Research Article

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Exploring the Mechanisms of Lipopolysaccharide-Induced Liver Damage in a Rat Model

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ABSTRACT

Liver injury is closely associated with changes in gut microbiota, leading to the release of bacterial endotoxins such as lipopolysaccharide (LPS). These endotoxins interact with toll-like receptors (TLRs), especially TLR4, expressed on liver immune cells. Although LPS is known to play a crucial role in liver pathogenesis, the progression of liver dysfunction following LPS exposure remains insufficiently explored. This study aimed to develop and characterize a rat model of LPS-induced liver injury. Animals were divided into five groups (n=6). Group I received saline (control), Group II received alcohol (1.24%, 0.4 ml), and Groups III–V were sensitized with increasing doses of LPS (1 µg, 10 µg, and 100 µg/rat, i.p.) for 10 days and challenged on day 21, with LPS (10 µg/rat, i.p.) which markedly increased the liver parameters. Biochemical parameters like SGPT, SGOT, ALP, bilirubin, total protein, globulin, and albumin were evaluated at multiple time points. Groups III and IV showed significant increased in AST, ALT and ALP level. The protein levels were significantly reduced in alcohol treated group. Bilirubin level was significantly increased in LPS treated and alcohol treated groups around 28th day of treatment, which indicates liver injury. This model demonstrates a reproducible method to study LPS-induced liver damage.

Keywords: Toll-like Receptors, Liver injury, TLR4, Lipopolysaccharide, Pathogen-associated molecular patterns

1. Introduction

The liver, particularly its resident macrophages known as Kupffer cells, plays a critical role in the innate immune response against bacteria and bacterial products that enter through the portal circulation. However, during liver injury, this immunological barrier is compromised. Endogenous gut-derived bacterial components, especially lipopolysaccharide (LPS), have been recognised as key contributors to the exacerbation of liver damage. LPS activates Kupffer cells, leading to the release of various proinflammatory cytokines, notably tumor necrosis factor-α (TNF-α), which have been implicated in the pathogenesis of hepatocyte injury. Recent studies suggest that this cytokine-mediated inflammatory response significantly contributes to the progression of liver injury, establishing a link between intestinal microbiota-derived products and hepatic inflammation [1].

TLRs are a family of pattern recognition receptors, expressed on immune cells and recognize various microorganism products including lipopolysaccharide, nucleic acid, carbohydrates, lipid, heat shock proteins etc. [2]. A total of 13 TLRs are identified till date. Out of 13, TLR4 has been reported to be present in almost all liver cells [3]. The liver, in its capacity as a regulator of innate immune responses, exerts the control through the modulation of Toll-Like Receptor (TLR) signalling, a phenomenon referred to as "Liver Tolerance". But when this tolerance mechanism is compromised, it can trigger inappropriate immune responses, which may subsequently lead to the onset of acute and chronic inflammatory liver diseases [3].

Lipopolysaccharide (LPS), a structural component of the outer membrane of Gram-negative bacteria, plays a pivotal role in mediating host immune responses. High-dose exposure to LPS—approaching lethal levels, such as those encountered during severe bacterial infections like colibacillosis—can induce significant liver injury. This is typically characterized by midzonal hepatocellular necrosis accompanied by an inflammatory response. In contrast, low-dose LPS exposure may trigger a minimal immune reaction and is generally non-injurious, lacking overt signs of inflammation [4,5].

LPS primarily targets hepatic macrophages—particularly Kupffer cells—through engagement with Toll-like receptors (TLRs), leading to the release of various inflammatory mediators. Among these interactions, the activation of TLRs on Kupffer cells by LPS is considered a central pathway in the development of liver injury. This process promotes the secretion of proinflammatory cytokines, which contribute to hepatocellular necrosis and tissue damage [6,7,8].

LPS also plays important roles in the activation of autophagy and release of damage-associated molecular patterns (DAMPs). DAMPs are released from injured or necrotic cells and act as ligands that can activate pattern-recognition receptors, such as TLRs, resulting in the activation of immune and inflammatory responses.12 TLRs are mainly expressed by macrophages and hepatocytes in the liver [9,10]

Figure 1 illustrates the pathological mechanisms by which lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls, contributes to hepatocellular dysfunction and thrombosis. Upon LPS exposure, Toll-like receptor 4 (TLR4) on monocytes, macrophages, and endothelial cells becomes activated, leading to the release of proinflammatory cytokines such as IL-1β, TNF-α, and IL-6. These cytokines stimulate neutrophils, which subsequently release neutrophil extracellular traps (NETs), enhancing platelet activation and promoting a prothrombotic state. Simultaneously, macrophages increase the expression of tissue factor (TF), which in combination with coagulation factor VII, accelerates thrombin generation and fibrin formation, contributing to clot development. Additionally, elevated PAI-1 and suppressed t-PA expression inhibit fibrinolysis,

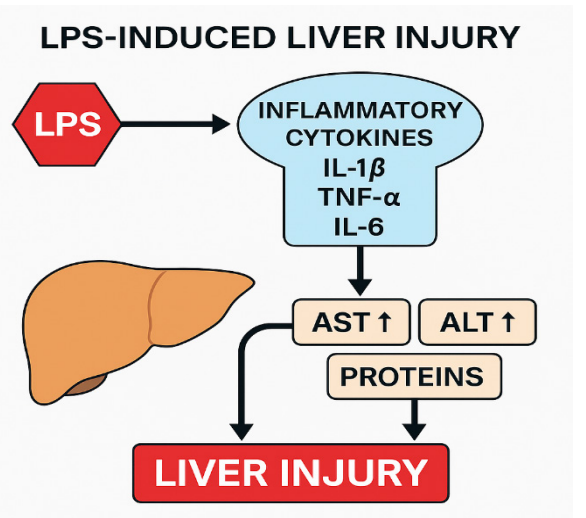

Figure 1. Mechanism of Lipopolysaccharide in liver injury [11]

further supporting thrombus formation. Endothelial dysfunction is also evident, marked by decreased expression of thrombomodulin (TM) and endothelial protein C receptor (EPCR), both of which play critical roles in anticoagulation. These processes collectively lead to reduced oxygen delivery to hepatocytes, aggravating tissue injury. Ultimately, the interplay of inflammation, coagulation, and impaired oxygenation culminates in severe hepatocellular dysfunction and systemic thrombosis, hallmarks of LPS-induced liver damage [11].

As per recent studies it has been reported that no studies describing the mutual interaction among macrophages, autophagy, and DAMPs in rat livers under the influence of LPS. Importantly, in mammals, the liver is exposed continuously to gut-derived LPS via the portal vein [12] therefore, exposure to a small amount of LPS may affect liver homeostasis. In order to clarify the interaction among macrophages, autophagy, and DAMPs in the biphasic functions (hepatocellular injury versus protection) of LPS, which may lead to liver homeostasis or diseases, the data were compared between rats treated with low-dose LPS and those treated with high-dose LPS.

2. Material and Methods

2.1. Materials

2.1.1. Drugs, kits and instruments

Lipopolysaccharide was purchased from Sigma Aldrich, New Delhi.

Kits for the determination of SGOT, SGPT, ALP, Total Protein, Albumin, and Bilirubin were purchased from Span Diagnostics Ltd., Surat, India

Cooling centrifuge - Remi instruments Mumbai, India was used to separate Serum or Plasma. and UV-visible spectrophotometer – Shimadzu, Japan.

2.2. Experimental protocol and procedure

2.2.1. Animals

Thirty healthy female Wistar rats having average body weight of 200-250 g were used in this study. Current experiment was approved by Institutional Animal Ethics Committee of the institute with protocol number KBIPER/11/276 dated 28 November 2011(KBIPER-K.B. Institute of Pharmaceutical Education and Research). The animals were housed in polypropylene cages at 25°C under 12 hrs dark/light cycle, with free access to a standard pellet diet and water ad libitum during the experiment.

2.2.2. Experimental design

Animals were divided into 5 groups of six animals each. Saline was injected intraperitoneally in group I animals for 10 days, while group II animals were received single dose of alcohol (1.24%, 0.4 ml) [13] orally which serve as model control. Group III, group IV and group V were sensitized with 1µg, 10µg, and 100µg dose per animal of lipopolysaccharide for 10 days intraperitonially [14]. On day 21, group III, group IV and group V animals were challenged with single dose of lipopolysaccharide (10µg/animal, i.p). SGOT, SGPT, ALP, Bilirubin, total protein, albumin, globulin, and ratio of AST to ALT were measured on day 0, 7, 14, 21, 28, 35. For validation, serum glucose, cholesterol, Hb gm%, total RBC, total WBC, bleeding time were measured on day 35.

2.2.3. Sample collection

Blood samples were collected via retro-orbital route into EDTA-containing and EDTA-free vials on days 0,7,14,21,28,35. Plasma was separated by centrifugation at 4000 rpm for 15 minutes using refrigerated centrifuge. Alanine transaminase (ALT), aspartate transaminase (AST), bilirubin, albumin and total protein were estimated from plasma. Alkaline phosphatase (ALP) activity was measured from serum. Blood samples collected in EDTA-containing vials were also used to determine hematological parameters.

2.2.4. Histopathological investigation

At the end of the experimental period, the animals were kept on overnight fasting. Subsequently, rats of each group were sacrificed and the liver was excised and washed with ice-cold saline. The excised liver tissue was fixed in 10 % buffered-formalin solution and sent to the Acutest laboratory, Ahmedabad, Gujarat (India) for histopathological analysis. Liver sections were stained using Haematoxylin and Eosin Stain (H and E Stain) and the extent of LPS-induced liver injury was evaluated by examining the morphological changes.

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Table 1. Experimental groups and treatment of animals

Group No.	Treatment	No. of Animals	Route of Administration
I	Normal control (Saline)	06	Intraperitoneal
II	Alcohol (1.25%, 0.4 ml) (Animal model control)	06	Orally
III	LPS (1µg/animal) followed by LPS (10µg/rat challenge)	06	Intraperitoneal
IV	LPS (10µg/animal) followed by (LPS 10µg/rat) challenge	06	Intraperitoneal
V	LPS (100µg/animal) followed by (LPS 10µg/rat) challenge	06	Intraperitoneal

2.2.5. Statistical analysis

Data were expressed as mean ± SEM. The data were analyzed by two-way ANOVA followed by Dunnet's Multiple Comparison Test using GraphPad Prism 10.5.0 trial version. A value of $p<0.05$ was considered statistically significant.

3. Results and Discussion

The present study was planned to investigate the effect of different doses of LPS on the liver injury. The injury was assessed by evaluating biochemical markers like AST, ALT, ALP, etc. (Figure 2) and histopathological (Figure 3) changes observed at different timepoints. These observations gave clear understanding of the liver injury by LPS. ALP level was markedly increase as compared to the treatment group treated with 10 and 100 µg LPS on day 7 and day 35. LPS could upregulates the ALP level by alleviated expressions of TLR-4 and other inflammatory mediators like TNF-α and interleukins. This results in to the damage of hepatocytes in the liver [15]. However, the chronic single dose of alcohol treated group showed non-significant increase in ALP level. AST activity was significantly increased from day 21 to 35 in both 10 µg & 100 µg LPS treated groups. Study shows that LPS can induce endotoxemia which releases of nitric oxide. The production of nitric oxide seems to be dose dependent and the increase in nitric oxide level may rise the AST level. The present finding supports the previous findings of the liver injury [16]. ALT level was significantly increase on day 35 in the rats treated with 100 µg LPS. The LPS elevates the proinflammatory cytokine factor tumor necrosis factor (TNF-α) and also upregulates the levels of inflammasomes like Nalp 1 and Nalp 3. This upregulation damages the hepatocyte so the level of ALT elevates significantly which indicates the liver injury [17]. AST/ALT ratio is considered as a clue to understand the alcoholic and non-alcoholic liver injury. Generally, the ratio greater or equal to 2 is considered as alcoholic liver damage and more than 1 is considered to be non-alcoholic liver injury. In the present work, LPS along with alcohol markedly increased the ratio [18]. Due to liver injury, the AST level is increased and ALT level is decreased which results in elevated AST/ALT ratio. Alcohol damages hepatocyte mitochondria which exhibits more AST activity than hepatocyte cytosol. This unlikely, increase the serum AST level which is confirmed in the present study. Another justification is given that liver injury damages hepatocytes and also ALT levels in the cells which in turn raises the AST/ALT ratio [19]. Total protein, albumin and globulin levels were significantly changed in the LPS and alcohol treated groups. Alcohol increases the permeability of the LPS in the bloodstream by disrupting the tight junctions of the intestine. Upon reaching in the liver, LPS activates the Kupffer's cells by bind with TLR4 and CD14. Activated Kupffer's cells releases the inflammatory mediators like IL-6, TNF-α. These inflammatory mediators can damage the liver cells and decreases the total protein and albumin levels [20-22]. Bilirubin is considered as a crucial biomarker in the liver injury. Elevated bilirubin level clearly indicates the chronic liver damage because the bilirubin is a byproduct of hemoglobin metabolism. If the liver is damaged, release of bilirubin in the bloodstream that reflects the liver injury [23]. Liver histology of alcohol treated and LPS treated groups give clear indication of the liver injury. Alcohol treated

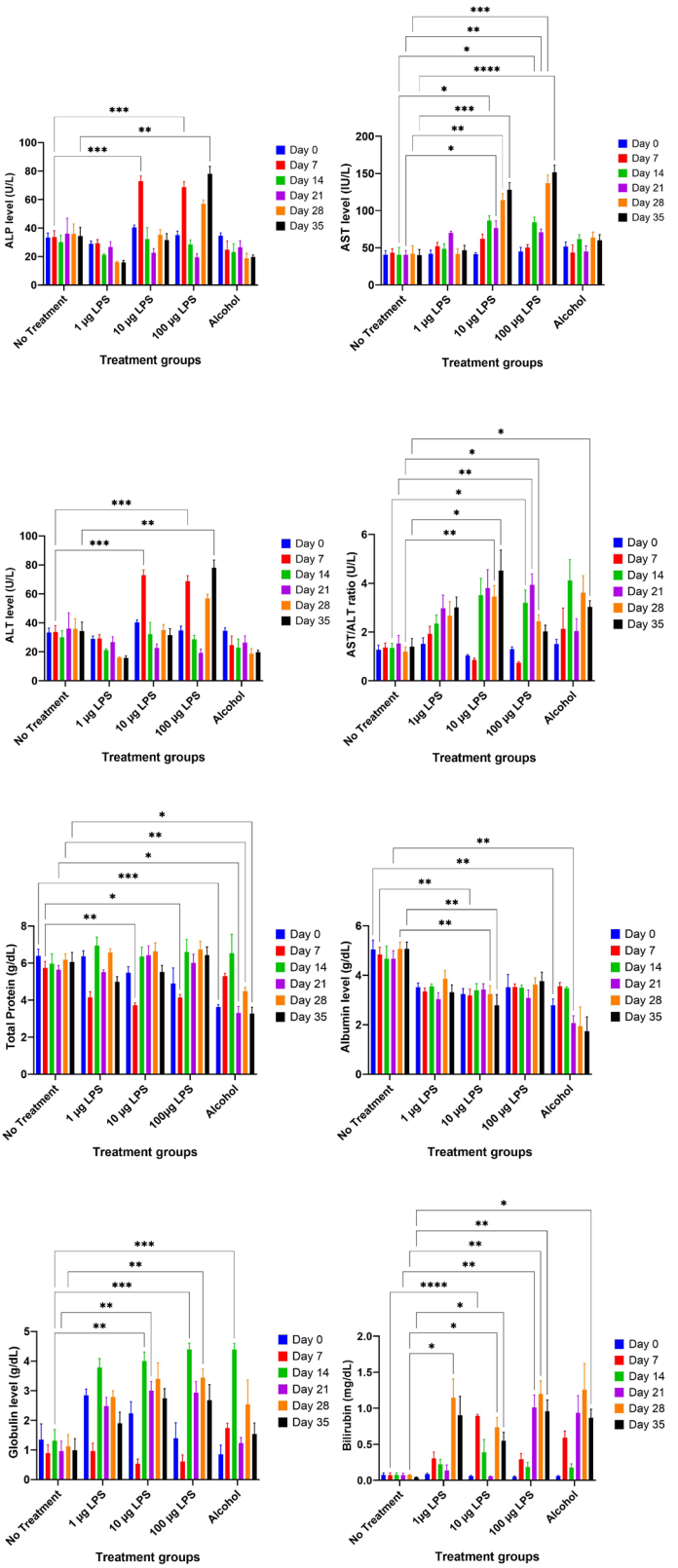

Figure 2. Change in various biochemical parameters in various treatment groups of rats given the different treatment. Data were expressed as mean ± SEM.
*: p-value < 0.05; **: p-value < 0.01; ***: p-value < 0.001; ****: p-value < 0.0001

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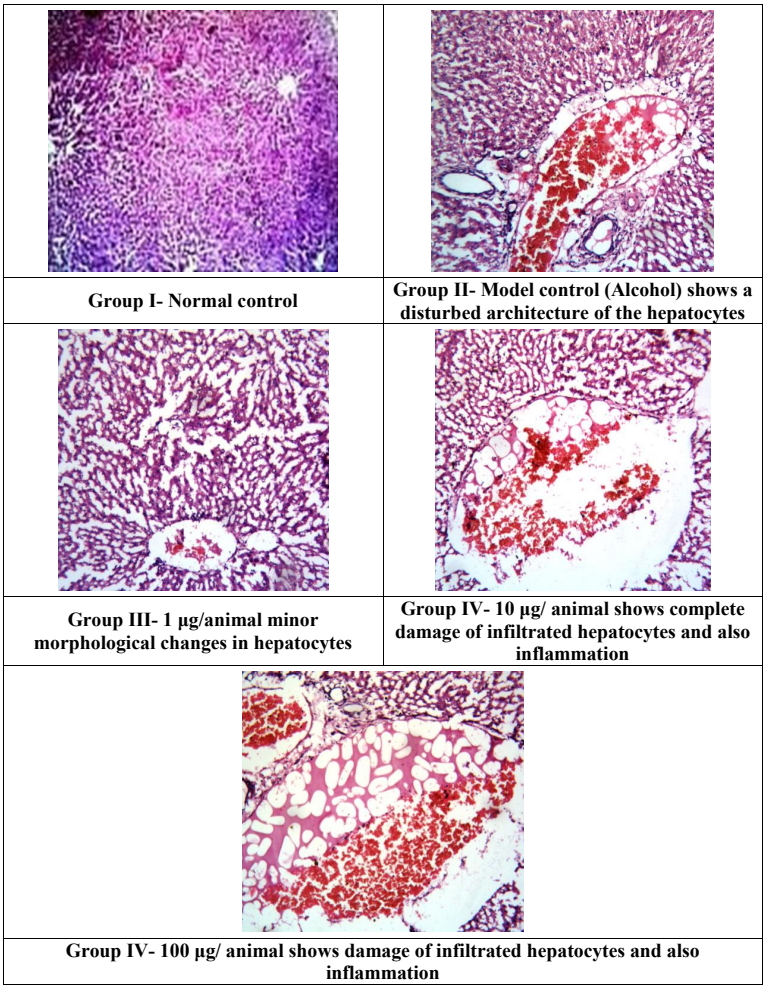

Figure 3. Histopathological study of the various groups of rats given with the different drugs. (1, 10 and 100 µg LPS, alcohol, and normal saline)

group showed disturbed architect of the hepatocytes and infiltration of the cellular structure or signs of hemorrhage. LPS also showed the dose-depended liver injury in the present findings. The severity of the hepatocyte infiltration, damage of hepatic cord and hepatocellular degeneration. The finding clearly supports the chronic liver injury at cellular level by LPS and alcohol [24].

4. Conclusion

Current experiment was approved by Institutional Animal Ethics Committee of the institute with protocol number KBIPER/11/276 dated 28 November 2011(KBIPER-K.B. Institute of Pharmaceutical Education and Research). Based on the results and discussion, it can be concluded that, lipopolysaccharide has potential to cause chronic liver injury and may involve TLR4 in pathogenesis of liver failure. The proposed animal model of chronic liver injury appears to be suitable for screening drugs intended for the treatment of liver failure and related disorders.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships

that could have appeared to influence the work reported in this paper.

Statement of Contribution of Researchers

Concept- G.S.M.C., G.S.; Design- G.S., M.C.; Supervision- G.S., M.C.; Resources- G.S., GS, M.C.; Materials – G.S., G.S., M.C.; Analysis and/or Interpretation – G.S., P.B.; Literature Search –G.S., M.C. P.B.; Writing –G.S.; Manuscript review and suggested changes in manuscript – G.S., P.B.

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