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RESEARCH ARTICLE

Effect of Mentofin and ASI-MIRUS on Humoral Immune Response and Tissue Changes in Infectious Bursal Disease Vaccinated Broiler Birds

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ABSTRACT

Infectious bursal disease (IBD) is a viral disease of poultry causing immunosuppression in young chicks. The present project was designed to evaluate the efficacy of two immunostimulants Mentofin and ASI-Mirus against IBDV vaccine. A total 300 broiler chicks were taken, divided in to six groups each having 50 birds and were replicated under controlled conditions. Birds of groups A, B and D were vaccinated with the IBD live virus vaccine. Birds of groups B and C were treated with Mentofin. In group B chicks were treated with Mentofin and group E were supplemented with ASI-MIRUS while F group served as negative control. The effect of immunostimulants on humoral immune response was evaluated by recording weekly serum antibody titer against IBDV through Enzyme linked immunosorbent assay (ELISA). The antibody titer was significantly increased ($P<0.05$) in group D supplemented with ASI-MIRUS as compared to group B supplemented with Mentofin and vaccinated. Significantly high bursa to body weight ratio was observed in vaccinated group D. Eucalyptus and peppermint oils increases bursa to body weight (B/BW) ratio as compared to untreated groups. In group B (Mentofin treated), bursal samples showed necrosis at medullary region of bursal follicles. Group D (ASI-MIRUS treated) showed the active follicle consist of lymphoid cells and shown no histopathological lesions. These results indicated volatile oils in ASI-MIRUS have effective immunomodulatory effects on humoral immune response against IBD virus in broiler chicks.

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INTRODUCTION

Poultry industry is facing many infectious diseases which are responsible for great economic losses (Mukhtar *et al.,* 2012; Abbas *et al.,* 2017a & b). The poultry meat contributes a significant share (28.0%) in the total meat production in the country, highlighting the potential of the poultry in meeting demands for the food, poverty alleviation and to promise future food security (Anonymous, 2013-2014). The major poultry viral diseases are Newcastle disease (ND), Infectious Bursal disease (IBD), Infectious bronchitis (IB), Avian influenza and Fowl pox (Khan *et al.,* 2009; Zahid *et al.,* 2015; Abbas *et al.,* 2015). Despite of vaccination, ND and IBD are still the most common diseases in Pakistan causing great economic losses (Numan *et al.,* 2005).

Infectious bursal disease (IBD) is an infectious viral disease of poultry which is highly contagious affecting the immune system of young chicken. It is characterized by the destruction of the lymphoid organs, mainly the bursa of Fabricius, in which B lymphocytes differentiate and mature (Van den Berg *et al.,* 2000). The causative agent of infectious bursal disease virus is a non-enveloped, bi-segmented ds RNA genome virus, belonging to Birnaviridae family. Suspected chicken flock (age 3rd to 6th weeks) shows the signs of reduced growth rate and death as well as excessive carcasses condemnation occur due to hemorrhages of skeletal muscles. IBD illustrated the attention of research workers for an active control due to its worldwide occurrence. Except vaccination, there is no effective control of IBD (Lukert and Saif, 2003).

A number of botanicals have been reported to have beneficial effects against infectious diseases of poultry

(Abbas *et al*., 2017c; Hussain *et al*., 2017; Idris *et al*., 2017; Mahmood *et al*., 2016). Eucalyptus leaf extract has anti-bacterial effects and which also has been reported to pathogenic bacteria isolated from patients of the problem in the respiratory tract (Ocak *et al.,* 2008). Eucalyptus and peppermint essential oils protect the respiratory tract of broilers to meet the challenges of avian influenza virus assessed by Barbour *et al.* (2006). Essential oils extracted from herbs and spices are a complex mixture of various compounds, which consist of aromatic and volatile substances (Alcicek *et al*., 2004).

Mentofin is one of natural herbal product which contain eucalyptus oil 10%, menthol 10%, liquid binder 33% and saponins 44% (Rehman *et al.,* 2013). ASI-MIRUS is a unique blend of natural oils which contain *Eucalyptus globulus* 100000 mg/lt, *Origanum vulgare* 5000 mg/lt, *Menthapiperita* 20000 mg/lt, Citric Acid 5000 mg/lt, Vitamin A 100000 IU/lt, Propylene Glycol 50000 mg/lt, Sorbitol 50000 mg/lt. Eucalyptus and peppermint oils have impact in boosting the cell-mediated and humoral immune response in chicken. These volatile oils have a strong immune effect and they stimulate the immune response in chickens (Awaad *et al.,* 2010). Therefore, we investigated the effect of Mentofin and ASI-MIRUS on humoral immune response as well as on tissue changes in broiler chicken.

MATERIALS AND METHODS

Experimental design: A total number of 300 day old chicks of broiler from commercial hatchery were reared into six groups mentioned as A, B, C, D, E and F, each group replicate with 50 birds/pen. Broiler birds in groups A, B and D were vaccinated with IBD live vaccine (Bur-706) of Merial at day zero (0 day). Chicks in groups B and C were treated with Mentofin (Ewabo Co., Germany) @ 0.25 ml/liter (commercially recommended dose) drinking water from 0-42 days of age. Group D and E were treated with ASI-MIRUS (ASIFAC VETPHARMA Co., Ltd. VIETNAM) @0.25 ml/liter drinking water from 0-42 days of age. Groups A (untreated), B (treated) and D (treated) were vaccinated groups. Groups C (unvaccinated treated) and E (unvaccinated treated) were positive control groups. Group F was negative control group (not treated and not IBD vaccinated). Table 1: represent the layout of experimental design. Chicks were housed under controlled environmental condition at University of Veterinary and Animal Sciences (UVAS), Lahore.

Sample collection: Blood samples were taken randomly from each group at 0, 7, 14, 21, 28, 35 and 42 days of age without adding EDTA through Syringe and serum was collected in serum tube to perform the in-direct ELISA for detecting the antibody response. Five chicks were slaughtered on every week and postmortem examination was done in order to identify any gross change in bursa. Bursa of the slaughtered birds was collected in order to calculate bursa to body weight ratio as well as to perform the histopathology for identification of any tissue change.

In-direct ELISA: In-direct ELISA were performed on weekly basis in order to check the antibody response in chicks. In order to detect antibody directed against IBDV a commercial ELISA kit, IDEXX Flock Chek standard (IDEXX Corporation, Westbrook, ME, USA) was used. The procedure was conducted as per manufacturer's recommendations (Zahid *et al.,* 2015).

Bursa to body weight ratio and histopathology: At every week, five chicks slaughtered by halal slaughtering method to collect the bursa for histopathology to calculate the bursal lesion scoring and bursa to body weight ratio. Bursa to body weight ratio was estimated as described by (Debnath *et al.,* 2005). For histopathology, five broiler chicks were selected randomly from each treatment groups and slaughtered at 0, 7, 14, 21, 28, 35 and 42 days of age. Bursa of Fabricus and spleen were observed for gross lesions. After postmortem examination, bursa of Fabricious was collected and tissue samples were fixed in 10% neutral buffered formalin to be used for histopathology. Tissue samples were embedded in paraffin and were stained through hematoxylin and eosin (Bancroft and Gamble, 2008). Bursal lesions like lymphocytic depletion and other histological changes in bursa of Fabricious were scored (Raue *et al.,* 2004). Lymphocytic depletion and bursal damage were scored according to following criteria: 0=Normal lymphoid follicles, 1=Mild depletion of lymphoid follicles shown by thinning of lymphocyte population without focal necrosis or notable edema, 2=Moderate depletion of lymphoid follicles accompanied by interfollicular edema and focal necrosis, 3=Severe depletion of lymphoid follicles having no lymphocyte but only reticular cell, and 4=Atrophy of follicles commonly with cystic spaces, in folding of epithelium and clear fibroplasias.

RESULTS

The present study was conducted to determine the effect of two ameliorating products (Mentofin and ASI-MIRUS) on humoral immunity, bursa to body weight ratio and tissue changes against IBD vaccinated broiler chickens. ELISA was used to measure the serum antibody titer against IBD on 0, 7, 14, 21, 28, 35 and 42 days of age. The antibody titer for these samples was examined by a commercial ELISA kit, IDEXX Flock Chek standard (IDEXX Corporation, Westbrook, ME, USA). After reading of samples in ELISA reader, the difference between positive control group and negative control group was greater than 0.14. The geometric mean (GM) ELSA antibody titer of different groups is represented in table 2. On all experimental days, group D had significantly ($P<0.05$) higher geometric mean titer (GMT) against IBD virus as compared to group B. In all vaccinated groups, antibody titer declined from day 14th day, as followed increasing by 21st day . Then titer gradually increased from 35th day of age and continued till 42 day of experiment. In all un-vaccinated groups, antibody titer gradually declined. Group E had considerably higher geometric mean titer (GMT) as compared to group C (Table 3). In control group, antibody titer declined from day 0 till day of completion of experiment.

Bursa to body weight (B/BW) ratio was significantly ($P<0.05$) high in unvaccinated group E (ASI-MIRUS treated) on all experimental days as compared to unvaccinated group C (Mentofin treated) and F (untreated

Table 1: Experimental design

Schedule	Group A	Group B	Group C	Group D	Group E	Group F
Vaccination	Vaccine against IBD	Vaccine against IBD	*	Vaccine against IBD	*	*
Treatment	*	Mentofin	Mentofin	ASI-MIRUS	ASI-MIRUS	*
Control	*	*	+	*	+	-
Remarks	Vaccinated untreated	Vaccinated treated	Unvaccinated treated	Vaccinated treated	Unvaccinated treated	Unvaccinated untreated

Note: * = Do nothing, + = +ve control, - = -ve control.

Table 2: Geometric mean ELISA antibody titers against infectious bursal disease virus (IBDV) vaccine

Groups	Days					
	7th day	14th day	21st day	28th day	35th day	42nd day
Group A (Vaccine only)	2944±31.4^{a}	845±22.9^{b}	1149±28.1^{b}	1958±33.1^{b}	2931±41.1^{b}	3814±31.1^{b}
Group B (Vaccine+ Mentofin)	4162±92.1^{b}	968±88.1^{a}	1402±77.9^{c}	2271±62.2^{b}	3233±42.8^{d}	4591±52.8^{c}
Group C (Mentofin)	3630±48.2^{a}	740±51.4^{a}	698±42.8^{d}	308±40.1^{b}	129±39.9^{c}	86±46.7^{c}
Group D (Vaccine + ASI-MIRUS)	4218±110.4^{b}	1059±90.4^{c}	1600±88.8^{a}	2392±62.4^{a}	3408±87.1^{a}	4767±89.1^{a}
Group E (ASI-MIRUS)	3880±62.2^{a}	893±48.1^{b}	749±38.1^{c}	432±62.1^{d}	190±60.9^{d}	110±35.1^{d}
Group F (Control)	2562±14.1^{a}	640±11.8^{a}	442±55.6^{a}	214±12.9^{a}	81±14.2^{b}	48±18.4^{b}

Values (mean+SE) having different superscripts in a column differ significantly (P<0.05).

Table 3: Comparision between vaccinated and treated groups with treated groups

Group B (Vaccine+ Mentofin)	Group C (Mentofin)	Group D (Vaccine + ASI-MIRUS)	Group E (ASI-MIRUS)
4162±92.1^{b}	3630±48.2^{a}	4218±110.4^{b}	3880±62.2^{a}
968±88.1^{a}	740±51.4^{a}	1059±90.4^{c}	893±48.1^{b}
1402±77.9^{c}	698±42.8^{d}	1600±88.8^{a}	749±38.1^{c}
2271±62.2^{b}	308±40.1^{b}	2392±62.4^{a}	432±62.1^{d}
3233±42.8^{d}	129±39.9^{c}	3408±87.1^{a}	190±60.9^{d}
4591±52.8^{c}	86±46.7^{c}	4767±89.1^{a}	110±35.1^{d}

Values (mean+SE) having different superscripts in a column differ significantly (P<0.05).

Table 4: Bursa/body weight ratio of different treatment groups at different days of age in experiment

Groups	Days					
	7th	14th	21st	28th	35th	42nd
A	0.19±0.10^{a}	0.24±0.15^{a}	0.8±0.21^{b}	0.97±0.12^{b}	1.04±0.46^{c}	1.24±0.51^{d}
B	0.28±0.22^{a}	0.44±0.24^{a}	1.01±0.31^{a}	1.11±0.22^{b}	1.59±0.41^{b}	1.83±0.45^{b}
C	0.21±0.21^{a}	0.35±0.22^{a}	0.91±0.31^{a}	1.04±0.20^{a}	1.24±0.14^{c}	1.66±0.13^{c}
D	0.31±0.34^{b}	0.5±0.41^{b}	1.08±0.44^{b}	1.20±0.51^{c}	1.64±0.43^{b}	1.90±0.71^{d}
E	0.23±0.25^{a}	0.39±0.24^{a}	0.98±0.33^{a}	1.09±0.41^{a}	1.30±0.22^{d}	1.75±0.11^{d}
F	0.20±0.12^{a}	0.28±0.23^{c}	0.9±0.14^{a}	1.01±0.13^{a}	1.19±0.14^{a}	1.28±0.19^{a}

Values (mean+SE) having different superscripts in a column differ significantly (P<0.05).

Table 5: Histopathological bursal lesion scoring of different treatment groups at different days of age in experiment

Groups	Histopathological bursal lesion scoring						
	Days						
	0th	7th	14th	21st	28th	35th	42nd
A	1	1	1	3	4	4	4
B	0	1	1	1	3	4	3
C	0	0	0	1	1	2	2
D	0	0	2	1	1	0	0
E	0	0	0	1	1	1	0
F	0	0	0	0	0	1	1

and unvaccinated). Significantly (P<0.05) high bursal weight observed in vaccinated group D (ASI-MIRUS treated) comparing with other vaccinated groups A (untreated and vaccinated) and B (Mentofin treated) (Table 4).

No gross lesions were observed in spleen, thymus and caecal tonsil. But bursa had large size in vaccinated groups as compared to non-vaccinated groups.

Tissue changes in bursa of Fabricius scored at 0, 7, 14, 21, 28, 35 and 42 days of age, revealed increased number of lymphocytes in ASI-MIRUS and Mentofin treated groups. Histopathologically, varying degree of lymphocytic depletion and bursal damage were observed in the bursa. Bursal lesions like lymphocytic depletion and other histological changes in bursa of Fabricious were scored (Table 5). Birds of Group-F (unvaccinated control) showed the active follicle consist of lymphoid cells with interfollicular tissue(Fig. 1A). At 5th and 6th week, group F showed follicular depletion and no obvious histopathological lesion (Fig. 1B). In Group B (Mentofin treated), bursal samples showed necrosis at medullary region of bursal follicle (Fig. 1C). Bursal samples from Group D (ASI-MIRUS treated) showed the active follicle consist of lymphoid cells and shown no obvious histopathological lesion (Fig. 1D). Moderate lymphocytic depletion was observed in lymphoid follicles in Group C.

DISCUSSION

IBD causes the considerable economic losses through high mortality and immunosuppression in poultry. In Pakistan commercially, available vaccines are abruptly used to control different viral diseases but unfortunately failure of these products occur from time to time. Therefore, present study was conducted to determine the immunostimulatory effect of two commercially available products in IBD vaccinated birds. This study showed the highest antibody titer in group D treated with ASI-MIRUS. It has been well defined previously that herbs have important effect on humoral immune response against IBD vaccines and weight gain of broiler chicken (Qayyum *et al.*, 2012).

Infectious bursal disease is a viral infection and responsible for degeneration of bursa of Fabricius of chicken that cause suppression of humoral immunity. Live virus (intermediate) vaccine was used for vaccinating broiler chicks. Although intermediate vaccines neutralize the maternal antibodies, this vaccine was administered at day old age of chicks because early vaccination causes replication of vaccine virus in the chicks and dissemination

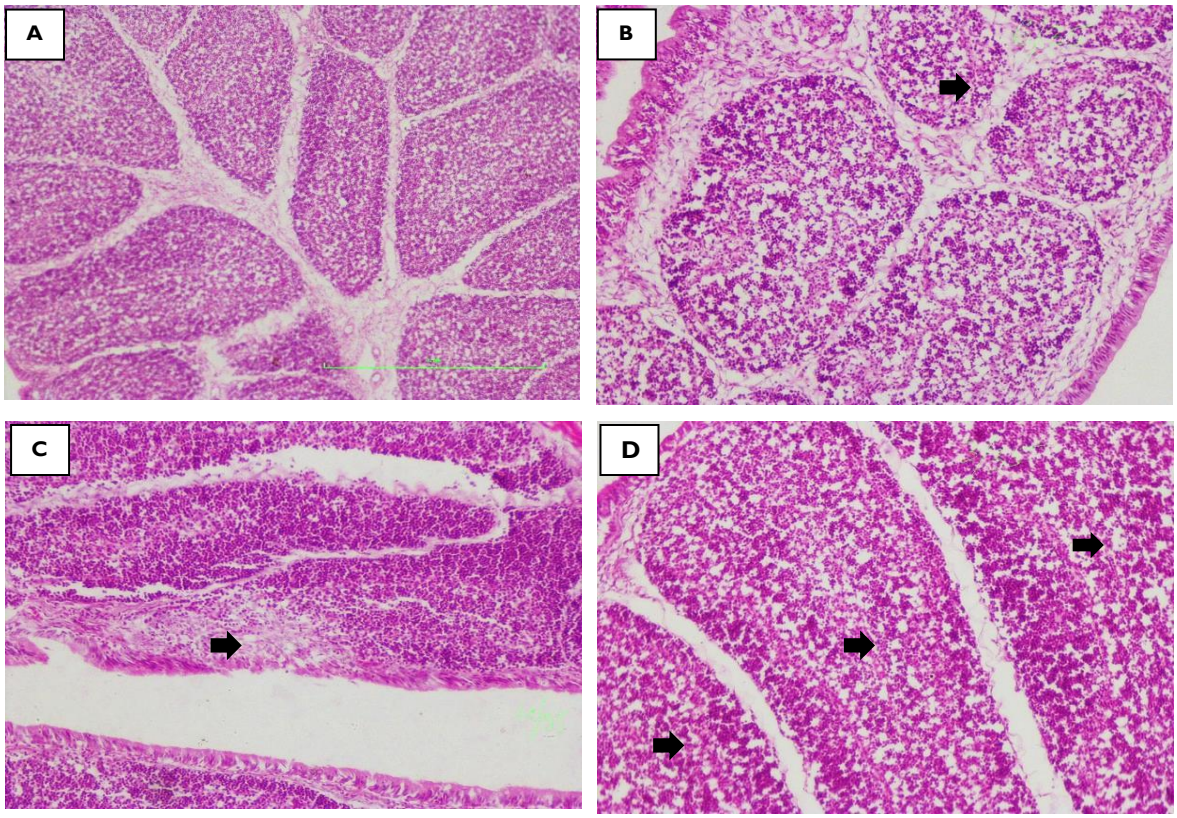

Fig 1: Histopathological changes in bursa **(A-D). A=**Bursa of group F (unvaccinated control) showed the active follicle consisting of lymphoid cells with interfollicular tissue and showed no histopathological changes. **B=** group F showed folicular depletion (arrow) and no pathological changes at 42 day, **C=**group B showed necrosis (arrow) at medullary region of bursal follicle, **D=**group D showed the active follicle consist of lymphoid cells (arrows) and shown no obvious histopathological lesion. H&E stain **(A)** 100X**, (B-D)** 200X.

of virus within the farm. This would, at least partially, provide indirect vaccination to the other chicks at a time (Van den Berg *et al.,* 2000). Immunogenic response and antibody titers were also higher due to live virus than killed virus. Intermediate strain vaccines (228-E & BUR-706) achieved better as this vaccine induced meaningfully higher antibody levels than mild strain vaccine (Al-Zubeedy, 2009).

In present study, the highest antibody titer against IBD vaccine and supplemented with ASI-MIRUS (contain Eucalyptus and Peppermint oils) detected in group D throughout the experiment compared to group B. Group B supplemented with Mentofin (contain Eucalyptus and Peppermint oils) showed protective serum antibody titer ($P<0.05$) compared to groups A, C, E and F. Humoral immune response and cell mediate immunity of broiler birds increased by Eucalyptus and Peppermint oils supplementation against viral antigens (Awaad *et al.*, 2010). Bursa and spleen are responsible for antibody production in chicks and their appropriate size are indicative of effective coverage against diseases, which can be confirmed by enhanced antibody titer (Nidaullah *et al.*, 2010).

Broiler chicken fed ASI-MIRUS (contain Eucalyptus and Peppermint oils) showed significant increases in bursa to body weight ratio as compared to groups B, A, C, E and F. Eucalyptus and peppermint oils increase bursa to body weight ratio compared with control group (Awaad *et al.*, 2010).

In the present study, bursa of Fabricius of all the birds of group A showed lymphoid necrosis at cortical and medullary regions. Infectious bursal disease virus appears first in other hematogenic organs like kidney, spleen and liver via the blood after infection followed by bursa where mass replication occur (Zhang *et al.*, 2002). Within bursa of Fabricius immature B-cells is believed to be first site of replication for IBD virus where virus causes damage to B lymphocytes in lymphoid follicles (Chen *et al.*, 2009). In Group B (Mentofin treated), bursal samples showed necrosis at medullary region of bursal follicle. Serious necrosis and atrophy of lymphoid follicles in IBD virus infected chicken has been observed in bursa (Mawgod *et al.*, 2014). Bursal samples from Group D (ASI-MIRUS treated) showed the active follicle consist of lymphoid cells and shown no obvious histopathological lesion. It has been reported that immunostimulants protected bursa and other lymphoid organs from damage in infections such as IBD (Mushtaq *et al.*, 2003).

In our study, Mentofin and ASI-MIRUS (contain eucalyptus and peppermint oils) which have the immune stimulatory effect were observed. Peppermint oil retains the structural integrity of immune cells because of its strong antioxidant activity which protects cell membrane from free radical oxidants, in that way subsequent in an amended immune response (Doughari *et al.*, 2012). Mekay and Blumberg, 2006 mentioned that peppermint oil has antiviral, antimicrobial, immunomodulating, antitumor and chemopreventive potential.

Conclusions: Our study showed that eucalyptus and peppermint oils in Mentofin and ASI-MIRUS are able to improve both tissue changes and humoral immune

response against infectious bursal disease virus vaccine in broiler chicken. It could be determined that these volatile oils have effective immune response and ameliorate the immune response of chicks.

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Authors contribution: MUS reared the broiler birds and performed ELISA. AA contributed data analysis. GM and MUS contributed in histopathology examination and executed the experiment. BZ and MSI contributed in collection of samples. All authors interpreted the data critically revised and analyzed the manuscript for important intellectual contents and approved the final version.

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