Preparation and application of oil emulsion adjuvant of animal vaccines containing flavonoid saponins

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Abstract. With the development of immunology, the application of vaccines plays an important role in controlling the epidemic of animal diseases, and the development of adjuvants is particularly important. Immune adjuvant is a kind of non-specific immune enhancer. It can effectively induce long-term and high-efficiency specific immune response and improve the self-protection ability of the body when injected with antigen or in advance. In this paper, a flavonoid saponin extracted from persimmon leaves was added on the basis of traditional oil adjuvant. The animal potency test showed that the oil emulsion adjuvant containing flavonoid saponin was more effective than single use, and could be widely used in the development of animal vaccine.

1. Introduction
With the development of modern animal husbandry, animal infectious diseases have dealt a heavy blow to animal husbandry. The application of vaccines plays a very important role in controlling the epidemic of animal diseases. With the development of immunology, many new animal disease resistant vaccines with good antigen specificity and low toxicity have emerged, but the immunogenicity is poor and the immune response induced by the body is not enough. Therefore, it is necessary to add a safe and efficient adjuvant to enhance its immunogenicity[1-2]. At present, the commonly used adjuvant of livestock vaccine is oil adjuvant. The oil adjuvant, which contains oil and emulsifier, is generally coated in the microstructures formed by oil and water when it is prepared with the antigen vaccine emulsion[3-4]. When the vaccine is injected into the animal body, it can form a certain storage bank, release the antigen in the oil and water microstructure slowly, stimulate the body to produce cellular immunity and humoral immunity[5]. Natural flavonoids have a broad spectrum of biological activities and are a natural immune enhancer. The application of flavonoids in medicine and immunology has been studied. Among them, the inhibitory effect of plant flavonoids on avian influenza virus has been disclosed. The flavonoids extracted from Folium Nelumbinis can be effectively used to prepare antioxidants and drugs against human cervical cancer cell line[6]. Therefore, a plant extracted flavonoid saponin is added to the
traditional oil adjuvant in this project, which can treat bacteria and virus cells. The growth of the plant is regulated. The immune adjuvant prepared by mixing the flavonoid saponin and oil emulsion adjuvant can enhance the immunogenicity of the antigen, which is more effective than the single oil adjuvant. The adjuvant can be widely used in the development of animal vaccines, reduce the economic losses caused by animal diseases, and have a profound impact on the development of animal husbandry.

2. Experimental

2.1. Materials.
The raw materials are as follows: ND antigen (inactivated newcastle disease virus) and A2 antigen (Inactivated avian influenza virus) were supplied by Ringpu Biology. Foot and mouth disease inactivated virus was obtained from JinYu Bio-Technology. BALB/c mice were purchased in the animal room of Hebei Medical University. Flavonoid saponins and reagents for ELISA were supplied by Institution of Biology, Hebei Academy of Sciences. The water was distilled.

2.2. Characterization
Enzyme linked immunosorbent assay (ELISA) was used to test and analyze the immune titer. The procedure includes the following steps: coating antigen, blocking, coating immune antibody, adding enzyme labeled secondary antibody, coloring and termination. The OD value was measured at 450 nm by enzyme labeling instrument. The OD450nm value of the negative control well was N, and each positive well to be tested was P. The titer was determined with P / N ≥ 2.1.

2.3. Preparation of Vaccine Emulsions
(1) Preparation of natural flavonoids adjuvant
A certain amount of flavonoid saponin was weighed and dissolved in absolute ethanol to form a suspension. The quantitative M-52 oil, polyoxyethylene dehydrated mannitol oleate and mannitol oleate were weighed in proportion for physical mixing. The quantitative ethanol solution of flavonoid saponins was added into the system, and mixed evenly by magnetic stirring at 500r/min. 30min later, the immune adjuvant containing flavonoid saponin was obtained.

(2) Preparation of vaccine emulsions
The immune adjuvant was sterilized at 121 ℃ for 30 min. A2, ND and FMDV antigens were added to the adjuvant slowly, respectively. And the dosage was 100% of that of flavonoids. After completion, magnetic stirring was carried out at 1000r/min for 30min. Then vaccine emulsions contain different antigens were obtained.
3. Results

3.1. Effect of natural flavonoids adjuvant on immune titer of mice

The vaccine emulsions were prepared by A2, ND antigen and adjuvant with different contents of flavonoids saponins of 0.001%, 0.003%, 0.005%, 0.007%, 0.009% and 0%, respectively. And immunization tests were carried out in mice. As shown in Fig.1 and Fig.2, the results of ELISA test showed that the titer of mice reached the highest when the content of flavonoid saponin was 0.005%, but the titer began to decrease after that content. Therefore, flavonoid saponins could obviously stimulate the immune system of mice and enhance their ability of secretory antibody.
3.2. Results of titer test of natural flavonoids adjuvant in clean pigs

![Graph showing IgG antibody titer](image)

**Fig.3** Vaccine emulsions were prepared by FMDV antigen and natural flavonoid adjuvant, respectively. IgG antibody titer after immunization in clean pigs was test(A- blood samples were collected before immunization; B-blood samples were collected from vena cava 3 weeks later immunization; C-blood samples were collected from vena cava 3 weeks later).

As shown in Fig. 3, the vaccine emulsions made from the natural flavonoid adjuvant made in this project is immunized in clean pigs. The titer of IgG was detected in pig vena cava blood collection at the third week of the first immunization. The OD450nm value was significantly higher than that in the blank group. After sixth weeks, the OD450nm value was all higher than 2.5, and the OD450nm value of the adjuvant group with 0.005% flavonoid saponin was higher than that of other groups, indicating that the addition of flavonoid saponin enhanced the immune performance of oil emulsion adjuvant, which fully demonstrated that the natural flavonoid adjuvant formed by flavonoid saponin and oil emulsion adjuvant combined with antigen could significantly improve the antibody level in serum.

3.3. Effect of flavonoid saponins on the titer of vaccine emulsions.

The mice were immunized with natural flavone adjuvant of 0.005% flavonoid saponin and oil emulsion adjuvant without flavonoid adjuvant. The immune content and IgG titer (OD450) were as follows:

| Table 1 | Comparison of titer of different adjuvant dosage in mice |
|---------|----------------------------------------------------------|
| OD450   | 0.6dose | 0.8dose | 1 dose |
| Adjuvant without flavonoid saponin | 2.86    | 3.11    | 3.23   |
| Adjuvant of 0.005% flavonoid saponin | 3.26    | 3.38    | 3.49   |

In this study, adjuvant of 0.005% flavonoids saponin was prepared with antigen in mass ratio of 1:1. 0.005% flavonoid adjuvant and adjuvant without flavonoids were selected to test the effect of their dosage on the titer. The results are shown in Table 1. When the dosage of 0.005% flavonoid saponin adjuvant is 0.6 dose, it is equivalent to 1 dose of adjuvant without flavonoid saponin content. In terms of economic benefits, the addition of flavonoids saponin significantly improves the titer of oil emulsion adjuvant, and the dosage is reduced by 20%, which greatly saves the production cost of vaccine.
4. Conclusion

The adjuvant was formulated with A2 and ND antigen respectively. The immune titer of the vaccine emulsion with 0.005% flavonoid saponin after mice immunization was the best. This adjuvant was collected to mix with FMDV antigen and compounded into a vaccine emulsion containing flavonoid saponins. The titer was tested in the clean grade pigs. The results showed that the 0.005% flavonoid saponin adjuvant had a higher immune titer. Therefore, the development of this adjuvant can be widely used in the development of livestock vaccines, and reduce the production cost of domestic vaccines, with high economic and social benefits.

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