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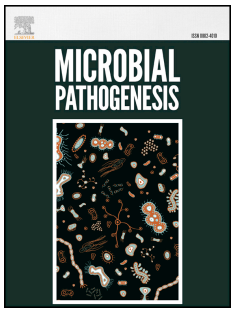

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The Taishan *Robinia pseudoacacia* polysaccharides enhance immune effects of rabbit haemorrhagic disease virus inactivated vaccines

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Abstract

Robinia pseudoacacia flower, a common component in traditional Chinese medicine, has long been well-known for its high pharmaceutical value. This study aimed to assess the immunopotentiating effects of Taishan *Robinia Pseudoacacia* polysaccharides (TRPPS) in rabbits inoculated with a rabbit haemorrhagic disease virus (RHDV) inactivated vaccine. The rabbits were administered with the RHDV vaccine in conjunction with varying concentrations of TRPPS, and their blood samples were collected at different time points to analyze the ratio and number of blood lymphocytes. In addition, sera were prepared and analyzed to determine the overall antibody titer and the level of IL-2, a cytokine commonly used as an indicator of immune activity. The various TRPPS-supplemented vaccines were shown to be more effective in enhancing the immune functions of the inoculated rabbits compared to their polysaccharide-free counterpart, with 200 mg/mL of TRPPS exhibiting the most pronounced benefits that were comparable to those of propolis. In addition, the TRPPS-supplemented RHDV inactivated vaccines could significantly improve the survival rates of the immunized rabbits against RHDV infection. Our studies offered convincing experimental evidence for the development of TRPPS as a new type of plant-derived immunopotentiator.

Keywords: *Robinia pseudoacacia* polysaccharide; rabbit haemorrhagic disease virus; vaccine; immunopotentiator.

1. Introduction

Polysaccharides are carbohydrate-based macromolecules that are characterized by their ubiquitous presence, diverse and complex structures, as well as species specificity. These natural compounds have recently attracted the attention of researchers due to their biocompatibility, biodegradability and potential therapeutic values [1]. Several fungal polysaccharides were found to play an important role in mediating cellular and humoral responses [2]. In another study, *Radix Isatidis* polysaccharides were shown to enhance non-specific immunological function and improve the level of hemolysin in immunosuppressive mice [3]. Xia and colleagues obtained a crude polysaccharide extract from *Dendrobium officinale* and demonstrated that it could significantly stimulate splenocyte proliferation and natural killer cell cytotoxicity [4]. *Robinia pseudoacacia*, a herb frequently used in traditional Chinese medicine recipes, is considered as pharmaceutically valuable, with its beneficial effects ranging from the improvement of capillary elasticity, alleviation of blood lipids and inhibition of tumor growth [5]. Despite its wide cultivation in China, *Robinia pseudoacacia*-related research has primarily focused on the extraction and analysis of the chemical components that it contains. In comparison, there have been very few literature studies on the potential role of Taishan *Robinia pseudoacacia* polysaccharides (TRPPS) as an immune potentiator in livestock and poultry industries.

Rabbit hemorrhagic disease (RHD), a highly contagious and fatal viral disease, first

emerged in China in 1984 and subsequent became prevalent around the world. Infected animals often develop acute necrotizing hepatitis and may also suffer from internal bleeding, particularly in the lungs, heart and kidneys, due to disseminated intravascular coagulation [6]. At present, there is no effective cure for RHD and disease management can only be achieved through preventative vaccine inoculation [7]. Unfortunately, the inactivated RHD virus vaccine needs to be administered multiple times due to its unsatisfactory stability and immunogenicity [8]. Although these drawbacks can be mitigated by formulating the vaccine in oil emulsions, this inevitably leads to significant cost increase and has been suggested in studies to have a negative impact on the quality of the fur and meat of the immunized animals [9]. More importantly, there is increasing evidence suggesting that vaccine-containing adjuvants could produce adverse effects such as the induction of autoimmunity and inflammation [10]. Currently, many herbal medicine-based immunopotentiators and adjuvants have been developed and demonstrated remarkable therapeutic efficacies on various animal diseases with reduced or no side effects. These findings warrant further studies on the development of new herbal polysaccharides-based treatment strategies.

In this study, we investigated the effects of different concentrations of TRPPS on the immune response of rabbits injected with an RHDV inactivated vaccine. The immune functions of the vaccinated rabbits were evaluated and compared to their non-immunized counterparts and also to those that were administered with the same vaccine but supplemented instead with propolis, which, as a key ingredient in many

traditional Chinese medicine recipes, has shown excellent immunostimulatory properties and been used as a vaccine adjuvant in numerous studies. [11, 12]. For example, propolis adjuvant has been found to significantly improve antibody titer and T lymphocyte proliferation when administered together with inactivated porcine parvovirus vaccine to guinea pigs [13]. Our results indicate that TRPPS, especially at the concentration of 200 mg/mL, could boost the protective effects of the RHDV vaccine in rabbits against the viral infection, suggesting that the polysaccharides could be used as a novel adjuvant to combat RHDV.

2. Materials and Methods

2.1 Ethics Statement

All animal procedures performed in this study were reviewed, approved, and supervised by Shandong Institute of Animal Husbandry and Veterinary (Permit No.: 2015749).

2.2 Extraction of Taishan *Robinia pseudoacacia* polysaccharides

Fresh, blooming *Robinia pseudoacacia* flowers were collected from Taishan region in Shandong Province of China. After removing the peduncles by sieving through a 200-mesh sieve, the rest of the flowers were dried in an oven at 70 ^{o}C. The *Robinia Pseudoacacia* pollens were then collected in filter paper and their fat content was extracted with ethyl ether using a Soxhlet extractor. The fat-free pollens were then combined with deionized water in a volume ratio of 1:25, followed by the addition of 0.8% cellulase (Solarbio, China) and pH adjustment of the resultant solution to 4.5.

The treatment was conducted at 45 ^{o}C for one hour and the supernatant was collected. The extraction was repeated four times until the polysaccharides were fully dissolved. The obtained supernatant was filtered, centrifuged at 10000 rpm for 10 min, and then concentrated under reduced pressure in a rotary evaporator. The concentrated solution was treated with sevage reagent twice to remove all protein contaminants. TRPPS were then precipitated by adding a four-fold volume of anhydrous ethyl alcohol to the protein-free concentrate and freeze-drying the resultant suspension. The yield and purity of the obtained TRPPS were determined by the anthrone-sulfuric acid assay based on a glucose standard curve [14]. The level of protein contaminants was measured by Bradford method [15].

2.3 Preparation of RHDV inactivated vaccine

RHDV NJ85 (Genebank AY268525) was selected as the vaccine of choice in the current study. RHD hepar tissues of high virus titers (256 HA units/ml) were mixed with ten volumes of physiological saline, fully homogenized and separately packed. Propolis, a natural immunostimulant with proven effectiveness, was used as a positive control. For the preparation of TRPPS-supplemented vaccine reagents, the prepared polysaccharides were added to a final concentration of 50, 100 or 200 mg/mL. For propolis-supplemented vaccine, propolis (Lufengyuan, China) was added to a final concentration of 40 mg/mL. The resultant solutions were then inactivated for 48 h by adding formaldehyde (Kangde, China) to a final concentration of 0.4%.

2.4 Animal experiment

A total of 72 healthy and nonimmune Rex rabbits weighing around 1 kg and aged 7-8 weeks were obtained from the Taishan Stud Rabbit Plant and randomly divided into six equal experiment groups (group I to VI, 12 per group). All rabbits were raised in isolation under the same conditions. Before the experiment, the absence of maternal RHDV antibodies in all animal subjects was confirmed by performing enzyme-linked immunosorbent assay (ELISA) using Rabbit hemorrhagic disease virus antibody (RHDV Ab) ELISA Kit (Jingmei, China).

Prime boost, in which the same or different vaccine shots with the same antigen are administered multiple times over a certain period of time, is one of the most commonly used vaccination techniques for effective immunization [16]. Nevertheless, we vaccinated our animal subjects only once during the current study so that the difference in immunization effectiveness between various vaccine-adjuvant combinations would not be amplified over the course of multiple administrations. For vaccination, the rabbits in groups I, II, III and IV were injected hypodermically with 1 mL of RHDV inactivated vaccine containing 0, 50, 100, 200 mg/mL of TRPPS, respectively. In contrast, the ones in group V received the same vaccine supplemented with 40 mg/mL propolis, and the control group VI was administered with an equal volume of physiological saline. Blood samples were drawn from the rabbit auricular vein on day 3, 7, 14, 21, 28, 35 and 42 after the vaccination. The sera were separated by allowing the blood samples to clot at 37 ^{o}C for 2 h and centrifuging the resultant mixture at 3000 rpm for 5 min. The obtain serum samples were then stored at -20 ^{o}C before being analyzed in a series of assays described below.

2.5 Determination of serum antibody titers

Antibody titers in the collected serum samples were evaluated by Rabbit hemorrhagic disease virus antibody (RHDV Ab) ELISA Kit (Jingmei, China) following the manufacturer's instructions. The titers were expressed as OD values at 450 nm.

2.6 Blood lymphocyte ratio and count

Two milliliters of fresh pre-diluted anticoagulant (Wokai, China) was mixed with 20 μL of each blood sample. Rabbit blood lymphocyte ratios and numbers were determined using an automated blood cell analyzer (Pukang, China).

2.7 Determination of IL-2 level

Rabbit IL-2 ELISA Kit (Sigma, USA) was used to quantify the serum levels of IL-2 in the immunized rabbits according to the manufacturer's instructions. The absorbance of each sample was measured on an automated ELISA reader (Costar, USA) at 450 nm.

2.8 Artificial challenge of the rabbits

On day 21 after vaccination, 20 rabbits from each group (grouping and vaccination according to the step 2.4) were challenged with 10 LD50 RHDV via hypodermic injection. Clinical symptoms and survival status of the rabbits were minitored and recorded during the 10 day period following the challenge. The challenge experiments were performed in triplicate.

2.9 Statistical analysis

All experimental data were expressed as mean ± SD. Duncan's multiple comparison analysis was performed using SPSS 17.0 software. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Extraction of TRPPS

TRPPS were extracted from the pollens of *Robinia pseudoacacia* flowers using an optimized protocol that involved the use of hot water as the solvent followed by ethanol precipitation. To ensure the quality of the extracted TRPPS, the lipid and protein contents were removed by ethyl ether extraction and treatment with the sevage reagent. The concentration of impurity protein is 13.6 μg/ml in TRPPS. The yield and purity of the extracted TRPPS were determined by the anthrone-sulfuric acid assay to be 4.93% and 94.8%, respectively.

3.2 Change of serum antibody titers

To investigate the effect of TRPPS on stimulating host immune response against RHDV, we inoculated different groups of non-immunized Rex rabbits with RHDV inactivated vaccine supplemented with varying concentrations of the polysaccharides (group I-IV, see Materials and Methods). Meanwhile, control rabbits were either immunized with propolis-containing RHDV vaccine (group V) or physiological saline (group VI). We measured the serum RHDV antibody titers at various time points following the immunization. The results showed higher antibody titers in groups II-V

compared to group I starting from day 3 after the vaccination. In particular, groups III-V exhibited significant increases of antibody levels between day 7 and day 42 ($P < 0.05$), suggesting that both TRPPS and propolis could exert an immune-enhancing effect against RHDV in rabbits (Fig. 1). Moreover, TRPPS were found to stimulate the antibody production in a dose-dependent manner as evidenced by elevated titers in group IV compared to groups II and III between day 7 and 28 ($P < 0.05$). The antibody titer in group VI was also shown to be higher than those in group V, which were administered with vaccine that contained 40 mg/mL propolis, during the period of day 3-42 post-vaccination. The various TRPPS groups all exhibited peak serum antibody levels on day 21, whereas similar phenomenon was observed in the propolis group (group V) on day 28 following the vaccine inoculation. Altogether, these results demonstrated that TRPPS, especially at the concentration of 200 mg/mL, could elicit significant immune response and promote antibody production in rabbits injected with RHDV inactivated vaccine.

3.3 Change of blood lymphocyte ratio

To further explore the immunogenic activity of TRPPS, we quantified the blood lymphocyte ratio, which serves as an indicator of the cellular immune response level. During the period between day 7 and 42, groups III-V showed significantly increased lymphocyte ratios than group I ($P < 0.05$, Table 1). Meanwhile, the lymphocyte ratio in group II also appeared to be higher than the vaccine-only group (group I), although the difference was found not to be statistically significant. Similar to the trend of antibody titers mentioned above, a higher dose of TRPPS resulted in an elevation in

the lymphocyte ratio as demonstrated by a comparison between group III and IV. In addition, the ratios in group IV and V were significantly higher than those of the other groups from day 7 to 28 after the vaccination ($P < 0.05$), with no significant difference observed between the two except on day 14. The lymphocyte ratios in the TRPPS and propolis groups (II-V) achieved peak levels on day 21, whereas a similar trend was detected in group I on day 28 following the vaccination.

3.4 Change in lymphocyte number

Changes in lymphocyte numbers were also monitored. The addition of TRPPS or propolis was found to boost the lymphocyte count in groups II-V compared to that in group I during the period between day 14 and day 21 (Table 2). The number of blood lymphocytes in group IV and V were higher than in other groups during day 3 to 42, with the former showing the highest value of all groups between day 7 and 42 following the vaccine administration, though the difference was not significant. The overall trend during the post-vaccination period was similar to that of the blood lymphocyte ratio, in which lymphocyte numbers of groups II-V and of group I become highest on day 21 and 28, respectively. These results suggested that TRPPS effectively promoted the generation of lymphocytes in the vaccinated rabbits.

3.5 Effects of TRPPS on IL-2 content

The observation that TRPPS improved the blood lymphocyte ratio and lymphocyte number in RHDV-vaccinated rabbits strongly implied an immunostimulatory role of the polysaccharides. To probe this possibility, the serum

level of IL-2, a cytokine produced by activated lymphocytes, was measured in the six groups of immunized rabbits. Consistent with the trends of the immune parameters described above, the IL-2 levels in group II-V were significantly un-regulated compared to that in group I from day 3 onward ($P < 0.05$) (Fig. 2). A comparison of the three TRPPS groups (II-IV) revealed that 200 mg/mL of the polysaccharides produced the most significant increase in the serum concentration of IL-2 between day 7 and 35 ($P < 0.05$). Group IV also exhibited a somewhat higher level of the cytokine compared to the propolis group V, with the difference again not being statistically significant ($P > 0.05$). The maximum IL-2 levels were observed on day 28 in all experiment groups after the inoculation. Taken together, the results confirmed that the supplementation of TRPPS in the RHDV inactivated vaccine could significantly boost the serum IL-2 concentration in immunized rabbits.

3.6 Protection against RHDV infection

Lastly, we examined whether TRPPS-supplemented RHDV inactivated vaccine could offer effective immune protection against the infection of the target virus in rabbits. The six groups of rabbits were first immunized as described above, and then each infected with a lethal dose of RHDV on day 21 following the administration of the vaccine. In the first 24 h following the viral infection, all immunized groups showed significantly higher survival rates compared to the blank control group VI ($P < 0.05$, Fig. 3). No statistically significant difference was observed between the survival rates of the five immunized groups. In comparison, the survival rate of group VI further dropped to 30% at 48 h after the infection. Groups I-V also showed varying

243 degrees of decline in the survival rate. It was shown that group IV had the lowest
244 number of rabbits that were killed as a result of RHDV infection in comparison to all
245 other immunization groups, including group V that used propolis ($P < 0.05$).
246 Furthermore, rabbits receiving 100 or 200 mg/mL of the extracts (groups III-IV)
247 displayed significantly higher survival rates than group I ($P < 0.05$), where the
248 percentage of rabbits killed by the virus increased to 70% at 2 day. No significant
249 change of survival rate was observed between 2 day and 10 day for any of the six
250 experiment groups. Taken together, these data offered convincing evidence that the
251 supplementation of TRPPS in the vaccine boosted the latter's protective effect on
252 rabbits against RHDV, with 200 mg/mL of the polysaccharides demonstrating the
253 most pronounced benefits of all concentrations tested.

254 **4. Discussion**

255 In the current study, we investigated the role of TRPPS in improving the immune
256 function of rabbits administered with an RHDV inactivated vaccine. A previous
257 chemical analysis of TRPPS indicated its composition to be a mixture of multiple
258 monosaccharides, including rhamnose, glucose, galactose and fructose [17]. To obtain
259 high-quality TRPPS, we first removed the lipid fraction from the pollens of Taishan
260 *Robinia pseudoacacia* flowers through ethyl ether extraction, and then used sevage
261 reagent to precipitate proteinous contaminants from the dissolved polysaccharides. On
262 the other hand, propolis, known as an efficient immunostimulant and immunoadjuvant
263 [18, 19], was used as a positive control to evaluate the efficacy of TRPPS in the
264 vaccinated rabbits. We measured and compared the levels of serum antibody titer,

lymphocyte ratios and counts, as well as the concentrations of IL-2, a cytokine that serves as a sensitive indicator of immune functions, in different experiment groups. Moreover, we performed viral infection experiments to examine whether TRPPS could enhance the protective effects of the RHDV inactivated vaccine against the target virus in the immunized rabbits.

There is mounting experimental evidence that plant polysaccharides can exert immunopotentiating effects in a variety of animal species. For example, *Polygonum cillinerve* (Nakai) Ohwi (PCCP) polysaccharides were found to improve the immune system in mice treated with cyclophosphamide, a commonly used immunosuppressant [20]. Polysaccharides extracted from Taishan *Pinus massoniana* pollens have been reported to promote antibody production and improve blood lymphocyte ratio in rabbits inoculated with polysaccharide-rabbit hemorrhagic disease tissue inactivated vaccine [21]. Very recently, *Artemisia annua* polysaccharides were tested as an alternative hepatitis C vaccine adjuvant and were shown to significantly enhance the immune functions in mice [22]. Our current finding that TRPPS could be employed as an effective adjuvant of RHDV vaccine constitutes a valuable new addition to the growing list of Chinese herbal polysaccharides with great pharmaceutical potentials in livestock and poultry industries.

Considerable research efforts have been directed at deciphering the mechanisms behind the immunomodulatory activities of natural polysaccharides. Investigation of a galactin-3-deficient mouse model revealed that certain lipopolysaccharides could be implicated in the maturation of dendric cells and resultantly the regulation of key

287 immune functions [23]. In another study, a mixture of polysaccharides extracted from
288 *Astragalus membranaceus* and *Codonopsis pilosulae* were demonstrated to provide a
289 boosting effect to a dendric cell-based tumor vaccine in a mouse model [24].
290 Subsequent bioinformatics analysis suggested that the polysaccharides, consisting
291 primarily of glucose units, could regulate the levels of key cytokines and chemokines,
292 which in turn could lead to events such as the alteration of lymphocyte proliferation
293 capacities. These findings were echoed by our results showing an increasing trend of
294 serum IL-2 level in rabbits that received RHDV vaccine in conjunction with TRPPS
295 adjuvant. IL-2 is a functionally versatile cytokine that has been established to
296 participate in the Th1-mediated immune response [25]. It can also promote T cell
297 proliferation, enhance natural killer cell activity and induce interferon secretion by
298 lymphocytes [26]. These immunoregulatory properties of IL-2 suggested that it could
299 serve as a useful immune function indicator. Meanwhile, the results provided evidence
300 that IL-2 could be mechanistically involved in the TRPPS's ability to enhance the
301 immunity of vaccinated rabbits against RHDV.

302 The lymphocyte ratio is defined as the percentage of lymphocytes in the total count
303 of leucocytes, the change of which can reflect the overall level of immune activity
304 [27]. The lymphocyte ratio in the current study was measured by an automatic blood
305 cell analyzer, which offered substantially better accuracy and speed than the
306 conventional method that relies on manual microscopic counting. In the current study,
307 the addition of TRPPS was indicated to improve both the serum lymphocyte ratio and
308 number in rabbits inoculated with the RHDV vaccine, compared to both the

non-immunized control and the ones that received the polysaccharide-free vaccine. In addition, the rabbits administered with 200 mg/mL TRPPS exhibited a higher lymphocyte ratio and count than the ones injected with 50 mg/mL of the polysaccharides, whereas the difference between the effects of 200 mg/mL and 100 mg/mL TRPPS was found not always to be statistically significant. Furthermore, the lymphocyte-stimulating effect of TRPPS was also shown to be on par with that of propolis. These data provided further experimental support for the beneficial impact of TRPPS on activating the immune response of the vaccinated rabbits to the target virus.

In summary, our results demonstrated that TRPPS improve the immune functions in rabbits inoculated with the RHDV inactivated vaccine. Specifically, the administration of TRPPS was shown to lead to increase in antibody production, blood lymphocyte ratio and number, as well as IL-2 level in the inoculated rabbits compared to the non-immunized control and those that were vaccinated without the polysaccharide supplement. The benefit of TRPPS was shown to be dose-dependent during most of the observation period following the vaccination. The immune-enhancing effects of TRPPS were also found to be on comparable levels to those of propolis, a commonly used immunopotentiator. Moreover, injection of the RHDV inactivated vaccine in conjunction with TRPPS resulted in a marked improvement in the overall survival rate of the immunized rabbits at 10 day after the vaccination, compared to the ones that were not administered with the polysaccharides, with 200 mg/mL TRPPS showing the best protective effect. Additional advantages of using TRPPS as a

vaccine adjuvant included its easy availability from plants, which can effectively lower the production cost of the vaccine, its relative innocuity and its efficient absorption by the host. The future work will focus on the elucidation of the molecular mechanism that underlies TRPPS's role as an immunostimulant against RHDV and other types of viruses.

Conflict of interest

The authors have no financial conflicts of interest.

Acknowledgments

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Figure captions

Fig. 1. The effects of different adjuvants on serum antibody titer in immune rabbits (OD_{450} value). Rabbits were treated with the RHDV inactivated vaccines utilizing different adjuvants. The adjuvant was 0, 50, 100, 200 mg/mL TRPPS and 40 mg/mL propolis in group I-V. Group VI (control) was injected with saline. Data are represented as mean ± SD at each time point. ($P < 0.05$, n=12)

Fig. 2. The effects of different adjuvants on IL-2 in immune rabbits (ng/L). Rabbits were treated with the RHDV inactivated vaccines utilizing different adjuvants. The adjuvant was 0, 50, 100, 200 mg/mL TRPPS and 40 mg/mL propolis in group I-V. Group VI (control) was injected with saline. Data are represented as mean ± SD at each time point. ($P < 0.05$, n=12)

Fig. 3. Survival rates of rabbits against RHDV infection (%). Rabbits were treated with the RHDV inactivated vaccines utilizing different adjuvants. The adjuvant was 0, 50, 100, 200 mg/mL TRPPS and 40 mg/mL propolis in group I-V. Group VI (control) was injected with saline. After 21 day post-vaccination, rabbits in groups I-VI were then infected with a lethal dose of RHDV. Data are expressed as the percentage of survival, and represented as mean ± SD at each time point. ($P < 0.05$, n=20)

Table 1

The effects of TRPPS on lymphocyte ratio in immune rabbits (%).

Group	Day post-vaccination (d)						
	3	7	14	21	28	35	42
I	33.43±2.56^{ab}	38.58±1.27^{b}	39.03±3.06^{b}	41.47±3.02^{b}	42.97±3.17^{b}	39.07±2.89^{b}	33.64±1.56^{b}
II	36.91±3.08 ^{bc}	39.31±2.63^{b}	41.98±1.75^{b}	46.94±2.01^{c}	45.43±3.98^{bc}	40.43±1.8^{bc}	35.18±1.68^{b}
III	38.45±1.27^{cd}	40.9±2.06 ^{b}	49.95±2.02^{c}	50.35±2.17^{cd}	47.21±1.89^{c}	42.15±1.56^{bcd}	36.35±2.42^{b}
IV	41.7±3.36^{d}	45.92±3.64^{c}	54.67±2.22^{d}	56.27±3.04^{f}	55.9±2.81^{e}	47.02±3.75^{e}	42.7±2.29^{c}
V	39.03±1.49^{cd}	45.88±2.33^{c}	50.78±3.23^{c}	54.72±2.44^{ef}	53.12±1.13^{de}	45.12±1.94^{de}	41.35±2.32^{c}
VI	30.75±0.59^{a}	31.53±1.92^{a}	30.02±1.2^{a}	32.65±2.69^{a}	31.17±1.85^{a}	29.17±2.31^{a}	28.23±1.65^{a}

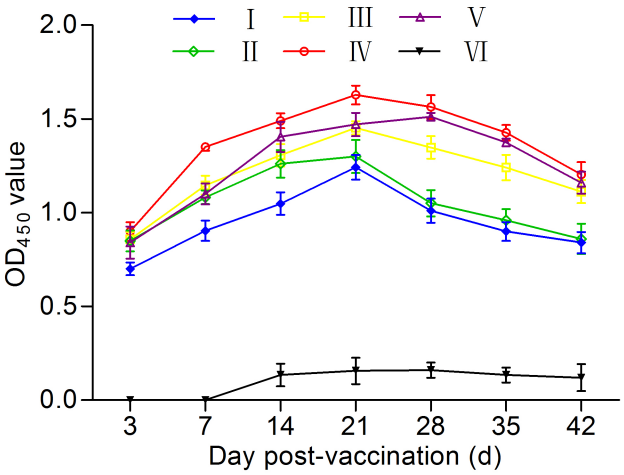
The data with different little letters show significant difference in the same column ($P < 0.05$).

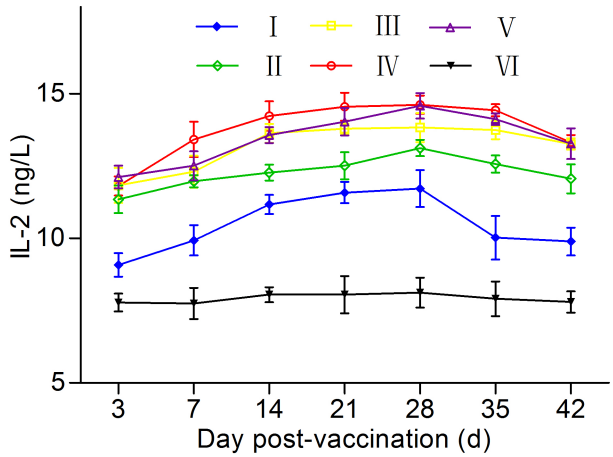
Table 2

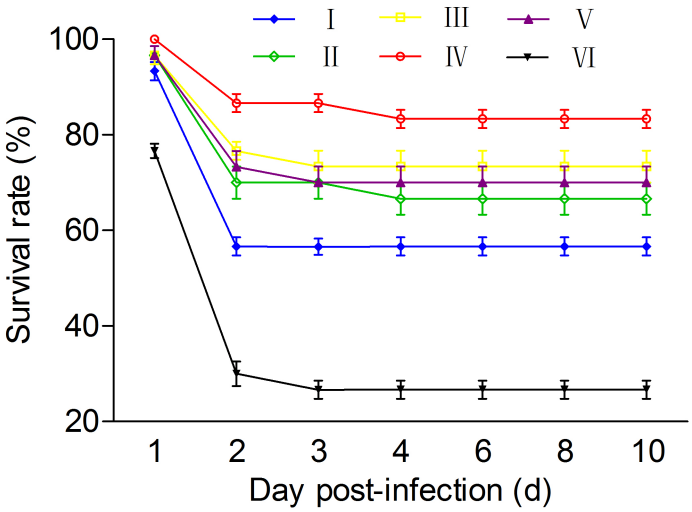
The effects of TRPPS on lymphocyte count in immune rabbits (10^9/L).

Group	Day post-vaccination (d)						
	3	7	14	21	28	35	42
I	2.35±0.19^{a}	3.05±0.15^{ab}	3.11±0.18^{ab}	3.15±0.12^{ab}	3.21±0.19^{ab}	2.6±0.12^{ab}	2.06±0.22^{a}
II	2.46±0.2^{a}	3.05±0.11^{ab}	3.53±0.15^{bc}	3.64±0.21^{bc}	3.43±0.18^{b}	2.75±0.11^{ab}	2.12±0.15^{a}
III	2.48±0.11^{a}	3.22±0.18^{ab}	3.85±0.19^{bc}	3.91±0.12^{bc}	3.62±0.18^{b}	3.1±0.16^{ab}	2.15±0.08^{a}
IV	2.54±0.15^{a}	3.77±0.16^{ab}	4.09±0.11^{c}	4.18±0.11^{c}	4.1±0.11^{b}	3.49±0.19^{b}	2.52±0.13^{a}
V	2.8±0.13^{a}	3.67±0.11^{b}	3.96±0.15^{bc}	4.15±0.14^{c}	4.07±0.22^{b}	3.23±0.25^{ab}	2.48±0.16^{a}
VI	2.43±0.17^{a}	2.5±0.18^{a}	2.45±0.14^{a}	2.6±0.07^{a}	2.47±0.09^{a}	2.45±0.17^{a}	2.4±0.15^{a}

The data with different little letters show significant difference in the same column ($P < 0.05$).







Highlights:

This study aimes to assess the immunopotentiating effects of TRPPS in rabbits inoculated with RHDV inactivated vaccine.

TRPPS is effective in enhancing the immune functions of the inoculated rabbits.

TRPPS-supplemented vaccines significantly improve the survival rates of the rabbits against RHDV infection.

TRPPS is a new type of plant-derived immunopotentiator.