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Utilization of Shark Cartilage Byproducts from Fisheries for Anti-Inflammatory Biomedical Applications in LPS-Induced Mice

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ABSTRACT

The utilization of marine byproducts, such as shark cartilage, presents a sustainable strategy for the development of novel biomedical applications. This study investigated the anti-inflammatory potential of shark cartilage extract derived from *Prionace glauca*, a byproduct of fisheries, in a lipopolysaccharide (LPS)-induced mouse model of inflammation. The cartilage was processed via maceration in distilled water (1:10 b/v) at 45°C for 8 hours, and its chondroitin sulfate (CS) content was quantified using High-Performance Liquid Chromatography (HPLC), yielding an average of 2.23%. Male BALB/c mice were assigned to seven groups, including an LPS-only group, a standard CS group, a commercial supplement group (Welmove), and three dosage groups of shark cartilage extract (50, 100, and 200%). Inflammation was induced through intraperitoneal injection of LPS (1mg/ 100mL PBS), and immune responses were assessed by analyzing TNF-α and IFN-γ expression in CD4+ T cells and NK cells via flow cytometry. The extract significantly reduced the percentages of $CD4^+TNF\text{-}\alpha^+$, $CD4^+IFN\text{-}\gamma^+$, and $NK^+IFN\text{-}\gamma^+$ cells in a dose-dependent manner ($P < 0.05$), with higher doses (D2 and D3) restoring cytokine expression toward baseline levels. These findings highlight the potential of shark cartilage byproducts as a valuable source of natural anti-inflammatory agents, supporting their further development in pharmaceutical and biomedical fields.

INTRODUCTION

Shark is among cartilaginous marine organisms that has long gained significant attention due to its body parts utilization to be processed in biomedical purposes **(Agustin *et al.*, 2023)**. One type of shark that has high therapeutic use is the blue shark (*Prionace glauca*), which is known as a member of the class Chondrichthyes, subclass Elasmobranchii, and belongs to the family Carcharhinidae, which includes around 465 species with diverse characteristics and inhabits vast coastal and oceanic areas of both

tropical and temperate regions around the world **(Lu *et al.*, 2022)**. The meat, skin, and fin of blue sharks have been the main byproduct targets in both longline and gillnet fisheries **(Li *et al.*, 2023)**. Apart from the extensive consumption of certain shark body parts, shark cartilage is often discarded, highlighting the need to explore its potential **(Agustin *et al.*, 2023)**. Recent findings have demonstrated that shark cartilage contains various important bioactive compounds, including collagen, glucosamine, and in particular, chondroitin sulphate (CS) **(Vázquez *et al.*, 2016; Bu *et al.*, 2017; Urbi *et al.*, 2022; Agustin *et al.*, 2023; Rakhmiyati *et al.*, 2023)**.

Chondroitin sulphate is a naturally occurring compound consisting of sulfated heteropolysaccharides. Its sulfate groups are covalently bound to the sugar molecules of glucuronic acid (GlcA) and N-acetyl-galactosamine (GalNAc) **(Bishnoi *et al.*, 2016)**. CS extracted from shark cartilage has been found to contain high levels of chondroitin-6-sulfate (CS-C) and chondroitin-2, 6-sulfate (CS-D) disaccharide units, whereas its biological activity is strongly influenced by its structure, which varies mainly in their disaccharide composition and molecular weight **(Yang *et al.*, 2023)**. Recent discoveries have highlighted the diverse potential of marine product-derived CS in alleviating osteoarthritis symptoms, promoting antiviral properties, and its applications in tissue engineering and skin care products **(Urbi *et al.*, 2022)**. In addition, CS has been shown to possess antioxidant, anti-coagulant, and anti-tumor properties **(Chen *et al.*, 2022)**. It underlines the possible significance of blue shark cartilage-derived CS as an anti-inflammatory agent alternative in the regulation of immune system.

Lipopolysaccharide (LPS) as part of strong pathogen-associated molecular pattern (PAMP) serves as the major component of the outer membrane of Gram-negative bacteria that could trigger an acute inflammatory response by inducing an excessive release of pro-inflammatory cytokines **(Tucureanu *et al.*, 2017; Fu *et al.*, 2021)**. This mechanism is mediated by Toll-like-receptor 4 (TLR4) via the TLR4/NF-κBp65 signaling pathways. Upon LPS binding, the activation of TLR4 will initiate the MyD88-dependent cascade, leading to an NF-κB p65 transcriptional activation and subsequent release of pro-inflammatory cytokines to help eliminate invading pathogens, which, in an acute milieu, could give rise to an inflammatory disorder due to imbalances **(Ciesielska *et al.*, 2021; Tang *et al.*, 2021)**. Therefore, the changes of pro-inflammatory cytokines levels become a crucial indicator in evaluating the efficacy of a novel agent in suppressing LPS-administered immune response.

Earlier bodies of research have many investigated natural anti-inflammatory agents derived from marine organisms, including those sourced from shark bone **(Merly & Smith, 2015; Agustin *et al.*, 2016a, b; Safari & Hassan, 2020; Tanna *et al.*, 2020)**. The studies suggested that CS from other sources exhibited anti-inflammatory activity by inhibiting several key pro-inflammatory cytokines and the NF-κβ pathways **(Yang *et al.*, 2020; Sharma *et al.*, 2022)**. However, ground research on examining the anti-

inflammatory activity of shark cartilage extract in LPS-mediated immune response has not yet been elucidated. The present study aimed to address this gap by evaluating shark cartilage extract as a natural potent anti-inflammatory agent. In this study, inflammation was induced in mice using LPS and the expression of the pro-inflammatory cytokine TNF-α and IFN-γ was assessed.

MATERIALS AND METHODS

Preparation and extraction of shark cartilage

Shark cartilage was obtained from the Fish Freezing Industry, Sidoarjo, Indonesia in frozen condition. Shark cartilage preparation was carried out based on **Agustin *et al*. (2023)**. First, the frozen shark cartilage was thawed with running water and cleaned of any remaining meat. Then, the cartilage was cut into pieces according to the bone segments and dried in a dryer for 2 days at a temperature of 50 ± 2°C (6 hours per day). The dried shark bones were then blended and sieved using an 80-mesh sieve, resulting in a sample in the form of bone flour.

During the extraction stage, the shark cartilage flour was soaked with hexane (1:10 w/v, 1 hour) and continuously stirred for 1 hour using a magnetic stirrer. Then, the shark cartilage powder was separated from the hexane and air-dried overnight. Next, the shark bone powder was extracted in distilled water (1:10 w/v) at a temperature of 45 ± 2°C for 8 hours. The extract was then centrifuged at a speed of 4000 rpm for 10 minutes, and the supernatant was collected.

Chondroitin sulphate content analysis

The content of chondroitin sulphate was analyzed using High-Performance Liquid Chromatography (HPLC). The HPLC system was assembled with a stabilizer, pump, refractive index detector (RID), modular components, and a computer equipped with a Central Processing Unit (CPU), and operated until a stable flat baseline was observed on the chromatogram. The mobile phase consisted of a potassium phosphate buffer solution adjusted to pH 3. This buffer was prepared by dissolving 1.36 grams of potassium dihydrogen phosphate in 800mL of distilled water, followed by the addition of phosphoric acid (H_3PO_4). The buffer was then mixed with acetonitrile in a 99.5:0.5 ratio. The flow rate was set at 1mL/ min. Chromatographic separation was carried out using a C18 column (4.6 × 250mm, 5µm particle size, Merck), maintained at a temperature of 28°C.

Animals

Six-week-old male BALB/c mice (*Mus musculus*) weighing approximately 25g were used in this study. The test animals were acclimatized for 7 days with *ad libitum* feeding and drinking. This study was divided into 7 treatment groups (Table 1) with 5 replicates each. All procedures in this study were approved by the Ethics Committee of

the Faculty of Dentistry, Hang Tuah University, Surabaya, Indonesia (No: EC/0138/KEPK-FKGUHT/XII//2024).

Table 1. Variety of treatment groups

Group	Treatment
Normal	Distilled water (10 µL)
LPS	LPS 1 mg/100 µL PBS on day 15
LPS+CS	Standard CS 1.54 mg/250 µL + LPS (1 mg/100 ml PBS) injected 100 µL on day 15
LPS+Welmove	Welmove 6.45 mg /250 µL distilled water + LPS (1 mg/100 ml PBS) injected 100µL on day 15
LPS+D1	Shark cartilage extract dose 1 (50%; 142 µL per 25 g body weight of mouse) + LPS (1 mg/100 ml PBS) injected 100µL on day 15
LPS+D2	Shark cartilage extract dose 2 (100%; 284 µL per 25 g body weight of mouse) + + LPS (1 mg/100 ml PBS) injected 100µL on day 15
LPS+D3	Shark cartilage extract dose 3 (200%; 568 µL per 25 g body weight of mouse) + + LPS (1 mg/100 ml PBS) injected 100µL on day 15

To determine the anti-inflammatory effects of shark cartilage extract, different doses (142, 284, and 568µL) were administered orally to the LPS+D1, LPS+D2, and LPS+D3 groups, respectively, for 14 consecutive days. The normal and LPS groups received distilled water orally for the same duration. On day 15, 100µL of LPS (1mg/ 100mL PBS) was injected intraperitoneally into the treatment groups (LPS+D1, LPS+D2, LPS+D3, LPS+Welmove, LPS+CS, and LPS).

Lymphocyte isolation and flow cytometry analysis

On the last day of the experiment, lymphocyte cell isolation was performed following the procedure described by **Adharini *et al.* (2020)**, with modifications. First, the mice were euthanized via cervical dislocation without the use of carbon dioxide or other chemicals. The mice were then dissected, and lymphocytes were isolated from the spleen by grinding the spleen using the base of a syringe and suspending the tissue in 10mL of PBS. The sample was centrifuged at 2,500 rpm at 10°C for 5 minutes. The supernatant was discarded, and the resulting pellet, containing lymphocytes, was used for antibody staining.

Antibodies were applied at a concentration of 0.005mg/ 100µL. Lymphocytes were stained with extracellular antibodies, namely 50µL of FITC-conjugated rat anti-

mouse CD4 and FITC-conjugated rat anti-mouse NK (BioLegend®, San Diego), and incubated for 20 minutes in an ice box (4°C). Intracellular cytokine staining was carried out by adding 50µL of Cytofix (BD Biosciences Pharmingen) to the samples and incubating them for 20 minutes in an ice box. Subsequently, 400– 500µL of Wash Perm Solution (WPS; BioLegend®, USA) was added, and the samples were centrifuged at 2,500 rpm at 10°C for 5 minutes. The supernatant was discarded, and the pellet was stained with 50 µL of PE-conjugated rat anti-mouse TNF-α and IFN-γ (BioLegend®, San Diego). Each sample was then transferred to a cuvette and analyzed using a flow cytometer (FACSCalibur; BD Biosciences, New Jersey, USA), with data acquisition performed using BD CellQuest Pro™ software.

Data analysis

Flow cytometry data were analyzed using BD CellQuest Pro™ software. Results were presented as mean ± standard deviation using Microsoft Excel 2013. Statistical analysis was performed using SPSS version 20.0 for Windows. Normally distributed data were tested using one-way ANOVA at a 95% confidence level ($\alpha = 0.05$). A *P*-value > 0.05 indicated no significant differences between treatments, while a *P*-value < 0.05 indicated a statistically significant difference. In such cases, the Least Significant Difference (LSD) test was conducted to identify which treatment groups differed significantly.

RESULTS

Chondroitin sulphate content

Chondroitin sulphate is a vital component of connective tissue, playing a critical role in maintaining the integrity and function of cartilage **(Bishnoi *et al.*, 2020)**. The chondroitin sulphate content obtained from the extract analyzed in this study using HPLC is presented in Table (2).

Table 2. Chondroitin sulfate content of shark cartilage extract

Replicates	Extract volume (mL)	Extract weight (gr)	Chondroitin sulphate (%)
1	79	77.58	1.6
2	84	79.60	1.7
3	76	74.58	4.0
4	79	78.07	1.7
5	80	79.60	2.0
6	78	77.58	2.4
Mean	79.3	77.83	2.23

The average CS content of shark cartilage extract in this study was 2.23%, indicating the potential of shark cartilage as a natural source of bioactive compounds.

However, this value is lower than that reported in a previous study by **Agustin *et al.* (2023)**, where the chondroitin sulphate content in shark cartilage extract pretreated with an absorber before freeze-drying reached 2.62%. In contrast, fresh shark cartilage contained only 0.49%, and extracts without freeze-drying yielded as low as 0.12%. These findings suggest that drying and the use of absorbers can enhance the concentration of chondroitin sulphate in the extract.

Chondroitin sulphate content varies depending on the source of raw material and the extraction method used. **Yang *et al.* (2021)** successfully extracted 23.7% chondroitin sulphate from the cartilage of Humboldt squid (*Dosidicus gigas*) using an ultrasonic-assisted extraction method. Similarly, **Urbi *et al.* (2022)** reported that various fish organs can serve as raw material sources for chondroitin sulphate production, with skin and cartilage being the most commonly utilized tissues. According to **Shen *et al.* (2023)**, chondroitin sulphate and peptides can be effectively separated from other tissue components through membrane filtration following enzymatic hydrolysis, and further purified using chromatographic column techniques.

Shark cartilage extract reduced $CD4^+TNF\text{-}\alpha^+$ level in LPS-induced mice

As can be seen in Fig. (1), the level of $CD4^+TNF\text{-}\alpha^+$ in response to LPS administration appears to be significantly higher than that in the normal group (20.08%). While CS substantially dampened $CD4^+TNF\text{-}\alpha^+$, its expression in response to Welmove treatment did not differ significantly compared to both the LPS and normal groups in this study. Furthermore, the effect of shark cartilage extract toward $CD4^+TNF\text{-}\alpha^+$ reduced significantly in dose-dependent manner ($P< 0.05$). Here, the administration of shark cartilage extract at dose 1 group was able to slightly reduce $CD4^+TNF\text{-}\alpha^+$ expression to 15.53%, although the difference was not statistically significant compared to the LPS group ($P> 0.05$). Nevertheless, there remains a significant difference discernible at the higher dose groups, dose 2 and dose 3, as they were shown to diminish $CD4^+TNF\text{-}\alpha^+$ level to 8.48 and 10.06%, respectively, compared to LPS group. Moreover, the levels of $CD4^+TNF\text{-}\alpha^+$ in both doses were not indifferent from the normal group. This implies a sufficient capacity of dose 2 and dose 3 in normalizing the level of $CD4^+TNF\text{-}\alpha^+$ in the LPS-induced inflammatory environment.

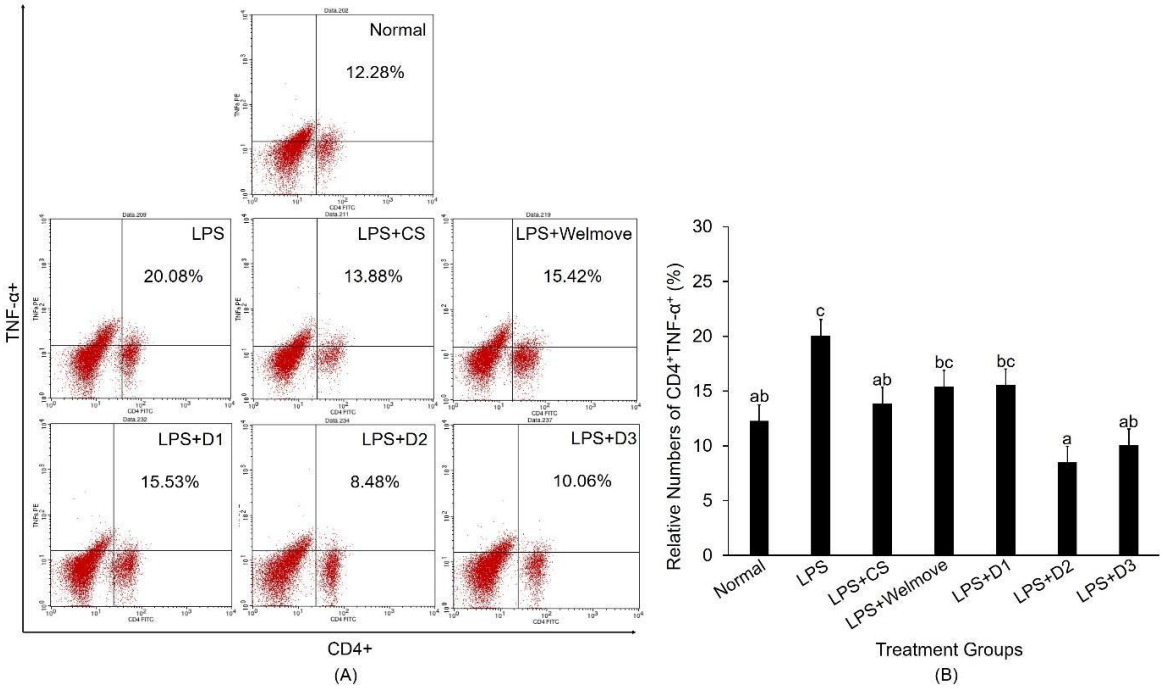

Fig 1. The modulation of $CD4^+TNF$-α expression by LPS, chondroitin sulfate, welmove, and shark cartilage extract doses

Shark cartilage extract reduced $CD4^+IFN$-γ^+ level in LPS-induced mice

As presented in Fig. (2), LPS administration led to a marked increase in the level of $CD4^+IFN$-γ^+ to 23.16%. Treatment with both CS and Welmove significantly reduced $CD4^+IFN$-γ^+ level compared to the LPS group, with levels of 10.42 and 8.24%, respectively. Although the reduction did not follow a clear dose-dependent manner, a comparable trend was observed across all shark cartilage extract groups. All shark cartilage extract treatment groups successfully decreased the level of $CD4^+IFN$-γ^+ significantly compared to the LPS group ($P< 0.05$). Notably, IFN-γ expression in the dose 2 and dose 3 groups were found to be relatively lower than those in the Welmove and CS groups, with only dose 2 falling below normal group, indicating a potentially greater ability for shark cartilage extract at these concentration to normalize $CD4^+IFN$-γ^+ level in LPS-induced inflammatory environment.

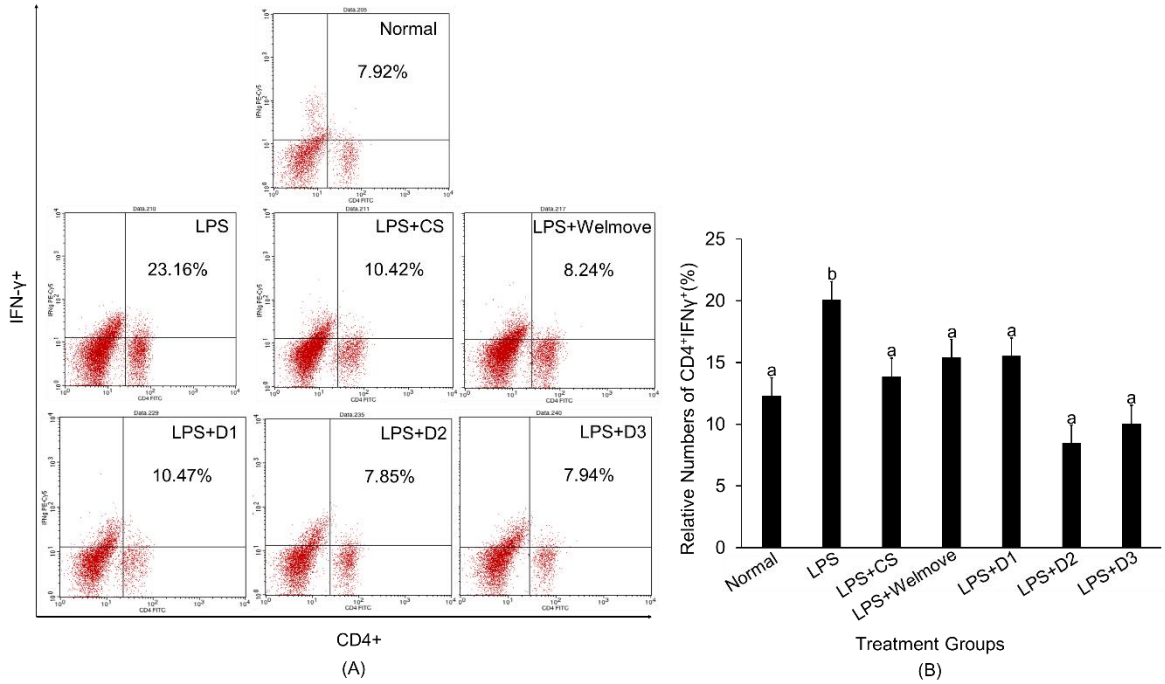

Fig. 2. Comparative effects of LPS, CS, welmove, and shark cartilage extract gradient on $CD4^{+}IFN\text{-}\gamma^{+}$ cell levels

Shark cartilage extract reduced $NK^{+}IFN\text{-}\gamma^{+}$ level in LPS-induced mice

Fig. (3) shows that, the administration of LPS significantly promoted an increase in $NK^{+}IFN\text{-}\gamma^{+}$ expression to 50.57% compared to the normal group (30.06%). Both CS and Welmove treatments failed to significantly decrease the $NK^{+}IFN\text{-}\gamma^{+}$ levels of 49.79 and 47.60%, respectively. Similarly, dose 1 treatment resulted in only a modest reduction, yielding an $NK^{+}IFN\text{-}\gamma^{+}$ level of 39.18%. In contrast, dose 2 and dose 3 exhibited significantly greater inhibitory effects, with $NK^{+}IFN\text{-}\gamma^{+}$ levels of 27.10 and 27.64%, respectively ($P< 0.05$) that remained comparable to the normal group. These findings suggest that both doses possess a promising capacity to effectively inhibit $NK^{+}IFN\text{-}\gamma^{+}$ expression under LPS-triggered inflammatory conditions.

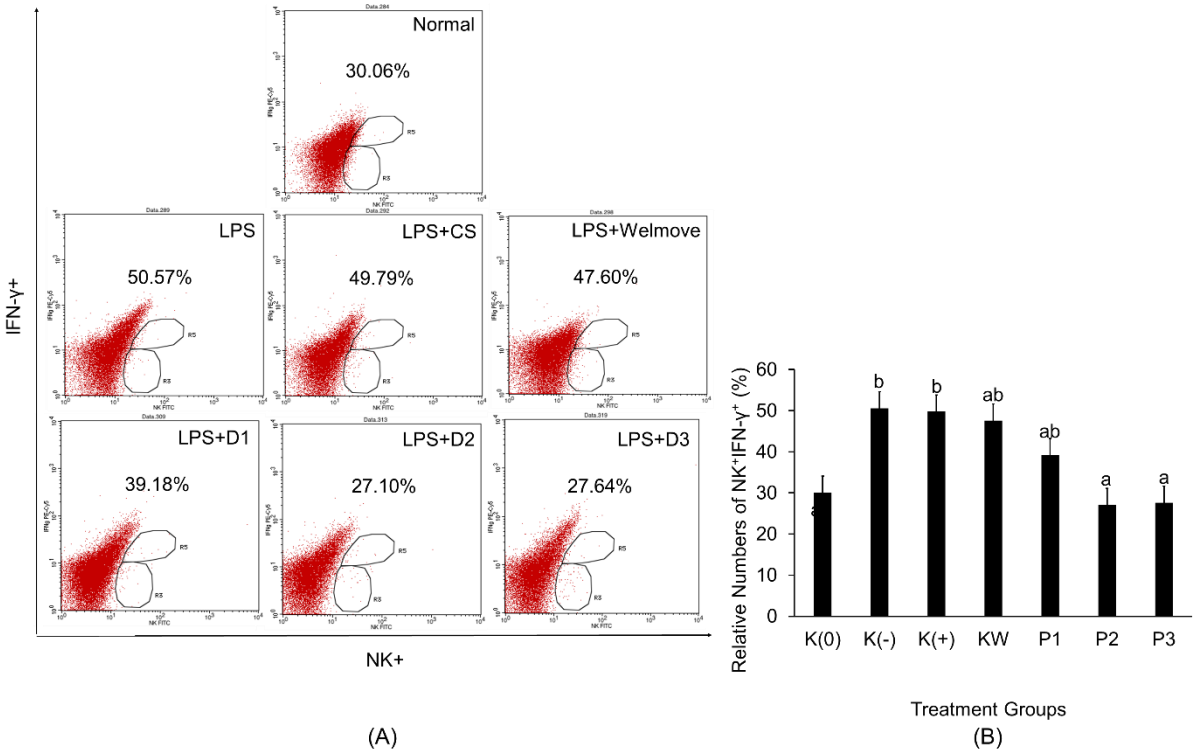

Fig. 3. $NK^+IFN\text{-}\gamma^+$ cell response to LPS, CS, welmove, and increasing doses of shark cartilage extract

DISCUSSION

The results of this study are supported by **Stabler *et al.* (2017)**, wherein LPS induces a strong inflammatory response by triggering TNF-α cytokine production through NFκB activation. NFκB is a transcription factor that can modulate the activation and differentiation of $CD4^+$ T cells and plays a crucial role in the inflammatory response as a central mediator of pro-inflammatory gene expression **(Liu *et al.*, 2017)**. In its inactive state, NFκB is bound to the inhibitory factor IκB within the cell cytoplasm. Activation of the cell by an extracellular ligand, such as LPS, result in the phosphorylation, ubiquitination, and eventual degradation of IκB by the proteasome. Once released from its inhibitor, the p65-NFκB subunit translocate from the cytoplasm to the nucleus, thereby initiating the transcription of target genes associated with immune and inflammatory responses, including the pro-inflammatory cytokine TNF-α **(Stabler *et al.*, 2017)**.

The ability of shark cartilage extract in inhibiting the secretion TNF-α at certain doses demonstrates its potential as an anti-inflammatory agent. According to **Zulhendri *et al.* (2022)**, natural anti-inflammatory agents work by inhibiting and reducing the levels of certain pro-inflammatory cytokines, one of which is TNF-α. Previous studies have

shown that CS can reduce TNF-α levels in mice with LPS-induced liver inflammation, indicating that CS has a regulatory effect on the expression of pro-inflammatory cytokine genes **(Song *et al.*, 2017)**. The anti-inflammatory activity of CS through the reduction of the pro-inflammatory cytokine TNF-α was also observed in LPS-induced astrocytes in mice **(Stabler *et al.*, 2017)**. According to the study, the downregulation of TNF-α secretion is likely due to the inhibition of NFκB activation, which ultimately inhibits NFκB translocation to the nucleus, preventing transcription of target genes and reducing the production of pro-inflammatory cytokine TNF-α **(Guo *et al.*, 2024)**. According to **Stabler *et al.* (2017)**, chondroitin-4-sulfate (C4S) and chondroitin-6-sulfate (C6S) can inhibit the inflammatory response involving NFκB activity by interacting with TLR-4. The reduction in TNF-α expression by CD4 T cells in this study following administration of shark cartilage extract which is known to have a high C6S content **(Novoa-Carballal *et al.*, 2017; Yang *et al.*, 2023)** may be attributed to the blocking of the TLR-4 receptor by CS. Therefore, the ability of CS to reduce the inflammatory response in LPS-induced mice in this study is presumed to occur through the mechanism of NFκB inhibition and reduction of TNF-α, which is likely caused by TLR-4 inhibition.

Importantly, this study highlights not only the pharmacological efficacy but also the biomedical value of marine byproducts, specifically shark cartilage from *Prionace glauca*, a species commonly caught in fisheries. By repurposing this biological waste into a high-value bioactive extract, this work contributes to sustainable resource utilization and circular bioeconomy principles. Rather than being discarded, shark cartilage can be transformed into an anti-inflammatory therapeutic candidate, adding both environmental and economic value to fishery operations.

This study assumed the inhibition of NFκB may not only suppressed TNF-α, but also other pro-inflammatory cytokine IFN-γ produced by CD4 and NK cells in LPS induced mice. LPS induction activated NKT cells mediate their effects by secreting proinflammatory cytokines such as IFN-γ and tumor necrosis factor-alpha TNF-α, while activated NK cells primarily produce IFN-γ **(Alanazi *et al.*, 2023)**. The administration of Welmove in this study was found to be not significantly different from the LPS group and the shark cartilage extract treatment groups at doses 1 and 3, although when looking at the average values, the TNF-α and IFN-γ levels in the Welmove group were lower compared to the LPS group. In comparison with the LPS+Welmove group and the 1-dose treatment, the 2- and 3-dose shark cartilage extracts exhibited stronger anti-inflammatory effects in suppressing TNF-α and IFN-γ levels in LPS-induced mice. Treatment groups 2 and 3 differed significantly from the LPS group but did not differ significantly from the normal group. Given the superior performance of shark cartilage extract at higher doses compared to the commercial supplement, it demonstrates potential as an alternative or complementary natural therapeutic for managing inflammatory and joint-related disorders. Welmove has been marketed for cartilage health and inflammation reduction

(Kantor *et al.*, 2021; Wang *et al.*, 2023). Therefore, given the greater anti-inflammatory effect of shark cartilage extract at certain doses (doses 2 and 3) compared to Welmove in reducing TNF-α levels, this extract may also have potential as an anti-inflammatory agent to help alleviate inflammation, particularly joint inflammation.

This study confirms the anti-inflammatory efficacy of shark cartilage extract from *Prionace glauca*, as demonstrated by its ability to reduce pro-inflammatory cytokine levels in LPS-induced mice. The extract, particularly at medium and high doses, significantly downregulated TNF-α and IFN-γ production by CD4+ T cells and NK cells, matching or surpassing the performance of commercial CS and joint supplements. These results suggest that the anti-inflammatory mechanism of action may involve inhibition of TLR4-mediated NF-κB activation. By demonstrating the pharmacological utility of fishery byproducts, this research reinforces the concept of biomedical valorization of marine waste, contributing both to health innovation and sustainable marine resource management.

CONCLUSION

This study demonstrates that shark cartilage extract, derived from *Prionace glauca* fisheries byproducts, effectively reduces inflammatory markers—$CD4^+TNF\text{-}\alpha^+$, $CD4^+IFN\text{-}\gamma^+$, and $NK^+IFN\text{-}\gamma^+$ cells—in a dose-dependent manner in LPS-induced mice. Higher doses (D2 and D3) successfully restored cytokine levels to near-normal conditions. These results underscore the potential of utilizing shark cartilage waste as a bioresource for anti-inflammatory biomedical applications, likely through modulation of NF-κB signaling pathways. This work supports the sustainable valorization of marine byproducts in the development of natural therapeutic agents.

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REFERENCES

Adharini, W. I.; Nilamsari, R. V.; Lestari, N. D.; Widodo, N. and Rifa'i, M. (2020). Immunomodulatory effects of formulation of *Channa micropeltes* and *Moringa oleifera* through anti-inflammatory cytokines regulation in type 1 diabetic mice. Pharmaceutical Sciences, 26(3): 3.

Agustin, T. I.; Risma, R.; Sari, R. and Setyawan, D. (2023). The microstructure and potential of chondroitin sulfate in shark cartilage extract. *Squalen Bulletin of Marine and* Fisheries Postharvest and Biotechnology, 18(2): 74–80.

Agustin, T. I.; Sulistyowati, W.; Arsiniati, A. and Erina, Y. (2016). Pemanfaatan tulang rawan hiu karet (*Prionace glauca*) sebagai suplemen radang sendi. *Prosiding Simposium Hiu dan Pari di Indonesia, Kerjasama Kementerian Kelautan dan Perikanan, Lembaga Ilmu Pengetahuan Indonesia dan WWF*.

Agustin, T. I.; Yatmasari, E. and Sulistyowati, W. (2016). Study on the bioactive compounds of shark (*Prionace glauca*) cartilage and its inflammatory activity. *International* Journal of PharmTech Research, 9(1): 171–178.

Alanazi, A.; Nagi, M. N.; Alhareth, D. Y.; Al-Hamamah, M. A.; Mahmoud, M. A.; Ahmad, S. F. and Attia, S. M. (2023). Crosstalk of TNF-α, IFN-γ, NF-kB, STAT1 and redox signaling in lipopolysaccharide/d-galactosamine/dimethylsulfoxide-induced fulminant hepatic failure in mice. Saudi Pharmaceutical Journal, 31(3): 370–381.

Bishnoi, M.; Jain, A.; Hurkat, P. and Jain, S. K. (2016). Chondroitin sulphate: A focus on osteoarthritis. Glycoconjugate Journal, 33(5): 693–705.

Bu, Y.; Elango, J.; Zhang, J.; Bao, B.; Guo, R.; Palaniyandi, K.; Robinson, J. S.; Geevaretnam, J.; Regenstein, J. M. and Wu, W. (2017). Immunological effects of collagen and collagen peptide from blue shark cartilage on 6T-CEM cells. Process Biochemistry, 57: 219–227.

Chen, J.; Chen, X.; Li, J.; Luo, B.; Fan, T.; Li, R.; Liu, X.; Song, B.; Jia, X. and Zhong, S. (2022). Preparation and characterization of nano-selenium decorated by chondroitin sulfate derived from shark cartilage and investigation on its antioxidant activity. Marine Drugs, 20(3): 3.

Ciesielska, A.; Matyjek, M. and Kwiatkowska, K. (2021). TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cellular and Molecular Life Sciences, 78(4): 1233–1261.

Fu, Y.; Xu, B.; Huang, S.; Luo, X.; Deng, X.; Luo, S.; Liu, C.; Wang, Q.; Chen, J. and Zhou, L. (2021). Baicalin prevents LPS-induced activation of TLR4/NF-κB p65 pathway and inflammation in mice via inhibiting the expression of CD14. Acta Pharmacologica Sinica, 42(1): 88–96.

Golovach, I.; Rekalov, D.; Akimov, O. Y.; Kostenko, H.; Kostenko, V.; Mishchenko, A.; Solovyova, N. and Kostenko, V. (2023). Molecular mechanisms and potential

applications of chondroitin sulphate in managing post-traumatic osteoarthritis. Reumatologia, 61(5): 395–407.

Guo, Q.; Jin, Y.; Chen, X.; Ye, X.; Shen, X.; Lin, M.; Zeng, C.; Zhou, T. and Zhang, J. (2024). NF-κB in biology and targeted therapy: new insights and translational implications. Signal Transduction and Targeted Therapy, 53(2024): 1–37.

Kantor, E. D.; O'Connell, K.; Du, M.; Cao, C.; Zhang, X.; Lee, D. H.; Cao, Y. and Giovannucci, E. L. (2021). Glucosamine and chondroitin use in relation to C-reactive protein concentration: Results by supplement form, formulation, and dose. The Journal of Alternative and Complementary Medicine, 27(2): 150–159.

Li, W.; Tian, S.; Dai, X.; Staples, K. W.; Chen, B.; Huang, H. and Tian, S. (2023). Blue Shark (*Prionace glauca*) Distribution in the Pacific Ocean: A Look at Continuity and Size Differences. Water, 15(7): 1324.

Liu, T.; Zhang, L.; Joo, D. and Sun, S.-C. (2017). NF-κB signaling in inflammation. Signal Transduction and Targeted Therapy, 2(1): 17023.

Lu, W. C.; Chiu, C. S.; Chang, Y. J.; Guo, T. P.; Lin, C. C.; Wang, P. C.; Lin, P. Y.; Mulio, A. T. and Li, P. H. (2022). An In Vivo Study to Evaluate the Efficacy of Blue Shark (*Prionace glauca*) Cartilage Collagen as a Cosmetic. Marine Drugs, 20: 633.

Merly, L. and Smith, S. L. (2015). Pro-inflammatory properties of shark cartilage supplement. Immunopharmacology and Immunotoxicology, 37(2): 140–147.

Novoa-Carballal, R.; Pérez-Martín, R.; Blanco, M.; Sotelo, C. G.; Fassini, D.; Nunes, C.; Coimbra, M. A.; Silva, T. H.; Reis, R. L. and Vázquez, J. A. (2017). By-products of *Scyliorhinus canicula*, *Prionace glauca* and *Raja clavata*: A valuable source of predominantly 6S sulfated chondroitin sulfate. Carbohydrate Polymers, 157: 31–37.

Rakhmiyati, R.; Widiyani, T. and Budiharjo, A. (2023). High performance liquid chromatography (HPLC) for detection of glucosamine and chondroitin sulfate compounds. Biology, Medicine, & Natural Product Chemistry, 12(1): 1.

Safari, E. and Hassan, Z.-M. (2020). Immunomodulatory effects of shark cartilage: Stimulatory or anti-inflammatory. Process Biochemistry, 92: 417–425.

Sharma, R.; Kuche, K.; Thakor, P.; Bhavana, V.; Srivastava, S.; Mehra, N. K. and Jain, S. (2022). Chondroitin Sulfate: Emerging biomaterial for biopharmaceutical purpose and tissue engineering. Carbohydrate Polymers, 286: 119305.

Song, Y. O.; Kim, M.; Woo, M.; Baek, J.-M.; Kang, K.-H.; Kim, S.-H.; Roh, S.-S.; Park, C. H.; Jeong, K.-S. and Noh, J.-S. (2017). Chondroitin sulfate-rich extract of skate cartilage attenuates lipopolysaccharide-induced liver damage in mice. Marine Drugs, 15(6): Article 6.

Stabler, T. V.; Huang, Z.; Montell, E.; Vergés, J. and Kraus, V. B. (2017). Chondroitin sulphate inhibits NF-κB activity induced by interaction of pathogenic and damage associated molecules. Osteoarthritis and Cartilage, 25(1): 166–174.

Tang, J.; Xu, L.; Zeng, Y. and Gong, F. (2021). Effect of gut microbiota on LPS-induced acute lung injury by regulating the TLR4/NF-kB signaling pathway. International Immunopharmacology, 91: 107272.

Tanna, P. D.; Fofandi, D. C. and Motivarash, Y. B. (2020). Processing and utilization of shark cartilage. Journal of Entomology and Zoology Studies, 8(1): 614–615.

Tucureanu, M. M.; Rebleanu, D.; Constantinescu, C. A.; Deleanu, M.; Voicu, G.; Butoi, E.; Calin, M. and Manduteanu, I. (2017). Lipopolysaccharide-induced inflammation in monocytes/macrophages is blocked by liposomal delivery of Gi-protein inhibitor. International Journal of Nanomedicine, 13: 63–76.

Urbi, Z.; Azmi, N. S.; Ming, L. C. and Hossain, Md. S. (2022). A concise review of extraction and characterization of chondroitin sulphate from fish and fish wastes for pharmacological application. Current Issues in Molecular Biology, 44(9): 3905–3922.

Vázquez, J. A.; Blanco, M.; Fraguas, J.; Pastrana, L. and Pérez-Martín, R. (2016). Optimisation of the extraction and purification of chondroitin sulphate from head by-products of *Prionace glauca* by environmental friendly processes. Food Chemistry, 198: 28–35.

Wang, X.; Liu, D.; Li, D.; Yan, J.; Yang, J.; Zhong, X.; Xu, Q.; Xu, Y.; Xia, Y.; Wang, Q.; Cao, H. and Zhang, F. (2023). Combined treatment with glucosamine and chondroitin sulfate improves rheumatoid arthritis in rats by regulating the gut microbiota. *Nutrition & Metabolism*, 20(1): 22.

Yang, J.; Shen, M.; Wen, H.; Luo, Y.; Huang, R.; Rong, L. and Xie, J. (2020). Recent advance in delivery system and tissue engineering applications of chondroitin sulfate. Carbohydrate Polymers, 230: 115650.

Yang, J.; Shen, M.; Wu, T.; Chen, X.; Wen, H. and Xie, J. (2023). Physicochemical, structural characterization, and antioxidant activities of chondroitin sulfate from *Oreochromis niloticus* bones. Food Science and Human Wellness, 12(4): 1102–1108.

Yang, K. R.; Tsai, M. F.; Shieh, C. J.; Arakawa, O.; Dong, C. D.; Huang, C. Y. and Kuo, C. H. (2021). Ultrasonic-assisted extraction and structural characterization of chondroitin sulfate derived from jumbo squid cartilage. Foods., 10(10): 2363.

Zulhendri, F.; Lesmana, R.; Tandean, S.; Christoper, A.; Chandrasekaran, K.; Irsyam, I.; Suwantika, A. A.; Abdulah, R. and Wathoni, N. (2022). Recent update on the anti-inflammatory activities of propolis. Molecules., 27(23): 23.