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Immunomodulatory Effects of Aluminium Potassium Sulphate and Saponin Adjuvants in Contagious Caprine Pleuropneumonia Vaccinated Rabbits

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ABSTRACT

Contagious caprine pleuropneumonia (CCPP) is a severe respiratory disease affecting small ruminants, and effective vaccination strategies are critical to preventing its spread. The current study was designed to evaluate the effects of aluminium potassium sulphate (APS) and saponin as adjuvants along with the CCPP vaccine in rabbits. All rabbits (n = 20) were randomly divided into five groups (4 rabbits/group): Group A (control), Group B (APS 5mg 1% adjuvant), Group C (Bacterin), Group D (Saponin 4.25mg 0.85%), and Group E (APS 2.5mg 0.5% + 2.5mg Saponin 0.5%). Both adjuvants were administered once along with the CCPP Vaccine (0.5 ml subcutaneously). Blood samples were collected on days 15, 45, and 75 to analyze albumin, globulin, total protein, WBC, RBC, hemoglobin, and antibody titer. Serum albumin levels significantly increased ($P<0.05$) in Group B on days 15 and 45, and in Group E on days 15, 45, and 75, with a lesser but still increasing ($P<0.05$) trend followed by D and C to the control. Serum globulin levels significantly increased ($P<0.05$) in Groups B and E on day 45, and in Group E on day 75, while the other groups and days showed non-significant changes ($P>0.05$) on days 15, 45, and 75. Total protein content of serum increased ($P<0.05$) in Group B across all three tie points (days 15, 45, and 75), while in Group E on days 45 and 75. A non-significant increasing trend ($P>0.05$) in total protein content was observed in groups C, D, and E on days 15, 45, and 75. The RBC count increased ($P<0.05$) in Group D on days 15, 45, and 75, whereas the remaining groups showed a nonsignificant ($P>0.05$) difference. WBC count increased ($P<0.05$) in Groups B, C, D, and E on day 45, with a non-significant increasing trend ($P>0.05$) observed in Groups B, D, and E on days 15 and 75, and Group C on day 75. In contrast, WBC count decreased ($P<0.05$) in Group C on day 15. Antibody titer increased ($P<0.05$) in all treated groups (B, C, D, and E) on days 15, 45, and 75. In conclusion, Group B (APS 5 mg 1%) and Group E (APS 2.5 mg 0.5% + Saponin 2.5 mg 0.5%) have shown better immunomodulatory effects, significantly increasing serum albumin, globulin, total protein, and antibody titers. APS, particularly in Group B, was the most effective in enhancing immune responses and protein levels, highlighting its potential as a potent adjuvant in vaccine formulations.

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AAJ and SB participated in the design of this research. RSB Formal analysis. MM and GAM analyzed the data. AAJ carried out the study and collected the data. MBA wrote the manuscript. SAS helped during the experiment and data collection.

Key words
Adjuvants, Aluminium Potassium Salphate, Contagious caprine pleuropneumonia, Saponin, Vaccine, Rabbit

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INTRODUCTION

Adjuvants play a crucial role in vaccine development. Adjuvants employed in licensed vaccines have rapidly evolved since 2010s (Goetz *et al*., 2021), However, some attenuated vaccines can induce mild infections in recipients, activating an immune response similar to innate immunity. Its ability to stimulate specific subsets

of T cells, such as CD4+ TH1 and TH2 cells, as well as CD8+ cells involved in cytotoxic responses are closely linked to adjuvanticity (Paiva *et al*., 2024). Vaccines with adjuvants play a pivotal role in enhancing and prolonging the immunogenicity of antigens, thereby modifying the immune response. This modulation is critical for extending the duration of immunity and reducing the required antigen dose, particularly in newborns, the elderly, and immunocompromised individuals (Wang and Xu, 2020). Adjuvants promote both humoral and cellular immune responses against antigens while simultaneously diminishing toxicity and improving immune efficiency in the host. Various adjuvants, such as Aloe Vera, liposomes, mineral salts, bacterial products, emulsions, nucleic acids, small molecules, micro-particles, saponin, and aluminium potassium sulphate (APS), are used in vaccines to enhance potency, increase shelf life, and extend the duration of immunity (Di Pasquale *et al*., 2015; Bastola *et al*., 2017).

APS (KAl $(SO_4)_2$, $12H_2O$) is known for its ability to activate the immune response by stimulating interleukin-1 converting enzyme (ICE) via nucleotide-binding domain and leucine-rich repeat-containing proteins 3 (NLRP3). It has been reported that APS synergizes with toll-like receptors (TLRs) facilitating the differentiation of inflammatory monocytes into dendritic cells (DCs), which act as messengers between the innate and adaptive immune systems (Esposito, 2016). Additionally, APS can regulate the immune response by activating NLRP3 (NACHT, LRR, and PYD domains-containing protein 3), leading to necrosis. APS has been found to activate phagocytosis by release of interleukin-1 (IL1) (Halle *et al*., 2008). Paiva *et al*. (2024) highlighted that APS stimulates the production of cytokines such as IL-1β and IL-18, which are pivotal in orchestrating an effective immune response. These studies underscore APS's role in modulating innate and adaptive immunity, leading to enhanced vaccine efficacy. The simultaneous release of the adjuvant aluminum as Al3+ aqueous might then act in a co-stimulatory manner, as previously observed for B cells (Gibb *et al*., 2017; Zhao *et al*., 2021).

Saponins structurally possess one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative (El-Aziz *et al*., 2019). Their adjuvant properties are exclusively associated with extracts from *Quillaja saponaria* Molina (Atim *et al*., 2016; Nguyen *et al*., 2020). The use of *Quillaja saponaria* extract (soapbark extract) as an adjuvant was initially described in the 1930s and later employed to enhance a foot-and-mouth disease vaccine (Crotty, 2015). In 1978, a saponin (Quill A) enriched mixture was derived from its extract, demonstrating stimulation of both humoral and cellular immunity and the induction of differential antibody isotypes in 1992. Saponins exhibit diverse physicochemical properties, serving as a frothing agent when mixed with water and undergoing saponification, making them widely applicable, including their extensive use in the beverage industry. Importantly, saponins can activate the mammalian immune system, leading to significant interest in their potential as vaccine adjuvants. The principal active ingredient of saponin is Q.S-21, as reported in studies (Güçlü-Üstündağ and Mazza, 2007). However, the use of saponins as vaccine adjuvants has been limited due to their toxicity, including the induction of site reactions (Fauzia *et al*., 2016). Studies have reported that Quill A or QS-21 increases serum antigen-specific Th1-associated immunoglobulin levels in foot-and-mouth disease and measles virus in mice-vaccinated animals (Moses *et al*., 2014). Additionally, Sun *et al*. (2009) reported that saponins enhance the activation of dendritic cells, leading to a more effective presentation of antigens and subsequent activation of T-cells. This property makes saponins particularly valuable for vaccines aimed at eliciting strong cellular immunity.

Despite extensive research on APS and saponins in various animal models, The findings will provide valuable insights into the potential of these adjuvants in enhancing vaccine efficacy in rabbits, a key laboratory animal mode The studies have demonstrated that APS and saponin exhibit significant effects as adjuvants in modulating immune responses against various vaccines in animals. studies specifically evaluating their adjuvant effects in rabbits are limited. This study aims to address this gap by assessing the impact of APS and saponins on immune responses and hematological parameters in rabbits vaccinated against contagious caprine pleuropneumonia (CCPP).

MATERIALS AND METHODS

Experimental animals and their management

Twenty healthy adult mixed-breed male and female rabbits aged 40-45 weeks and weighing 1.8-2 kg were used in this study. The animals were housed in a controlled environment with a temperature of 25°C and humidity between 60-70% at the vaccine production unit in Tandojam. The rabbits were housed in cages and provided *ad libitum* access to water and a standard commercial diet (Brand name: Brit animals, Adult rabbit). The diet composition included alfalfa, herbs (nettle, dandelion, plantain), dried apples, barley, carrot, corn, wheat, linseed, brewer´s yeast, mannan-oligosaccharides (75 mg/kg), fructo-oligosaccharides (50 mg/kg), milk thistle (50 mg/kg), *Yucca schidigera* extract (45 mg/kg). Before the start of the experiment, all animals were acclimatized to the environment for one week.

Experimental design

The twenty rabbits were divided into five groups, (A, B, C, D, and E) each group containing four rabbits. Group A was unvaccinated (control), fed on a normal diet, and had access to tap water. The remaining sixteen rabbits were vaccinated with 5 million bacteria of CCPP vaccine (*Mycoplasma capricolum* PG3 strain) each of the immunized rabbits, Group B was administrated 5mg APS with 1% adjuvant and dilution (NaCl 0.85% + formalin 0.85) subcutaneously for 75 days, Group C was administered with 0.5 ml dilution (NaCl 0.85% + formalin 0.65) subcutaneously for 75 days without any adjuvant. D group was administered with 4.25 mg saponin with 0.85% adjuvant and 0.5 ml dilution (NaCl 0.85% + formalin 0.65%) subcutaneously for 75 days, while E group was administered with a combination of APS 2.5 mg and saponin 2.5 mg as adjuvants and 0.5 ml dilution (NaCl 0.85% + formalin 0.65%).

After administering adjuvants/vaccines as a single subcutaneous dose to different groups, blood samples were collected from all rabbits on days 15, 45, and 75 post-treatment. The blood samples were obtained from the ear vein, cephalic vein (forelimb antebrachium region), and lateral saphenous vein (hindlimb region). Blood was collected using a BD (Becton, Dickinson) 1 ml syringe with a 30-gauge, 0.5-inch needle, then transferred into plain BDH (British Drug Houses) vacutainers and allowed to clot at room temperature. The serum was separated by centrifugation at 5000 rpm for 10 min. Biochemical parameters were measured from serum and whole blood. Serum was stored at -20°C and whole blood was stored at 4°C until further analysis. A complete blood count was analyzed using a hematology analyzer at a commercial laboratory. Serum albumin, globulin, and total protein were measured using a kit from CYGNUS Technologies, following the manufacturer's procedure. Antibody titers were measured from blood serum using an ELISA kit (Catalogue no: 99-56231) IDEXX Laboratories, following the procedure provided by the company.

Statistical analysis

The values were presented as means ± SD for different groups. Statistical analysis was performed using SPSS software (version 8.1). A one-way analysis of variance (ANOVA) was applied, and the least significant difference (LSD) test was used for multiple comparisons between groups. Results were considered statistically significant when $P<0.05$.

RESULTS

Effect on serum albumin and globulin concentration

The serum albumin and globulin levels showed significant variation among the treated groups over different days. In group B, treated with APS, a significant increase ($P<0.05$) in albumin concentration was observed on day 45, while levels decreased on days 15 and 75 compared to the control (group A). In Group C, treated with the bacterin, albumin and globulin levels significantly increased ($P<0.05$) on day 45 but decreased on days 15 and 75 relative to the control. As shown in Table I, the administration of saponins in group D resulted in no significant change in albumin levels compared to the control group but significant increase ($P<0.05$) in globulin and albumin levels on days 45 and 75, while globulin levels decreased on day 15, compared to group A. Co-administration of APS and saponin in group E resulted in a significant increase ($P<0.05$) in albumin and globulin levels on days 45 and 75, with a decrease observed in albumin level and constant globulin level on day 15, compared to the control group.

Table I. Effect of aluminium potassium sulphate (APS), saponin as adjuvant and bacterin of CCPP vaccine on serum albumin and globulin, and total protein concentration in rabbit.

Treated groups	Day 15th	Day 45th	Day 75th
Albumin (g/dL)			
A (Control)	3.16 ± 0.04^{b}	2.98±0.00^{c}	3.43±0.02^{c}
B (APS)	5.45±0.08^{a}	5.79±0.01^{a}	5.15±0.01^{a}
C (Bacterin)	2.95±0.02^{c}	3.81±0.00^{b}	3.35±0.00^{c}
D (Saponin)	3.01±0.00^{c}	3.29±0.00^{b}	3.13±0.00^{c}
E (APS+Saponin)	3.36± 0.00^{b}	3.86±0.01^{b}	3.61±0.00^{c}
LSD (0.05): 0.0238; SE± 0.0487			
Globulin (g/dL)			
A (Control)	3.99±0.00^{c}	3.92±0.01^{c}	5.42±0.00^{a}
B (APS)	4.30±0.00^{c}	6.13±0.00^{a}	5.30±0.00^{a}
C (Bacterin)	4.03±0.00^{c}	5.03±0.03^{b}	5.07±0.00^{b}
D (Saponin)	3.71±0.00^{c}	5.10±0.00^{b}	4.71±0.00^{b}
E (APS+Saponin)	4.44±0.00^{b}	5.04±0.00^{b}	5.44±0.00^{a}
LSD (0.05): 7.868; SE± 0.0161			
Total protein (g/dL)			
A (Control)	7.15±0.00^{b}	6.60±0.36^{c}	8.85±0.00^{a}
B (APS)	9.69±0.00^{a}	11.02±0.00^{a}	10.43±0.00^{a}
C (Bacterin)	7.00±0.00^{b}	8.83±0.00^{b}	8.16±0.00^{b}
D (Saponin)	6.71±0.00^{c}	7.95±0.06^{c}	8.05±0.00^{b}
E (APS+Saponin)	7.79±0.00^{b}	9.29±0.00^{b}	9.04±0.00^{b}
LSD (0.05): 0.0789; SE± 0.2922			

^{a,b} and ^{c} varies significantly ($P<0.05$) difference.

Effect on serum total protein content in rabbit

Table I shows that APS treatment in group B induced a significant increase (P<0.05) in serum total protein levels on days 15, 45 and 75 compared to the control (Group A). In group C, treated with the bacterin, a significant increase (P<0.05) in serum total protein levels was observed on days 45 and 75, while levels remained normal on day 15 compared to the control group. In group D, treated with saponin, total protein levels significantly increased (P<0.05) on days 45 and 75, while remaining normal on day 15 compared to group A. Co- administration of APS and saponin in group E resulted in a significant increase (P<0.05) in total protein levels on days 45 and 75, while a decrease was observed on day 15 compared to the control group values.

Effect on hemoglobin concentration

Analysis of variance, as shown in Table II, indicated that hemoglobin levels remained normal on days 15, 45 and 75 in group B treated with APS, compared to the control group. In group C, treated with the bacterin, hemoglobin levels significantly increased (P<0.05) on days 45 and 75, while remaining normal on day 15 compared to the control group. In group D, treated with saponin, hemoglobin levels were significantly higher (P<0.05) on days 15, 45 and 75 compared to group A. Co-administration of APS and saponin in group E resulted in hemoglobin levels remaining normal on days 15, 45 and 75 compared to the control group.

Effect on RBCs and WBC counts

Subcautious administration of APS to rabbits in group B resulted in a significant increase (P<0.05) in RBC and WBC counts on days 45 and 75, while a decrease was observed on day 15 compared to the control group (Table II). In contrast, treatment with the bacterin in group C did not cause significant changes in RBC levels, which were significantly higher (P<0.05) on days 15, 45 and 75 as compared to the control group. The WBC counts on the other hand were significantly higher (P<0.05) on day 45 and 75 in group C, treated with the bacterin, but showed a marked decrease on day 15 compared to the control group. In group D, treated with saponin, WBC counts were significantly increased (P<0.05) on day 75, while a decrease was observed on days 15 and 45 compared to the control group. In group E, co-administration of APS and saponin resulted in a significant increase (P<0.05) in RBC counts on days 15, 45, and 75 and WBC counts compared to the control group.

Effect on antibody titer PI values

The administration of APS in group B resulted in a highly significant increase (P<0.05) in antibody titer levels on days 15, 45, and 75 compared to the control group (Table III). Similarly, in group C, treated with the bacterin, a significant increase (P<0.05) in antibody titer levels also showed a significant increase (P<0.05) on days 15 45, and 75 compared to the control group. Coadministration of APS and saponin in group E resulted in a significant increase (P<0.05) in antibody titer levels on days 15, 45 and 75 compared to the control group.

Table II. Effect of aluminium potassium sulphate (APS), saponin as adjuvants and bacterin of CCPP vaccine on hemoglobin concentration, and RBC and WBC counts in rabbits.

Treated groups	Day 15th	Day 45th	Day 75th
Hemoglobin concentration (g/dL)			
A (Control)	11.54±0.00^{c}	11.56±0.00^{c}	11.06±0.00^{d}
B (APS)	10.74±0.00^{c}	11.68±0.01^{c}	10.64±0.00^{e}
C (Bacterin)	10.34±0.00^{c}	12.11±0.00^{b}	10.87±0.01^{d}
D (Saponin)	15.48±0.01^{a}	17.10±0.01^{a}	15.85±0.01^{a}
E (APS+Saponin)	11.33±0.01^{c}	11.83±0.00^{c}	11.49±0.00^{c}
LSD (0.05): 4.089; SE± 8.376			
RBC counts (10^{6}/µL)			
A (Control)	5.84±0.00^{a}	6.15±0.00^{ab}	6.14±0.00^{ab}
B (APS)	6.27±0.00^{b}	6.32±0.00b^{c}	6.33±0.00b^{c}
C (Bacterin)	6.03±0.00^{a} I	6.25±0.08^{ab}	5.93±0.08^{a}
D (Saponin)	8.48±0.00^{a}	8.88±0.01^{b}	8.66±0.00^{ab}
E (APS+Saponin)	6.33±0.00^{a}	6.41±0.00^{b}	6.49±0.00^{b}
LSD (0.05): 0.0217; SE± 0.0445			
WBS counts (10^{3}/µL)			
A (Control)	8.57±0.00^{a}	9.13±0.00^{ab}	8.36±0.00^{a}
B (APS)	9.49±0.01^{b}	15.33±0.00^{c}	9.67±0.00^{b}
C (Bacterin)	5.20±0.10^{a}	14.90±0.10^{c}	7.80±0.05^{b}
D (Saponin)	7.43±0.01^{a}	13.17±0.00^{c}	7.82±0.00^{a}
E (APS+Saponin)	7.93±0.00^{a}	9.43±0.00^{ab}	8.08±0.00^{a}
LSD (0.05): 0.0389; SE± 0.0797			

^{a,b,c} and ^{d} varies significantly (P<0.05).

Table III. Effect of aluminium potassium sulphate (APS), saponin as adjuvants and bacterin of CCPP vaccine on antibody titer PI (percentage of inhibition) values in rabbits.

Treated groups	Day 15th	Day 45th	Day 75th
A (Control)	0.0±0.00	0.0±0.00	0.0±0.00
B (APS)	29.53±0.00^{a}	58.20±0.00^{c}	31.50±0.10^{b}
C (Bacterin)	15.50±0.10^{ab}	30.22±0.01^{d}	19.33±0.01^{c}
D (Saponin)	21.53±0.01^{c}	41.73±0.01^{c}	22.30±0.01^{bc}
E (APS+Saponin)	26.86±0.02^{b}	50.46±0.03^{b}	26.26±0.01^{abc}
LSD (0.05): 0.1158; SE± 0.2373			

$^{a, b, c, d, ab,}$ and ^{abc} varies significantly (P<0.05).

DISCUSSION

Vaccination is an essential strategy for preventing disease, however, live-attenuated, inactivated, and subcellular vaccines often fall fail of producing a strong immune response similar to that produced by live microbes (Wang *et al*., 2016; Tesgera *et al*., 2017). Adjuvants are commonly used in vaccinations to improve their immunogenicity. An ideal adjuvant must improve vaccine efficacy, guarantee safety, and stimulate the immune system efficiently while avoiding notable side responses. Antibodies are a particular type of immunoglobulins that include IgG, IgM, IgA, IgE, and IgD, each of which has a unique function in pathogen neutralization. It is crucial to distinguish between immunoglobulins and antibodies (Yagnik *et al*., 2019).

APS and saponins stimulate the immune system in several immunomodulatory mechanisms. It makes antigens more accessible to immune cells and increases antigen deposition at the injection site, thereby delaying antigen release and increasing immunological exposure. Furthermore, APS facilitates the recruitment and activation of antigen-presenting cells, including macrophages and dendritic cells. These APCs are necessary for antigen processing and presentation to T cells, which triggers adaptive immunological responses. Moreover, APS increases the synthesis of pro-inflammatory cytokines, such as IL-1 and IL-6, that activate T-helper cells and enhance humoral immunity in addition to cell-mediated immunity. subsequently, APS promotes the development of B cells into plasma cells, resulting in the release of IgG specific to antigens and supports humoral immunity over the long term (He *et al*., 2015; Kim *et al*., 2021).

Saponins facilitate the formation of immune complexes between antigens and antibodies, thereby improving antigen recognition and uptake by antigen-presenting cells, such as dendritic cells. Additionally, saponins promote the production of Th1 cytokines, notably interferon-gamma (IFN-γ), which activates macrophages and cytotoxic T cells, augmenting the cellular immune response. Furthermore, saponins stimulate B cell activation and differentiation into plasma cells, resulting in increased production of antigen-specific antibodies, thereby amplifying humoral immunity. Moreover, their membrane-disruptive properties enhance APC activation and antigen presentation, further optimizing adaptive immune responses (Den Brok *et al*., 2016).

In this study, the administration of APS and saponin as adjuvants with the CCPP vaccine demonstrated immunomodulatory effects in rabbits. The albumin-to-globulin ratio, a crucial indicator of health and immune status, can be altered during infections. In our findings, inoculation with APS and saponin, along with the CCPP vaccine, resulted in a significant increase ($P<0.05$) in albumin levels in group B on day 45, whereas no significant change was observed in other treated groups on days 15, 45, and 75 ($P>0.05$). Previous studies have shown that albumin levels increase during infections, which can also be linked to an immune response post-vaccination (Pedersen *et al*., 2019). This suggests that vaccination, acting as a controlled infection, might temporarily elevate albumin levels in response to adjuvants.

Similarly, the study observed a significant increase ($P<0.05$) in globulin levels in group B on day 45 and group E on days 45 and 75 post-treatment, indicating the role of globulin in the immune response. Previous literature supports this observation, showing that serum immunoglobulin levels rise following antigen exposure (Bartsch *et al*., 2020). The increased globulin production might be due to a shift toward IFN-γ secreting Th1-like helper cells, which are crucial for immune responses (Ahmadi *et al*., 2017; Gottstein *et al*., 2015).

Our study also demonstrated a significant increase ($P<0.05$) in total protein levels in group B across all days and in group E on days 45 and 75. Total protein elevation, influenced by serum albumin and globulin, supports findings from previous studies where vaccine adjuvants increased serum protein production by modulating these two components (Tiwari *et al*., 2017).

Saponin, used as an adjuvant, significantly increased ($P<0.05$) hemoglobin levels in group D. This increase could be attributed to the stimulation of erythropoiesis, as saponin has been reported to enhance hemoglobin production, consistent with earlier studies (Etim *et al*., 2014). The increased hemoglobin levels may also be a compensatory mechanism of the respiratory system in response to the vaccine's effect on lung function (Lloyd and Marsland, 2017). Similarly, the RBC count increased significantly ($P<0.05$) in group D on days 15, 45, and 75, which is consistent with previous reports of enhanced erythrocyte production following vaccination (Ejelonu *et al*., 2017; Shahzad *et al*., 2016).

WBC count significantly increased ($P<0.05$) in groups B, C, D, and E on day 45, whereas a significant decrease was observed in group C on day 15. These results align with the literature suggesting that adjuvants can stimulate WBC production by enhancing immune responses (Chen *et al*., 2018; Pernow *et al*., 2019). APS has been shown to activate B cells and T cells, leading to cell-mediated immune responses, including enhanced phagocytosis and WBC proliferation (Bode *et al*., 2012).

Lastly, the antibody titer increased significantly ($P<0.05$) in all treated groups on days 15, 45, and 75, with the highest titers observed on day 45, followed by

a decline on day 75. This pattern aligns with earlier studies where adjuvants, particularly APS, were found to increase antibody production and sustain long-term immune responses (Slifka and Amanna, 2019). The role of adjuvants in enhancing humoral immunity, particularly through the activation of dendritic cells and macrophages, has been well documented, supporting the findings of this study (Bénard *et al*., 2018).

The observed immune responses in terms of antibody production, WBC proliferation, and the modulation of albumin and globulin levels indicate that APS and saponin, when used as adjuvants with the CCPP vaccine, effectively enhance both humoral and cellular immunity. This supports the use of these adjuvants in promoting a stronger and more sustained immune response in vaccinated animals.

CONCLUSION

All three treatments, namely APS alone, saponin alone, and the combination of APS with saponin demonstrated immunomodulatory effects with APS showing better effects than the other treatments. APS exhibited a dose-dependent effect, where higher doses resulted in an enhanced level of immune responses. Saponin significantly increased RBCs and hemoglobin levels, an effect not observed with the other treatments. APS proved to be safer and more effective in inducing rapid and prolonged immunity by increasing antibody titer, WBC count, albumin, and globulin levels, as compared to saponin when administered with the CCPP vaccine.

DECLARATIONS

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IRB approval

The research study was approved by the departmental board of studies (Ref no: PHAR-104-2015), Sindh Agriculture University, Tandojam

Ethical approval

The protocols applied in this study were approved Research Ethics Committee of the Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan, Approval Certificate No. DAS-256-2016.

Generative AI and AI-assisted technology statement

The authors have declared that no generative AI or AI-assisted technologies were used to create this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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