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Hepato-Renal Protective Effects of Krill Oil and Atorvastatin in a Caecal Ligation and Puncture Model of Sepsis in Mice

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Abstract
Sepsis induces significant hepatic and renal dysfunction due to systemic inflammation and impaired resolution mechanisms. Effective therapies that preserve liver and kidney function are critical for improving sepsis outcomes. This study investigated the protective effects of krill oil (KO), atorvastatin (ATR), and their combination on liver and kidney injury in CLP-induced septic mice. Sepsis was induced in Swiss albino mice via cecal ligation and puncture. Treated groups received KO, ATR, or KO+ATR. Serum liver and kidney biomarkers (ALT, BUN, creatinine) were measured, and hepatic and renal tissues were examined histologically to assess cellular injury, congestion, and structural integrity. CLP-induced sepsis caused significant elevations in ALT, BUN, and creatinine, accompanied by hepatocellular swelling, vacuolation, and renal tubular degeneration with glomerular congestion. Monotherapy with KO or ATR partially attenuated these biochemical and histopathological alterations. Notably, combination therapy normalized ALT, BUN, and creatinine levels and restored near-normal hepatic and renal architecture. Improvements in biochemical parameters closely aligned with histopathological preservation, highlighting a direct link between functional recovery and tissue integrity. The findings demonstrate that KO and ATR exert protective effects on liver and kidney during sepsis, with combined therapy showing additive benefits. Restoration of biochemical homeostasis and tissue architecture suggests that these interventions mitigate sepsis-induced organ injury, likely through modulation of inflammation and enhancement of resolution pathways.

Keywords: Sepsis, Liver, Kidney, Krill Oil, Atorvastatin, Organ Protection

Introduction
Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection, and is a major cause of mortality worldwide. The kidneys and liver are among the most vulnerable organs in sepsis: acute kidney injury (AKI) occurs in a large proportion of septic patients and is tightly linked to elevated mortality [1]. Similarly, sepsis-induced hepatocellular injury is reflected in elevations of alanine aminotransferase (ALT) and histopathological lesions of the liver [2, 3]. In animal models of polymicrobial sepsis (such as CLP), significant increases in ALT have been documented, confirming that the liver is an early target of injury in this syndrome [3].
In sepsis, the mechanisms of hepatic and renal injury are multifactorial: inflammation and oxidative stress, microcirculatory dysfunction, mitochondrial injury, apoptosis, and immune-mediated damage all contribute. AKI in sepsis is characterised by tubular injury, microvascular dysfunction, and cell cycle arrest [1]. Liver injury in sepsis may present as centrilobular necrosis, sinusoidal congestion, and inflammatory cell infiltration, and is associated with worse outcomes [2, 3]. Biochemically, elevations of ALT correlate with the severity of the septic insult. Furthermore, impaired kidney and liver function tests (increased BUN/creatinine, increased ALT) serve as key markers of organ dysfunction and prognostic indicators in septic patients [2].
Given the paramount role of inflammation-resolution pathways in limiting organ damage,

interventions that modulate these processes may confer protection against sepsis-induced organ injury. Krill oil is a marine-derived lipid extract rich in long-chain omega-3 polyunsaturated fatty acids (PUFAs) bound in phospholipids and has demonstrated enhanced bioavailability and anti-inflammatory properties compared with conventional fish oil [4, 5]. These attributes suggest krill oil may bolster endogenous resolution of inflammation and protect organs such as the liver and kidney from septic insult. Meanwhile, atorvastatin, a widely used HMG-CoA reductase inhibitor, has pleiotropic anti-inflammatory and endothelial-protective effects beyond lipid lowering, and emerging data show statins may accelerate formation of specialized pro-resolving mediators (SPMs) and reduce end-organ damage in critical illness [6, 7].

Despite the individual promise of krill oil and atorvastatin, relatively little research has investigated their combined effect on sepsis-mediated hepatorenal dysfunction. In particular, few studies have focused on the measurement of ALT, blood urea nitrogen (BUN), and creatinine, together with histopathological assessment of liver and kidney in a CLP-induced mouse model of sepsis. The present study therefore aims to evaluate the therapeutic potential of krill oil and atorvastatin—alone and in combination—in modulating liver and kidney functional parameters (ALT, BUN, creatinine) and corresponding histopathological damage in mice subjected to polymicrobial sepsis via CLP.

Materials and Methods

Experimental Animals and Ethical Approval

Adult male Swiss albino mice (25–35 g) were procured from the Laboratory Animal Medicine facility, Tamil Nadu Veterinary and Animal Sciences University, Chennai. All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (IAEC Approval No.: 31/SA/IAEC/2024), in compliance with CPCSEA guidelines for the care and use of laboratory animals. Mice were maintained under controlled environmental conditions (20–24 °C, 50–60% humidity, 12 h light/dark cycle) with ad libitum access to food and water and acclimatized for one week prior to experimentation.

Induction of Sepsis

Sepsis was induced using the cecal ligation and puncture (CLP) model, following the procedure described by Wichterman *et al*. (1980). Under ketamine–xylazine anesthesia (80 µg/g + 10 µg/g, i.p.), a midline laparotomy was performed to exteriorize and ligate the cecum just distal to the ileocecal valve. The ligated cecum was punctured twice with a 20-gauge needle, returned to the abdomen, and the incision closed in layers. Sham-operated (SO) mice underwent identical procedures except ligation and puncture. Post-surgical fluid resuscitation was performed with 1 ml isotonic saline (s.c.).

Experimental Groups and Treatments

Mice were randomized into five groups (n = 7/group):

- Group I (SO): Sham-operated control
- Group II (CLP): Sepsis control
- Group III (Krill oil + CLP): Pre-treated with krill oil (250 mg/kg p.o.) for 14 days before CLP
- Group IV (Atorvastatin + CLP): Pre-treated with atorvastatin (1 mg/kg i.p.) for 3 days before CLP
- Group V (Atorvastatin + Krill oil + CLP): Combination pre-treatment as above

The doses were adapted from Piscitelli *et al*. (2011) and Salic *et al*. (2016) with minor modifications.

Serum Biochemical Analysis

Blood was collected via cardiac puncture 20 ± 2 h after surgery. Serum was separated by centrifugation (2000 rpm, 10 min) and analyzed using an automated clinical chemistry analyzer (A15 Biosystems) with commercial diagnostic kits. Hepatic and renal functions were assessed by measuring alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine levels.

Histopathological Examination

Liver and kidney tissues were excised, rinsed in phosphate-buffered saline, and fixed in 10% neutral buffered formalin. After dehydration and paraffin embedding, 4 µm sections were cut and stained with hematoxylin and eosin (H&E). Slides were examined under a light microscope for structural alterations such as hepatocellular necrosis, sinusoidal congestion, tubular epithelial degeneration, and glomerular changes.

Statistical Analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using IBM SPSS v2.0. Intergroup differences were evaluated using one-way ANOVA followed by Tukey's post hoc test. A value of $p < 0.05$ was considered statistically significant.

Results

Serum Biochemical Parameters

Biochemical alterations reflecting hepatic and renal function following CLP-induced sepsis and subsequent therapeutic interventions are summarized in Table 1.

The CLP (sepsis) group demonstrated a marked elevation in serum alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine concentrations compared with the sham-operated control group ($p < 0.05$), indicating significant hepatic and renal dysfunction secondary to septic insult.

Treatment with krill oil, atorvastatin, or their combination substantially mitigated these elevations. Among all treated groups, the krill oil + atorvastatin (K + A) group exhibited the greatest reduction in ALT, BUN, and creatinine levels, with values approaching those of the control mice. Krill oil alone conferred notable hepatoprotective and nephroprotective effects, whereas atorvastatin monotherapy provided moderate improvement.

These results suggest that combined administration of krill oil and atorvastatin synergistically protected against sepsis-induced hepatic and renal injury, potentially through modulation of inflammatory and oxidative stress pathways.

Table 1: Serum biochemical parameters

Group	ALT (U/L)	BUN (mg/dL)	Creatinine (mg/dL)
SHAM	33.05 ± 1.33^{a}	20.07 ± 1.41^{a}	0.728 ± 0.049^{a}
CLP	87.14 ± 4.41^{c}	50.53 ± 3.24^{c}	1.051 ± 0.082^{b}
KO	67.25 ± 2.99^{b}	39.98 ± 2.90^{ab}	0.837 ± 0.053^{ab}
AT	74.97 ± 3.11^{bc}	43.09 ± 2.24^{bc}	0.879 ± 0.064^{ab}
K + A	64.95 ± 3.04^{b}	35.52 ± 2.46^{ab}	0.784 ± 0.037^{a}

Values represent mean ± SE (n = 7 per group). Different superscripts (a, b, c) indicate significant differences ($p < 0.05$) among groups by One-Way ANOVA followed by Tukey's post hoc test.

Histopathological Evaluation

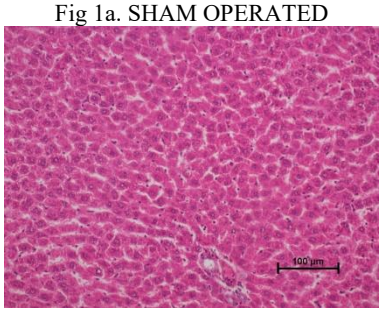
Liver

Microscopic examination of liver sections (Fig. 1) from the CLP group revealed severe hepatocellular degeneration, with swollen hepatocytes exhibiting cytoplasmic vacuolation, fine granularity, and nuclear rarefaction. Marked sinusoidal congestion and portal vascular engorgement were also evident, reflecting extensive hepatic inflammation and vascular compromise typical of sepsis-induced injury.

Treatment with krill oil or atorvastatin alone showed partial improvement, with mild to moderate reduction in hepatocellular degeneration and congestion. However, complete architectural recovery was not achieved.

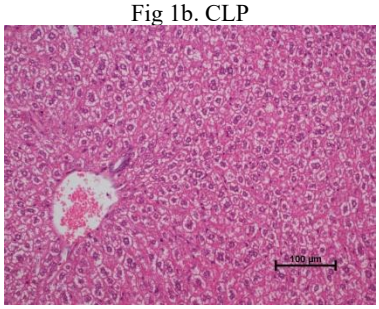
In contrast, liver sections from the krill oil + atorvastatin group displayed nearly normal hepatic cords, intact cellular morphology, and well-preserved sinusoidal architecture, comparable to the sham group. These findings indicate a synergistic hepatoprotective effect of the combined therapy in attenuating sepsis-mediated hepatic damage.

Fig 1a. SHAM OPERATED



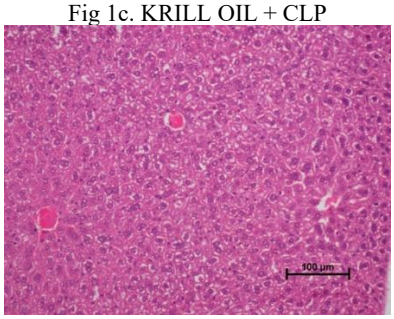
Normal histology

Fig 1b. CLP



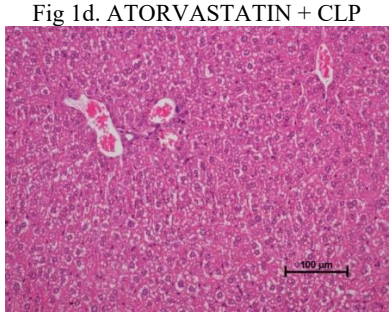
Severe degeneration of hepatocytes and highly engorged portal vessels

Fig 1c. KRILL OIL + CLP



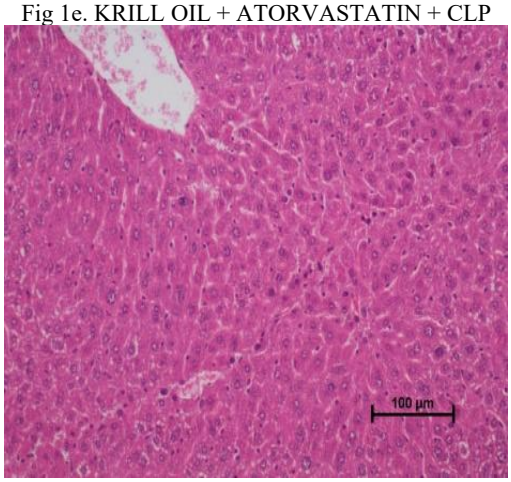
Mild to moderately swollen hepatocytes and engorged portal vessels.

Fig 1d. ATORVASTATIN + CLP



Moderately swollen hepatocytes and engorged portal vessels.

Fig 1e. KRILL OIL + ATORVASTATIN + CLP



Mild degeneration of hepatocytes.

Fig 1: Histopathology- Liver

Kidney

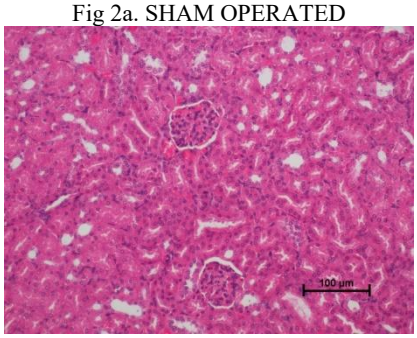
Kidney sections (Fig. 2) from the CLP group demonstrated severe tubular epithelial degeneration, desquamation, and vacuolar changes in renal tubules, accompanied by glomerular congestion and interstitial vascular engorgement, signifying acute renal injury induced by sepsis.

Animals treated with krill oil or atorvastatin exhibited moderate attenuation of renal lesions, with partial restoration of tubular epithelial integrity and reduced vascular congestion.

Notably, the krill oil + atorvastatin combination group showed preserved glomerular and tubular structures, minimal congestion, and absence of necrosis or desquamation, closely resembling normal histoarchitecture seen in the sham control. These observations highlight the potent nephroprotective potential of the combined treatment against sepsis-induced renal damage.

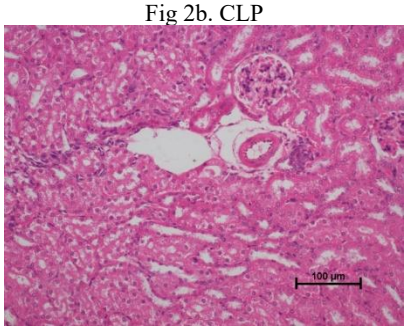
Collectively, the biochemical and histopathological findings demonstrate that CLP-induced sepsis leads to pronounced hepatic and renal dysfunction, which was effectively ameliorated by krill oil and atorvastatin, particularly when used in combination. The synergistic effects observed in the combination group suggest enhanced modulation of inflammatory and oxidative pathways, contributing to the restoration of organ integrity and function.

Fig 2a. SHAM OPERATED



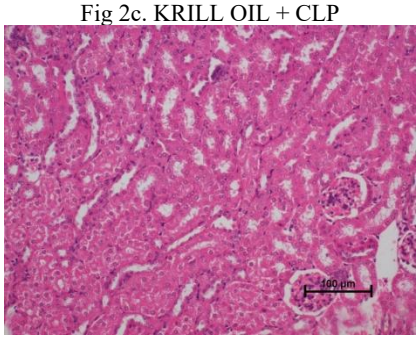
Normal histology

Fig 2b. CLP



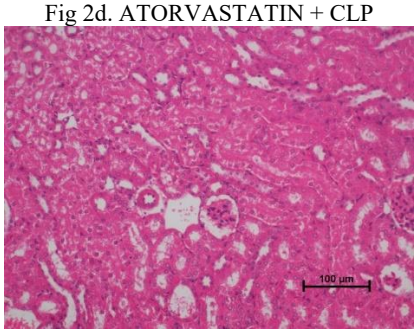
Severe degeneration of tubular epithelial cells with highly engorged glomeruli and blood vessels.

Fig 2c. KRILL OIL + CLP



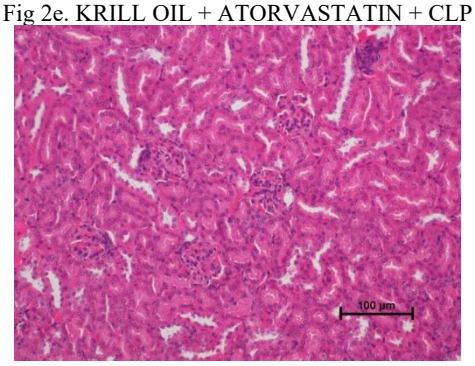
Mild to moderate degeneration of tubular epithelial cells with engorged glomeruli

Fig 2d. ATORVASTATIN + CLP



Moderate degeneration of tubular epithelial cells with engorged blood vessels.

Fig 2e. KRILL OIL + ATORVASTATIN + CLP



Close to normal histology.

Fig 2: Histopathology- Kidney

Discussion

Polymicrobial sepsis induced via the cecal ligation and puncture (CLP) model resulted in pronounced hepatic and renal dysfunction in Swiss albino mice, as evidenced by significant elevations in serum alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine levels, alongside histopathological alterations. Hepatic sections from CLP mice demonstrated hepatocellular swelling, vacuolation, and vascular congestion, indicative of ongoing inflammatory injury and cellular stress. Similarly, renal histopathology revealed severe tubular epithelial degeneration, desquamation, and glomerular congestion, consistent with acute kidney injury secondary to systemic inflammation. These structural changes closely corresponded to the biochemical markers, confirming that the observed elevations in ALT, BUN, and creatinine reflected true organ injury rather than isolated enzymatic fluctuations [11, 12].

Treatment with krill oil, atorvastatin, and particularly their combination, significantly ameliorated both biochemical and histopathological derangements. Monotherapy partially restored hepatocyte integrity and attenuated tubular epithelial damage, whereas combination therapy nearly normalized tissue architecture. The hepatoprotective effects of omega-3 polyunsaturated fatty acids (PUFAs) present in krill oil are well-established, involving inhibition of Kupffer cell activation, reduction of neutrophil-mediated injury, and attenuation of oxidative stress, which collectively preserve hepatocyte viability and vascular integrity [13,14]. Atorvastatin exerts additional protection through pleiotropic effects, including modulation of endothelial function, reduction of microvascular inflammation, and promotion of anti-inflammatory mediator signaling [15]. The combination therapy appears to act synergistically, providing substrate for specialized pro-resolving mediator (SPM) biosynthesis (via EPA/DHA from krill oil) while enhancing receptor-mediated resolution pathways (via atorvastatin), thereby accelerating recovery from sepsis-induced organ injury.

Renal protection mirrored hepatic findings. Combination treatment preserved tubular and glomerular architecture, which correlated with reduced BUN and creatinine levels. SPMs, particularly resolvins and lipoxins, play a key role in renal repair by enhancing macrophage efferocytosis, suppressing pro-inflammatory cytokines, and maintaining microvascular flow [16,17]. These effects likely underlie the improved renal histology and biochemical indices observed in the current study.

Overall, the results demonstrate that CLP-induced sepsis leads to significant hepato-renal injury, which is closely reflected in serum biochemical markers. Both krill oil and atorvastatin contribute to organ protection, with combination therapy

offering superior restoration of liver and kidney structure and function. The findings underscore the importance of targeting both inflammation resolution and endothelial stabilization to mitigate sepsis-induced multi-organ damage.

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Conflict of Interest
The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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