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Research Article

Development of pharmaceutical compositions based on animal and plant-derived squalene

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Abstract

Objective: This study aimed to develop and evaluate stable pharmaceutical compositions based on squalene derived from both animal (shark liver) and plant (amaranth oil) sources.

Methods: Oil-in-water nanoemulsions were prepared and stored at 4 °C for six months to evaluate stability. Key stability parameters, including droplet size and zeta potential, were determined using Dynamic Light Scattering (DLS). The immunogenicity of VLP-based rotavirus vaccine formulations containing these adjuvants was evaluated in a guinea pig model. Humoral and cellular immune responses were quantified using Enzyme-Linked Immunosorbent Assay (ELISA) for IgG titers, Neutralization Assays (NA) for virus-neutralizing activity, and the Lymphocyte Blast Transformation Reaction (LBTR) to assess cell-mediated immunity.

Results: Both formulations exhibited high stability at 4 °C. Mean droplet diameters were 157 ± 3 nm (animal-derived) and 149 ± 3 nm (plant-derived), which were smaller than the commercial reference (174 ± 4 nm). Zeta potential values (-35 to -39 mV) confirmed robust colloidal stability. Immunization elicited potent responses; no statistically significant differences were observed between the two squalene sources regarding specific IgG titers (GM = 126 267) or virus-neutralizing activity (VNA GM = 640 vs 452.5, $p > 0.05$). Cellular immunity was induced by both adjuvants, yielding Proliferation Stimulation Index (PSI) values of 3.11 for the plant-derived formulation and 2.10 for the animal-derived variant.

Conclusion: Plant-derived squalene from amaranth oil is a highly effective, sustainable alternative to shark-derived components, providing comparable stability and immunogenic potency for vaccine delivery.

Keywords: pharmaceutical compositions, adjuvants, squalene, amaranth oil, nanoemulsions, virus-like particles, VLPs, vaccine formulations.

INTRODUCTION

Adjuvants play a critical role in modulating the immune response, enabling the induction of specific pathways and tailoring the host's defense mechanisms. Beyond enhancing potency, adjuvant integration facilitates dose-sparing strategies, reducing both the required antigen concentration and the frequency of administrations. This is particularly vital for improving vaccine efficacy in populations with reduced immunological reactivity. To be clinically viable, however, these agents must satisfy rigorous safety profiles: they must be stable, biodegradable, and capable of eliciting a targeted immune response tailored to specific pathogen-protection requirements[1-3].

Adjuvants are traditionally classified into mineral, bacterial, emulsion-based, combined, and synthetic categories, based on their physicochemical and biological properties. Among these, oil-in-water (O/W) emulsions—alongside combined and synthetic variants—represent the most prevalent group[4]. These emulsions serve as highly effective vaccine delivery systems by optimizing antigen presentation. Furthermore, O/W emulsions elicit immune responses that significantly surpass those achieved with traditional aluminum-based salts, establishing them as superior candidates for modern vaccine development. Critically, the biphasic nature of these emulsions allows for the effective solubilization of lipophilic compounds by incorporating them into the hydrophobic oil phase[5-8]. Beyond providing a sustained-release profile for incorporated compounds, the particulate nature of these emulsions extends their biological residence time. This increases the likelihood of phagocytosis by antigen-presenting cells (APCs), thereby enhancing the cellular uptake of vaccines or therapeutics. These adjuvants function through a dual mechanism: acting as delivery

vehicles that facilitate interaction with the immune system while simultaneously exerting immunostimulatory effects that trigger various immune pathways. Within this category, nanoemulsions have gained significant prominence; these systems typically feature oil droplet diameters ranging from 10 to 1000 nm, with most effective formulations falling within the 20–600 nm range. Particle size is a decisive factor in both the colloidal stability and immunogenicity of emulsion-based adjuvants. Research indicates that droplet diameter critically influences biological activity and cellular antigen uptake. Specifically, particles within the 150–600 nm range appear optimal for maximizing immune responses, as their dimensions facilitate superior tissue penetration and rapid trafficking to the draining lymph nodes. Notably, emulsions with a droplet size of approximately 160 nm have been shown to elicit particularly potent responses. Unlike traditional aluminum salts, emulsion adjuvants generate a more robust and balanced response, polarizing toward both Th1 and Th2 phenotypes. Well-established examples of this class include the licensed squalene-based emulsions MF59 and AS03, which are currently utilized in seasonal and pandemic influenza vaccines[9-18].

Squalene ($C_{30}H_{50}$; 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) is a linear triterpene and an analogue of provitamin A. While historically sourced from shark liver oil, squalene is also found in various vegetable oils. Notably, plants of the genus *Amaranthus* represent the most abundant botanical source, containing 6 % to 9 % squalene (1040 to 60 000 µg/100 g), and serve as a viable sustainable substrate for industrial production. Given its highly hydrophobic nature, the utilization of squalene as a vaccine adjuvant necessitates its formulation into stable oil-in-water emulsions to ensure compatibility with hydrophilic biological environments. Achieving optimal biological efficacy requires the precise calibration of emulsification techniques to produce submicron droplets, ideally within the 80–200 nm range. Such microfluidized squalene emulsions have been shown to elicit robust humoral and cellular immune responses while maintaining long-term stability at room temperature. Research indicates that these adjuvants polarize the immune response toward a Th1-type profile — characterized by high IFN-γ production — while simultaneously enhancing the generation of specific IgG1 and IgG2a antibodies, thereby demonstrating a balanced Th1/Th2 immunogenic potential[3,9,14,16,18,19-22].

Virus-like particles (VLPs) represent a promising therapeutic tool for the prevention of infections caused by rotavirus A, which is one of the most common causes of severe diarrhea in infants and children under 5 years of age. VLP-based vaccines are considered safer than inactivated or attenuated viruses, as they eliminate the possibility of incomplete inactivation and restoration of viral replication in host cells. These nanostructures mimic the virion morphology while lacking the viral genetic material; therefore, they are incapable of infecting the host cell, making VLPs safe for use in vaccines. These particles can be modified to improve their immunogenic properties and targeted antigen delivery, enhancing vaccine efficacy. The use of VLP-based vaccines opens new prospects in controlling RV, particularly in regions with high disease incidence. Previously, we described a technology for producing VLPs based on rotavirus A proteins — VP2, VP6, as well as VP4 and VP7 of the most prevalent genotypes in the Russian Federation and conducted a comparative analysis of the efficacy of various adjuvants in VLP-based rotavirus vaccines in BALB/c laboratory mice, based on which a squalene-based adjuvant was selected. It was demonstrated that the VLP-based vaccine possesses specific immunogenicity and efficacy for the prevention of RVI caused by certain rotavirus A genotypes and exhibits a high degree of safety[23-29].

MATERIALS AND METHODS

Materials

Animals. A guinea pig model was employed to evaluate the induction of immunity against RVI, utilizing 24 female animals (270–350 g) obtained from the Stolbovaya Branch of the Scientific Center for Biomedical Technologies (FMBA, Russia).

Bioethics. All animal studies were conducted in strict adherence to international ethical standards and regulatory guidelines, including the Guide for the Care and Use of Laboratory Animals (National Academy Press, 2011), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123), and Directive 2010/63/EU. The experimental protocol was formally approved by the Biomedical Ethics Committee of the N.F. Gamaleya National Research Center for Epidemiology and Microbiology (Protocol #08/2023). All procedures, including euthanasia, were carried out according to established ethical standards to ensure animal welfare.

Rotavirus proteins. The antigenic component of the formulations comprised purified recombinant virus-like particles (VLPs) assembled from nucleocapsid proteins (VP2, VP6) and surface proteins (VP4, VP7). These VLPs represented six rotavirus type A genotypes (G1, G2, G4, G9, P[4], and P[8]) and were produced using a baculovirus expression system[30].

Adjuvants. Experimental adjuvants included squalene-based O/W emulsions of animal and plant origin, while a commercial animal-derived nanoemulsion (adjuvant of foreign manufacture) served as a reference for comparison.

Viruses and cell lines. Attenuated rotavirus A G1P[8] was sourced from the State Collection of Viruses at the N.F. Gamaleya National Research Center for Epidemiology and Microbiology (Ministry of Health of the Russian Federation). Propagation was conducted in the MARC-145 continuous cell line — a rhesus macaque (*Macaca mulatta*) embryonic kidney line — provided by the Russian National Collection of Cell Cultures at the same institution.

Methods

Enzyme-linked immunosorbent assay (ELISA). To quantify antigen-specific IgG, 96-well microtiter plates were

sensitized with 5 µg/mL of purified recombinant VP2/6 proteins in carbonate-bicarbonate buffer (pH 9.5) for 18 h at 4 °C. Following incubation, non-specific binding sites were blocked with 1 % gelatin for 1 h at 37 °C. Guinea pig sera were added in a twofold serial dilution prepared in PBS-T containing 0.5 % BSA and incubated for 1 h at 37 °C. Immune complexes were visualized using a 1:60 000 dilution of polyclonal HRP-conjugated rabbit anti-guinea pig IgG (A5545, Sigma), followed by the addition of 3,3',5,5'-tetramethylbenzidine (TMB) substrate. The enzymatic reaction was terminated with 1M sulfuric acid, and the optical density (OD) was recorded at 450 nm. The antibody titer was defined as the highest serum dilution yielding an OD at least twofold higher than the negative control.

Virus neutralization assay (NA). To evaluate virus-neutralizing activity elicited by the VLP vaccine, a classic *in vitro* micro-neutralization technique was employed utilizing the attenuated human rotavirus A strain Wa (G1P). The indicator system consisted of MARC-145 rhesus macaque kidney cells cultured in DMEM supplemented with 5 % fetal bovine serum (FBS). Prior to the assay, the viral challenge agent was standardized to an infectious dose of 100 per well (initial titer: 5.5 lg/0.1 mL). Animal sera were heat-inactivated at 56 °C for 30 min and subjected to twofold serial dilutions prepared in DMEM. To allow for neutralization, these dilutions were mixed with the standardized virus and incubated at 37 °C for 1 h prior to cell inoculation. Following a 4–5day incubation on 80–90 % confluent MARC-145 monolayers, plates were examined via microscopy for cytopathic effect (CPE). The neutralization titer was defined as the reciprocal of the highest serum dilution providing complete inhibition of viral CPE.

Lymphocyte blast transformation reaction (LBTR). To evaluate the cell-mediated immune response, functional lymphocyte activity was assessed via the LBTR. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using a single-step Ficoll density gradient (ρ = 1.077 g/cm^3), washed twice in RPMI-1640 medium, and seeded into 96-well culture plates at a density of 10^5 cells/well. Cells were maintained in complete RPMI-1640 medium (supplemented with 20 % FBS, 2 mM glutamine, 4.5 g/L glucose, 50 µg/mL gentamicin, and 0.2 U/mL insulin) at 37 °C in a 5 % CO_2 atmosphere. Under sterile conditions, cells were stimulated with 100 µl/well of specific antigens, including a VLP suspension (50 µg/mL) and inactivated Rotavirus A (3 lg $TCID_{50}$/mL, determined by the Reed-Muench method). Concanavalin A (5 µg/mL) and inactivated Crimean-Congo hemorrhagic fever (CCHF) virus served as positive and non-specific controls, respectively. Additionally, experimental O/W adjuvants (animal- and plant-derived squalene) were evaluated at a 1:100 dilution in comparison to a commercial adjuvant. Following a 4–5 day incubation period, lymphocyte proliferation was assessed via inverted microscopy (400× magnification). Results were expressed as the Proliferation Stimulation Index (PSI), defined as the ratio of the mean number of blasts in stimulated wells to those in unstimulated control wells. A PSI ≥ 2 was established as the threshold for a positive response.

Preparation of pharmaceutical compositions based on squalene

The production process for squalene-based pharmaceutical compositions is illustrated in Fig. 1.

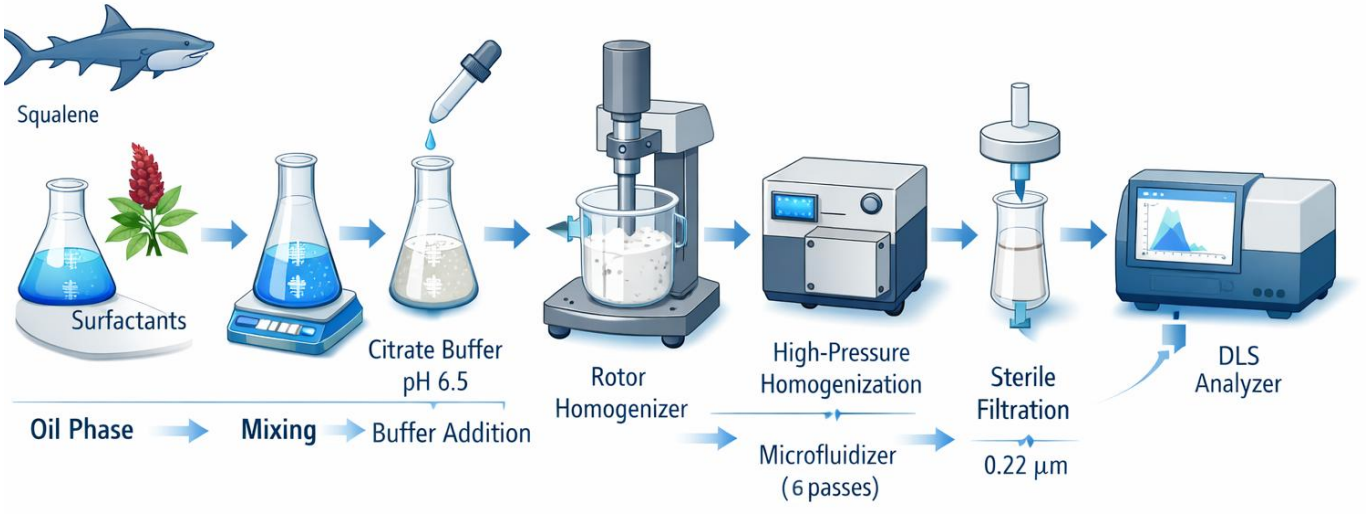


Figure 1: Technology for preparation of pharmaceutical compositions based on squalene

Two different sources of squalene were utilized: animal-derived (shark liver; ≥ 98% purity, CAS 111-02-4, Sigma) and plant-derived (amaranth oil; kindly provided by D.I. Mendeleev University of Chemical Technology of Russia[31]). The lipid components of the emulsion formulations were combined by weight (w/v%). Specifically, the oil, surfactant, and squalene were vortexed, after which the resulting mixture was brought to its final volume with a citrate buffer solution (pH 6.5). The resulting mixtures were pre-emulsified until a

uniform, opalescent appearance was achieved. The crude emulsion was then processed via high-pressure homogenization for up to six passes to reach the target droplet size. The microfluidized emulsions were passed through a 0.22 µm membrane for sterilization, after which droplet size was determined via DLS using a Malvern Zetasizer Nano ZSP (UK).

Determination of adjuvant dispersion degree

Adjuvant stability was monitored at periodical intervals over a six-month storage period at 4 °C, with initial baseline assessments performed on the day of preparation. The droplet size of the dispersed phase was characterized via DLS using a Zetasizer Nano ZSP (Malvern Instruments, UK). All measurements were conducted at room temperature using a fixed scattering angle of 173° (backscattering mode). Data were collected in triplicate for each sample, and the resulting mean values were utilized for subsequent statistical analysis.

Experimental design and immunization protocol

The experimental design for evaluating the immunogenicity of the squalene-based formulations is summarized in Figure 2. Groups were established to compare the efficacy of animal- and plant-derived adjuvants against a commercial reference.

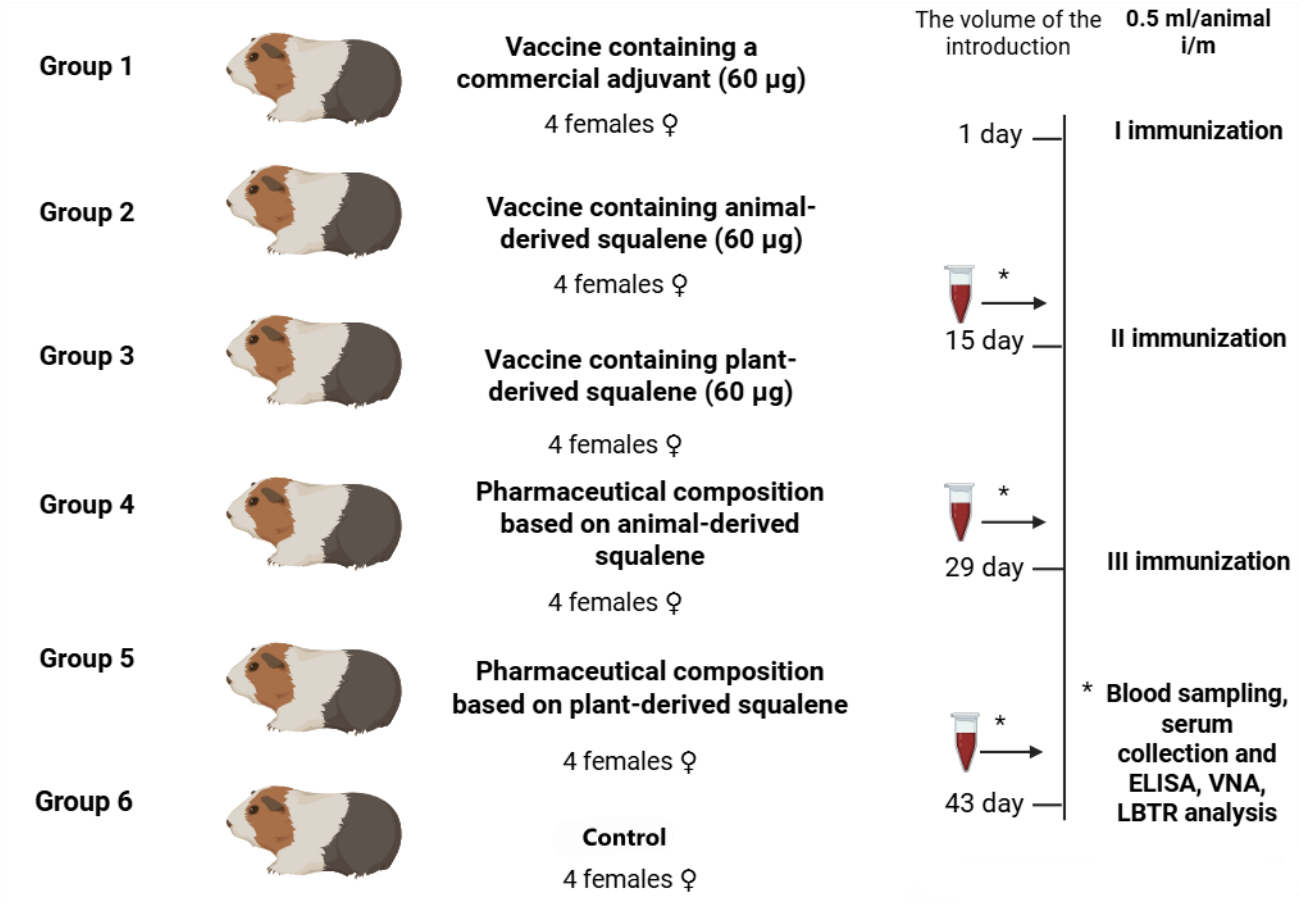


Figure 2: Study design for evaluating the immunogenicity of squalene-based pharmaceutical formulations in a guinea pig model. Schematic created using BioRender.com

Guinea pigs were assigned to experimental groups and received three intramuscular administrations of VLP-based vaccine formulations (60 µg antigen) containing either a commercial adjuvant or experimental pharmaceutical compositions at 14 day intervals. Control groups administered experimental compositions based on animal- and plant-derived squalene (without VLP) or a 0.9 % sodium chloride solution via the same route. Blood samples were collected 14 days following the second and third immunizations, and the obtained sera were stored at –18 °C or below until further analysis.

The magnitude of the humoral immune response was assessed by quantifying IgG levels and virus-neutralizing antibody (VNA) titers via ELISA and NA, respectively. The methods are described in more detail in[32,33]. Concurrently, cell-mediated immunity was evaluated by assessing functional lymphocyte activity via the LBTR, as described in the previous section.

Statistical analysis

Statistical processing of the data was executed via GraphPad Prism 10 (GraphPad Software, USA). The normality of the data distribution was assessed using the Shapiro–Wilk test. The stability of emulsion particle sizes over time was evaluated using two-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for intergroup comparisons. The stability of the emulsions was confirmed by the absence of a significant influence

of the time factor on the particle diameter. Antibody titers were analyzed following logarithmic transformation (log10) of the data to achieve a normal distribution. Comparison of geometric mean (GM) titers between groups was performed using one-way ANOVA, followed by Dunnett's multiple comparisons test (for comparisons with the control group) or Tukey's test. In the case of a non-parametric distribution, the Kruskal–Wallis test was employed. Results are presented as the mean ± standard deviation (Mean ± SD) or as the median with interquartile range. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Physicochemical characterization and stability of squalene-based formulations

In vaccine development, emulsion stability is a critical parameter optimized through the precise selection of lipid phases and surfactants. While squalene is susceptible to oxidative degradation upon prolonged oxygen exposure, it also possesses intrinsic antioxidant properties that can protect lipids from peroxidation [11, 34].

Various established methods exist for the physicochemical characterization and stability monitoring of squalene-based emulsions. Traditional approaches include visual inspection, accelerated destabilization (via high temperature or centrifugation), and morphological characterization using optical or electron microscopy. Furthermore, particle surface charge (zeta potential) obtained by microelectrophoresis, as well as viscometry and rheometry, remains standard for assessing rheological behavior. A distinctive requirement for VLP vaccines, however, is the implementation of additional quality control methods, specifically dispersion testing. Determining VLP dispersion is critical for controlling the multi-stage isolation and purification process. In this context, DLS has emerged as a primary tool for measuring particles in the nanorange and characterizing their stability via electrokinetic potential[11,30].

In the present study, we have successfully developed pharmaceutical compositions via high-pressure homogenization, yielding nanometer-scale droplet diameters consistently within the 100–200 nm range. Characterization via DLS established mean droplet diameters of 155 nm for the animal-derived formulation and 149 nm for the plant-derived formulation — both of which are notably smaller than the commercial reference (176 nm). Over a six-month storage period at 4 °C, these dimensions remained remarkably stable, with final mean diameters of 157 ± 3 nm and 149 ± 3 nm, respectively (Fig. 3).

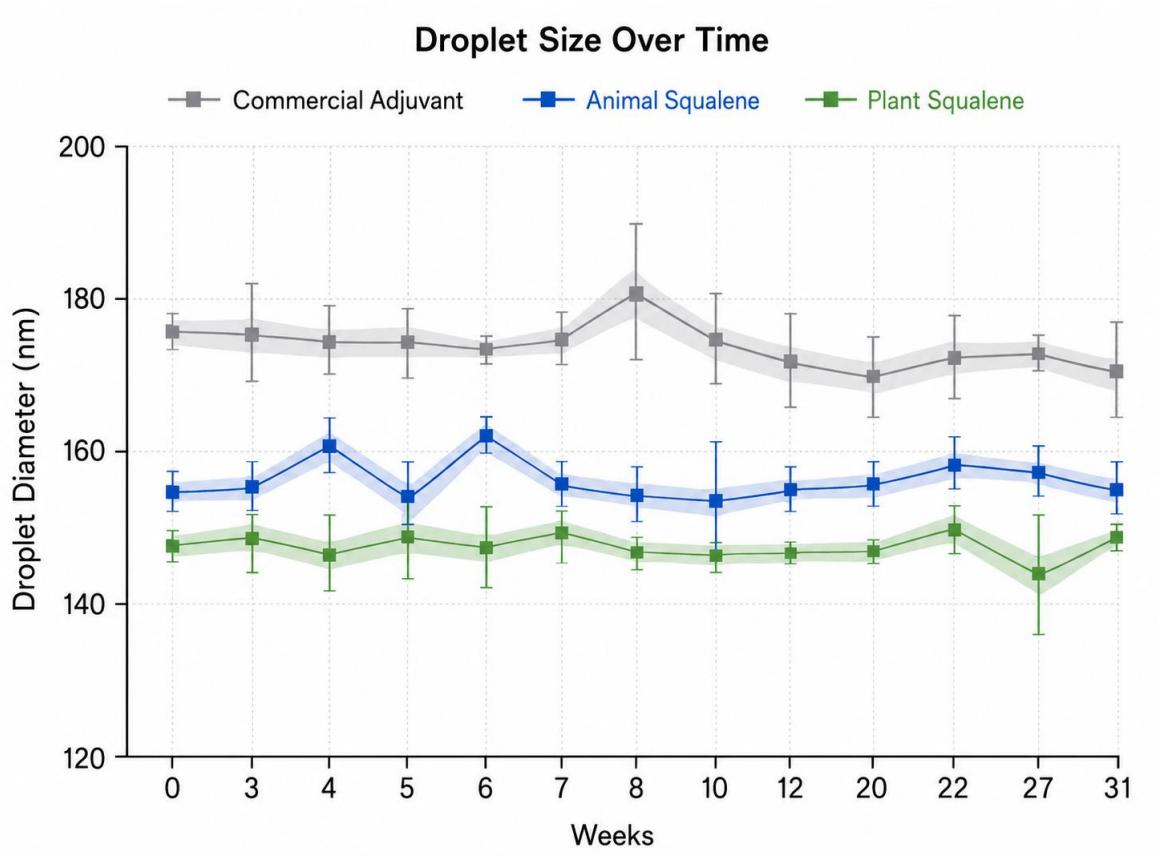


Figure 3: Stability profile of squalene-based oil-in-water nanoemulsions during storage at 4 °C. Mean droplet diameters (nm) were determined by dynamic light scattering (DLS) over a 31-week storage period. The X-axis represents storage duration (weeks), and the Y-axis shows mean droplet diameter (nm). Data are presented as mean ± standard deviation (SD). The commercial adjuvant was used as a reference formulation. The experimental nanoemulsions based on animal- and plant-derived squalene remained within the nanometer range throughout storage, confirming their physicochemical stability under refrigerated conditions.

Furthermore, particle surface charge determination confirmed the robust colloidal stability of the test samples over time. Zeta potential values were −35 ± 4 mV for the animal-derived composition, −37 ± 6 mV for the plant-derived composition, and −39 ± 6 mV for the commercial adjuvant. Such high surface charges provide the electrostatic repulsion necessary to prevent droplet coalescence and maintain a uniform dispersion.

The observed mean droplet diameters (149–155 nm) for our developed compositions are consistent with established requirements for high-performance vaccine adjuvants. Research published in [17] indicates that squalene emulsions with particle sizes below 150 nm maintain superior stability at 5 °C and 25 °C compared to larger droplets (200–250 nm), which are more prone to temperature-dependent phase separation. Furthermore, our zeta potential values (−35 to −37 mV) align with the physical properties of marketed formulations, confirming that our plant-derived alternative provides the necessary electrostatic repulsion for long-term storage. These findings align with previous research indicating that smaller droplet sizes (typically < 200 nm) enhance both colloidal stability and immunogenicity.

Immunogenicity of VLP-based vaccine formulations

The characterized compositions were evaluated as experimental adjuvants in a VLP-based rotavirus vaccine using a guinea pig model. Studies have shown that guinea pigs are susceptible to RV and capable of producing high levels of rotavirus-specific antibodies, as confirmed by results of previous experiments based on serological data[32]. It was established that immunization of guinea pigs with purified rotavirus A VLPs elicits immunity in mammals against homo- and heterotypic rotavirus. Previously, we demonstrated that the rise of RVA-specific IgG antibodies in blood serum is a marker of systemic immunity formation against rotavirus infection, which, along with local immunity, ensures animal survival after experimental challenge[23,35].

Humoral immune response

A significant increase in IgG titers was observed across all vaccinated groups (Groups 1–3) following the second immunization. The highest geometric mean (GM) titer was recorded in Group 3 (plant-derived squalene; GM = 42 089), surpassing both the commercial adjuvant (Group 1; GM = 31 981) and the animal-derived composition (Group 2; GM = 31 981). After the third dose, Groups 2 and 3 reached identical peak titers (GM = 126 267), while Group 1 remained lower (GM = 95 942). Despite these numerical differences, no statistically significant variations were found between Groups 1, 2, and 3. However, Groups 2 and 3 demonstrated a highly significant increase compared to control groups (Groups 4–6) after the third dose ($p<0.001$; Fig. 4).

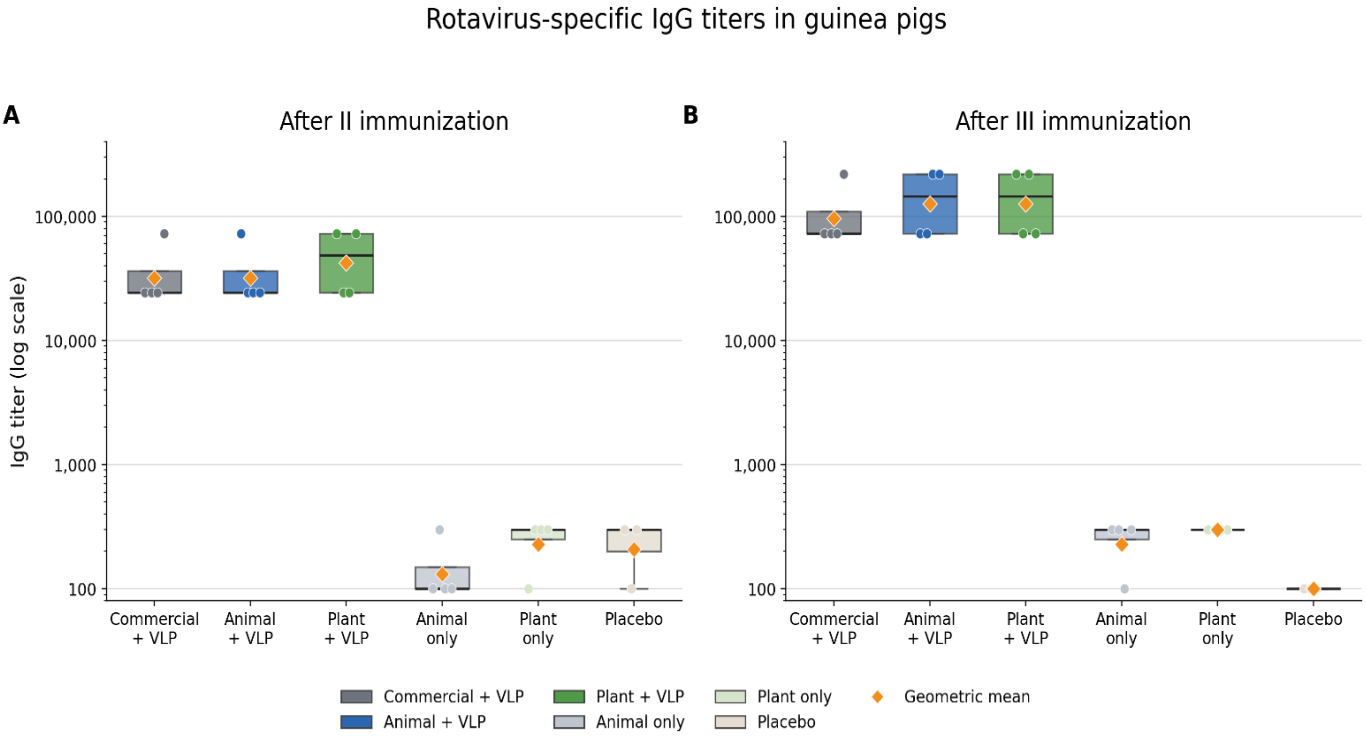


Figure 4: Rotavirus-specific IgG titers in guinea pigs after the second and third immunizations. Serum IgG responses induced by VLP-based vaccine formulations containing a commercial adjuvant, an animal-derived squalene composition, or a plant-derived squalene composition were evaluated after the second and third immunizations. Panel A shows the results obtained after the second immunization; Panel B shows the results obtained after the third immunization. The Y-axis represents IgG titers on a logarithmic scale. Individual data points are shown for each animal, boxplots indicate data distribution within each group, and the diamond marker denotes the geometric mean (GM). Groups receiving adjuvant formulations without VLPs and the placebo group served as controls.

Virus-neutralizing assay (VNA)

Virus-neutralizing antibodies were detectable following the second immunization in all VLP-vaccinated groups. Group 3 (plant-derived) exhibited the highest neutralizing capacity after both the second (GM = 320) and third (GM = 640) doses. In comparison, Groups 1 and 2 yielded lower VNA titers (GM = 226.3 and 113.1 after the second dose; GM = 452.5 after the third). While differences between the three adjuvant groups were not statistically significant, all three (Groups 1–3) significantly outperformed the controls ($p<0.05$; Fig. 5).

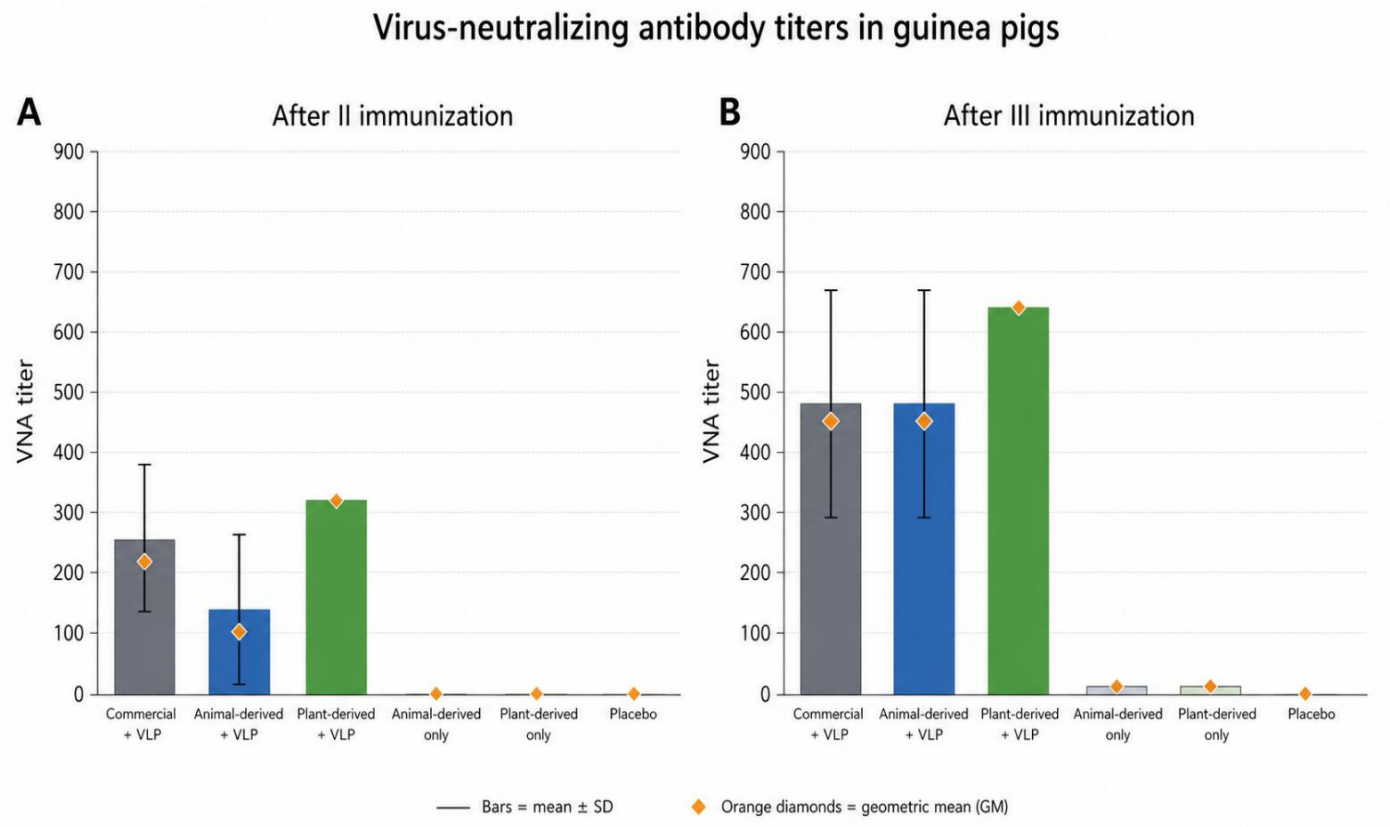


Figure 5: Virus-neutralizing antibody (VNA) titers in guinea pigs after the second and third immunizations. Virus-neutralizing activity induced by VLP-based vaccine formulations containing a commercial adjuvant, an animal-derived squalene composition, or a plant-derived squalene composition was assessed after the second and third immunizations. Panel A shows the results obtained after the second immunization; Panel B shows the results obtained after the third immunization. Bars represent arithmetic mean ± standard deviation (SD), while diamond markers indicate geometric mean (GM) values. Groups receiving adjuvant formulations without VLPs and the placebo group served as controls. The highest neutralizing activity was observed after the third immunization, with the plant-derived squalene formulation demonstrating the strongest response.

Cell-mediated immune response

Assessment of cell-mediated immunity via LBTR confirmed that VLP-adjuvant combinations successfully stimulated lymphocyte proliferation. In all VLP-containing groups, the proliferation stimulation index (PSI) exceeded the positivity threshold, whereas adjuvant-only control formulations remained below this level. The strongest cellular response was observed after three administrations of the plant-derived squalene formulation (Group 3; PSI = 3.11), compared with PSI values of 2.10 for Groups 1 and 2. These results indicate that VLP-adjuvant combinations induced a detectable cell-mediated immune response, with the highest PSI value observed for the plant-derived squalene formulation (Fig. 6).

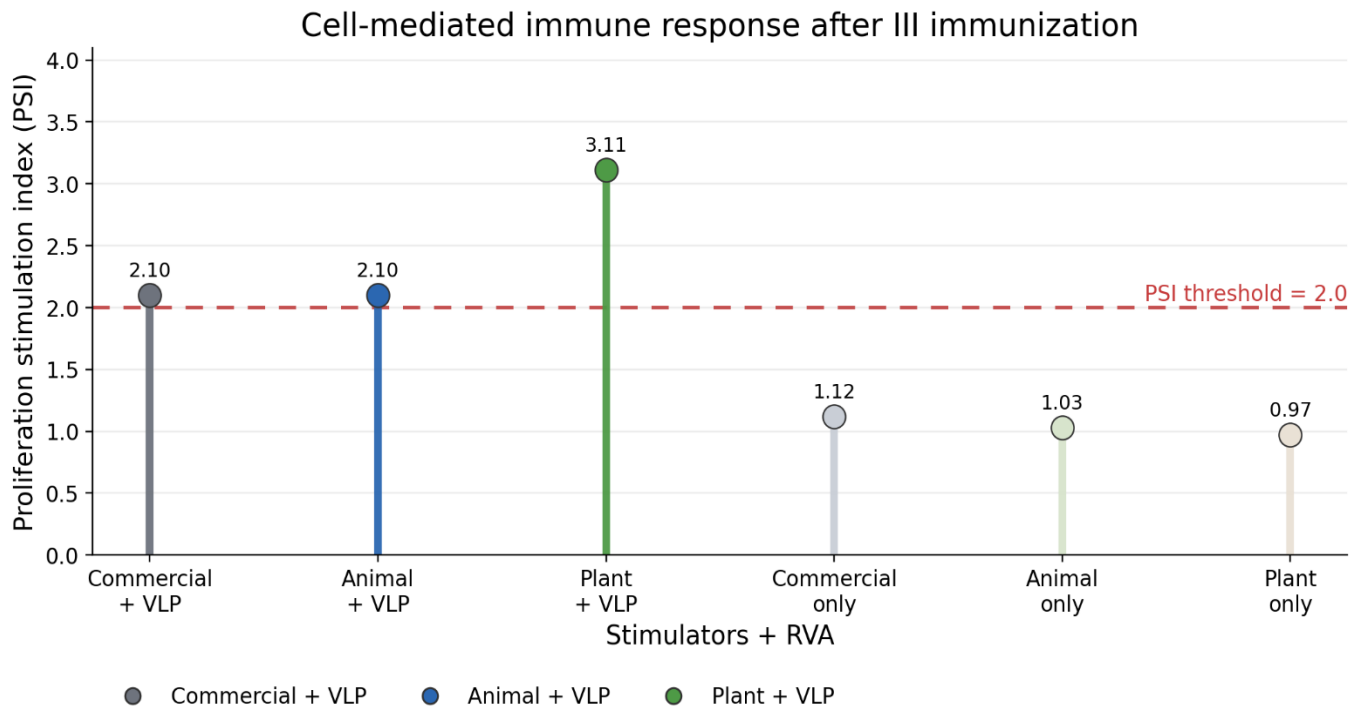


Figure 6: Cell-mediated immune response assessed by the lymphocyte blast transformation reaction (LBTR) after the third immunization. The proliferation stimulation index (PSI) was determined after stimulation with inactivated rotavirus A (RVA) antigen. The X-axis identifies VLP-based vaccine formulations containing a commercial adjuvant, an animal-derived squalene composition, or a plant-derived squalene composition, as well as the corresponding adjuvant-only control formulations. The Y-axis shows PSI values. The dashed horizontal line indicates the positivity threshold for lymphocyte proliferation (PSI = 2.0). All VLP-containing formulations exceeded the positivity threshold, whereas the adjuvant-only control formulations remained below this level. The highest PSI value was observed for the plant-derived squalene formulation.

These data demonstrate that the proposed pharmaceutical compositions, particularly the plant-derived squalene formulation, elicit a robust immune response comparable to commercial adjuvant. This includes strong antigen-specific IgG production, functional virus neutralization, and a potent cell-mediated response, all of which are critical for protection against rotavirus infection. These results align with findings that plant-derived squalene is immunologically comparable to shark-derived sources[36]. The efficacy of such emulsions is well-documented in studies regarding squalene-based O/W adjuvants[37]. Further validation of plant-based alternatives may confirm their use as sustainable and effective adjuvant components.

CONCLUSION

In conclusion, the developed pharmaceutical compositions based on animal and plant-derived squalene demonstrated stability for 6 months (storage at 4 °C). For the animal-derived composition, the mean droplet diameter was 157 ± 3 nm, while for the plant-derived composition, it was 149 ± 3 nm, which is smaller than the mean droplet diameter of the commercial reference adjuvant (174 ± 4 nm). The zeta potential values were –35 ± 4 mV, –37 ± 6 mV, and –39 ± 6 mV, respectively, which also indicates high stability of the resulting adjuvant compositions.

These pharmaceutical compositions have been used as experimental adjuvants in various VLP-based vaccine formulations for the prevention of rotavirus infection. Assessment of the humoral immune response in a guinea pig model demonstrated that incorporation of the developed adjuvants resulted in the production of specific IgG and VNA both when using the experimental plant-derived squalene adjuvant (IgG GM = 126 267 and VNA GM = 640) and when using experimental animal-derived squalene (IgG GM = 126 267 and VNA GM = 452.5). In comparison, the commercial reference adjuvant achieved an IgG GM of 95 942 and a VNA GM of 452.5. A pronounced cellular immune response was also observed (PSI = 3.11 and PSI = 2.10, respectively, compared to PSI = 2.10 for the commercial adjuvant). These results highlight the potential of plant-derived squalene as a sustainable and highly effective alternative to traditional animal-sourced components in next-generation vaccine development.

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Conflicts of interest: The authors declare no conflicts of interest.

Authors' contributions: All authors have read and approved the final manuscript. The individual contributions were as follows: M.M. Silaenkova: conducted experimental work, performed statistical analysis, analyzed and interpreted data, and drafted/translated the manuscript; T.M. Garaev: conducted experimental work, analyzed data, and drafted the manuscript; I.I. Yudin: conducted experimental work and provided manuscript translation; O.V. Ledenev: conducted analyzed, visualizated and interprited data; O.V. Eliseeva: conceived and designed the study, performed data collection and interpretation; O.E. Latyshev: conducted experimental work; A.V. Syroeshkin: conceived the study design and interpreted data; T.V. Grebennikova: conceived the study design, performed data interpretation, and provided final approval of the version to be published.

Data availability statement: The raw data supporting the findings of this study are available from the corresponding author upon reasonable request.

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