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AMELIORATION OF PATHOLOGICAL EFFECTS OF NEWCASTLE DISEASE EFFECTED BROILER CHICKS BY FEEDING PROPOLIS

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Poultry industry is playing an important economic role in Pakistan. Newcastle disease is causing major economic loss to poultry industry. Propolis, a honey bee product, possessing many pharmacological properties and which is widely used in folk medicine. In current study, a total of 90 broiler chicks were purchased. These chicks were divided into six experimental groups: A, B, C, D, E and F (15 birds in every group). Chicks in group A were negative control (without infection and without supplementation). B group chicks were positive control for ND Infection (experimentally infected without any supplementation). The chicks in group C were positive control for propolis and were given 500mg propolis per kilo gram (kg) of diet and were not given ND infection. Group D, E and F were supplemented with propolis at rate of 250, 500 and 750 mg/kg in diet, respectively. Supplementation of propolis was provided from 1st. Infection was given on day 14. On day 1, 13 (pre-exposure), 17, 19 and 21 (post-exposure) blood was collected for Heamagglutination Inhibition (HI) test against ND. Group F shows protective antibody titer (GMT of Lag_2 of HI = 3.25) as compared to positive control group B (GMT of Lag_2 of HI = 0.00) antibody titre. The average weight gain was highest for group F. Mortality rate in positive control group was 100% while in group F, mortality rate was 26.67%. Gross pathological lesions were also significantly different with respect to proventricular haemorrhages, button ulcers in intestine, tracheal lesions and haemorrhages in the brain. Histopathology of bursa, spleen, thymus, cecal tonsils, trachea and lungs shown significant differences from group B to other groups. It was concluded that propolis have immuno modulatory effects. Propolis protects the bird from ND virus by interfering with its multiplication process. It also promotes growth performance of broiler birds and help the birds to survive against lethal ND infection.

Keywords: Newcastle Disease, Propolis, GC-MS, Heamagglutination Inhibition, Histopathology

Poultry farming is thought to be the profitable business but its progress is hampered by occurrence of variety of infectious diseases. ND is among the primary important diseases as it may cause 100% mortality in infected flock. The common name of this disease is “Ranikhet”. This disease is highly contagious, communicable and it is widely distributed in the world. Newcastle disease virus (NDV), belongs to the genus Avulavirus, within the family, Paramyxoviridae (Eze et al., 2014) can cause 100 % mortality depending upon the pathogenicity of virus. There are three pathotypes of NDV according to virulence. Low virulence NDV is termed as lentogenic, moderate virulence NDV is termed as mesogenic and high virulence NDV as velogenic. Propolis, a honey bee product, possessing many pharmacological properties and which is widely used in folk medicine (Yildirim et al., 2016). t has been reported to possess therapeutic and prophylactic properties against inflammation, hepatotoxicity, diabetes mellitus, heart disease, and cancer (Banskota et al., 2001; Burdock, 1998). Antiviral activity of propolis were revealed the properties of involving anti-BBMV (Mohamed and Owayss, 2005), anti-HSV (Amoros et al.,

1994; Amoros et al., 1992; Huleihel and Isanu, 2002; Nolkemper et al., 2010; Schnitzler et al., 2010), anti-poliovirus (Búfalo et al., 2009), anti-r IBDV, anti-reovirus (El Hady and Hegazi, 2002), anti-HIV (Gekker et al., 2005; Ito et al., 2001).

MATERIALS AND METHODS

Geolocation, Birds and diets

Ninety broiler chicks (Hubbard) were purchased and reared in poultry experimental shed, Department of Pathology, University of Veterinary and Animal Sciences (UVAS), Lahore. Standard management conditions were adopted. The birds were divided into six experimental groups *i.e.* A, B, C, D, E and F. Every group contained fifteen chicks. Chicks were housed in pens of identical size. The respective amount of propolis was mixed in feed and offered to respective groups throughout the experiment. Feed (pre-starter, started and grower) in form of crumbs.

Source of Virus

ND virus was obtained from Quality Operations Laboratory (QOL), UVAS Lahore. Egg Infectivity titer of ND virus was $10^{7.5}$.

Source of Propolis

Freshly prepared propolis by honey bees (*Apis mellifera*) was collected from the hives held in Honey Bee Research Institute (HBRI) Rawalpindi.

Propolis preparation and identification

Sample was prepared in Post Graduate Laboratory, Department of Pathology, UVAS, Lahore. Propolis was grated and then mixed with 70% ethanol (1:10, w/v) for 24 hours at room temperature. The solution was evaporated to get the dry extract. 2.5 milligram (mg) of dried propolis extract was prepared for gas chromatography mass spectrometry (GC-MS). 2.5 mg dried extract of propolis was derivatized with 50 microliter (µl) pyridine and 100 µl bis (trimethylislyl) triflouracetamide (BSTFA) at 100^0C for 30 minutes. Ingredient were identified by gas chromatography-mass spectrometry (GC-MS) in Pakistan Council of Scientific and Industrial Research (PCSIR), Lahore as described by Mahmud et al., 2015 (Mahmoud et al., 2015)

Experimental Design

Broiler birds in group A were negative control (without infection and without supplementation), in group B only ND Infection was given (experimentally infected birds without any supplementation). The birds in group C were positive control for Propolis and were given 500mg propolis per kilo gram (kg) of diet and were not given ND infection. Group D, E and F were supplemented with different concentrations of propolis i.e. 250, 500 and 750 mg/kg in diet respectively (Mahmoud et al., 2014; Mahmoud et al., 2015). Supplementation of propolis in feed was started from 1st day to the end of the experiment. Infection was given on day 14. Blood samples were collected on day 1 and 13 17, 19 and 21 to check the antibodies titer against ND by Heamagglutination Inhibition (HI) test. On day 17, 19 and 21 lymphoid organs were collected (Gharaibeh and Mahmoud 2013).

Challenge with live virus

The ND virus obtained from QOL, UVAS Lahore. The virus was diluted in PBS to 10^3 dilution. 0.1 ml of diluted virus was used for each bird. At day 14 group B, D, E and F were given infection with live ND virus through intranasal and intraocular routes. Before the challenge to avoid spread of infection the other groups were transferred to a remote shed away from the challenged birds.

Heamagglutination Inhibition Test

HA and HI performed according to standard protocol to check the antibody titers against ND virus. The geometric mean titers (GM) Log_2 of different groups were calculated.

Weight gain

Weight gain of birds was calculated weekly with the help of weighing balance.

Mortality rate:

Mortality rate of birds was noted before and after the NDV infection within all groups. Percentage of mortality rate was calculated using formula:

Percentage of Mortile Birds = (No. of dead birds in a group/ Total No. of birds) × 100

Gross pathological lesions:

Postmortem was performed at postmortem block UVAS Lahore. Following gross

pathlogical lesions were noted (Saif et al., 2008).

1. Hemorrhages on proventriculus
2. Button like ulcers in intestine
3. Tracheal lesions and brain lesions

Histopathology of Organs

After inoculation of virus, four birds were randomly selected from each group and postmortem examination was performed on 17, 19 and 21 day, respectively. Lymphoid organs (bursa, thymus, spleen, and caecal tonsils along with trachea and lungs) were isolated and processed for gross pathological lesions. The weight of the lymphoid organs (thymus, bursa, and spleen) were also determined (Machaca and Compton 1993). These organs were preserved in 10% Neutral Buffered Formalin (NBF) after postmortem examination. For further processing the samples were brought to the histopathology laboratory QOL, University of Veterinary and Animal Science (UVAS), Lahore (Luna, 1968; Bancroft and Gamble 2008)

Effect of Propolis Extract on infectivity titer of NDV in chicken embryos

A total of ten (10) embryonated eggs, which were Nine (9) days old, purchased from Veterinary Research Institute (VRI), Lahore. These eggs were divided into two (2) groups: A and B. In group A NDV infection and propolis was given simultaneously. In group B only NDV infection was given. After giving 4 day of incubation time. CAM fluid was extracted and HA was perfomed to check the virus quantity.

RESULTS AND DISCUSSION

The current study is performed to check the amelioration of pathological effects of Newcastle disease by feeding them propolis. This study is in agreement with (Hegazi et al., 1994) which reported influence of propolis on Newcastle disease virus. This study is also in agreement with (Drago et al., 2007) who reported that propolis have well known activity against bacteria, yeasts, viruses and parasites, which has been demonstrated both in in-vitro and in-vivo studies

Analysis by GC-MS shown that the main components of ethanol extract of propolis (EEP) are flavonoid, esters and aromatic phenyl-carboxylic acid. In temperate zone-the propolis samples contain flavonoids, phenolic acid and esters of phenolic acid (Marcucci, 1995; Burdock, 1998; Schnitzler et al., 2010). The differences in constituents of ethanol extract of propolis could be due to difference in geographical and botanical origin.

HI titers at day 1 and day 13 (pre-exposure) were high non-significantly which is in agreement with study of (Rahman et al., 2002). Rahman et al., 2002, reported that day old chicks from vaccinated parent flock contained high level of maternally derived antibodies. These antibodies gradually decrease with the passage of time. The reason is that all the birds were from the same vaccinated parent flock and all the birds had same amount of maternal antibodies. In Groups C, D, E and F level of antibodies was little higher, it was because of presence of propolis in the feed. The propolis shows immunostimulatory effect thus it resists the antibody titer to decrease.

Post-exposure HI titer of Group A and Group C were not effected as these both groups did not receive any challenge and their titers continue to decrease. The decrease in the titers of these groups was due to decrease in the level of maternal antibodies in the blood this study confirms the work previously reported by (Rahman et al., 2002) which demonstrated the decrease of maternal antibodies within 15 to 20 days of age. Post-exposure of ND virus, HI titer of Group B is severely affected as Group B is positive control and did not receive any treatment. There was significant difference for Group F from other groups as its HI titers were remain protective. This study is in agreement with previously performed work of (Valle et al., 2005). This was due to the higher concentration of propolis in diet because propolis has flavones which has immunostimulatory effect as studied by (Fan et al., 2011). Over all post-exposure HI results shows that there is significant difference of propolis feeding groups than non propolis feeding group. The groups D, E and F resist the replication of ND virus as propolis contains triterpenoids which have antiviral activity as studied by (Ito et al.,

Post exposure GMT±SD of Log_2 of antibody titers by HI test

Days	Groups						p Value
	A negative control	B ND positive control	C Propolis positive control	D 250mg propolis+ ND infection	E 500mg propolis+ ND infection	F 750mg propolis+ ND infection	
17	2.50 ± 0.58^{ab}	1.50 ± 0.58^{b}	2.5 ± 0.58^{ab}	2.5 ± 0.58^{ab}	2.75 ± 0.5^{a}	3.25 ± 0.5^{a}	0.01
19	1.00 ± 0.00^{bc}	0.25 ± 0.5^{c}	1.25 ± 0.50^{bc}	1.5 ± 0.58^{b}	2.75 ± 0.50^{a}	3.25 ± 0.50^{a}	0.00
21	1.00 ± 0.00^{b}	00.0 ± 0.0^{c}	1.00 ± 0.00^{b}	1.5 ± 0.58^{b}	3.25 ± 0.5^{a}	3.05 ± 0.58^{a}	0.00

$^{a, b,}$ and ^{c} significantly different from each other in a row.
p value will be considered significant if $p<0.05$.
Infection was given at day 14.

2001) and show significant difference from group B. Among the groups D, E and F, group F shows maximum antiviral properties because it was feeding higher concentration of propolis.

Weekly weight gain in Group A was normal and it was also observed by other scientists. Among the experimental Groups D, E and F the weight of Group F was observed significantly higher. There was significantly loss of weight in group B. The current study is also in agreement with (Ezema et al., 2016) which describes that there is weight loss during ND infection. This increased weight was significant at day 21 because as days passed by the amount of propolis birds eat become more and more. The more propolis the birds eat the more they grow as described by (Mahmoud et al., 2015). This weight loss is due to decrease in water and feed consumption, depression and diarrhea. It shows that propolis feeding in broiler bird significantly improves the weight gain when infected with ND virus.

Mortality rate in Group A and Group C was minimum. This was because of the fact that these groups did not receive any challenge or any stress during experiment. Mortality rate was observed highest in positive control Group B and it was also reported by (Swayne and King 2003). This was because of fact that the Group B did not receive any treatment in the experiment. The mortality in Group D was higher than in Group E and F because Group D was consuming lowest concentration of propolis. In Group F mortality rate was minimum because this group was consuming maximum amount of propolis and propolis has antiviral effect as it interferes with the replication process of virus as described by (Schnitzler et al., 2010). Another study reported that the mortality was highest on the day 3rd of post infection and birds continue to die afterwards. This was due to incubation period of ND virus. The incubation period of ND virus is from 2 to 15 days (Eisa and Omer, 1984).

Lesion in ND infection also include hemorrhages in trachea and brain. In some cases the mucus in trachea also observed. In present study there was significant difference between the Group F and other groups as Group F did not show the lesions in trachea or brain. This study is in agreement with (Cohen et al., 2004) which reported that propolis preventing respiratory tract infection in children.

Weight of lymphoid organs (thymus, bursa, spleen) were also observed as parameter for infectivity of ND virus. The weight of thymus and bursa in Group A and Group C was decreasing because these organs regress as bird become older. But in other groups thymus and bursa were not decreasing instead the weight of thymus and bursa was increased due to infection of ND virus. This study is in agreement with (Alexander et al., 2004). These gross pathological changes were due to ND virus infection.

Splenomegaly was observed in Group B, D, E and F because these groups were infected by ND virus. There was atrophy of lymphoid organs which was also observed by (Bwala et al., 2012) in the ND infected birds. Less

lesions were observed in group F which was feeding on higher concentration of propolis.

Histopathological lesions induced by NDV have been observed by (Shaheen et al., 2005). The present study confirms the work in which microscopic lesions were more severe with the NDV and generally characterized by extensive depletion and necrosis of lymphoid organs and aggregate as studied by (Mohammadamin and Qubih 2011).

Egg inoculation of propolis and NDV shows that there is significant difference in HA titers of fluid obtained from chorio-allontois. This was also reported by (Hegazi et al., 1994) for ND virus and (Manolova et al., 1985) for influenza virus. (Vilela et al., 2011) also reported antiviral effect of propolis in embryonated eggs. The decrease in HA titer may be due to the interference of propolis in replication and propagation of ND virus.

The current study shows that the propolis has indirect antiviral activity against ND virus. This study is agreement with (Urushisaki et al., 2011) who reported that there is not any direct impact of extracts of propolis on herpes simplex virus. It may induce internal cellular modifications that can affect the viral replication. This study is also in agreement with (Hegazi et al., 1994; Búfalo et al., 2009) who observed antiviral properties of propolis. This may be due to antiviral activity of flavonoids, which are constituent of propolis. In another study it was observed that epimedium polysaccharide and propolis flavone possessed synergistical action, EP-PF prescription could significantly inhibit the cellular infectivity of NDV (Fan et al., 2011). This might be due to containing high concentration of antiviral constituents of flavons. Antiviral activity of propolis also observed by (Amoros et al., 1992) who observed antiviral activity of french propolis against herpes simples type 2 virus and vesicular stomatitis virus. (Schnitzler et al., 2010) also showed antiviral activity or popolis.

The mechanism of antiviral activity was reported by (Schnitzler et al., 2010). According to this study propolis extract interfare with virion envelop proteins and masking viral compounds which are necessory for adsorbtion or entry into the host cell but in another study (Urushisaki et al., 2011) reported that propolis extract has no any direct effect on herpes simples virus instead it may induce internal cellular changes that can effect replication of virus. (Nolkemper et al., 2010) reported that antiviral activity of propolis is not due to any single chemical compound instead it is a synergistic effect of many compounds in propolis.

The present study revealed that propolis has significant impact on humoral immune response and histopathological changes in chicken against NDV infection. It could be concluded that there is significant immunomodulatory effect of propolis against ND infection.

REFERENCES

1. Alexander DJ, Bell JG, Alders RG. A technology review: Newcastle disease, with special emphasis on its effect on village chickens. Food & Agriculture Org.; 2004.
2. Amoros M, Sauvager F, Girre L, Cormier M. 1992. In vitro antiviral activity of propolis. Apidologie. 23 (3): 231-240.
3. Búfalo M, Figueiredo A, De Sousa J, Candeias J, Bastos J, Sforcin J. 2009. Anti-poliovirus activity of Baccharis dracunculifolia and propolis by cell viability determination and real-time PCR. Journal of applied microbiology. 107 (5): 1669-1680.
4. Burdock G 1998. Review of the biological properties and toxicity of bee propolis (propolis). Food and Chemical toxicology. 36 (4): 347-363.
5. Bwala DG, Clift S, Duncan NM, Bisschop SP, Oludayo FF. 2012. Determination of the distribution of lentogenic vaccine and virulent Newcastle disease virus antigen in the oviduct of SPF and commercial hen using immunohistochemistry. Research in veterinary science. 93 (1): 520-528.

6. Cohen HA, Varsano I, Kahan E, Sarrell EM, Uziel Y. 2004. Effectiveness of an herbal preparation containing echinacea, propolis, and vitamin C in preventing respiratory tract infections in children: a randomized, double-blind, placebo-controlled, multicenter study. Archives of pediatrics & adolescent medicine. 158 (3): 217-221.
7. Drago L, De Vecchi E, Nicola L, Gismondo M. 2007. In vitro antimicrobial activity of a novel propolis formulation (Actichelated propolis). Journal of applied microbiology. 103 (5): 1914-1921.
8. Ezema WS, Eze DC, Shoyinka SVO, Okoye JOA. 2016. Atrophy of the lymphoid organs and suppression of antibody response caused by velogenic Newcastle disease virus infection in chickens. Tropical Animal Health and Production. 48 (8): 1703-1709.
9. Fan Y, Liu J, Wang D, Hu Y, Yang S, Wang J, Guo L, Zhao X, Wang H, Jiang Y. 2011. Epimedium polysaccharide and propolis flavone can synergistically inhibit the cellular infectivity of NDV and improve the curative effect of ND in chicken. Int J Biol Macromol. 48 (3): 439-444.
10. Fan Y, Liu J, Wang D, Hu Y, Yang S, Wang J, Guo L, Zhao X, Wang H, Jiang Y. 2011. Epimedium polysaccharide and propolis flavone can synergistically inhibit the cellular infectivity of NDV and improve the curative effect of ND in chicken. International journal of biological macromolecules. 48 (3): 439-444.
11. Hegazi A, El Berdiny F, El Assily S, Khashabah E, Hassan N, Popov S. 1994. Studies on some aspects of antiviral activity. 1-Influence of propolis on Newcastle disease virus.
12. Ito J, Chang F-R, Wang H-K, Park YK, Ikegaki M, Kilgore N, Lee K-H. 2001. Anti-AIDS agents. 48. 1 Anti-HIV activity of moronic acid derivatives and the new melliferone-related triterpenoid isolated from Brazilian propolis. Journal of Natural Products. 64 (10): 1278-1281.
13. Kidd M 2004. Nutritional modulation of immune function in broilers. Poultry Science. 83 (4): 650-657.
14. Mahmoud, AM M, Abdel-Mohsein HS, Farghali MR. 2015. Antioxidant Properties of Chinese Propolis in Ross Broilers Exposed to Heat Stress in Egypt. Open Journal of Veterinary Medicine. 5 (09): 197.
15. Manolova N, Maximova V, Gegova G, SERKEDJIEVA Y, UZUNOV S, MAREKOV N, BANKOVA V. 1985. ON THE ANTI-INFLUENZA ACTION ON FRACTIONS FROM PROPOLIS. DOKLADI NA BOLGARSKATA AKADEMIYA NA NAUKITE. 38 (6): 735-738.
16. Marcucci MC 1995. Propolis: chemical composition, biological properties and therapeutic activity. Apidologie. 26 (2): 83-99.
17. Mohammadamin O, Qubih T. 2011. Histopathology of virulent Newcastle disease virus in immune broiler chickens treated with IMBO®. Iraqi Journal of Veterinary Sciences. 25 (1): 9-13.
18. Nolkemper S, Reichling J, Sensch KH, Schnitzler P. 2010. Mechanism of herpes simplex virus type 2 suppression by propolis extracts. Phytomedicine. 17 (2): 132-138.
19. Rahman MM, Bari A, Giasuddin M, Islam M, Alam J, Sil G, Rahman M. 2002. Evaluation of maternal and humoral immunity against Newcastle disease virus in chicken. Int. J. Poult. Sci. 1 (5): 161-163.
20. Schnitzler P, Neuner A, Nolkemper S, Zundel C, Nowack H, Sensch KH, Reichling J. 2010. Antiviral activity and mode of action of propolis extracts and selected compounds. Phytotherapy Research. 24 (S1): S20-S28.
21. Shaheen S, Anjum A, Rizvi F. 2005. Clinico-pathological observations of pigeons (Columba livia) suffering

from Newcastle disease. Liver. 2 7.14.
22. Swayne DE, King DJ. 2003. Avian influenza and Newcastle disease. Journal of the American Veterinary Medical Association. 222 (11): 1534-1540.
23. Urushisaki T, Takemura T, Tazawa S, Fukuoka M, Hosokawa-Muto J, Araki Y, Kuwata K. 2011. Caffeoylquinic acids are major constituents with potent anti-influenza effects in brazilian green propolis water extract. Evidence-Based Complementary and Alternative Medicine. 2011.
24. Valle P, Anunciado S, Lapitan R. 2005. Effects of Aloe vera on the white blood cell count and antibody titer of broiler chickens vaccinated against Newcastle disease. Philip. J. Vet. Med. 42 (1): 49-52.
25. Vilela CO, Fischer G, de Castro CC, Nunes CF, Huuml SO, Raffi MB, Salles SE, Anciuti MA, Vargas GD. 2011. Virucidal activity of green propolis against avipoxvirus in chorioallantoic membrane of embryonated chicken eggs. African Journal of Microbiology Research. 5 (9): 1075-1082.

