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Research Article

AMELIORATING EFFECT OF TULSI (OCIMUM SANCTUM) LEAF POWDER ON PATHOLOGY OF SALMONELLA GALLINARUM INFECTION IN BROILER CHICKENS

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ABSTRACT

The present study was undertaken to investigate the effects of dried Tulsi (*Ocimum sanctum*) leaf powder on pathology of *Salmonella gallinarum* in chickens. A total of 160, day-old unvaccinated *Salmonella* free broiler chicks were randomly divided into two groups (I and II), each group had 80 chicks. Group I was given normal feed and water while chicks in group II were given feed supplemented with Tulsi leaf powder @ 5g/kg feed. At 7 days of age, chicks in each group were divided into 2 subgroups of 30 and 45 chicks thus forming four groups viz. A and B (from Group I) and C and D (from Group II). Chicks in groups A and C (each having 30 chicks) were kept as uninfected groups while chicks in groups B and D (each having 45 chicks) were infected with ID_{50} dose of *S. gallinarum* by the subcutaneous route. Maximum mortality (18/45, 40.0%) was recorded in group B (given normal feed and infected), whereas in group D (given feed supplemented with Tulsi dried leaf powder and infected), overall mortality was comparatively low (10/45, 24.44 %). Gross lesions observed in group B chicks were typically of fowl typhoid. Initially there was congestion and haemorrhages in the most of visceral organs. Liver and spleen were enlarged and had necrotic foci. Heart showed fibrinous pericarditis, myocarditis and necrotic nodules. Chicks of group D revealed similar lesions with mild intensity. Number and size of necrotic foci were less in liver, heart and spleen of Tulsi supplemented chicks (group D). Microscopically, necrotic foci in liver, spleen and heart, fibrinous pericarditis and myocarditis, vascular and degenerative changes in lungs and intestine and depletion of lymphoid tissue and focal necrotic areas in bursal follicles were observed in chicks of group B. Similar lesions of less severity were observed in Tulsi supplemented chicks (group D) where recovery from the disease was earlier than the group B birds.

Key words: *Salmonella gallinarum*, chickens, pathology, Tulsi

Fowl typhoid is an acute septicaemic disease of chickens affecting all age groups and is caused by *Salmonella gallinarum*. Poultry industry is facing great economic losses in form of morbidity, mortality, reduced growth rate, reduced feed conversion efficiency, drop in egg production and low fertility and hatchability due to this disease. Kumar *et al.* (2010) while studying epidemiology of fowl typhoid from 2005-2008, reported 227 outbreaks of this disease in broiler chicks in Haryana state. They reported more outbreaks in chicks of 7 to 9 days old and in extreme weather conditions. Maximum mortality and case fatality rate were found in birds of 1-2 weeks age.

In ethnoveterinary practice, various herbal and medicinal plants have been used to manage poultry diseases (Dold and Cocks, 2001; Waihenya *et al.*, 2002). Tulsi (*Ocimum sanctum*) has been reported to possess various medicinal properties like antibacterial, anti-inflammatory, immuno-modulatory and hepato-protective properties (Gupta *et al.*, 2002). There is paucity of literature pertaining to use of Tulsi leaves in broiler chickens as antimicrobial and immunomodulator. The present study was planned to investigate the effects of dried Tulsi leaf powder on pathology of *Salmonella gallinarum* in chickens.

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MATERIALS AND METHODS

Salmonella strain: *S. gallinarum* strain used for inducing experimental fowl typhoid in the present study was obtained from Indovax Diagnostic Laboratory, Hisar. The characterization of strain was carried out by growth characteristics, morphology, staining and biochemical tests as per methodology described by Cruickshank *et al.* (1965). The strain was serially passaged through 2 to 6 weeks old broiler chickens four times by subcutaneous route to enhance its pathogenicity before using it for experimental reproduction of disease.
Tulsi leaf powder: Fresh leaves of Tulsi were plucked, washed and then dried in shade. These dried leaves were then powdered with a mixer. Tulsi dry leaf powder (DLP) thus obtained was kept in packets of 25 gms each till use.

Infective dose (ID_{50}): The ID_{50} of *S. gallinarum* in one week old broiler chickens was determined to be 8.2 x 10^8 organisms/ml of BHI broth by subcutaneous route as per the method of Reed and Muench (1938).

Experimental design: A total of 160, day old unvaccinated *Salmonella* free broiler chicks were randomly divided into two groups (I and II) with 80 chicks in each group. Group I was given normal feed and water while chicks in group II were given feed supplemented with DLP Tulsi @ 5g/kg feed. At 7 days of age, chicks in each group were divided into 2 subgroups of 30 and 45 chicks thus forming four groups viz. A and B (Group I) and C and D (Group II). Chicks in groups A and C (each having 30 chicks) were kept as uninfected groups while chicks in groups B and D (each having 45 chicks) were infected with ID_{50} dose of *S. gallinarum* by the subcutaneous route.

Pathological studies: To study the sequential gross and histopathological changes, three randomly selected broiler chickens from each group were sacrificed at 0, 3, 5, 7, 10, 14, 21, 28 and 35 days post infection (DPI). Mortality occurring post inoculation was recorded in all experimental groups. Detailed post mortem examination of chickens that died naturally during the course of study or sacrificed at above mentioned intervals was carried out. Gross lesions particularly in liver, heart, spleen, lungs, trachea, oesophagus, intestine, kidneys, bursa of Fabricius and brain were recorded. Representative pieces from different organs were collected and fixed in 10 per cent formal saline for histopathological studies. The tissues were processed for paraffin embedding and sections of 4 µ thickness were cut. These sections were stained by haematoxylin and eosin stains (Luna, 1968).

RESULTS AND DISCUSSION

Following inoculation, maximum mortality (18/45, 40%) was recorded in group B (given normal feed and infected) which occurred upto 5 DPI and continued upto 10 DPI. Whereas in group D (given feed supplemented with Tulsi DLP and infected), overall mortality was comparatively low (10/45, 24.44%). No mortality was observed in uninfected chicks of groups A and C.

Pathological changes

Gross changes: Following *S. gallinarum* infection, typical lesions of fowl typhoid were observed in Tulsi DLP supplemented (group D) and non supplemented (group B) chickens. Lesions in most of the visceral organs suggested systemic dissemination of *S. gallinarum* after initial colonization. These included septicaemic changes like congestion and haemorrhages in various visceral organs along with hepatomegaly and splenomegaly during early stages of infection. Later, necrotic foci were evidenced on liver (Fig. 1), spleen and heart. Gall bladder was distended, kidneys were swollen and lungs showed congestion and consolidation. Bursa of Fabricius was atrophied. The lesions, in general, were of milder intensity in Tulsi DLP supplemented chicks (group D) as compared to non supplemented chicks (group B). Gross changes like splenomegaly, hepatomegaly and necrotic foci on liver, heart and spleen have been reported by Hafeeji *et al.* (2000), Shivaprasad (2000), Prasanna *et al.* (2001), Sujatha *et al*. (2003) in chicks affected with *Salmonella*. However, the bronze discolouration of liver was not observed in the present study.

Histopathological studies: Histopathological alterations were observed principally in liver, spleen, heart, lung, intestine and bursa of Fabricius. Changes were similar in both infected groups (groups B and D) but were less severe and persisted for lesser period of time in group D than group B.

Liver: Initial changes in liver comprised of congestion, haemorrhages, degenerative changes, infiltration of heterophils (Fig. 2) along with few minute foci of necrosis. The intensity of necrosis and cellular reaction increased with the progression of disease. The cellular infiltration changed with increased number of lymphocytes, plasma cells and macrophages on 14 DPI and continued till the end of observation period. Large necrotic foci were observed on 7, 10 and 14 DPI which persisted upto 28 DPI (Fig 3A). On 14 DPI, well defined granulomae of varying sizes were observed in parenchyma. Liver necrosis in group D chicks was noticed on 7 DPI that persisted upto 21 DPI (Fig. 3B). Kupffer cells were found increased on 7 DPI and marked hyperplastic activity was observed on 14 DPI in chicks of group B. In chicks of group B, Kupffer cell hyperplasia was more severe than group D on 14 DPI. Similar degenerative, necrotic and infiltrative changes in liver of chickens affected with fowl typhoid have been reported by Shivaprasad (2000) and Prasanna *et al.* (2001).

Spleen: Histopathological changes in spleen included focal necrosis and depletion of lymphoid tissue which

was prominent in group B chicks on 7 DPI (Fig. 4A) that persisted upto 28 DPI. In Tulsi DLP supplemented chicks (group D), minute necrotic foci were observed on 7 DPI (Fig. 4B) that persisted till 14 DPI. Many secondary lymphoid follicles were observed in splenic parenchyma on 14 DPI in group D. Similar histopathological changes including focal necrosis, RE cell hyperplasia and secondary lymphoid follicles have been reported by Shivaprasad (2000).

Bursa of Fabricius: The microscopic changes in bursa of Fabricius included degeneration and necrosis of lymphoid tissue in follicles, RE cell hyperplasia (Fig. 5) increase in interfollicular connective tissue and hyperplastic changes in bursal epithelium. The changes in bursa in chicks of group D were less marked and milder in intensity. Loss of lymphoid tissue from follicles and cystic degeneration of bursa of Fabricius has been reported by Garren and Barber (1955). These workers suggested that changes in bursa resulted from adverse physiological conditions like anorexia, dehydration, anaemia etc.

Heart: Heart in chicks of group B revealed degeneration and necrosis of myocardial muscle fibres and severe leucocytic infiltration on 7 DPI that persisted till the end of the experiment (Fig. 7). Pericardium showed evidence of pericarditis. Similar but milder lesions were observed in heart of group D birds. Necrotic areas and intense leucocytic infiltration appeared on 7 DPI that persisted upto 14 DPI. Similar vascular and cellular changes along with necrosis of myocardial tissue have been reported by Kokosharov *et al.* (1997), Singh *et al.* (1998) and Shivaprasad (2000).

Kidney: Histopathologically, kidneys in chicks of group B revealed congestion, haemorrhages, degeneration of renal tubular epithelium, necrotic foci and infiltration of heterophils and mononuclear cells indicative of interstitial nephritis. These changes were prominent on 5 DPI and persisted till 35 DPI. Similar changes of milder intensity were observed in group D chicks that were prominent between 5 and 21 DPI. Similar vascular, degenerative and infiltrative changes in kidneys of birds affected with fowl typhoid have been described by Kokosharov *et al.* (1997) and Shivaprasad (2000).

Lungs: Histopathological examination of lungs in chicks of group B revealed severe congestion, haemorrhages and perivascular serofibrinous exudate along with heterophilic and mononuclear cell infiltration in interlobular tissue during early stages of infection. Later on, well defined granuloma was seen in one case on 10 DPI (Fig. 6). These changes persisted upto 21 DPI. Leucocytic aggregates were observed in submucosal area and bronchial lumen contained exudate comprised of desquamated epithelial cell, RBCs and leucocytes. Similar but milder changes were observed in group D chicks supplemented with Tulsi DLP and infected with *S. gallinarum*. Almost similar lesions have been reported by Kaushik *et al.* (1986), Kokosharov *et al.* (1997) and Shivprasad (2000) in fowl typhoid affected birds.

Intestine: The intestine in chicks of group B revealed evidence of catarrhal enteritis. Mucosal villi showed degeneration and desquamation. Goblet cells were hyperplastic. Cellular infiltration comprising of heterophils, lymphocytes and plasma cells was observed in mucosa and between intestinal glands. These changes were observed between 5 and 21 DPI. In group D birds, milder intestinal changes were observed between 5 to 14 DPI. The histopathological changes in affected birds were in congruence with the observations of Hafeeji *et al.* (2000) and Prasanna *et al.* (2001). Smith (1955) reported severe catarrhal and haemorrhagic enteritis. *Salmonella* induced diarrhoea is multifactorial. The onset of fluid secretion is preceded by a massive influx of inflammatory cells leading to release of prostaglandins that stimulate intestinal adenylcyclase mediated fluid secretion (Giannella, 1979).

Proventriculus: Proventriculus of the birds in group B revealed congestion, haemorrhages and degenerative changes along with heterophilic infiltration in mucosa and submucosa. Necrosis of mucosal epithelium and adherence of fibrinous necrotic material was observed in few chicks on 7 DPI. The intense cellular reaction comprising heterophils, lymphocytes and a few macrophages was observed on 7 DPI which persisted till 21 DPI. In group D, similar but milder lesions were observed. Mucosal epithelium and submucosal glands showed degeneration and leucocytic infiltration that persisted till 14 DPI. Thereafter, proventriculus appeared almost normal. Minute submucosal haemorrhages were observed in both infected groups (B and D) as also reported by Rao *et al.* (1952) but these haemorrhages were not seen in the form of band as described by these workers.

Pancreas: Changes in pancreas included congestion, haemorrhages and focal degenerative and necrotic changes in pancreatic acinar cells along with heterophilic and mononuclear aggregates in the form of nodules.

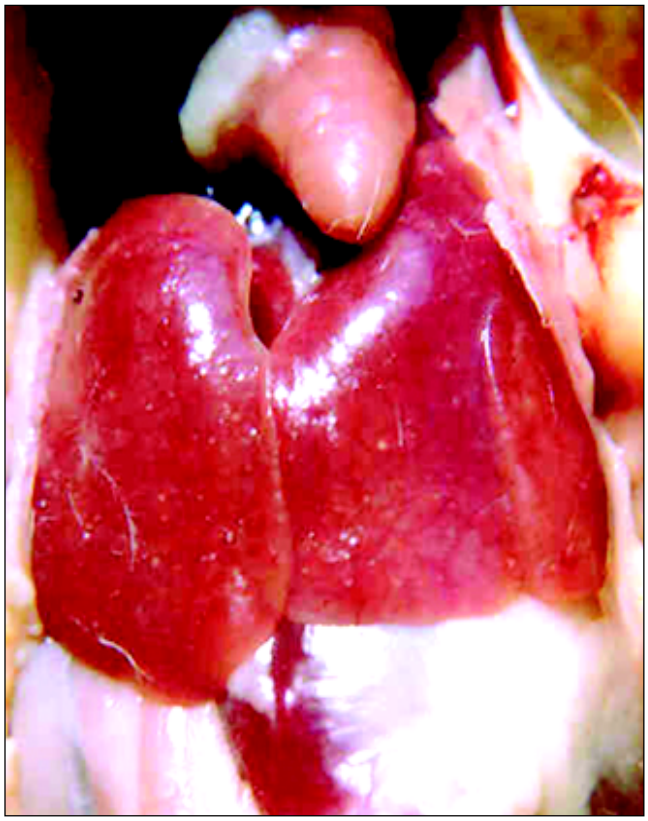

Fig 1. Necrotic foci on liver in a bird from Group B at 7 DPI.

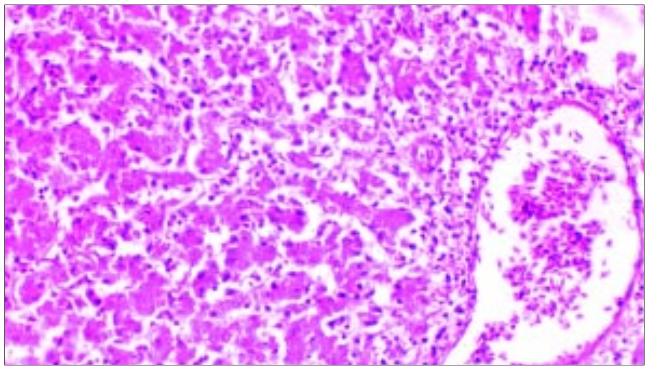

Fig 2. Congestion, degenerative changes and leucocytic infiltration in liver section in Group B at 5 DPI. (H. & E. x 66)

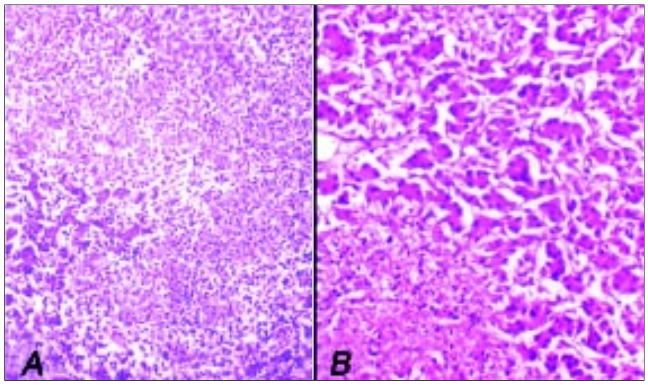

Fig 3 A. Large necrotic areas in hepatic parenchyma with leucocytic infiltration in Group B at 10 DPI. (H. & E. x 33)

B. Necrotic areas infiltrated with leucocytes along with Kupffer cell hyperplasia in liver in Group D at 10 DPI. (H. & E. x 66)

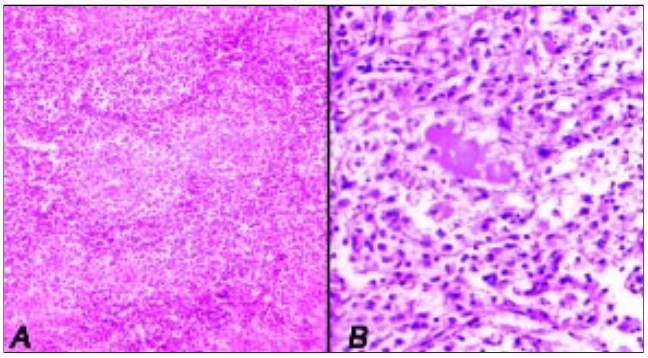

Fig 4 A. Multiple areas of necrosis and lymphoid depletion in white pulp in spleen in Group D at 7 DPI. (H. & E. x 33)

B. Distrinct necrotic areas in splenic corpuscles along with RE cell hyperplasia in Group D at 7 DPI. (H. & E. x 33)

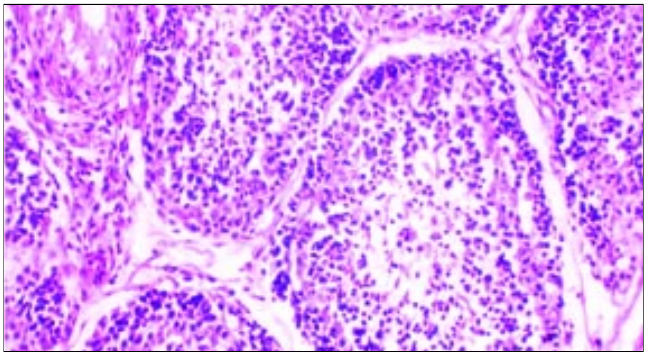

Fig 5. Lymphoid tissue depletion and RE cell hyperplasia in medulla of bursal follicles in Group B at 14 DPI. (H. & E. x 66)

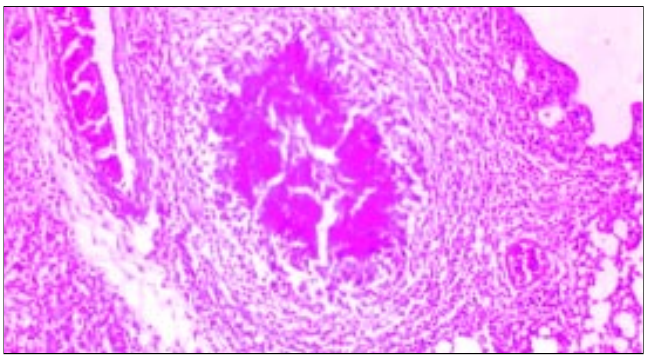

Fig 6. Formation of well defined granuloma with leucocytic infiltration in interstitium of lung in Group B at 10 DPI. (H. & E. x 33)

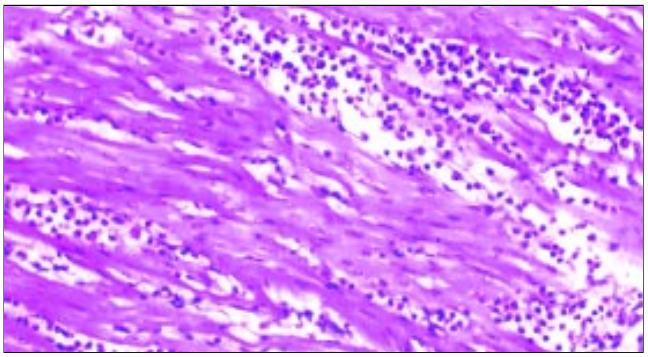

Fig 7. Degenerative changes in myocardial muscle fibres along with heterophilic infiltration in Group B at 10 DPI. (H. & E. x 66)

The changes were milder during initial stages but moderate to severe changes were observed on 14 and 21 DPI. Histopathological changes in pancreas of birds of group D were very mild in the form of mild degenerative and few minute necrotic foci in glandular acinar tissue. The microscopic changes in pancreas were similar to those reported by Madhuri (1997).

Oesophagus: Microscopic examination of oesophagus from birds of group B did not reveal any salient change except mild congestion and haemorrhages in initial stages and submucosal aggregate of lymphocytes on 10 DPI. No appreciable microscopic changes were observed in oesophagus of group D birds.

Brain: Histopathological changes observed in cerebrum of chickens infected with *S. gallinarum* revealed mild degenerative changes in form of perivascular and perineuronal edema along with congestion and minute haemorrhages. On 10 DPI, there was evidence of satellotosis and neuronophagia. No appreciable changes were observed in cerebrum of chicks of group D.

Tulsi leaves contain essential oils that yield eugenol, methyl eugenol and caryophyllene along with other substances. The therapeutic potential of essential oils is largely due to eugenol, a phenolic compound. Its anti-inflammatory effect is due to dual inhibition of arachidonic acid metabolism by blocking both cyclooxygenase and lipooxygense pathways (Singh *et al.,* 1996). Based upon the present investigation, it may be concluded that Tulsi dry leaf powder had protective effects on pathology of experimental *S. gallinarum* infection in broiler chickens as evident from reduced severity of gross and histopathological lesions in chicks fed Tulsi leaves and inoculated with *S. gallinarum*.

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