Veterinary Integrative Sciences 2026; 24(1): e2026024-1-17. DOI: 10.12982/VIS.2026.024

Veterinary Integrative Sciences
ISSN; 2629-9968 (online)

Research article

Immunoprotective properties of *Scaphium scaphigerum* fruits in cyclophosphamide-induced immunosuppressed mice targeting humoral and cell-mediated immunity

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Abstract

Scaphium scaphigerum has been used in Thai traditional medici[illegible]or ce[illegible]es. T[illegible]alidate its traditional use, this study investigated the effects of *S. scaphigerum* f[illegible]s on ad[illegible] immune responses in a cyclophosphamide-induced immunosuppressed mouse mod[illegible] inistration o[illegible] *S. scaphigerum* at 78 mg/kg significantly enhanced delayed-type hypersensitivity reaction[illegible]and a[illegible]dy production. These enhanced immune responses were corroborated by increased leukocyte and lymphocyte c[illegible]s, as well as elevated levels of CD4+ and CD8+ T lymphocytes. Furthermore, notable lymph[illegible] activation was observed in the germinal centers of the spleen's white pulp. These findings provide initia[illegible]vidence supporting the immunoprotective potential of *S. scaphigerum* fruits.

Keywords: Immunoprotection, Malva nut, Pangdahai, S[illegible] *caphium scaphigerum*.

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Article history; *received manuscript[illegible]ry 2025,*
revised manuscri[illegible]April[illegible]5,
accepted manu[illegible]pt. 9 J[illegible]2025
published online[illegible]
Academic editor; *Korakot N[illegible]vongp[illegible]*

INTRODUCTION

Modulating immune responses to address diseases associated with immune suppression has become a topic of scientific interest. Numerous conditions threatening human health, including chronic and recurrent viral and fungal infections, allergies, and cancer, are believed to result from dysregulated immune system function (Mishra, 2004; Lommatzsch and van Eeden, 2020; Roth-Walter et al., 2021; Sangeetha Vijayan et al., 2024). Study on plant-derived polysaccharides, such as those from *Aloe barbadensis*, *Glycerhiza glaba*, *Anaccychus pyrethrum* and *Alpinia galangal* have demonstrated beneficial effects on immune function (Ramamoorthy et al., 1996; Nose et al., 1998; Bendjeddou et al., 2003; Yang et al., 2020; Poles et al., 2021).

Scaphium scaphigerum (G. Don) Guib. and Pierre, a small tree known scientifically as *Sterculia lychnophora* Hance and *Scaphium macropodum* (Mast.) Pierre, has various common names, including Malva nut, Samrong, and Pangdahai. This plant is native to Thailand and other Asian countries, including Vietnam, Malaysia, Indonesia, and China (Yamada et al., 2007). In traditional medicine across these regions, it is widely claimed to relieve sore throat, cough, pharyngitis, and conjunctivitis. (Ai et al., 2012; Kanlayavattanakul et al., 2017; Oppong et al., 2018).

A thin layer pericarp of *S. scaphigerum* (SS) fruits contains large amount of high molecular weight gum which forms a jelly-like consistency upon absorbing water. The chemical composition of this gum includes 62% total carbohydrates, 8.3% protein, 8.4% ash, and 7.8% moisture (Pantipa et al., 2006). Among its carbohydrate components, sterculia polysaccharide PP III has been identified. The primary monosaccharides include arabinose, galactose, and rhamnose, along with smaller amounts of uronic acid, glucose, and xylose (Chen et al., 1996). Structural analyses revealed that polysaccharides contain terminal L-arabinofuranose (L-Araf), 1-3 linked L-Araf, 1-4 linked D-galactopyranose (D-Galp), and minor binding units such as 1,2,4-linked D-Galp and 1,2,3,4-linked rhamnopyranose (Rhap) (Pantipa et al., 2006).

SS has been utilized in food, beverages, and traditional herbal medicine. Pharmacological studies have demonstrated that its polysaccharides exhibit antioxidant (Phlicharoenphon et al., 2017; Huang et al., 2024), anti-inflammatory (Huang et al., 2024; Oppong et al., 2024), anti-diabetic (via alpha-glucosidase inhibition and glucose entrapment) (Palanuvej et al., 2009), and anti-obesity (Zhao et al., 2018) properties all without observed toxicological effects (Phlicharoenphon et al., 2017; Huang et al., 2024). However, to date, no studies have investigated immunological properties of SS in animal models. Therefore, this present work aims to evaluate the immunological activity of SS fruits in immunosuppressed mice.

MATERIALS AND METHODS

Animals

Female ICR mice, aged 5-6 weeks and weighing between 25-35 g, were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhon Pathom. The mice were housed under standard conditions, with a temperature of 25 ± 2°C, a 12-hour light/dark cycle, and were provided with a standard pellet diet and tap water ad libitum. The animals were acclimatized for one week prior to being used in the experiments. The experiments were approved by Animal Ethical Committee of the Faculty of Veterinary Science, Chulalongkorn University (No. 001/Date 15-03- 2006).

Plant material

The SS fruits were sourced and authenticated by the Thailand Institute of Scientific and Technological Research. The dried fruits were rehydrated by immersing them in distilled water at room temperature for 15 minutes, during which

any contaminants were removed. The rehydrated SS fruit gel was thoroughly rinsed, then boiled in distilled water at 100°C for 60 minutes. After boiling, the gel was drained through a sieve and further pressed through a fabric filter. The gel was spread evenly on a stainless-steel tray and dried in a hot air oven at 65–70°C for 8 hours. Once dried, the gel was ground into a fine powder and sieved. For the animal study, the SS gel powder was mixed with normal saline to achieve three different concentrations, which were freshly prepared for daily treatment.

Chemicals

Levamisole hydrochloride (TP drug, Thailand), cyclophosphamide (Baxter, Germany) Fluorothiocynate (FITC) labeled CD3 anti-mouse monoclonal antibody, Phycoerytherin (PE) labeled CD4 anti-mouse monoclonal antibody, PE labeled CD8 anti-mouse monoclonal antibody, FACS lysing solution, FACS sheath fluid (Becton Dickinson, USA).

Equipment

Agilent 7890A GC system (Agilent Technologies, CA, USA)., FACScan (Becton Dickinson, USA) with Cell Quest Program was used for data analysis, dial caliper (Ozazi, Japan)

Antigenic stimulus

Sheep red blood cells (SRBC) in Alsever's solution were washed with phosphate-buffered saline (PBS). In a 15-mL centrifuge tube, 1 part of SRBC was mixed with 2 parts of PBS and centrifuged at 1000 × g for 10 minutes at 4°C. The supernatant was carefully discarded, and the pellet was resuspended in PBS. This washing step was repeated three more times. Finally, SRBC were resuspended at a concentration of 0.5 × 10^9 cells/mL for the sensitization phase or 5 × 10^9 cells/mL for the challenge phase.

Experimental design

Mice were divided into six groups, each containing seven animals. Group I served as the immunocompetent control. Groups II-VI were immunosuppressed with cyclophosphamide (CP) and varying concentrations of SS or levamisole. Specifically:

Group II: Immunosuppressed mice (CP) without SS treatment
Group III: CP with SS at 19.5 mg/kg (SS1)
Group IV: CP with SS at 39 mg/kg (SS2)
Group V: CP with SS at 78 mg/kg (SS3)
Group VI: CP with Levamisole at 25 mg/kg (Lev)

For the first two weeks, SS or levamisole was administered orally. On day 10, during the pretreatment period, all mice were intraperitoneally injected with 1 × 10^8 SRBC (0.2 mL) for sensitization. At the end of the pretreatment phase, immunosuppression was induced by administering cyclophosphamide at 50 mg/kg body weight for three consecutive days to all groups except Group I (the immunocompetent control), which received intraperitoneal normal saline instead. One week after SRBC sensitization, all mice were challenged with SRBC at the footpad to assess the delayed-type hypersensitivity response. The test was performed the following day, after which all mice were euthanized for blood collection and histopathological examination.

Methods

Characterization of SS gel

The dried seeds of SS were obtained, thoroughly cleaned, and finely ground to a uniform powder. 10 g of SS gel powder was subjected to extraction using two different solvents: distilled water and ethanol. For the ethanolic extraction, 10 g of the SS gel powder was mixed with 100 mL of ethanol and allowed to macerate at

room temperature for 24 hours. The mixture was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C. The final extract was stored at 4°C until further analysis. The water extraction followed a similar procedure with distilled water as the solvent. The hydrolysis technique of SS polysaccharide was described by (Trabelsi et al., 2021) with some modification. Briefly, 10 mg of the extract was dissolved in 1 mL of 2 M trifluoroacetic acid (TFA) at 100°C for 8 h. The hydrolysates were dried under nitrogen gas and derivatized using alditol acetate derivatization. Reduction was performed with sodium borohydride ($NaBH_4$), and acetylation was carried out using acetic anhydride and pyridine at 100°C for 1 hour. The resulting derivatized samples were reconstituted in ethyl acetate and filtered before analysis.

GC-MS analysis was conducted using an Agilent 7890A GC system coupled to a 5975C mass spectrometer (MSD). A DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was used, with helium as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed as follows: initial temperature at 150°C (held for 2 min), increased to 250°C at 4°C/min, and held for 10 min. The injector temperature was set at 280°C, and a split injection mode (split ratio 10:1) was used. The MS detector was operated in electron ionization (EI) mode at 70 eV, scanning from m/z 50–600. Identification of monosaccharides was performed by comparing the retention times and mass spectra of peaks in the chromatograms of both water and ethanolic extracts with standard monosaccharides. The resulting chromatograms revealed the presence of rhamnose, arabinose, ribose, xylose, fructose, galactose, glucose, sucitol, mannitol, erythritol, and inositol in varying proportions across both extracts.

Delayed-type hypersensitivity test

The delayed-type hypersensitivity (DTH) response was assessed in all mice one week after sensitization with SRBC. Prior to the SRBC challenge, the thickness of the left hind footpad was measured using a dial caliper. Mice were then subcutaneously injected with 20 µL of a 5×10^9 SRBC suspension into the left hind footpad. After 24 hours, footpad thickness (FT) was measured again. The change in footpad thickness was calculated as the percentage of inflammation using the following formula:

$$\text{Inflammation (\%)} = \frac{[\text{FT after challenge} - \text{FT before challenge}]}{\text{FT before challenge}} \times 100$$

Hemagglutination assay

The hemagglutination assay (HA) was performed to assess the ability of animals to produce antibodies in response to an antigen. as described by Puri et al. (1992) and Coligan (2005). Briefly, serum was separated by centrifugation, and two-fold serial dilutions were prepared by mixing 25 µL of serum with an equal volume of normal saline in a 96-well U-bottom plate. To each well, 25 µL of 1% sheep red blood cell (SRBC) suspension was added. After gentle mixing, the plates were incubated at room temperature for 2 hours and then examined for hemagglutination. The antibody titer was determined as the reciprocal of the highest dilution of serum that still produced agglutination.

Leukocyte and lymphocyte estimation

Leukocyte and lymphocyte counts were performed using the Coulter Act Diff automated hematology analyzer. A 20 µL aliquot of EDTA-treated blood was aspirated, and the sample was analyzed for total leukocyte count and differential cell count. The analyzer provides the percentage and absolute count of lymphocytes, based on their size and scatter properties. Results were expressed as cells per microliter (cells/µL) of blood.

T lymphocyte subsets estimation (CD4⁺ and CD8⁺ T lymphocyte)

Fluorochrome-labeled mouse monoclonal antibodies against the co-receptors CD3, CD4, and CD8 were used to quantify T lymphocyte subsets via flow cytometry. FITC-conjugated anti-mouse CD3 and PE-conjugated anti-mouse CD4 or CD8 antibodies were employed to identify and determine the percentage of CD4+ and CD8+ T lymphocytes. Separate tubes were used for the detection of each subset. A 100 µL aliquot of whole blood was mixed with 10 µL of FITC-labeled anti-CD3 and 10 µL of PE-labeled anti-CD4 or anti-CD8 monoclonal antibodies. The tubes were incubated in the dark for 30 minutes at room temperature. After incubation, 2 mL of 1× FACS Lysing Solution was added, and the mixture was gently mixed and incubated for an additional 10 minutes at room temperature. The samples were then centrifuged at 1500 rpm for 5 minutes, and the supernatant was carefully aspirated. The cell pellet was washed with PBS. FACS sheath fluid. The stained cell pellet was resuspended in 500 µL of 1% paraformaldehyde and analyzed using a flow cytometer (FACScan®, Becton Dickinson). Data analysis was performed directly on the flow cytometer using CellQuest software.

Spleen weight and histopathological examination

At the end of the study, animals were humanely euthanized by cervical dislocation. The spleens were then carefully excised and weighed. Two spleen samples from each group were randomly selected for histopathological analysis and fixed in 10% buffered formalin for tissue preparation.

Statistical analysis

Data are expressed as mean ± SE of each group then compared using One-way ANOVA statistical method followed by Least significant difference multiple comparison tests. Statistical significance was set at $P<0.05$.

RESULTS

Characterization of SS gel

The chromatogram of the water extract from SS reveals several significant sugar and sugar alcohol compounds. The most prominent peaks correspond to high concentrations of erythritol and glucose, with other notable components including arabinose, rhamnose, ribose, D-fructose, galactose, mannitol, and inositol. These results suggest that the extract is rich in sugars and polyols, which could contribute to its functional properties in applications such as traditional medicine or as a natural sweetener (Figure 1). In contrast, the ethanolic extract of SS (Figure 2) displays a distinct profile, with higher relative abundances of rhamnose, arabinose, and ribose. These differences suggest that ethanolic extracts a different set of components, likely favoring sugars and sugar alcohols that are more soluble in non-aqueous solvents. This profile indicates that the ethanolic extract may possess unique bioactivities, potentially beneficial for applications targeting anti-inflammatory, antioxidant, and metabolic effects.

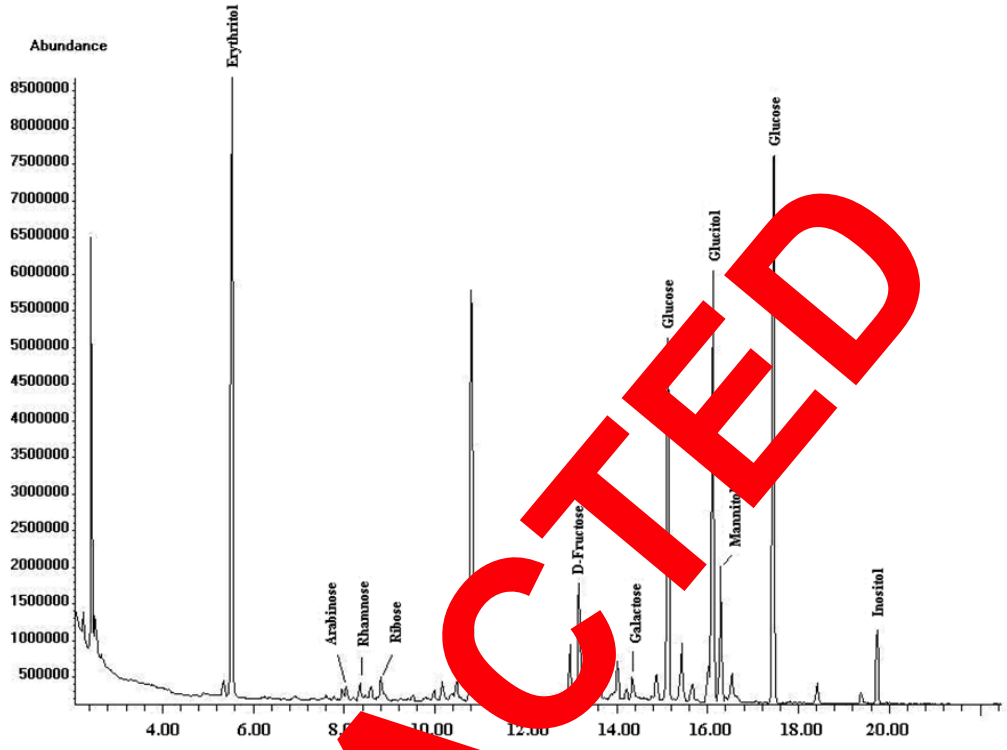


Figure 1 GC-MS chromatogram from [illegible]water extract. The chromatogram of SS water extract revealed a detailed profile [illegible] and [illegible]ar alcohol content based on peak retention times and relative abundances.

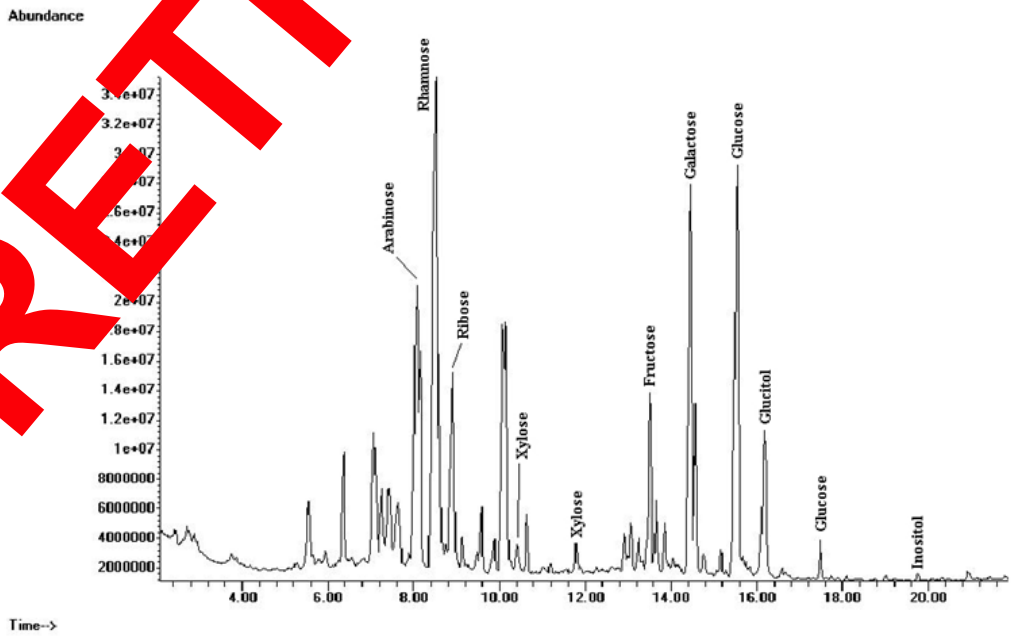
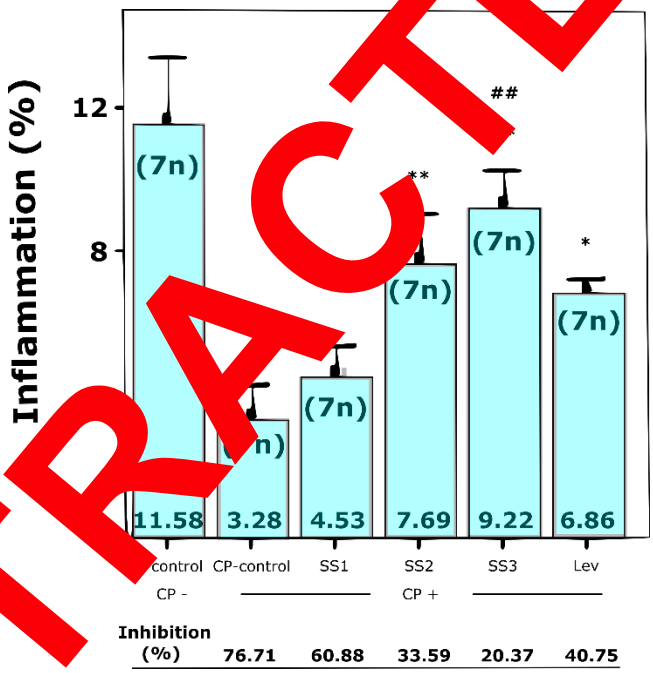


Figure 2 GC-MS chromatogram from SS ethanolic extract. The chromatogram of the SS ethanolic extract presents a complex profile of sugars and polyols.

DTH test

The effect of SS on cell-mediated immune responses was assessed by measuring the percentage of inflammation in a delayed-type hypersensitivity (DTH) model. After 24 hours of SRBC challenge, footpad thickness was re-measured. The normal immune response was observed in the NS-control group. Cyclophosphamide (CP), an immunosuppressive drug, significantly inhibited immune responses, as indicated by a marked reduction in the percentage of inflammation in CP-treated mice (CP+). Notably, the CP-only group (CP-control) showed the greatest inhibition, with a 76.71% reduction in inflammation. Oral administration of SS mitigated the immune suppression induced by CP in a dose-dependent manner. Significant differences in inflammation were observed at 39 and 78 mg/kg doses ($p < 0.01$) compared to the CP-control group, with the 78 mg/kg dose also showing a significant difference when compared to the 19.5 mg/kg dose. The Levamisole-treated group also showed significant results ($p < 0.05$) compared to the CP-control group (Fig



Figu ct of S on DTH reaction in immunosuppressed mice as assessed by footpad swelling . Inf mation% was measured on the left footpad for determining cell-mediated ne res e. Data are mean± SE of 7 mice. * $p<0.05$ and ** $p<0.01$ as compared to CP-con l; ## $p<0.01$ as compared to SS1 analyzed by one-way ANOVA (Least significant dif ultiple comparison test). The %inhibition of CP was shown below the figure.
+ = cyclophosphamide treated groups; NS-control = normal saline control; CP-control = n l saline (PO); SS1 = 19.5 mg/kg; SS2 = 39 mg/kg; and SS3 = 78 mg/kg; Lev = 25 mg/kg levam sole.

Hemagglutination assay

Antibody titers were expressed on a log scale. The immunocompetent group, which mounted a normal immune response to SRBC, exhibited an antibody titer of 6.71 ± 0.28. CP treatment resulted in a significant suppression of immune responses, as indicated by a 42.47% reduction in antibody titer compared to the immunocompetent group. Oral administration of SS alleviated this suppression in a dose-dependent manner, with significant improvements observed at the highest dose of SS. However, even at the highest dose, SS did not fully restore the antibody titer to levels seen in the immunocompetent group. Mice treated with levamisole

also showed a slight increase in antibody titer compared to the CP-control group, but the difference was not statistically significant. (Figure 4)

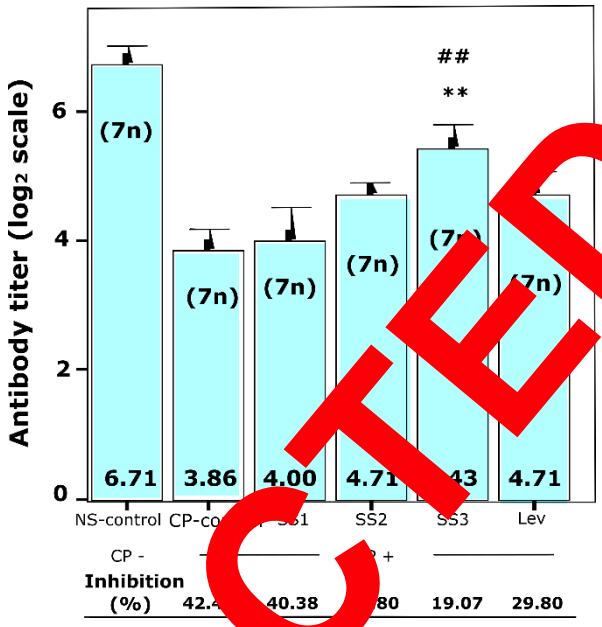


Figure 4 Effect of SS on antibo production in immunosuppressed mice as assessed by HA test. Antibody titer was expres scale. Data are mean± SE of 7 mice. ** p<0.01 as compared to CP-control; ## p<0. as ared to SS1 analyzed by one-way ANOVA (Least significant difference multiple com on test). The %inhibition of CP was shown below the figure. CP+ / CP- = cyc opham treated / untreated; NS-control = normal saline control; CP-control = normal ine ( ); SS1 19.5 mg/kg; SS2 = 39 mg/kg; and SS3 = 78 mg/kg; Lev = 25 mg/kg levamis

eukocy and lymphocyte estimation

Leukocyte and lymphocyte counts are presented in Figures 5. Mice in a norm ealth state exhibited an average leukocyte count of 3886 ± 369 cells/µL, with ly hocytes constituting the predominant cell population (3362 ± 339 cells/µL). Consequently, alteration in lymphocyte numbers corresponded with ch ges in the total leukocyte count. Treatment with CP induced a marked d rease in both leukocytes and lymphocytes, with reductions of 78.66% and 6.71%, respectively, compared to the immunocompetent control group. Administration of SS attenuated the CP-induced leukocyte reduction in a dose-dependent manner, with the highest SS dose demonstrating a statistically significant increase in both leukocyte and lymphocyte counts compared to the CP-treated group. Similarly, levamisole produced comparable effects.

Two types of lymphocytes were separated by a CD3 marker, CD3+ T lymphocyte and CD3- lymphocyte which was supposed to be B lymphocyte. The both cell types were increased in those SS groups however, the increase of CD3-lymphocyte showed no statistical significance (Figure 6).

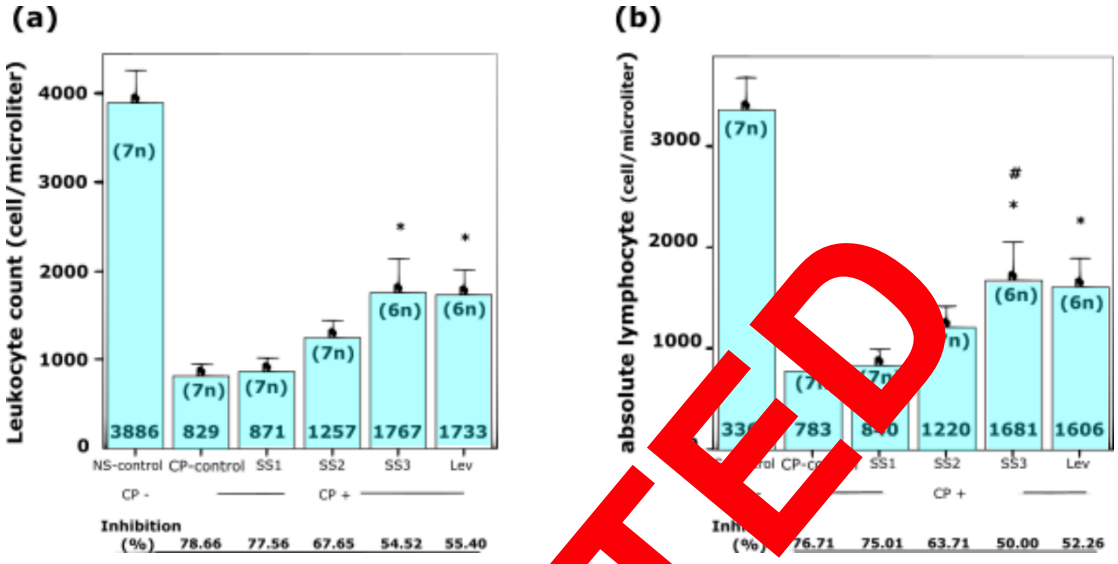


Figure 5 Effect of SS on leukocyte and lymphocyte count in immunosuppressed mice. (a) and (b) represent leukocyte count and lymphocyte number respectively. Data are mean± SE of 7 mice. * p<0.05 as compared to CP-control; # p<0.05 as compared to SS1 analyzed by one-way ANOVA (Least significant difference multiple comparison test). The %inhibition of CP was shown below the figure. CP+ / CP- = cyclophosphamide treated / untreated; NS-control = normal saline control; CP-control = normal saline (PO); SS1 = 19.5 mg/kg; SS2 = 39 mg/kg; and SS3 = 78 mg/kg; Lev = 25 mg/kg levamisole.

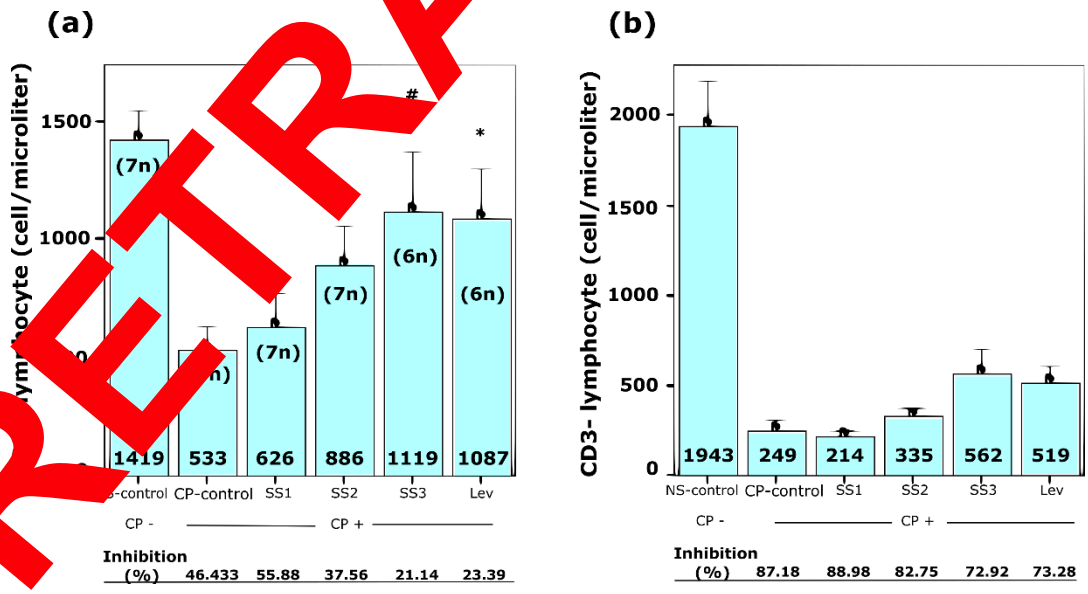


Figure 6 Effect of SS on CD3 lymphocyte number in immunosuppressed mice. (a) and (b) represent CD3+ lymphocyte and CD3- lymphocyte number respectively. Data are mean± SE of 7 mice. * p<0.05 as compared to CP-control; # p<0.05 as compared to SS1 analyzed by one-way ANOVA (Least significant difference multiple comparison test). The %inhibition of CP was shown below the figure. CP+ / CP- = cyclophosphamide treated / untreated; NS-control = normal saline control; CP-control = normal saline (PO); SS1 = 19.5 mg/kg; SS2 = 39 mg/kg; and SS3 = 78 mg/kg; Lev = 25 mg/kg levamisole.

T lymphocyte subsets estimation (CD4⁺ and CD8⁺ T lymphocyte)

The absolute numbers of CD4+ and CD8+ T lymphocytes were determined based on their respective ratios to the total T lymphocyte count in the blood. In general, CD4+ T lymphocytes were more abundant than CD8+ T lymphocytes in murine blood. Mice in normal health exhibited an aver[illegible] of 1075 ± 101 cells/μL for CD4+ T lymphocytes and 361 ± 28 cells/μL for CD[illegible] T ly[illegible]hocytes. Similar to the effect observed on total leukocytes, treatment [illegible]n CP resu[illegible]d in a significant reduction of both CD4+ and CD8+ T lymphocytes, w[illegible] decrea[illegible]s of 60.83% and 68.42%, respectively. Following SS administr[illegible]on, a d[illegible]de[illegible]ndent increase in both CD4+ and CD8+ T lymphocytes was [illegible]serv[illegible]d, wit[illegible]e most pronounced effect seen in the high-dose group. Similar[illegible] eva[illegible]sole treatment also significantly enhanced the numbers of both CD4+ a[illegible] C[illegible] lym[illegible]cytes (Figure 7).

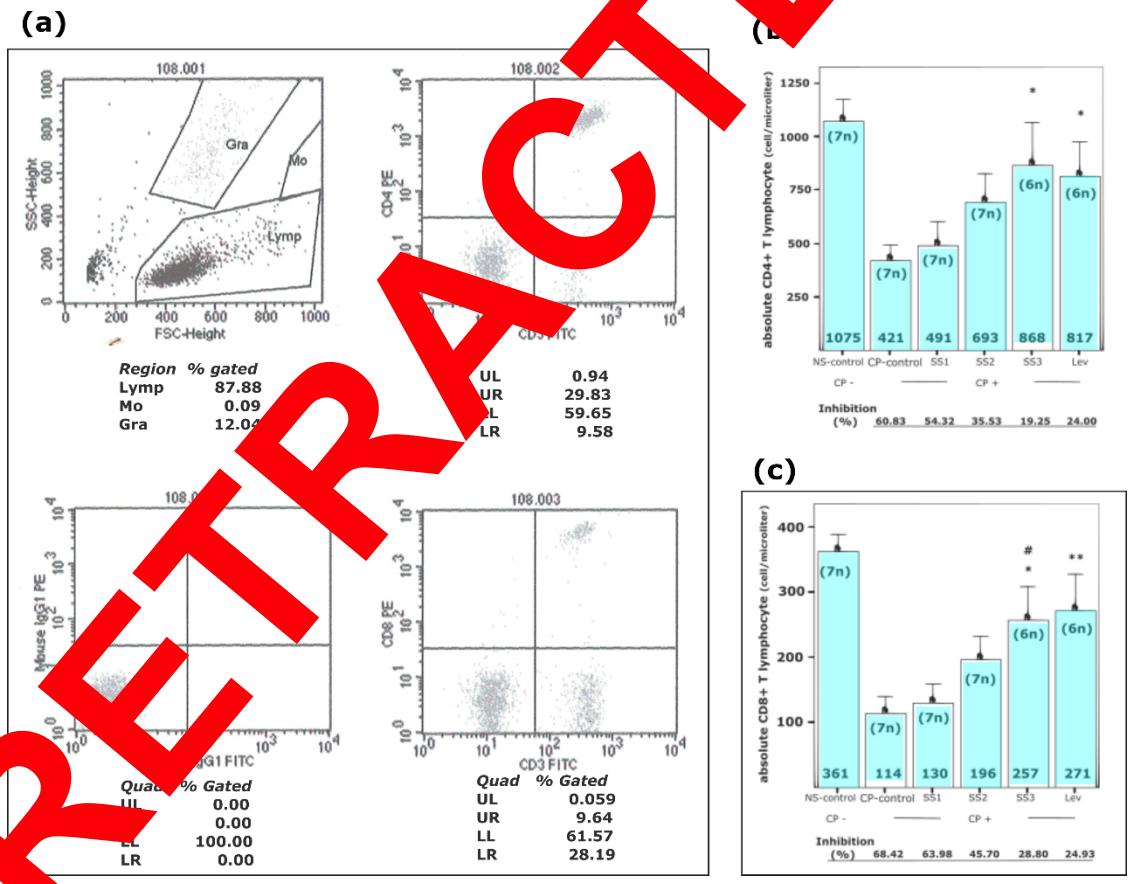


Figure 7 Effect of SS on T lymphocyte subsets estimation in immunosuppressed mice as assessed by flow cytometric assay. CD4⁺ T lymphocyte and CD8⁺ T lymphocyte were counted separately. Each group contained 7 samples excepted SS3 and Lev group had 6 samples. (a) demonstrates the cell gating strategy for lymphocytes, subsequent CD4+ T cell and CD8+ T cell in one normal mouse while (b) and (c) represent the absolute number of CD4+ T lymphocyte and CD8+ T lymphocyte respectively. Data are mean± SE * $p<0.05$ ** $p<0.01$ as compared to CP-control; # $p<0.05$ as compared to SS1 analyzed by one-way ANOVA (Least significant difference multiple comparison test). The %inhibition of CP was shown in parentheses.
CP+ / CP- = cyclophosphamide treated / untreated ; NS-control = normal saline control; CP-control = normal saline (PO); SS1 = 19.5 mg/kg; SS2 = 39 mg/kg; and SS3 = 78 mg/kg; Lev = 25 mg/kg levamisole.

Splenic weight and histopathological examination

After conducting the DTH reaction test and collecting blood samples, the spleens were removed from the bodies of all mice, weighed, and the average spleen weight for each group was calculated. The results in Figure 8 revealed that the immunocompetent animals (NS-control) had an average spleen weight of 150±10 mg, which was higher than all groups that received CP treatment. The weights for the CP groups were as follows: CP-control = 82±3, SS1 = 86±3, SS2 = 94±6, SS3 = 102±3, and Lev = 86±5. It was observed that the CP-control group, which did not receive any test substance, had the lowest average spleen weight, 45.33% less than the NS-control group. The group that received SS at a dose of 78 mg/kg (SS3) had the highest average spleen weight, with a statistically significant difference at $p<0.05$ when compared to the CP-control group. No statistically significant difference was found in the average spleen weight of the Lev group when compared to the CP-control group.

Figure 9 and 10 showed the histopathological findings of the spleens. The spleens from the NS-control group exhibited a prominent expansion of lymphoid follicles, with clear boundaries of the germinal centers in the white pulp, and the lymphocytes in this area were densely arranged. The spleens from the CP-control group were clearly different from those of the NS-control group. The boundaries and distribution of the lymphoid follicles were less pronounced compared to the other groups, no germinal centers were observed, and the arrangement of lymphocytes in the white pulp was less dense than in the other groups. In the groups that received immunosuppressive treatment and were administered SS, the boundaries and distribution of the lymphoid follicles increased in proportion to the dose of SS given. The SS3 group exhibited prominent lymphoid follicles and a higher density of lymphocytes in the white pulp compared to the SS1 and SS2 groups. In the Lev group, changes similar to those observed in the SS3 group were noted.

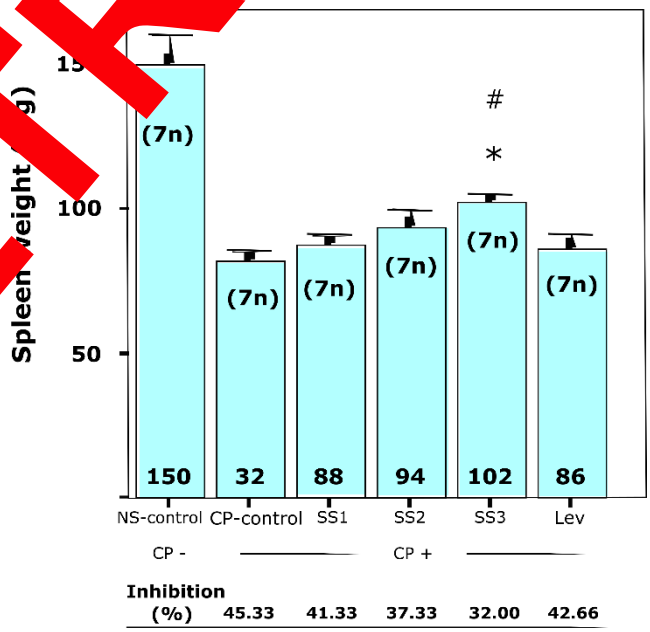
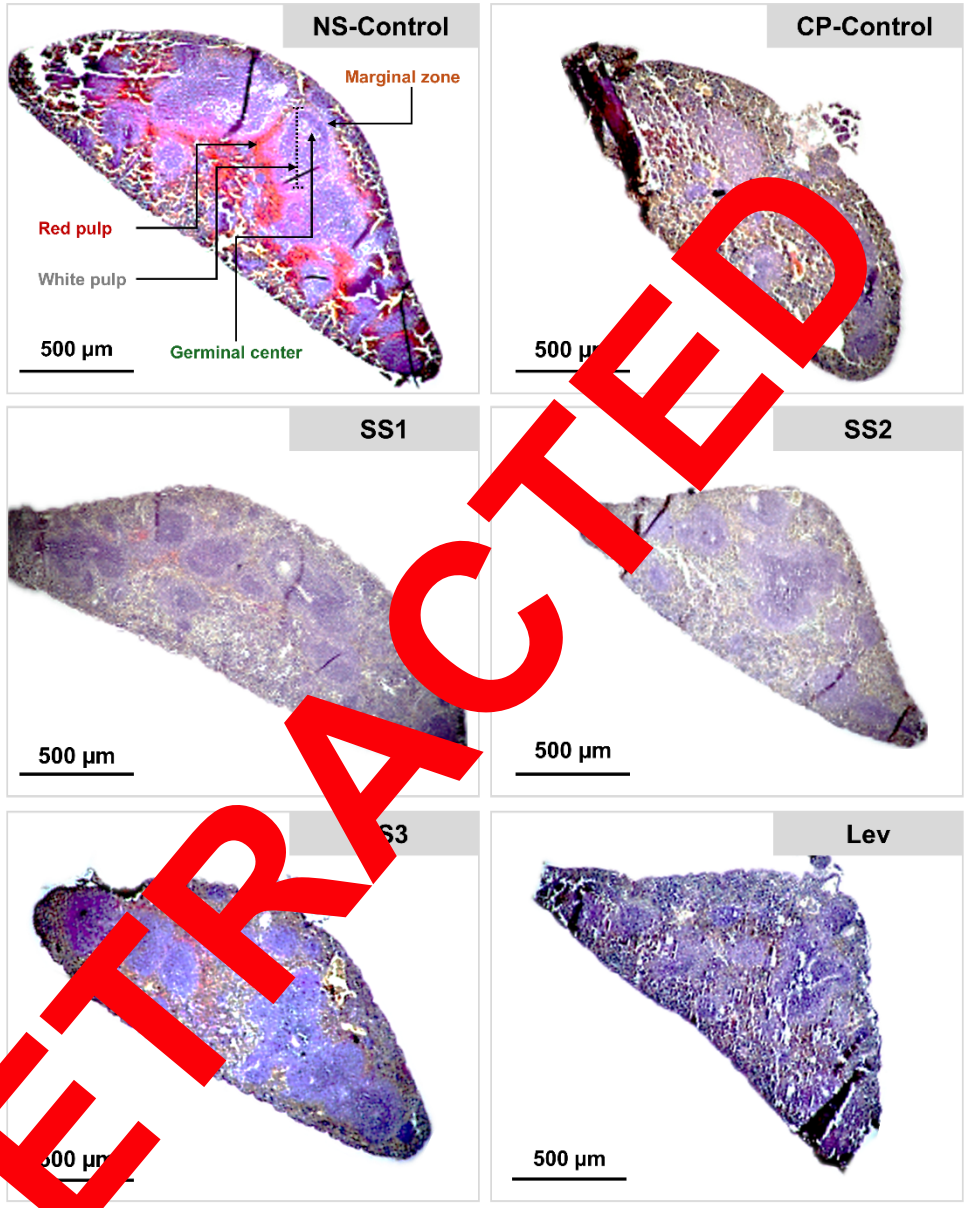


Figure 8 Effect of SS on splenic weight in immunosuppressed mice. Data are mean± SE of 7 mice. * $p<0.05$ as compared to CP-control; # $p<0.05$ as compared to SS1 analyzed by one-way ANOVA (Least significant difference multiple comparison test). The %inhibition of CP was shown below the figure. CP+ / CP- = cyclophosphamide treated / untreated; NS-control = normal saline control; CP-control = normal saline (PO); SS1 = 19.5 mg/kg; SS2 = 39 mg/kg; and SS3 = 78 mg/kg; Lev = 25 mg/kg levamisole.



F stopathological analysis of spleen tissue sections from immunosuppressed mice eated with different doses of SS. NS-Control: normal saline control group; CP-Control: ophosphamide control group; SS1, SS2, SS3: treatment groups receiving 19.5 mg/kg, 39 mg/kg, and 78 mg/kg of SS, respectively; Lev: positive control treated with levamisole. Sections stained with hematoxylin and eosin (H&E).

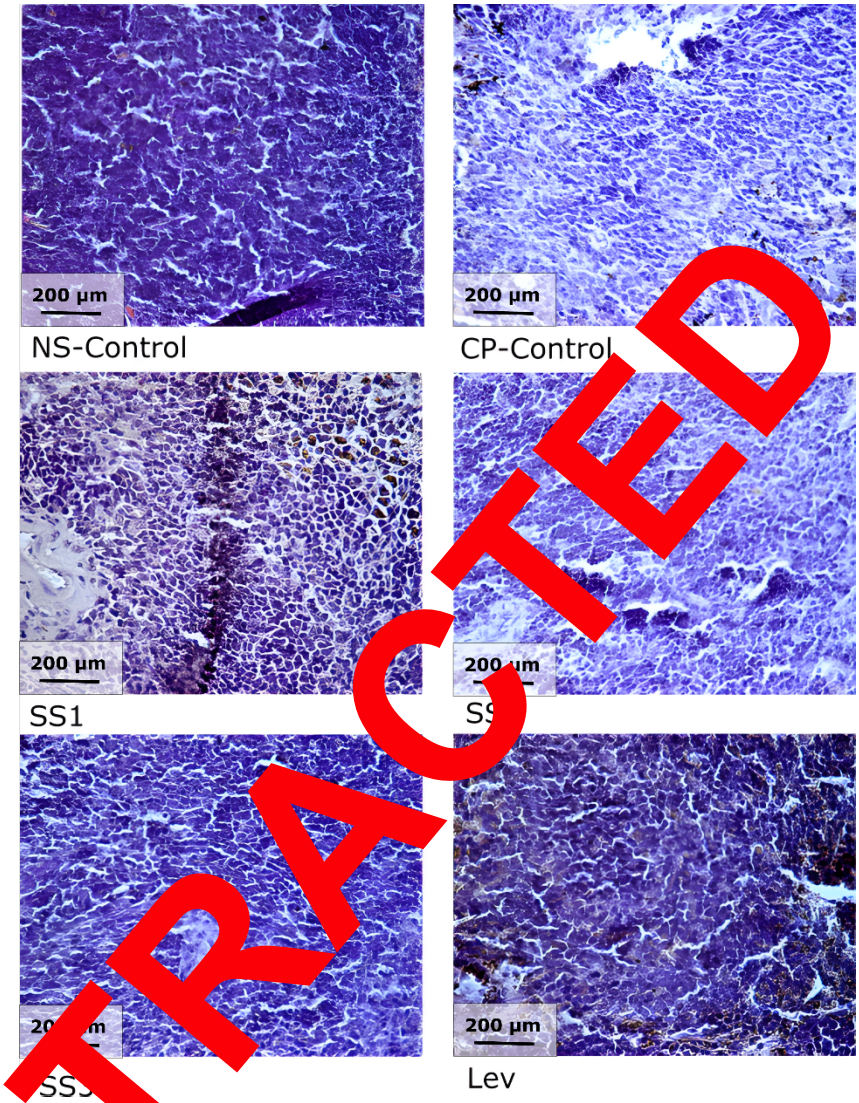


Figure 2.0 High magnification histopathological analysis of spleen white pulp from immunosuppressed mice treated with different doses of SS. NS-Control: normal saline control group; CP-Control: cyclophosphamide control group; SS1, SS2, SS3: treatment groups receiving 19.5 mg/kg, 39 mg/kg, and 78 mg/kg of SS, respectively; Lev: positive control treated with Levamisole. Variations in lymphocyte density and structural integrity of white pulp are evident. Sections stained with hematoxylin and eosin (H&E).

DISCUSSION

Dysregulation or suppression of the immune system can result from various factors, including genetic defects, infections, chronic stress, exposure to toxins, and certain medical treatments (Shirani et al., 2015; Delmonte, 2020; Kharrazian, 2021; Alotiby, 2024). Recently, there is a rise in using natural products for health promotion, which could help prevent various diseases (Chiavaroli and Brunetti, 2023). The aim of this study was to investigate the immunological effect of SS in immunosuppressed conditions. The results of oral administration of SS reveal the enhancement of both humoral and cell-mediated immunity in immunocompromised animals.

Chemical analysis of SS pericarp, a gel-like substance after hydration, identifies polysaccharide structures in both aqueous and alcoholic extracts (Burana-Osot et al., 2010; Ai et al., 2012; Kanlayavattanakul et al., 2017; Huang et

al., 2024). These extracts are composed of different proportions of common sugars, including erythritol, glucose, arabinose, rhamnose, ribose, D-fructose, galactose, mannitol, and inositol (Burana-Osot et al., 2010). Apart from polysaccharides, the alcoholic extracts additionally contain lipophilic bioactive compounds, such as pinoresinol, tiliroside, 3-cinnamoyltribuloside, and 3,4-dihydroxybenzoic acid (protocatechuic acid), which show potent anti-inflammatory properties in vitro (Oppong et al., 2024). However, the water-extracted polysaccharides exhibit more pronounced antioxidant a[illegible]han the ethanolic extract. Polysaccharides from medicinal plants are o[illegible]sionally [illegible]nd to possess immunological properties. However, Burana-Osot et [illegible](2010) r[illegible]orted that SS polysaccharides failed to stimulate T lympho[illegible]e pro[illegible]ation. Therefore, the present study has provided new evidence reg[illegible]ing the im[illegible]logical effects of SS.

The inflammation on the animal's foot[illegible] assoc[illegible]ed with the delayed-type hypersensitivity reaction (DTH), whi[illegible]s me[illegible]ed [illegible]T helper 1 lymphocytes (Th1). By releasing critical cytokines[illegible]er antige[illegible]cognition, T lymphocytes enhance macrophage and immune cell [illegible]ctions to exhibit significant immune responses. T lymphocytes eventually achiev[illegible]onal expansion under the influence of the autocrine, cytokine IL-2. S[illegible]equently, a [illegible]ecific clone of T cells traffics and infiltrates the target site, resul[illegible]g in a firmly inflamed lesion, a distinct characteristic of the DTH reaction (M[illegible]phy et [illegible], 2017). Our results showed that immunosuppressed mice ex[illegible]ited re[illegible]ced DTH reactions at their footpads compared to immunocompet[illegible], indicating the cytotoxic drug (CP) diminished immune[illegible]nses in these animals. However, SS administration mitigated this effect, [illegible]sulti[illegible]creased footpad inflammation.

Similar to the hu[illegible]ra[illegible]mmune response, animals receiving the highest dose of SS exhibit[illegible]a sig[illegible]ant increase in antibody production against SRBC, indicating t[illegible]b[illegible] T and [illegible] cell-mediated responses were partially restored from CP-induc[illegible] imm[illegible]osuppression. This finding is supported by leukocyte profile data, wh[illegible]d[illegible]ons[illegible]ed that the reductions in total leukocyte count, $CD3^+$ lym[illegible]cytes, $CD3^-$ lymphocytes, $CD4^+$ T cells, and $CD8^+$ T cells were ameliorated fol[illegible]ing SS tr[illegible]ent. However, despite the observed improvement, leukocyte [illegible]n SS-treated animals remained markedly lower than those in healthy controls [illegible]the established normative range of 3.84–6.23 × 10^3/μL for laboratory mice (Silv[illegible]antana et al., 2020). This suggests that while SS exhibited i[illegible]munoprotective effects, it may not have been sufficient to fully counteract the [illegible]mun[illegible]suppressive impact of CP under the conditions of this study.

[illegible]he enhanced immune response, resulting from an increased number of [illegible]mocytes, was further supported by histopathological examination of the s[illegible]een. In immunosuppressed mice, no explicit response to antigens was observed in the white pulp or lymphoid follicles of the spleen, where germinal centers serve as sites for recognition and activation of antigen-presenting cells and T lymphocytes. This activation is followed by clonal expansion (Abbas et al., 2024), visible as dense clusters of lymphocytes in these regions. Mice administered with high doses of SS fruits exhibited prominent germinal centers and increased lymphocyte density in the white pulp.

Previous studies have demonstrated that water-extracted components, such as polysaccharides, and organic solvent-extracted components, such as flavonoids and alkaloids, from the fruit pulp of SS exhibit distinct pharmacological activities (Oppong et al., 2018; Oppong et al., 2024). Polysaccharides are generally associated with enhanced innate immune cell activity (Sindhu et al., 2021), likely due to the activation of pattern recognition receptors including Toll-like receptor, scavenger receptor, ß glucan receptor, complement receptor type 3, mannose receptor, and mannan binding lectin (Leung et al., 2006; Ying and Hao, 2023), while organic solvent extracts display anti-inflammatory and antioxidant effects (Wang et al., 2003; Phlicharoenphon et al., 2017; Huang et al., 2024; Oppong et al., 2024). In this study, we did not separate the components of the fruit pulp. Nevertheless, the

findings reveal that SS pulp exhibits immunostimulatory properties. These effects might not be entirely due to polysaccharides, as the anti-inflammatory and antioxidant properties of organic solvent-extracted compounds could also help reduce lymphoid tissue damage caused by CP. This combined effect likely enhances the overall immune response.

Despite these promising findings, this study has certain limitations. First, the immunomodulatory effects of SS were evaluated in a CP-induced immunosuppressed mouse model, which may not fully represent other forms of immune suppression caused by infections, chronic diseases, or aging. Second, the specific bioactive compounds responsible for the observed immune-enhancing effects were not identified. Additionally, the study primarily focused on adaptive immune responses, whereas the effects on innate immunity, cytokine profiles, and long-term immune memory remain unexplored. Future studies should focus on isolating and characterizing the active compounds in SS that contribute to its immunomodulatory properties. Additionally, further research on the mechanisms of cytokine regulation and antigen presentation could provide deeper insights into the pathways underlying its immunoprotective effects.

In conclusion, the fruit pulp of SS exhibits significant immunoprotective effects by counteracting CP-induced suppression of humoral and cell-mediated immunity. This immunomodulatory activity may contribute to reducing the susceptibility of animals to infections, thereby supporting overall health and productivity. The findings support further exploration of SS as a functional ingredient in food or beverage formulations aimed at promoting immune health and offering a natural adjunct or alternative to conventional therapies.

ACKNOWLEDGMENTS

The authors thank Dr. Saranyou Oontawee for improving image quality.

AUTHOR CONTRIBUTIONS

Thanapoom Siriarchavatana: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (equal); methodology (lead); visualization (lead); writing-original draft (lead); writing-review & editing (lead). **Naowarat Kanthamnatpong**: Conceptualization (equal); investigation (equal); project administration (lead); supervision (equal); writing-review & editing (supporting). **Wanaree Limpanasitthikul**: Conceptualization (equal); funding acquisition (lead); resource (lead); supervision (equal); writing-review & editing (supporting).

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How to cite this article;
Parkpoom Siriarchavatana, Naowarat Suthamnatpong, Wacharee Limpanasitthikul. Immunoprotective properties of *Scaphium scaphigerum* fruits in cyclophosphamide-induced immunosuppressed mice targeting humoral and cell-mediated immunity. Veterinary Integrative Sciences. 2026; 24(1): e2026024-1-17.