



Original Article



The Effect of Ferulic Acid on Inflammation in a Myocardial Ischemia-Reperfusion Experimental Model

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ABSTRACT

Cardiovascular disease is a leading cause of death globally, with ischemia-reperfusion injury (IRI) causing tissue damage through oxidative stress and inflammation. Interleukin-6 (IL-6) plays a role in IRI-related myocardial damage. Ferulic acid, a plant-derived phenolic compound, shows potential as an anti-inflammatory and antioxidant therapy to reduce IRI. **Objectives:** To investigate the dose-dependent protective effect of ferulic acid in an experimental myocardial ischemia-reperfusion injury model, focusing on its impact on serum IL-6 levels. **Methods:** This experimental study was conducted over 6 months by the Department of Pharmacology at Rehman College of Dentistry, Peshawar, Pakistan, utilizing healthy male Sprague Dawley rats weighing 300-350 grams (g). Rats showing signs of illness were excluded from the study. Twenty rats were randomly allocated to control (n=10) and treatment (n=10) groups. Preceding the induction of ischemia-reperfusion injury, the treatment group was administered oral ferulic acid for 15 days; controls were untreated. Data were analyzed using SPSS version 23.0. Results are presented as mean \pm standard deviation, and the mean difference was compared by an independent sample t-test, taking $p < 0.05$. **Results:** IL-6 levels in serum were measured using ELISA, with control rats showing higher IL-6 (66-80 ng/l) than ferulic acid-treated rats (46-55 ng/l). There was a significant reduction in IL-6 levels in the treated group. This indicates ferulic acid lowers inflammation in myocardial ischemia-reperfusion injury. **Conclusions:** It was concluded that the ability to lower the IL-6 level and modulate oxidative-inflammatory pathways, ferulic acid can be a promising therapeutic agent to attenuate myocardial inflammatory responses and reperfusion injury.

INTRODUCTION

Cardiovascular disease (CVD) is a major cause of death in Southeast Asia as well as globally [1,2]. Myocardial infarction (MI), which afflicts over a million people per year in the United States alone [3], frequently results in ischemia-reperfusion injury (IRI) an unexpected tissue injury caused by the reinstatement of blood flow to ischemic myocardium [4, 5]. IRI is a complex process in which oxidative stress, mitochondrial injury, calcium overload, apoptosis, and substantial inflammation are

implicated [6, 7]. Inflammation is closely involved in the process of IRI. For pro-inflammatory cytokines, interleukin-6 (IL-6) is particularly relevant because of its association with myocardial dysfunction, infarct expansion, and adverse remodeling [8-10]. Increased concentration of IL-6 has been associated with acute coronary syndromes, heart failure, and poor cardiac outcomes [11]. In addition, IL-6 stimulates C-reactive protein (CRP) production and thus exacerbates the



inflammatory cascade [12]. Modulation of the inflammatory response during reperfusion is important for enhancing recovery of the heart. Novel therapies now include anti-inflammatory and antioxidant treatments to alleviate IRI [13]. Of these, ferulic acid (FA) has demonstrated potential as a phenolic compound found in plant sources such as rice bran, sweet corn, and tomatoes. FA has a significant antioxidant and anti-inflammatory capacity against free radicals, initial inhibition of NF- κ B, and reduced expression of IL-6, TNF- α , and COX-2 [14, 15]. Along with the antioxidant effects of Ferulic Acid, FA also provides neuroprotective, hepatoprotective, cardioprotective, and antidiabetic actions, hence making it a pleiotropic compound as compared to more narrowly acting phenolics [16].

This study aims to investigate the dose-dependent protective effect of ferulic acid in an experimental myocardial ischemia-reperfusion injury model, focusing specifically on its impact on serum IL-6 levels using a rat ELISA assay.

METHODS

This experimental study was conducted at the Department of Pharmacology, Rehman College of Dentistry, Rehman Medical Institute, Peshawar, Pakistan. All experimental procedures were conducted in compliance with institutional ethical guidelines, and ethical consent was obtained from the Institutional Review Board of Rehman Medical College of Dentistry, Peshawar, Pakistan (Ref. No. RCD-09-22-1135). The study was conducted over six months, from January to June 2023. The first two months were dedicated to randomization and intervention, followed by the next two months, during which intervention and laboratory analysis were carried out alongside preliminary statistical analysis. The fifth and sixth months were focused on data handling, detailed statistical analysis, and preparation of the final report. This study consisted of 2 major experimental phases that included the dose-response activity of ferulic acid as well as its protective effect against myocardial ischemia/reperfusion in rats. Male Sprague Dawley (SD) rats (300–350 g) were used in the present study. The healthy adult male rats that were negative for infection or pre-existing disease were selected as the inclusion criteria. Rats that displayed altered behavior, including signs of disease or body weight outside the ideal range, were not included in the study. Ferulic acid and Rat IL-6 Enzyme-linked immunosorbent assay (ELISA) Kit were purchased from Sigma-Aldrich® RAB0114. Eppendorf Tubes® and square Petri dishes (120 mm × 120 mm) were obtained from Thermo Scientific™, and the Eppendorf pipette used in the experiment was from RochG*Power 3.1. was used to determine the sample size. For the dose-response phase (n

= 5 per group): For a one-way ANOVA with $\alpha=0.05$, power $(1-\beta)=0.80$, and $k=4-5$ groups, $n=5$ per group provides 80% power to detect a large effect size (Cohen's $f \approx 0.78-0.84$; $\eta^2 \approx 0.38-0.41$). Treatment phase ($n=10$ per group): For a two-sample comparison (two-tailed t-test, $\alpha=0.05$, power=0.80), $n=10$ per group provides 80% power to detect a large effect size (Cohen's $d \approx 1.32$). Our pilot data for IL-6 indicated an expected effect size of $d \approx 1.6$, exceeding this threshold. Randomization was carried out by using a simple random number generator to avoid selection bias. To determine the optimal dose of ferulic acid, 30 rats were randomly divided into six groups of five rats each. Group 1 received only a standard rat diet, while the other groups received ferulic acid orally at doses of 10, 20, 30, 40, and 50 mg/kg diluted in water for 15 days, five times per week. The animals were kept in cages under a controlled environment with a constant temperature of 22–24 °C and were given pellet diets ad libitum. After one hour of oral administration on the final dosing day, 0.5 mL of blood was collected from the lateral tail vein of each rat into sterile anticoagulated tubes and stored at -80 °C. Samples were centrifuged at 3500 rpm for 15 minutes. The concentration of ferulic acid in the serum was measured using HPLC at a UV wavelength of 320 nm. To induce ischemia-reperfusion injury, 20 rats were randomly assigned to either a control group ($n=10$) or a treatment group ($n=10$). The treatment group received the optimized dose of ferulic acid orally for 15 days, while the control group received no treatment. Anesthesia was induced using 5% isoflurane in 50% oxygen via a facial mask and maintained with 2% isoflurane during the procedure. The left anterior descending (LAD) coronary artery was ligated for 30 minutes, followed by 120 minutes of reperfusion to simulate ischemia-reperfusion injury. After completion of the reperfusion period, 1 mL of blood was collected via cardiac puncture using a 23G1 needle and a 5 mL syringe. Serum IL-6 levels were quantified using the Rat IL-6 ELISA kit to assess inflammation. Data were analyzed using SPSS version 23.0. Data were expressed as mean \pm standard deviation (SD). Normality of IL-6 values was assessed using the Shapiro-Wilk test. For comparison between groups: If normally distributed, the independent-samples t-test (two-tailed) was applied. If not normal, the Mann-Whitney U test was used. For Effect sizes, the Parametric: Cohen's d with 95% confidence intervals (CI) was used. The confidence intervals for mean differences were calculated by using SD and the t-distribution. The Significance was set at $\alpha=0.05$. Exact p-values are reported; $p < 0.001$.

RESULTS

This research analyzed 135 patients treated at the Civil Hospital Karachi's Department of Medicine, Pakistan, who met the study's inclusion and exclusion criteria. The

participants' ages varied from 30 to 60 years, with a mean age of 46.71 ± 6.82 years. Regarding the gender distribution, 55 (40.7%) were male and 80 (59.3%) were female. Regarding the status of residence, 43 (31.9%) were residing in urban locations, while 92 (68.1%) were in rural locations. Regarding hospital stay, most of the patients stay for ≤ 5 days (66.67%) (Table 1).

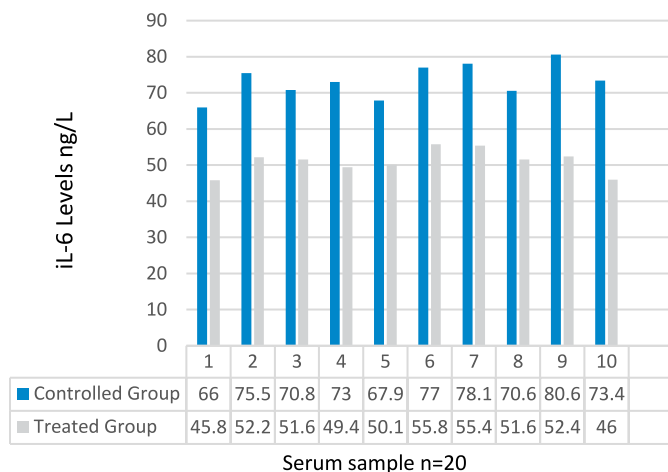


Figure 1: Comparison of IL Levels Between the Treated and Control Groups

Descriptive statistics, the Shapiro-Wilk test indicated that IL-6 values in both the groups were normally distributed (Control: $W=0.96$, $p=0.61$; and Treatment group: $W=0.94$, $p=0.48$) (Table 1).

Table 1: Descriptive Statistics and Normality Testing of IL-6 Concentrations

Groups	Number (n)	Mean \pm SD (ng/L)	95% CI (ng/L)	Shapiro-Wilk W (p)
Control	10	71.2 ± 3.2	68.9–73.5	0.96 ($p=0.61$)
Treatment	10	49.2 ± 2.1	47.7–50.7	0.94 ($p=0.48$)

The treatment group demonstrated a significant decrease in IL-6 as compared with the Control, with a mean difference of 22.0 ng/L (95% CI: 19.0–25.0), $p<0.001$. The standardized effect size was Cohen's $d=8.18$ (95% CI: 5.10–11.25), which reflected a large treatment effect. Serum IL-6 levels between the control group and the ferulic acid-treated group following myocardial ischemia-reperfusion injury. The control group showed significantly higher IL-6 levels (mean 71.2 ± 3.2 ng/l) compared to the treated group (mean 49.2 ± 2.12 ng/l). Statistical analysis revealed a significant difference between the 2 groups ($p<0.001$). This indicates that ferulic acid administration effectively reduced IL-6 levels, suggesting a potent anti-inflammatory effect in the experimental model. These findings support the role of ferulic acid in attenuating inflammation associated with ischemia-reperfusion injury (Table 2).

Table 2: Comparison of Serum IL-6 Levels between Control and Ferulic Acid-Treated Groups in Myocardial Ischemia-Reperfusion Injury Model

Comparison	Mean differences (95% CI)	Statistical Test	Effect Size (Cohen's d, 95% CI)	p-Value
Control vs Treatment Group	22.0 (19.0 – 25.0)	Independent t-test	8.18 (5.10 – 11.25)	<0.001

DISCUSSIONS

Plant polyphenol ferulic acid is effective at inhibiting inflammation triggered by myocardial I/R injury in rats. Serum interleukin-6 (IL-6) levels were significantly lower in the treated group compared to the controls, suggesting that ferulic acid had a potential protective effect on the attenuation of inflammation damage post-reperfusion. IL-6 is a major pro-inflammatory cytokine involved in tissue edema, neutrophil infiltration, and oxidative injury; hence, its inhibition is good evidence for its anti-inflammatory action. Multiple earlier studies validate these findings. Aneja *et al.* noted that epigallocatechin-3-gallate (EGCG) mitigated myocardial injury by blocking the NF- κ B and AP-1 signaling pathways, which resulted in decreased IL-6 and neutrophil activity during I/R injury in rats [17]. Likewise, Chen *et al.* found that rosmarinic acid lessened cardiomyocyte apoptosis and improved heart function by activating PPAR γ and suppressing inflammation via NF- κ B [18]. Lv *et al.* reported that total flavones of *Abelmoschus manihot* lowered serum IL-6 and TNF- α and reduced oxidative stress (MDA), while increasing antioxidant enzymes (SOD), thus conserving myocardial tissue from I/R injury [19]. Furthermore, Zhao *et al.* identified a synergistic drug combination from traditional Chinese medicine, including ferulic acid that effectively reduced IL-6, TNF- α , and oxidative markers, and enhanced myocardial structure and antioxidant defenses [20]. Their mechanistic findings highlighted activation of PPAR γ and inhibition of NF- κ B and Akt signaling, consistent with polyphenols' known bioactivity. Additional work by Sahu *et al.* emphasized the therapeutic role of polyphenols such as quercetin and ferulic acid in cardio protection by reducing inflammatory cytokines, inhibiting neutrophil activation, and attenuating oxidative stress following I/R injury [21]. In another animal study, Luo *et al.* demonstrated that ferulic acid significantly decreased infarct size and myocardial inflammatory infiltration, associated with downregulation of IL-6, IL-1 β , and TNF- α , and upregulation of SOD and catalase activity [22]. Oxidative stress and inflammation are correlated in the pathophysiology of I/R injury. During reperfusion, excessive production of reactive oxygen species (ROS) exacerbates myocardial damage and promotes cytokine release. Polyphenols like ferulic acid act as free radical scavengers, modulate transcription factors (e.g., NF- κ B), and enhance endogenous antioxidant systems such as SOD

and catalase, thereby mitigating both oxidative and inflammatory injury [22]. Moreover, the narrow standard deviation may be because all rats were approximately the same age and weight (300–350 g), kept under standardized environmental conditions, and all the selected rats were free from infection, which likely had reduced biological variability. Also, serum IL-6 measurements were performed using a high-sensitivity ELISA kit with technical replicates, which might have contributed to consistency in the readings. Despite the results, several limitations must be considered. The study had a relatively small sample size, which limited the generalizability and statistical reliability. Since only male rats were used in the study, the study excluded sex-based responses to ferulic acid. Moreover, the treatment duration was short, which may not reflect long-term efficacy or safety. Finally, a comparative reference group using standard antioxidants or anti-inflammatory drugs was not included, which limits the ability to position the effects of ferulic acid relative to established therapies.

CONCLUSIONS

In conclusion, the current study aligns with a growing body of evidence suggesting that ferulic acid has significant cardioprotective effects in the context of I/R injury. By reducing IL-6 levels and modulating oxidative-inflammatory pathways, ferulic acid may serve as a valuable therapeutic agent to attenuate myocardial inflammation and limit reperfusion injury. Future studies are needed to address the gaps by including both male and female rats, increasing the sample sizes, and experimenting with ferulic acid in relevant disease models. Increasing the duration of treatments and dose-response studies will further help to clarify the mechanisms of action. Comparative trials with other standard antioxidants or anti-inflammatory agents are also recommended to establish its relative efficacy.

Authors Contribution

Conceptualization: NA,

Methodology: NA, AIQ, MZY

Formal analysis: MT, MS

Writing review and editing: AIQ, MZY, MT, MS

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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