et al.* 1997, White *et al.* 2000, Van den Bogaard *et al.* 2001, Li *et al.* 2007). Alternatively use of herbal medicines is encouraged as these are ecologically safe. *Ocimum sanctum* (tulsi) is very important for its therapeutic potentials, and its leaf powder/extract has shown to possess antibacterial 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.

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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 perihepatitis 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 fibrinous 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 perihepatitis and hepatitis along with degenerative changes particularly fatty changes in hepatocytes and dilatation of sinusoids at different intervals of post infection. Perihepatitis 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 perihepatitis 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 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-lipoperoxidative 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 pneumonic lesions at later stage of the infection i.e. 7 DPI onwards (Fig. 4(a)). However, pneumonic lesions in OSL supplemented infected

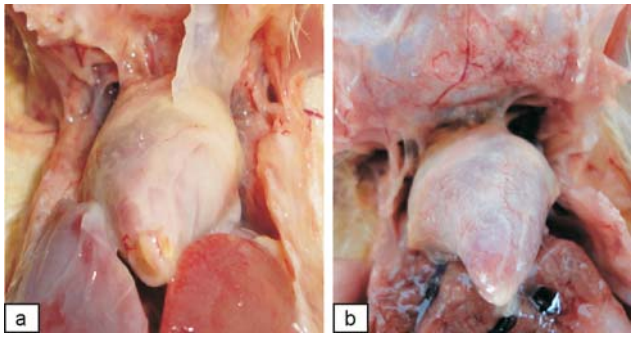

Fig. 1 (a-b). **(a)** Group B1 (4DPI): Thick fibrinous mass on the surface of heart (pericarditis). **(b)** Group A1 (4 DPI): Thin fibrinous mass on the surface of heart.

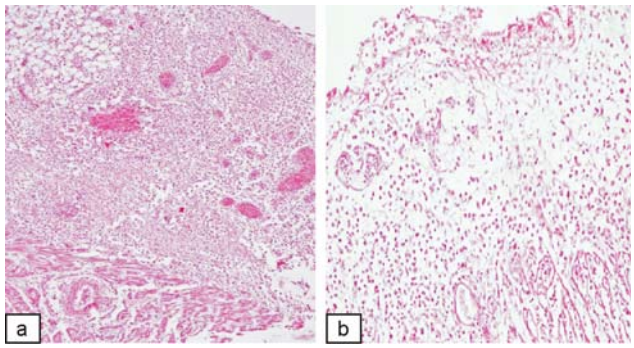

Fig. 2 (a-b). **(a)** Heart (group B1, 7DPI): Severe pericarditis characterized by fibrin and large number of heterophils and mononuclear cells. H & E 50×. **(b)** Heart (group A1, 7DPI): Moderate pericarditis characterized by infiltration of heterophils and fibrin. H & E 100×.

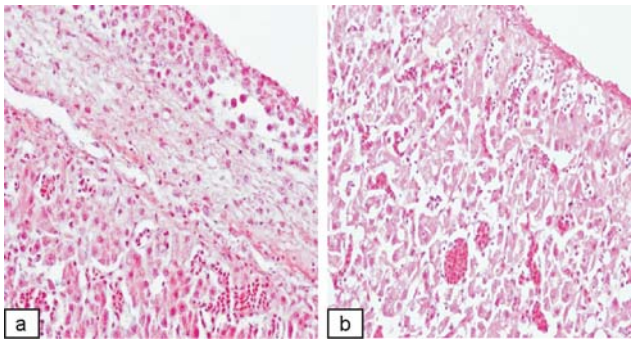

Fig. 3 (a-b). **(a)** Liver (group B1, 4DPI): Perihepatitis characterized by large amount of fibrin and infiltration of heterophils along with congestion in the sinusoids. H & E 200×. **(b)** Liver (group A1, 4DPI): Mild fibrin and presence of fatty changes and congestion in sinusoids. H & E 200×.

group were mild in nature as compared to non-OSL supplemented group (Fig. 4b). Intestines revealed hemorrhagic enteritis in broiler chicks infected with *E. coli* though the lesions in non-OSL supplemented infected group appeared early i.e. 2 DPI and in OSL supplemented infected group at a later stage i.e. 4 DPI. Furthermore, the lesions at different intervals in OSL supplemented infected group were not as severe as in non-OSL supplemented infected group. Proventriculus, in the present study revealed proventriculitis characterized by infiltration of heterophils and mononuclear

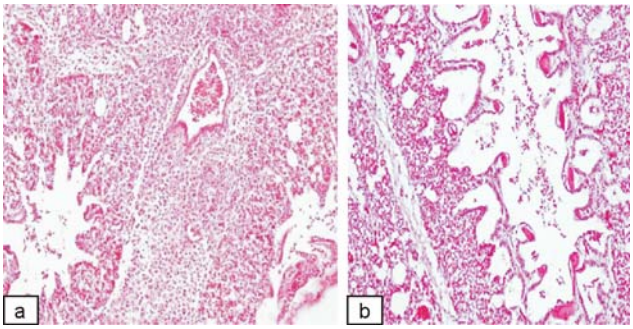

Fig. 4(a-b). **(a)** Lung (group B1, 14DPI): Severe pneumonia characterized by infiltration of lymphocytes and few heterophils around the bronchiole along with congestion in blood vessels. H & E 100×. **(b)** Lung (group A1, 14DPI): Congestion and mild pneumonia characterized by presence of mononuclear cells infiltration in the alveoli. H & E 100×.

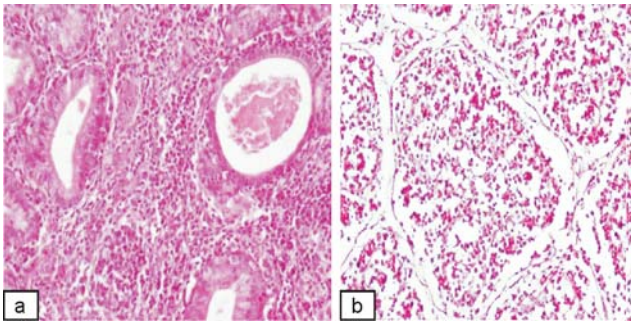

Fig. 5 (a-b). **(a)** Group B1, 14DPI): Cyst like structures in the bursal follicles along with inflammatory cells in the interfollicular space. H & E 200×. **(b)** Bursa of Fabricius (group A1, 14DPI): Mild depletion of lymphocytes in the bursal follicles. H & E 200×.

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. 1*993, 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 classical pathway observed in mice model.

Colibacillosis in the present study caused severe

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

Lesion score	Groups	Overall per cent mean scores in different organs irrespective of post infection period					
		Heart	Liver	Spleen	Bursa	Lungs	Intestine
GLS	Group A1	35.71	27.14	22.86	20.00	20.71	19.28
	Group B1	47.14	41.43	30.00	33.57	30.00	33.57
HLS	Group A1	30.00	27.14	27.86	23.57	22.86	20.00
	Group B1	50.71	40.00	40.00	41.43	31.43	28.57

GLS: Gross lesion score; HLS, histopathological lesion score

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

Lesion score	Groups	Overall per cent mean lesion scores irrespective of post infection period and organs	Per cent protective effect due to *Ocimum sanctum* leaf powder supplementation
GLS	Group A1	24.28	32.43
	Group B1	35.95	
HLS	Group A1	25.24	34.76
	Group B1	38.69	

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

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

Baliarsingh S K, Rao A G and Mishra P R. 1993. Pathology of experimental colibacillosis in chicks. *Indian Veterinary Journal* **70**: 808–12.

Bass L, Liebert C A, Lee M D, Summers A O, White D G, Thayer S G and Maurer J J. 1999. Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **43**: 2925–29.

Batra M. 2004. 'Studies on effect(s) of *Ocimum sanctum* (Tulsi) leaf powder on pathology and pathogenesis of experimentally induced hydropericardium syndrome in broiler chickens.' M.V.Sc. thesis, CCS Haryana Agricultural University, Hisar.

Batra M and Gupta R P. 2006. Effects of *Ocimum sanctum* leaf on pathology and immune response in chickens experimentally infected with hydropericardium syndrome. *Indian Journal of Veterinary Pathology* **30**: 1–4.

Bharath B K, Anjaneyulu Y and Srilatha Ch. 2011. Immuno-modulatory effect of *Ocimum sanctum* against endosulfan induced immunotoxicity. *Veterinary World* **4**: 25–27.

Bharavi K, Reddy A G, Rao G S, Reddy A R and Rao S V R. 2010. Reversal of cadmium-induced oxidative stress in chicken by herbal adaptogens *Withania somnifera* and *Ocimum sanctum. Toxicology International* **17**: 59–63.

Bhattacharya S K, Bhattacharya A, Das K, Murunganadam A A and Sairam K. 2001. Further investigation on the antioxidant activity of *Ocimum sanctum* using different paradigms of oxidative stress in rats. *Journal of Natural Remedies* **1**: 6–16

Blanco J E, Blanco M, Mora A and Blanco J. 1997. Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. *Journal of Clinical Microbiology* **35**: 2184–85.

Chattopadhyay R R, Sarkar S K, Ganguly S, Medda C and Basu T K. 1992. Hepatoprotective activity of *Ocimum sanctum* leaf extract against paracetamol induced hepatic damage in rats. *Indian Journal of Pharmacology* **24**: 163–65.

Chauhan D K, Singh V K, Govil J N and Singh G. 2002. Some potential herbal drugs for AIDS. Recent progress in medicinal plants. *Ethnomedical and Pharmacognosy* **2**: 239–41.

Geeta, Vasudevan D M, Kedlaya R, Deepa S and Ballal M. 2001. Activity of *Ocimum sanctum* (the traditional Indian medicinal plant) against enteric pathogens. *Indian Journal of Medical Science* **55**: 434–38.

Godhwani S, Godhwani J L and Vyas D S. 1987. *Ocimum sanctum*: an experimental study evaluating its anti-inflammatory, analgesic and antipyretic activity in animals. *Journal of*

Ethnopharmacology **21**: 153–63.

Hegazy M, Abd-El Samie L K and El Sayed E M. 2010. The immunosuppressive effect of *E. coli* in chickens vaccinated with infectious bronchitis (IB) or infectious bursal disease (IBD) vaccines. *Journal of American Sciences* **6**: 762–67.

Hemalatha R, Babu K N, Karthik M, Ramesh R, Kumar B D and Kumar P U. 2011. Immunomodulatory activity and Th1/Th2 cytokine response of *Ocimum sanctum* in myelosuppressed Swiss Albino mice. *Trends in Medical Research* **6**: 23–31.

Hooda A. 2009. 'Etio-pathological studies on poultry mortality with special reference to gastrointestinal tract disorders.' M.V.Sc. thesis, CCS Haryana Agricultural University, Hisar.

Kabir S M L. 2010. Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *International Journal of Environmental Research and Public Health* **7**: 89–114.

Kelm M A, Nair M G, Strasburg G M and DeWitt D L. 2000. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. *Phytomedicine* **7**: 7–13.

Kim S C, Magesh V, Jeong S J, Lee H J, Ahn K S, Lee E O, Lee M H, Kim J H and Kim S H. 2010. Ethanol extract of *Ocimum sanctum* exerts anti-metastatic activity through inactivation of matrix metalloproteinase-9 and enhancement of anti-oxidant enzymes. *Food and Chemical Toxicology* **48**: 1478–82.

Lahon K and Das S. 2011. Hepatoprotective activity of *Ocimum sanctum* alcoholic leaf extract against paracetamol-induced liver damage in Albino rats. *Pharmacognosy Research* **3**: 13–18.

Li X S, Wang G Q, Du X D, Cui B A, Zhang S M and Shen J Z. 2007. Antimicrobial susceptibility and molecular detection of chloramphenicol and florfenicol resistance among *Escherichia coli* isolates from diseased chickens. *Journal of Veterinary Science* **8**: 243–47.

Luna L G. 1968. *Manual of Histologic Staining Method of Armed Forces Institute of Pathology*. 3rd edn. Mc Graw Hill Book Company, New York.

Mamta. 2004. 'Studies on effects of *tulsi* (*Ocimum sanctum)* leaf powder on pathology and pathogenesis of experimental Fowl Typhoid in broiler chickens.' M.V.Sc. thesis, CCS Haryana Agricultural University, Hisar.

Manimaran K, Singh S D and Shivachandra S B. 2003. Haematobiochemical and pathological changes in experimental *Escherichia coli* infection in broiler chicks. *Indian Journal of Animal Sciences* **73**: 960–62.

Panda V S and Naik S R. 2009. Evaluation of cardioprotective activity of *Ginkgo biloba* and *Ocimum sanctum* in rodents. *Alternative Medicine Review* **14**: 161–71.

Rahman M A, Samad M A, Rahman M B and Kabir S M L. 2004. *In vitro* antibiotic sensitivity and therapeutic efficacy of experimental salmonellosis, colibacillosis and pasteurellosis in broiler chickens. *Bangladesh Journal of Veterinary Medicine* **2**: 99–102.

Reddy L S S V P, Thangavel A, Leela V, Narayana and Raju K V S. 2009. Antioxidant enzyme status in broilers: role of dietary supplementation of tulasi (*Ocimum sanctum*) and selenium. *Journal of Veterinary and Animal Science* **5**: 251–56.

Renu. 2010. 'Pathological investigation of the diseases affecting gastrointestinal tract of poultry.' M.V.Sc. thesis, CCS Haryana Agricultural University, Hisar.

Sadul R R, Gidde M R and Bipinraj N K. 2012. Comparative study of antimicrobial activity and phytochemical screening of aqueous and alcoholic leaf extract of *Ocimum sanctum* on *E.coli. Journal of Environmental Research Development* **7**: 312–20.

Sharma M K, Kumar M and Kumar A. 2002. *Ocimum sanctum* aqueous extract provides protection against mercury induced toxicity in Swiss albino mice. *Indian Journal of Experimantal Biology* **40**: 1079–82.

Shetty S, Udupa S and Udupa L. 2008. Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of *Ocimum sanctum* Linn in rats. *Evid. Based Complement. Alternative Medicine* **5**: 95–101.

Singh B and Jaggi R K. 2003. Antiinflammatory effect of *Ocimum sanctum* Linn. and its cultures. *Indian Journal of Pharmaceutical Sciences* **65**: 425–28.

Singh S. 1998 Comparative evalution of antiinflammatory potential of fixed oil of different species of *Ocimum* and its possible mechanism of action. *Indian Journal of Experimantal Biology* **36**: 1028–31.

Van den Bogaard A E, London N, Driessen C and Stobberingh E E. 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. *Journal of Antimicrobial Chemotherapy* **47**: 763–71.

White D G, Piddock L J, Maurer J J, Zhao S, Ricci V and Thayer S G. 2000. Characterization of fluoroquinolone resistance among veterinary isolates of avian *Escherichia coli*. *Journal of Antimicrobial Agents and Chemotherapy* **44**: 2897–99.

Witter R L. 1982. Protection by attenuated and polyvalent vaccines against highly virulent strains of Mareks disease virus. *Avian Pathology* **11**: 49–62.