Journal of Cell and Animal Biology Vol. 6(10), pp. 160-163, 31 May, 2012
Available online at http://www.academicjournals.org/JCAB
DOI: 10.5897/JCAB12.023
ISSN 1996-0867 ©2012 Academic Journals

Full Length Research Paper

Evaluating the effect of *Hypericum perforatum* on antibody titers obtained from B1 and La Sota vaccines in broiler chicks with HI test

Adel Feizi [1]* and Farhad Dadian[2]

[1]Department of Clinical Sciences, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
[2]Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

Accepted 30th May, 2012

Nowadays using of live and killed vaccines is usually done to prevent Newcastle disease of poultry; however, some of the poultry farms are being encountered with this disease, because the available vaccines do not produce enough antibody titres. In this research, an attempt was made to investigate the effect of using an immune stimulator named *Hypericum perforatum* on antibody production against Newcastle vaccine. 450 broiler chicks (Ross 308) were divided into five groups and three replicates of 30 chicks per replicate. For six weeks, various doses of dry extract (16, 20/5, 24/5 and 28/5 mg/kg) of *H. perforatum* were administered in drinking water to four treatment groups and placebo was administered to the control group. All groups received Newcastle vaccines on days: 11, 19 and 38. Subsequently, on days 10, 25, 34 and 42 blood samples were taken from each group and Newcastle antibody titres were defined by HI test. This experiment showed that the use of *H. perforatum* in each of the foregoing doses, had increasingly effects on antibody titres and this fact is significant between the control group and treatment groups. By using Duncan multiple range test, it was determined that this effect is significant in the case of 1st, 2nd and 3rd groups at 25th days results, but at 34th and 42nd days results, all groups show the same range of titres.

Key words: *Hypericum perforatum*, B1, La Sota, antibody titers, HI test and broiler chicks.

INTRODUCTION

Newcastle disease is one of the important diseases in poultry industry that its intensity is different depending on virus strain, species and the age of host, immunity condition, coincident infections with other organisms and so on (Saif, 2003). Viscerotropic velogenic Newcastle disease which is the most severe form of the disease is prevalent in Iran and treats country's farms. Therefore, an immunity stimulant was used in order to enhance immunity system. The herb *Hypericum perforatum* or St John's Wort is one of the Hypericaceae families (Re et al., 2003). Medical effects of the herb are antibiotic (Mennini and Gobbi, 2004), antiviral (Meruelo et al., 1988), antioxidant (Benedi et al., 2004), anti-stress (Franklin et al., 2004), anticancer (Hostanska et al., 2003), anti-depression, and some other effects on natural killer cells (Helgason et al., 2000).

In some *in vitro* experiments, it was found that Hypericin available in this herb has antiviral activity against several viruses (Jacobson et al., 2001; Lau et al., 1998). Increased activity of natural killer cells under the effect of the herb has been proven by *in vitro* experiments. Regarding that, these cells are of inherent immunity and considered as the first defensive layer against infected cells by virus; so the herb is efficient in enhancing one of the prominent elements of inherent immunity system (Lau et al., 1998). Immunoglobulin or secreted antibodies by these cells are fundamentals for

*Corresponding author. E-mail: a.feizi.iaut@gmail.com. Tel: +98 914 116 0795.

Table 1. The rate of dried herb wer administered in drinking water in treatment groups (mg/kg).

Treatment groups	T-group 1	T-group 2	T-group 3	T-group 4
Herb dosage	16	20/5	24/5	28/5

humoral immunity. Antibodies exist in most body fluids mainly, in serum or blood plasma. These antibodies enter reaction with microorganisms and causes to their removal (Chauhan, 1993; Mayahi and Bouzarghmehrifard, 2000). This study aimed to investigate the effect of dried extract of *H. perforatum* on antibody titer obtained from Newcastle vaccination in broiler chicks and its relationship with humoral immunity as well as evaluating the rate of serum antibodies by HI test.

MATERIALS AND METHODS

450 Ross 308 broiler chicks were used in this study. They were divided into 5 groups and each group was replicated thrice with 30 birds per replicate. Four groups were selected as treatment groups and 1 group as the control group. The chicks were distributed in 3 m^2 pens which floors was covered with straw.

Keeping and rearing

Pens, straw and equipment were disinfected with formaldehyde gas. Rearing has been in standard condition and its duration was 42 days. Feeding method for all groups was conducted as free access.

Vaccination

B1 vaccine, with serial number 1210t and dead vaccine, with serial number P118306, both made by Razi research and serum producing institute, were used as eye drop and subcutaneous injection respectively on 11th day. La sota vaccine, made by Veternia Co., with serial number 5245046 was administered on 19th day for Newcastle vaccine. Newcastle vaccine, made by Veternia Co., with serial number 5225053 was used on 38th day.

Medicine prescribing

Dried extract of *H. perforatum* obtained from Saha Co. Iran, was used in 4 different periods in treatment groups. Distilled water instead of the herb in identical condition was used for control group. The extract was standardized based on the rate of chikuric acid and minimum acid content was 1.4%. Also total bacterial count and total mould and yeast was in standard level and free from *Staphylococcus aurous*, *Pseudomonas aeroginosa*, *Salmonella* and *Escherichia coli*. Used dosages of the herb were as shown in the Table 1.

FCR calculating

The grain was weighed at special hours, daily and was placed in pens, separately. After removing the remaining grains of previous day, total remaining grains were weighed separately in order to calculate consumed grain by chicks. The chicks from each group were individually weighed weekly for calculating FCR carefully. Note that in each group calculating the total amount of feed and divide it by live weight FCR was obtained.

Sampling

Blood sampling was conducted a day before the first vaccination and then, after three times vaccination (blood sampling was conducted by cutting off the chicks' head followed to sampling from wing area on 10th day). The samples were transferred to the laboratory and the samples' serum separated by 3000 rpm centrifuging for 15 min in order to do the HI test on serum samples. Altogether, samplings were done 4 times; so 420 samples were obtained for experiments.

RESULTS

Different superscripts on means show significant difference (P < 0.05)

The results of HI titration on 10th day showed that there is no meaningful difference between treatment and control groups (P > 0.05). This result demonstrates the identical antibodies in all groups before Newcastle vaccine prescribing. HI titration on 25th day was so after B1 vaccine prescribing and dead vaccine on 11th day followed by blood sampling on 25th day, there is no significant difference among all groups (P > 0.05). The results showed that all dosages have similar effect on HI titration increase resulted by B1 and dead vaccine reactions. Based on Orthogonal experiments, there was a significant difference between treatment and control groups (P < 0.05); suggesting boosting antibody titer in treatment groups compared with control group. Based on data analysis by Duncan Test for determining relationship between different levels of medication and HI titer resulted from B1 and dead vaccine reactions, first, second and third dosages had meaningful difference with fourth dosage.

Based on data obtained from HI test using one-way variance analysis of variance on 34th day, the mean serum antibody was not meaningful among all groups (P > 0.05) but based on Orthogonal Test there was significant difference between treatment and control groups (P < 0.05). Based on data analysis using Duncan Test in order to determine the relationship among different levels of medication and titer obtained by La sota vaccine, there was no meaningful relationship among HI titer of different levels of medication. The results of HI titration on 42nd day following vaccination on

Table 2. The rate of Newcastle HI antibody titer obtained from groups on 10th, 25th, 34th, 42nd day (Mean ± SD).

Group / Day	T-group 1	T-group 2	T-group 3	T-group 4	Control group
10	2/212±0/14[a]	2/220±0/02[a]	2/212±0/14[a]	2/211±0/08[a]	2/226±0/03[a]
25	5±0/66[a]	5/17±0/21[a]	5/323±0/17[a]	4/751±0/83[a]	3/942±0/15[b]
34	5/898±0/44[a]	5/810±0/34[a]	6±0/14[a]	5/799±0/20[a]	5/132±0/12[b]
42	6/514±0/21[a]	6/122±0/25[a]	6/817±0/16[a]	6/211±0/22[a]	5/534±0/17[b]

Table 3. Comparative study of Mortality percentage and FCR of treatment and control group.

Function / Groups	Mortality percentage	FCR
T – group 1	1/5[a]	1/602[a]
T – group 2	2[a]	1/638[a]
T – group 3	1/5[a]	1/644[a]
T – group 4	2[a]	1/631[a]
Control group	3/85[b]	1/764[b]

38th day are as follows: one-way analysis of variance showed that the mean serum antibody after vaccination was not significantly different among groups ($P > 0.05$), but based on Orthogonal Test, significant difference was observed between treatment and control groups ($P < 0.01$). Based on data analysis using Duncan Test for determining the relationship among different levels of medication and antibody titer obtained from La sota vaccine, there was no meaningful difference among the ones obtained in HI titers from different levels of medication and all treatment groups had increased antibody titer in similar extent (Table 2).

Different superscripts on means show significant difference ($P < 0.05$)

Table 3 demonstrates mortality percentage in treatment and control groups. Based on statistical analysis using one-way analysis of variance, there was meaningful difference among all groups ($P < 0.05$). Furthermore, based on comparative analysis of Orthogonal Test, the difference between treatment and control groups was meaningful ($P < 0.01$).

Based on data analysis using Duncan Test in order to determine the relationship among different level of medication and mortality percentage among treatment groups, there is more difference about groups 1 and 3. The FCR between treatment and control has been shown in Table 3. Based on statistical studies there was no meaningful difference among all groups ($P > 0.05$) but the difference between treatment groups and control group is meaningful ($P < 0.05$).

DISCUSSION

Newcastle disease is one of the important diseases in poultry industry that its intensity is different depend on virus strain, species and the age of host, immunity condition, coincident infections with other organisms, and so on (Alexander, 2003). Viscerotropic velogenic Newcastle disease which is most severe form of the disease, is prevalent in Iran and treats country's farms. Most of the vaccination programs produce no complete immunity against Newcastle disease; so for obtaining high antibody titer in order to prevent birds from the disease, strengthening immunity compounds are suggested. Helgason et al. (2000) showed by *in vitro* experiments that, *H. perforatum* increases activity of natural killer cells as the first defensive layer against viral infected cells; therefore has effective influence on inherent immunity system (Helgason et al., 2000). Hostanska et al. (2003) showed the herb's effects on malignant cancer cells in human body. Prince et al. (2000) proved antivirus effects of the herb against cattle diarrhea virus by *in vitro* experiments. Meruelo et al. (1988) identified antivirus effects of the herb against leukemia. Tang et al. (1990) showed that Hypercin available in the extract of the herb is an antivirus against leukemia. Kraus et al. (1990) proved Hypercin effect on horses' infectious anemia. Lavie et al. (1989) identified that Hypercin is an antivirus against attenuator virus of mice immunity; also, Lenard et al. (1993) proved Hypercin antiviral function against vesicoloestomatit. The role of *H. perfortum* extract in stimulating immunity system for increasing antibody titer because of vaccination, which was investigated in the present study on *in vitro* models (human and mouse) conforms to Helgason et al. (2000) studies in the case of increased immunity stimulation. Based on studies conducted by Mennini et al. (2004) antiviral and antibiotic effects of the herb have been proved; therefore, meaningful reduction in mortality rate of treatment groups compared with control group accounts for the herb's properties because the control of bacterial infections especially *E. coli* have an important role in reducing mortality rate.

Trofimiuk et al. (2005) showed that the herb reduces the mice disorders resulted by chronic stresses, also El-sherbing et al. (2003) proved anti-stress effects of the herb on mice brains. It was found in Franklin et al. (2004)

studies on rats that the extract of the herb can reduce brain's cortisol and corticosterone. Mentioned findings about rats stress reduction conform to the present study findings; such that the herb's extract caused mortality and FCR reduction in treatment groups. Based on results obtained from the present study, the effect of any four dosages of the herb was observed in increased rate of HI antibody titer. Furthermore, mortality percentage reduction and FCR improvements was seen in treatment groups compared with control group; that the least rate of mortality related to treatment groups 1 and 3, and the best FCR related to treatment group 1. Then it can be concluded that the use of the herb's extract leads to increase immunity level and the rate of antibody titer obtained from vaccination against Newcastle disease. The extract causes to reduce the complications and mortality rate of the disease as well as stress reduction that leads to increase immunity and disease reduction. Therefore, we suggest that the rate of 24/5 mg/kg *H. perforatum* dried extract should be used in broiler chicks drinking water because among the groups studied in this research, the highest Newcastle HI antibody titer and best performance is to T – group 3, that this dose was used in drinking water.

REFERENCES

Alexander DJ (2003). Newcastle disease and other avian pneumoviruses. Disease of poultry. Editied by Dennis J. Alexander. (11th ed.) Iowa state university Press, Ames, IA, pp. 63-87.

Benedi J, Arroyo C, Romeo S, Martin A, Villar AM (2004). Antioxidant properties and protective effects of a standardized extract of *Hypericum perforatum* on hydrogen peroxide-induced oxidative damage in PC12 cells. Life science, 75: 1263-1276.

Chauhan HVS (1993). Poultry Diseases. diagnosis and treatment, willey Eastern Limited, New Delhi, pp. 44-48.

El- Sherbing DA, Khalifa AE, Attia A, Eldenshany EEDS (2003). *Hypericum perforatum* extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amestic dose of scopolamine. Pharmacol. Biochem. Behavior., 76: 525-533.

Franklin M, Reed A, Murck H (2004). Sub- chronic treatment with an extract of hypericum perforatum significantly reduces cartisol and corticosterone in the rat brain. European Neurop syehapharmacol., 14: 7-10.

Helgason CM, Wiesler Frank JL, Johnson DR, Frank MG, Hendricks SE (2000). The effect of st. John's wort on NK cell activity in vitro. Immunopharmacol., pp. 251-274.

Hostanska K, Reichling J, Bommer S, Weber M, Reinhard S (2003). Hyperforin a constituent of st John's wort (*Hypericum perforatum*) extract induces apoptosis by triggering activation of cases and with hypericin synergistically exerts cytotoxicity towards human malignant cell lines. Eur J Pharm Biopharm., 56: 121-132.

Jacobson JM, Feinman L, Liebes L, Ostrow N, Koslowski V, Tobia A, Cabana BE, Lee DH, Sprizler J, Prince AM (2001). Pharmacokinetics, Safety, and Antiviral Effects of Hypericin, a Deivative of St. John's Wort Plant, in Patients with chronic Hepatitis C Virus Infection. Am. Soc. Microbiol. DOI, 45(2): 517-524.

Kraus GA, Pratt D, Tossberg J, Carpenter S (1990). antiretroviral activity of synthetic hypericin and related analogies. Biochem. Biophys. Res. Commun., 172: 149-153.

Lau DT, Kleiner DE, Ghany MG, Schmid P, Hoofagle JH (1998). Sustained virologic response to interferon alpha (IFN-α) in chronic hepatitis is associated with long-term histologic improvement and lack of hepatic HCV RNA. Gastroenterol., 114: A1284.

Lavie G, Valentine F, Levin B, Mazur Y, Gallo G, Lavie D, Weiner D, Meruelo D (1989). Studies of the mechanisms of action of the antiretroviral agents hypericin and pseudohypericin. Proc. Natl. Acad. USA., 86: 5963-5997.

Lenard J, Rabson A, Vanderoef R (1993). Photodynamic inactivation of infectivity of human immunodeficiency virus and other enveloped viruses using hypericin and rose Bengal. Proc. Natl. Acad. Sci. USA., 90: 158-162.

Mayahi M, Bouzarghmehrifard MH (2000). The effect of infectious bursal disease virus and cyclophosphamide on immune respone against Newcastle disease vaccination in broiler chicks. Indian J. Anim. Sci., 70: 8-10.

Mennini T, Gobbi M (2004). The antidepressant mechanism of *Hypericum perforatum*. Life science. 75: 1021-1027.

Meruelo D, Lavie G, Lavie D (1988). Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin. Proc. Natl. Acad. Sci., 85: 5230-5234.

Prince AM, Pascual D, Meruelo D, Liebes L, Mazur Y, Dubovi E, Mandel M, Lavie G (2000). Strategies for evaluation of enveloped virus inactivation in red cell concentrates using hypericin. Photochem. Photobiol., 71(2): 188-195.

Recorneli M, Sturani E, Paolucci G, Rossini F, Leon OS, Martinez G, Bordicchia M, Tomassetti Q (2003). Effects of Hypericum extract on the acetylcholine release: a loose patch clamp approach. Pharmacol. Res., 48: 55-60.

Rodriguez-Landa JF, Contreras CM (2003). A review of clinical and experimental observations about antidepressant actions and side effects produced by *Hypericum perforatum* extracts. Phytomedicine, 10: 688-699.

Saif YM, (2003). Disease of poultry. Iowa State press. 11th edition, pp. 63-87.

Tang J, Colacino M, Spizer W (1990). Virucidal activity of hypericin against enveloped and non-enveloped DNA and RNA viruses. Antivir. Res., 13: 313-326.

Trofimiuk E, Walesiuk A, Braszko JJ (2005). St Jon's wort (*Hypericum perforatum*) diminishes cognitive impairment caused by the chronic restraint stress in rats. Pharmacol. Res., 51: 239- 246.