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Micellar Nanoformulation of Berberine to Mitigate Doxorubicin-induced Cardiotoxicity: A Cell-line Study

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ABSTRACT

Background: Doxorubicin-induced cardiotoxicity (DIC) is a major complication of cancer chemotherapy. Thus, developing effective myocardial protection strategies during doxorubicin (Dox) therapy is a medical necessity.
Objectives: To evaluate and compare the cardioprotective effectiveness of free berberine (Ber) and berberine loaded in micelles (mBer) against DIC.
Materials and methods: The study, which was conducted in 2023, employed the H9c2 cell line, derived from embryonic cardiomyocytes, as a model. The study included a control group and six experimental groups: the Ber-treated group, the mBer-treated group, the Dox-treated group, the Ber-Dox combination-treated group, and the mBer-Dox combination-treated group, as well as the void micelles-treated group. The study evaluated the alterations in several cardiotoxicity markers with triplicate measurements: [lactate dehydrogenase (LDH), creatine kinase myocardial band (CK-MB), and cardiac troponin I (cTn-1)], lipid peroxidation indicator (malondialdehyde (MDA), oxidative stress markers [Reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), inflammatory cytokines (interleukin-1β (IL-1β) and interleukin-6 (IL-6)], and the activity of the apoptosis proteins caspases 3/7.
Results: The DOX group demonstrated significant increases in cardiotoxicity enzyme indices, lipid peroxidation, generation of free radicals, inflammatory cytokines, and caspase 3/7 activity relative to the control group. When Ber, or mBer, was co-delivered with Dox, the levels of LDH, CK-MB, cTn-1, and MDA significantly decreased. Whereas the activities of SOD and CAT were significantly improved when Ber, or mBer, was co-delivered with Dox. They reduced the elevation in both IL-β and IL-6 levels as well as the activities of caspases 3 and 7 induced by Dox. Importantly, the utilization of the micellar formulation of Ber in conjunction with Dox significantly enhanced the cardioprotective efficacy of Ber against DIC in H9c2 cells.
Conclusion: Our results suggest that mBer offers a novel Ber delivery approach and prospective therapeutic strategy for the treatment of DIC.
Keywords: Berberine, Doxorubicin, Cardiotoxicity, Polymeric nanomicelle, H9c2.

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INTRODUCTION

Despite the pervasive use of Doxorubicin (Dox) as a chemotherapeutic agent to treat patients with a wide variety of cancer types, the drug's propensity for heart problems poses a significant barrier to its clinical application [1]. Although the precise mechanism of DIC remains unknown, oxidative stress, apoptosis, and other inflammatory mechanisms are believed to be involved. DIC is also associated with disturbances in microRNA modulation, iron regulatory protein, topoisomerase activity, Ca^{+2} homeostasis, gene expression, and structural changes in heart muscle [2].

Oncologists and cardiologists face an ongoing problem as they deal with the potential trade-off between mitigating Dox-induced cardiotoxicity (DIC) through dose reduction and the

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consequent decrease in chemotherapy efficacy [3].

Dexrazoxane has been approved in many countries as a cardioprotective agent to reduce DIC [4]. However, its use is restricted due to worries that it may impede Dox's effectiveness, induce suppression of bone marrow and liver toxicity, and increase the risk of future malignancies [5]. Despite attempts to reduce the DIC through the development of the liposomal formulation of Dox, research has shown that it is just as likely to cause cardiac events [6]. Although extensive investigations have been done to develop an effective treatment for DIC, no clinically approved effective treatment is presently available [7]. Thus, combining Dox with cardioprotective agents is still a pressing clinical necessity.

Various phytochemicals (plant-derived small molecules) have been tested in preclinical studies and showed cadioprotective effects against DIC [8]. Among such natural products is berberine (Ber), an active alkaloid derived from the roots and foliage of Berberis species plants, which has various applications [9]. A combination of in-vitro experiments and in-vivo investigations using various animal models collectively provide evidence supporting the capacity of Ber to regulate cardiovascular functions and safeguard cardiac tissues from the detrimental effects of Dox [10]. The limited water solubility, bioavailability, and permeability across biological membranes of Ber have posed significant challenges in its application within therapeutic contexts [11]. To maximize Ber's therapeutic potential, it is necessary to resolve problems with solubility and bioavailability by creating effective dosage forms or implementing novel drug delivery techniques.

Our hypothesis suggests that the incorporation of Ber within the hydrophobic core of Pluronic F127 micelles may potentially improve its solubility, stability, and ability to pass through cellular membranes. The generated micelles have the potential to be delivered concurrently with Dox with the ultimate aim of resolving Ber solubility challenges and enhancing the cardioprotective activity of Ber against DIC by reducing oxidative stress and mitigating inflammation and apoptosis that are commonly associated with Dox therapy, utilizing the embryonic cardiomyocyte cell line H92c as a model for the study. The primary objective of this study was to evaluate and compare the cardioprotective efficacy of free Ber and berberine encapsulated in micelles (mBer) against DIC.

MATERIALS AND METHODS

This experimental study was conducted from February to July 2023 at the Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan, Iran.

Chemicals

Supplies for cell culture, including Dulbecco's Modified Eagle's Medium (DMEM), penicillin/streptomycin, amphotericin B, trypsin ethylenediaminetetraacetic acid (EDTA), sodium pyruvate, glutamine, and fetal bovine serum (FBS), as well as chemicals of analytical grade, including Dox, Ber hydrochloride, and pluronic F127 were sold from Sigma Aldrich (USA). The thin-film hydration technique was used to develop Ber-loaded pluronic F127 nano-micelles (mBer) [12].

Tissue culture

The H9c2 rat embryonic cardiomyoblast cell line (ATCC® CRL-1446TM) was grown in cell culture plates with 96 wells with a flat bottom containing DMEM medium supplemented with 10% FBS, 1% penicillin, 10,000 international units of streptomycin, and 25 μg of amphotericin B. In addition, sodium pyruvate)1 mM(plus glutamine)2 mM(were added to the culture mixture for maintenance purposes. The cells adhered to the bottom of the wells 24 hours after being seeded at a density of 5.0×10^3cells per well and incubated at 37^{o}C in an air environment containing 5% CO_2.

Experimental groups

H9c2 cells were subdivided into seven treated groups based on the received treatment, and each group was treated separately with 1 μL of Dimethylsulfoxide (DMSO) at a final concentration of 0.5% in each well. In this study, the experimental groups included the control group, Ber-treated group, Ber-loaded pluronic F127 micelles (mBer)-treated group, Dox-treated group, Ber-Dox combination, and mBer-Dox combination-treated groups at 1:1 molar ratio, as well as the Ber-unloaded pluronic micelles-treated group (EM). All groups received treatment and were subsequently incubated for 48 hours. The treatment with the 1 μM concentration of Ber or mBer and the 1 μM cardiotoxic concentration of Dox was followed as a treatment regime for the next set of experiments in this study.

Evaluation of the effect of mBer on the viability of H9c2 cells treated with Dox

evaluate the preventive efficacy of Ber or mBer against DIC, the 3–(4, 5–dimethylthiazol–2–yl)–2, 5–diphenyl–2H–tetrazolium bromide (MTT) assay was utilized to determine the viability of cells, which was performed in triplicate. MTT assay works on the basis that only living cells, owing to their active metabolism, can transform MTT into purple formazan, which has the highest absorbance at 492 nm. The quantity of the formed formazan is quantified spectrophotometrically, and it is directly correlated with cell viability [13]. Briefly, the assay was conducted by adding 10 μL of a 5 mg/ml MTT reagent to each well containing cultured cells, followed by a 4 hours incubation under similar conditions. After removing the supernatant with a multichannel pipette, the cells were lysed, and formazan crystals were fully solubilized by adding 150 μl of DMSO and 25 μl of glycine to each well with good mixing. A microplate (ELISA) reader was used to compare the absorbance at 492 nm between test and control cells to determine the percentage of cells that were inhibited, assuming that control cells treated with the vehicle alone would have a survival rate of 100%. The following formulas were used to estimate treated cells' percentage inhibition:

Percentage of Inhibited Cells = 100 - (Absorbance of Treated Cells / Absorbance of untreated Cells) × 100

Percentage of Cell viability =100 - Percentage of Inhibited Cells

Estimation of cardiomyocytes toxicity

Measurement of lactate dehydrogenase (LDH)

The leakage of LDH from the H9c2 cells was used to determine the cytotoxicity. After the cells were treated and incubated for 48 hours, the culture media was collected, and the levels of LDH in a cell culture medium were measured with the use of the LDH activity colorimetric assay kit purchased from Thermo Fischer Scientific, USA (Cat. NO.: C20300).

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Determination of creatine kinase myocardial band (CK-MB)

CK-MB levels were calculated in the culture medium used to grow the cells utilizing ELISA Kit specific for this enzyme purchased from Elabscience, China (Cat. NO.: E-EL-R1327).

Determination of Cardiac Troponin I (cTn-I) release

cTn-I was estimated in the cell culture supernatant utilizing ELISA Kit specific for this enzyme in rats purchased from Elabscience, China (Cat. NO.: E-EL-R1253).

Evaluation of oxidative stress status

Measurement of malondialdehyde (MDA) level

MDA concentration (nmol/mg protein) was determined colorimetrically based on its reaction with thiobarbituric acid using the MDA Content Assay Kit (Sunlong Biotech Co., China, Cat. No.: AK0662-50T-48S).

Assessment of Reduced glutathione (GSH) level

GSH was assessed using the GSH Assay Kit (Sigma-Aldrich Co., USA, Cat. No.:MAK364).

Evaluation of catalase (CAT) activity

CAT activity was quantified in accordance with the manufacturer's instructions (Sunlong Biotech Co., China, Cat.No. AK0580).

Determination of Superoxide dismutase (SOD) activity

In accordance with the manufacturer's guidelines (Sunlong Biotech Co., China, Cat. No.: AK0584), the SOD activity was measured using spectrophotometry.

Measurement of some pro-inflammatory cytokines levels

The level of some inflammatory cytokines including interleukin-1β (IL-1β) and interleukin-6 (IL-6) was determined in the cell culture supernatant using specific ELISA kits purchased from Beyotime Institute of Biotechnology, China (Cat. NO.: PI303 and PI328 for IL-1β and IL-6 respectively).

Assessment of Caspase-3/7 activity in H9c2 cells

Based on the manufacturer's instructions (Promega, USA, Cat. No.: G8090), Caspase-Glo® 3/7 reagent was used to estimate the caspase 3/7 activity utilizing a GloMax® microplate reader.

Ethical consideration

The researchers obtained ethical approval from the research Ethical Approval Committee of the College of Veterinary Medicine, University of Al-Qadisiyah, with document number 426 on February 5, 2023. There were neither humans involved in this study to obtain their consent, nor animals involved in this study.

Statistical analysis

The results obtained were statistically analyzed using the statistical software GraphPad Prism, specifically version 9.5.0. This study's data followed a normal distribution. A one-way analysis of variance (ANOVA) test was used to analyze the data. Subsequently, the least significant difference (LSD) test was conducted as a post hoc analysis to ascertain any statistically significant differences. A significance level of P-value < 0.05 was employed to determine statistical significance.

RESULTS

Cell viability

Cell viability after treatment with either empty micelles, Ber, or mBer was not statistically significant (P-value > 0.05) different from the control, demonstrating that neither polymeric micelles nor Ber in its free or micellar form per se produces cytotoxicity in H9c2 cells. Treatment of H9c2 cells with a 1 μM concentration of Dox, resulted in significantly lower viability (P-value < 0.05) compared to the control cells. Dox-induced cell death was significantly (P-value < 0.05) mitigated by pretreatment with free Ber, which increased cell viability. Our evaluation of the protective effects of mBer against DIC in H9c2 cells revealed that the cell viability was significantly (P-value < 0.05) greater with the mBer-Dox combination compared to Ber- Dox combination (Figure 1).

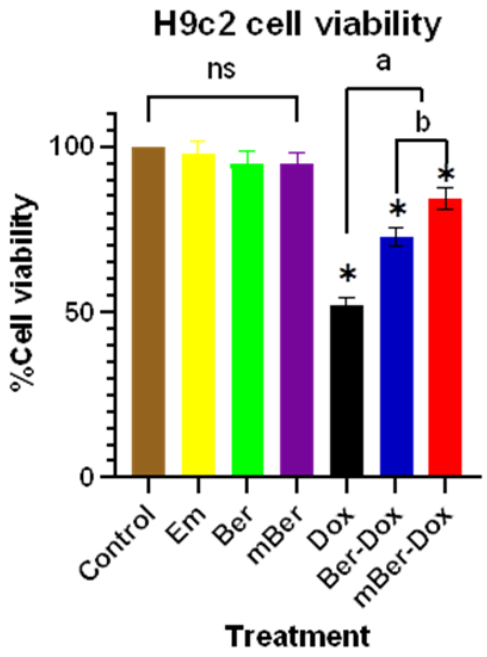

Figure 1. H9c2 cell viability using the MTT reduction assay. The data are presented in the form of a mean value standard deviation derived from three independent trials. ns: not statistically significant (P-value > 0.05) compared to control; *: significantly different (P-value < 0.05) compared to control; a: significantly different (P-value < 0.05) compared to Dox; and b: significantly different (P-value < 0.05) compared to Ber-Dox. Em: empty blank micelles; Ber: berberine; mBer: berberine-loaded pluronic F127 micelles; and Dox: doxorubicin.

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The mBer attenuates DIC by reducing LDH, CK-MB, and cTn-1 release

LDH, CK-MB, and cTn-1 release levels were utilized as indicators of cardiomyocyte damage. The control group exhibited the lowest cytotoxicity, while the Dox group exhibited the highest release level of the markers. The release levels of the studied markers from cells treated with either void micelles, free Ber, or mBer were not significantly different (P-value $>$ 0.05) from the control, indicating that these compounds do not cause cytotoxicity in H9C2 cells. Treatment with either free Ber or mBer in combination with Dox significantly (P-value $<$ 0.05) reduced Dox injury to cultured cardiomyocytes, as evidenced by the reduced release of LDH, CK-MB, and cTn-1 compared to Dox-treated cells. mBer was significantly (P-value $<$ 0.05) more effective than free Ber in this regard (Figure 2).

The mBer mitigates the oxidative stress induced by Dox

Afterward, the amounts of variables linked to oxidative stress were assessed. MDA production was dramatically increased by Dox treatment in the H9c2 cells, whereas Ber co-treatment significantly (P-value $<$ 0.05) reduced the Dox-induced increase in MDA level. Compared to Ber-Dox-treated cells, mBer-Dox-treated cells demonstrated a significant reduction (P-value $<$ 0.05) in MDA levels. The levels of MDA in Em-treated cells, Ber-treated cells, and mBer-treated cells are not significantly different from those in control-treated cells (Figure 3a).

Moreover, anti-oxidative status in H9c2 cells was evaluated by looking at indicators including GSH content as well as CAT and SOD activities. GSH level and CAT and SOD activities significantly (P-value $<$ 0.05) declined in Dox-treated cells after 48 hours of incubation relative to the control group. In contrast, the GSH level and CAT and SOD activities of cells that were treated with both Dox and Ber were significantly (P-value $<$ 0.05) lower than those assessed in the cardiomyocytes that were only treated with Dox. This demonstrates how Ber can lessen the oxidative stress that Dox causes in H9c2 cells. Comparing cells treated with Ber-Dox to those treated with mBer-Dox, the levels of GSH, CAT, and SOD activities were significantly (P-value $<$ 0.05) greater in those who received mBer-Dox. The studied anti-oxidant parameters neither significantly (P-value $<$ 0.05) differ between the control and EM treated cells nor between the Ber and mBer treated cells (Figures 3b, 3c, and 3d).

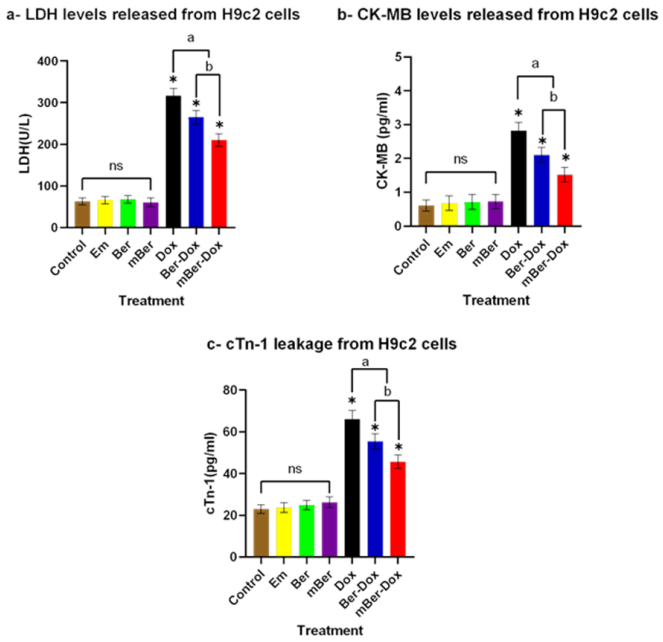

Figure 2. The effects of mBer on Dox-induced cardiomyocyte toxicity markers Leakage in H9c2 cells. Cardiotoxicity parameters included a: release of lactate dehydrogenase (LDH), b: Creatine kinase myocardial band (CK-MB) leakage, and c: Cardiac troponin I (cTn-I) release. The data are presented as the mean value ± standard deviation based on three independent samples. ns: not statistically significant (P-value $>$ 0.05) versus the control; *: statistically significant (P-value $<$ 0.05) versus the control; a: statistically significant (P-value $<$ 0.05) versus Dox; and b: statistically significant (P-value $<$ 0.05) versus Ber-Dox. Em: empty micelles; Ber: berberine; mBer: pluronic F127 micelles loaded with berberine; and Dox: doxorubicin.

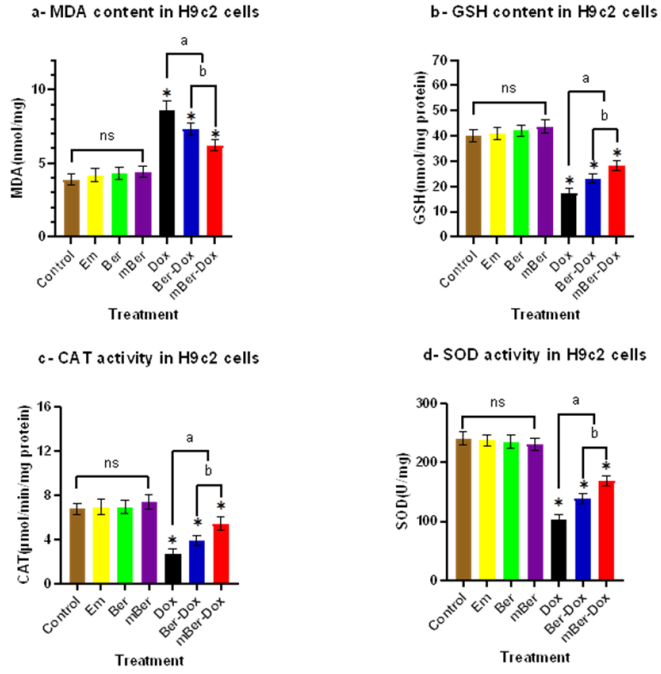

Figure 3. mBer suppresses Dox-induced oxidative stress. The oxidative stress parameters in H9c2 cells were: a: Lipid peroxidation indicated by malondialdehyde (MDA) level; b: Reduced glutathione (GSH) level; c: Catalase (CAT) activity; and d: Superoxide dismutase (SOD) (GPx) activity. Based on triplicates, the data are shown as mean ± standard deviation. ns: not statistically significant (P-value $>$ 0.05) compared to the control; *: statistically significant (P-value $<$ 0.05) compared to the control; a: statistically significant (P-value $<$ 0.05) compared to Dox; and b: statistically significant (P-value $<$ 0.05) compared to Ber-Dox. Em stands for empty micelles, Ber for berberine, mBer for pluronic F127 micelles containing berberine, and Dox for doxorubicin.

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The mBer alleviates cardiac cytokines secretion in Dox-treated H9c2 cells

After 48 hours of exposure to 1μM Dox, the levels of IL-1β and IL-6 in the culture medium of Dox-treated cardiomyocytes were significantly (P-value < 0.05) higher than those of control cells. A rise in cytokines levels caused by Dox was shown to be significantly (P-value < 0.05) lessened by combining treatment with either Ber or mBer. However, combination treatment with mBer resulted in an additional significant reduction (P-value < 0.05) in cytokines than did combined treatment with Ber. Again, Em, Ber, and mBer do not have any significant (P-value > 0.05) effect on the level of the studied cytokines that are released into the culture medium of cardiomyocytes (Figure 4).

The mBer protects H9c2 cells from Dox-induced cell apoptosis

Dox-treated cells exhibited fivefold more caspase 3/7 activity than untreated control cells. Ber-Dox or mBer-Dox significantly (P-value < 0.05) reduced caspase 3/7 activity in H9c2 cells compared to Dox treatment alone while maintaining apoptotic activity beyond the control level. Notably, there is a significant (P-value < 0.05) reduction in caspase 3/7 activity in cells treated with mBer-Dox as compared to those treated with Ber-Dox. Em, Ber, and mBer have no discernible effect on the activity of Caspase 3/7in H9c2 cells (Figure 5).

DISCUSSION

Cell viability

We used a concentration of 1 μM of Dox in this study because this concentration is representative of what is seen in human plasma after therapeutic doses of Dox have been delivered [14]. In clinical practice, after Ber was given to patients, its maximum concentration in the plasma could reach 1 μM [15]. In accordance with this, we find that Ber at this concentration does not display clear cellular toxicity in cultured cardiomyocytes. The significant improvement in the cardioprotective activity of mBer over the free form of Ber reported in this study can be attributed to the ability of the micelle to preserve insoluble hydrophobic compounds such as Ber. Micelles' structure and function mirror the characteristics of biological transport systems [16]. Thus, loading Ber into the amphiphilic polymeric Pluronic F127 micelle permits the release and availability of Ber nanoparticles readily in the treated cells. This is consistent with a previous study indicating that the hypoglycemic effect of Ber can be enhanced by using F127 micelles as a carrier [17].

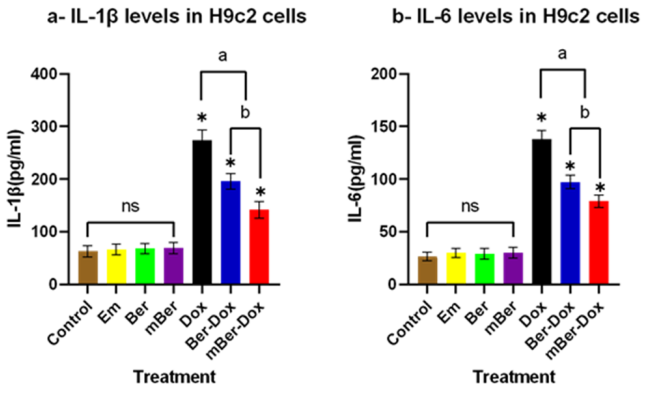

Figure 4. The effect of mBer on Dox-induced proinflammatory cytokine elevation in H9c2 cells. The studied inflammatory markers included a: interleukin-1β (IL-1β) and b: interleukin-6 (IL-6). Data presented as mean ± standard deviation based on three independent samples. ns: not statistically significant compared to the control (P-value > 0.05); *: statistically significant compared to the control; a: compared to Dox; and b: compared to Ber-Dox (P-value < 0.05).

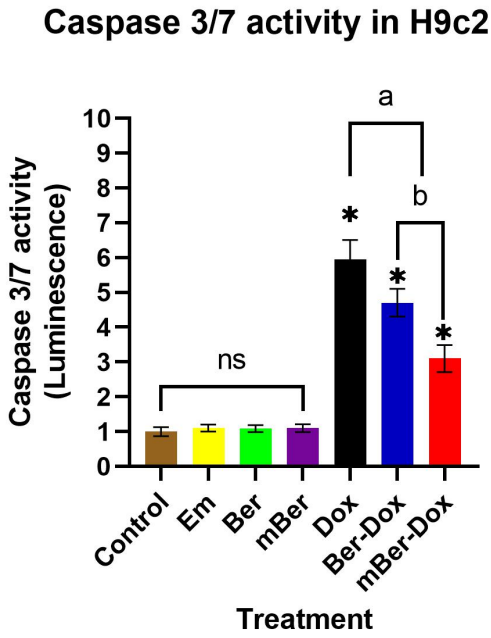

Figure 5. mBer inhibits Caspase 3/7 activity in Dox-treated H9c2 cells. The presented data illustrates the mean ± standard deviation from three distinct biological replicates. The notation "ns" denotes no statistically significant difference when compared to the control group, while "*", "a", and "b" signify significant differences in relation to the control group, Dox and Ber-Dox, respectively (P-value < 0.05).

LDH, CK-MB, and cTn-1 analysis in cardiomyocyte toxicity

The elevation in the studied cardiotoxicity markers after Dox treatment is consistent with previous research indicating that Dox compromises the integrity of myocardial cell membranes [18]. Interestingly, simultaneous treatment with free Ber or mBer reduced LDH, CK-MB, and cTn-I release from Dox-treated H9c2 cells. Ber's ability to reduce the release of cardiac LDH, CK-MB, and cTn-I might be due to Ber's antioxidant properties and/or interaction with cell signaling pathways.

Antioxidative status indices

Cardiomyocytes are more susceptible to reactive oxygen species (ROS)-induced oxidative injury due to their relatively

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lower levels of antioxidant enzymes [19]. Our findings validated that Dox exhibited a notable elevation of MDA levels and a significant reduction in the GSH content and performance of CAT and SOD, which unequivocally point to the presence of severe oxidative stress, which is consistent with previous study [20]. The treatment with Ber resulted in a significant improvement in the studied oxidative stress parameters, which is consistent with prior investigations [20]. One possible mechanism for Ber antioxidant activity is that Ber is a type of metal chelator that can chelate transition metals, including iron, which is particularly significant as iron has been shown to contribute to the initiation of ROS. Thus, treatment with Ber results in a decrease in the generation of ROS [21]. The cardioprotective properties of Ber may be attributed to its antioxidant potential. Importantly, an enhancement in antioxidant activity is evident in H9c2 cells receiving mBer-Dox treatment groups in comparison to the Ber-Dox-treated group. Our findings suggest that the incorporation of Ber within the micelles enhances the antioxidant capacity of this phytochemical.

Anti-inflammatory activity

Notably, the expression of inflammatory cytokines is directly induced by oxidative stress, which further escalates inflammatory processes [22]. Dox is capable of causing significant cardiac toxicity via stimulation of the synthesis of multiple pro-inflammatory cytokines, such as IL 1β, and IL-6 which are involved in cardiomyocytes inflammatory damage [23]. Therefore, prior studies have shown that reducing cardiomyocyte mortality and cardiac dysfunction after Dox administration is possible via the regulation of cardiac inflammation [24]. The anti-inflammatory properties of Ber in cardiac cells have been previously linked to its ability to impede the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway. This, in turn, leads to a decrease in the release of several pro-inflammatory cytokines, including IL-6, IL-1β, and tumor necrosis factor (TNF-α), in cardiomyocytes [25]. The findings suggest that Ber could have the ability to mitigate the inflammatory response triggered by Dox in cardiomyocytes, and this ability was improved when Ber was loaded into pluronic micelles.

Caspase 3/7 activity implies apoptosis.

Various anticancer agents, such as Dox, can induce apoptosis through the activation of caspases 3/7, implying either an intrinsic or extrinsic pathway for ultimate cell death. Therefore, evaluating caspase 3/7 may provide insights into ultimate cell death regardless of the specific apoptotic mechanism being used [26]. A substantial increase in caspase 3/7 activity after Dox treatment is consistent with prior reports about Dox in cultured cardiomyocytes [27]. The antagonistic interaction between Ber and Dox in H9c2 cells in terms of apoptosis may be caused by a reduction in both the activity of caspase 3/7 and reactive oxygen species in cardiomyocytes induced by Ber treatment. This is corroborated by previous reports that Ber treatment reduced apoptotic and oxidative stress in response to ischemia injury by lowering caspase-3 activity and MDA levels [28]. However, Ber or mBer co-delivery did not completely inhibit Dox-induced caspase 3/7 activation and return to normal levels (untreated), indicating that DIC is not completely mitigated. A possible rationale for this occurrence is that Dox-induced caspase 3/7 activation may involve other mechanisms that might not be fully affected by Ber treatment at the current concentration.

One limitation of the current study is its exclusive focus on assessing the effectiveness of the cardioprotective effect of mBer in vitro. To validate these findings, further in vivo studies need to be undertaken to further test this approach. Additionally, more research is needed to determine the long-term efficacy of mBer's as a cardioprotective agent as part of a Dox treatment regimen to establish the comprehensive utility of this approach.

CONCLUSION

The present study demonstrates that the efficacy of Ber in the treatment of cardiomyocytes exposed to Dox is improved when administered in its nanomicellar formulation. The utilization of mBer presents a more suitable option compared to conventional free Ber in mitigating cardiotoxicity and the elevated levels of oxidative stress, inflammation, and apoptosis-inducing factors in cardiomyocytes exposed to Dox treatment. Hence, the properties of the mBer formulation can be harnessed as a promising protective agent against DIC.

ETHICAL DECLARATIONS

Acknoweldgements

We appreciate the Iraqi Center for Cancer Research laboratory personnel for their valuable advice.

Ethics Approval and Consent to Participate

The study was approved by the Ethical Approval Committee of the College of Veterinary Medicine, University of Al-Qadisiyah, with a reference number of 426 on February 5, 2023. There were neither humans involved in this study to obtain their consent nor animals involved in this study.

Consent for Publication

Not applicable (no individual personal data included).

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

Funding

No funding.

Authors' Contributions

All stated authors contributed significantly, directly, and intellectually to the work and consented it to be published.

REFERENCES

[1] Tanawat Attachaipanich, Siriporn C Chattipakorn, and Nipon Chattipakorn. Potential Roles of Melatonin in Doxorubicin-Induced Cardiotoxicity: From Cellular Mechanisms to Clinical Application. *Pharmaceutics*, 15(3):785, 2023.

[2] Pushkar Singh Rawat, Aiswarya Jaiswal, Amit Khurana, Jasvinder Singh Bhatti, and Umashanker Navik. Doxorubicin-induced cardiotoxicity: An update on the molecular mechanism and novel therapeutic strategies for effective management. *Biomedicine & Pharmacotherapy*, 139:111708, 2021.

[3] Joerg Herrmann. Adverse cardiac effects of cancer therapies: cardiotoxicity and arrhythmia. *Nature Reviews Cardiology*, 17(8):474–502, 2020.

[4] Brian B. Hasinoff and Eugene H. Herman. Dexrazoxane: How it works in cardiac and tumor cells. Is it a prodrug or is it a drug? In *Cardiovascular Toxicology*, volume 7, pages 140–144, 2007.

[5] David S Monahan, Eimhear Flaherty, Aamir Hameed, and Garry P Duffy. Resveratrol significantly improves cell survival in comparison to dexrazoxane and carvedilol in a h9c2 model of doxorubicin induced cardiotoxicity. *Biomedicine & Pharmacotherapy*, 140:111702, 2021.

[6] Judith A. Smith *et al.* Is it equivalent? Evaluation of the clinical activity of single agent Lipodox® compared to single agent Doxil® in ovarian cancer treatment. *Journal of Oncology Pharmacy Practice*, 22(4):599–604, 2016.

[7] Sruthi Sritharan and Nageswaran Sivalingam. A comprehensive review on time-tested anticancer drug doxorubicin. *Life sciences*, 278:119527, 2021.

[8] Shreesh Ojha *et al.* Cardioprotective potentials of plant-derived small molecules against doxorubicin associated cardiotoxicity. *Oxidative medicine and cellular longevity*, 2016, 2016.

[9] Inder Pal Singh and Shivani Mahajan. Berberine and its derivatives: a patent review (20092012). *Expert opinion on therapeutic patents*, 23(2):215–231, 2013.

[10] Yun Cai *et al.* A new therapeutic candidate for cardiovascular diseases: Berberine. *Frontiers in pharmacology*, 12:631100, 2021.

[11] Chang Shun Liu, Yu Rong Zheng, Ying Feng Zhang, and Xiao Ying Long. Research progress on berberine with a special focus on its oral bioavailability. *Fitoterapia*, 109:274–282, 2016.

[12] Jiangxiu Niu *et al.* Berberine-loaded thiolated pluronic f127 polymeric micelles for improving skin permeation and retention. *International Journal of Nanomedicine*, 15:9987–10005, 2020.

[13] Victor Kuete, Ouzhan Karaosmanolu, and Hülya Sivas. Anticancer Activities of African Medicinal Spices and Vegetables. In *Medicinal Spices and Vegetables from Africa: Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases*, pages 271–297. Elsevier, 2017.

[14] Christian Siebel, Claudia Lanvers-Kaminsky, Gudrun Würthwein, Georg Hempel, and Joachim Boos. Bioanalysis of doxorubicin aglycone metabolites in human plasma samplesimplications for doxorubicin drug monitoring. *Scientific Reports*, 10(1):1–7, 2020.

[15] Y J Wu, L F Li, and J H Meng. Study on the Pharmacokinetics of Berberine. *Journal of Mathematical Medicine*, 21:217–219, 2008.

[16] M Ghezzi *et al.* Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. *Journal of Controlled Release*, 332:312–336, 2021.

[17] Zi Wang, Peiran Chen, Min Guo, Xiaoting Yang, Wei Song, and Fengjie Huang. Physicochemical Characterization of Berberine-loaded Pluronic F127 Polymeric Micelles and In Vivo Evaluation of Hypoglycemic Effect. *Journal of Pharmaceutical Innovation*, pages 1–10, 2022.

[18] Muhammad Hassaan Shahid, Irfan Anjum, Muhammad Naveed Mushtaq, and Saba Riaz. Cardioprotective effect of boswellic acids against doxorubicin induced myocardial infarction in rats. *Pakistan Journal of Pharmaceutical Sciences*, 34, 2021.

[19] Goncalo C Pereira, Ana M Silva, Catia V Diogo, Filipa S Carvalho, Pedro Monteiro, and Paulo J Oliveira. Drug-induced cardiac mitochondrial toxicity and protection: from doxorubicin to carvedilol. *Current pharmaceutical design*, 17(20):2113–2129, 2011.

[20] Yan Zhao Wu, Lan Zhang, Zi Xiao Wu, Tong Tong Shan, and Chen Xiong. Berberine Ameliorates Doxorubicin-Induced Cardiotoxicity via a SIRT1/p66Shc-Mediated Pathway. *Oxidative Medicine and Cellular Longevity*, 2019, 2019.

[21] Firouzeh Gholampour and Samaneh Keikha. Berberine protects the liver and kidney against functional disorders and histological damages induced by ferrous sulfate. *Iranian journal of basic medical sciences*, 21(5):476, 2018.

[22] Jong-Seok Moon *et al.* NOX4-dependent fatty acid oxidation promotes NLRP3 inflammasome activation in macrophages. *Nature medicine*, 22(9):1002–1012, 2016.

[23] Alexander Riad *et al.* Tolllike receptor4 deficiency attenuates doxorubicininduced cardiomyopathy in mice. *European journal of heart failure*, 10(3):233–243, 2008.

[24] Yu-Pei Yuan *et al.* CTRP3 protected against doxorubicin-induced cardiac dysfunction, inflammation and cell death via activation of Sirt1. *Journal of Molecular and Cellular Cardiology*, 114:38–47, 2018.

[25] Zhu Qin-Wei and L I Yong-Guang. Berberine attenuates myocardial ischemia reperfusion injury by suppressing the activation of PI3K/AKT signaling. *Experimental and therapeutic medicine*, 11(3):978–984, 2016.

[26] M Olsson and B Zhivotovsky. Caspases and cancer. *Cell Death & Differentiation*, 18(9):1441–1449, 2011.

[27] Lisa Janssen Carlson, Brianna Cote, Adam W G Alani, and Deepa A Rao. Polymeric micellar co-delivery of resveratrol and curcumin to mitigate in vitro doxorubicin-induced cardiotoxicity. *Journal of pharmaceutical sciences*, 103(8):2315–2322, 2014.

[28] Abeer Abdulredha, Munther Abosaooda, Fadhil Al-Amran, and Najah R Hadi. Berberine protests the heart from ischemic reperfusion injury via interference with oxidative and inflammatory pathways. *Medical Archives*, 75(3):174, 2021.

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