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Evaluation of the Effect of Gallic Acid on QT Interval Prolongation and Serum Billirubin in Rat Model of Liver Cirrhosis.

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ABSTRACT

Cirrhotic cardiomyopathy is a type of clinical syndrome in patients with liver cirrhosis, among the factors that increase the QT interval are important in cirrhotic cardiomyopathy. Cirrhotic patients are known with cardiovascular complications due to the release of free radicals in the body. In this study, gallic acid (GA) has been used as an antioxidant to prevent cardiovascular complications of liver cirrhosis in rat model of liver cirrhosis. Male Sprague Dawley rats (200-250 g) were divided in four groups (n=8); Sham (Animals underwent false surgery cirrhotic), Cirrhosis group, Gallic acid group (30 mg/kg/day, gavaged for 28 days), Cirrhosis group receiving Gallic acid. Biliary cirrhosis was induced in a Cirrhosis group by 28 days Bile Duct Ligation (BDL). At the first day and 28 days after surgery animals were anesthetized, electrocardiogram (lead II) recorded and QT interval, voltage of QRS, heart rate and bilirubin investigated. 28 days after induction of BDL, increased QRS voltage, reduction of QTc interval, bilirubin reduction in the cirrhotic group receiving GA was shown significantly. Gallic acid as an antioxidant agent can reduces some electrocardiological complications and laboratory parameters in cirrhotic patients.

Key words: Cirrhotic cardiomyopathy, Gallic acid, QT interval, QRS, Bilirubin, Rat.

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INTRODUCTION

Cardiovascular disease independent of sexes, ethnicity, and economic status is one of the major causes of death in the world and a large financial cost to society imposes [1]. Cirrhosis, final stage of liver disease, is a chronic irreversible hepatic parenchyma injury [2]. Patients with cirrhosis are at risk of progression of many complications such as the cardiovascular complications [3].

Patients with cirrhosis have high dynamic blood flow, which increases heart rate, lowers blood pressure and cardiac output and peripheral vascular resistance. Clinical signs of hyper-dynamic circulation are warm skin, spider angioma, arrhythmia, and pulse jumper [4].

In cirrhosis of liver, cell regeneration of the fibrous scar is irreversible, therefore scar fibrosis and disruption of liver structure; function disorder can creates disorder in vascular function and lead to an increase in pressure inside the liver shunt. Because of insufficient blood supply and the resulting damage from the direct effects of toxins, inflammation, or metabolic factors on liver cells, liver cells is disrupted normal work [5].

Peripheral vascular vasodilatation associated with cirrhosis originated many of the clinical manifestations in patients with cirrhosis [6]. Extensive fibrosis associated with the formation of the generative nodules. Autonomic dysfunction is a common complication of cirrhosis, so that the autonomic dysfunction of the cardiovascular system raises the risk of death in these patients and is associated with a worse prognosis in liver disease [7].

Cardiac output increases in cirrhotic patients and systolic and diastolic function decreases. These disorders are called cirrhotic cardiomyopathy [8]. Cirrhotic cardiomyopathy has shown the structural and functional abnormalities in heart. These disorders include changes in systolic and diastolic function, electrophysiological changes (such as QT prolongation), and hypertrophy of the heart chambers and are shown microscopic and macroscopic changes [8].

Gallic acid, the chemical name 3.4.5 three-hydroxyl benzoic acid, is a polyphenol component that found in many fruits and vegetables, tea, oak, red grapes, strawberry, apple peel and other different herbs found [9].

Studies have shown that fruits and vegetables are rich in antioxidants such as carotenoids, polyphenols, glutathione and vitamin E and C. As a result, the protective effect of these antioxidants is against cardiovascular disease, cancer and neurological [10]. Gallic acid has antioxidant properties and may protect against oxidative stress and necrosis lysosomal membrane and heart muscle morphology [11, 12]. Gallic acid by increasing the activity of superoxide dismutase, catalase and glutathione peroxidase may be removal free radicals [13, 14] and acts as an antioxidant to protect cells from oxidative damage. Gallic acid also acts as anti-bacterial, anti-inflammatory, anti-viral [15].

Flavonoid compounds, including gallic acid by preventing the inflammatory reaction after ischemia can prevent and reduce the harmful effects of ischemia [13, 16]. Gallic acid by enhancing the activity of the membrane of lysosomes and decrease lysosomal enzymes lead inhibition of lipid peroxidation and against with damage caused by ischemia [17]).

Effective treatments postpone deaths from cardiovascular diseases is very expensive. Therefore special attention to the prevention and treatment of cardiovascular disease is required. Given that cirrhotic patients with cardiovascular complications have been reported, and the effects of free radicals in the body, therefore, in this study gallic acid as an antioxidant to prevent cardiac complications of cirrhosis will be used.

MATERIALS AND METHODS

Chemicals

Gallic acid was purchased from Sigma-Aldrich Co. (USA), ketamine hydrochloride (10 %) and xylazine (2 %) were obtained from Alfasan Co. (Netherlands).

Animals

Thirty-two male Sprague-Dawley rats (Weighting: 200-250 g), were obtained from animal breeding center of Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran). Animals were housed in standard cages at a temperature of 22±2 °C and 12 h light and dark cycle. Rats had free access to food and water. The research protocol approved with the Animal Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (No. ajums APRC-93-06).

Study procedures

Animals were randomly divided in 4 groups, containing eight rats in each. The treatments were as follow:

A) Sham: Animals in this group underwent false surgery cirrhotic; Rats were anesthesized (50 mg/kg Ketamine and 10 mg/kg xylazine, i.p); After anesthesia, the middle part of the abdomen shaved, cut and the common bile duct was dissected from the circumambient tissue and the animals were permitted to recover [18].

B) Cirrhosis group: Animals in this group underwent surgery the same as in the Sham group and after finding bile duct, billiary ligation in animals (extrahepatic cholestasis) induced at two separate points using Size 3/0 surgical silk and then released into the abdomen and abdominal wall muscle and skin in two layers with silk stitched to form consecutive of stitches, and the animals were permitted to recover [18].

C) Gallic acid group: Animals similar to the Sham group underwent surgery (without cirrhosis) and received gallic acid (30 mg/kg/daily gavage to 4 weeks) [19].

D) Cirrhosis group receiving gallic acid: Animals like the Cirrhosis group underwent surgery (with cirrhosis) and received gallic acid (30 mg/kg/daily gavage to 4 weeks) [19].

Evaluation of QT interval

In the all groups after 4 weeks from the time of surgery, animals (like before) was anesthetized and lead II electrocardiogram recorded and connected to the evaluation of QT interval [20] using the Bio Amp and Power lab system (AD-Instruments, Australia). QT interval varies with heart rate and the most frequently-used formula to correct QT for heart rate (QTc) has been proposed by Bazett [21].
Bazett's formula:

$$QTc\ (QT\ corrected\ for\ HR) = QT\ interval / \sqrt{RR\ interval}$$

Heart rate and voltage of QRS recording

In the all groups, heart rate and voltage of QRS were measured in the duration of the ECG recording using the Bio Amp and Power lab [21].

Measuring bilirubin

After opening the chest, heart blood sample (to measure bilirubin concentration in plasma as a marker to indicate the occurrence of cholestasis) was prepared [18]. Pars azmon kit for measuring bilirubin test was performed and direct and total bilirubin was measured.

Statistical Analysis

Results are shown as Mean±SEM for eight animals in each group. The comparisons between multiple groups were made by One-Way ANOVA followed by LSD. The comparisons between two groups were analyzed using Paired t-test. Differences were statistically considered when $P<0.05$.

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RESULTS

Effect of gallic acid on the QTc interval

In the all groups, at the first day of experiment, QTc interval was not shown significant differences. Four weeks after induction of BDL, QTc interval was significantly increased in cirrhosis group and in the BDL group treated with gallic acid (Figure 1, p<0.001) in comparison with the first day, however, QTc interval in Cirrhosis group receiving gallic acid, showed a significantly reduction in comparison with cirrhosis group (Figure 1, P< 0.01).

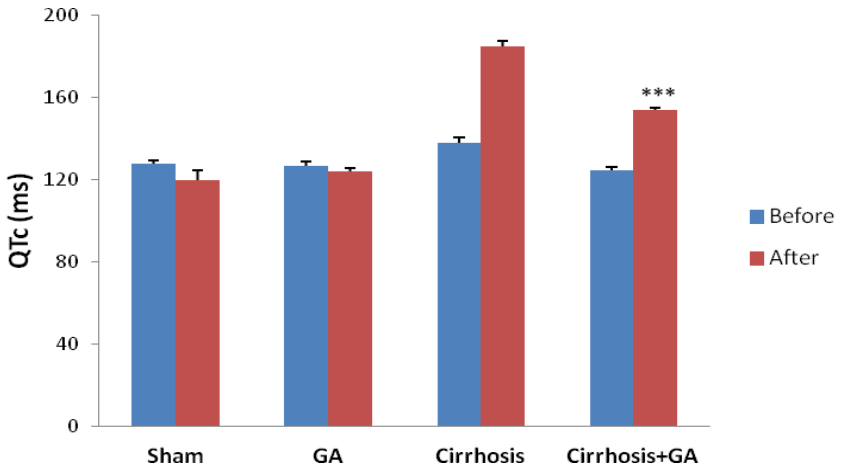

Figure 1: Comparison of QTc interval (msec) in different groups [Sham, GA (Gallic acid), Cirrhosis and Cirrhosis+ GA (Cirrhosis+ Gallic acid)], before and four weeks after surgery in rat (n=8). Results are expressed as Mean±SEM. One-way ANOVA followed by LSD test and Paired t-test was used. ***P<0.001 vs. Sham group, ##P<0.01 vs. Cirrhosis group.

Effect of gallic acid on the QRS

At the first day of experiment, the voltage of QRS complex did not show a significant difference in the all groups. 28 days after induction of BDL, the voltage of QRS complex was significantly reduced in cirrhosis group (Figure 2, p<0.001) but it was increased in the BDL group treated with gallic acid (Figure 2, P<0.01).

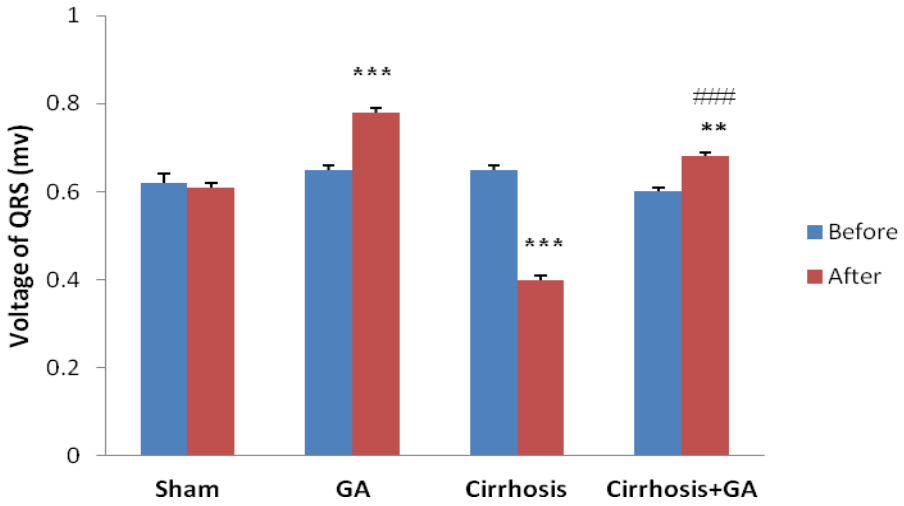

Figure 2: Comparison of voltage of QRS complex (mv) in different groups [Sham, GA (Gallic acid), Cirrhosis and Cirrhosis+ GA (Cirrhosis+ Gallic acid)], before and four weeks after surgery in rat (n=8). Results are expressed as Mean±SEM. One-way ANOVA followed by LSD test and Paired t-test was used. ***P<0.001 and **P<0.01 vs. Sham group, ###P<0.001 vs. Cirrhosis group.

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Effect of gallic acid on heart rate (HR)

At the first day of experiment and at the last day of experiment, HR did not show a significant difference in the all groups except in the animals that received gallic acid which showed significantly decrease in HR (Figure 3, P<0.05).

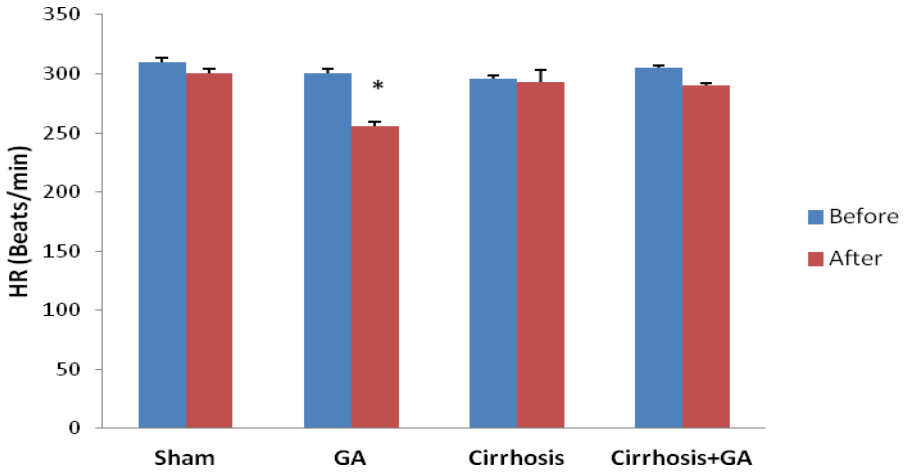

Figure 3: Comparison of heart rate (Beats/min) in different groups [Sham, GA (Gallic acid), Cirrhosis and Cirrhosis+ GA (Cirrhosis+ Gallic acid)], before and four weeks after surgery in rat (n=8). Results are expressed as Mean±SEM. One-way ANOVA followed by LSD test and Paired t-test was used. *P<0.05 vs. the first day of experiment.

Effect of gallic acid on Total and Direct Bilirubin

Total and direct (conjugated) bilirubin in cirrhotic group increased significantly in cirrhosis group and in cirrhosis group receiving gallic acid in comparison of Sham group (Figures 4 and 5, P<0.001), however, total and direct (conjugated) bilirubin in Cirrhosis group receiving gallic acid, showed a significantly reduction in comparison with cirrhosis group (Figures 4 and 5, P<0.001),

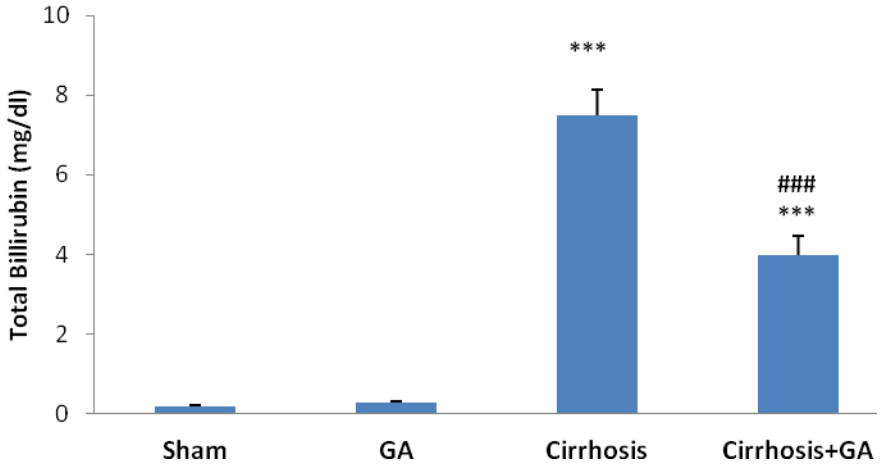

Figure 4: Comparison of total bilirubin level (mg/dl) in different groups [Sham, GA (Gallic acid), Cirrhosis and Cirrhosis+ GA (Cirrhosis+ Gallic acid)], four weeks after surgery in rat (n=8). Results are expressed as Mean±SEM; One-way ANOVA followed by LSD test was used. ***P<0.001 vs. Sham group, ###P<0.001 vs. Cirrhosis group.

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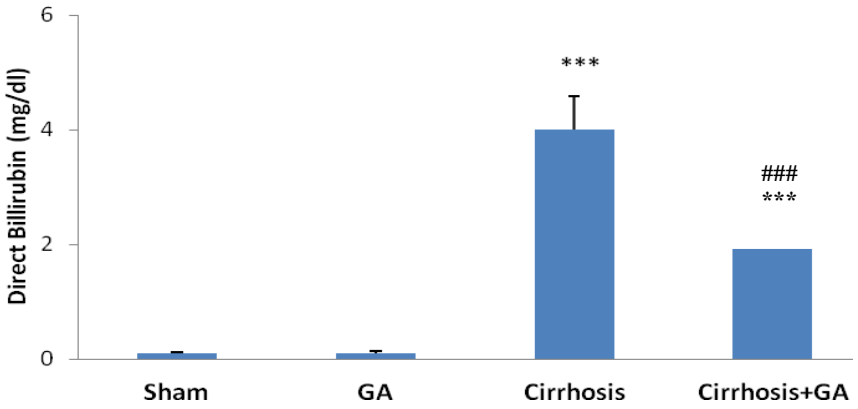

Figure 5: Comparison of direct bilirubin level (mg/dl) in different groups [Sham, GA (Gallic acid), Cirrhosis and Cirrhosis+ GA (Cirrhosis+ Gallic acid)], four weeks after surgery in rat (n=8). Results are expressed as Mean±SEM; One-way ANOVA followed by LSD test was used. ***P<0.001 vs. Sham group, ###P<0.001 vs. Cirrhosis group.

DISCUSSION

In this study, the effect of gallic acid was studied on QTc interval, QRS voltage, HR and serum bilirubin in cirrhosis rats. Gallic acid has shown an increased QRS voltage, reduction of QTc interval, reduction of bilirubin levels (Conjugated and total) four weeks after BDL in Rats.

Canbek et al. (2011) examined the effect of gallic acid against oxidative stress induced by ischemic/reeperfusion in the liver and kidneys of rats, and its protective effect against this damage was demonstrated [22]. The Sutra et al. (2008), preventive effect gallic acid on cardiac fibrosis associated with the metabolic syndrome study and sugessted that gallic acid reduces insulin resistance and increased blood prevented [23]. In 2011, Patel et al. in a study on the protective effect gallic acid against heart problems in type I diabetes induced by STZ, and concluded that the gallic acid for the treatment of myocardial damage subsequent diabetes is useful [24]. Nair (2013) showed that oral intake of gallic acid improves antioxidant enzyme activity in rat liver [25]. Considering that, cirrhotic patients with cardiovascular complications have been reported and causes of free radicals in the body; so in this study of gallic acid as an antioxidant to prevent complications of liver cirrhosis have been used.

Cirrhosis is main and most common non-neoplastic liver dysfunction, which liver disease leads to death. Cardiac dysfunction in patients with liver cirrhosis has been reported [26]. Hyperdynamic blood flow and increase in cardiac output associated with decreased peripheral vascular resistance is known. Kowalski reported cardiovascular disorders in cirrhosis patients [27]. In recent studies showed that blood flow in cirrhotic patients is especially decompensated cirrhosis has been hyperdynamic, leading to increasing the flow of the heart and cardiac output and reduce peripheral vascular resistance and peripheral arterial pressure is decreased. Among the factors that increase the QT interval are important in cirrhotic cardiomyopathy [28].

BDL is one of the known models to induction of liver disease in rats such as cirrhosis [29]. Liver cirrhosis reflects with a significant increase in bilirubin (Conjugated and total) levels [29]. In the present study, bilirubin levels have increased in cirrhosis animals' and decreased by gallic acid treatment. Previous studies had shown that the liver damage markers were increased by BDL in cirrhosis [30]. Several studied have shown decreases liver damage following BDL in animals treated by free radicals [20]. This study has shown reduction levels of bilirubin in cirrhosis rats treated with gallic acid.

A prolonged QT interval is important described in animals with liver disease [31]. Changes in the QT interval seem to revent with improved cirrhosis, abnormality in liver disease with ventricular arrhythmic risk [31]. The systemic circulation in liver diseasewas shown with hyperdynamic and characterized with increased heart rate and reduces QRS voltage [32]. Gallic acids as an antioxidant were administered to cirrhotic rats. Significant decreased QTc interval and increase QRS voltage was shown in cirrhosis animals treated with

Gallic acid but has not affected heart rate in intact rats. Pervious study was shown that gallic acid as an antioxidant factor increased activation glutathione which act as a strong agent to scavenging reactive oxygen species (ROS), and hydroxyl radicals and superoxide onions [13]. Gallic acid attenuated the cardiac enzymes the impaired both function and structure of cardiac.

CONCLUSION

The results show that the gallic acid can improve cardiac effects such as QTc prolongation, decreased QRS and increase in bilirubin levels in the cirrhosis rats. The results of this study suggest a protective effect of gallic acids on cardiac disorders in cirrhotic patients.

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