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Research Paper: The Anti-Inflammatory and Antioxidant Activity of Aspirin in Septic Animal Models

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ABSTRACT

Background: Sepsis is a systemic body reaction to invasive microorganisms, such as bacteria and fungi. Furthermore, it is one of the top ten main causes of death among all patients admitted to the hospital. Multiple potential drug therapies have been investigated in this area; however, an effective pharmacotherapy for sepsis remains undiscovered. Therefore, we explored the effect of Aspirin or Acetylsalicylic Acid (ASA) on the treatment outcomes and reduction of sepsis complications concerning the parameters involved in the oxidative damage of liver tissue. To perform an in vivo experiment, an experimental inflammatory model Cecal Ligation and Puncture (CLP) was performed in rats.

Methods: The investigated rats were divided into 4 groups (n=40), as follows: 1. Controls; 2. Laparotomy (LAP) group; 3. CLP group; and 4. The treatment group with aspirin 2 mg/kg bw for 48 h after CLP induction. Then, the explored rats were anesthetized and blood samples were collected from their hearts. Next, the animals were sacrificed and the liver tissue was separated for histopathologic and biochemical studies.

Results: The obtained data suggested that the treatment of animals with aspirin was effective in adjusting the antioxidant and inflammatory parameters. Pathological studies also indicated that sepsis led to injuries in the liver tissues, which could be improved by interventions.

Conclusion: In conclusion, sepsis caused oxidative damage in the liver tissue, and using aspirin was effective in preventing and improving these injuries.

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1. Introduction

The liver is a multifunctional organ with diverse metabolic activities. It plays a central role during sepsis and concerning the regulation of immune defense during systemic infections. Such a target is achieved by some mechanisms, such as bacterial clearance, cytokine production, and metabolic adaptation to inflammation. Additionally, the liver is a target for sepsis-related injury in critically ill patients admitted to the Intensive Care Unit (ICU). Sepsis is characterized by life-threatening organ dysfunction induced by a dysregulated host response to infection [1, 2]. It is an essential medical problem globally, as the most frequent cause of death among critically ill patients [3].

There exist some models for the induction of sepsis, such as Cecal Ligation and Puncture (CLP), polymicrobial model, Colon Ascendents Stent Peritonitis (CASP), and bolus injection models Lipopolysaccharide (LPS) or bacteria) [4]. However, among all models, the CLP method is a surgery contributing to polymicrobial peritonitis and a golden standard model for inducing sepsis [5]. A reason for the widespread use of CLP is its versatility. Besides, it is possible to tailor the severity of sepsis to use the CLP model for investigating acute as well as chronic sepsis [6].

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are prevalently used for treating pain and fever; however, their impact during bacterial infections remains controversial. Aspirin or Acetylsalicylic Acid (ASA) is a non-selective inhibitor of Cyclooxygenase Enzyme (COX), i.e., subject to significant first-pass metabolism; most of its action occurs in the portal circulation of the liver [7-9].

Considering the importance of the liver as a modifier and target of sepsis and in drug metabolism, this study aimed to report the effect of aspirin on sepsis-induced liver injury.

2. Materials and Methods

Male Wistar rats (Mean±SD body weight= 150±30 g) were obtained from the Pasteur Institute, Iran. The animals were maintained in a controlled environment (12/12 h light/dark cycle with a temperature of 20° C -25° C, & a relative humidity of 50%). Water and food were provided to the rats ad libitum. Animal experiments were performed according to the Ethics Committee and Institutional Animal Care guidelines. The investigated rats were divided into 4 groups, including control, Laparotomy (LAP), CLP, and CLP surgery with aspirin treatment (orally at doses of 2 mg/kg bw) groups. For CLP surgery, the explored rats were anesthetized, a 2-3 cm of abdominal incision was made, and the cecum was exposed.

The ligated portion of the cecum was punctured twice with a 20-gauge needle [10]. In the LAP group, the cecum was exposed, manipulated, and returned to the peritoneal cavity without being punctured. After 48 h, blood samples were collected by heart puncture and centrifuged at 3000 g for 10 min. Accordingly, the liver samples were transferred to ice-cold containers and homogenized in phosphate buffer (100 mM, pH 7.0). Then, the obtained samples were used to measure the biochemical parameters.

Malondialdehyde (MDA) level was measured spectrophotometrically based on the procedure described by Buege and Aust [11]. The Glutathione (GSH) content was expressed as nmol/mg protein according to the procedure of Ellman [12]. Tissue Myeloperoxidase (MPO) activity was evaluated according to Bradley et al.'s procedure [13]. The activities of Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), and Bilirubin were determined in serum according to the protocol of Pars Azmoon kits (Pars Azmoon Co, Iran).

To determine Cyclooxygenase-2 (COX-2) gene expression, total Ribonucleic Acid (RNA) was isolated from the liver tissues by the RNA total kit (GeneAll, Korea). Moreover, complementary DNA (cDNA) was conducted with the PrimeScript TM RT reagent kit (Takara Bio Inc, Japan) and oligo dt primers (Takara Bio Inc, Japan). Real-time Polymerase Chain Reaction (PCR) was performed using SYBR® Green Master Mix (Amplicon) by real-time PCR System (Rotor-Gene Q-QIAGEN). The specific primer is presented in Table 1. The threshold cycle (Ct) was calculated and each Messenger RNA (mRNA) expression value was normalized against a housekeeping gene Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH). The fold change was calculated by the formula $2^{-\Delta\Delta Ct}$.

For the histopathological assay, the liver sections were stained with hematoxylin and eosin and were analyzed using a light microscope (Olympus CX31RBSF).

The analyzed data were presented as the mean and standard error of mean and the statistical significance level was set at $P<0.05$.

3. Results

In Tables 2 and 3, the levels of oxidative enzyme MDA and MPO activity, liver enzymes AST and ALT were markedly increased in the CLP and LAP groups, compared with the controls (P<0.05). Furthermore, the levels of GSH diminished in CLP and LAP groups, compared to the control group (P<0.05). The 2 mg/kg bw aspirin dose inhibited MDA, MPO, and GSH levels, as well as AST and ALT liver enzymes, in comparison to the CLP group. On the other hand, ALP and Bilirubin levels demonstrated no comparable changes in all groups even after being treated with Aspirin (P>0.05) (Table 1).

Moreover, COX-2 level increased in the CLP group (Figure 1), compared with the control and LAP groups. The administration of Aspirin to rats significantly decreased COX-2 gene expression in them (P<0.05) (Table 1).

Histopathological findings revealed a mild infiltration of neutrophils in the liver parenchyma and portal tracts in the LAP group (Figure 2). However, in the CLP group, severe congestion, the infiltration of neutrophils in the liver parenchyma, and necrotic hepatocytes with pyknotic or karyolytic nuclei were observed (Figure 2).

4. Discussion

Through influencing cellular integrity with a mechanism of ROS, oxidative stress significantly impacts sepsis. Such a process involves the interactions between subcellular membranes and unsaturated fatty acids of cellular [14]. This leads to peroxidation of Subsequently, membrane lipids are peroxidated, and highly-cytotoxic products, including MDA, are released which eventually cause cytotoxicity. This mechanism is among the fundamental models of free radicals-induced tissue damage [15]. The process of lipid peroxidation accompanied by excess production of MDA has been well documented in sepsis; it could lead to the mortality of affected subjects [16].

Additionally, upon oxidative stress activation, neutrophils produce various ROS via Myeloperoxidase (MPO) that catalyze the H_2O_2-dependent formation of Hypochlorous Acid (HClO). H_2O_2 generates the hydroxyl radical (HO·) that could directly cause Lipid Peroxidation (LP) [17, 18]. Besides, the hepatocellular liver enzymes AST and ALT have been regarded as markers of liver injury [19]. Our collected data (Table 3) demonstrated that sepsis increased liver enzymes caused by liver damage. These data together with the histological findings (Figure 2) supported the pathophysiological alternations in the liver tissue. Toscano et al. [20] also documented

Table 1. The primers used in the current assay

Primers	Sequence (5′ 3′)	Product Length
COX2 forward	ACCTCTGCGATGCTCTTC	188 bp
COX2 reverse	AGGAATCTCGGCGTAGTAC	
GAPDH forward	TGCCAGCCTCGTCTCATAG	197 bp
GAPDH reverse	ACTGTGCCGTTGAACTTGC	

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Table 2. Biochemical markers in the liver tissue

Groups	Mean±SD		
	MDA	GSH	MPO
Control	7.23±0.78	14.33±0.75	6.32±0.3
LAP	10.48±1[a]	11.42±0.58[a]	9.43±0.6[a]
CLP	17.51±1.28[b]	7.21±0.67[b]	21.14±0.7[b]
CLP+ASA	11.23±0.45[c]	11.23±0.8[c]	8.25±0.3[c]

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a. P<0.05 is considered as significantly different between the control and LAP groups; b. P<0.05 is considered as significantly different between the LAP and CLP groups; c. P<0.05 is considered as significantly different between the CLP and CLP+aspirin (ASA) groups.

Table 3. The liver enzymes

Group	Mean±SD			
	AST	ALT	ALP	Bilirubin
Control	94±8.6	53±6.17	32±0.2	0.51±0.02
LAP	120±9.58[a]	76±5.35[a]	37±0.3	0.54±0.03
CLP	217±13.58[b]	136±8.76[b]	39±0.3	0.6±0.02
CLP+ASA	131±8.76[c]	70±4.58[c]	38±0.3	0.58±0.03

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a. P<0.05 is considered as significantly different between the control and LAP groups; b. P<0.05 is considered as significantly different between the LAP and CLP groups; c. P<0.05 is considered as significantly different between the CLP and CLP+aspirin (ASA) groups.

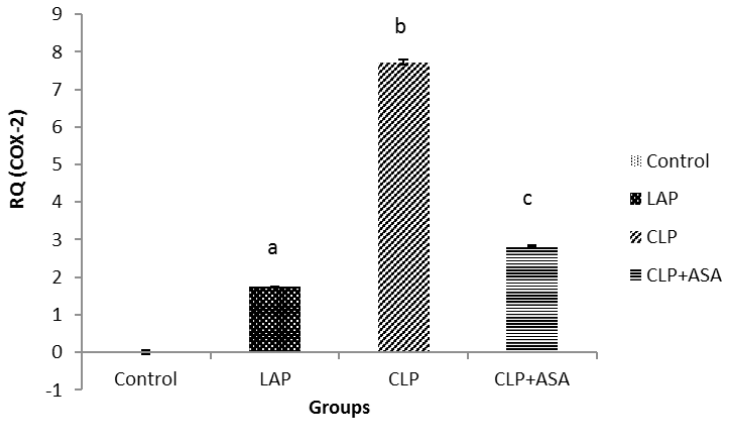

Figure 1. The gene expression of COX-2

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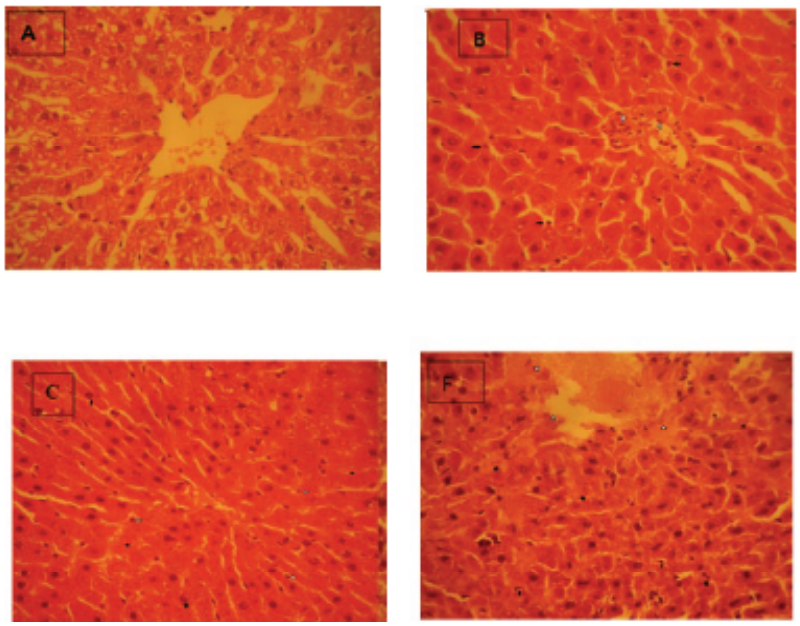

Figure 2. Histopathological findings

A. Control group; B. LAP group; C. CLP groups; F. CLP+ASA group; H&E. 400*

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that the oxidative stress conditions in sepsis are directly related to organ damage and injuries in CLP models.

Moreover, the present research findings suggested that oxidative stress and proinflammatory response contribute to the liver injury that occurs in sepsis. Studies indicated that sepsis could create oxygen-free radicals. The radicals cause oxidative stress and multi-organ failure, such as liver and lung impairments [21, 22]. A study reported that CLP could alter antioxidant enzymes and increase PGE2 and COX-2, as an inflammatory factor 24 h after CLP. COX-2 impacts inflammatory responses which could initiate oxidative damage on tissue [23]. Furthermore, the achieved results were consistent with those of Rasooli and associates [10]. This study suggested that CLP could change the gene expression of CD177 and MPO, as an inflammatory factor.

Additionally, Aspirin, in the dose of 2 mg/kg bw could protect the liver by restoring the ideal concentration of inflammatory factor and antioxidant parameter. Floyd and Ferro [9] expressed that Aspirin could manipulate the processes in sepsis by the inhibition of COX. Another study demonstrated that Aspirin was effective in the murine models of sepsis with Salmonella enteritidis endotoxin pre-treatment [24]. Our results were supported by histopathological findings (Figure 2).

5. Conclusion

In conclusion, CLP caused oxidative and pro-inflammatory insult in the liver to aggravate the survival rate and mean survival time. Aspirin reduced the oxidative stress by reversing the lipid peroxidation, MPO, and GSH levels, as well as COX-2 gene expression, as an inflammatory factor. Thus, Aspirin could be of potential use for sepsis treatment to improve liver injury.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee and the Research Council of the University of Medical Sciences Ethics Code: IR.MAZUMS.REC.95.2509).

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Authors' contributions

All authors were equally contributed in preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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