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Original Article

The Effects of Vanillic Acid on the Q-T Interval and the Knee Joint Vascular Response in Rats With Liver Cirrhosis

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ABSTRACT

Background: Liver cirrhosis often leads to various cardiovascular complications. Given the protective role of antioxidant agents in cardiovascular diseases, vanillic acid (VA) may play a preventive role due to its antioxidant properties. In this experiment, we examined the effects of VA on the ECG and vascular responses in the knee joints of rats with cholestasis-induced cirrhosis.

Methods: Thirty-two male Sprague-Dawley rats were divided into 4 groups: sham, cirrhosis, cirrhosis + VA, and VA. Liver cirrhosis was induced by chronic (4-week) bile duct ligation (BDL). ECG (lead II) recordings were obtained on the first day and 4 weeks post-surgery. Laser Doppler flowmetry was employed 4 weeks after the surgery to assess blood flow changes in the knee joints of the animals.

Results: On the first day, no significant differences were observed in the QRS complex voltage and QTc interval. However, 4 weeks post-BDL induction, the QTc interval significantly increased, while the QRS complex voltage and the blood vessel response decreased in the cirrhosis group. VA was found to have a mitigating effect on these parameters.

Conclusions: Cardiovascular complications in cirrhotic patients may result from an increase in free radicals. Therefore, it can be inferred that the reduction in cardiovascular complications observed in the cirrhosis group treated with VA is likely attributable to its antioxidant properties. ***(Iranian Heart Journal 2024; 25(4): 13-21)***

KEYWORDS: Liver cirrhosis, QRS complex voltage, QTc interval, Knee joint vascular responses, Vanillic acid

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As the body's largest organ, the liver plays a crucial role in regulating various functions, including metabolism. [1] Cirrhosis (Ci) represents the final stage of numerous chronic liver diseases, such as viral hepatitis and excessive alcohol consumption. [2,3] One potential complication of Ci is femoral head necrosis, which can arise from several circulatory system alterations. [4] Common complications of Ci include portal hypertension, encephalopathy, renal failure, ascites with spontaneous bacterial peritonitis, and bleeding from esophageal varices. [5,6]

Liver Ci is often accompanied by various cardiovascular complications, such as

cirrhotic cardiomyopathy. This condition is characterized by one or more of the following changes: 1) normal or enhanced systolic function at rest, with a diminished contractile response to stress; 2) altered diastolic relaxation [7]; 3) structural anomalies in cardiac chambers [8,9]; 4) electrophysiological modifications, like Q-T interval prolongation; [10] and 5) low systemic blood pressure indicative of hyperdynamic circulation. [5,6] Biliary Ci is a form of Ci distinguished by the presence of scar tissue surrounding the bile ducts. [11]

Bile duct ligation (BDL) in rats serves as an experimental model for rapidly progressive biliary fibrosis. The early stages of this model are marked by acute cholestasis, a process in which oxidative stress and inflammation play crucial roles. [12] Herbal medicines have demonstrated both preventive and therapeutic potential for various ailments. Their availability, affordability, and reduced side effects make them particularly significant in research, positioning them as viable alternatives to conventional chemical medications. [13]

Vanilla extract contains various compounds, with vanillin (4-hydroxy-3-methoxy benzaldehyde) being a common non-toxic food additive that appears as white or slightly yellow crystals. Vanillin is metabolized in the liver, forming vanillic acid (VA) (3-methoxy-4-hydroxybenzoic acid), which is primarily excreted as conjugates and, to a lesser extent, as a free metabolite in urine. [14,15] The conversion of dietary vanillin into VA, the main urinary metabolite, has been confirmed in both rats and humans. [14-16]

VA is one of the known phenolic acids in plants such as *Melilotus metagenesis,* [17] *C. murale,* [18] *garden grass,* [19] and *Juglans regia L.* [20] VA possesses antioxidant properties that can potentially serve as a safer alternative to synthetic antioxidants with potentially toxic effects. [20] Given the important role of antioxidant factors in reducing complications associated with Ci, the current study aimed to explore the impact of VA on cardiovascular complications resulting from BDL in an experimental model.

METHODS

Chemicals

Ketamine HCl (10%) and xylazine (2%) were acquired from Alfasan Co (Netherlands), while VA, phenylephrine, acetylcholine, and sodium nitroprusside were procured from Sigma-Aldrich Co (Germany).

Animals and Grouping

In this study, 32 male Sprague-Dawley rats (200–250 g) were acquired from the Animal Center of Jundishapur University of Medical Sciences in Ahvaz. The rats were handled per established animal care guidelines, housed at a temperature of 22±2 °C under a 12-hour light-dark cycle, and provided with food and water ad libitum. The experimental protocol was approved by the Animal Ethics Committee of Jundishapur Ahvaz University of Medical Sciences (APRC-93-10).

Experimental Design

The 32 male Sprague-Dawley rats were divided into 4 groups: sham, Ci, cirrhosis treated with vanillic acid (Ci + VA), and VA. Biliary Ci (extrahepatic cholestasis) was induced in the animals via chronic (28-day) BDL. For the procedure, anesthesia was induced by intraperitoneal injection of a combination of 50 mg/kg of ketamine hydrochloride and 10 mg/kg of xylazine. [21] After a midline abdominal incision near the sternum, the common bile duct was identified, and a double suture was applied using 3/0 silk. [22] The sham group underwent a laparotomy procedure, during which only the tissue surrounding the common bile duct was dissected. The animals were then returned to their cages with ad libitum access to water and food. In the Ci + VA and VA groups, VA (10 mg/kg) was administered

daily via gavage for 28 days. [23] The sham and Ci groups received an equivalent volume of normal saline as a control. [24,25]

The Q-T Interval and QRS Complex Voltage Recording

Under anesthesia, ECG (lead II) recordings were obtained for all groups on the first day before surgery and day 28 using a BioAmp and Power Lab system (AD Instruments, Australia). ECG recordings were acquired over 15 minutes. The QRS complex (representing inotropic properties) and the Q-T interval (indicating dromotropic properties) were derived from the ECG tracings. Since the Q-T interval is influenced by heart rate, the corrected Q-T (QTc) was calculated using Bazett's formula (QTc = Q-T interval / √RR).

Measurement of Changes in Blood Flow

On day 28, the rats were anesthetized using the same procedure as before. Once deep anesthesia was confirmed through the absence of hindlimb withdrawal reflex upon painful paw stimulation, an oval-shaped section of skin was excised from the anterior-medial region of the knee. Relative changes in blood flow were then evaluated using a laser Doppler flowmeter (MBF3D, Moor Instruments, Axminster, UK). This technique has been previously validated for assessing blood flow changes in the knee joints of various animal species, including rabbits, cats, and mice. The laser Doppler flowmetry probe was positioned vertically over the exposed anterior-medial region of the knee joint capsule. Prior research has demonstrated the effectiveness of this probe placement for accurately detecting synovial blood flow alterations. [26] Different amounts of vasoconstrictor drugs (phenylephrine with doses of 10^{-5}-10^{-7}) and vasodilators (acetylcholine and sodium nitroprusside with doses of 10^{-6}-10^{-8}) were administered randomly and locally on the bare area of the knee joint in a volume equal to 0.1 mL. Subsequently, alterations in knee joint blood flow across the various rat groups were assessed using the laser Doppler flowmeter and compared with the sham group.

At the conclusion of the experiment, the rats were euthanized with an overdose of anesthesia. Their biological zero was then measured and subtracted from the baseline blood flow value before the calculation of percentage changes. Biological zero represents a numerical value that reflects the change in light frequency upon interaction with tissues other than blood, as detected by the laser Doppler flowmeter under conditions of animal death and cessation of blood flow. Subtracting this value from the baseline blood flow ensures a more precise calculation of flow alterations. [27]

Statistical Analysis

Data are presented as mean ± SEM for 8 animals per group. The percentage change in blood flow between the groups was analyzed using a 2-way repeated measures analysis of variance, while the QTc interval and QRS complex comparisons were performed using a 1-way analysis of variance test followed by an LSD test. Statistical significance was defined as a *P* value below 0.05.

RESULTS

Dromotropic Properties (QTc Interval) in Experimental Groups

As depicted in Figure 1, there was no significant difference in the QTc interval among the groups at the outset of the experiment (day 1). Nonetheless, at the conclusion of the study (day 28), a significant increase in the QTc interval was observed in the Ci group and the Ci + VA group compared with their respective pre-BDL values ($P < 0.001$ and $P < 0.05$, respectively). Additionally, the administration of VA appeared to reduce the QTc interval in the Ci + VA group compared with the Ci group ($P < 0.05$).

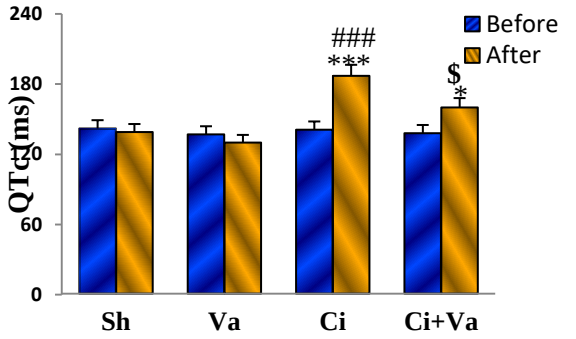

Figure 1: The image compares the QTc interval (ms) among the experimental groups on day 1 and day 28 post-bile duct ligation in rats (n=8). ***P < 0.001 and *P < 0.05 indicate significant differences between pre-surgery and 4-week post-surgery values. $^{###}P$ < 0.001 denotes a significant difference between the Ci and Sh groups. $^{\$}P$ < 0.05 signifies a significant difference between the Ci + VA and Ci groups.
Sh: sham, Ci: cirrhosis, VA: vanillic acid

Inotropic Properties (QRS Complex Voltage) in Experimental Groups

As illustrated in Figure 2, there was no significant difference in the QRS complex voltage among the groups before BDL induction. On day 28, a significant decrease in the QRS complex voltage was observed in the Ci group (P < 0.01), while a significant increase was noted in the VA group (P < 0.01) when compared with their respective pre-BDL values. Additionally, VA administration appeared to increase the QRS complex voltage in the Ci + VA group compared with the Ci group on day 28 (P < 0.01).

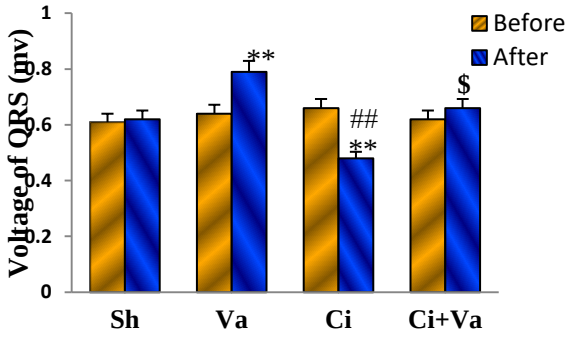

Figure 2: The image compares the QRS complex voltage (mV) among the experimental groups on day 1 and day 28 post-bile duct ligation in rats (n=8). **P < 0.01 indicates significant differences between pre-surgery and 4-week post-surgery values. $^{##}P$ < 0.01 denotes a significant difference between the Ci and Sh groups. $^{\$\$}P$ < 0.01 signifies a significant difference between the Ci + VA and Ci groups.
Sh: sham, Ci: cirrhosis, VA: vanillic acid

Vasoconstriction Response of the Knee Joint to Various Phenylephrine Concentrations in Experimental Groups

Figure 3 illustrates the effects of VA on the vascular response of the knee joint in the different groups. The comparison of the contractile response of knee joint vessels to varying concentrations of phenylephrine (10–5 to 10–7) revealed a significant decrease in the Ci group compared with the sham group (P < 0.01 and P < 0.001). The administration of VA (10 mg/kg) for 4 weeks resulted in a significant increase in the vascular response of the Ci + VA group compared with the Ci group (P < 0.05, P < 0.01, and P < 0.001). No significant difference in vascular response was observed between the Ci + VA and sham groups.

Vasodilation Response of the Knee Joint to Various Acetylcholine Concentrations in Experimental Groups

As depicted in Figure 4, the vasodilator response of the knee joint to different concentrations of acetylcholine (10–6 to 10–8) was significantly reduced in the Ci group compared with the sham group (P < 0.001). Administration of VA (10 mg/kg) for 4 weeks resulted in a significant increase in vascular response in the Ci group receiving VA compared with the Ci group without VA treatment (P < 0.001).

Vasodilation Response of the Knee Joint to Various Sodium Nitroprusside Concentrations in the Experimental Groups

As demonstrated in Figure 5, the vasodilator response of the knee joint to different concentrations of sodium nitroprusside (10–6 to 10–8) was significantly reduced in the Ci group compared with the sham group (P <

0.01 and $P < 0.001$). Administration of VA (10 mg/kg) for 28 days led to a significant increase in vascular response in the Ci + VA group compared with the Ci group without VA treatment ($P < 0.05$ and $P < 0.001$).

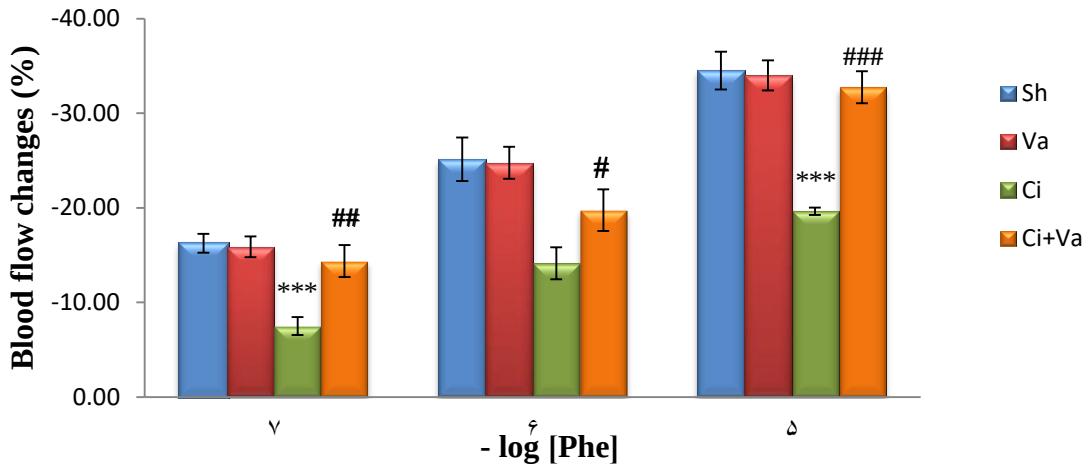

Figure 3: The image compares contractile responses of knee joint vessels to different concentrations of phenylephrine in the different groups (Sh, VA, Ci, and Ci + VA) based on the percentage of blood flow changes (n=8). $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate significant differences between the Ci and Sh groups. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ denote significant differences between the Ci + VA and the Ci groups.
Sh: sham, Ci: cirrhosis, VA: vanillic acid

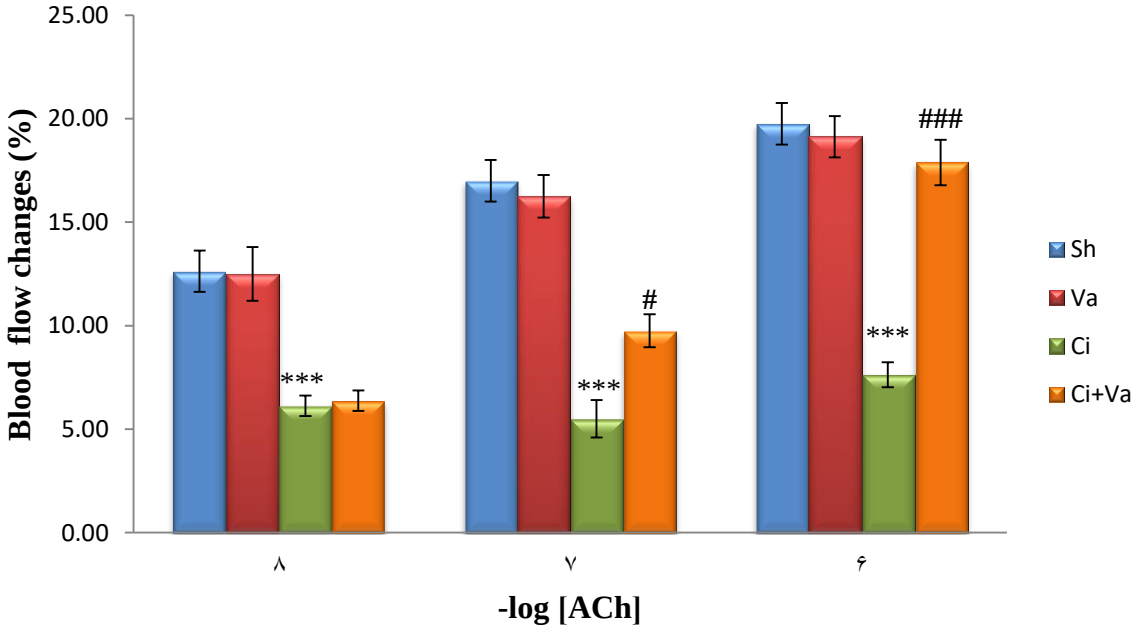

Figure 4: The image compares knee joint vasodilator responses to different concentrations of acetylcholine (10-6 to 10-8) in the different groups (Sh, VA, Ci, and Ci+ VA) based on the percentage changes in blood flow (n=8). $^{***}P < 0.001$ indicates a significant difference between the Ci and Sh groups. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ denote significant differences between the Ci + VA and Ci groups.
Sh: sham, Ci: cirrhosis, VA: vanillic acid

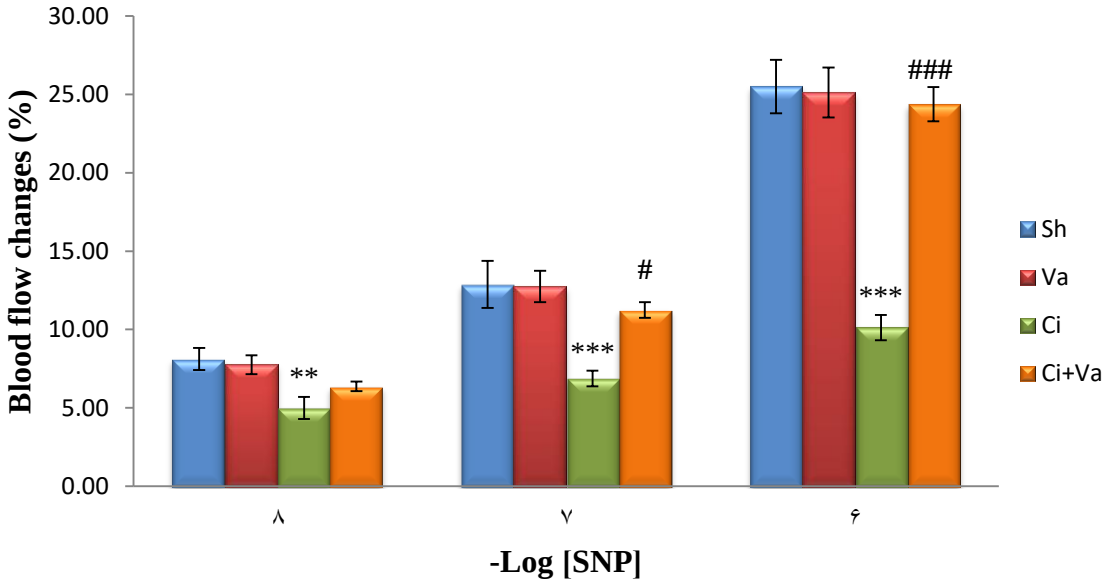

Figure 5: The image compares knee joint vasodilator responses to different concentrations of sodium nitroprusside (10-6 to 10-8) in the different groups (Sh, VA, Ci, and Ci + VA) based on the percentage changes in blood flow (n=8). $^{**}P < 0.01$ and $^{***}P < 0.001$ indicate significant differences between the Ci and Sh groups. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ denote significant differences between the Ci + VA and Ci groups.
Sh: sham, Ci: cirrhosis, VA: vanillic acid

DISCUSSION

In this study, VA, a recognized antioxidant, was employed to investigate its effects on the QTc interval (dromotropic properties), the QRS complex (inotropic properties), and the vascular response of the knee joint in a rat model of Ci. Cardiovascular complications are known to occur in patients with Ci due to the generation of free radicals within the body. Consequently, we utilized VA, which possesses antioxidant and free radical scavenging properties, as a potential intervention to alleviate the cardiovascular complications associated with Ci.

Previous research has demonstrated that VA enhances the activity of non-enzymatic substances such as glutathione, a potent scavenger of reactive oxygen species (ROS), superoxide anions, and hydroxyl radicals. [29] These effects may be attributed to the phenolic properties of VA, [30] which enable it to scavenge harmful substances like free radicals and inhibit the release of ROS.

BDL in animals is a widely recognized model for inducing Ci [22] Previous research has shown that BDL-induced Ci results in increased levels of liver damage markers. [31] Numerous studies have demonstrated that administering antioxidants significantly attenuates liver damage following BDL in rats. [32] Ci is the primary and most prevalent form of non-neoplastic liver dysfunction, often leading to mortality. Cardiac dysfunction has been documented in patients with liver Ci, accompanied by characteristic hemodynamic alterations such as hyperdynamic blood flow, elevated cardiac output, reduced peripheral vascular resistance, and decreased arterial pressure. [33] Cirrhotic cardiomyopathy is significantly influenced by an increase in the Q-T interval. [34] Electrophysiological abnormalities and systolic and diastolic dysfunction are recognized complications of cirrhotic cardiomyopathy. Kowalski [35] was the first to report cardiovascular disorders in

patients with Ci. Despite this, the significance of heart disease in Ci has not been adequately highlighted. Consequently, the present study aimed to investigate the effects of VA on cardiovascular complications in a rat model of Ci. Prior studies have demonstrated that biliary Ci results in prolonged Q-T interval, reduced QRS complex voltage, and diminished cardiac contractility. [36,37] Consistent with these findings, our experiment revealed increased QTc interval and decreased QRS complex voltage in animals with Ci. Administration of VA significantly ameliorated these parameters in the Ci group receiving VA, suggesting its therapeutic potential for addressing these factors.

While previous studies have reported vascular disorders of the knee joint in Ci, [34] the potential role of antioxidants such as VA in improving these complications has not been extensively explored. In light of the known antioxidant properties of VA and its demonstrated efficacy in reducing pain-related behavior in knee osteoarthritis rats, [37] it may be worth investigating its potential therapeutic effects on vascular disorders associated with Ci. The present study revealed that Ci led to a reduced vascular response of the knee joint to both vasoconstrictor and vasodilator drugs in animals with Ci. Notably, VA administration significantly enhanced this vascular response in the Ci group receiving VA compared with the untreated Ci animals. This result demonstrates that VA had a positive effect on the knee joint response in Ci rats. As vasodilation has been recognized as a complication of Ci, [38] the reduced vascular response observed in these conditions may be attributed to a decrease in contractile response. The administration of VA was found to improve this vascular response, indicating its potential therapeutic role in addressing vascular complications associated with Ci.

CONCLUSIONS

BDL was found to effectively induce Ci in this study. The results demonstrated that BDL led to significant cardiovascular complications, likely due to an increase in oxidative factors in these animals. Furthermore, the antioxidant properties of VA were found to significantly mitigate these complications.

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