RESEARCH ARTICLE

AMELIORATIVE EFFECTS OF FOUR HERBS (Withania somnifera, Tinospora cordifolia, Azadirachta indica AND E CARE SE HERBAL) ON THE PATHOGENESIS OF CHICKEN INFECTIOUS ANAEMIA VIRUS

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ABSTRACT

The present study was conducted to assess the ameliorative effect of four herbal preparations namely Withania somnifera, Tinospora cordifolia, Azadirachta indica and E Care Se Herbal on the pathogenesis of chicken infectious anaemia virus (CIAV) in chicks. Ninety day old specific pathogenic free (SPF) chicks were randomly divided into six groups (A to F) in which the first four groups were fed with W. somnifera (1% pure extract), T. cordifolia (1% pure extract), A. indica (0.2% pure extract) and E Care Se Herbal (0.1% in drinking water), respectively, from the first day. Groups E and F were treated as positive and negative controls respectively. Groups A to E were intra-muscularly on 14th day of age with 40 times of 50% chicken infectious dose (CID50) of CIAV A strain. Chicks of each group were sacrificed on 14th day post infection (DPI), the lymphoid organs collected and ameliorative effects of the four herbal preparations were evaluated by assessing the degree of gross and histopathological changes. The chicks of all the herbal treatment groups showed ameliorative effects as revealed by reduced pathological lesions and changes when compared with the positive virus control group. The herbal preparations evaluated in this study can be recommended for immunomodulatory, prophylactic and therapeutic purposes against CIAV induced immunosuppression so as to reduce mortality and secondary infections associated with this virus.

INTRODUCTION

Poultry industry worldwide has been undergoing continuous genetic selection and intensive productions systems that made the birds highly susceptible to various stresses. Such a stressed poultry population is exposed to the opportunistic microorganisms, which evolve into higher pathogenicity levels and the existing pathogens which enhance their virulence. Chicken infectious anaemia (CIA), caused by chicken infectious anaemia virus (CIAV), is an emerging disease of poultry, especially of young chicks, causing considerable economic losses to the poultry industry worldwide (Kataria et al., 1999; Todd, 2000; Dhama et al., 2002, 2008a, 2011; Verma et al., 2005; Schat, 2009). The CIAV causes considerable economic loss to the poultry industry by producing severe immunosuppression, aplastic anaemia and generalized lymphoid atrophy in chicks (McNulty, 1991; Smyth et al., 1993; Schat, 2003). This virus is ubiquitous in all major chicken producing countries of the world as evidenced by serology, virus isolation and molecular detection studies (Schat, 2003; 2009; Dhama et al., 2003, 2008a; Natesan et al., 2006a,b; Toro et al., 2006; Kim et al., 2010; Bhatt et al., 2011a; Snoeck et al., 2012; Nayabian and Mardani, 2013). Since CIAV is a potent immunosuppressive agent for very young unprotected chicks, it increases their susceptibility to secondary infections, viz. viral, bacterial and fungal agents, and depresses vaccinal immunity and production performance in the field situations (Pope, 1991; Hu et al., 1993; Liu et al., 1997; Adair, 2000; Schat, 2003; Miller and Schat, 2004; Basaraddi et al., 2013; Gowthaman et al., 2013). Like other viral infections there is no specific therapeutic approach for the treatment of CIAV infected birds; however, broad spectrum antibiotics are generally used to control or avoid secondary bacterial infections. Birds in convalescent stages can be provided with immunostimulants and hematinsics so as to boost the immune system and the process of haematopoiesis, respectively (Schat, 2003; Dhama et al., 2008a, 2011). Effective immunomodulatory and therapeutic regimen needs to be evaluated to counter the massive immunosuppression produced by the disease so as to reduce the economic losses produced by this economically important poultry pathogen (Bhatt et al., 2011b, 2013; Shyma, 2013). The present study was therefore, undertaken to assess the ameliorative potential of selected herbal preparations viz., Withania somnifera, Tinospora cordifolia, Azadirachta indica and E Care Se Herbal on reducing the pathology induced by CIAV virus in chicks.

MATERIALS AND METHODS

Specific Pathogen Free (SPF) chicks

A total number of 100 specific pathogen free (SPF) embryos, certified free from various poultry pathogens, were kindly
supplied by M/S Venkateshwaras Hatcheries Group Pvt. Limited (VHL), Pune, Maharashtra. These eggs were incubated in an isolated setter and hatcher at the Hatchery Unit of Central Avian Research Institute (CARI), Izatnagar. The hatched out White leghorn layer chicks were maintained in experimental infection and control sheds of Avian Diseases Section, Division of Pathology, IVRI, Izatnagar.

Chicken Infectious Anaemia Virus (CIAV)

Virus used in this study was Indian field isolate of CIAV A strain (Pune, Maharashtra) of chicken infectious anaemia virus (CIAV) with the accession no: AY583755, which was maintained in Avian Diseases Section, IVRI, Izatnagar.

Herbal preparations

Four herbal preparations were used in this study in order to assess their immunomodulatory and ameliorative potential against CIAV. These were the pure extracts of Withania somnifera (Ashwagandha), Tinospora cordifolia (Guduchi) and Azadirachta indica (Neem) (Natural Remedies Pvt. Ltd., India) and one commercial herbal preparation of poultry named E Care Se Herbal (Provimi India Pvt. Ltd. India).

Experimental design

Ninety (90) clinically healthy day-old SPF chicks were used in the experimental studies. These were divided randomly into six groups A-F, having equal number of chicks (n=15). Groups A to D were fed with respective immunomodulatory herbal preparations as presented in Table 1 while groups E and F were maintained as virus positive and negative controls for viral infection to be given later at 14th day of age. Ashwagandha, Guduchi and Neem were mixed with feed as per the experimental designs of Yamada et al. (2011), Rajkumar et al. (2009) and Sadekar et al. (1998), respectively. E Care Se Herbal was mixed with drinking water as per the product information. All the experimental groups of chicks were reared separately under strict isolated conditions and fed autoclaved feed and water, supplemented with vitamins and minerals, ad libitum. The chicks of all the groups were also given antibiotic Lixen (Cephalexin, Glaxo India Ltd.) for five days in prophylactic doses to prevent secondary bacterial infections. They were regularly monitored for clinical signs of disease and mortality, if any. Initially, the chicks of all the groups were kept in Control Experimental Shed, Avian Diseases Section, IVRI, Izatnagar.

RESULTS

Gross changes

The gross lesions observed in various lymphoid organs of CIAV infected birds at 14th DPI were suggestive of acute form of the disease (Fig. 1). The lesions were severe in group E (virus positive control) with the characteristic generalized lymphoid atrophy. Macroscopically, major findings in chicks of group E revealed atrophied thymus, pale fatty bone marrow, mild to moderately atrophied spleen and bursa, and pale swollen liver. The extent and degree of the gross lesions in the herbal treated groups were lesser compared to the CIAV positive control, suggestive of the protective/ameliorative effects and a resistance to the viral pathogenesis. Organ: body weight ratio taken at 14 DPI in chicks of different experimental groups also indicated ameliorative and immunomodulatory effects of the herbal preparations on CIAV pathogenesis (Table 2).

Histopathology

Histopathological lesions were recorded in various organs viz., liver, thymus, bursa and spleen of CIAV infected chicks, sacrificed at 14th DPI and are shown in Fig. 2. Lymphoid organs of all the infected groups demonstrated CIAV lesions but the lesions were comparatively milder in herbal treatment groups. In CIAV infected control (Group E), thymus showed atrophy and depletion of lymphocytes in cortex and medulla. In spleen and bursa, there was mild to moderate depletion of lymphocytes. In liver, there was mild degeneration of hepatocytes, dilated blood capillaries and focal lymphoid aggregation. While, Group A treated with W. somnifera showed reduction in pathological changes in the tissues examined with mild lymphoid depletions in the thymus and spleen but comparatively no changes in liver and bursa on 14th DPI. Group B treated with Tinospora showed much reduction in pathological changes in the tissues with comparatively normal architecture of thymus, mild lymphoid depletion in spleen but comparatively no changes in liver and bursa on 14th DPI. Group C treated with A. indica showed mild lymphoid depletion of cortex and severe congestion of medulla in thymus, mild lymphoid depletion in spleen with reduction in white pulp and reticular cell hyperplasia, but comparatively no changes in liver and bursa on 14th DPI. Group D showed comparatively less lesions in thymus and liver and the tissues retained normal architecture. Spleen showed mild lymphoid depletion

<table>
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<th>Table 1. Experimental Design</th>
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<td>Group</td>
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<td>A. Withania somnifera (Ashwagandha)</td>
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<td>B. Tinospora cordifolia (Guduchi)</td>
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<td>C. Azadirachta indica (Neem)</td>
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<td>D. E Care Se Herbal</td>
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<td>E. Positive control</td>
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<td>F. Negative control</td>
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At 2 weeks of age, groups A to E were shifted to Experimental Infection shed and were given infection by intramuscular inoculation of 40 times of the 50% chicken infectious doses (CID50) of CIAV A strain. Three chicks from each group were humanely sacrificed on 14th day post infection (DPI) and lymphoid organs (thymus, spleen, liver and bursa of Fabricius) were collected for assessing pathological changes and for confirmation of the establishment of the virus infection by polymerase chain reaction (PCR) testing. Genomic DNA was isolated from the blood and tissues of chicks from all the six groups and DNA was extracted using ® Blood and Tissue Kit (QIAGEN, Germany) following manufacturer’s instructions. The primers used for amplification of the VP2 gene of CIAV were F 5' ATG CAC GGG AAC GGC CCA C 3' (forward) and R 5'TCA CAC TAT ACG TAC CCG GG 3' (reverse) generating an amplicon size of 651 bp.

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<th>Table 2. Organ : body weight ratio at 14 DPI in chicks of different experimental groups</th>
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<td>Organ</td>
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<td>Liver</td>
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<td>Thymus</td>
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<td>Spleen</td>
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Values are presented as mean of organ (g): body weight (g) of three chicks
around peri-arteriolar lymphoid sheath (PALS) with reticular cell proliferation. Bursa showed moderate lymphoid depletion in cortex and medulla.

Bone: Group E (virus positive control) depicts the characteristic pale and thin femur bone whereas all the herbal treated groups (A to D) showed lesser degree of pathological changes. Liver: In group E, pale swollen and mottled liver observed but the pathological changes were less severe in all the treatment groups. Spleen: In group E, enlarged spleen depicting acute viral infection whereas in treatment groups almost normal spleen with only mild enlargement seen. Thymus: Group E showed severely atrophied thymus which is the characteristic lesion of CIA, but in all the treatment groups lesser degree of thymic atrophy observed.

**Fig. 1.** Gross lesions in target organs of the experimental groups.

Thymus: Chicks of Group E (virus positive control) showed generalized lymphoid atrophy of thymic cortex, while in all the herbal treatment groups there was lesser degree of thymic atrophy. Spleen and Bursa: In group E, there was acute lymphoid depletion whereas in treatment groups only mild lymphoid depletion observed.

**Confirmation of virus in target organs by PCR**

All the CIAV infected groups (A to E) were found positive for CIAV DNA at 14th DPI. PCR amplified products showed a distinct virus specific DNA band of 651 bp in length in 1% agarose gel electrophoresis (Fig. 3). There was no amplification of DNA from uninfected control chicks (Group F), which was included as negative control.

**Fig. 2.** Microscopic lesions in target organs in experimental groups

**DISCUSSION**

Chicken infectious anaemia (CIA) is an emerging disease of poultry, especially of young chicks, causing considerable economic losses to the poultry industry worldwide (Dhama et al., 2002, 2008a; Bhatt et al., 2011a). The CIAV causes severe immunosuppression, aplastic anaemia, lymphoid atrophy and predisposes birds to secondary infections (Schat, 2003; Dhama et al., 2008a; Dhama et al., 2011; Snoeck et al., 2012). CIAV possesses specific tropism for lymphocytes, which is responsible for lymphoid depletion in affected birds (Todd, 2000; Schat, 2003, 2009). In addition to immature T cells, mononuclear cells from the spleen are also susceptible to infection. Gross lesions include generalized lymphoid atrophy particularly of thymus, which is the most consistent lesion; and pale and fatty bone marrow, which is the most characteristic lesion (McNulty, 1991; Schat, 2003, 2009; Hoerr, 2010). Liver becomes swollen and mottled with pale/yellowish discoloration. Thymic atrophy sometimes results in an almost complete absence of thymic lobes (Dhama et al., 2002, 2008a, 2011; Basaraddi et al., 2013).

**Fig. 3.** CIAV PCR testing of thymus from chicks of each group on 14th DPI

Lane M: 100 bp ladder; Lane 1-4: VP2 amplified products from chicks of herbal treated Groups (A to D, respectively); Lane 5: Negative control (Group F); Lane 6: virus positive control (Group E).

In the present study, group E, representing the virus positive control, depicted the characteristic pathological changes of CIAV infection. Gross and histopathological changes revealed a mild to moderate degeneration and depletion of lymphocytes in the lymphoid organs examined in chicks of group E on 14th DPI, which is in accordance with the findings of Dhama (2002) and Basaraddi (2011). Earlier studies suggest that the primary target cells of CIAV include haematopoietic precursor (haemocytoblasts) and thymic precursor (lymphoblasts) cells in the bone marrow and thymus cortex, respectively (Dhama et al., 2002, 2008a; Schat, 2003, 2009; Oluwayelu 2010). Ratio of organ : body weight ratio at 14 DPI also revealed ameliorative potential of the herbal preparations CIAV induced pathogenesis (Table 2). VP2 gene of CIAV was amplified from blood and tissues of all the five infected groups (A-E) confirming the virus multiplication and establishment of the virus infection in experimental chicks. PCR has been reported to be a very useful molecular tool for diagnosis of CIAV (Dhama et al., 2004, 2008a; Kataria et al., 2005; Natesan et al., 2006a; Oluwayelu, 2010; Basaraddi et al., 2013). All the four herbal treated groups have shown better immunomodulatory response against CIAV infection as evidenced by the reduction of gross and histopathological changes in target lymphoid organs. *W. somnifera* has its action on immune system in selectively skewing the immune response towards Th1 response cells rather than Th2 cells by increased IFN gamma, IL-2 versus IL-4 cytokines levels (Bani et al., 2006) and it modulates immune response by augmenting the counts of CD4+ cells, CD8+ cells, and natural killer (NK) cells (Davis and Kuttan, 2002; Khan et al., 2006). These actions exerted by *W. somnifera* are attributed to effectively resisting the viral multiplication and pathogenic effects in...
lymphoid tissues, resulting in significantly reduced viral load and pathogenic lesions in lymphoid organs. Group B treated with T. cordifolia revealed less lymphoid lesions which can be correlated with the property of this plant in augmenting the myeloid differentiation of bone marrow progenitor cells and the recruitment of macrophages (Rege et al., 1999; Dhanukar et al., 2000; Singh et al., 2006), thereby it can enhance the cell mediated immune response in chicks. This can be accounted for the lesser degree of pathological changes in group B compared to that of group E.

Reduced histopathological lesions and viral load of CIAV in tissues of A. indica fed chicks (Group C) points to the potent antiviral properties and immunopotentiating effect of this plant which coincides with the findings of Haq et al. (1999) and Ansari et al. (2012) that A. indica was found to be effective on humoral and cell mediated immune responses, in broilers which had survived an outbreak of infectious bursal disease and Newcastle disease. The findings are also in accordance with Renu et al. (2003) who concluded the enhanced cell mediated immune response of Neem leaf extract by delayed type of hypersensitivity (DTH) in terms of increased skin thickness to 2, 4-dinitro-chlorobenzene in skin contact sensitivity test and enhanced humoral immune response against NDV antigen by indirect ELISA. Along with herbal remedies, early and rapid diagnosis as well as effective and novel vaccines and therapeutics for combating chicken infectious anemia virus and other poultry pathogens is required. This strategy would help in combating CIAV, an economically important immunodepressive disease of poultry and other associated secondary infections, which would help alleviate economic losses to the poultry farmers and producers/industry (Dhma et al., 2004; Kataria et al., 2005; Dhma et al., 2008a,b; Mahima et al., 2012; Dhma et al., 2013a,b,c,d; Tiwari et al., 2013).

Conclusion

The present study revealed that the herbal preparations viz., W. somnifera, T. cordifolia, A. indica and E Care Se Herbal were effective in the amelioration of CIAV induced pathological lesions in chicks. Combination of these herbal preparations may also be evaluated in future for use against CIAV.

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