



DMF Ameliorate Myocardial Damage in Rat Model of Polymicrobial Sepsis Induced by CLP

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Abstract: Sepsis leads to life-threatening multiorgan failure, representing a serious healthcare problem with significant morbidity and mortality rates worldwide. Cecal ligation and puncture (CLP) in rodents is one of the most widely used models for experimental polymicrobial sepsis. In this study, we investigate the role of dimethyl fumarate (DMF), an FDA-approved anti-oxidative and anti-inflammatory agent, in mitigating the devastating consequences of sepsis on heart tissues. Four groups of rats were used in this work: the sham group (no sepsis), the control group (CLP only), the solvent group (DMSO i.p. injected before CLP), and the treatment group (DMF i.p. injected before CLP). Our data revealed that DMF lowered the pro-inflammatory markers NF- κ B, TNF- α , and TLRs, compared to the solvent-only group or the control group. Histopathological examination of the heart tissues revealed favorable tissue integrity in the DMF-pretreated group compared to the solvent-only group or the control group. The results suggest that DMF could potentially lower the risk of heart tissue damage due to sepsis.

Key Words: sepsis, cecal ligation and puncture, dimethyl fumarate, inflammation, heart tissue damage, polymicrobial sepsis, NF- κ B

I. INTRODUCTION

Sepsis is the result of systemic infection which leads to malfunction of the immune system, septic shock, and finally death. Sepsis leads to life-threatening organ failure, and it represents a serious healthcare problem with significant morbidity and mortality rates worldwide. Therefore, researchers have aimed at developing models for studying sepsis and trying different approaches to prevent or limit the occurrence and progression of sepsis [1]. Cecal ligation and puncture (CLP) in rodents is one of the most widely used models for experimental polymicrobial sepsis as the cecum contains different types of microbes that live in a symbiotic relationship with the human's gut. Perforation of the cecum allows microbes from the gut to be translocated into the blood compartment. When the CLP model is used in rodents, they show disease patterns with typical symptoms of sepsis [1], [2]. In this study, we investigate the role of dimethyl fumarate, an FDA approved anti-oxidative and anti-inflammatory agent, in the protection given to rats with CLP-induced sepsis.

II. MATERIALS AND METHODS

A. MATERIALS

Dimethyl fumarate (DMF) (Cat No. 242926) was purchased from Sigma Aldrich, Germany. DMF was dissolved in a stock solution (29 mg / 1 ml) of DMSO/water [3], [4].

B. ANIMALS

Sixteen mature Swiss albino male rats weighing 180-200 grams and with an average age of 8-12 weeks were purchased from the Animal Shelter in the Science's College at Kerbala University, Iraq. The rats were maintained in the animal shelter at Al-Zahrawi University College, Iraq, at 25°C with a humidity of 60-65% and a 12-hour light:dark cycle.

C. STUDY DESIGN

The study was carried out at the Al-Zahrawi University College Department of Pharmacy, as well as the Middle Euphrates Unit for Cancer Research, Iraq. The rats were divided into four groups (n=6 per group):

- 1) Sham group (Sham): Rats were anesthetized and underwent laparotomy surgery but did not have CLP.
- 2) Sepsis (CLP) group: Rats were operated on with CLP.
- 3) Vehicle group (Vehicle): Rats received dimethyl sulfoxide (DMSO) intraperitoneally (i.p.) half an hour before CLP treatment.

- 4) Dimethyl fumarate group (DMF): Rats received DMF (50 mg/Kg) i.p. half an hour prior to CLP.

D. EXPERIMENTAL PROCEDURE

In brief, rats have been anesthetized i.p. with 100 mg/kg ketamine and 10 mg xylazine. Incision (1.5 cm) in the midline was used for abdominal laparotomy, and the cecum was exposed. The cecum was ligated right below the ileocecal valve then doubly punctured with a 22-gauge needle. The ligated cecum was gently pushed to expel some of the feces material it was placed back into the abdominal cavity. The abdomen has been then sutured with a 5/0 surgical suture size [1]. Rats were returned to their cages with unlimited access to water and food and they were checked every 4 hours for 24 hours with [2]. After 24 hr, the animals anesthetized, sacrificed, and the samples heart was harvested. The heart was divided into two pieces, rinsed with 0.9% saline, and placed in a 0.9% NaCl or 10% formalin and stored at -20 °C until further analysis.

E. TISSUE PREPARATION FOR NF- κ B P65 MEASUREMENT

The heart tissues were cut into very small pieces under cold conditions, followed by homogenization with a homogenization solution containing PBS, protease inhibitor cocktail, and Triton X-100 for 20 min (5s each time) using a high-intensity ultrasonic liquid processor at 4 °C. The mixture was then centrifuged at 2,000 - 3,000 r.p.m for 20 min at 4°C and stored at -80°C until analysis [5].

F. MEASUREMENT LEVELS OF TLR2 AND TLR4 THROUGH IMMUNOHISTOCHEMISTRY TECHNIQUE

The heart tissues obtained from the groups were collected to count the cells labeled with TLR2 and TLR4, HO-1 antibodies according to the manufacturer's protocol (see Table 1). The immunohistopathological scoring scale was calculated according to the following equation [6]:

$$QQ = P \times I,$$

where Q is the quick score, P is the percentage of positive cells, and I is the intensity.

G. TISSUE SAMPLE PREPARATION FOR HISTOPATHOLOGY

To eliminate red blood cells or clots, heart tissues obtained following rat sacrifice were rinsed with cold isotonic sodium chloride solution (0.9%). The tissue was then fixed in a 10% formalin solution and processed into paraffin blocks. Following fixation, the specimens were dehydrated by immersing them in ethanol for two hours each (70%, 80%, 90%, and 100%) to remove any remaining formalin or H₂O from the samples. The heart tissues were then cleaned with xylene to remove alcohol before embedding in paraffin wax [7]. Histological sections from all groups were evaluated to semi-quantify the differences in heart damage. The histopathology

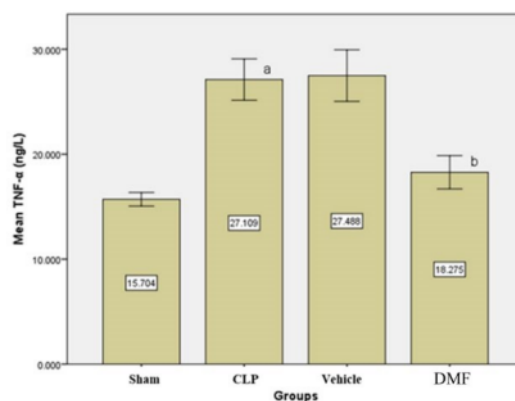


FIGURE 1: TNF- α (ng/L) Mean \pm SEM level in heart tissue in the groups; sham vs. sepsis (pvalue=0.00001), dimethyl fumarate (DMF) vs. sepsis (CLP) or vehicle (DMSO) (p-value=0.0001)

examination was carried out at magnifications ranging from X100 to X400 (13) and assessed through the following score of tissue damage: (0) normal architecture, (1) mild: 25% damage, (2) moderate: 25-50% damage, (3) severe: 50-75% damage, and (4) highly severe: 75-100% damage.

III. STATISTICAL ANALYSIS

For statistical analysis, SPSS version 26 was used. The data has been presented as Mean \pm Standard Error of the Mean (SEM). For multiple group comparisons, ANOVA was used, followed by a post-hoc test with Bonferroni correction. The Kruskal-Wallis test was performed to examine if the statistical difference between the groups was significant as the mean score for histological alterations in heart tissue. A p-value of 0.05 was considered statistically significant.

IV. RESULTS

A. DIMETHYL FUMARATE REDUCES INFLAMMATORY TNF- α AND NF- κ B MARKERS IN HEART TISSUE

The sepsis group (CLP only) and vehicle group (DMSO) showed significantly higher TNF- α and NF- κ B levels compared to the sham group. TNF- α and NF- κ B levels in the heart tissues were significantly lower in the dimethyl fumarate group (DMF) compared to the CLP group or the vehicle group (Figures 1 and 2).

B. DIMETHYL FUMARATE LOWERED TLRs MARKER IN HEART TISSUE

The TLRs levels in the heart tissues were significantly increased ($p < 0.05$) in the sepsis (CLP) group compared to the sham group. When comparing with the sepsis group, the DMF pre-treated group showed a significantly lower TLRs expression (Figure 3).

Score	0	1+	2+	3+	4+
Positive Cells	<10%	10-25%	25-50%	50-75%	>75%
Score	1	2	3		
Intensity of Staining	weak staining	moderate staining	strong staining		

TABLE 1: The heart tissues obtained from groups

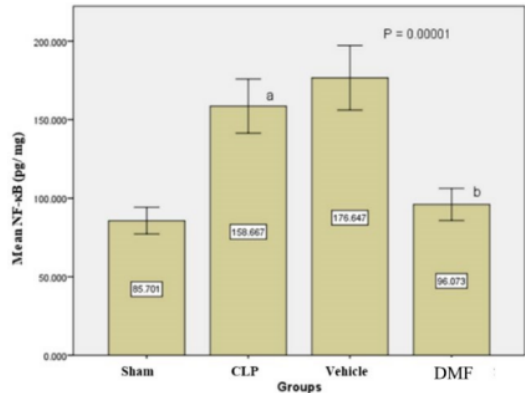


FIGURE 2: NF-κB (pg/mg) Mean ± SEM level in heart tissue in the groups; in comparing sham vs. sepsis (p-value = 0.00001), dimethyl fumarate (DMF) vs. sepsis (CLP) or vehicle (DMSO) (p-value = 0.0001)

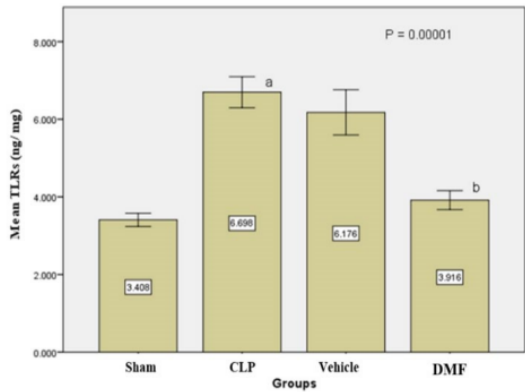


FIGURE 3: TLRs (ng/mg) Mean ± SEM level in the heart tissue in the groups; sham vs. sepsis (pvalue=0.00001), DMF vs. sepsis or vehicle (p-value=0.0001)

C. DIMETHYL FUMARATE MINIMIZED HEART INJURY

Histopathological examination was used to evaluate the integrity of the heart tissue architecture. Normal heart tissue histology was observed in the sham group. In contrast, the heart tissues of the sepsis (CLP) and vehicle (DMSO) groups showed the development of congestion, perivascular inflammation, interstitial edema, and localized extravasations of RBCs. The DMF pre-treated group exhibited moderate architectural alterations in comparison with the sham group (Figure 4).

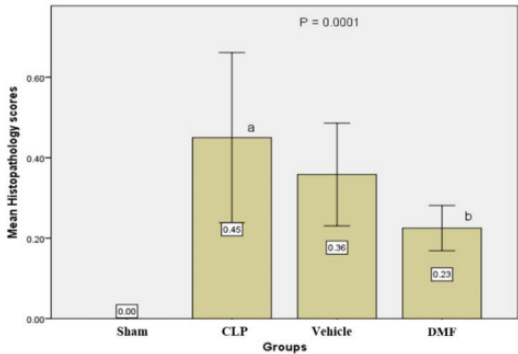


FIGURE 4: Histopathological Score of the four test groups. Sham vs. sepsis (CLP) and vehicle (DMSO) (pvalue=0.0001), DMF vs. sepsis (CLP) (p-value=0.0001), DMF vs. sepsis (p-value=0.0001)

V. DISCUSSION

The onset of cardiac dysfunction is one of the deadliest effects of sepsis, and the degree of myocardial damage is a critical indicator of survival in sepsis patients [8]. In intensive care units (ICUs) both at home and abroad, the mortality rate of sepsis and associated comorbidities has been steadily rising in recent years.

According to statistics, there are annually more than 600,000 sepsis cases in North America, with a 30-50% fatality rate. Sepsis is also prevalent in ICUs in China, consuming a significant amount of medical resources and often leading to unfavorable clinical outcomes [9]. Sepsis, a major cause of death, results from multiple-organ failure due to immune system dysfunction. The urgent need to develop and implement therapies to protect against or mitigate the impact of septicemia is evident. The cecal ligation and puncture (CLP) procedure has been the gold standard for creating a multi-microbial sepsis model. In our study, we evaluated the potential of DMF (an antioxidative and anti-inflammatory agent) to protect heart tissues in a CLP rat model. Our findings indicate that, as anticipated, CLP led to an increase in the production of TNF-α and NF-κB, resulting in severe tissue damage [10], [11].

We observed that pre-administration of DMF significantly reduced the levels of the pro-inflammatory markers TNF-α and NF-κB, leading to improved tissue integrity overall. Our results suggest that DMF has the potential to reduce the severity of heart tissue damage following sepsis.

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