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Antimicrobial activity of *Cuminumcyminum* extract against avian cholera in chicken embryo

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ABSTRACT

Avian cholera outbreaks are a serious threat to the health of poultry and overall stock viability resulting in terrible economic losses. Pasteurella multocida is the causative agent of avian cholera. This study was conducted to evaluate the antimicrobial activity of the seed extracts of *Cuminumcyminum*, an aromatic plant within the Apiaceae family, against avian cholera in broiler chickens. Five hundred fertilised chicken eggs were divided into three groups, control (C; n=300), second group (O; n=100) and the third group (O+U; n=100) and were injected with distilled water, P. multocida and P. multocida + *Cuminumcyminum* seed extract respectively. After 14 days of incubation, the embryos were extracted and the tissues processed for hematoxylin and eosin staining to study the histopathological changes in the stomach, intestine and spleen tissues. The tissues of P. multocida infected embryos demonstrated lesions, degeneration, atrophy, hemorrhage, necrosis, karyolysis, congestion and desquamation whereas the control groups exhibited normal tissue histology. Interestingly, in spite of being infected by P. multocida, the tissues treated with *Cuminumcyminum* seed extract also demonstrated normal histology. The present study thus showed that *Cuminumcyminum* had an antibacterial role and protected the tissues from the damage induced by the avian cholera infection.

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INTRODUCTION

The etiological agent of avian cholera, also known as fowl cholera, avian pasteurellosis, avian hemorrhagic septicemia, is the Gram-negative coccobacillus *Pasteurella multocida*. It can cause disease in a wide range of animal species and is the causative agent of numerous, economically important diseases, including avian/fowl cholera, bovine haemorrhagic septicaemia, bovine mastitis, enzoonotic pneumonia and swine atrophic rhinitis and (Harper *et al.,* 2006, Wilkie *et al.,* 2012). Avian cholera is a highly contagious disease affecting domestic and wild birds worldwide, the susceptibility being host-dependent (Wilkie *et al.,* 2012, Petersen *et al.,* 2001). The route of infection is nasal (respiratory system) or oral (digestive system), the sources being nasal exudates, excreta, carcasses, contaminated soil and water supplies and mechanical transfer via contaminated shoes and equipment. The factors integral to the outbreaks of avian cholera include complex interactions between the bacterial agent, density of birds and bacteria, and the ambient environment (Qin *et al.,* 2017). The climate of a place is the major determinant of the ambient environment; outbreaks occur in cold and wet weather (in late summer, fall and winter).

Avian cholera is the most common pasteurellosis of poultry (Herath *et al.,* 2010), leading either too high morbidity and mortality or chronic, localised infections. Outbreaks of avian cholera have been

reported worldwide, such as Australia, Europe, Africa and Asia (Qin *et al.,* 2017, Kwon and Kang 2003, Wang *et al.,* 2009). Avian cholera causes high economic losses in chicken and turkey breeders, especially in broiler breeders, due to high mortality, low production of hatching eggs and reduction of fertility (Huberman and Terzolo 2016).

Although drugs are available for the control of avian cholera, mortality may resume after discontinuation of treatment, thus necessitating exploration of alternative methods for control of the disease. *Cuminumcyminum*, a flowering plant of Apiaceae family, was native to Egypt and has been cultivated in the Middle East, India, China and Mediterranean countries (Boning 2010). *Cuminumcyminum* is composed of alkaloid, coumarin, anthraquinone, flavonoid, glycoside, protein, resin, saponin, tannin and steroid and exerts a multitude of pharmacological activities, such as antimicrobial, insecticidal, anti-inflammatory, analgesic, antioxidant, anticancer, antidiabetic, antiplatelet aggregation, hypotensive, bronchodilatory, immunological, contraceptive, anti-amyloidogenic, anti-osteoporotic, aldose reductase, alpha-glucosidase and tyrosinase inhibitory effects, protective and central nervous effects (Al-Snafi 2016). The seed extracts have shown antibacterial activity against 10 Gram-positive and Gram-negative bacteria (Sheikh *et al.,* 2010).

It has been reported that a combination of the aqueous extracts of *Petiveriaalliacea, Cestrum lanatum, Coutareahexandra* and *Jatropha curcas* prevented avian cholera (Carrillo *et al.,* 1997). Another study reported the efficacy of blackberry and blueberry pomace extracts and citrus oil in the inhibition of *P. multocida* growth (Salaheen *et al.,* 2014). In this study, the bactericidal activity of *Cuminumcyminum* was investigated for the first time against *P. multocida* in avian cholera infected broiler chickens.

METHODS

Cumin extract

Capsules containing cumin seeds were procured from the iHerb company, USA. For the purpose of injection into infected chicken embryos, one capsule is diluted in 5 ml of distilled water to obtain the solution of powdered *Cuminumcyminum* seeds.

Management of birds

Chickens (broilers) were obtained from flocks which were afflicted with avian cholera and were in the process of being depopulated. The poultry, Al-Watania Poultry Company in

Al-Qassim (Buridah), Saudi Arabia had declared the birds to be infected based on clinicopathological findings and history of mortality. All the chickens (n=5) were seven-week-old broilers, obtained from a flock size of 45,000 that had experienced 35% mortality over the past six days. Clinical symptoms observed were depression, ataxia, swelling of the combs and wattles.

Experimental design

To study the effect of *Cuminumcyminum* on the histopathology of avian cholera infected tissues, fertilised chicken eggs (n=500) were divided into three groups. Eggs in each group were given different types of intervention, the first / control group (C; n=300), the second group (O; n=100) and the third group (O+U; n=100) were treated with distilled water, *P. multocida* and *P. multocida* + *Cuminumcyminum* extract respectively. 0.5 ml/egg of the respective intervention solution was injected into the yolk sac (air space) on the fifth day of incubation (first week) according to standard techniques (Tavakkoli and Salandari 2014) and incubated at 37.5ºC and 55% relative humidity. The embryos were extracted after day 14 of incubation and tissue specimens of the stomach, small intestine and spleen were dissected out. The samples were processed by routine paraffin embedding technique. Briefly, the tissues were fixed in 10% formaldehyde, dehydrated in ascending grades of ethanol, cleared with xylene and embedded in paraffin wax followed by sectioning. Thin sections of 5 µm were stained with Haematoxylin-Eosin stain (H&E) and observed under a light microscope.

RESULTS

Histopathology of stomach

Histopathological examination of the stomach tissues from the three groups revealed that the control group was normal with all the layers, namely mucosa, submucosa, muscularisexterna and serosa, the lumen and the gastric glands being intact (Figure 1a, b). In contrast, infection with avian cholera caused lesions in the tissues to a great extent (Figure 1 c, d and e). The surface of the stomach, serosa, was found to be desquamated. The mucosa and muscularisexterna exhibited irregularities such as degeneration and atrophy while most of the cells of the mucosal epithelium were haemorrhaged. The submucosa was absent, and the gastric glands were shrunken. Notably, treatment with *Cuminumcyminum* helped in maintaining the normal histological structure (Figure 1 e, f).

Histopathology of the small intestine

The small intestine tissues of the control group appeared normal under light microscopic, where all the layers, serosa, muscularisexterna, submucosa

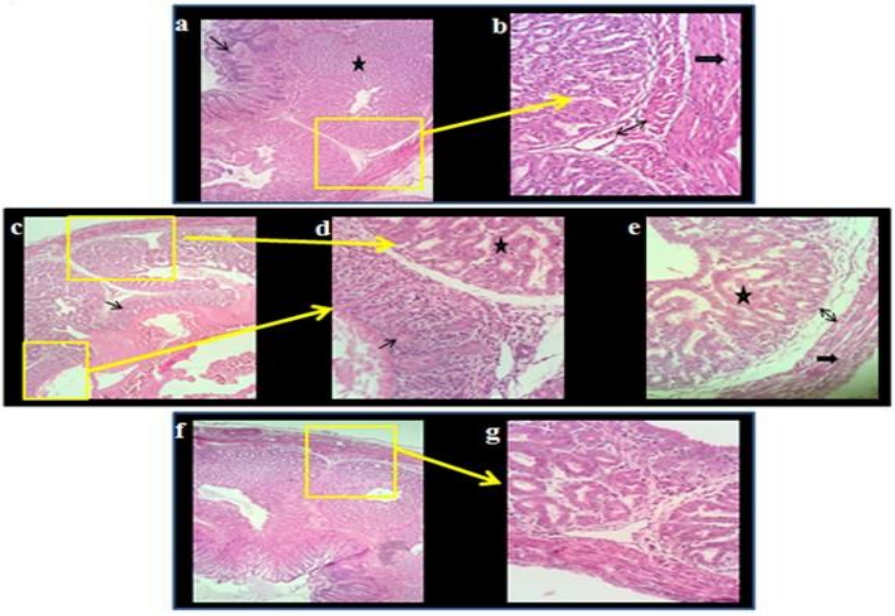

Figure 1: Examination of stomach tissue with light microscopic. Panels a and b represent the control group histological structure that contains lumen, mucosa, mucosa epithelium (↑), gastric glands (✶), submucosa (⇔), muscularis mucosae, muscularisexterna (⇨), serosa. Panels c, d and e represent the lesions caused by avian cholera; stomach mucosal arrangement, irregular and degenerated (↑), absence of submucosa (⇔), atrophy, shrunken muscularis mucosae (⇔), muscularisexterna, hemorrhage in most of the mucosal epithelium (↑), shrunken and damaged gastric glands (✶), edema interstitial muscularis mucosae, muscularisexterna (⇨). Panels f and g represent the tissues treated with *Cuminumcyminum* that are similar to the control group. The boxes and arrows (yellow) indicate the portion that is enlarged in the next figure in that row. The magnification at which the images are taken is, 100X (a, c, f) and 400X (b, d, e, g).

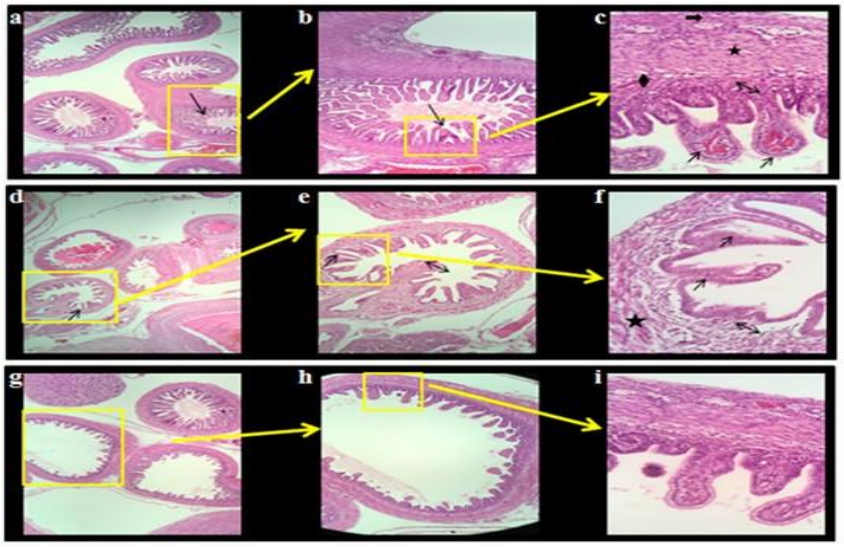

Figure 2: Examination of intestine tissue with light microscopic. Panels a, b and c representing the control group show normal histological structure, containing intestinal lumen, mucosa, mucosal epithelium (↑), villi (↑), intestinal crypts (⇔), lamina propria, blood capillaries, muscularis mucosae, submucosa (♦), muscularisexterna (✶), serosa (⇨). Panels d, e and f represent the infected tissues where lesions were observed in sections of small intestine, thinner with marked degeneration, shrunken, atrophy, desquamation, vacuolization in most cells. The tips of the villi (↑) were degenerated, shrunken, karyolitic and necrotic. Muscularis mucosa and muscularisexterna (✶) showed degeneration, atrophy and fibrosis. Intestinal crypts (⇔) showed necrosis, and blood capillaries of lamina propria exhibited haemorrhage. Panels g, h and I represent the tissues treated with *Cuminumcyminum* that show similar histology as that of the control group. The boxes and arrows (yellow) indicate the portion that is enlarged in the next figure in that row. The magnification at which the images are taken are, 40X (a, d, g), 100X (b, e, h) and 400X (c, f, i).

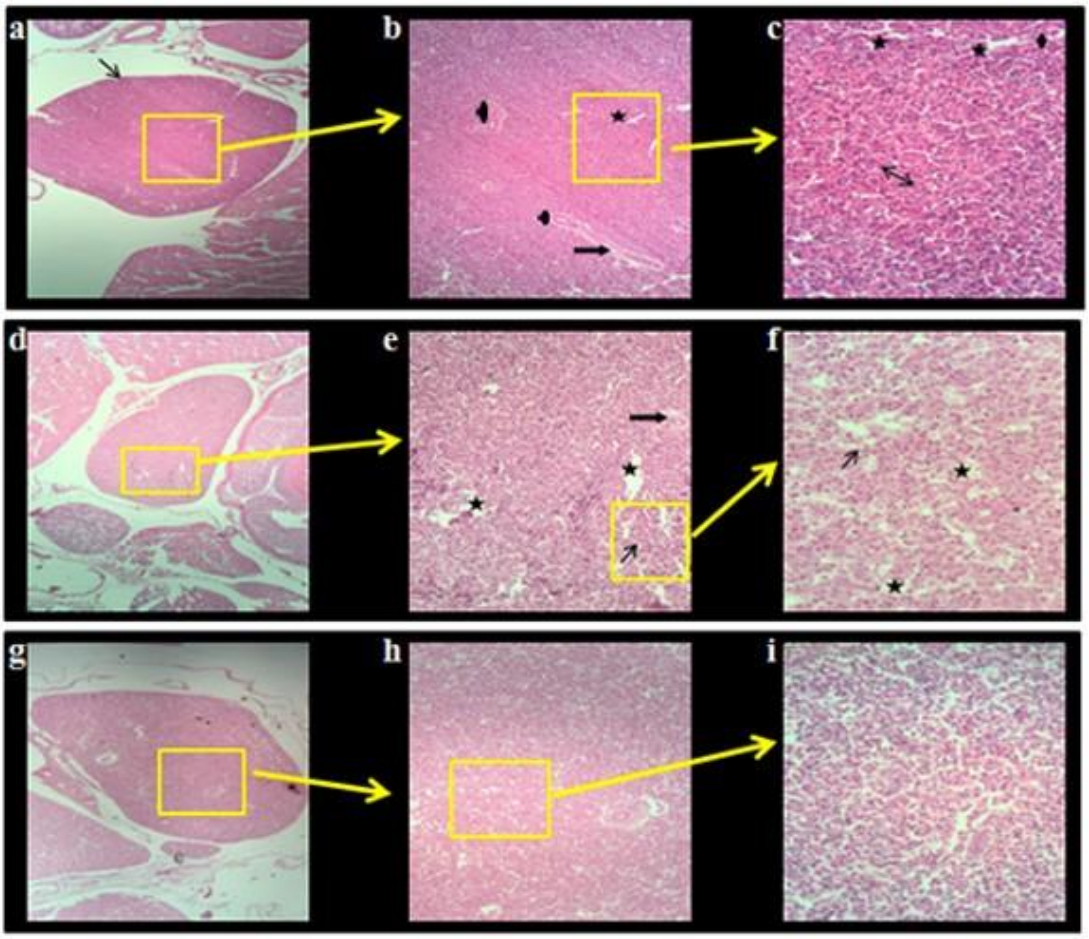

Figure 3: Examination of spleen tissue with light microscopic. Panels a, b and c are the control group. Uninfected spleen shows the normal histological structure of splenic tissue, containing capsule (↑), white and red pulp areas(⇔), central arteries, endothelial cells of central arteries(♦), trabecular arteries(✶) and connective trebeculaes(⇨). Panels d, e and f represent the tissues of the infected group that shows degeneration (↑), edema and necrosis in most of the cells of white and red pulp areas along with nuclear debris and vacuoles (✶). Damage was observed in most of the central arteries with endothelial cells and trabecular arteries (⇨). Panels g, h and I represent the *Cuminumcyminum* treated tissues having normal histology. The boxes and arrows (yellow) indicate the portion that is enlarged in the next figure in that row. The magnification at which the images are taken are, 40X (a, d, g), 100X (b, e, h) and 400X (c, f, i).

and mucosa, were without any lesions (Figure 2a, b and c). Avian cholera infected tissues showed lesions. Most of the villi tips, present on the mucosa, appeared to be shrunken and atrophied. Karyolisis and necrosis were observed in both villi and intestinal crypts. Both the muscularisexterna and mucosa exhibited atrophy and fibrosis. Desquamation was observed in the luminal epithelium while the submucosa was congested and haemorrhaged (Figure 2d, e and f). However, it was observed that the sections of small intestine treated with *Cuminumcyminum* showed normal histology of the tissues (Figure 2 g, h and i).

Histopathology of spleen

The sections of spleen from the control group exhibited normal morphology for all the components, white and red pulp, splenic capsule and the trabeculi (Figure 3a, b and c). However, lesions were observed in sections of spleen infected with avian cholera. The white and red pulp areas were shrunken and showed marked degenerated. Vacuolization, edema and necrosis were also observed. The thickness of the splenic capsule was reduced, the number of trabeculi was increased, and the splenic and central arteries were damaged (Figure 3d, e and f). The effect of *Cuminumcyminum* was similar to stomach and intestine (Figure 3g, h and i) where normal tissue morphology was retained in spite of bacterial infection.

Examination with light microscopic, control spleen sections showed normal splenic tissue. The spleen was a rounded, reddish – brown organ which lies close to the right side of the junction between the

proventriculus and gizzard. It was a mixed lymphoid tissue, having both the white and red pulp areas. The spleen of the broiler was surrounded by a thick splenic capsule, and there was a small number of trabeculi. The red pulps were less distinct, and these were scattered distributed within the white pulp. The white pulp was composed of a network of reticular cells and reticular fibers within which small, medium and largely sized lymphocytes and plasma cells were diffusely distributed. It contained sheathed arteries and lymphatic nodules. The red pulp of the spleen was formed from venous sinuses and anastomosing cord of reticular cells, macrophages, lymphocytes and blood cells. The network of the splenic tissue consisted of a network of reticular cells and fibers (Figure 3 a, b and c).

DISCUSSION

This study demonstrated that the antibacterial properties of the seed extract of the natural herb, *Cuminumcyminum* (cumin) has the potential to kill the bacteria *Pasteurella multocida* in chickens infected with avian cholera, evident from the lack of histopathology in spite of infection with the bacteria. The stomach, intestine and spleen tissues infected with the pathogen presented pathological lesions such as atrophy, degeneration, haemorrhage, congestion, necrosis and desquamation; a similar pathology was observed with 12-15 weeks old *P. multocida* infected birds (Sood and Verma 2016).

The justification for including antimicrobial compounds in the treatment of infectious diseases, drugs and feed of animals is to provide them with protection and ensure increased economic production of farm animals. Knowledge regarding the natural medicinal plants has been established since centuries. In recent times, the major concern in disease treatment is drug resistance that has led to the search for alternative treatments using natural resources. The plant-derived extracts and essential oils are recognized for being natural and safe antibacterial, antifungal, anticarcinogenic, antioxidant, analgesic, anticoccidial, and insecticidal agents (Dadashi *et al.,* 2016, Hussain *et al.,* 2008, Roomiani 2013).

Spices used worldwide for enhancing the flavour and taste of food are being identified for their potential medicinal, antimicrobial, antioxidant and the food is stabilizing properties (Dua *et al.,* 2013). It has been reported that *Cuminumcyminum* inhibits the growth of many pathogens including *Escherichia coli*, *Staphylococcus aureus* (Dua et al 2013, Chaudhry and Tariq 2008), *Klebsiella pneumonia*, *Enterococcus faecalis* (Saee *et al.,* 2016), *Pseudomonas aeruginosa*, *Bacillus pumilus* (Dua *et al.,* 2013), *Salmonella sp*ecies (Stefanini 2001), *Bacillus cereus* and *Aspergillusniger* (Das 2012). It has been observed that the antimicrobial activity of *Cuminumcyminum* extracts takes place due to cell damage; disruption of cell membranes and leakage of intracellular nucleotides and proteinaceous materials (Dua *et al.,* 2013). Similar inhibitory effects have been observed when *Petroselinumcrispum* (parsley) and *Coriandrumsativum* (cilantro) leaves and stem extracts were used against other infecting pathogens (Wong and Kitts 2006). Another study conducted using the aqueous methanolic extract of the roots of *Glycyrrhizaglabra* against mixed *Eimeria* species infection in broiler chickens demonstrated the ability of the extract in controlling avian coccidiosis (Hussain *et al.,* 2017).

The method of preparation of the plant extract is of paramount importance as the antimicrobial activity against the bacterial strains is dependent on the different bioreactive compounds in the extracts. There are reports that the essential oils have enhanced antimicrobial activity, owing to their composition, compared to the hydro-extract, methanolic extract and powder of plants (Saee *et al.,* 2016). In this study, it was found that cumin essential oil had a significant antibacterial effect on Gram-negative bacteria and cumin extract had excellent activity against both Gram-positive and -negative bacteria. The chemical structure of the ingredients, especially aromatic rings decompose the outer membrane of Gram-negative bacteria and increase the permeability of the inner cytoplasmic membrane (Saee *et al.,* 2016).

Among the disease syndromes caused by *P. multocida*, avian cholera is caused largely by strains of capsular type A, F and very rarely D; the virulence factors being the capsule, LPS, fimbriae, filamentous haemagglutinin, iron acquisition proteins and sialic acid uptake (Wilkie *et al.,* 2012). Epidemiological studies have stated that the apparent source of the bacterium is other birds and during outbreaks, it is spread by sharing water troughs or ponds. Though the likelihood of susceptibility to the more virulent strains is more, the susceptibility to a given *P. multocida* isolate is dependent on the species of bird. Reports suggest that out of the domesticated poultry species, turkeys are the most susceptible while chickens are the most resistant (Wilkie *et al.,* 2012). Our study checked for lesions in the stomach, intestine and spleen as the routes of entry are the respiratory and the digestive tracts and spleen being an important lymphoid organ, helps in fighting infection. It has already been reported that *P. multocida* after its entry establishes in the lung and multiplies in tissues, particularly liver and spleen (Wilkie *et al.,* 2012). Most of the

tissue damage could be due to the host's granulocytes as the avian cholera strains of *P. multocida* do not produce any known toxins (Bojesen *et al.,* 2014); the degree of tissue damage being dependentent on the dose of the bacterium, progression of the disease and individual host response. Our report isin agreement with another study where haemorrhages on the intestinal peritoneum and the sur- faces of other viscera were found to be common.

CONCLUSION

In the present study showed that *Cuminumcyminum* seed extract has antibacterial activity against avian cholera in chickens. However, further studies are needed to establish the best method of extract preparation and to characterise the active compounds of *Cuminumcyminum* involved in enhancing the antimicrobial potential against avian cholera. The mode of action in controlling bacterial replication also needs to be elucidated. The knowledge from these studies can then be extended for future investigation and application against other diseases caused by *P. multocida*.

REFERENCE

Al-Snafi AE. The pharmacological activities of *Cuminumcyminum*-A review. IOSR J Pharmacy, vol. 6, 2016 pp. 46-65.

Bojesen AM, Petersen KD, Nielsen OL, Christensen JP, Bisgaard M. Pasteurellamultocida infection in heterophil-depleted chickens. Avian Dis, vol. 48, 2014 pp. 463-470.

Boning CR. Florida's best herbs and spices: native and exotic plants grown for scent and flavour: Pineapple Press Inc., Florida, United States, 2010.

Carrillo C, Chinchilla E, Gonzalez L, Toledo R, Zambrana H. The prevention of fowl cholera, with aqueous extracts of plants, in poultry production. Agronomia Mesoamericana, vol. 8, 1997 pp. 152-158.

Chaudhry NMA, Tariq P. In vitro antibacterial activities of kalonji, cumin and poppy seed. Pakistan J Botany, vol. 40, 2008 pp. 461.

Dadashi M, Hashemi A, Eslami G, Fallah F, Goudarzi H, *et al.,* Evaluation of antibacterial effects of ZatariamultifloraBoiss extracts against ESBL-producing Klebsiellapneumoniae strains. Avicenna J phytomed, vol. 6, 2016 pp. 336.

Das S, Anjeza C, Mandal S. Synergistic or additive antimicrobial activities of Indian spice and herbal extracts against pathogenic, probiotic and food-spoiler micro-organisms. Int Food Res J, vol. 19, 2012 pp. 1185-1191.

Dua A, Gaurav G, Balkar S, Mahajan R. Antimicrobial properties of methanolic extract of cumin (*Cuminumcyminum*) seeds. Int J Res Ayurveda Pharm, vol. 4, 2013 pp. 104-107.

Harper M, Boyce JD, Adler B. Pasteurellamultocida pathogenesis: 125 years after Pasteur. FEMS microbial let, vol. 265, 2006 pp. 1-10.

Herath C, Kumar P, Singh M, Kumar D, Ramakrishnan S, *et al.,* Experimental iron-inactivated Pasteurellamultocida A: 1 vaccine adjuvanted with bacterial DNA is safe and protects chickens from fowl cholera. Vaccine, vol. 28, 2010 pp. 2284-2289.

Huberman Y, Terzolo H. Fowl Cholera in poultry. Argentina, Experimental Balcarce INTA EEA Balcarce, 2016.

Hussain AI, Anwar F, Sherazi STH, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (Ocimumbasilicum) essential oils depends on seasonal variations. Food Chem, vol. 108, 2008 pp. 986-995.

Hussain K, Iqbal Z, Abbas RZ, Khan MK, Saleemi MK. Immunomodulatory Activity of Glycyrrhizaglabra Extracts against Mixed Eimeria Infection in Chickens. Int J Agri Biol, vol. 19, 2017 pp. 928-932.

Kwon Y, Kang M. Outbreak of fowl cholera in Baikal teals in Korea. Avian Dis, vol. 47, 2003 pp. 1491-1495.

Petersen KD, Christensen JP, PerminA, Bisgaard M. Virulence of Pasteurellamultocida subsp. multocida isolated from outbreaks of fowl cholera in wild birds for domestic poultry and game birds. Avian Pathol, vol. 30, 2001 pp. 27-31.

Qin H, Xiao J, Li J, Gao X, Wang H. Climate Variability and Avian Cholera Transmission in Guangxi, China. Revista Brasileira de CiênciaAvícola, vol. 19, 2017 pp. 211-220.

Roomiani L. Antibacterial activity of *Cuminumcyminum* essential oil and Nisin on Streptococcus inaie in lab and fillets of rainbow trout. Iranian Scient Fisheries, vol. 22, 2013 pp. 50-58.

Saee Y, Dadashi M, Eslami G, Goudarzi H, Taheri S, Fallah F. Evaluation of antimicrobial activity of *Cuminumcyminum* essential oil and extract against bacterial strains isolated from patients with symptomatic urinary tract infection. Novelty in Biomed, vol. 4, 2016 pp. 147-152.

Salaheen S, Almario J, Biswas D. Inhibition of growth and alteration of host cell interactions of Pasteurellamultocida with natural byproducts. Poultry Sci, vol. 93, 2014 pp. 1375-1382.

Sheikh MI, Islam S, Rahman A, Rahman M, Rahman M, *et al.,* Control of some human pathogenic bacteria by seed extracts of cumin (*Cuminumcyminum* L.). Agriculturae Conspectus Scientificus, vol. 75, 2010 pp. 39-44.

Sood S, Verma P. Pathology of Pasteurellamultocida infection in chickens. Indian J Anim Res, vol. 40, 2016 pp. 15-19.

Stefanini M, Figueiredo R, Ming LC, Júnior A. Antimicrobial activity of the essential oils of some spicy herbs. In the International Conference on Medicinal and Aromatic Plants (Part II), vol. 597, 2001 pp. 215-216.

Tavakkoli H, Salandari S. Pathological Evaluation of Essential Amino Acid Injection for Chicken Embryonated Eggs. Int J AdvBiol Biomed Res, vol. 2, 2014 pp. 2874-2879.

Wang C, Wu Y, Xing X, Hu G, Dai J, He H. An outbreak of avian cholera in wild waterfowl in Ordos wetland, Inner Mongolia, China. J Wildl Dis, vol. 45, 2009 pp. 1194-1197.

Wilkie I, Harper M, Boyce J, Adler B. Pasteurellamultocida: diseases and pathogenesis. In Pasteurellamultocida, pp. 1-22 Springer, 2012.

Wong PY, Kitts DD. Studies on the dual antioxidant and antibacterial properties of parsley (Petroselinumcrispum) and cilantro (Coriandrumsativum) extracts. Food Chem, vol. 97, 2006 pp. 505-515.