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RESEARCH PAPER

The protective effects of curcumin and curmumin nanomicelle against cirrhotic cardiomyopathy in bile duct-ligated rats

Mohammad Sheibani [1, 2], Ahmad Reza Dehpour [1, 2], Sadaf Nezamoleslami [1, 2], Seyedeh Elaheh Mousavi [1], Mahmoud Reza Jafari [3], Seyed Mahdi Rezayat Sorkhabadi [1]*

[1]*Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[2]*Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran*
[3]*Department of Pharmaceutics, Mashhad University of Medical Sciences, Mashhad, Iran*

ABSTRACT

Objective(s): Cirrhotic cardiomyopathy refers to cardiac muscle dysfunction caused by liver cirrhosis. Seemingly, free radicals and inflammatory factors play a critical role in the pathophysiology of cardiomyopathy. Curcumin has the anti-inflammatory, antioxidant, and anticancer properties . However, the therapeutic indications of this compound are limited due to its low absorption, rapid metabolism, and low bioavailability. Curcumin nanomicelle is a form of nanoparticle developed to overcome the poor kinetic profile of curcumin and enhance its bioavailability and therapeutic effects. The present study aimed to develop an experimental model of cirrhosis induced by biliary duct ligation in rats.

Materials and Methods: The animals were kept until 28 days after the bile duct ligation and received curcumin or curcumin nanomicelle via oral gavage at various doses during days 7-28. After the intervention, the effects of curcumin and curcumin nanomicelle on cardiovascular function, some inflammatory and antioxidant biomarkers, and histopathological changes were assessed.

Results: According to the findings, cardiac electrophysiology function and contractile force improved only in the curcumin nanomicelle groups. In addition, curcumin nanomicelle significantly reduced inflammatory factors and increased antioxidant enzymes. In the histopathological studies, cardiac tissue damage and destruction were observed to decrease in the curcumin nanomicelle groups.

Conclusion: Therefore, it was concluded that curcumin nanomicelle plays a protective role in cirrhotic cardiomyopathy by reducing inflammatory and oxidative factors and improving the cardiac function. Furthermore, curcumin nanomicelle exhibited more significant therapeutic effects compared to the curcumin treatment groups.

Keyword: *Cirrhosis, Cardiomyopathy, Curcumin, Nanomicelle, Inflammation*

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INTRODUCTION

Cardiomyopathy refers to cardiac muscle damage and ventricular dysfunction [1], which may be associated with electrophysiological abnormalities and symptoms of heart failure, such as including dyspnea, edema, fatigue, and death [2]. Cardiomyopathy is of several types, including dilated, restrictive, hypertrophic, and cirrhotic cardiomyopathy [2, 3]. Ample evidence suggests that regardless of etiology, liver cirrhosis could cause cardiac dysfunction and cardiomyopathy [4-7]. This phenomenon has been observed in alcoholic and non-alcoholic individuals with liver damage [4, 5]. In the past, the main cause of cardiomyopathy was liver cirrhosis due to alcohol consumption, while recently, this issue has been reported to be common in non-alcoholic individuals as well [6, 7].

Various mechanisms have been proposed for the development of cirrhotic cardiomyopathy, such as the increased production of mediators (e.g., nitric oxide, carbon monoxide, hydrogen sulfide, and endocannabinoids) [8, 9]. Changes in the membrane fluidity and density of the beta-adrenergic receptors in myocytes have

* *Corresponding Author Email: rezayat@sina.tums.ac.ir*
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also been reported in the pathophysiology of cirrhotic cardiomyopathy [10, 11]. Furthermore, some studies have suggested that inflammatory cytokines such as interleukin (IL-1β) and tumor necrosis factor alpha (TNF-α) may play a critical role in the pathophysiology of cirrhotic cardiomyopathy [12, 13].

To date, several studies have been focused on various models for the treatment and control of cirrhotic cardiomyopathy. Nevertheless, no definitive treatments have been proposed for the clinical management of this extrahepatic complication of cirrhosis. Among various experimental models, the induction of cirrhotic cardiomyopathy by bile duct ligation has proven efficient in this regard [12, 14].

Curcuminoids are turmeric molecules, which are composed of curcumin, dimetoxycurcumin, and bisdimetoxycurcumin compounds. These compounds have exhibited anti-inflammatory, antioxidant, antifungal, and antiviral effects in various studies [15]. Curcuminoids are extracted from the roots of *Corcuma longa* and were used as coloring compounds and spices, as well as for therapeutic purposes, in the past [15, 16]. Curcumins are polyphenol compounds, the anti-inflammatory effects of which have been widely investigated in various inflammatory models, and their protective effects on hepatotoxicity and liver damage are most notable [15, 17, 18]. In some cardiac injury models (e.g., diabetic cardiomyopathy and ischemia), the protective effects of curcumins have also been demonstrated [19, 20]. Despite the positive effects of curcumins, their therapeutic use is limited due to poor bioavailability, poor oral absorption, and rapid hepatic elimination [21]. Drug nanoformulations could enhance the pharmacokinetic profile of medicines, while also reducing drug side-effects and toxicity. In addition, some of their dosages could improve the therapeutic effects of drugs [22, 23]. Nanomicelles are widely used in various pharmaceutical forms in nanotechnology. These compounds have a spherical shape and are composed of a hydrophobic inner part, and formulated drugs are placed inside this layer. The outer layer is hydrophilic, which facilitates the dissolution of the compound, thereby increasing the absorption and enhancing its solubility and therapeutic effects [24]. Several studies have reported the importance of curcumin nanoformulations as effective therapeutic agents in several inflammatory experimental models [25, 26]. The present study aimed to investigate the protective and therapeutic effects of curcumin nanomicelle on the animal model of cirrhotic cardiomyopathy.

MATERIALS AND METHODS

Ethical considerations

The protocol of this experimental study was approved by the Ethical Committee of Tehran University of Medical Sciences and performed in accordance with the ethical guidelines for animal studies (IR.TUMS.VCR.REC.1398.003). Based on the International Guiding Principles for Biomedical Research Involving Animals (1985), male Wistar rats weighing 250-300 grams were kept in standard cages within a 12-hour light/dark cycle at the temperature of 22±2°C. The animals had access to food and water *ad libitum*.

Experimental drugs

Curcumin and curcumin nanomicelle were purchased from Exir Nano Sina Industrial Company (Tehran, Iran) and dissolved in almond oil and saline, respectively. All the medications were administered via oral gavage (P.O.).

Study group design

The animals were allocated to seven groups of eight. Group I included normal rats (without procedure), group II included sham-operated rats (only abdominal surgery and receiving vehicle without bile duct ligation), group III (control animals with bile duct ligation), group IV (rats with bile duct ligation and oral gavage of curcumin [300 mg/kg]), group V (animals with bile duct ligation and oral gavage of curcumin [600 mg/kg]), group VI (animals with bile duct ligation and oral gavage of curcumin nanomicelle [100 mg/kg]), and group VII (animals with bile duct ligation and oral gavage of curcumin nanomicelle [200 mg/kg]).

After seven days of bile duct ligation (BDL), the treatment groups received the treatment daily via oral gavage for 28 days [27, 28]. Before the main experiment, a pilot study was performed using various doses of curcumin and curcumin nanomicelle were administered to the animals. After the analyses, the doses yielded the optimal results and were selected for the main study.

Surgical procedure

Initially, the rats were anesthetized with

ketamine (80 mg/kg), their abdominal skin was shaved, and prep and drep were performed with betadine for the disinfection of the surgery site. The surgical procedure continued in sanitary conditions using microsurgical instruments. The common bile duct was exposed through a midline abdominal incision and ligated into two parts, including a distal portion (before entrance to the pancreas) and a proximal portion (below the hepatic duct junction) with a non-absorbable polypropylene suture (0-7). Afterwards, the common bile duct was dissected in the middle in order to prevent re-canalization. Finally, the abdominal wall was closed in two layers [29, 30].

After seven days of BDL surgery, the animals in the treatment groups were administered with curcumin and curcumin nanomicelle daily via oral gavage. All the experiments were carried out 28 days after the BDL [31]. Following that, the animals were sacrificed, and blood samples and heart tissues were obtained for analysis. To confirm liver cirrhosis, the histopathological changes in the spleen weight (Fig 1) and liver tissue were investigated [32].

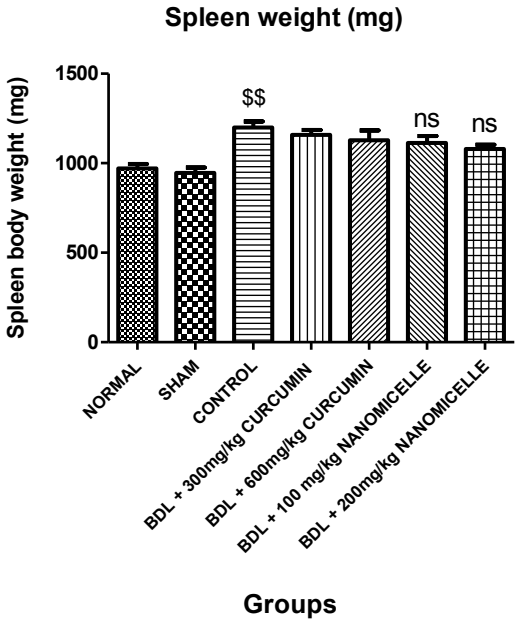

Fig 1. Spleen Weight of Animals in Study Groups (Results expressed as Mean±SEM; number of animals in each group: 8; $$P<0.01 control group compared to normal group; treatment groups not statistically significant compared to control)

Spleen weight measurement

The spleen weight is correlated with liver fibrosis and cirrhosis [33]. In the current research, the spleen of each animal was dissected and weighed after washing with normal saline.

Inflammatory cytokine assay

TNF-α and IL-1β were assessed in the serum samples of the rats as the key inflammatory factors using the rat tumor necrosis factor-α ELISA kit (RAB0479, Sigma-Aldrich, USA) and rat IL-1 β ELISA kit (RAB0277, Sigma-Aldrich, USA), respectively. In addition, the absorbance of the samples was measured at 450 nanometers using an ELISA reader (Bio-Tek Synergy HT, USA). The levels of TNF-α and IL-1β were expressed as pg/ml. The assessment of IL-10 as an anti-inflammatory cytokine was also carried out using the ELISA kit in accordance with the instructions of the manufacture.

Evaluation of lipid peroxidation and oxidative stress

At this stage, malondialdehyde (MDA) was produced as a marker to measure the level of oxidative stress in various organisms [34]. The heart ventricle that was separated from the sacrificed animals was preserved at the temperature of -80̊C to assess the tissue levels of MDA. The procedure was performed based on the described protocol in the references [35].

Evaluation of anti-oxidative enzymes

Superoxide dismutase (SOD) is an antioxidant molecule, which plays a key role in the reduction of oxidative stress and inflammatory markers [36]. In this study, the collected tissues were centrifuged at 15,000x grams and temperature of 4°C for 20 minutes after using a mechanical homogenizer. Following that, the supernatant was separated and used for the analysis of the total cardiac tissue SOD activity using the ELISA kit (ab65354).

The contents of total protein and reduced glutathione [GSH] were measured in the heart tissues. In addition, the heart sections of the study groups were homogenized and centrifuged at 100,000x grams and temperature of 4°C for 30 minutes in phosphate-EDTA buffer solution containing 25% HPO_3. Afterwards, 4.5 milliliters of the phosphate-EDTA buffer was added to the collected supernatant. Two milliliters of the final reaction mixture (containing the phosphate-EDTA buffer, diluted tissue supernatant, and o-phthalaldehyde solution) were mixed and incubated at room temperature for 15 minutes.

Finally, the absorbance of the samples was detected at 350 nanometers via fluorescence [37].

Evaluation of the left ventricular papillary muscle contractile force

At this stage, the animals were anesthetized via the intraperitoneal administration of ketamine. The heart of the animals were rapidly separated and washed with saline. The left ventricular papillary muscles were exposed and isolated to be placed in a physiological salt solution as described by Yarmohammadi et al. [38, 39].

Electrocardiography (ECG)

Before the animals were sacrificed, ECG was performed using the PowerLab data acquisition system (Chart 5.5, AD Instruments, PowerLab, UK). The rats were anesthetized with ketamine (80 mg/kg I.P.; Sigma, USA). Electrode needles were placed under the skin to record the electrophysiological function of the heart for the evaluation of the ECG markers, such as QRS complexes, RR, and QT intervals.

Histological studies

For the histopathological studies, the tissue blocks of the left ventricle were separated from the heart. Tissue samples (thickness: 4 µm) were stained using the H&E stain, and the heart tissue sections were studied under the microscope (20x magnification). According to the histopathological evidence for cardiomyopathy, cardiomyocytes were analyzed in terms of tissue damages such as cytoplasmic vacuolization, intracellular edema, tissue disarrangement, neutrophil infiltration, and rupture of the myocardial fibers [40].

Statistical analysis

The obtained data were expressed as mean and standard error of the mean (SEM). Data analysis was performed using one-way analysis of variance (ANOVA) in the GraphPad Prism software version 5, followed by Tukey's post-hoc test. The differences in the obtained values were considered significant at $P<0.05$ in all the statistical analyses.

RESULTS

Effects of bdl on the histopathological changes in the spleen weight and liver

Fig 1 depicts the effect of BDL on the spleen weight. Compared to the normal group, the spleen weight significantly increased in the control animals ($P<0.001$), which confirmed cirrhosis in these rats. Moreover, yellowish pigmentation was observed in the ears, skin body, and urine of the cirrhotic rats. According to the findings, curcumin nanomicelle had no significant effects on the changes in the spleen weight of the animals.

The histopathological study of the liver indicated no evidence of inflammation, confluent necrosis or steatosis. In the H&E evaluation, no interface activity and significant fibrosis were observed, and the hepatic architecture was preserved in the normal group (Fig 2-A), while in the control group, the microscopic assessment revealed that the liver architecture was distorted due to BDL-induced damage, and marked ductular proliferation were observed as well.

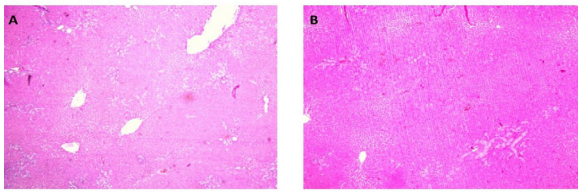

Fig 2. Liver Histopathological H&E Staining Studies in A) Normal Group and B) Control Group (Based on H&E evaluation, no interface activity and significant fibrosis were observed, and hepatic architecture was preserved in normal group; in control group, liver architecture was distorted by BDL damage, and marked ductular proliferation were observed; mild microvesicular changes, foci of pericentral confluent necrosis, bile plugs, and neutrophil infiltration were identified; stage of fibrosis in control group was estimated at 6/6, equivalent to modified healthcare-associated infections [HAI] stage)

Furthermore, mild microvesicular changes, foci of pericentral confluent necrosis, bile plugs, and neutrophil infiltration were detected. The stage of fibrosis in the control group was estimated at 6/6, which was equivalent to the modified healthcare-associated infections (HAI) stage (Fig 2-B).

Inflammatory and anti-inflammatory cytokine assay

The serum level of TNF-α was evaluated as an important inflammatory marker. As is depicted in Fig 3-A, TNF-α significantly increased in the control group compared to the normal group ($P<0.001$). On the other hand, the serum levels of TNF-α significantly decreased in the groups administered with 100 and 200 mg/kg of curcumin nanomicelle compared to the control group ($P<0.05$ and $P<0.01$, respectively). In the treatment groups administered with 300 and 600 mg/kg of curcumin, the reduction was not

considered significant compared to the controls, while a significant difference was observed in the serum levels of TNF-α between the treatment groups administered with 200 mg/kg of curcumin nanomicelle and 600 mg/kg of curcumin (P<0.05).

The analysis of the IL-1β serum levels indicated a significant increase in the control group compared to the normal group (P<0.001). In the treatment groups administered with 100 and 200 mg/kg of curcumin nanomicelle, serum IL-1β levels were similar to the normal group (P<0.01 and P<0.001, respectively).

In addition, the comparison of the treatment groups administered with 200 mg/kg of curcumin nanomicelle and 600 mg/kg of curcumin indicated that the reduction in the serum levels of IL-1β was only significant in the curcumin nanomicelle group (P<0.001) (Fig 3-B).

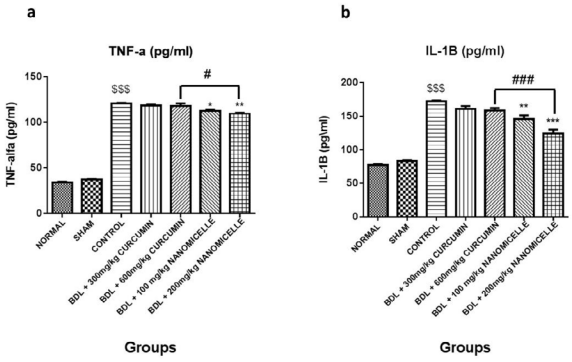

Fig 3. Serum Levels of A) TNF-α and B) IL-1β in Study Groups (Results expressed as Mean±SEM; number of animals in each group: 8; $$$P<0.001 control group compared to normal group; *P<0.05, **P<0.01, and ***P<0.001 compared to control; #P<0.05 and ###P<0.001 BDL + 200 mg/kg of nanomicelle compared to BDL + 600 mg/kg of curcumin)

In the assessment of the protective effects of curcumin and curcumin nanomicelle, IL-10 was considered an anti-inflammatory cytokine. As is shown in Fig 4, the IL-10 levels in the control, normal, and sham groups had no significant changes, while in the animals receiving 600 mg/kg of curcumin and 100 and 200 mg/kg of curcumin nanomicelle, the serum levels of IL-10 were higher compared to the controls (P<0.05, P<0.05, and P<0.001, respectively).

Furthermore, curcumin nanomicelle administration at the concentration of 200 mg/kg exerted more potent effects on the serum levels of IL-10 compared to the curcumin concentration of 600 mg/kg (P<0.05).

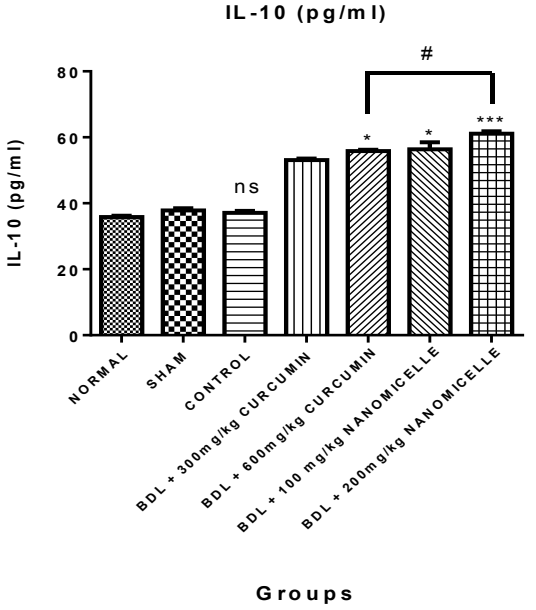

Fig 4. Serum Levels of IL-10 in Study Groups (Results expressed as Mean±SEM; number of animals in each group: 8; control group not statistically significant compared to normal group; *P<0.05 and ***P<0.001 compared to control; #P<0.05 BDL + 200 mg/kg of nanomicelle compared to BDL + 600 mg/kg of curcumin)

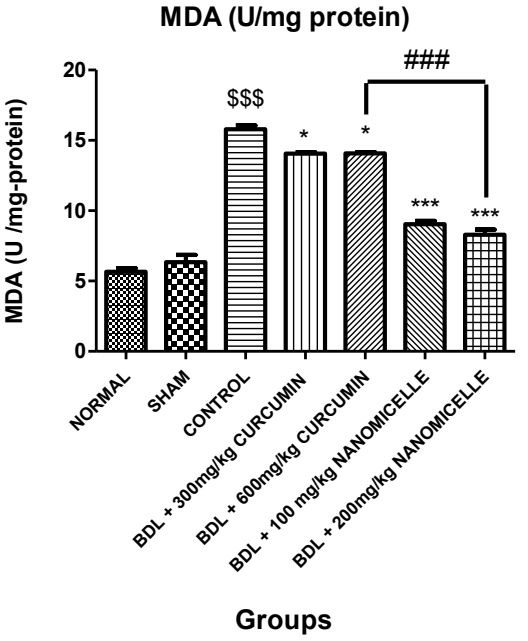

Fig 5. Cardiac Tissue Levels of MDA in Study Groups (Results expressed as Mean±SEM; number of animals in each group: 8; $$$P<0.001 control group compared to normal group; *P<0.05 and ***P<0.001 compared to control; ###P<0.001 BDL + 200 mg/kg of nanomicelle compared to BDL + 600 mg/kg of curcumin)

Oxidative stress analysis

In the assessment of the oxidative pathways, the activity of the tissue levels of MDA was also analyzed.

As is depicted in Fig 5, the MDA level was higher in the control BDL group compared to the normal group (P<0.001), while this trend significantly decreased in the treatment groups receiving 100 and 200 mg/kg of curcumin nanomicelle (P<0.001).

In the animals receiving 300 and 600 mg/kg of curcumin, a significant reduction was observed in the MDA levels compared to the controls (P<0.05). In addition, a significant difference was observed between the treatment groups receiving 200 mg/kg of curcumin nanomicelle and 600 mg/kg of curcumin regarding the tissue levels of MDA (P<0.001). In fact, the animals treated with 200 mg/kg of curcumin nanomicelle had the most positive response to the reduction of the MDA levels.

Antioxidants Enzyme Evaluation

SOD is an important antioxidant enzyme in numerous pathophysiological states. The results of the present study indicated a significant decrease in the tissue activity of SOD in the BDL group (Fig 6-A).

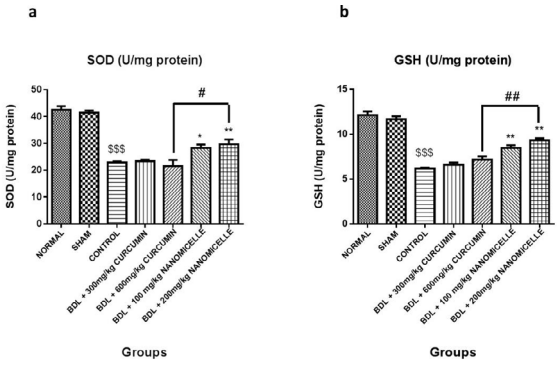

Fig 6. Cardiac Tissue A) SOD Levels and B) GSH Levels in Study Groups (Results expressed as Mean±SEM; number of animals in each group: 8; $$$P<0.001 control group compared to normal group; *P<0.05 and **P<0.01 compared to control; #P<0.05 and ##P<0.01 BDL + 200 mg/kg of nanomicelle compared to BDL + 600 mg/kg of curcumin)

Moreover, the SOD level significantly increased in the animals administered with 100 and 200 mg/kg of curcumin nanomicelle compared to the controls (P<0.05 and P<0.01, respectively). This difference was also considered significant between the treatment groups receiving 200 mg/kg of curcumin nanomicelle and 600 mg/kg of curcumin (P<0.05).

In the current research, the GSH level was assessed as an important antioxidant enzyme. The obtained results in the control group indicated that the production of this biomolecule significantly reduced compared to the normal group (P<0.001). In the treatment groups administered with 100 and 200 mg/kg of curcumin nanomicelle, the GSH level significantly increased in the heart tissues compared to the controls (P<0.01). In addition, the GSH level was significantly higher in the animals administered with 200 mg/kg of curcumin nanomicelle compared to those receiving 600 mg/kg of curcumin (Fig 6-B). Therefore, it could be concluded that the optimal response to increased GSH was obtained with the curcumin nanomicelle concentration of 200 mg/kg (Fig 6).

Papillary Muscle Contraction and Excitation

The contractile force (NM^2) of the isolated left ventricular papillary muscle was measured using an organ bath following the electrical stimulation of the isolated papillary muscle. As is shown in Fig 7-A, the contractile force of the heart due to the heart damage caused by BDL significantly decreased in the control group compared to the normal group (P<0.001).

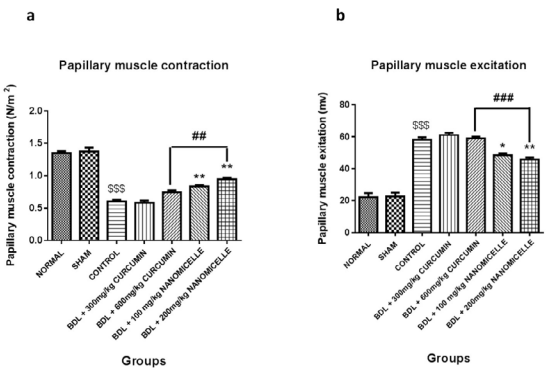

Fig 7. Papillary Muscle Contraction in Study Groups (Results expressed as Mean±SEM; number of animals in each group: 8; $$$P<0.001 control group compared to normal group; **P<0.01 and *P<0.05 compared to control; ##P<0.01 and ###P<0.001 BDL + 200 mg/kg of nanomicelle compared to BDL + 600 mg/kg of curcumin)

In the treatment groups administered with 100 and 200 mg/kg of curcumin nanomicelle, the contractile force of the heart muscle significantly enhanced compared to the controls and was similar to the value obtained in the normal group (P<0.01).

We also investigated the excitation threshold

required to stimulate the papillary muscle for muscle contraction in the organ bath (Fig 7-B). According to the findings, higher voltage was required for the contraction of the papillary muscles of the heart in the control (BDL) group compared to the normal group (P<0.001). In the treatment groups receiving 100 and 200 mg/kg of curcumin nanomicelle, the papillary muscle of the heart was contracted with a lower electrical voltage compared to the controls (P<0.05 and P<0.01, respectively). On the other hand, the comparison of treatment with 200 mg/kg of curcumin nanomicelle and 600 mg/kg of curcumin indicated a significant difference in the excitation threshold (P<0.001).

Electrophysiological Change Analysis

The electrophysiological activity of the heart of the animals was investigated to assess the protective effects of curcumin nanomicelle and curcumin on cardiac conductivity (Table 1).

The QRS, QT, and RR intervals in the control (BDL) group significantly increased compared to the normal group (P<0.001). However, no significant changes were observed in ECG in the treatment groups administered with 300 and 600 mg/kg of curcumin, while the QRS, QT, and RR intervals significantly decreased in the animals receiving 200 mg/kg of curcumin nanomicelle (P<0.01). In the group treated with 100 mg/kg of curcumin nanomicelle, only the QT and QRS intervals improved significantly compared to the control group (P<0.05). Moreover, comparison of the animals treated with 200 mg/kg of curcumin nanomicelle and 600 mg/kg of curcumin group in terms of the ECG showed the only significant difference in the QT and RR intervals (P<0.05).

Histopathological Studies

After sacrificing the animals, their heart tissues were separated and examined histologically to assess tissue injury in the study groups.

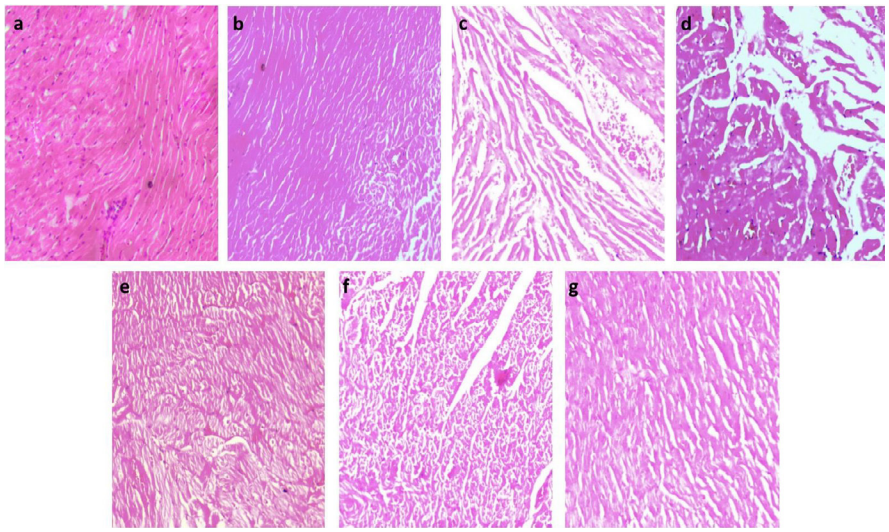

Fig 8. A, B) No Edema, Disarrangement, Hemorrhage or Tissue Damage in Normal and Sham Groups; C) Hemorrhage, Moderate-to-severe Intercellular Edema, Tissue Degradation, Neutrophilic Infiltration, and Eosinophilia In control (BDL) Group; D) No Significant Difference between Control Group and Curcumin Treatment Group (300 mg/kg); E) Lower Neutrophilic Infiltration, Tissue Disarrangement, and Hemorrhage in Curcumin Treatment Group (600 mg/kg); F) Minimized Tissue Damage and No Significant Intercellular Tissue Edema, Hemorrhage, and Neutrophilic Infiltration in Curcumin Nanomicelle Treatment Group (100 mg/kg); G) Minimized Tissue Damage Near to Normal/Sham Groups and No Significant Intercellular Tissue Edema, Hemorrhage, and Neutrophilic Infiltration in Curcumin Nanomicelle Treatment Group (200 mg/kg)

Table 1. Electrophysiological Changes in Study Groups

	Normal	Sham	Control	BDL + Curcumin (300 mg/kg)	BDL + Curcumin (600 mg/kg)	BDL + Curcumin Nanomicelle (100 mg/kg)	BDL + Curcumin Nanomicelle (200 mg/kg)
QT interval	56.5±1.55	58.25±1.37	100.8±3.68	97.75±3.42	91.5±2.02	87±2.12*	83.5±1.84**#
QRS	16.5±0.64	17.25±0.94	24±0.81	23±0.81	21.75±0.85	20±0.40*	21.10±0.50*
RR Interval	159.5±3.37	161.3±3.77	249.8±5.40	245.3±5.41	240.3±4.40	236.5±2.75	222.5±2.63**#

Results expressed as Mean±SEM; number of animals in each group: 8; *P<0.05 and **P<0.01 compared to control; #P<0.05 compared to curcumin treatment (600 mg/kg)

No edema, disarrangement, hemorrhage or tissue damage was observed in the normal and sham groups, while hemorrhage, moderate-to-severe intercellular edema, tissue degradation, neutrophilic infiltration, and eosinophilia were observed in the control (BDL) group. In the treatment groups administered with 300 and 600 mg/kg of curcumin, no significant differences were observed with the control group, while the rates of neutrophilic infiltration, tissue disarrangement, and hemorrhage were lower comparatively. In the treatment groups administered with 100 and 200 mg/kg of curcumin nanomicelle (especially at the dose of 200 mg/kg), tissue damage was minimized and was highly similar to the normal and sham groups. Furthermore, no significant intercellular tissue edema, hemorrhage, and neutrophilic infiltration were observed (Fig 8).

DISCUSSION

The present study aimed to investigate the anti-inflammatory, antioxidant, and cardioprotective effects of curcumin nanomicelle on a rat model of cirrhotic cardiomyopathy. Curcumin nanomicelle with administered at the concentrations of 100 and 200 mg/kg, and curcumin with administered at the doses of 300 and 600 mg/kg via gavage after seven days of BDL induction. According to the obtained results, curcumin nanomicelle (especially at the dose of 200 mg/kg) exerted significant protective effects on the cardiac function, while also reducing inflammatory cytokines and inducing antioxidant enzymes. The comparison of the treatment groups receiving curcumin nanomicelle (200 mg/kg) and curcumin (600 mg/kg) indicated the significant positive effects of curcumin nanomicelle on all the evaluated factors. Moreover, histopathological changes were observed to decrease in the animals treated with curcumin nanomicelle compared to the control group.

Cirrhotic cardiomyopathy is a cardiovascular abnormality in cirrhotic patients, which is combined with liver dysfunction. It has been described as a combination of electrophysiological disorders and systolic and diastolic dysfunction. In cirrhotic rats, QT interval prolongation and ventricular electrical dysfunction may occur due to the reduction of the potassium currents in the ventricular myocytes [41]. In this regard, some studies have indicated lower papillary muscle contractile force compared to the rates with induced cirrhotic cardiomyopathy [42]. The other factors that have been reported to be involved in cardiomyocyte impairment and apoptosis include inflammatory factors (e.g., nitric oxide, carbon monoxide, and endocannabinoids) and cytokines (e.g., TNF-α) [4]. Several experimental and clinical studies have investigated the interventional [43] and anti-inflammatory approaches to the treatment of cirrhotic cardiomyopathy [44]. For instance, Nagarkatti P. et al. (2008) reported that the inhibition of inflammation enhanced the myocardial contractility of cirrhotic rats.

The beneficial anti-inflammatory properties of curcumin have been demonstrated in numerous experimental and animal models. Curcumin has been reported to reduce the production of pro-inflammatory cytokines (e.g., TNF-α and IL-1β) and increase the expression of anti-inflammatory cytokines (e.g., IL-10) (45, 46). In a study in this regard, Siddiqui et al. (2006) stated that curcumin could inhibit the endotoxin-induced increment in TNF-α expression in an experimental sepsis model. Various pharmacological functions have also been attributed to curcumin, such as antioxidant properties [47]. In addition, studied have shown curcumin to be highly capable of interacting with some of the molecular targets that are involved in inflammation [48].

According to clinical trials, curcumin may have the potential to be used as a therapeutic compound in the treatment of some disorders, such as arthritis, inflammatory bowel disease, and anterior uveitis, as well as some malignancies [49]. However, a major limitation with the application of curcumin is its low bioavailability [49]. Some data have indicated that nanomicelles could improve the solubility, metabolism, oral bioavailability, and stability of curcuminoids [50]. The formulation of curcumin nanomicelle could significantly enhance the therapeutic efficacy and bioavailability of curcumin [50, 51]. Furthermore, curcumin nanoparticles have exhibited cardioprotective effects against cardiomyocyte apoptosis and aging-related diseases. Nanomicelle formulation also has remarkably improved *in-vitro* and *in-vivo* pharmacological properties compared to curcumin. Some studies have suggested that nanocurcumin will become an effective treatment strategy in the near future [52, 53].

In cirrhotic patients, the overexpression of cytokines (interleukins and TNF-α) may exert inhibitory effects on normal myocardium contractility [54]. On the other hand, the bacterial

translocation in cirrhotic patients could lead to the increased levels of endotoxin and pro-inflammatory cytokines, such as interleukins and TNFs [54, 55]. According to the findings of the current research, curcumin nanomicelle decreased cardiac damage possibly through the suppression of inflammatory mediators. Furthermore, curcumin nanomicelle (especially at the dose of 200 mg/kg) demonstrated beneficial effects through the reduction of TNF-α and IL-1β. Therefore, it could be suggested the cardiac effects of curcumin nanomicelle were associated with the suppression mechanisms of inflammatory mediators.

IL-10 is an anti-inflammatory cytokine, which has been recognized in previous studies due to its ability to reduce pro-inflammatory cytokines and apoptosis [56, 57]. Presumably, IL-10 has suppressive effects on tissue fibrosis [58-60]. On the other hand, IL-10 could inhibit the synthesis of pro-inflammatory and pro-fibrogenic cytokines, such as IL-6, TNF-α, and TGF-β [61]. According to the results of the present study, serum IL-10 levels increased in the animals treated with curcumin nanomicelle in response to cirrhotic cardiomyopathy injury. In addition, IL-10 may serve a protective function in the presence of cirrhotic cardiomyopathy through the modulation of pro-inflammatory factors.

Lipid peroxidation products may act as markers for the assessment of oxidative stress pathways. In general, lipid peroxidation is described as a process associated with the overproduction of oxidants such as free radicals species [62]. Free radicals produce the lipid peroxidation metabolite in various organisms, and increased levels of free oxygen radicals lead to the overproduction of MDA, which is known as a reliable indicator of oxidative stress [63]. Furthermore, lipid peroxidation leads to the identification of antioxidants as the protective enzymes that could decrease oxidative functions and stimulate the scavenging of reactive oxygen species (ROS) before cell damage [64].

Tissue MDA levels were also evaluated in the current research, and the findings indicated that in the treatment groups with curcumin nanomicelle [especially at the dose of 200 mg/kg), curcumin nanomicelle exerted antioxidant effects through decreasing the MDA levels. Therefore, it could be concluded that curcumin nanomicelle administration in the cirrhotic cardiomyopathy model may have antioxidant effects. Antioxidant molecules are protective and reducing agents that are able to react with destructive oxidants. Moreover, antioxidants could act as radical scavengers [65].

GSH is considered to be the most essential and prominent molecule among various endogenous antioxidants, which allows the scavenging of ROS [66, 67]. According to the result of the present study, curcumin nanomicelle treatment in the cirrhotic rats increased the GSH content, confirming the antioxidant effects of this compound on the inflammatory model. It has also been suggested that GSH may support cellular defense mechanisms through the inhibition of lipid peroxidation and protecting cardiomyocytes against oxidative injuries.

Curcumin nanomicelle may reduce free radicals by elevating tissue SOD levels, thereby protecting cells against the damage induced by oxidative stress. In the current research, curcumin nanomicelle increased the antioxidant and anti-inflammatory effects of SOD on the cirrhotic myocytes, which confirmed the cardioprotective effects of curcumin nanomicelle through the overproduction of SOD. SOD is a potent antioxidant and detoxification enzyme in cells, as well as an endogenous antioxidant enzyme that could protect cells against ROS [36]. Moreover, SOD removes superoxide and decreases oxidative stress and tissue injuries [68]. The SOD3 enzyme is the major antioxidant factor against cardiovascular diseases and inflammatory models such as ischemia-reperfusion injury. Potent associations have been denoted between SOD deficiency and several pathological conditions [69].

In the present study, we investigated the effects of curcumin and curcumin nanomicelle on the cardiac function of cirrhotic rats, as well as the isolated left ventricular papillary muscles in the rats. According to the obtained results, the papillary muscle contractile response reduced in the control groups to the stimulation level. In addition, the inotropic response of the papillary muscles significantly improved in the animals receiving curcumin nanomicelle compared to the control group. Therefore, it could be concluded that curcumin nanomicelle could improve impaired cardiac contractility through the suppression of inflammatory factors and induction of antioxidant enzymes or directly affecting cardiomyocytes. In the electrophysiological assessment, various concentrations of curcumin nanomicelle were

observed to reduce the QT, QRS, and RR intervals in the ECG, which could be attributed to the anti-inflammatory and antioxidant effects of curcumin nanomicelle.

CONCLUSION

According to the results, curcumin nanomicelle exerted anti-inflammatory, antioxidant, and cardioprotective effects on a rat model of cirrhotic cardiomyopathy. Moreover, curcumin nanomicelle had more significant cardioprotective properties through directly affecting the cardiac function or modulation of the inflammatory pathways compared to curcumin.

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