Evaluation of the Efficacy of Tofacitinib, a JAK Inhibitor, in Alleviating Sepsis-Induced Multiple Organ Dysfunction Syndrome

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ABSTRACT

Sepsis, a life-threatening condition triggered by an uncontrolled response to infection, results in a systemic inflammatory response syndrome (SIRS) and the failure of multiple organs leading to multiple organ dysfunction (MODS). In the present study, we investigated the therapeutic potential of tofacitinib (TOFA), an FDA-approved inhibitor of JAK1 and JAK3 against sepsis, using a mouse model induced by cecal ligation puncture (CLP). Swiss albino mice were employed to replicate the CLP-induced sepsis model and were randomly divided into four groups: control, CLP, 150 mg/kg TOFA, and 300 mg/kg TOFA. Six hours after the last TOFA dose, we collected blood and tissue samples from the liver, lungs, kidneys, and spleen for histological analysis. Blood samples were used to assess granulocyte and lymphocyte percentages. Throughout the experiment, we monitored body weight and short-term survival. Our comparative histological analysis revealed that 150 mg/kg TOFA had a protective effect against multiple organ damage. Conversely, the study highlighted the harmful effects of 300 mg/kg TOFA, primarily due to liver and renal toxicity within this group. In summary, our findings demonstrate that tofacitinib at an optimal dose of 150 mg/kg showed promise as a potential therapeutic intervention for sepsis-induced multiple organ failure. However, caution is warranted when considering higher dosages.

Highlights

- Sepsis is an uncontrolled response to infection which leads to Multiple organ dysfunction syndrome (MODS)
- Tofacitinib (TOFA) is an FDA-approved JAK inhibitor used in autoimmune diseases
- Tissue damage in sepsis induced MODS was reduced by Tofacitinib
- However, a higher dose of Tofacitinib can cause toxicity in kidney and liver
- Tofacitnib can be a potential drug candidate against sepsis induced MODS

Introduction

Sepsis, a life threatening condition, emerges from severe viral, bacterial, or fungal infections, triggering a systemic inflammatory response syndrome (SIRS). It manifests symptoms like fever, reduced blood pressure (hypotension), and possibly fatal multiple organ dysfunction or failure, that can even cause mortality [1]. In 2017, an estimated 49 million people were impacted, contributing to around 11 million potentially preventable global deaths (WHO). Information from India remains limited, predominantly focusing on infection epidemiology (both community and hospital acquired), rather than sepsis as a host's infection response [2].

The progression of sepsis unfolds across three stages: SIRS, severe sepsis, and septic shock. Initial suspicion of sepsis arises from noticeable shifts in body temperature (extremely high or low), leukopenia, elevated respiratory and heart rates. These manifestations

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collectively signify SIRS, which can escalate to sepsis if an infection is present. As the infection disseminates, it triggers acute organ dysfunction, leading to more severe outcomes like laboured breathing, reduced platelet count, decreased urine production, and heightened bilirubin levels in the liver-hallmarks of "severe sepsis." Ultimately, this cascade culminates in septic shock—the most critical sepsis stage. Septic shock is characterized by profound circulatory, metabolic, and cellular anomalies, accompanied by hypotension necessitating vasopressor intervention. This stage carries a higher mortality risk compared to sepsis alone [3]. However, sepsis-induced immunosuppression through Compensatory Anti-Inflammatory Response Syndrome (CARS) contributes to secondary infections and delayed mortality [4]. Importantly, both SIRS and CARS responses in sepsis are orchestrated via the JAK-STAT signalling pathway.

Sepsis leading to multiple organ dysfunction syndrome (MODS) is one of the primary causes of death in sepsis patients. During sepsis, the kidneys, lungs, and liver are more susceptible to damage and functional impairment [5]. While most septic patients begin with the failure of one organ system, such as acute kidney damage requiring renal replacement treatment, the failure of another organ system frequently follows, and MODS ensues. There is a substantial correlation between the number of malfunctioning organ systems and patient outcome [6]. The exact pathogenic processes that contribute to the development of MODS during sepsis are still unknown.

Ruxolitinib, Barcitinib, Fligocitinib, Tofacitinib (TOFA) etc. are among some of the JAK inhibitors which were tested for clinical trials for the regulation of immune mediated diseases [7]. Tofacitinib is a FDA (Food and Drug Administered) approved inhibitor of JAK, being used in the treatment of rheumatoid arthritis which obstructs the signal transduction of inflammatory cytokines by blocking the JAK STAT signalling pathway [8]. Among the four classes of JAK family (JAK1, JAK2, JAK3 and TYK2) TOFA preferentially inhibits JAK1 and JAK3 [9]. Earlier, studies showed that the TOFA has been an effective drug in inflammation related disorders such as ulcerative colitis [10], psoriasis [11], pemphigus vulgaris [12] etc. However, the potential benefits of TOFA treatment in the context of sepsis induced multi-organ failure remain relatively unexplored. Present study is an attempt to investigate the protective role of TOFA against the multiple organ dysfunction (liver, kidney, lungs and spleen) in the CLP model of sepsis.

Materials and Methods

Molecular Docking

Molecular docking is a structural and computational tool to find out the ligand-protein binding conformations. In the present study docking was used to predict the binding energy of JAK1 and JAK3 with tofacitinib. The chemical structures and associated data of Tofacitinib (ligand), was downloaded from Pubchem in SDF format [13]. Further, SDF format was converted into PDB format by using the open babel tool [14]. And finally, it was changed into PDBQT format. Structures of the receptor JAK1 (6N7A) and JAK3 (5LWM) were downloaded from the PDB database [15–17]. Missing loops in the structures were modulated by modeller which is a plugin of chimera molecular visualization tool [18, 19]. JAK1 and JAK3 were converted into PDBQT format from PDB format. This was achieved by adding charge and H bonds using AutoDock-4 [20]. Now, both ligand and receptor were compatible for docking by Autodock vina [21]. Furthermore, tofacitinib was docked against JAK1 and JAK3 individually with 32 exhaustiveness after the preparation of the grid which enabled the selection of docking sites. Further, the H bonds, binding energy and interaction of amino acids in JAK1 and JAK3 with tofacitinib were recorded. Tofacitinib docked location within the active site was represented by ribbon, hydrophobic surface images, and interaction with JAK1 and JAK3 amino acids is illustrated by 2D ligplot graph [22].

Mice study in CLP model

a) *Reagents and equipments*

Reagents employed in this study included Dimethyl sulphoxide (DMSO), Tofacitinib Citrate, Phosphate buffer saline (PBS), Tween 20, Ketamine (95 mg/kg body weight), Xylazine (12.5 mg/kg body weight), Sterile saline solution (0.9 % (wt/vol) saline), as well as protective gear such as latex gloves and a face mask. Additional items included were Hair removal cream, Autoclaved gauze pads, Non-absorbable surgical suture, Surgical blades, Syringes, and a set of Surgical instruments comprising dissection scissors, microdissection scissors, straight surgical forceps, straight anatomical forceps, needle holder, Wax tray and an Oral gavage 20 were also utilized throughout this investigation.

b) *Animals*

Swiss albino mice were purchased from NIPER (National institute of pharmaceutical education & research) Mohali, (Punjab) and were acclimatized at room temperature (25–27 °C) with 12-h light and dark cycles for 1 week before surgery. The mice were supplied with standard laboratory feed and water. The experimental procedures used in the current study were approved by the Departmental Ethical Committee, University of Rajasthan, Jaipur.

CLP (Cecal ligation puncture) model creation

Swiss albino mice of about seven to nine weeks, weighing 30–35 g were anaesthetized by using ketamine (95 mg/kg) and xylazine (12.5 mg/kg) via intraperitoneal injection and fixed on a wax tray. After disinfecting the abdominal skin with 70 % ethanol, hair removal cream was applied and left for 2–3 minutes. Then cream was removed with a cotton swab and a longitudinal incision of approximately 1.5–2 cm was made in the skin with a scalpel along the vertical line above the pubic bone and further with the use of small scissors, peritoneal cavity was reached, the cecum was identified and was carefully taken out with the use of blunt anatomical forceps. Cecum was punctured using a sterilized needle and it was then squeezed gently in order to expel a small amount of faeces from the site of perforation. The cecum was placed back to the peritoneal cavity and incision was stitched with the help of non-absorbable suture. Saline was injected to the mice for resuscitation. Mice were then placed back into the cages with proper water and food availability.

Experimental Design

Twenty Swiss albino mice weighing between 30–35 g were randomly allocated into four groups, each consisting of five mice: the

control group, the CLP group (mice subjected to cecal ligation puncture), the 150 mg/kg TOFA group (CLP mice treated with 150 mg/kg of tofacitinib), and the 300 mg/kg TOFA group (CLP mice treated with 300 mg/kg of tofacitinib). In the treatment groups, mice were administered with the first dose of TOFA (dissolved in PBS and tween 80) 150 mg/kg or 300 mg/kg via oral gavage, six hours after the CLP surgery. Subsequently, three additional doses were administered at 12-hour intervals. Throughout the experiment, the body weights of mice were recorded and their short term survival were monitored. Following a six-hour interval from the last TOFA dose, the experimental mice were euthanized, blood was collected by the heart puncture method from all the groups, and tissues (Liver, lungs, kidney and spleen) were collected and stored in 10 % formalin for further histopathological examination (▶ **Fig. 1**).

Histological slide preparation and analysis

To facilitate histological analysis, all collected tissues (including the liver, lung, spleen, and kidney) underwent embedding in molten wax to create paraffin wax blocks. These blocks were prepared following a dehydration process, involving a progressive alcohol concentration series to remove formalin and water from the tissues. Subsequently, organic solvent (xylene) was employed to eliminate residual alcohol during the clearing process, allowing for effective infiltration of paraffin wax. Once solidified, the paraffin blocks were meticulously sectioned into 5µm-thin sections using a microtome. These thin sections were then carefully transferred onto slides and subjected to staining with haematoxylin and eosin. The resulting stained slides were subsequently examined under a light microscope for detailed histological analysis.

To quantify the availability of alveolar airspace, we employed ImageJ software [23]. This software allowed for a detailed analysis by separating the specific area representing alveolar airspaces. We achieved this through color deconvolution, which essentially isolates the channel containing only the white color – the color typically used to represent airspaces in these images. Following this isolation, a threshold was applied to define a clear distinction between the airspaces and surrounding tissue. This created a mask, effectively filtering out any unwanted signal. Finally, the image occupied by this mask (representing the white airspace area) was measured. This process was repeated for each image, and the mean value was calculated to provide a quantitative measure of alveolar air space availability across the samples.

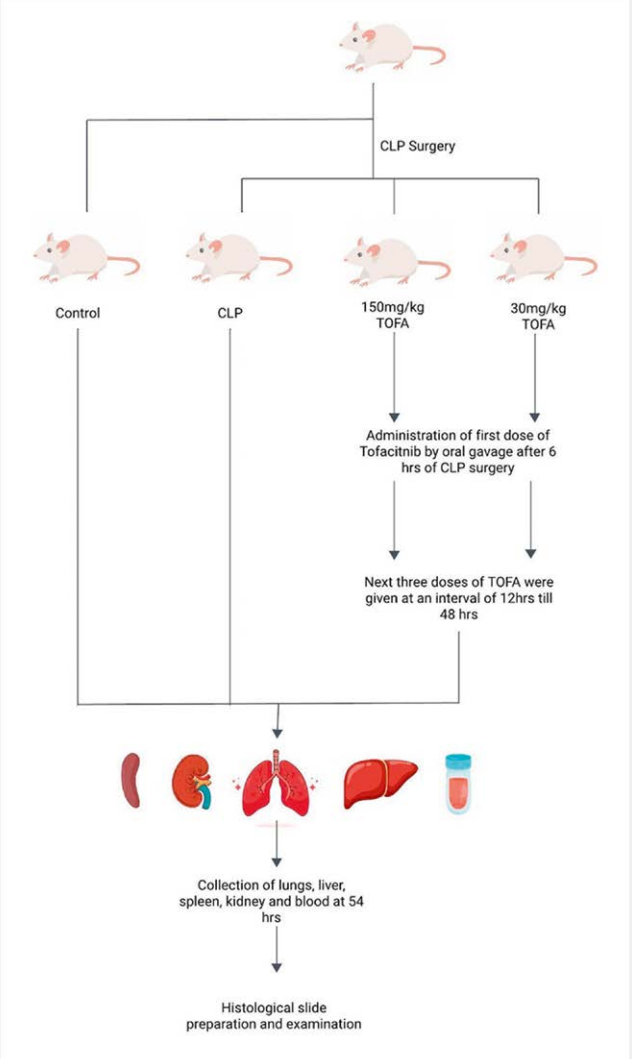
Statistical Analysis

GraphPad Prism5 software was utilized for data analysis and figure preparation. To evaluate statistical differences among the different treatment groups, one-way ANOVA with post hoc Tukey's test was employed, and the differences between the groups were considered to be statistically significant when P value was less than 0.05 (95 % confidence level).

Results

a) *Molecular Docking*

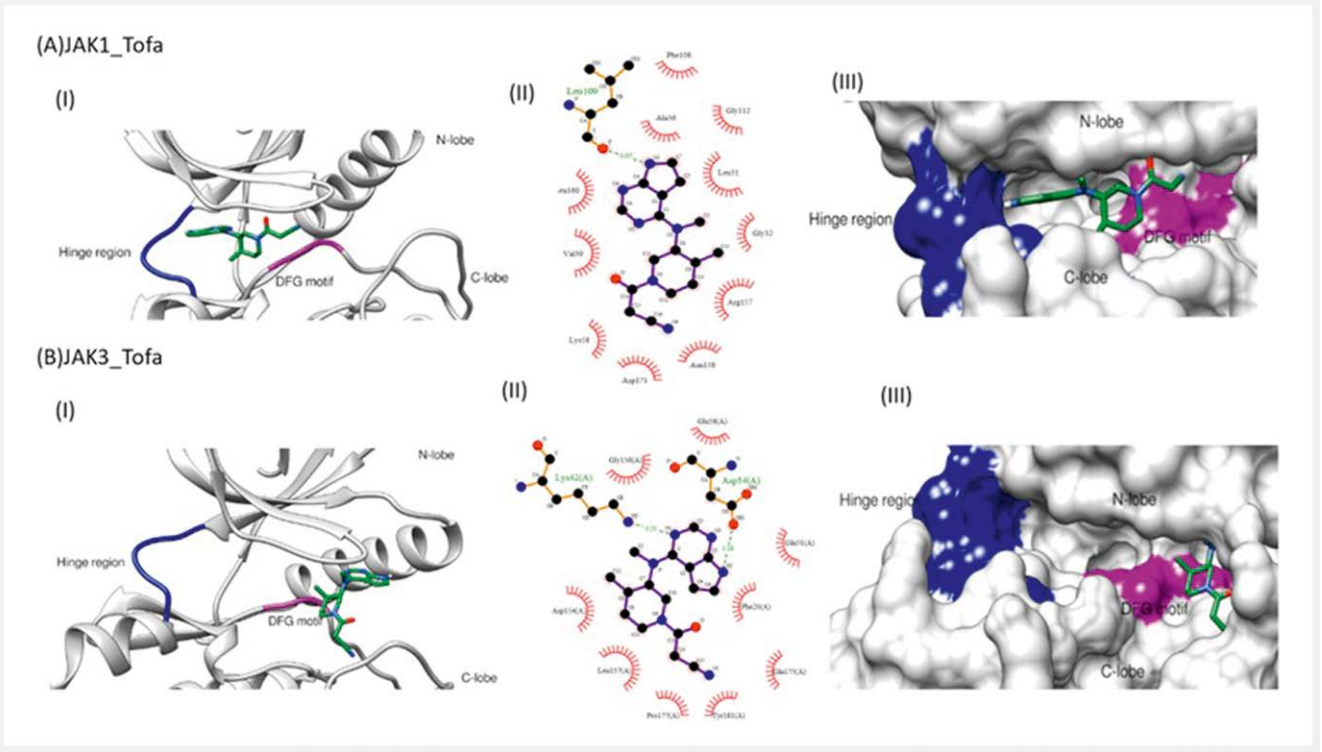
The binding energies of tofacitinib with JAK1 and JAK3 were −8.0 and −7.9 Kcal/mol, respectively (▶ **Table 1**). Tofacitinib formed an H-bond with leu 109 at 3.07 Å and a hydrophobic contact with Phe (108), Ala (56), Gly (112), Leu (31), Gly (32), Arg (137), Asn (158), Asp (171), Lys (58), Val (39), Leu (160) residues in JAK1's active



▶ **Fig. 1** The schematic illustration of experimental design.

▶ **Table 1** Docking binding energy and amino acid interaction with Tofacitinib through H-bond and hydrophobic interaction within binding pocket.

Docked Complex	Binding Energy	H-bond	Hydrophobic Interaction
JAK1_Tofa	−8.0	Leu (109)	Phe (108), Ala (56), Gly (112), Leu (31), Gly (32), Arg (137), Asn (158), Asp (171), Lys (58), Val (39), Leu (160)
JAK3_Tofa	−7.9	Lys (42), Asp (54)	Gly (156), Glu (58), Gln (51), Phe (20), Gln (175), Tyr (181), Pro (177), Leu (157), Asp (154)



▶ **Fig. 2** Interactions of compounds dext-linkng molecular docking (**a**) JAK1 and Tofacitinib (**b**) JAK3 and Tofacitinib and (I) Ribbon surface image (II) 2D ligplot interaction (III) Hydrophobic surface image.

site. Tofa formed two hydrogen bonds with JAK3 protein, one with Lya (42) and the other with Asp (54) that had bond lengths of 3.21 Å and 3.18 Å, respectively. The amino acids Gly (156), Glu (58), Gln (51), Phe (20), Gln (175), Tyr (181), Pro (177), Leu (157), and Asp (154) acted hydrophobically (▶ **Table 1**). Ribbon and hydrophobic pictures demonstrate that Tofa fits into the active site of JAK1,JAK3, and interacts with it. The DAF motif binding has a function in modulating enzyme kinase activity (▶ **Fig. 2**).

b) *Granulocyte and Lymphocyte Percentage*

Granulocyte and lymphocyte counts were estimated from the blood samples obtained during autopsy using the Accurex Automated Double Chamber Haematology Analyzer CBC-360 Plus blood analyzer. A significant increase in granulocyte percentage was observed in both the CLP and 300 mg/kg TOFA groups when compared to the control group ($P < 0.05$) (▶ **Fig. 3a**). Interestingly, the administration of TOFA at a concentration of 150 mg/kg mitigated this increase in granulocyte levels when compared with control and decreased when compared with CLP group, but these findings were not statistically significant. Conversely, no significant change was seen in the impact of TOFA on agranulocytes, particularly lymphocytes, across all groups (▶ **Fig. 3b**).

c) *Experimental observations*

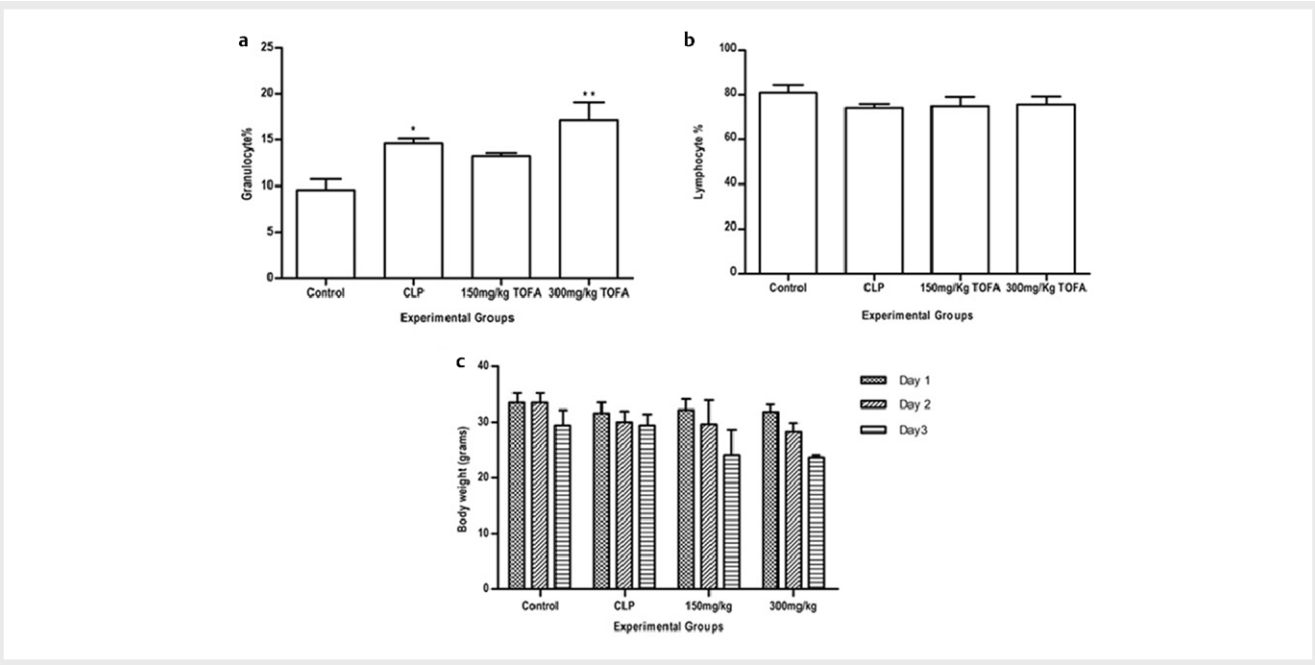
When compared to the control group, mice in the CLP groups demonstrated symptoms such as shivering and jerky movements following CLP surgery which indicates the successful creation of the model. However, in treatment groups, these symptoms have decreased. Beside this, during organ collection via dissection, fragile spleen and liver were found in the CLP group. In addition, spleen enlargement was seen in the CLP group. Body weight measurements were also recorded for all three days of the experiment, plotted on graph and analyzed using one-way ANOVA. The results demonstrated a significant decrease in body weight on both days 1 and 3 of the experiment ($P < 0.05$) across all groups. ▶ **Fig. 3c** illustrates the decline in body weight throughout the duration of the experiment in the CLP group and as well as in treatment groups which is attributed to sepsis.

d) *Short Term Survival Assessment Throughout the Experiment*

Throughout the experiment, we closely monitored all groups to assess the effectiveness of this sepsis model development process and the impact of TOFA treatment. Notably, all mice in the control and 300 mg/kg TOFA groups survived throughout the investigation. Conversely, in both the CLP and 150 mg/kg TOFA groups, one mouse per group succumbed, resulting in a mortality rate of 20 % in both of these groups.

e) *Histological Analysis*

After CLP surgery, mice were administered tofacitinib at two different concentrations, and tissue samples (lungs, liver, kidney, and spleen) were harvested at 48 hours after the treatment. These tissue specimens were processed and stained with hematoxylin-eosin. Subsequently, the stained slides of these tissue specimens were observed under light microscope for histological examination to investigate the influence of tofacitinib on CLP induced septic mouse models.



▶ **Fig. 3** Effect of Tofacitinib on CLP induced sepsis in mice. **a**) Percentage of granulocytes (P = 0.0044) **b**) Percentage of Lymphocytes **c**) Comparison of body weight dext-linkng the experiment. *(P < 0.05) vs control group. Statistical significance was assessed by one-way analysis of variance (ANOVA) with post hoc tukey's test. Data were represented as mean ± standard deviation (SD), *n* = 5.

i). *Lungs*

In the control group, normal airways with thin alveolar lining and no neutrophil infiltration were observed. On the other hand, the CLP group exhibited patchy neutrophil infiltration, thickened alveolar walls, and signs of congestion. Remarkably, the 150 mg/kg TOFA group displayed a reduction in neutrophil infiltration, alveolar thickness, and reduced congestion in comparison to the CLP group. At the 300 mg/kg TOFA concentration, neutrophil infiltration decreased, while alveolar thickness remained relatively constant (▶ **Fig. 4**). Further, semi-quantitative analysis of alveolar airspace availability using ImageJ software revealed a significant decrease in alveolar air space in the CLP group compared to the control group. Conversely, treatment with TOFA (150 mg/kg and 300 mg/kg) in the CLP group resulted in a notable increase in available alveolar airspace, as shown in ▶ **Fig. 5**.

ii). *Liver*

Liver tissue from the control group mice showed a normal hepatic architecture. In contrast, the CLP-induced group displayed cytoplasmic degradation, neutrophil infiltration, and reduced hepatocyte regeneration. The 150 mg/kg TOFA group showed a lower degree of degeneration compared to its regeneration, along with decreased neutrophil infiltration. However, the 300 mg/kg TOFA group displayed increased hepatocyte degeneration (hepatocellular apoptosis) relative to its regeneration, coupled with neutrophil infiltration (▶ **Fig. 6**).

iii). *Splee*n

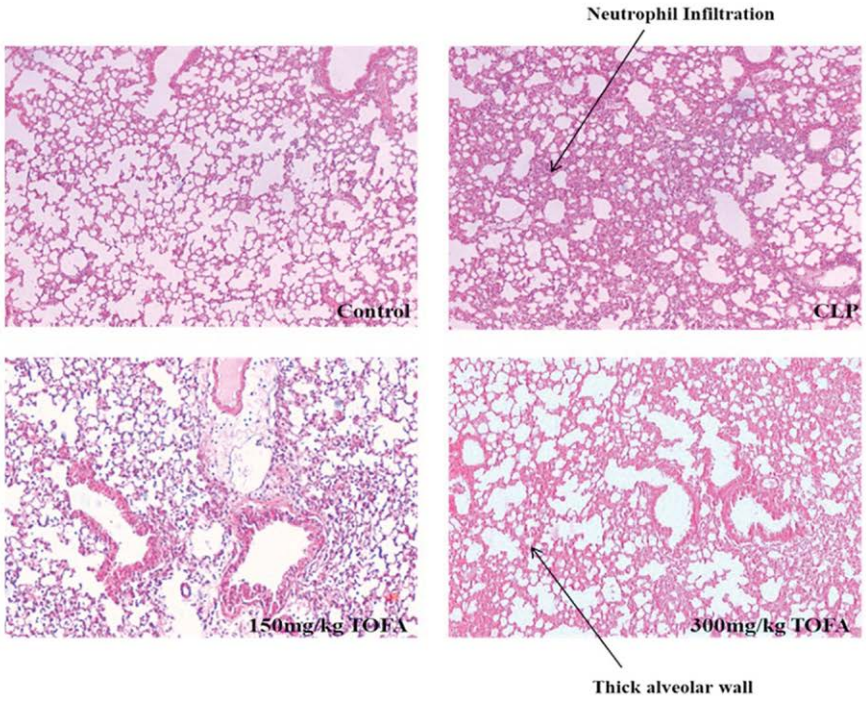
The splenic tissue of the control group exhibited a normal red and white pulp architecture. In the CLP group, cytoplasmic degeneration was evident, and during autopsy, spleen dilation and enlargement were also observed in the CLP group. In the 300 mg/kg TOFA group, degeneration decreased, but clear differentiation between red and white pulp was not discernible. In contrast, the 150 mg/kg TOFA group exhibited a distinct partition between red and white pulp, and there was an increase in the area of red pulp compared to the CLP group (▶ **Fig. 7**).

iv). *Kidney*

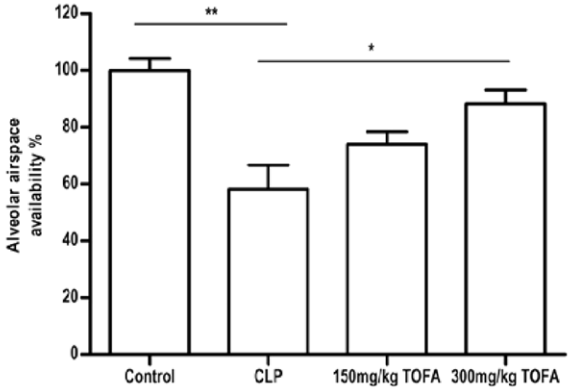
The control group displayed a normal renal histological appearance without signs of congestion or inflammatory infiltration. In the CLP group, renal tubular changes, glomerular atrophy, and necrosis were observed. Notably, the 150 mg/kg TOFA group showed some tubular dilation with tubular vacuolization, while the glomerular structure remained intact compared to the CLP group. However, the 300 mg/kg TOFA group exhibited a higher degree of degeneration and necrosis relative to all other groups, along with glomerular atrophy (▶ **Fig. 8**).

Discussion

Sepsis, a life-threatening condition leading to multi-organ damage, remains a major concern despite advancements in research focused on its treatment [24]. Tofacitinib, an FDA-approved JAK inhibitor for rheumatoid arthritis [8], has shown promising protecting effects against sepsis. Recent studies have highlighted its potential benefits in sepsis, such as its preventive effect against LPS-induced acute kidney injury [25] and its ability to reduce acute lung injury and improved survival, in septic rats via JAK/STAT signalling pathway targeting [26]. Few previous studies have demonstrated the multifaceted effects of tofacitinib. Jarneborn et al. observed that



▶ **Fig. 4** Histological changes in lung parenchyma in septic mice after 48 hrs of treatment by Tofacitinib (H&E stain, 100X).

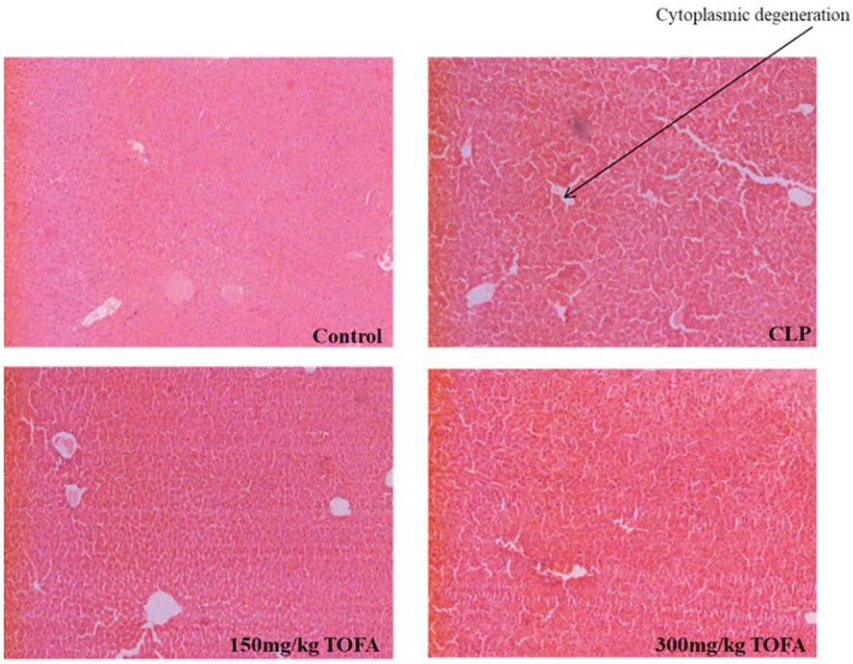


▶ **Fig. 5** Quantification of available airspace in the lungs of all the groups. Statistical significance was assessed by one-way analysis of variance (ANOVA) with post hoc tukey's test. Data were represented as mean ± standard deviation (SD), $n = 5$.

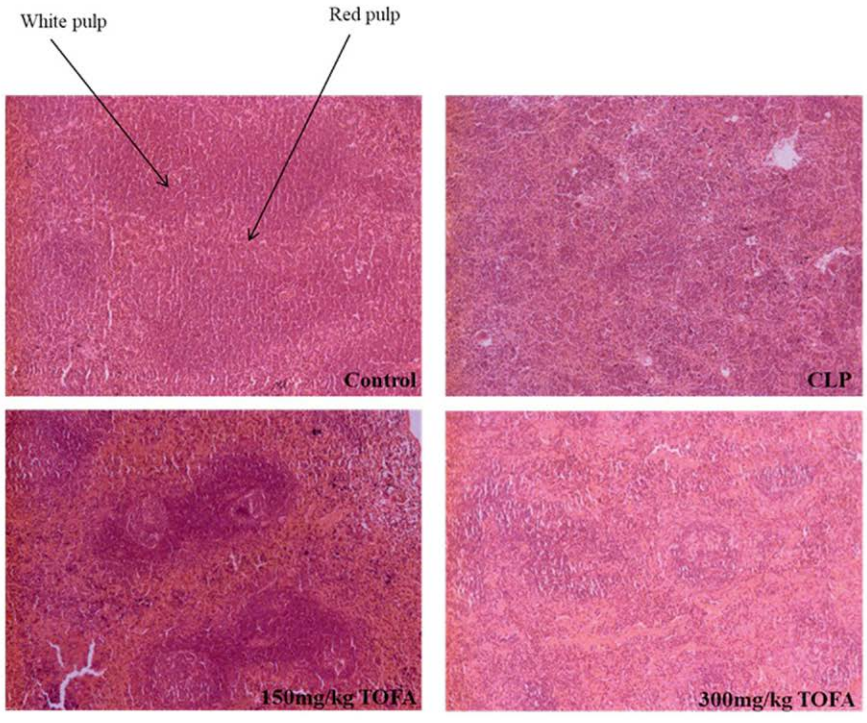
tofacitinib pretreatment significantly improved survival in a staphylococcal sepsis mice model, highlighting its potential role in sepsis treatment [27]. Additionally, tofacitinib's immunomodulatory properties through JAK1 inhibition have been widely explored in regulating inflammatory bowel disease (IBD) [28]. These findings collectively suggest the therapeutic potential of tofacitinib against various immunological disorders by regulating inflammation.

However, the current understanding of tofacitinib's efficacy in established sepsis with multiple organ dysfunction syndrome (MODS) remains limited. This study addresses this gap by investigating the protective effects of orally administered tofacitinib in a CLP-induced septic mice model, focusing on its ability to mitigate MODS and improve survival. While previous studies have documented the safety and efficacy of lower-dose tofacitinib regimens (twice or once daily) in reducing inflammation, we chose a higher dose specifically for patients with established MODS, a critical factor contributing to sepsis mortality. This investigation aimed to explore the potential of higher-dose tofacitinib in regulating tissue damage and prolonging survival in this severe sepsis scenario. By analyzing tissue histopathology at two different tofacitinib concentrations, we sought to elucidate its role in combating MODS and improving outcomes in septic mice. So, 150 mg/kg or 300 mg/kg TOFA doses were administered four times to septic mice, at an interval of 12 hrs in order to evaluate its effect. Six hours after the last dose, tissue samples were collected for histological analyses.

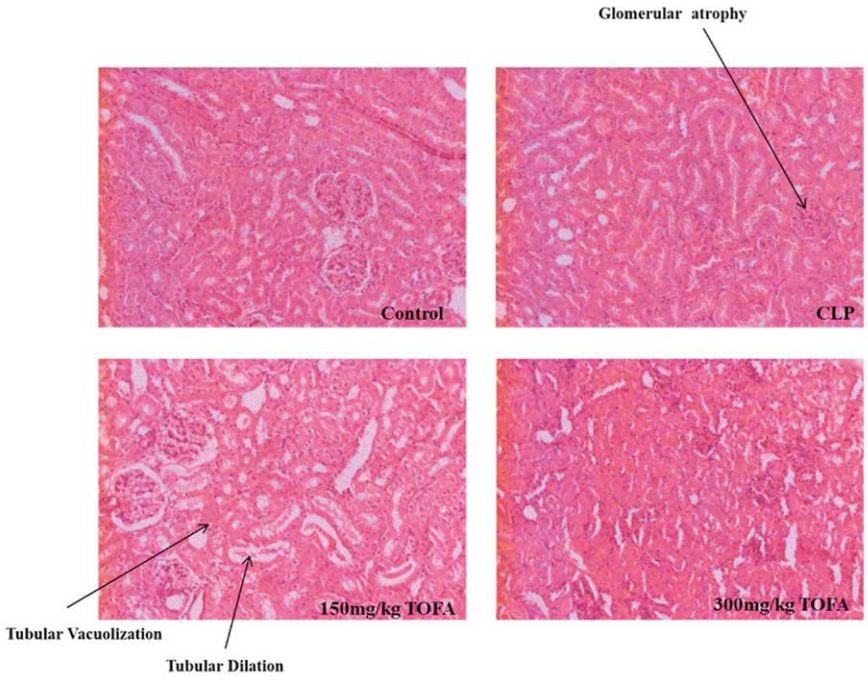
An elevation in the total white blood cell (WBC) count is often associated with inflammation and infection. This count can fluctuate in various clinical scenarios, including non-infectious inflammatory conditions such as rheumatoid arthritis, lupus, and cancer [29, 30]. However, in certain sepsis cases, the WBC count may remain within normal range or even decrease. Consequently, the total WBC count exhibits low specificity, which limits its effectiveness as a sepsis biomarker [31]. In addition, several studies have reported that lymphocyte counts tend to decline during the initial phase of sepsis and are associated with adverse outcomes [32, 33]. There-



▶ **Fig. 6** Histological changes in liver parenchyma in septic mice after 48 hrs of treatment by Tofacitinib (H&E stain, 100X).



▶ **Fig. 7** Histological changes in spleen parenchyma in septic mice after 48 hrs of treatment by Tofacitinib (H&E stain, 100X).



▶ **Fig. 8** Histological changes in kidney parenchyma in septic mice after 48 hrs of treatment by Tofacitinib (H&E stain, 200X).

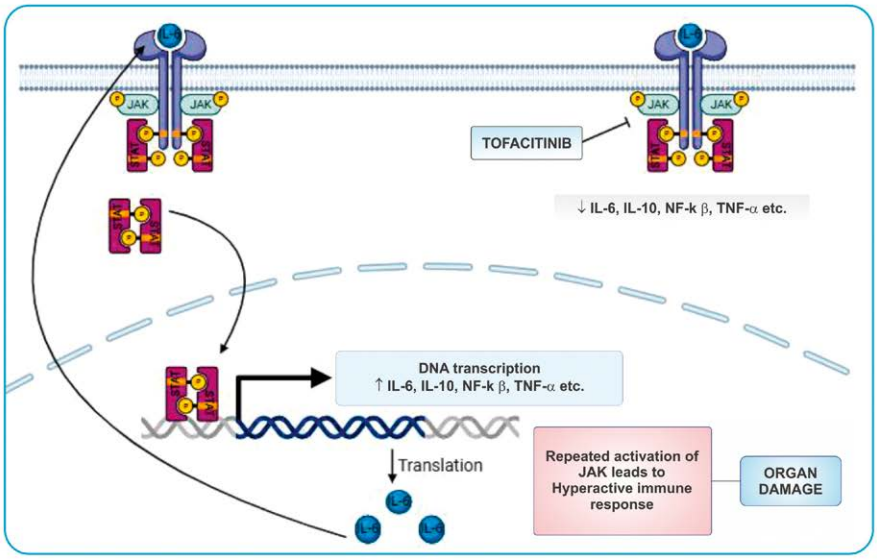
fore, we conducted an analysis of lymphocyte and granulocyte percentages in each group.

Our study revealed elevated granulocyte percentages in the CLP group and 300 mg/kg TOFA group, indicating persistent infection and potential TOFA-related hazards at this dose respectively. Conversely, a decrease in granulocyte percentage at the 150 mg/kg TOFA concentration suggests TOFA's potential in reducing infection. Lymphocyte count patterns showed no significant changes among groups, except for a slight decrease in lymphocytes in all groups compared to the control. Additionally, a significant drop in mouse body weight was observed on days 1 and 3 of the experiment.

The surgical CLP procedure induced tissue damage in the lung, liver, spleen, and kidney, affirming the successful establishment of the septic model. Lung tissue from the control group served as a representation of normal pulmonary physiology in the absence of infection. Microscopic comparison between the control and CLP groups revealed notable neutrophil infiltration in the alveolar spaces of the lungs, resulting in a reduction of the functional surface area for gaseous exchange. Conversely, the other two groups receiving 150 mg/kg TOFA and 300 mg/kg TOFA exhibited remarkably diminished neutrophil infiltration and reduced pathological severity. Interestingly, earlier studies [26] have also reported comparable effects of TOFA on CLP-induced acute lung injury in septic rat models, further supporting our findings. It can be inferred that TOFA administration plays a role in mitigating neutrophil infiltration at both concentrations. However, it is noteworthy that at the 300 mg/kg concentration, an unexpected thickening of the alveolar walls with dilated airspaces was observed. This increased alveolar thickness can potentially impair the diffusion capacity of gases between the bloodstream and air, leading to respiratory difficulties.

Comparatively, the CLP group exhibited signs of acute liver injury characterized by cytoplasmic degeneration, hepatocellular degeneration, and regeneration when contrasted with the control group. The liver possesses remarkable regenerative capabilities, allowing it to recover and repair following damage. However, in the CLP group, the extent of hepatocyte degeneration surpassed that of regeneration, indicative of liver damage. Additionally, the presence of a substantial influx of neutrophils in the CLP group corroborates the occurrence of infection, aligning with previous studies that elucidate the pathogenesis of liver injury in sepsis. Muftuoglu et al.'s histological findings have also underscored hepatocellular damage in CLP-induced septic models [34]. Conversely, in the 150 mg/kg TOFA group, reduced neutrophil infiltration into the liver sinusoidal cavities suggests a reduction in infection, possibly attributable to the influence of TOFA. However, at the 300 mg/kg TOFA concentration, a notable increase in hepatocellular apoptosis was observed. This phenomenon may be explained as a combined effect of sepsis and the potential toxicity of tofacitinib."

The spleen plays a pivotal role in bolstering phagocytosis and serves as a vital defense against infections in a healthy body. Individuals with compromised spleen function are notably more susceptible to developing fulminant sepsis, an ailment characterized by a high fatality rate [35]. Sepsis patients often exhibit significant apoptosis in spleen tissue, a detrimental occurrence that, if prevented, could potentially enhance survival rates [36]. In line with the findings of our current study, the splenic tissue of the CLP group displayed cytoplasmic degenera-



▶ **Fig. 9** Binding of inflammatory cytokines to the receptor leads to the dimerization of the receptor and phosphorylation of JAK. Activated JAK then phosphorylates the STAT and phosphorylated STAT then forms a dimer which enters to the nucleus in order to regulate gene expression by binding to the promoter.

tion, coupled with an indistinct separation of the red and white pulp, indicative of the sepsis-induced infection. Conversely, the 150 mg/kg TOFA group exhibited a clear demarcation between the red and white pulp, signifying TOFA's role in re-establishing the marginal zone and showcasing its protective function. However, within the 300 mg/kg TOFA group, there was a limited recovery of the marginal zone. Notably, aside from these adjustments, there was minimal observable tissue damage within the spleen.

The association between sepsis and acute kidney injury (AKI) has been thoroughly documented and extensively studied. Sepsis is linked to up to 50 % of AKI cases, and as many as 60 % of sepsis patients may concurrently experience AKI [37]. In our present study, we observed several effects indicative of AKI within the CLP group, including renal tubular dilation, tubular vacuolization, and glomerular atrophy. Renal tubular dilation can result from pathogenic processes that interfere with absorption and secretion. Furthermore, cytoplasmic vacuolization in renal tubules is generally associated with degeneration. Similar histological changes have been observed in a study by Tiwari et al. conducted on a C57BL/6 mice model of LPS-induced sepsis, where LPS induced tubular degeneration, mild brush-border loss, and vacuolization in the early segments of proximal tubules of the kidney [38]. Additionally, glomerular atrophy, possibly attributed to infection, was also noted within the CLP group. All these forms of tissue damage were notably reduced in the 150 mg/kg TOFA group, which exhibited a relatively normal renal architecture when compared to the CLP group. In stark contrast, the 300 mg/kg TOFA group displayed more severe tissue damage than the CLP group, suggesting that at this concentration, TOFA may induce kidney toxicity.

Upon comprehensive analysis of the results, it is evident that tofacitinib exhibits a protective effect against multiple organ damage at a concentration of 150 mg/kg. Nevertheless, at the higher dosage of 300 mg/kg, tofacitinib's toxicological impact on the liver and kidney was observed, rendering it unsuitable for further evaluation. Intriguingly, despite these observed toxic effects, no fatality was observed within the 300 mg/kg TOFA group, though the underlying explanation for this phenomenon remains unclear. Considering the results, it is quite difficult to say whether the toxicity is against septic models or in overall healthy mice. In order to claim that TOFA is toxic at 300 mg/kg concentration in sepsis models further investigation is required in comparison with mice only administered with TOFA without CLP surgery.

The molecular docking of tofacitinib against JAK1 and JAK3 shows that Tofa binding with JAK3 has two H-bonds, although JAK-1 only has one H-bond in the docked position and TOFA interacts with the DFG motif in both the JAK1 and JAK3 enzymes. DFG-motif is the activation loop (A-loop), a stretch of 20–30 residues, which serves as the regulator of kinase activities [39]. Thus, our molecular docking study indicates that TOFA's protective effects are due to its inhibitory interactions with JAK1 and JAK3, which regulate the JAK/STAT inflammatory signalling pathway and lower cytokine production by inhibiting JAKs [26].

The JAK-STAT signalling pathway holds immense significance in various pathological conditions such as cancer [40], immunological disorders [24], and infectious diseases like sepsis. Moreover, the association and the identification of the multiple organ failure in sepsis patients has been previously documented in a study [41]. Inflammatory cytokines (IL-6, IL-10, and TNF-α) activate the JAK/STAT pathway, initiating receptor dimerization and JAK phosphorylation. Subsequently, phosphorylated JAK in turn activates the STAT by adding phosphate to it and then two phosphorylated STAT binds to each other to form a dimer. This dimer translocates to the

nucleus, regulating gene expression (▶ **Fig. 9**). Targeting JAK receptors can limit JAK/STAT activation, rendering it a potential therapeutic strategy for autoimmune and inflammatory conditions, including sepsis [42].

The present study underscores the protective potential of TOFA at a concentration of 150 mg/kg, suggesting its ability to modulate the JAK/STAT signalling pathway. Future research employing immunohistochemistry and western blotting can offer deeper insights into CLP induced cytokine rise in sepsis and TOFA's role in reducing this cytokine production. Assessing cytokine expression levels will provide a more comprehensive understanding of TOFA's mechanism of action within the JAK/STAT signalling cascade during sepsis. Additionally, to evaluate TOFA's long-term effects and survival outcomes in this sepsis model, it is imperative to maintain and monitor the experimental mice over an extended period.

Conclusion

In conclusion, the results of this study showed that administering TOFA at a dosage of 150 mg/kg can effectively mitigate tissue damage in CLP induced septic mice. TOFA, on the other hand, has toxic effects in the liver and kidney at concentrations of 300 mg/kg. It was shown that TOFA plays a protective role in the multiple organ dysfunction in CLP-induced sepsis models. Given TOFA's proven status as an FDA-approved JAK inhibitor, these findings suggest that the drug is a good therapeutic candidate for the treatment of MODS in sepsis.

Ethics approval and consent to participate

Study is approved by the Departmental Ethical Committee.

CRediT Author Statement

Vaishnavi Singh: Experimentation, Methodology, Writing- Original draft preparation. Kavita Joshi: Experimentation, Methodology, Writing- Original draft preparation. Samit Chatterjee: Methodology, Reviewing and Editing. Sameer Qureshi: Data curation, Investigation. Snigdha Siddh: Data curation, Investigation. Vandana Nunia: Conceptualization, Supervision, Methodology.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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