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Immunostimulatory Effect of Indian Herbs *Tinospora cordifolia* and *Asparagus racemosus* on Ranikhet Disease Vaccination in Chickens

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ABSTRACT

Ranikhet disease (RD) is important infectious disease of chicken. Although vaccination available, sometimes immunosupression induced by factors like stress lead to vaccination failures. Immunostimulation by using of herbs in Ayurveda for enhancing the general host immune status is well documented. Present study is aimed to investigate the immunostimulatory effect of important Indian herbs Guduchi (*Tinospora cordifolia*) and Shatavari (*Asparagus racemosus*) on RD vaccination in chickens. Stem dry powder of Guduchi and root dry powder of Shatavari were mixed in regular poultry feed to different groups of birds. All the groups of birds were vaccinated with primary, 1st booster and 2nd booster doses of RD vaccine and the sera samples were tested for antibody levels by hemagglutination inhibition (HI) test. Significant higher ($p < 0.001$) HI antibody titers were found in Shatavari supplemented group and in both the herbs supplemented groups of chickens after 1st and 2nd booster doses of RD vaccination in comparison to RD vaccinated control group. However, Guduchi didn't show any significant immunostimulating properties on RD vaccination after 1st booster and 2nd booster doses of vaccination.

HIGHLIGHTS

- Supplementing the root powder of Indian herb Shatavari in layers had resulted in significant increase antibody titre in response to RD vaccination.
- Guduchi stem power supplementation has low response in immune stimulating properties against RD vaccination in layers.

Keywords: Layers, Guduchi, Immunomodulation, Shatavari and Vaccination failure

Ranikhet disease (RD) is one of the most important infectious diseases of chickens caused by most virulent strains Avian Paramyxovirus-1 (Ashraf and Shah, 2014) which is resulting in huge economic losses to poultry industry. The disease is characterized by rapid in onset, respiratory distress and nervous manifestations in chickens. Commercially available RD vaccines offer reasonably good protection against the disease in vaccinated birds. Inspite of vaccination, sometimes it is unable to protect 100% birds from Newcastle disease (Priya *et al.,* 2022) due to stress induced immunosuppression in birds, occurrence of other immunosuppressive diseases and also other factors. Immunosuppression in chickens is responsible for increased susceptibility to other diseases and also leads to vaccination failures.

Immunostimulation is gaining importance for increasing the immunocompetence of the host. India is traditionally rich with the knowledge of Ayurveda, where in many

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herbs have been routinely used in ayurvedic preparations as immunostimulatory. Research reports are available about the Immunostimulation properties of various Indian herbs in animals and humans (Tiwari *et al.,* 2018). The Immunostimulation properties of these herbs could be utilized for improving the general immune status of the host. Commonly available Indian herbs like Guduchi (*Tinospora cordifolia*) and Shatavari (*Asparagus racemosus*) are reported to exhibit Immunostimulation properties in humans and animals (Jain, 2021; Subaihawi andAbbas 2021; Mohanambal *et al*., 2018; Bhat *et al.,* 2012).It is already reported that application of immunostimulators coupled with vaccination improved the efficacy of vaccination in chickens (Shaha and Bellankimath, 2017). Therefore, the present study is aimed to investigate the immunostimulatory effect of Indian herbs *Tinospora cardifolia* and *Asparagus racemosus* on RD vaccination in chickens.

MATERIALS AND METHODS

Herbal Powders

Stem dry powder of *Tinospora cordifolia* (Guduchi) and root dry powder of *Asparagus racemosus* (Shatavari) were purchased from commercial manufacturer M/s Agri Gold Organics Pvt. Ltd, Herbs & Health Care Division, Vijayawada, Andhra Pradesh. and were used in this study.

Experimental birds

Day old white leg horn layer chicks of the same sex (female) were purchased from M/s Srinivasa Hatcheries Limited, Vijayawada for this study. All these birds were housed in three tire cage system in the Department of Poultry of the college; poultry feed and clean drinking water were provided to these birds *ad libitum*. The birds were maintained till the completion of experimental study (80 days).

The experimental birds were divided into four groups. Each group consisted of twenty-four birds.

- 1st Group : Normal controls (fed with regular poultry feed).
- 2nd Group : Birds fed with *Tinospora cordifolia* powder mixed poultry feed.
- 3rd Group : Birds fed with *Asparagus racemosus* powder mixed poultry feed.
- 4th Group : Birds fed with *Tinospora cordifolia* powder and *Asparagus racemosus* powder mixed poultry feed.

The herbal powders were mixed in the poultry feed for the respective groups of birds and fed right from day one as per their weekly average body weight. The quantity of the herbal powder mixed was calculated as per the JavaScript based allometric interspecies dosage scaling calculator (http://home.fuse.net/clymer/minor/allometry.html).

RD vaccine and vaccination schedule

Lasota strain of RD virus was used for the primary dose and 1st booster dose. These two doses of vaccine were administered intranasally to all the groups of chickens. R2B strain of RD virus was used for the 2nd booster dose and this was administered intramuscularly. The RD vaccines manufactured by M/s *Indovax Pvt. Ltd.* India were used in this study (Courtesy: PVS Lab. Pvt. Ltd., Vijayawada). Primary dose of the RD vaccine was given on 7th day of age, 1st booster dose was given on 28th day of age and 2nd booster dose was given on 70th day of age.

Collection of sera samples

Sera samples were collected from all the groups of chickens 14 days after primary dose of vaccine, 10 days after 1st booster dose and 7 days after 2nd booster dose. Collected sera samples were stored at -20 ºC till further use.After primary dose of vaccination, the sera samples were collected from only five birds in each group. However, after 1st booster and 2nd booster doses the sera samples were collected from all the twenty-four birds in each group.

Haemagglutination Inhibition (HI) test

The antibody titers of the sera samples were assessed by standard method of HI test as per the protocol of Terrestrial Animal Health Manual of Office International des Epizootis (OIE) (2016) with slight modifications.

Briefly, the HI test was performed in 96 well ‘V’ shaped microplates. Two-fold dilutions of sera samples were

prepared in 50 µl of normal saline solution. Fifty µl of 4 HA units RDV was added to each well containing the sera dilutions. Then 50 µl of 1% of chicken RBC was added to all the wells containing dilutions of sera samples treated with RD virus. The test plates were incubated for half an hour at 37ºC. The highest dilution of sera sample causing complete inhibition of 4 HA units of RD virus was taken as HI titer for that sera sample. Appropriate positive and negative controls were maintained in the HI test.

STATISTICAL ANALYSIS

The mean HI antibody titers of RD vaccination were calculated for each group of birds. The data pertaining to all groups of birds were subjected to one-way analysis of ANOVA and comparisons of mean values between different groups of birds were made using Tukey's multiple range test. (Snedecor and Cochran, 1994). The mean HI antibody titers in normal control groups were compared with those of 2nd, 3rd and 4th groups of birds.

RESULTS AND DISCUSSION

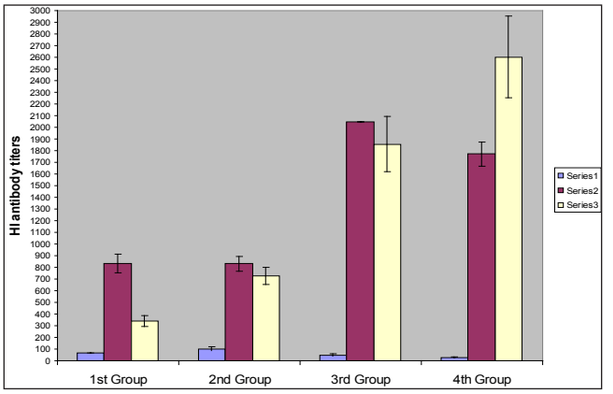
The HI antibody titer for each serum sample is calculated. The mean HI antibody titers ± standard error of the serum samples in each group the chickens is summarized in Table 1.

Table 1: HI antibody titers of different groups of chickens (Mean± SE)

Groups	Primary Immunization*	1st Booster dose**	2nd Booster dose**
1st	64 ± 0.0	832.67 ± 80.14	341.33 ± 45.36
2nd	102.4 ± 15.67	832 ± 64	725.33 ± 74.96
3rd	48 ± 10.11	2048 ± 0.0	1856 ± 238.35
4th	28.8 ± 3.2	1770.67 ± 102.11	2602.67 ± 352.63

* n=5; ** n=24.

The level of significance with regard to HI antibody titers among the different groups of chickens is presented in Table 2. The HI antibody titers after primary immunization, 1st booster and 2nd booster are depicted in Fig. 1.



Series 1 = Primary dose of RD vaccination; Series 2 = 1st booster dose of RD vaccination; Series 3 = 2nd booster dose of RD vaccination.

Fig. 1: HI antibody titers after primary, 1st booster and 2nd booster doses of RD vaccination

Immunoprophylaxis is practiced as a preventive measure to control RD in chickens. Although vaccination offers protection against the disease, immunosupression induced by stress and other diseases decreases the immune response to the vaccination, thus leads to vaccination failures. As immunostimulation coupled with vaccination helps to tide over the problem of immunosupression, Indian herbs *Tinospora cordifolia* (Guduchi) and *Asparagus racemosus* (Shatavari) that are reported to have immunostimulating activity (Sharma and Sharma 2013; Subaihawi and Abbas, 2021) were tested in this study for their immunostimulating effect on RD vaccination. Commercially available Guduchi stem dry powder and Shatavari root dry powder were

Table 2: Level of significance with regard to HI antibody titers among different groups of chickens

Groups	Primary	Immunization	1st	Booster dose	2nd	Booster dose
1st Vs 2nd	*	$p < 0.05$	ns	$p > 0.05$	ns	$p > 0.05$
1st Vs 3rd	ns	$p > 0.05$	***	$p < 0.001$	***	$p < 0.001$
1st Vs 4th	ns	$p > 0.05$	***	$p < 0.001$	***	$p < 0.001$
2nd Vs 3rd	**	$p < 0.01$	***	$p < 0.001$	***	$p < 0.001$
2nd Vs 4th	***	$p < 0.001$	***	$p < 0.001$	***	$p < 0.001$
3rd Vs 4th	ns	$p > 0.05$	*	$p < 0.05$	ns	$p > 0.05$

mixed in the poultry feed either separately or together and fed to experimental chickens right from day one, followed by RD vaccination. The herbal powders were mixed in the poultry feed as per the formula of allometric scaling, keeping in view the body metabolic rate of the chickens with the comparable animal species.

The HI test was performed with the sera samples to measure the protective antibodies after RD vaccination. After primary immunization the sera samples were collected form only five birds per group. Since it was difficult to collect required volume of blood from the wing vein at three weeks of age and also collection of blood from all the birds from the heart might lead to risk of death, only five birds per group were chosen for sera collection after primary immunization. However, after the 1st and 2nd booster doses sera was collected from all the 24 birds in each group.

From the results it is observed that after primary immunization although the immunostimulant Guduchi could able to induce significantly increased HI antibody titers, when compared to normal controls ($p<0.05$), it could not show any significant immunostimulating properties on RD vaccination after 1st booster and 2nd booster doses of vaccination (Table 1 and 2; Fig. 1). However, since only few birds (n=5) were selected for sera sampling after primary immunization, it is difficult to conclude the immunostimulating activity of Guduchi after primary immunization with RD vaccine. The inability of Guduchi to act as immunostimulant after first and second booster doses of RD vaccination is contrary to the earlier findings on immunostimulating activity of Guduchi (Bharadwaj*et al.,*2012)where author indicated marked overall promising and significant effect with Guduchi aqueous extract. Whereas aqueous extract of Guduchi stem has shown significant increase in INFγand IL levels in chicken peripheral blood mononuclear cells against IBD (Infectious Bursal disease) (Sachan *et al.,* 2019) and significant immunomodulatory effect of hydro alcoholic stem extract of *Tinospora cardifolia* has been documented by Nety *et al.* (2017).

Another immunostimulant Shatavari was found to exhibit immunostimulant properties after 1st and 2nd booster doses of RD vaccination. Highly significant raise ($p < 0.001$) in the HI antibody titers were found in Shatavari fed group of chickens after 1st and 2nd booster doses of RD vaccination, when compared with the normal control RD vaccinated birds (Table 1 and 2; Fig. 1). These findings are in conformity with the earlier reports on immunostimulating properties of Asparagus sps. (Kumari *et al.,* 2012; Sharma *et al*., 2012). Although HI antibody titers to RD vaccination in Shatavari fed chickens were not increased after primary immunization, the titers were significantly increased after 1st and 2nd booster doses. This phenomenon may be attributed to the adaptogenic and immunostimulating properties of the Shatavari (Shaha and Bellankimath 2017; Mishra *et al.,* 2017).

The fourth group of birds was fed with both Guduchi and Shatavari, where in after the primary immunization no significant raise in HI antibody titers were found ($p > 0.05$) compared to normal control RD vaccinated birds. However, after 1st and 2nd booster doses there was highly significant raise in HI antibody titers in this group ($p < 0.001$) compared to normal controls (Table 1 and 2; Fig. 1). It is observed from the present study that the Guduchi didn't exhibit significant immunostimulating activity when fed to the birds. However, when both the Guduchi and Shatavari were fed to chickens, there was significantly increased immunostimulating activity on RD vaccination, which was manifested by increased HI antibody titers. Findings of the present study indicated that feeding of herbal powders in different extracts form or different combination of herbal powders may also show the varied results of immunomodulation. This is in agreement with the earlier findings (Bharadwaj *et al.*, 2012; Subaihawi and Abbas, 2021). In conclusion it is summarized that Shatavari acted as good immunostimulator, whereas Guduchi induced immunostimulation significantly when it was fed to chickens along with Shatavari. Therefore, from this study it may be recommended that the two Indian herbal immunostimulators Guduchi and Shatavari may be fed along with feed at the rate proportional on allometric scaling, to improve the general immune status of chickens and also to improve the efficacy of vaccines routinely administered in poultry. Further studies are required to probe the molecular events involved in inducing immunostimulation by these two herbs.

CONCLUSION

It can be concluded that, supplementation of Shatavari root powder alone or in combination with Guduchi stem

powder is able improve immune stimulant response against Newcastle disease vaccine poultry there by reducing the vaccination failures. However, Guduchi stem powder alone is unable to improve the immune response against Newcastle disease vaccination compared to control.

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