Short Communication

HISTOMORPHOLOGICAL STUDIES OF ATORVASTATIN TREATMENT IN MOUSE MODEL OF SEPSIS

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ABSTRACT

This study aimed to assess the protective effects of atorvastatin in a mouse model of sepsis induced by caecal ligation and puncture (CLP). Eighteen male Swiss albino mice were divided into three groups: sham operation (n=6), CLP alone (n=6) and atorvastatin-treated CLP (n=6). Atorvastatin was administered 18h and 3h prior to CLP induction. After 20 hours of CLP, vital organ tissues (liver, lungs, spleen, kidneys, and brain) were collected for histopathological analysis. The CLP group exhibited severe damage, including congestion, hemorrhage and organ-specific abnormalities. Conversely, the atorvastatin-treated CLP group showed no observable lesions. Histological findings from this study suggested that atorvastatin pretreatment in sepsis prevents cellular damage, modulates inflammatory responses and preserves vascular integrity. The study underscores the potential of atorvastatin as a preventive measure against multiorgan damage in acute sepsis, filling a gap in existing literature on atorvastatin's role in sepsis prevention.

Key words: Atorvastatin, caecal ligation and puncture, histopathology, mice, multiorgan, sepsis

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INTRODUCTION

Sepsis, characterized by uncontrolled systemic inflammation or a 'cytokine storm,' is a complex condition triggered by microbial infections, often leading to systemic inflammatory response syndrome (SIRS) and organ failure (Bosmann and Ward, 2013). With a significant mortality rate in critical care patients, sepsis remains a global health concern, causing approximately 11 million deaths annually, particularly impacting children (WHO,

2023). The mortality rate was 1.89 per 1000 people globally in 2014, emphasizing its severity and impact on healthcare (Nezic *et al.*, 2020).

Pathogen and damage-associated molecular patterns activate toll-like receptors during sepsis, inducing inflammation and the release of pro-inflammatory cytokines, such as TNF-alpha and IL-beta. The dysregulation of pro- and anti-inflammatory responses, coupled with endotheliopathy, forms the immunological basis of sepsis (Frencken *et al.*, 2008; Gao *et al.,* 2008). Moreover, sepsis contributes to dysfunction in vital organs, including the kidneys, liver, and lungs, through inflammatory pathways (Chen *et al.,* 2021).

The CLP model is widely acknowledged for effectively mimicking organ damage induced by sepsis, providing a robust foundation for research in this field (Wichterman *et al*., 1980; Dejager *et al*., 2011).

Statins, inhibitors of hydroxyl methylglutaryl coenzyme A reductase (HMG CoA-reductase), have shown positive effects in preventing sepsis. Atorvastatin, a statin used to manage cholesterol levels and prevent cardiovascular diseases, has garnered attention for its potential benefits. Recent evidence suggests that statins, as HMG CoA-reductase inhibitors, prevent the down-regulation of the alpha-1-adrenoceptor in septic myocardium (Ramasamy *et al.*, 2014). Beyond lipid-lowering properties, statins exhibit immunomodulatory and anti-inflammatory effects (Pruefer *et al.*, 2002). Therefore, this study hypothesizes that pretreatment with atorvastatin in a CLP-induced mice model of sepsis may minimize tissue damage in multiple organs and present histological findings to support this hypothesis.

MATERIALS AND METHODS

Healthy adult male Swiss Albino mice (30-35 g) were obtained from the Laboratory Animal Medicine Unit at the Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Chennai. The mice were housed in polypropylene cages with unrestricted access to food and water. All animal handling procedures were conducted as per the approved protocols of the Institutional Animal Ethical Committee at the Madras Veterinary College, Chennai, India (IAEC Approval No.: 07/SA/IAEC/2023). After a 7-day acclimatization period, the mice were categorized into three groups: (a) sham-operated (SO), (b) CLP, and (c) Atorvastatin (ATR) + CLP. Atorvastatin treatment involved dissolving ATR in ethanol to achieve 10 mg/mL concentration and then further diluting it with 0.9% NaCl to attain a ratio of 1:1000. For the placebo group, 0.9% NaCl based carrier solution without statin was prepared in ethanol at a concentration of 1:1000. Mice were administered either vehicle or ATR (0.2 mg/g body weight) intraperitoneally at 18 and 3 hours before surgery, following a dosage based on a previously reported study (Ramasamy *et al.,* 2014). Sepsis was induced using the caecal ligation and puncture procedure as described by Wichterman *et al* (1980). Mice

were fasted overnight with access to water before surgery. Anesthesia was induced by intraperitoneal injections of xylazine (10 mg/g body weight) and ketamine (80 mg/g body weight), and a 2-cm ventral midline incision was made. The cecum was exposed, ligated just distal to the ileocecal valve with 3-0 silk, punctured twice with a 20-gauge needle, and then returned to the abdomen. Sham-operated mice underwent the same surgical procedure without CLP. All mice received a subcutaneous injection of normal saline (1 mL) to prevent dehydration. Following sacrifice, vital organs such as heart, lungs, liver, kidneys and brain were dissected, washed with phosphate-buffered saline and fixed in 10% neutral buffered formalin. The tissues were dehydrated, paraffin-embedded, sectioned at 4 µm thickness and stained with haematoxylin and eosin for histopathological examination.

RESULTS AND DISCUSSION

Comparison of Sepsis Severity in Mice:

This study investigates the protective potential of atorvastatin against acute organ damage in mice subjected to the CLP model of sepsis, employing histopathological examinations of various internal organs. Mice in the CLP group exhibited decreased activity, lethargy, hypothermia, reduced water and food intake, dyspnea and diarrhea. Additionally, observable clinical signs included conjunctival mucous membrane congestion and upright fur. Conversely, mice in the ATR+CLP group displayed normal activity, consumed regular feed and water and showed no apparent clinical signs.

Histopathological Studies:
Liver:

In the sham-operated group, the liver presented normal histology. Contrastingly, in the CLP group, the liver exhibited congestion in veins and sinusoids, accompanied by diffuse vacuolar degenerative changes in the hepatocytes, central lobular cell swelling and fatty alterations in hepatocytes. Additionally, focal subcapsular hemorrhage was observed. However, in the ATR+CLP group, the liver displayed normal histology, devoid of the alterations seen in the CLP group, as illustrated in Figure 1(a), 1(b) & 1(c). Severe acute abdominal sepsis in CLP is triggered by the release of polymicroflora from caecal contents, leading to a systemic inflammatory response syndrome characterized by acute inflammatory responses, hypercoagulation, immunosuppression and multiorgan failure (Kruger *et al.*, 2013). Statins are recognized for their potential to reduce tissue or organ damage in local inflammation or sepsis induced by endotoxins or CLP, primarily through their pleiotropic effects on various cell signaling pathways (Merx *et al.*, 2004). While hepatic dysfunction is common in sepsis, statins are underutilized in liver diseases due to concerns about hepatotoxicity. However, recent preclinical and clinical research indicates their beneficial effects on the liver (Vargas *et al*., 2017). In our study, the observed congestion and degenerative changes in the hepatocytes in the CLP group are associated with hypoxic or oxidative stress caused by free radicals in sepsis. Early in sepsis, liver detoxification mediated by cytochrome P450 is reduced (Gao *et al.*, 2008). However, pretreatment

with ATR+CLP demonstrated no observable lesions in liver histology is indicative of the hepatoprotective effect of atorvastatin in sepsis, despite its metabolism relying on cytochrome P450 in the liver. Statins might have mitigated hepatic injury severity by enhancing vascular stability and preventing hepatic fat infiltration. The protective effect of atorvastatin in the liver is attributed to decreased acute inflammatory response and antioxidant effects, as reported in previous studies on sepsis (Stolf *et al*., 2012).

Spleen:

The spleen displayed normal histology in the sham-operated group. In the CLP group, histological observations revealed lymphoid hyperplasia in the white pulp, moderate depletion of lymphocytes in periarteriolar lymphoid sheaths, severe congestion, hemorrhage, erythrocytic and megakaryocytic hyperplasia, and an increased presence of diffuse hemosiderin-laden macrophages in the red pulp. However, the ATR+CLP group demonstrated an enhanced splenic histology characterized by dense lymphoid follicles, minimal hemorrhage, hemosiderosis, and a hematopoietic response, as depicted in Figure 2(a), 2(b) & 2(c). The spleen, being a highly vascular organ, plays a crucial role in responding to acute systemic infections and sepsis. In the CLP group, observed lymphoid hyperplasia, lymphoid cell depletion in periarteriolar lymphoid sheaths, and severe vascular damage indicated an attempt for erythropoietic and megakaryocytic responses. In contrast, pretreatment with ATR+CLP demonstrated vascular stability in the spleen without severe damage. Statins prevent spleen damage by promoting T-cell proliferation and reducing T-cell apoptosis (Zang *et al.*, 2016). They also reduce cytokine secretion from splenocytes in sepsis and enhance the expression of CD4+ and CD8+ T cells, acting as immune stimulators by improving T cell responses in sepsis (Kong *et al.,* 2018).

Lungs:

Lungs histology appeared normal in the sham-operated group. In the CLP group, the lungs exhibited blood-mixed exudate in bronchiolar lumens, hyperplasia of epithelial and goblet cells in bronchiolar epithelium, and cellular exudate in the lumen. Severe congestion, edema in alveoli and interstitium, hemorrhage and moderate neutrophilic infiltration were observed. However, in the ATR+CLP group, lung histology showed a notable improvement, with a reduction in the aforementioned lesions, as illustrated in Figure 3(a), 3(b) & 3(c). Lungs, with their rich vascularity, are highly predisposed to injury in acute sepsis. The CLP group exhibited increased vascular stasis and permeability, hemorrhage, epithelial and goblet cell hyperplasia in bronchioles. These findings are attributed to hypoxic and oxidative stress-induced vascular leakage and endothelial damage in acute sepsis. Pretreatment with atorvastatin in CLP attenuated the damage by exerting anti-inflammatory and antioxidant effects, maintaining the integrity of vascular endothelial cells. Previous studies have

reported that atorvastatin reduced lung inflammatory infiltration in Klebsiella sp.-induced lung infection in mice (Jing *et al*., 2016; Paula *et al.*, 2018).

Kidneys:

The kidneys exhibited normal histology in the sham-operated group. In the CLP group, the kidneys manifested glomerular and interstitial congestion, multifocal extensive interstitial hemorrhage, infiltration of lymphoplasmacytic cells, swelling, vacuolar degenerative changes and coagulative necrosis of tubular epithelial cells. But, in the ATR+CLP group, the kidneys demonstrated reduced tubular damage, absence of hemorrhage and inflammation (Figure 4(a), 4(b) & 4(c)). Kidneys are prone to acute injury in sepsis due to glomerular ischemia, hypoxia, increased vascular permeability and endothelial damage. The histopathological changes in the CLP group indicated vascular and tubular epithelial damage with inflammation. However, pretreatment with atorvastatin in CLP showed no adverse effects of sepsis-induced injury in renal tissues. The renoprotective effect of statins arises from their antioxidant and anti-inflammatory effects, improving renal perfusion and vascular integrity in sepsis (Santos *et al*., 2018).

Brain:

The brain exhibited normal histology in the sham-operated group. In the sepsis group, meningeal blood vessels exhibited congestion, diffuse vacuolation of neutrophils, perivascular edema in the cerebral cortex, chromatolysis, nuclear pyknosis in scattered neuronal cell bodies and mild gliosis. The atorvaststin pretreated CLP group showed no histological changes (Figure 5(a), 5(b) & 5(c)). The brain undergoes cerebral injury due to hypoxia and blood-brain barrier dysfunction in systemic infection in sepsis. The histological changes in the brain in the CLP group were more apparent in the cerebral cortex, with neurophilic vacuolation, perivascular edema, neuronal cytoplasmic vacuolation, chromatolysis, nuclear pyknosis and mild gliosis. However, pretreatment with atorvastatin in sepsis group showed no such histological changes, indicating the beneficial effects of atorvastatin attributable to its anti-inflammatory, immunoregulatory and antioxidant effects, maintaining the integrity of the blood-brain barrier and making it non-permissive to proinflammatory cytokines (Catalao *et al.*, 2022; Tian *et al.,* 2019). Limited studies are available on the histological changes induced by sepsis in the brain of mice.

Statins have been extensively studied preclinically and clinically for their pleiotropic effects in sepsis. While various statins have shown therapeutic effects in multiorgan damage in experimental and clinical sepsis, literature on the histological changes in vital organs during sepsis and the pretreatment of atorvastatin in sepsis is limited. Hence, our findings in the CLP mouse model of sepsis provide evidence that pretreatment with atorvastatin is protective against multiorgan damage in sepsis.

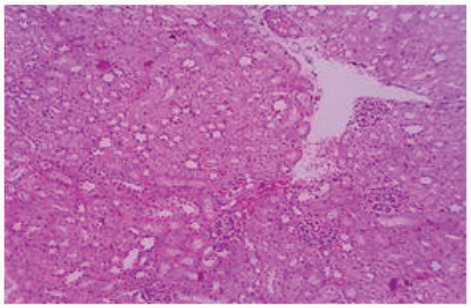

Figure 1(a) Liver, Sham-operated, Mice, Normal, H&E (100X).

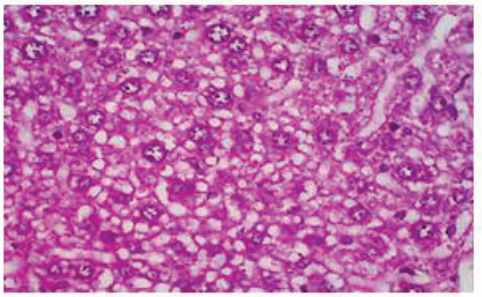

Figure 1(b) Liver, CLP Group, Mice, Diffuse fatty changes in hepatocytes, H &E (400X).

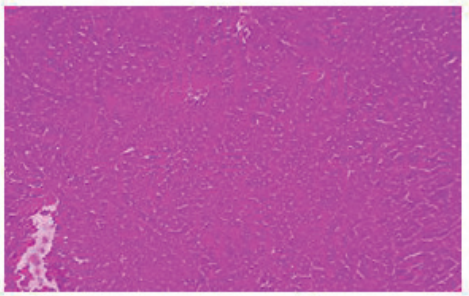

Figure 1(c) Liver, CLP+ATRGroup, Mice, Restoration to normal, H &E (100X).

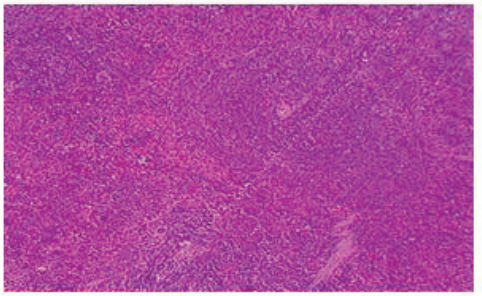

Figure 2(a) Spleen, Sham-operated, Mice, Normal, H&E (100X).

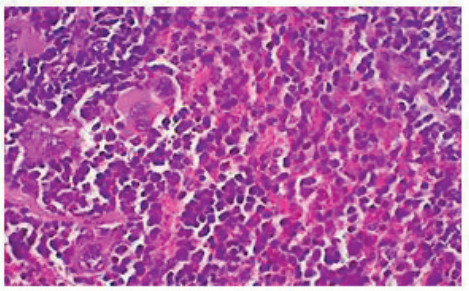

Figure 2(b) Spleen, CLP Group, Mice, Haemorrhages in sinusoids with megakaryocytes, H &E (400X).

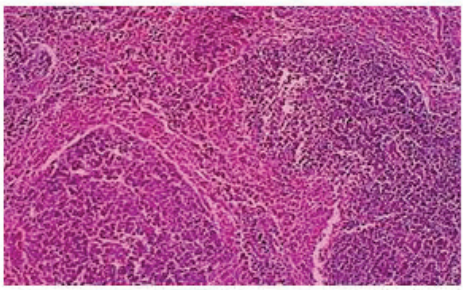

Figure 2(c) Spleen, CLP+ATR Group, Mice, Restoration to normal, H &E (100X).

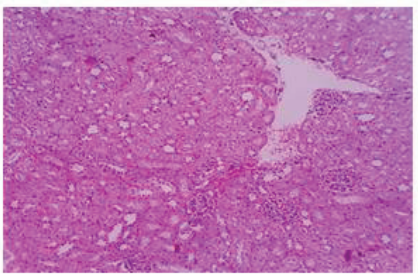

Figure 3(a) Kidney, Sham-operated, Mice, Normal, H&E (100X).

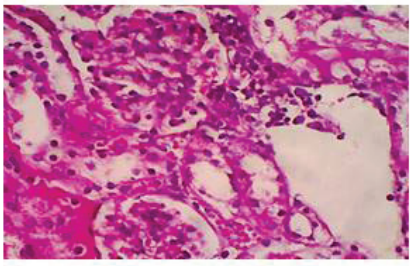

Figure 3(b) Kidney, CLP Group ,Mice, Focal interstitial haemorrhages in cortex, H &E (400X).

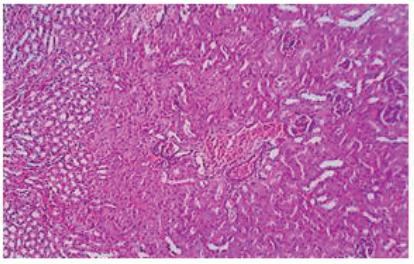

Figure 3(c) Kidney, CLP+ATR Group, Mice, Restoration to normal, H &E (100X).

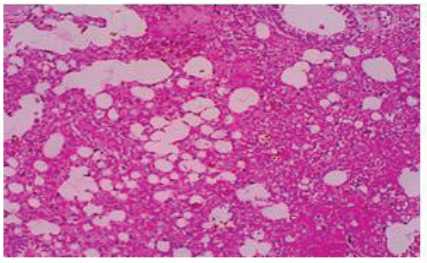

Figure 4(a) Lung, Sham-operated, Mice, Normal, H&E (100X).

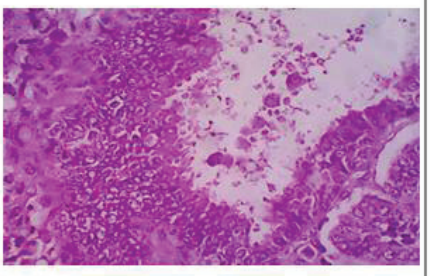

Figure 4(b) Lung, CLP Group, Mice Bronchiolar hyperplasia and sloughing, H &E (400X).

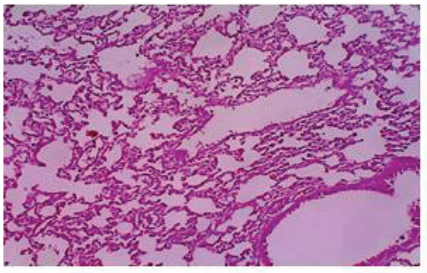

Figure 4(c) Lung, CLP+ATR Group, Mice, Restoration to normal, H &E (100X).

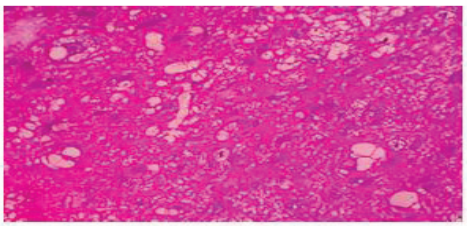

Figure 5(a) Brain, Sham-operated, Mice, Normal, H&E (400X).

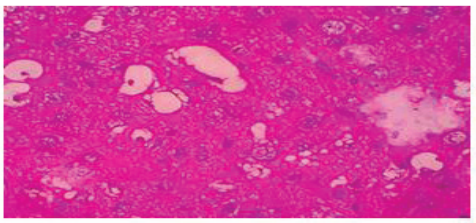

Figure 5(b) Brain, CLP Group, Mice, Perivascular edema, astrocytes swelling and gliosis, H &E (400X).

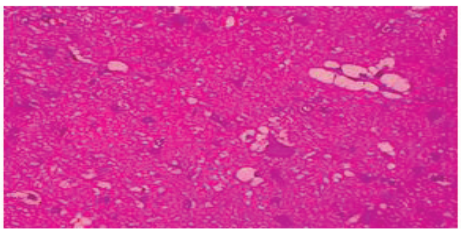

Figure 5(c) Brain ,CLP+ATR Group, Mice, Restoration to normal, H &E (400X).

CONCLUSION

In summary, this study demonstrates that atorvastatin has a protective effect against multiorgan damage in a mouse model of sepsis induced by caecal ligation and puncture. Through histopathological analysis of vital organs, atorvastatin pretreatment was found to mitigate severe organ damage, preventing cellular damage, modulating inflammatory responses and preserving vascular integrity during sepsis. These findings highlight the potential of atorvastatin as a preventive measure for multiorgan damage in acute sepsis, expanding its therapeutic role beyond cholesterol management.

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