Immunological Role Of The Egyptian Bee Glue On Vaccination Against Newcastle Disease In Broilers

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ABSTRACT

Bee glue (Propolis) was collected from local bee cells at summer season , 1996 in Sharkia Governorate to study the Physical properties, immunological effects and clinical evaluation of ethanolic extract of propolis (EEP) for vaccination against Newcastle disease in Broiler chickens.

Egyptian propolis possesses considerable antiviral and immunological activities against Newcastle disease (ND) infection in chickens especially when administered with ND vaccines at the periods of first day through its effect on cell mediated (mainly) and humoral immunities. The potential of propolis in the prevention or treatment of Newcastle disease infection is worth, further extensive evaluation to confirm these effects, formulate recommendations for the practitioners and practical applications of such natural product as its possible anti Newcastle disease immunostimulent in poultry field required more study.

INTRODUCTION

Propolis is a resinous hive product collected by honeybees from many plant sources. It can be yellow, green or brown depending on its source and collected season, *(1)*.

Recently, it has been reported to possess versatile biologic activities as an antibacterial*(2)*, antiviral*(3)*, and fungicidal*(4)*.

Although the immunostimulant effect of propolis on some viral diseases of humans has been demonstrated, few or may be negligible paper studied its effects on the immune response generally in the veterinary and specially in the poultry fields, therefore, the activity of propolis against ND is a matter of controversy. Therefore, the aim of our work was to investigate:

1) Some physicochemical composition and properties of Egyptian propolis.
2) The immunological role of using EEP on the cell mediated phagocytic activity, humeral immune response as well as its action on body weight gain, weight of lymphoid organs after vaccination against NDV of broiler chickens.

MATERIAL AND METHODS

Preparation of the extract

A bee glue sample was collected from Attala village near to Minia El Kamh city at Sharkia governorate in summer season of 1996. The EEP was extracted according to the method previously described *(5)*.

Newcastle disease viruses

a) NDV vaccines

All Newcastle disease virus vaccinal strains employed in vaccination experiments were obtained from Sanofi – Animal Health, Inc. USA. The vaccines were delivered by the agency (commercial company) as follow:

a) Hitchiner B1 vaccine with a titre of $10^{9.7}$ $EID_{50} = 10^{9.7}$
b) La Sota vaccinal strain with a titre of $10^{9.4}$ $EID_{50} = 10^{9.4}$

b) Challenge NDV

Newcastle disease virus Field strain (virulent velogenic viscerotropic), it was obtained from Serum and Vaccine Institute, Abbasia, Cairo with $10^{6.4}$ ELD_{50}

Titration of live NDV vaccines

Live Newcastle disease virus (NDV) vaccines were titrated in 9-11 day old chicken embryos according to standard technique after *(6)*. The end points were calculated after *(7)*.

Experimental design

Chickens

Two hundred one day old Hubbard chickens were obtained from commercial company, raised on the floor until 7-day of age where later were randomly separated to four equal groups ,each group 50 chickens as follows

G 1: birds were vaccinated with Hitchiner B1 NDV (vaccinated).

G 2: birds were vaccinated with Hitchiner B1 NDV and injected intraperitoneal (1/p) with 30 mg of EEP /bird (vaccinated and treated).

G 3: birds were injected 1/p with EEP in a dose of 30 mg/bird (treated and non vaccinated).

G 4: served as control (non vaccinated and non treated).

ND vaccines administrated by intraocular route (100 ul volume) with a full dose (10^6 EID_{50} / bird), at seven days of age with Hitchiner B1 strain and revaccinated with La Sota vaccine at 16, 24 and 35 day of age in G 1 and G 2 only.

Five chickens from each group were weighted weekly, the body weight was recorded, and then bird's sacarified and the lymphoid organs (bursa of fabricius, thymus, and spleen and caecal tonsils) were collected, examined and weighted.

Humeral immune responses

Humeral antibodies were detected with Haemagglutination inhibition test (HI test) ***(8)***.

The cell mediated immunity

Was assayed with lymphocyte transformation test ***(9)***, and with delayed hypersensitivity test ***(10)***.

Challenge test

At 28 days of age all groups were challenged I/M with 0.2ml suspension field virulent velogenic viscerotropic NDV strain. All chickens were observed for 49 days for any abnormal sings, mortality rate and post – mortem lesions were recorded.

Statistical analysis

Test for significance was made by calculating student (T) Table ***(11)***

RESULTS AND DISSCUSSION

Physico- chemical properties of propolis

Showed that Propolis is a resin being yellowish green to dark brown in color with a pleasant flavor of poplar buds, honey, wax and vanilla but it can also have a bitter taste. When burnt, it exhibits a smell of aromatic resins of great value. It is hard and brittle when cold, but becomes soft and very sticky when warm. It can be likened to aromatic glue, depending on its source, age and changed according to the geographical zones ***(1)***.

Chemical study revealed that the propolis constituents were phenolic acid esters (74.9%), dihydrochalcones (5.8%) flavones (5.2%), aliphatic acids (2.7%), flavanones (2.1%), Chalcones (1.5%), tetrahydrofuran derivatives (0.9 %) and phenolic acids (0.8%)***(4)***. It was clear that phenolic acid esters were present in a major quantity (74.9%). Differed from that reported by ***Ghisalberti (1)*** who reported that chemical studies conducted with propolis extracts revealed the existence of a very complex mixture of different naturally-occurring constituents with more than 300 constituents identified to date. Such results tend to confirm that Propolis composition varies with season and geographic region; such extraordinary variability among samples from different sources leads to variation of the pharmacological properties of propolis.

Therefore, the chemical composition of propolis is still insufficiently known, and need more researches to detect the existing chemical composition of propolis and their constitutions, ***(12)***. So it is advice for evaluation of the different methods of bee glue extraction as resin extracted after trituration, resin extracted as small pieces, grain alcohol and maceration, as well as the effect of these different methods on the chemical composition of the yield.

The effect of EEP on body weight

Table 1 showed the effect of EEP on body weights of chicken under experiments, there was no significant differences in all tested groups up to 7 DPI. After 7 DPI up to

the end of the experiment there were significant differences in body weight, where the final body weight was1954.4±47.9 gm of G 2,1771.6±67.4,1784.1±54.8 and 1754.7± 39.9 of G I,3 and 4 respectively.

These data pronounced the effects induced by EEP on body weight gain of chickens of group 2 allover the experiment, where they were 15.7%,15%, 15.9%, 29.6%, 13.3% and 11.4% for 14,21,28,35,42, and 49 days respectively with a cumulative increase in the body weight 16.1%. This may be due to tissue-regenerative and growth stimulatory properties of propolis, in addition the propolis extract has an anabolic effect,*(13).* Propolis stimulated mammalian tissue regeneration, as it caused strong activation of mitosis of cells cultured *in vitro* and it enhanced protein biosynthesis *(14)*. Spectrophotometric study showed that propolis contains large amount of flavonoids and proteins *(14)*.

The effect of EEP on lymphoid organs weights

The lymphoid organs of G 2 revealed a pronounced increase in thymus weight with significant differences from the second week PI up to the end of the experiment. While spleen weight slightly affected without any significant changes allover the experiment, but bursa and caecal tonsil weights were increased without statistical alteration at the first 3weeks PI and with significant differences at the last 4 weeks PI Table 2. Propolis has an anabolic effect and stimulated the immunologic processes *(15)*.

Evaluation of the chickens immune response

Data in Table 3 showed that HI maternal antibodies of non vaccinated and non treated G 4, decreased gradually by time and was much lower, while increase HI titer in G 1and 2 due to vaccination, where G2 (vaccinated and treated) showed the highest HI titers with significant rise among other groups 1,3 and 4 from the 3rd week until the end of the experiment. These results revealed that EEP enhances antibodies response, *(16).* Immunomodulating properties of ethanolic extract of bee glue on body immune response, could be attributed to the immunostimulating effect of EEP through a significant increase in leucocytic parameters and lymphocytes production and its reflect on the globulin increase , *(16)* . Administration of propolis extract to chickens caused a marked increase in muscle total protein, the myofibril, protein fraction, and gamma globulins and suggested that propolis has an anabolic effect, and that it stimulated the body's immune response when compared to corresponding control *(17)*.

Evaluation of the chickens immune responses by the phagocytic activity

Data presented in Table 4 showed that Propolis induced increase in macrophages and phagocytic activity, where the highest phagocytic activity percentage was 69.7%% in chickens of G2 vs. 55.8% of the control group at 14 DPI up to end of 7 weeks, this results reports the possibility of using EEP as a biological response modifier. The treatment with propolis of NDV infected chickens induced increase in the antibody titers and phagocytic percentage *(18)*. This may be due to the direct effect of EEP on macrophages, which increase the enzymatic function, and or that propolis could strengthen macrophagocyte phagocytosis in the abdominal-cavity.

Evaluation of the chickens immune responses by stimulation index

There was increase in the stimulation index of the peripheral lymphocytes as detected by stimulation index of lymphocyte transformation in case of G 2 2.5 vs. 0.8 at 14 DPI, Table 4, this may be due to the effect of EEP on the T-cell function. EEP increased T-lymphocyte and produce activation of mitosis as well as activation of granulocytes *(19)*. Also propolis as well as its constituents are capable of dose-dependently suppressing phytohemagglutinin (PHA)-induced DNA synthesis and T cells *(20)*. These data convincingly demonstrated that propolis has a direct regulatory effect on basic functional properties of immune cells.

Table 1. The effect of Egyptian propolis on chicken's body weight of broiler chickens.

Days	Mean ±SE of body weights / g			
	G1	G2	G3	G4
1day	41.3±3.2	43.7 ±2.4	41.8±3.6	46.7±1.9
7 day	127.8±10.5	135.8±9.4	136.3±11.6	138.2 ±13.9
14 day	339.8±21.8	364.1±14.8*	338.5±16.6	314.8 ±13.0
21 day	614.4±45.9	692.7±23.7*	662.3±36.0	602.4±30.5
28 day	969.3±49.9	1101.8±37.4*	941.7±60.4	951.0±37.3
35 day	1117.2±91.9	1314.3±90.4*	1219.0±100.8	1014.4±59.5
42 day	1623.1±109.7	1793.6 ±102.8*	1735.0±143.9	1583.6 ±79.2
49 day	1771.6±67.4	1954.4 ±47.9*	1784.1±54.8	1754.7 ±39.9

Table 2. The effect of administration of propolis at different ages on the lymphoid organs weight of Broiler chickens.

Weights/gm	G1	G2	G3	G4
Bursa weight at 7 D.	0.20±0.01	0.21±0.2	0.19±0.02	0.17±0.01
Bursa weight at 7 W.	4.9±0.3	5.11±0.2*	4.91±0.4	4.37±0.2
Caecal tonsil weight at 7 D.	0.13±0.02	0.14±0.01	0.14±0.01	0.12±0.01
Caecal tonsil weight at 7 W.	0.38±0.03	0.41±0.02**	0.38±0.01**	0.33±0.02
Spleen weight at 7 D.	0.070±0.006	0.067±0.005	0.068±0.008	0.066±0.005
Spleen weight at 7 W.	0.80±0.09	0.94±0.04	0.81±0.07	0.75±0.06
Thymus weight at 7 D.	0.44±0.03	0.42±0.02	0.40±0.03	0.39±0.04
Thymus weight at 7 W.	4.54±0.3	4.90±0.2**	4.65±0.4	3.99±0.3

The delayed hypersensitivity, skin reaction test

Table 4 showed that the delayed hypersensitivity skin reaction test using the sensitizing antigens "extract or antigens NDV" gave a positive specific stimulation after 72 hours after inoculation with specific antigen. It was observed that the EEP gave the typical delayed hypersensitivity when inoculated to the sensitized chickens. The index was 1.37 mm thickness for G 2 if compared with non-sensitized control G 4, 0.19 mm thickness, while the NDV showed also the typical delayed hypersensitivity skin reaction. The reaction was more pronounced in case of chicken of G2, "3.43 mm thickness". Propolis contact allergy is not caused by one main allergen, but by several allergens varying in chemical composition *(21)*. The presence of these in propolis depends on the nature of source plant and place as well as time of bees collection.

Table 3. Demonstrate the HI antibodies response post NDV vaccination up to 49 days old.

Age/day	G1 mean HI titer $\log_2$	G2 mean HI titer $\log_2$	G3 mean HI titer $\log_2$	G4 mean HI titer $\log_2$
1	9.7± 0.6	9.6 ± 0.7	9.7 ± 0.8	9.5 ± 0.8
7	6.9 ± 0.5	6.9 ± 0.5	6.8 ± 0.4	6.6 ± 0.5
14	5.2 ± 0.4	7.1 ± 0. 3***	5.4 ± 0.5	4.1 ± 0.4
21	6.6 ± 0.5***	7.3 ± 0.5***	4.3 ± 0.4***	2.7 ± 0.1
28	7.8 ± 0.4***	8.4 ± 0.4***	3.1 ± 0.2***	1.1 ± 0.2
35	6.9± 0.6***	8.3 ± 0.7***	1.9 ± 0.1***	0
42	7.1 ± 0.6***	8.8 ± 0.5***	0	0
49	8.5 ± 0.7***	9.2 ± 0.6***	0	0

NB: ND-HI titer $\log_2^{3.75}$ gives 90% protection against ND challenge,
Proposed and recommended protection % post ND vaccination (European and America Pharmacopeias) on the basis of **Allan et al; (1978)**.

Table 4. Evaluation of chicken immune response of different Group under experiments.

TEST	**G 1**	**G 2**	**G 3**	**G 4**
Phagocytic activity% at 14 DPI	62 4%	69.7%	64.1%	55.8%
Stimulation index of lymphocyte transformation at 14 DPI	1.2	2.5	2.8	0.8
Thickness index after 72 hours of skin inoculation of delayed hypersensitivity of EEP(mm)	0.67	1.37	0.92	0.19
Thickness index after 72 hours of skin inoculation of delayed hypersensitivity of NDV(mm)	2.94	3.43	0.32	0.22
Mortality rate (%)	20%	10%	80%	90%

The inoculation of different antigens in the footpad of sensitized and non-sensitized chickens produced varying degrees of footpad thickness as well as cellular and vascular reaction depending on the type of inoculation with the antigen of NDV, ***(18)***. In this work, the reaction was pronounced as typical Arthus type especially in G2 where the reaction was different. This could be due to that the lymphocytes appeared to play the main role in this reaction which became a delayed type of hypersensitivity. Propolis is rich in biochemical constituents including variety of flavonoids and free amino acid which able to induce an immunogenic effect as measured by delayed hypersensitivity skin reaction ***(14)***, , used honey and propolis as were used management of chronic skin ulcers ***(22)***.

The level of protection against VVND after EEP adminestration

Low mortality rate was observed (10 %) in G 2, when compared with (20%, 80%&90%) groups 1 & 3 and 4 respectively . Table 4, explained that the treatment with propolis before NDV infection induced increase in the antibody titers and phagocytic percentage. These results revealed clearly the immunostimulant effect of propolis and protection against NDV infection. Propolis acts actively as antiviral agent than honey ***(3,23)***.

It could be concluded that, the use of propolis as non specific-immunostimulant increased vaccination immune response with good protection against NDV in poultry farms.

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الملخص العربي

الدور المناعي لشمع النحل المصري على التحصين ضد مرض النيوكاسل في دجاج التسمين

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خلال فصل الصيف عام ١٩٩٦ تم جمع شمع (صمغ) النحل وهو منتج طبيعي من إحدى القرى بمحافظة الشرقية لدراسة بعض خصائصة الفيزيائية وتقييم الصفات المناعية لة سريريا للتحصين ضد مرض النيوكاسل فى دجاج التسمين.

وقد كَشفتْ النَتائِج أن شمع النحل يمتلك ميزتين وهما:

الأولى: أنة محفز لكل من نوعى المناعة الخلوية بصفة خاصة وكذا الدموية وذلك عند إعطائة فى الأيام الأولى لعمر الكتاكيت مقترنا بلقاح النيوكاسل.

والثانية: أنة قلل من وفيات الدجاج المصاب بفيروس مرض النيوكاسل كما بينت النتائج، ومن ثم يُمْكِنُ أن يُلعب دورا هاما فى الوقاية من أو للتحكم فى العدوى بفيروس مرض النيوكاسل.

وما آنف من النتائج تستحق وتتطلب المزيد من التقييم لشمع النحل من أماكن مختلفة من الجمهورية لاختيار أفضل الأنواع والأهم مقارنة طرق الاستخلاص الكيميائية المختلفة للحصول على أحسن النتائج حتى يمكن أن نوصى بها العاملين بمجال صناعة الدواجن للعمل بها، ونأمل أن يكون هذا فى المستقبل القريب.