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RESEARCH PAPER

Berberine nanomicelles attenuate cirrhotic cardiomyopathy in rats: Possible involvement of the NO-cGMP signaling

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ABSTRACT

Objective(s): In cirrhotic cardiomyopathy, a rise in pro-inflammatory cytokines results in the up-regulation of inducible nitric oxide synthase (iNOS), and the overproductions of nitric oxide (NO) and cyclic guanosine 3', 5' monophosphate (cGMP). Berberine (BBR), an isoquinoline-derived alkaloid isolated from Rhizoma coptidis, possesses anti-inflammatory, anti-oxidative, and cardioprotective properties. In this study, the effect of BBR-loaded micelles in a rat model of cirrhotic cardiomyopathy resulted from bile duct-ligation (BDL) was examined. Further, a possible role for NO-cGMP signaling was clarified.

Materials and Methods: Cirrhotic rats were orally treated with BBR-loaded micelles (50 mg/kg), free BBR (50 and 100 mg/kg) and silymarin (100 mg/kg). A selective iNOS inhibitor, aminoguanidine (AG) 100 mg/kg, i.p., was administered. iNOS expression and nitrite concentration were calculated using immunohistochemistry (IHC) and Griess reagent methods, respectively. Besides, ventricular tumor necrosis factor-alpha (TNF-α), cGMP, and serum interleukin -1beta (IL-1β) were measured using ELISA kits.

Results: TNF-α and IL-1β, nitrite, cGMP, and the expression of iNOS increased significantly in BDL rats. However, BBR (100 mg/kg), nanoBBR (50 mg/kg), and silymarin markedly lowered the levels of these markers. Notably, AG increased the nanoBBR effect.

Conclusion: This cardioprotective effect of nanoBBR probably mediated at least in part by down-regulations of the NO-cGMP pathway, and the inflammatory mediators.

Key words: *Bile duct-ligation, Cardiomyopathy, Nanoberberine; Rat, The NO- cGMP pathway*

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INTRODUCTION

In cirrhosis, the baseline cardiac output increases and ventricular contractile responses to pharmacological, surgical or physiological, and stressful stimuli blunt. This attenuated cardiac contractile responsiveness is known as cirrhotic cardiomyopathy [1].

The complex alterations of carbon monoxide and nitric oxide (NO) are important factors in the pathogenesis of cirrhotic cardiomyopathy [2, 3]. NO which is produced in the endothelial cells plays a crucial role in vascular functions and it has anti-inflammatory and anti-apoptotic properties [4]. In cirrhotic cardiomyopathy, the rise in pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) resulting in stimulation of inducible nitric oxide synthase (iNOS) and excessive production of NO, a known vasodilator [5]. There is evidence of increased cytokinemia and NO activity in cirrhotic patients, and animal models [6, 7]. The intracellular messenger, the cyclic guanosine monophosphate (cGMP), which is produced by the activation of enzyme guanylate cyclase, mediates various modulatory effects. Several modulatory effects of NO donors have been stated to occur via the cGMP pathway, and NO acts to stimulate soluble guanylate cyclase

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(sGC) to produce cGMP [8]. For example, evidence suggests that NO attenuates cGMP-mediated cardiac pacemaker activity [9]. The NO-cGMP signaling plays a critical role on smooth muscle tone, platelet activity, cardiac contractility, renal function, and cell growth [10]. In fact, cGMP is a necessary intracellular second messenger, which regulates fundamental physiological processes in the myocardium.

Berberine (BBR) is an isoquinoline-derived alkaloid isolated from *Rhizoma coptidis*. BBR has been extensively used in clinic owing to its wide range of pharmacological activities [11, 12] including antioxidant, antidiabetic, antihyperlipidemia, antiinflammatory, antitumor, and cardioprotective properties [13, 14]. BBR hydrochloride is an effective antioxidant and free radical scavenger preventing formation of reactive oxygen species (ROS) and exerting protective effects on cardiac, hepatic and renal functions [15]. The cardioprotective activities of BBR have been widely documented [16-20] for example, in murine heart failure models [21]. Several studies have also showed that certain BBR derivatives exert cardioprotective effect by reducing oxidative damages [22, 23]. Furthermore, BBR was shown to improve survival in congestive heart failure [24]. Nevertheless, clinical use of BBR has been greatly reduced due to its limited bioavailability [25]. Therefore, new formulations and nano-carriers have been developed to enhance its efficacy and therapeutic availability. For instance, to improve BBR delivery to tumors, micelles showed incredible stability and boosted water solubility with very low toxicity [26]. Notably, increased bioavailable BBR attenuated cerebral ischemia/reperfusion (I/R) injury in rats [27]. Liposome-encapsulated BBR diminished cardiac dysfunction following myocardial infarction (MI) [28].

Silymarin, an antioxidant flavonoid complex derived from the herb milk thistle (*Silybium marianum* L.) [29], showed cardioprotective effects in various animal models [30, 31]. Though BBR does not share any structural similarities with silymarin, they seem to enhance the cellular antioxidant defense and modulate the immune system function [32].

Recently, our colleagues showed the protective effect of BBR nanomicelles on hepatic cirrhosis in bile duct-ligated (BDL) rats [33]. In the current study, we hypothesized that the encapsulation of BBR into nanomicelles may improve its therapeutic availability and efficacy in cirrhotic cardiomyopathy induced by BDL in rats. In addition, we clarified the role of NO-cGMP pathway.

MATERIALS AND METHODS

Chemicals

Berberine hydrochloride, silymarin and aminoguanidine were purchased from Sigma-Aldrich (Sigma, St. Louis, MO, USA). Nanomicelles were developed in Nanotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. They contained 5% berberine, and berberine nanomicelles have the average diameter of 2.7 nm.

Animals

Male rats (250-280 g) were obtained from the Laboratory Animal Centre of Tehran University of Medical Sciences, Tehran, Iran. The animals were allowed to acclimate for one week, and were fed with standard pellet diet and water ad libitum. The animal room was at a temperature of 20-25°C under a 12-hr light/dark cycle. Food was withdrawn one day before the experiment, but water continued to be provided.

All animal experiment protocols complied with the guidelines of the Laboratory Animal Centre of the University of Tehran. The animals were processed (including drug treatment and sacrifice) in accordance with the international guidelines for laboratory animals.

Common Bile duct-ligation (BDL)

BDL was carried out to induce cirrhosis as described previously [34]. Briefly, the common bile duct was exposed by a midline abdominal incision under deep anesthesia. The duct was doubly ligated, and sectioned between the ligatures. Sham operated rats were treated in the same manner without ligating the bile duct [35].

Animal treatments

Forty-eight rats were divided into eight groups of six. All the treatments were administered three days following BDL or sham operation, and continued for twenty-eight days. In addition, the treatments were administrated via the oral route (p.o.) except for aminoguanidine (AG) which was given the intraperitoneal (i.p.). Sham-operated and BDL groups received the vehicle. Four BDL groups received silymarin (100 mg/kg, p.o.), berberine (50 and 100 mg/kg, p.o.), and nanoberberine (50

mg/kg, p.o.) [33]. Moreover, a BDL group received AG (100 mg/kg, i.p.), and a BDL group started receiving AG in combination with nanoberberine (50 mg/kg), on days 14-28.

Cytokine measurements: TNF-α and IL-1 β quantifications

To measure tissue TNF-α and serum IL-1β, Enzyme-linked immunosorbent assay (ELISA) kits were employed [(BioSource, Camarillo, CA; RAB0479-1KT)]. The left ventricular samples were homogenized in ice-cold phosphate-buffered saline (PBS) and centrifuged at 14,200 g (30 min). After that, 50 µl of the samples and the standard (bovine serum albumin) were pipetted into a 96-well plate pre-coated with rat specific antibodies. Following the addition of 50 µl biotinylated anti-TNF-α solution to each well plate, it was incubated for 90 min at room temperature. Then, the wells were washed four times with wash buffer. After that, streptavidin-peroxidase (100 µl) was added to each well, and incubated for 45 min at room temperature, and washed 4 times with PBS. Next, it was exposed to stabilized chromogen (100 µl) and incubated for 20 min. Lastly, for spectrophotometrically analysis at λ=450 nm, the stop solution (100 µl) was added to each well [2, 36]. Furthermore, to measure IL-1β, the ELISA kit was utilized using the serum samples according to the manufacturer's protocols.

Measurement of plasma nitrite concentration

The levels of nitrite/nitrate in plasma samples were measured, as indicators of NO production, using Griess reagent assay [37, 38]. Briefly, the samples were loaded in a 96-well microtiter plate (100 µl). Saturated solution of 100 µl of vanadium (III) chloride (VCl_3) was added to the wells then followed by the Griess reagent (100 µl each). The plates were measured using an ELISA standard plate reader at 540 nm and 30 min incubated at 37 °C. The obtained value represented the amount of plasma nitrite/nitrate. The results are expressed as micromoles.

Ventricular cGMP assay

Tissue level of Guanosine 3', 5'-cyclic monophosphate (cGMP) was measured using a commercially available ELISA kit (Amersham Life Science). The left ventricles were homogenized in ice-cold modified Hanks' balanced salt solution and centrifuged at 4000 for 10 min. The protein concentration in the supernatant was determined with Bio-Rad protein assay using bovine serum albumin as standard.

The non-acetylation assay method used. Briefly, the same amount of protein from each sample (approximately 2 mL of supernatant) was treated with concentrated trichloroacetic acid to precipitate the protein (final concentration of trichloroacetic acid, 6%), mixed, and centrifuged at 2000 g for 15 min. The supernatant was washed with water-saturated diethyl ether, and the upper ether layer discarded after each wash. Under a stream of nitrogen, the extract was dried at 60°C and then dissolved in 800 µL of assay buffer, before the analysis.

Immunohistochemistry (IHC)

For IHC examination, the left ventricle samples were immediately fixed in ice-cold freshly prepared 10% formalin. The heart pieces then were embedded in paraffin. After deparaffinizing, sections of the left ventricle muscle (10 µm) in xylene, and rehydrating in specific decreasing concentrations of ethanol, treated with 3% hydrogen peroxide in methyl alcohol for 5 min to block endogenous peroxidases activity. IHC staining was based on the Avidin-Biotin peroxidase method, and the positive control was human tonsil tissue. The reaction with polyclonal rabbit anti-iNOS (1:2000 dilution) antibody for 1 hr. followed by incubation with the secondary HRP-conjugated rabbit anti-rabbit immunoglobulin G antibody (1:2000 dilution) for 30 min at room temperature. After washing the sections, they were consecutively treated three times with Tris buffer (pH 7.4). The sections were incubated with diaminobenzidine-hydrogen peroxide solution for 10 min and 5% $CuSO_4$ for 5 min. Eventually, they counterstained with hematoxylin and eosin (H & E). Quantification of iNOS positive cells under light microscopy was performed obtaining brown-colored precipitation of cells per high-power field (20 ×).

Statistical analysis

Statistical analyses were calculated by SPSS software (version 21). The results are expressed as mean ± SEM. In the case of three or more experimental groups an analysis of variance (one-way ANOVA) was performed which followed by Tukey's post hoc test. In case of two variables (combination therapy), the analysis was performed

via two-way ANOVA followed by Bonferroni post hoc test. A P-value less than 0.05 was considered statistically significant.

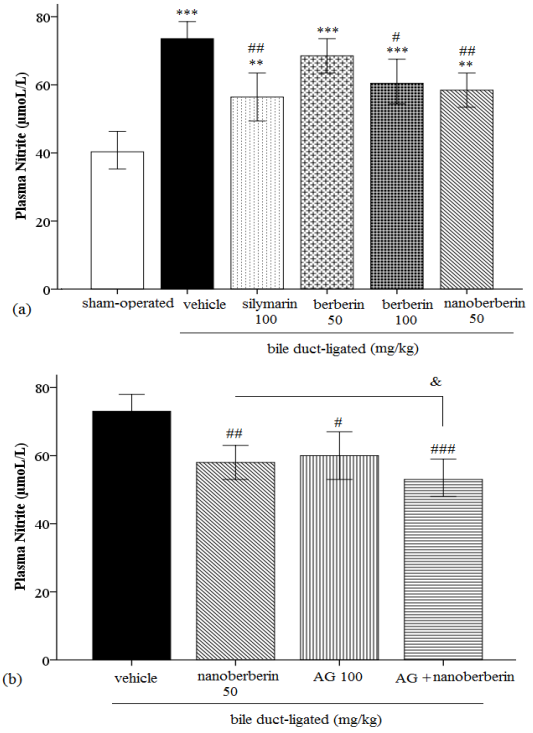

Fig 1. Effect of silymarin (100 mg/kg, p.o.), berberine (50 mg and 100 mg/kg, p.o.) and nanoberberine (50 mg/kg, p.o.) treatments on plasma nitrite level in BDL rats (n=6). ** P<0.01, *** P<0.001 significantly different from the sham-operated group. # P<0.05, ## P<0.01 significantly different from the vehicle group (Fig. 1a). Effects of aminoguanidine (AG) 100 mg/kg, i.p. alone or in combination with nanoberberine 50 mg/kg, p.o., on plasma nitrite level in the BDL rats (n=6). # P<0.05, ## P<0.01 and ### P<0.001 significantly different from the vehicle group. & significantly different from nanoberberine 50 mg/kg (Fig 1b)

RESULTS

Plasma nitrite and ventricular cGMP levels

As can be observed in Fig 1, plasma nitrite concentration increased significantly in BDL rats treated with vehicle compared to sham-operated group [F (5, 30) = 15.616; P<0.000)]. Moreover, BDL rats treated with silymarin (100 mg/kg), BBR (50 and 100 mg/kg) and nanoBBR (50 mg/kg) still had higher plasma nitrite levels compared to sham-operated group [F (5, 30) = 15.616; P<0.006)], [F (5, 30) = 15.616; P<0.000)], [F (5, 30) = 15.616; P<0.000)] and [F (5, 30) = 15.616; P<0.002)], respectively. On the other hand, silymarin (100 mg/kg), BBR (100 mg/kg) and nanoBBR (50 mg/kg) treatments markedly lowered nitrite levels compared with vehicle group [F (5, 30) = 15.616; P<0.003)], [F (5, 30) = 15.616; P<0.03)] and [F (5, 30) = 15.616; P<0.008)], respectively (Fig 1a). Effects of AG (100 mg/kg, i.p.), or AG in combination with nanoBBR (50 mg/kg, p.o.) on plasma nitrite level in BDL rats (n=6) are shown in Fig 1b. Notably, nanoBBR 50 mg/kg, AG 100 mg/kg and AG in combination with nanoBBR 50 mg/kg markedly decreased nitrite levels in comparison with vehicle group, [F (3, 20) = 8.744; P<0.007], [F (3, 20) = 8.744; P<0.02] and [F (3, 20) = 8.744; P<0.000], respectively.

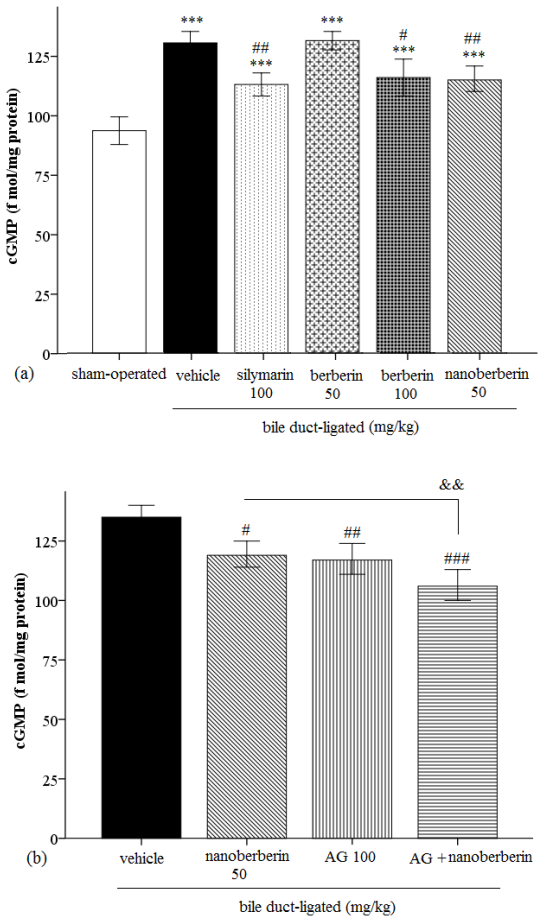

Fig 2. Effect of silymarin (100 mg/kg, p.o.), berberine (50 mg and 100 mg/kg, p.o.) and nanoberberine (50 mg/kg, p.o.) treatments on cardiac tissue cGMP level in the BDL rats (n=6). *** P<0.001 significantly different from the sham-operated group. # P<0.05, ## P<0.01 significantly different from the vehicle group (Fig. 2a). Effects of AG (100 mg/kg, i.p.) alone or in combination with nanoberberine (50 mg/kg, p.o.) on cardiac tissue cGMP level in the BDL rats (n=6). ## P<0.01, ## P<0.01 and ### P<0.001 significantly different from the vehicle group. && P<0.01 significantly different from nanoberberine 50 mg/kg (Fig 2b)

Moreover, AG in combination with nanoBBR 50 mg/kg was significantly different compared with nanoBBR 50 mg/kg and had lower nitrite level [F (3, 20) = 14.653, P<0.04].

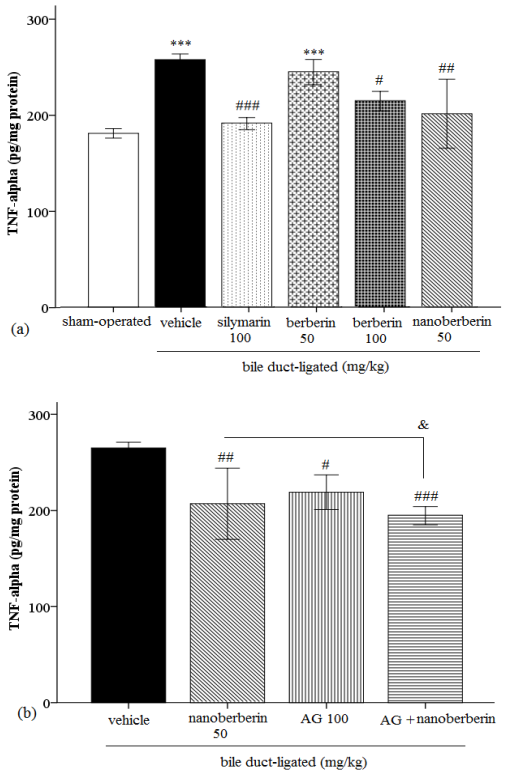

Fig 3. Effect of silymarin (100 mg/kg, p.o.), berberine (50 mg and 100 mg/kg, p.o.) and nanoberberine (50 mg/kg, p.o.) treatments on cardiac tissue TNF-α level in the BDL rats (n=6). *** P<0.001 significantly different from the sham-operated group. # P<0.05, ## P<0.01 and ### P<0.001 significantly different from the vehicle group (Fig. 3a). Effect of AG (100 mg/kg, i.p.) alone or in combination with nanoberberine (50 mg/kg, p.o.) on cardiac tissue TNF-α level in the BDL rats (n=6). # P<0.05, ## P<0.01 and ### P<0.001 significantly different from the vehicle group. & P<0.05 significantly different from nanoberberine 50 mg/kg (Fig 3b)

As can be noticed in Fig 2, tissue cGMP concentration increased significantly in BDL rats treated with vehicle compared to sham-operated group [F (5, 30) = 24.354; P<0.000)]. BDL groups treated with silymarin, BBR (50 and 100 mg/kg) and nanoBBR (50 mg/kg) had higher cGMP levels compared to sham-operated group [F (5, 30) = 24.354; P<0.000)]. On the other hand, silymarin (100 mg/kg), BBR (100 mg/kg) and nanoBBR (50 mg/kg) treatments markedly lowered cGMP levels compared with vehicle group [F (5, 30) = 24.354; P<0.002)], [F (5, 30) = 24.354; P<0.01)] and [F (5, 30) = 24.354; P<0.008)], respectively (Fig 2a). Effects of AG 100 mg/kg, i.p. alone or in combination with nanoBBR (50 mg/kg, p.o.), on cGMP level in BDL rats (n=6) are shown in Fig. 2b. As it is illustrated, nanoBBR 50 mg/kg, AG 100 mg/kg and AG in combination with nanoBBR 50 mg/kg groups markedly decreased cGMP levels in comparison with vehicle group [F (3, 20) = 14.653, P<0.01] and [F (3, 20) = 14.653, P<0.004] and [F (3, 20) = 14.653, P<0.000], respectively. Moreover, AG in combination with nanoBBR 50 mg/kg was significantly different compared with nanoBBR 50 mg/kg and had lower cGMP level [F (3, 20) = 14.653, P<0.004].

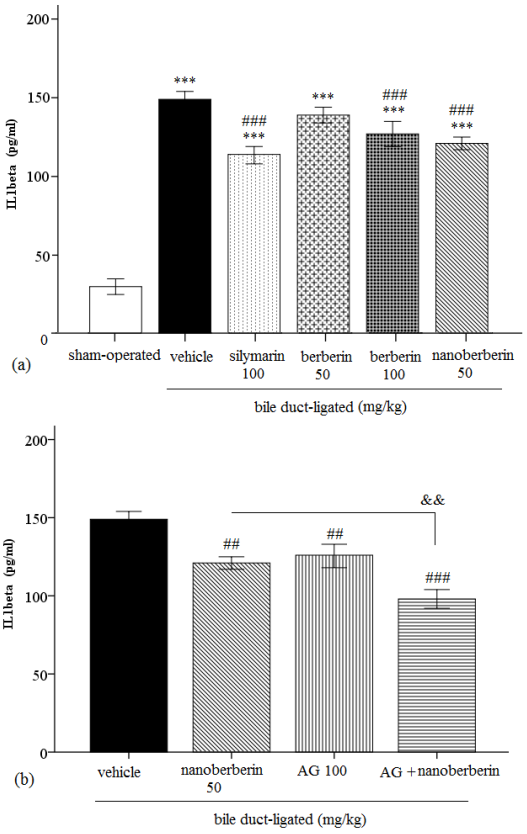

Fig 4. Effect of silymarin (100 mg/kg, p.o.), berberine (50 mg and 100 mg/kg, p.o.) and nanoberberine (50 mg/kg, p.o.) treatments on serum IL-1β level in the BDL rats (n=6). *** P<0.001 significantly different from the sham-operated group. ### P<0.01 significantly different from the vehicle group (Fig. 4a). Effect of AG (100 mg/kg, i.p.) alone or in combination with nanoberberine (50 mg/kg, p.o.) on serum IL-1β level in BDL rats (n=6). ## P<0.01 and ### P<0.001 significantly different from the vehicle group. && P<0.01 significantly different from nanoberberine 50 mg/kg (Fig 4b)

Cytokine levels

Cardiac tissue concentration of TNF-α increased significantly in BDL rats treated with

vehicle compared to that of sham-operated group [F (5, 30) = 13.469; P<0.000)].

BDL group treated with BBR (50 mg/kg) also had higher TNF-α levels compared to sham-operated group [F (5, 30) = 13.469; P<0.000)] (Fig 3).

Conversely, silymarin (100 mg/kg), BBR (100 mg/kg) and nanoBBR (50 mg/kg) treatments markedly lowered TNF-α levels compared with vehicle group [F (5, 30) = 13.469; P<0.000)], [F (5, 30) = 13.469; P<0.01)] and [F (5, 30) = 13.469; P<0.001)], respectively (Fig 3a). Effects of AG (100 mg/kg, i.p.) alone or in combination with nanoBBR (50 mg/kg, p.o.) on TNF-α levels in BDL rats (n=6) are shown in Fig. 3b. As can be observed, nanoBBR 50 mg/kg, AG 100 mg/kg and AG in combination with nanoBBR 50 mg/kg markedly decreased TNF-α levels in comparison with vehicle group [F (3, 20) = 8.346, P<0.006], [F (3, 20) = 8.346, P<0.03] and [F (3, 20) = 8.346, P<0.000], respectively.

Serum IL-1β concentration increased significantly in BDL rats treated with vehicle compared to that of sham-operated group [F (5, 30) = 234.018; P<0.000)]. BDL groups treated with silymarin, BBR (50 and 100 mg/kg) and nanoBBR (50 mg/kg) had higher IL-1β levels compared to sham-operated group [F (5, 30) = 234.018; P<0.000)] (Fig 4). In contrast, silymarin (100 mg/kg), BBR (100 mg/kg), and nanoBBR (50 mg/kg) treatments markedly lowered IL-1β levels in BDL rats compared with vehicle group [F (5, 30) = 234.018; P<0.000)] (Fig. 4a). Effects of AG (100 mg/kg, i.p.) alone or in combination with nanoBBR (50 mg/kg, p.o.) on IL-1β level in BDL rats (n=6) are shown in Fig. 4b. As it is obvious, nanoBBR 50 mg/kg and AG 100 mg/kg markedly decreased IL-1β levels in comparison with vehicle group [F (3, 20) = 56.787, P<0.005]. Also, AG in combination with nanoBBR 50 mg/kg had significantly lower level [F (3, 20) = 56.787, P<0.000]. Moreover, AG in combination with nanoBBR 50 mg/kg was significantly different compared with nanoBBR 50 mg/kg and has lower IL-1β level [F (3, 20) = 56.787, P<0.005].

Ventricular iNOS protein expression

To identify which cells express the NOS2 enzyme, a representative IHC localization of iNOS proteins in the left ventricles was performed. As shown in Fig 5, iNOS protein staining was observed in the cardiomyocytes of cirrhotic rats. The results showed significant increase of iNOS protein expression in cirrhotic heart in comparison with sham-operated group. Conversely, a significant decreased in iNOS immunostaining was observed in BBR and silymarin-treated ventricular tissues comparison with cirrhotic hearts. Notably, a very highly significant decrease in iNOS immunostaining was observed in nanoBBR-treated ventricular tissues in comparison with cirrhotic hearts. Besides, AG markedly strengthened the protective effect of nanoBBR, consequently, the iNOS expression reduced significantly.

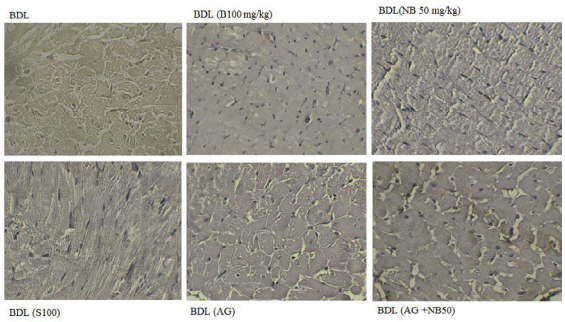

Fig 5. Immunohistochemical staining of iNOS protein in the cirrhotic hearts. iNOS-positive cells are localized in the cardiomyocytes of the left ventricle. Note the increased immunostaining of iNOS in the myocytes of the rats with cirrhosis. In contrast, BBR and silymarin (100 mg/kg) treatments resulted in significant decreases in iNOS protein staining. Notably, nanoBBR (50 mg/kg) caused a highly significant decreased in iNOS immunostaining in the ventricles and no marked immunostaining was observable on the slide. (Original magnification 40 ×)

DISCUSSION

In the present study, we induced BDL in rats as it reliably produces biliary cirrhosis with cardiovascular disturbances that reflect cirrhosis in human. Moreover, it avoids the use of any toxin that might complicate interpretation of the outcomes. Our results illustrated that BDL increased plasma nitrite, serum IL-1β, ventricular TNF-α, ventricular cGMP, and iNOS ventricular expression. On the other hand, nanoBBR treatments markedly restored the cardiovascular disturbances and down-regulated NO-cGMP activity. In addition, AG markedly strengthened the protective effect of nanoBBR. It should be noted that theses protective effect of nanoBBR markedly outweighed that of BBR, and were comparable to silymarin.

Consistent with our experiment, TNF-α in the cardiac tissue was increased in cirrhotic rats [39]. Similarly, in a murine model of cirrhotic cardiomyopathy it was suggested that TNF-α

mediated part of its cardio-depressant effect by augmenting oxidative stress [40]. Moreover, the increase in NO synthesis in the cardiac tissues of cirrhotic mice, an underlying mechanism for cirrhosis, is attributed to elevated TNF-α level [41]. In another comparable study, serum cytokine levels were increased in rats with cirrhosis. The negative inotropic effect of IL-1β reversed by pre-incubation of isolated papillary muscle with an NOS inhibitor [2]. Likewise, it has shown that increased NO synthesis in BDL rats causes bradycardia [42, 43]. In fact, cholestasis is known to be associated with endotoxemia [44], which induces inducible NOS (iNOS) [45]. Further, NOS inhibition improved vascular responsiveness of BDL rats, suggesting a role for NO overproduction in cholestasis [46]. Likewise, studies demonstrated that abnormal cardiac chronotropic function is associated with increased nitration of cardiac proteins in rats with biliary cirrhosis. The plasma nitrite/nitrate levels decreased after administration of a NOS inhibitor, L-nitro-arginine methyl ester (L-NAME) [47], or another NOS inhibitor, L-NMMA, improved blunted contractility in an isolated working heart [48]. It was observed that cardiac and serum levels of TNF-α and IL-1β were elevated in BDL rat, and that the negative inotropic effect of IL-1β could be reversed by pre-incubation with L-NAME. Then it was demonstrated that L-NAME treatment restored the depressed isolated papillary muscle contractile responsiveness in BDL rats. Besides, in the ventricles, increases were distinguished in soluble cGMP levels along with the content of iNOS mRNA and protein. These experiments demonstrated the role of iNOS conclusively in the pathogenesis of cirrhotic cardiomyopathy [2, 49].

BBR has shown protective effects in different cardiovascular disorders. For example, BBR exhibited protective effects against doxorubicin (DOX) induced acute cardiomyopathy and heart tissue free radical injury in rats [50] and mice [51]. Moreover, BBR significantly inhibited production of inflammatory cytokines in the cardiomyocytes following cardiomyocyte ischemia [52]. Protective effects of BBR on acute myocardial ischemia in rats were stated. BBR (30 and 60 mg/kg, p.o.) decreased serum level of TNF-α [21]. Likewise, BBR reduced oxidative stress and exhibited cardioprotection during ischemia-reperfusion injury (IRI) in rat [53], and mice [54]. Similarly, BBR (100 mg/kg/d) significantly reduced myocardial ischemia reperfusion (MI/R)-induced myocardial infarct size, improved cardiac function, and suppressed myocardial apoptosis and oxidative damage in a rat model of type 2 diabetes [55]. Furthermore, BBR attenuated MI/R injury in rat. BBR significantly improved post-MI/R cardiac function recovery possibly due to its strong anti-oxidative and anti-inflammatory activity [56].

In a rat model of hyperglycemia and hypercholesterolemia, chronic BBR (30 mg/kg/day) treatment remarkably improved the cardiac dysfunction [57]. In addition, BBR prevented hyperglycemia-induced endothelial injury and enhanced vasodilatation via adenosine monophosphate-activated protein kinase (AMPK) and endothelial NOS (eNOS) [58]. BBR also showed safe cardioprotective effects in patients with congestive heart failure [24, 59].

Several recent studies illustrate new formulations of BBR in different animal models. For instances, liposome-encapsulated BBR treatment attenuated cardiac dysfunction after myocardial infarction (M.I.). This shows that delivery of BBR-loaded liposomes significantly improves its therapeutic availability and identifies BBR-loaded liposomes as potential treatment [28]. Recently, our colleagues showed effect of BBR nanomicelles on hepatic cirrhosis in BDL rats. BBR nanomicelles (50 mg/kg, p.o.) and silymarin (100 mg/kg, p.o.) markedly decreased the hepatic markers while enhanced glutathione (GSH) level. BBR nanomicelles (50 mg/kg, p.o.) significantly lowered TNF-α level and prevented liver cirrhosis in the histopathologic analysis [33].

CONCLUSION

To conclude, our results illustrated that BDL enhance plasma nitrite, serum IL-1β, ventricular tissue TNF-α, cGMP levels, and iNOS expression in rats. Conversely, nanoBBR treatments markedly restored the pathologically increased markers and down-regulated NO-cGMP activity. Notably, effects of nanoBBR markedly outweigh those of silymarin and BBR. In addition, AG and nanoBBR showed synergistic effects on cirrhotic cardiomyopathy in rats. As a result, it is demonstrated that the NO-cGMP pathway may participate in cirrhotic cardiomyopathy resulted from BDL.

NanoBBR may improve impaired cardiac markers in cirrhotic rats and probably this effect could be mediated at least in part by down regulations of the inflammatory mediators and as a consequence the NO-cGMP signaling. It may

prevent nitration of cardiac proteins.

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