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[4] Methodology; Formal analysis

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Abstract

Kynurenine Pathway (KP) is the dominant metabolic route of tryptophan which is catalyzed by indoleamine-2,3-dioxygenase (IDO). This pathway is upregulated in liver disease where the level of KP metabolites correlates with the severity of disease. Cirrhosis is associated with cardiac dysfunction, which manifests itself during severe physiological challenges such as liver transplantation. Cardiac dysfunction in cirrhosis is linked to systemic inflammation and impaired cardiac beta-adrenergic signaling pathways. The KP pathway is involved in modulation of cardiac signaling and is upregulated by systemic inflammation. Therefore, this study aimed to evaluate the effect of IDO inhibition on development of cardiac dysfunction in an experimental model of cirrhosis. Cirrhosis was induced by bile duct ligation (BDL). Experimental groups were given either 1-methyl tryptophan (1-MT, 1, 3, 9 mg/kg), or saline. 28 days after BDL, cardiac chronotropic response to epinephrine was assessed *ex vivo*. HPLC was employed to measure hepatic and cardiac levels of tryptophan, kynurenine and kynurenic acid. Cirrhosis in rats was associated with impaired cardiac chronotropic responsiveness to adrenergic stimulation. 1-MT dose-dependently improved cirrhosis-induced chronotropic dysfunction as well as elevated serum levels of CRP and IL-6 in BDL rats. Hepatic and cardiac kynurenine/tryptophan ratio were elevated in cirrhotic rats and were reduced following 1-MT administration. Chronic administration of 1-MT could also reduce hepatic inflammation, fibrosis and ductular proliferation. 1-MT attenuates cardiac dysfunction in rats with biliary cirrhosis. This protective effect is not limited to the cardiac function as liver histopathologic changes were also improved following chronic 1-MT administration.

Keywords: Kynurenine; Tryptophan; Indoleamine-2,3-dioxygenase; 1-Methyl tryptophan; Cirrhosis; Cardiomyopathy

1. Introduction

Cirrhotic cardiomyopathy (CCM) is a complication of cirrhosis regardless of the etiology of liver disease. It is characterized by impaired inotropic and chronotropic response to physiological or pharmacological stress, altered diastolic function and electrophysiological abnormalities (Møller and Lee, 2018). The proposed mechanisms include impaired β-adrenergic receptor signaling, cardiac myosin isoform shift, elevated level of endogenous opioids and endocannabinoids (Abbasi et al., 2017; Honar et al., 2020; Tam et al., 2011; Wiese et al.; Zardi et al., 2010). Despite the mentioned alterations, signs and symptoms of myocardial dysfunction in cirrhosis is often masked clinically due to the presence of peripheral vasodilation and reduced peripheral vascular resistance. As a result, CCM remains silent for many years and the diagnosis of this functional cardiomyopathy is often missed or delayed. Nevertheless, CCM may manifest under severe physiological challenges during liver transplantation procedure such as clamping portal vein and inferior vena cava, massive blood transfusions and ischemia-reperfusion (Rahman and Mallett, 2015). Thus, 70% of liver transplant recipients confront cardiovascular complications following transplantation (Sampathkumar et al., 1998).

Kynurenine pathway (KP) accounts for 95% of tryptophan metabolism, which generates metabolites such as kynurenine, kynurenic acid, 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), quinolinic acid and finally nicotinamide adenine dinucleotide (NAD). The pathway is activated by tryptophan 2,3-dioxygenase (TDO) which is mostly expressed in brain and liver, or indoleamine-2,3-dioxygenase (IDO), the rate-limiting enzyme which is up-regulated under pathological conditions such as systemic inflammation (Badawy, 2020). KP activation has been linked to neurodegenerative and neuro-inflammatory diseases such as Alzheimer, multiple sclerosis, motor neuron diseases and seizure (Guillemin and Brew,

2002; Havelund et al., 2017; Lovelace et al., 2016; Nemeth et al., 2004); however, the contribution of KP in liver and cardiovascular diseases has been proposed (Song et al., 2017). The activation of KP in liver failure and the correlation of KP metabolites with the stages of liver diseases has been reported in recent studies (Clària et al., 2019; Kardashian et al., 2019). On the other hand, studies have confirmed KP metabolites as early biomarkers of cardiac dysfunction including heart failure (Baumgartner et al., 2019; Konishi et al., 2016; Ristagno et al., 2014). Also the accumulation of kynurenine has been reported to have correlation with the stages of dilated cardiomyopathy (Rudzite et al., 1992). KP metabolites have several targets and interact with different signaling pathways. For example, the interaction of kynurenic acid and kynurenine with β-adrenergic receptor signaling and endocannabinoid pathways, which are involved in pathophysiology of CCM, is documented by recent studies (Dadvar et al., 2018; Zádor et al., 2019). Also, CCM is associated with systemic inflammation, a condition that is linked with increased IDO activity. According to previous studies, IDO acts as an immune-modulator in different disorders (Mondanelli et al., 2020). However, the contribution of KP in the pathogenesis of CCM has not been investigated so far.

Taking this information into consideration, the purpose of this study was to evaluate the contribution of tryptophan-kynurenine pathway in an experimental model of cirrhosis-induced cardiac dysfunction. We also investigated the effect of IDO inhibition by 1-methyl L-tryptophan (1-MT) on cardiac chronotropic responses, tissue levels of kynurenine/tryptophan and liver function in an experimental model of cirrhosis.

2. Materials and methods

2.1 Animals

54 male Wistar rats (250-300 g) were provided from the animal house of Department of Pharmacology, Tehran University of Medical Sciences. All of the study procedures were conducted according to the 'Principles of Laboratory Animal Care' (NIH publication 82–23, revised in 1985 and further implemented in 1996) and legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU), and institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran). Animals were kept under 12 h light/dark and the temperature of 20-23 °C in plastic cages (4 rats in each cage). During experiments, animals had free access to food and water. Rats were randomly divided into 9 groups (N=6): 1. Control, 2. Sham, 3. Bile duct ligated (BDL), 4. BDL+ 1 mg/kg 1-MT, 5. BDL+ 3 mg/kg 1-MT, 6. BDL+ 9 mg/kg 1-MT, 7. Sham+ 1 mg/kg 1-MT, 8. Sham + 3 mg/kg 1-MT, 9. Sham + 9 mg/kg 1-MT.

2.2. Cirrhosis induction

In order to induce biliary cirrhosis, the bile duct was isolated and doubly ligated under ketamine (100 mg/kg ip) and diazepam (5 mg/kg ip) anesthesia as described (Mani et al., 2006). Sham-operated group had the same operation without any ligation. All the tests studies were carried out 28^{th} days post operation.

2.3. Drug administration

1-MT (Sigma-Aldrich) was dissolved in alkaline water (pH=11) in order to prepare 1, 3 and 9 mg/ml solutions. In treatment groups, 1 ml/kg of 1-MT solutions were injected intraperitoneally (ip) in such a way that rats received 1, 3 and 9 mg/kg 1-MT once a day at 9 a.m. on 7^{th}-28^{th} days (Jiang et al., 2018). Sham and BDL groups received alkaline water instead.

2.4. Electrocardiography and assessment of cardiac chronotropic response

A continues electrocardiography (ECG) was recorded under ketamine (100 mg/kg ip) and diazepam (5 mg/kg ip) anesthesia on 28th day and basal heart rate and corrected Q-T interval (cQT) were calculated (LabChart software, ADInstruments).

Moreover, the chronotropic response of heart to cumulative concentration of epinephrine was evaluated as described (Mani et al., 2006). For this purpose, the spontaneously beating atria of anesthetized rats were isolated and kept in physiological solution containing NaCl, 112; KCl, 5; CaCl2, 1.8; MgCl2, 1; NaH2PO4, 0.5; KH2PO4, 0.5; NaHCO3, 25; glucose, 10; and EDTA, 0.004 mM, 95% O2 and 5% CO2 and pH=7.4. The spontaneous atrial beating was measured under 1 g force and against cumulative concentrations of epinephrine from 10^{-9} to $3x10^{-6}$ mol/L.

2.5. Liver and kidney function tests

The collected blood samples were centrifuged 2000 x g for 10 min, 4 C˚ and the serum was used in order to evaluate the aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin and creatinine and sodium levels for evaluating liver and kidney functions respectively (Huang et al., 2006; Westwood, 1991). In addition, the spleen weight was evaluated to test the effect of pharmacological inhibition of IDO on the process of cirrhosis-induced splenomegaly.

2.6. KP metabolites measurement

The levels of tryptophan, kynurenine and kynurenic acid in liver and heart tissues were measured by high performance liquid chromatography (HPLC). For this purpose, the HPLC standards were purchased from Sigma-Aldrich (447439-T0254-K3375). Isolated liver tissue was homogenized using 0.14 M and 20 mM K2HPO4 (pH=6.5) buffer (Bartosiewicz et al., 2017). Cardiac tissue was homogenized using 5mM Tris-acetate buffer (pH=7.4), containing 50 µM pyridoxal 5ˈ-phosphate and 10 Mm mercapto-ethanol (Baran et al., 1997). Both samples were sonicated and

centrifuged at 44000 x g for 10 min at 4 C˚. The prepared samples were separated on a C18 (150x4.6 mm, 5µL) column by a HPLC system (Knauer smartline) linked to a UV detector. Tryptophan, kynurenine and kynurenic acid were detected at 280, 333 and 360 nm by UV detector. The mobile phase included; 0.1M acetic acid, 0.1M ammonium acetate (pH 4.6) containing 1.8% of acetonitrile, and it was pumped at a flow-rate of 0.2 ml/min.

2.7. Inflammatory markers measurement

With the aim of investigating the effect of cirrhosis and IDO inhibition on inflammatory biomarkers, C-reactive protein (CRP) and interleukin-6 (IL-6) levels were measured in serum samples by (ab108827) and (ab100772) Abcam ELISA kits according to the kit instructions.

2.8. Histopathological studies

Liver and heart tissues were isolated and kept in formalin 10% for histopathological studies. The sectioned tissues were stained by hematoxylin and eosin (H&E) as well as Masson trichrome. The stained liver tissues were used for the evaluation of fibrosis, ductular proliferation and inflammation as described (Hajiasgharzadeh et al., 2014). In brief, fibrosis (0, no fibrosis; 1, expansion of portal tract without linkage; 2, portal expansion with portal to portal linkage; 3, extensive portal to portal and focal portal to central linkage; 4, cirrhosis), ductular proliferation and inflammation (0, absent; 1, mild; 2, moderate; 3, severe) (Hajiasgharzadeh et al., 2014). On the other hand, the stained heart tissues were studied for histopathological changes as shown in table.1 (Sachdeva et al., 2014).

2.9. Statistical analysis

The data was analyzed by GraphPad Prism 6 through one-way analysis of variance (ANOVA) with post-hoc Tukey tests. Two-way ANOVA was also applied where the effect of two independent variables on a dependent variable was evaluated. In this study, $P<0.05$ was

considered a significant difference. The results are presented as mean ± standard error of the mean (S.E.M) unless stated otherwise.

3. Results

3.1. Cardiac parameters

The results of basal heart rate and cQT intervals are presented in Fig. 1. Heart rate decreased significantly in BDL group compared with control group ($P<0.001$). However, 1-MT administration normalized cirrhosis-induced bradycardia at 3 mg/kg and 9 mg/kg doses ($P<0.001$) (Fig. 1A). 1-MT (9 mg/kg) could also reverse the effect of biliary cirrhosis on alteration of cQT interval ($P<0.05$) (Fig. 1B).

Also the results of two-way ANOVA showed that there was a significant interaction between the effect of BDL induction and 1-MT administration on heart rate and cQT ($P<0.01$and $P<0.05$ for heart rate and cQT respectively) and in contrast to BDL+1-MT groups, heart rate and cQT did not differ significantly in sham+1-MT groups (1, 3, 9 mg/kg). (Figure not shown)

The chronotropic response of isolated atria against increasing concentrations of epinephrine (10^{-9} to $3x10^{-6}$ M) is illustrated in Fig. 2. Table.2 shows basal rate of spontaneous beating atria in the organ bath along with maximum response (R_{max}) and half maximal effective concentration (EC_{50}) of epinephrine. There was not any significant difference of basal atrial rate between groups ($P>0.05$). The impaired chronotropic response was observed in BDL group, since there was a significant increase in EC_{50} of epinephrine required for evoking the same response ($P<0.0001$), and decreased R_{max} of atria against stimulation ($P<0.0001$). In contrast, the 1-MT (3 mg/kg) decreased the EC_{50} ($P<0.0001$) and increased the R_{max} significantly ($P<0.05$). In addition,

1-MT (9 mg/kg) improved the chronotropic response by normalizing the EC50 ($P<0.0001$) and increasing the R_{max} significantly ($P<0.001$).

3.2. Liver and kidney function results

Regarding the impaired liver function in cirrhosis, hepatic transaminases (AST and ALT) and bilirubin levels were measured. As presented in Fig. 3A, the level of AST enzyme increased significantly in BDL group ($P<0.0001$). In contrary, in rats receiving all the mentioned doses of 1-MT, a significant reduction of AST was observed ($P<0.0001$). Similarly, BDL induction, raised the ALT level ($P<0.0001$) and 1-MT administration lowered the enzyme level significantly ($P<0.0001$) (Fig. 3B). Moreover, the level of total and direct bilirubin elevated in BDL group significantly ($P<0.0001$) and 1-MT brought it down to approximately control level ($P<0.0001$) (Fig. 3C and 3D).

In addition, there was a significant interaction between the effect of BDL induction and 1-MT administration on AST, ALT, total and direct bilirubin levels ($P<0.0001$). In contrast to BDL+1-MT groups, the level of mentioned parameters did not differ significantly in sham+1-MT groups (1,3,9 mg/kg) (Figure not shown).

Concerning the renal dysfunction in cirrhosis (hepatorenal syndrome), serum creatinine and sodium levels were measured. As shown in Fig. 4A, an elevated creatinine level was observed in BDL group ($P<0.0001$). In contrast the 9 mg/kg 1-MT decreased it significantly ($P<0.01$. Also, a significant hypothermia was seen in BDL group ($P<0.0001$) (Fig. 4B). Although the 3 and 9 mg/kg 1-MT increased the sodium level the difference was not statistically significant ($P>0.05$). Changes in spleen weight was investigated by calculating the ratio of spleen weight to body weight and the results are illustrated in Fig. 5. A remarkable splenomegaly was noticed in BDL group ($P<0.0001$). Contrarily, all three doses of 1-MT reduced this ratio significantly ($P<0.001$).

3.3 Levels of KP metabolites

Concentrations of tryptophan, kynurenine and kynurenic acid in liver and heart tissues are presented in Table 3. As shown in this table, the level of tryptophan increased in liver of BDL group ($P<0.0001$) however, 1-MT administration significantly decreased it in all the groups ($P<0.0001$). Similarly, the liver level of kynurenine and kynurenic acid elevated significantly in BDL group ($P<0.0001$, $P<0.01$). 1-MT reduced the kynurenine level in all treatment groups ($P<0.0001$). However, the hepatic kynurenic acid level was decreased in groups receiving 3 and 9 mg/kg 1-MT ($P<0.05$, $P<0.0001$).

The level of tryptophan decreased in heart tissue of BDL group ($P<0.0001$). Although 3 and 9 mg/kg of 1-MT elevated the tryptophan level ($P<0.0001$), there was a decrease in the group receiving 1 mg/kg 1-MT ($P<0.0001$). Cardiac kynurenine level increased in BDL group. However, this elevation was reversed by 1-MT (9 mg/kg) ($P<0.05$). In contrary, the cardiac kynurenic acid level increased in BDL group ($P<0.0001$) and 1-MT administration reversed it in two groups ($P<0.001$, $P<0.01$ in 3 and 9 mg/kg 1-MT respectively).

Considering the kynurenine/tryptophan ratio as an important indicator of IDO enzyme activity, the comparison of this ratio in liver and heart tissues of groups are shown in Fig 6. There was a remarkable increase of kynurenine/tryptophan ratio in liver and heart tissues of BDL group ($P<0.001$, $P<0.0001$) (Fig. 6A). While, the 1-MT significantly reduced the ratio at the doses of 3 and 9 mg/kg in both tissues, the lower dose of 1-MT (1 mg/kg) only decreased it in heart tissue ($P<0.01$) (Fig. 6B).

3.4. Serum inflammatory markers

Serum levels of CRP and IL-6 were measured in all experimental groups. CRP levels was elevated in BDL group ($p<0.0001$). 1-MT (9 mg/kg) was able to lower serum CRP level

significantly (P<0.0001) (Fig. 7A). While an elevated level of IL-6 was observed in BDL group (P<0.0001), the 3 and 9 mg/kg 1-MT reduced it significantly (P<0.05, P<0.001) (Fig. 7B).

Also a significant interaction between the effect of BDL and 1-MT administration on CRP and IL-6 was observed (P<0.0001, P<0.01). Although in BDL+1-MT groups the level of CRP and IL-6 decreased significantly, there was not any significant change in CRP and IL-6 of sham+1-MT (1,3,9 mg/kg) groups (Figure not shown).

3.5. Histopathology results

The quantitative results and images of histopathology studies of H&E and Masson trichrome staining are shown in Fig. 8,9 and 10 respectively. As demonstrated in Fig. 8A, a dramatic liver inflammation occurred in of BDL group (P<0.001) and 9 mg/kg 1-MT attenuated the inflammation (P<0.01). Likewise, ductular proliferation was highly observed in BDL group (P<0.0001), it was declined in groups receiving 3 and 9 mg/kg 1-MT (P<0.05, P<0.001) (Fig. 8B). Moreover, a considerable liver fibrosis was seen in BDL group (P<0.0001). All the three doses of 1-MT were able to reduce the fibrosis in liver tissue (P<0.05, P<0.05, P<0.0001) (Fig. 8C).

As presented in images, there was a severe ductular proliferation and moderate liver inflammation in BDL group (Fig. 9C). Also in this group, remarkable liver fibrosis was observed compatible with development of cirrhosis (Fig. 9D). However, 1-MT reduced the level of ductular proliferation and inflammation to mild degree (Fig. 9E), and attenuated the liver fibrosis compared to BDL group.

There was not any significant difference in cardiac specimens of control and BDL groups (Fig. 10).

4. Discussion

In the present study, we evaluated possible contribution of tryptophan-kynurenine pathway in cirrhosis-induced cardiac chronotropic dysfunction. The KP is the major route of tryptophan metabolism which is activated by TDO or IDO enzymes (Stone and Darlington, 2002). Under inflammatory conditions IDO, which converts the tryptophan to kynurenine, is the dominant enzyme responsible for KP activation (Davis and Liu, 2015). In this study the level of three important metabolites of KP such as tryptophan, kynurenine and kynurenic acid and also the kynurenine/tryptophan ratio, a potential indicator of IDO activity, was measured in the liver and cardiac samples of healthy and cirrhotic rats. In addition, the effect of 1-MT, an IDO inhibitor, was investigated in cirrhosis and complications including cardiac and renal dysfunction.

Development of cirrhosis was confirmed by the manifestation of elevated AST, ALT and bilirubin levels along with extensive liver fibrosis and inflammation and enhanced ductular proliferation. In addition, splenomegaly, a consequence of portal hypertension, was observed in BDL rats. Furthermore, hyponatremia and increased serum creatinine level in BDL group, goes along with development of cirrhosis-induced renal dysfunction (hepato-renal syndrome) which is a well-known complication of cirrhosis (Betrosian et al., 2007).

Our results showed that, cardiac chronotropic response was impaired following cirrhosis *ex vivo*, a phenomenon that was parallel with development of bradycardia in our experimental model. These results corroborate with cirrhosis-induced cardiac dysfunction that has been reported as a result of impaired β-adrenergic receptor signaling in BDL rats (Fede et al., 2015). Although previous studies have attributed these changes to increased production of NO, endocannabinoids and other endogenous mediators, the precise mechanism of cirrhosis-induced cardiac dysfunction has remained elusive (Møller and Henriksen, 2002). In the present study, a reduction in

kynurenine and kynurenic acid levels were associated with a marked improvement in development of cirrhosis-induced cardiac chronotropic dysfunction. This finding is novel and suggests a role for the KP in the pathogenesis of extrahepatic manifestations in cirrhosis. KP metabolites such as kynurenine and kynurenic acid have multiple physiological functions though interaction with their targets. For instance, most of the effects of kynurenic acid are reported to be mediated through interaction with GPR35 and N-Methyl-D-aspartate (NMDA) receptors. The activation of a G_{ai}-coupled GPR35 can impair the β-adrenergic signaling (G_{as}-coupled receptor) by reducing the formation of the second messenger cyclic adenosine monophosphate (cAMP), which in turn dampens the chronotropic response (Dadvar et al., 2018). In addition, reciprocal interaction of cannabinoids and KP metabolites have been reported (Jenny et al., 2009; Nagy-Grócz et al., 2017). IDO can be induced by cannabinoids, while kynurenine have a cross talk with cannabinoid receptor type 1 mediated signaling (Jenny et al., 2009; Nagy-Grócz et al., 2017). Moreover, the kynurenine elevation can result in cardiac depression and vasodilation through stimulating the NO formation in the endothelium (Nagy et al., 2017). Furthermore, the direct toxic effects of KP metabolites on cardiomyocytes including impaired NADH-driven complex I activity and diminished respiratory rate is another suggested mechanism through which kynurenine and kynurenic acid elevation leads to cardiac dysfunction (Baran et al., 2003; Baran* et al., 2016). In our study, the parallel increase of kynurenine and kynurenic acid level and the chronotropic dysfunction are similar to studies indicating the abnormal KP in other cardiovascular diseases. The early activation of KP predicts the mortality of cardiac arrests (Ristagno et al., 2014). In addition, accumulation of kynurenine is associated with the development of cardiomyopathy and higher plasma kynurenine and kynurenic acid levels are detected in heart failure (Dschietzig et al., 2019; Rudzite et al., 1992). Also it has shown that,

IDO inhibition limits the cardiac complications post-myocardial infarction (Melhem et al., 2021). Taken this evidence together we can suggest that the KP and its metabolites may be mechanistically associated with the pathophysiology of CCM. However, detailed mechanism of their contribution is not studied in the present investigation and awaits further research. In the present study, we surprisingly observed that the pattern of changes in the concentration of kynurenine and kynurenic acid differed in liver and heart tissues in rats given 1-MT. This might be related to impaired metabolic pathways following induction of liver regeneration and fibrosis. The KP is a linked series of chemical reactions with diverse expression of enzymes in different tissues and pathologic circumstances. For example, kynurenine aminotransferase, the key enzyme in kynurenine to kynurenic acid conversion might express differently in liver and heart tissues (Wenzel et al., 2003). In addition, liver cirrhosis is associated with altered hepatic tryptophan metabolism (Dabos et al., 2011), a phenomenon that may affect response to 1-MT when hepatic and extra-hepatic tissues are compared. Given the interlinked nature or tryptophan metabolism in the liver, understanding the exact mechanisms by which cirrhosis alters the activity of KP enzymes and metabolites, requires further research. Likewise, although the level of tryptophan was expected to increase by IDO inhibition in both liver and heart tissues, 1-MT reduced tryptophan levels in the liver while it increased tryptophan concentration in the cardiac tissue. We did not investigate this further in the present study; however, we can hypothesize that reduced hepatic tryptophan may reflect hepatoprotective effect of 1-MT in BDL rats. It is known that cirrhosis impairs tryptophan metabolism in the liver (Dabos et al., 2011). Therefore, the reduction of hepatic tryptophan level by 1-MT may be secondary to improved liver function which is confirmed by approximately normalized liver enzymes and bilirubin levels in BDL rats given 1-MT. While the focus of our research was on development of CCM in cirrhotic rats, we

unexpectedly observed that pharmacological inhibition of IDO could attenuate the severity of liver fibrosis in BDL rats. Therefore, the protective effect of 1-MT on cardiac dysfunction might be secondary to improvement of the severity of liver cirrhosis and its complications rather than a direct effect on cardiac chronotropic function. Likewise, 1-MT administration could also improve renal function by improving serum creatinine level, a phenomenon that can be secondary to improved portal hypertension and cardiovascular function in cirrhosis. The results are in agreement with the fact that, IDO is highly expressed in HCV-induced cirrhosis and hepatic fibrosis and high level of kynurenines are linked to oxidative stress and inflammation in end-stage renal diseases (Pawlak et al., 2009; Yang et al., 2019). Also it has been reported that, the arginine-dependent biosynthesis of polyamines, which is enhanced in cirrhosis, acts as an inducer of IDO activity (Mondanelli et al., 2017; Raunio et al., 1986).

Furthermore, our results showed that, inhibition of IDO was associated with a significant reduction in circulating CRP and IL-6 concentrations in BDL groups. CRP level markedly correlates with the severity of cirrhosis and IL-6 is highly associated with the cirrhosis mortality and the rate of CCM occurrence (Gregolin et al., 2020; Lahdou et al., 2013). Therefore, the reduction of CRP and IL-6 can be proposed as an additional protective effect of 1-MT on cirrhosis complications. Systemic inflammation is an important pathogenic mechanism in cirrhosis and its complication including hepatic encephalopathy. Our results on serum IL-6 and CRP levels are also in line with previous reports showing that IDO inhibition improves hepatic encephalopathy and behavioral changes in cirrhotic rats (Jiang et al., 2018).

Taken together, we can conclude that 1-MT attenuates cardiac dysfunction in rats with biliary cirrhosis. This protective effect is not limited to the cardiac function as liver histopathologic changes were also improved following chronic 1-MT administration. Future studies will pave the

way to understand the mechanisms by which the Kynurenine/IDO pathway is involved in these protective effects of 1-MT in experimental cirrhosis.

Limitations and suggestions

This study enjoyed the novelty of evaluating KP intervention in cirrhotic cardiomyopathy; however, it was a preliminary study and requires further investigations in order to find out the mechanism by which 1-MT reduces hepatic dysfunction in our experimental model and also why the level of metabolites is altered differently in studied tissues. Given the widespread effect of 1-MT on cirrhosis, it is possible that the KP may play as a hub in the network of mediators involved in pathophysiology of cirrhosis and its complications such as cardiomyopathy, hepatorenal syndrome, encephalopathy and susceptibility to hepatocellular carcinoma (HCC) (Sideras et al., 2017).

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Conflicts of interest

None.

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Fig 1. Comparison of heart rate and cQT interval (cQT) among control, sham-operated, BDL and 1-MT (BDL+1, 3 or 9 mg/kg 1-MT) groups (n=6). Data are presented as Mean ± S.E.M. (A) Basal heart rate (beats/min) results taken from ECG recording (***P<0.001 compared to control group). (### P<0.001 compared to BDL group). (B) cQT interval in different experimental groups (*P<0.05 compared to control group). (#P<0.05 compared to BDL group).

Fig 2. Chronotropic response to epinephrine stimulation in control, sham-operated, BDL and 1-MT (BDL+1, 3 or 9 mg/kg 1-MT) groups (n=6). Data are presented as Mean ± SD.

Fig 3. Liver function test results of control, sham-operated, BDL and 1-MT (BDL+1, 3 or 9 mg/kg 1-MT) groups (n=6). Data are presented as Mean ± S.E.M. (A) AST level (****P<0.0001 compared to control group). (####P<0.0001 compared to BDL group). (B) The ALT level (****P<0.0001 compared to control group). (####P<0.0001 compared to BDL group). (C) The total bilirubin level (****P<0.0001 compared to control group). (####P<0.0001 compared to BDL group). (D) The direct bilirubin level (****P<0.0001 compared to control group). (####P<0.0001 compared to BDL group).

Fig 4. Kidney function test results of control, sham-operated, BDL and 1-MT (BDL+1, 3 or 9 mg/kg 1-MT) groups (n=6). Data are presented as Mean ± S.E.M. (A) The serum creatinine level (**** P<0.0001 compared to control group). (## P<0.01 compared to BDL group). (B) The serum sodium level (**** P<0.0001 compared to control group).

Fig 5. Comparison of spleen weight between control, sham-operated, BDL and 1-MT (BDL+1,3,9 mg/kg 1-MT) groups (n=6). Data are presented as Mean ± S.E.M. (**** P<0.0001 compared to control group). (### P<0.001 compared to BDL group).

Fig 6. Comparison of kynurenine/tryptophan ratio in liver and heart tissues of control, BDL and 1-MT (BDL+1,3,9 mg/kg 1-MT) groups (n=6). Data are presented as Mean ± S.E.M. (A) Kynurenine/tryptophan ratio in liver. (*** P<0.001 compared to control group). (#### P<0.0001 compared to BDL group). (B) Kynurenine/tryptophan ratio in heart. (**** P<0.0001 compared to control group). (## P<0.01, #### P<0.0001 compared to BDL group).

Fig 7. Serum inflammatory marker levels of control, sham-operated, BDL and 1-MT (BDL+1, 3 or 9 mg/kg 1-MT) groups (n=6). Data are presented as Mean ± S.E.M. (A) Serum CRP level (**** P<0.0001 compared to control group). (#### P<0.0001 compared to BDL group). (B) Serum IL-6 level (**** P<0.0001 compared to control group) (# P<0.05, ### P<0.001 compared to BDL group).

Fig 8. Histopathological assessment of control, BDL and 1-MT (BDL+1, 3 or 9 mg/kg 1-MT) groups (n=6). Data are presented as Mean ± S.E.M. (A) Hepatic inflammation results (*** P<0.001 compared to control group) (## P<0.01 compared to BDL group). (B) Ductular proliferation comparison (**** P<0.0001 compared to control group) (# P<0.05, ### P<0.001 compared to BDL group). (C) Liver fibrosis comparison (**** P<0.0001 compared to control group) (# P<0.05, #### P<0.0001 compared to BDL group).

Fig 9. Representative hepatic histopathology images of control, BDL and 1-MT (BDL+ 9 mg/kg 1-MT) groups. (A) Liver tissue of a control rat (x100 H&E). (B) Liver tissue of a control rat (x100 Trichrome). (C) Liver tissue of a BDL rat (x 200 H&E). (D) Liver tissue of a BDL rat (x200 Trichrome). (E) Liver tissue of a BDL rat given 1-MT (9 mg/kg) (x 100 H&E). (F) Liver tissue of a BDL rat given 1-MT (9 mg/kg) (x 100 Trichrome).

Fig 10. Representative cardiac histopathology images of control and BDL groups. (A) Cardiac tissue in a control rat (x 200 H&E). (B) (C) Cardiac tissue in a control rat (x 200 Trichrome). (C) Cardiac tissue in a BDL rat (× 400 H&E). (D) Cardiac tissue in a BDL rat (×200 Trichrome).

Table 1. Grading system of histopathologic changes of heart tissue

Score	Myocardial damage (histopathological)
0	No lesions
0.5	Slight derangement of muscle fibers, few inflammatory cells and vacuoles
1	Focal lesions of the sub-endocardial portion of the apex and mid-ventricle, inflammatory cells, interstitial edema, vacuolization of myocytes
1.5	Focal lesions of the sub-endocardium of the apical and mid ventricular region with right ventricular involvement
2	Focal lesions extending over a wider area of both ventricles
2.5	Focal lesions extending over a wider area of both ventricles, extensive inflammatory cell infiltration, interstitial edema, rupture of myofibers
3	Confluent lesions of the apex, mid-left ventricle and right ventricle, extensive inflammatory cell infiltration, profuse edema
4	Confluent lesions throughout the heart

Table 2. The EC_{50} of epinephrine, basal atrial beating rate and R_{max} of groups.

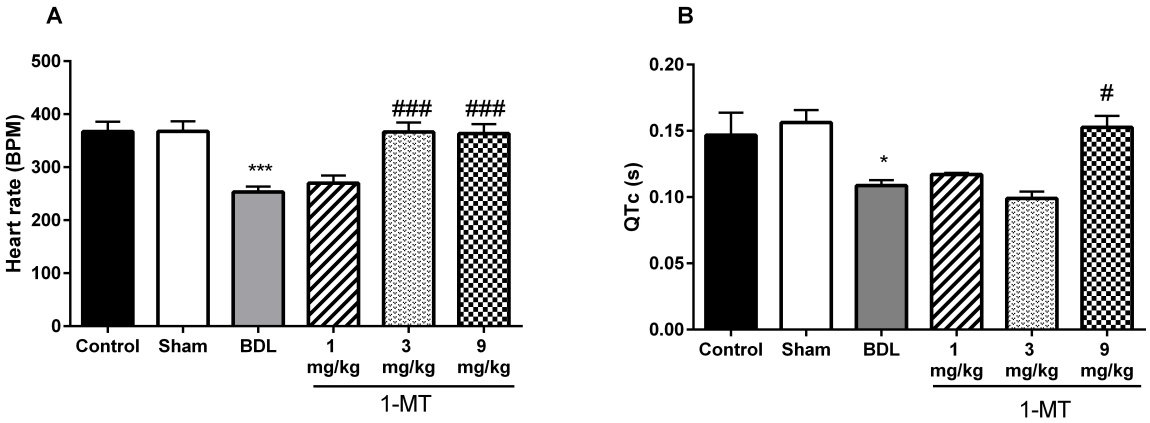
Group	log[EC_{50}]	Basal atrial beating rate (Beats/min)	R_{max} (Beats/min)
Control	-7.94 ± 0.014	357.53 ± 2.26	510.01 ± 1.44
BDL	-7.64 ± 0.023^{aaaa}	355.13 ± 1.18	475.03 ± 2.89^{aaaa}
1-MT (1 mg/kg)	-7.67 ±0.017	354.43 ± 3.83	477.99 ± 0.57
1-MT (3 mg/kg)	-7.92 ± 0.017^{bbbb}	363.13 ± 0.80	488.60 ± 0.88^{b}
1-MT (9 mg/kg)	-8.02 ± 0.031^{bbbb}	360.06 ± 1.79	495.60 ± 3.84^{bbb}

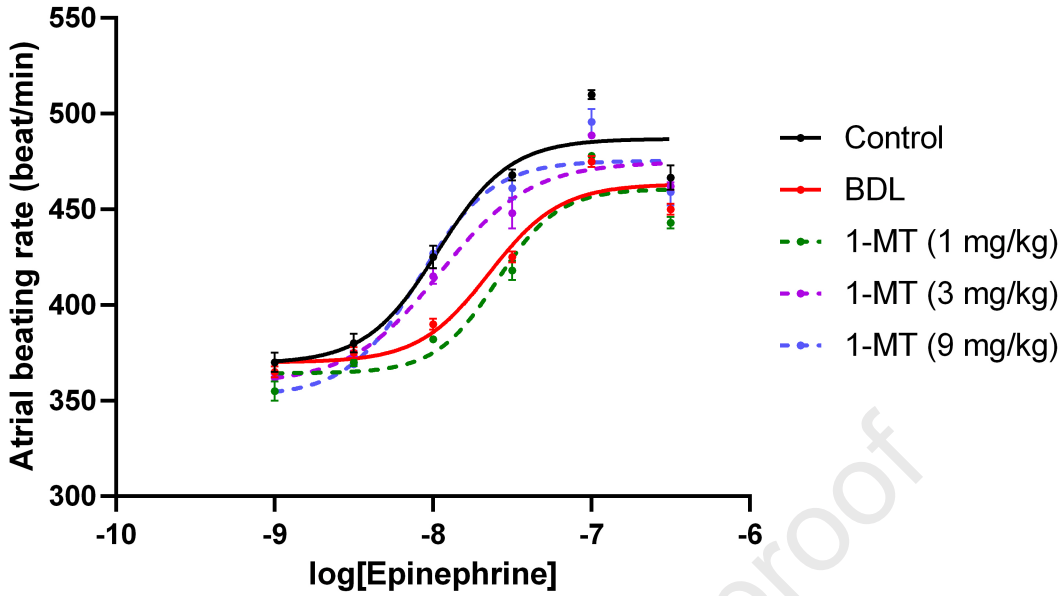
(^{aaaa} P<0.0001 compared to control group), (^{b} P<0.05, ^{bbb} P<0.001, ^{bbbb} P<0.0001 compared to BDL group). Data are presented as Mean ± S.E.M, (n=6).

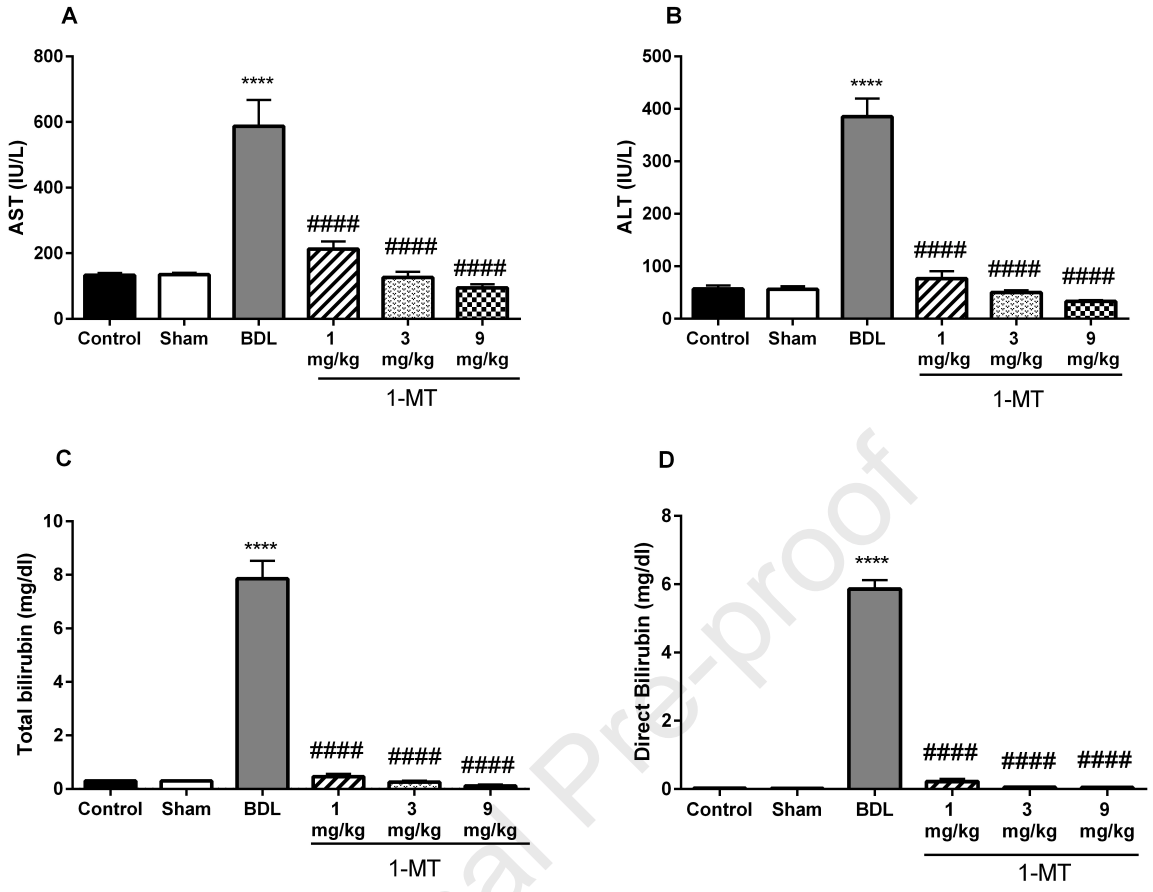
Table 3. The level of KP metabolites in liver and heart tissues measured by HPLC.

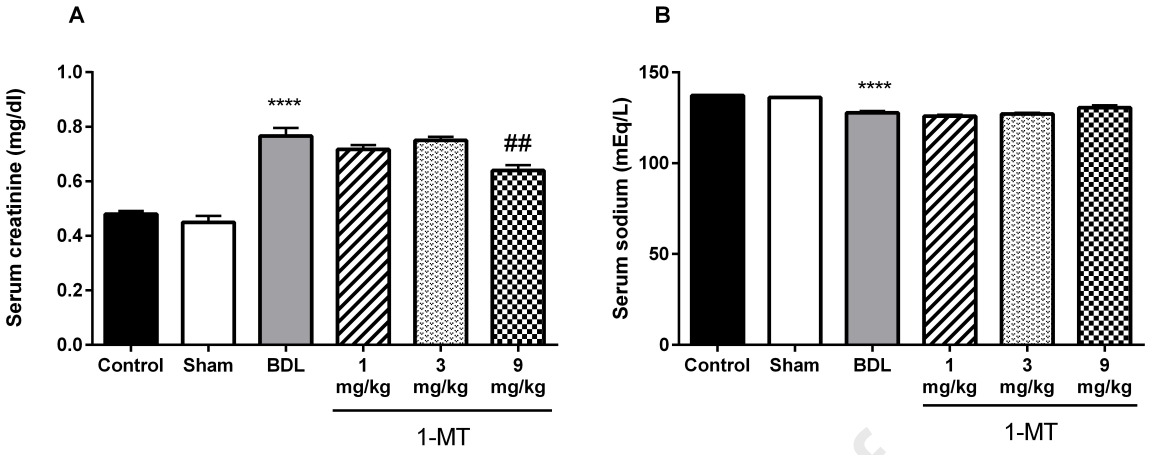
Groups	Liver			Heart		
	Trp (mg/ml)	Kyn (mg/ml)	KA (mg/ml)	Trp (mg/ml)	Kyn (mg/ml)	KA (mg/ml)
Control	0.049 ± 3.1E-03	0.00145 ± 1.45E-05	0.00166 ± 2.68E-05	0.013 ± 4.05E-04	0.00040 ± 1.79E-06	0.00148 ± 1.70E-05
BDL	0.211 ± 6.1E-03 ^{aaaa}	0.01000 ± 2.9E-04^{aaaa}	0.00173 ± 4.62E-06^{aa}	0.009 ± 1.16E-04^{aaaa}	0.00046 ± 1.74E-06^{aa}	0.00176 ± 1.59E-05 ^{aaaa}
BDL+ 1MT (1mg/kg)	0.121 ± 3.5E-03 ^{bbbb}	0.00689 ± 1.1E-04^{bbbb}	0.00171 ± 3.57E-06	0.001 $^{\pm}$ 1.74E-05 ^{bbbb}	0.00050 ± 1.65E-05	0.00177 ± 1.81E-05
BDL+ 1 MT (3 mg/kg)	0.029 ± 2.1E-04^{bbbb}	0.00045 ± 1.59E-05^{bbbb}	0.00179 ± 4.08E-06^{b}	0.011 ± 2.36E-04^{bbbb}	0.00049 ± 1.77E-06	0.00160 ± 1.76E-05 ^{bbb}
BDL+ 1 MT (9 mg/kg)	0.028 ± 2.3E-04 ^{bbbb}	0.00067 ± 1.16E-05^{bbbb}	0.00189 ± 2.09E06^{bb} ^{bb}	0.014 ± 1.76E-04^{bbbb}	0.00050 ± 2.89E-06^{b}	0.00163 ± 1.69E-05^{bb}

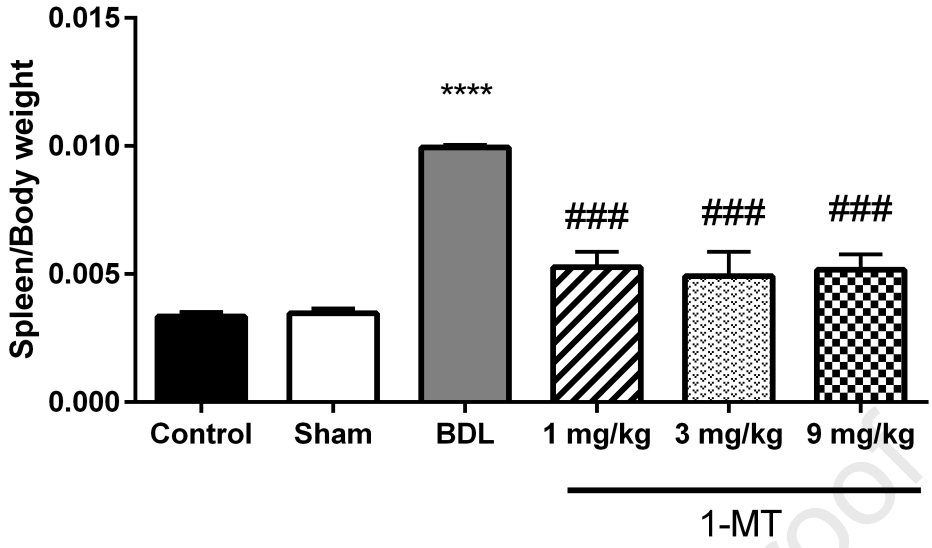
Trp: Tryptophan, Kyn: Kynurenine, KA: Kynurenic acid. (^{aa} P<0.001, ^{aaaa} P<0.0001 compared to control group). (^{b} P<0.05, ^{bb} P<0.01, ^{bbb} P<0.001, ^{bbbb} P<0.0001 compared to BDL group). Data are presented as Mean ± S.E.M, (n=6)

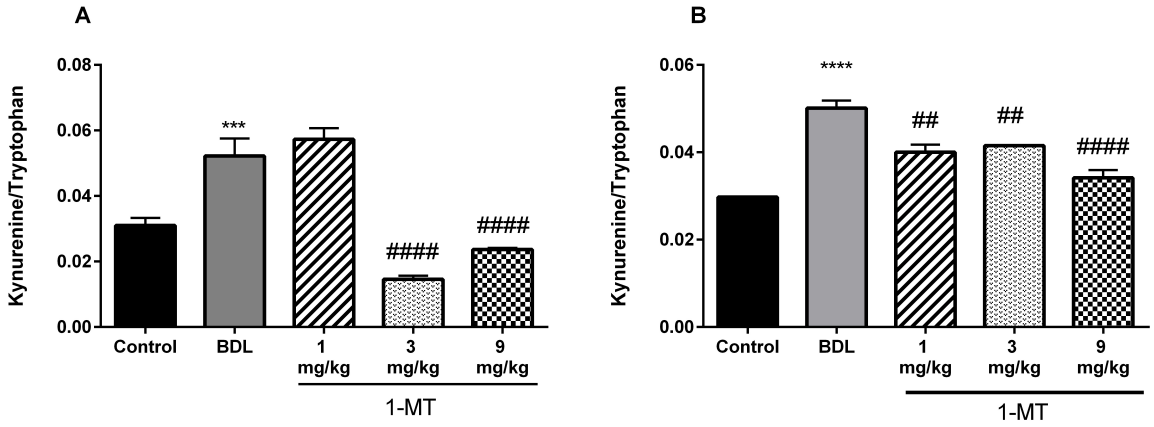


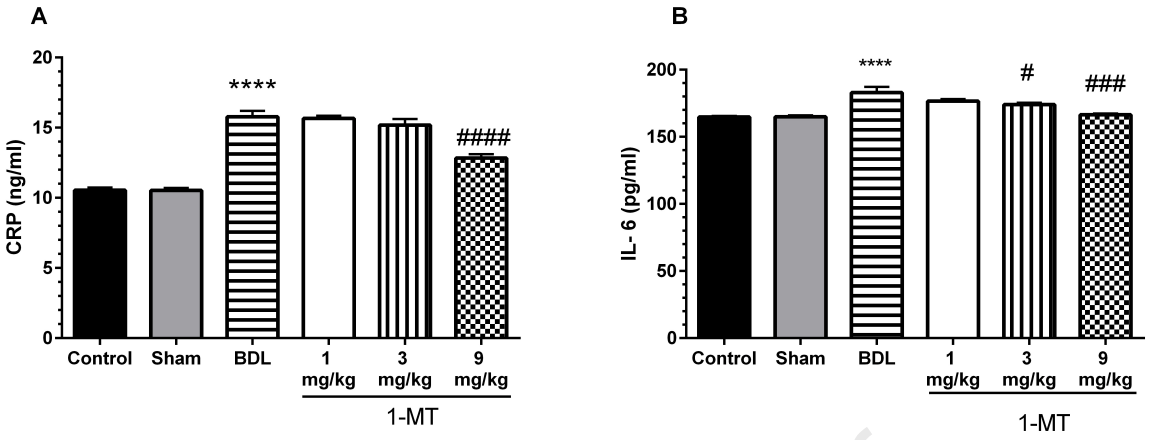


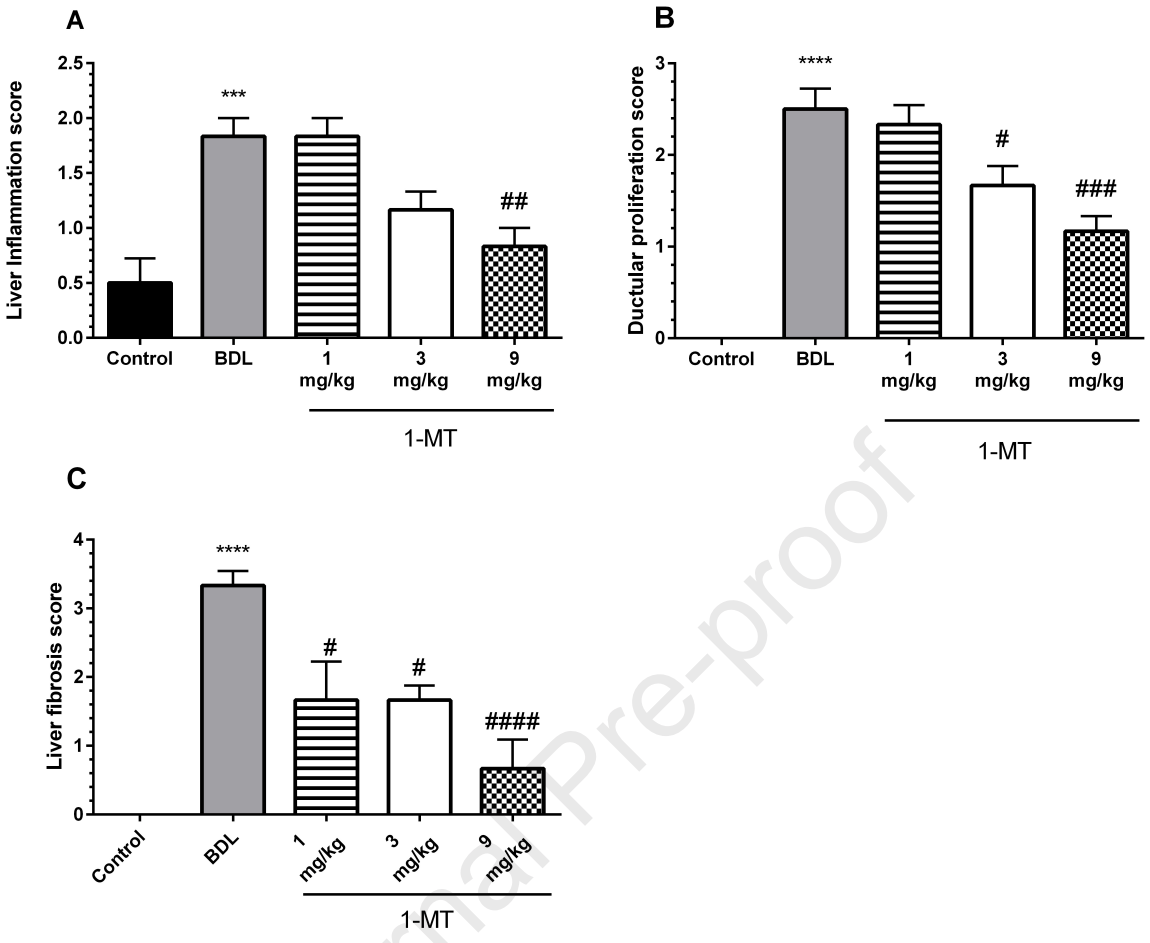


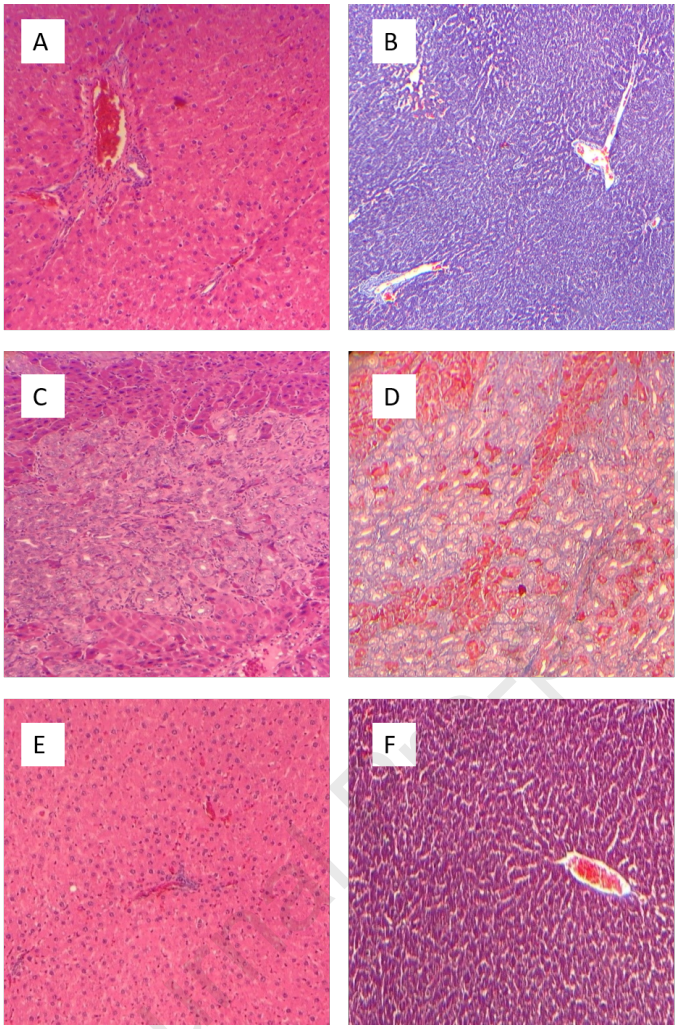


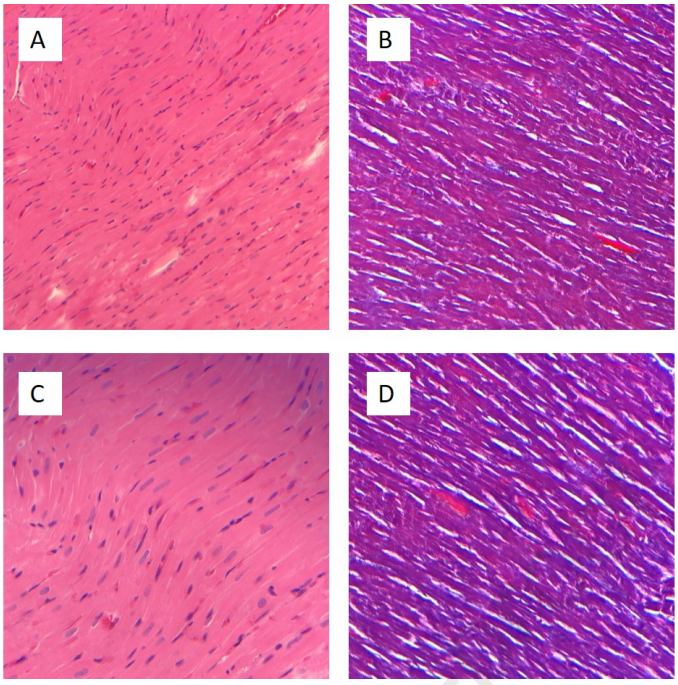












Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Amir Rashidian, 2021/07/02

Hedyeh Faghir-Ghanesefat, 2021/07/02

Ali R. Mani, 2021/07/02

Seyed Mohammad Tavangar, 2021/07/02

Ahmad R. Dehpour, 2021/07/02