Cardiovascular Toxicology
https://doi.org/10.1007/s12012-021-09651-4

Bacterial Lipase Neutralized Toxicity of Lipopolysaccharide on Chicken Embryo Cardiac Tissue

Afsaneh Bagherzadeh[1] · Hamidreza Vaziri[1] · Fatemeh Sokouti Nasimi[2] · Shahin Ahmadian[2] · Adel Feyzi[3] · Mehrdad Farhadi[4] · Fariba Yahyavi[2] · Behnam Hashemi[2] · Reza Rahbarghazi[2,5] · Mahdi Mahdipour[2,6]

Received: 26 February 2021 / Accepted: 7 April 2021

Abstract

It has been shown that near all organs, especially the cardiovascular system, are affected by bacterial lipopolysaccharide via the activation of Toll-like receptor signaling pathways. Here, we tried to find the blunting effect of bacterial lipase on lipopolysaccharide (LPS)-induced cardiac tissue toxicity in chicken embryos. 7-day fertilized chicken eggs were divided randomly into different groups as follows; Control, Normal Saline, LPS (0.1, 0.5 and 1 mg/kbw), and LPS (0.1, 0.5 and 1 mg/kbw) plus 5 mg/ml Lipase. On day 17, the hearts were sampled. The expression of genes such as GATA4, NKX2.5, EGFR, TRIF, and NF-ƙB was monitored using real-time PCR analysis. Using western blotting, we measured NF-ƙB protein level. Total antioxidant capacity, glutathione peroxidase, and Catalase activity were also studied. Microvascular density and anterior wall thickness were monitored in histological samples using H&E staining. High dose of LPS (1 mg/kbw) increased the expression of TRIF but not NF-ƙB compared to the control group ($p < 0.05$). We found a statistically significant reduction in groups that received LPS + Lipase compared to the control and LPS groups ($p < 0.05$). Western blotting revealed that the injection of Lipase could reduce LPS-induced NF-ƙB compared to the control group ($p < 0.05$). The expression of GATA4, NKx2.5, and EGFR was not altered in the LPS group, while the simultaneous application of LPS and Lipase significantly reduced GATA4, NKx2.5, and EGFR levels below the control ($p < 0.05$). We found non-significant differences in glutathione peroxidase, and Catalase activity in all groups ($p > 0.05$), while total antioxidant capacity was increased in groups that received LPS + Lipase. Anterior wall thickness was diminished in LPS groups and the use of both lipase and LPS returned near-to-control values ($p < 0.05$). Despite a slight increase in microvascular density, we found statistically non-significant differences in all groups ($p > 0.05$). Bacterial lipase reduces detrimental effects of LPS on chicken embryo heart induced via Toll-like receptor signaling pathway.

Keywords Chicken embryo heart · Toll-like receptor signaling · Lipopolysaccharide · Lipase · Toxicity

Handling Editor: Vittorio Fineschi.

✉ Reza Rahbarghazi
Rezarahbardvm@gmail.com; rahbarghazir@tbzmed.ac.ir

✉ Mahdi Mahdipour
mahdipourm@tbzmed.ac.ir; mahdi.mahdipour@gmail.com

[1] Department of Biology, Faculty of Science, Guilan University, Rasht, Iran

[2] Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[3] Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Tabriz Branch, Tabriz, Iran

[4] Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[5] Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

[6] Department of Reproductive Biology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

Published online: 15 April 2021

Introduction

Pregnancy is a unique period in which a single cell embryo develops into a multi-organ organism [1]. Embryonic development normally occurs during the pre and post-implantation period until birth. Because of embryo exposure to non-genetic and environmental risk factors such as infectious agents and pathogens, it is postulated that these conditions can lead to the lack of embryonic growth and development [2, 3]. Noteworthy, very early weeks of gestation period are considered the most critical times as any distracting factors can later cause irreversible anatomical abnormalities and structural birth defects known as teratogenesis [2, 3]. Within the uterus, the crosstalk between the mother and the embryo is disturbed due to the presence of inflammation and infectious pathogens [4]. Lipopolysaccharide (LPS) is Gram-negative bacteria endotoxin and the main component of the outer membranes. LPS has a molecular weight of more than 100 kD and consists of three components including a lipid A, core oligosaccharide, and hydrophilic polysaccharide named O antigen [5]. Lipid A is composed of a phosphorylated N-acetyl glucosamine dimer attached to 6 or 7 fatty acids [6]. In the structure of O antigen, 3 to 5 sugars are detectable. All of these structures participate in LPS bioactivities in in vivo conditions. It was suggested that the lipid A portion of LPS can lead to toxicity, while O antigen provokes the immune-related responses and immunogenicity [6]. LPS is shown to play a critical role in the pathogenesis of bacterial infections and confronts the host defense system [7]. High concentrations of LPS may lead to systemic sepsis that targets all organs, especially the cardiovascular system [5, 8–11]. The activation of both humoral and cellular immunities can lead to the detoxification of LPS via enzymatic degradation or complement-base activity [12]. Besides, blood can neutralize the effects of circulating LPS and limit the distribution of LPS to the remote sites. For instance, it has been shown that serum factors such as apolipoproteins, lipoproteins, and LPS-transferring proteins [LPS-binding protein (LBP), soluble CD14 and phospholipid transfer protein] can attach LPS [13]. Upon the activation of immune system, serum levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin-1 (IL-1), and -6 are elevated [5, 7]. It has been confirmed that wide types of plasma membrane receptors such as pathogen-associated molecular patterns (PAMPs), LBP, CD14, MD-2, and TLR4 are actively involved in the identification of LPS [8, 14]. To this end, the inhibition of LPS activity is an important therapeutic strategy to reduce Gram-negative infection complications [5]. To the best of our knowledge, different aspects of LPS activity have not been fully investigated during the gestation period.

Lipases (triacylglycerol acyl hydrolase) can hydrolyze carboxyl ester bonds in triacylglycerol [15]. The most important of commercial microbial lipases are produced by bacteria [16]. In an experiment conducted by our group, we found that bacterial lipase can reduce the detrimental effect of LPS on rat cardiomyoblasts in in vitro culture system [17].

In this study, the chicken embryo was used as a laboratory-animal model to assess the possible effect of LPS on cardiac tissue. We further analyzed the blunting effect of bacterial lipase on LPS-induced cardiac injury in the chicken embryos. LPS and lipase were injected into the albumen section and cardiac tissue was assessed in pre-hatched condition by histological examination and monitoring the expression of different genes participate in the inflammatory response (TRIF and NF-ƙB) and the development of cardiomyocytes (*i.e.*, GATA4, NKvx2.5, and EGFR).

Materials and Methods

Chicken Embryo and Ethical Issue

All the experiments conducted in this project were approved by the Local Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1397.082). Fertilized chicken eggs (Ross strain) were purchased from Local Chicken Hatcher Incubator Company and transferred to the laboratory in an enclosed container. After sterilization using 70% EtOH, eggs were immediately placed in an automatic hatcher incubator instrument set (Easy-Bator 1, Automatic Incubator, Urmia, Iran) at 60% humidity and 38 °C.

LPS and Lipase Stock Preparation

We purchased *E. Coli* LPS (Cat no: L2880; Sigma-Aldrich) and Lipase from *Pseudomonas cepacia* (Cat no: 62309; Sigma-Aldrich). Both LPS and Lipase were dissolved in sterile normal saline and aliquots were stored at − 80 °C until use. LPS was injected into the 7-day fertilized eggs at final concentrations of 0.1 and 1 mg/kbw. In groups that received the combination of LPS and Lipase, different doses of LPS were co-incubated with 5 mg/ml Lipase at 38˚C overnight to inactivate the LPS.

Inoculation of LPS and LPS + Lipase

At the first 7 days of incubation inside an automatic hatcher incubator instrument set, fertilized eggs were divided randomly into different groups (each in 6 eggs) as follows; Control, Normal saline (Vehicle), LPS (0.1 and 1 mg/kbw), and LPS (0.1 and 1 mg/kbw) + Lipase (5 mg/ml). Before injection, the surface of the eggs was

disinfected using 70% EtOH. Then, a small hole was generated at the site of injection using a sterile needle gauge 20. In ovo injection volume was near 100 µl solvent per egg. After the completion of the injection, the hole was sealed properly using melted paraffin drops, and eggs were immediately transferred into the incubator and maintained for additional 10 days.

Real-Time PCR Analysis

16-day-old chicken embryos were euthanized humanely and cardiac tissues were sampled under sterile conditions. On this basis, hearts were washed in sterile phosphate-buffered saline (PBS) and used for histological examination, gene expression analysis, and western blotting. For gene expression assay, RNA extraction was done in the heart samples using an RNA extraction kit (Cat No: FABRK001; Yektatajhiz Co.) according to the instructions given by the manufacturer. The quality and concentration of RNAs were assessed using the Picodrop spectrophotometer system (Model No: PICOPET01) and reverse-transcribed to cDNA (Cat. No: YT4500; Yektatajhiz kit). *Gallus gallus* species-specific primers such as GATA4, NKx2.5, EGFR, TRIF, and NF-ƙB were designed using online software (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and purchased from Metabion, Germany (Table 1). The expression of each gene was calculated based on the $2^{-\Delta\Delta CT}$ method after normalization to GAPDH. In this study, PCR reactions were performed in 45 cycles of 95 °C for 10 s (denaturation), 60 or 62 °C for 10 s (annealing), and 72 °C for 10 s (extension) using Roche LightCycler96 instrument. All the PCR reactions were performed with three biologically related samples for each group in two replicates.

Hematoxylin–Eosin Staining

To this end, heart samples were fixed in 10% formalin solution and embedded in paraffin blocks. Thereafter, 5-µm thick sections were prepared using Leica microtome system and stained with Hematoxylin and Eosin (H&E) solution. In this study, the existence of inflammatory responses, cardiomyocytes were monitored in left ventricles. To assess microvascular intensity, the number of microvesicles was randomly counted in 10-high-power fields. We also measured average anterior wall thickness randomly in four to five points per slide using ImageJ software (version 1.4, NIH) and results were compared among the groups. Left ventricle/slide section area was also calculated and expressed as mm^2.

Measuring Antioxidant Capacity

To assess whether in ovo injection of LPS and LPS + Lipase alter the oxidative status in chicken hearts, we measured the total activity of Catalase (Cat no: 707002; Cayman Chemical Company), Glutathione peroxidase (GPx, Cat No: RS 504, Randox laboratories, UK) and total antioxidant capacity (TAC, Cat No: NX 2332, Randox). Heart samples were lysed using protein lysis buffer consisted of 150 mM NaCl, 0.1% SDS, 50 mM Tris–HCl, 2 mM EDTA, and 1% NP-40 enriched with an anti-protease cocktail. TAC was calculated using the following formula:

Table 1 List of primers

Genes	Name and accession number	Sequence	Annealing ™ °C
GATA4	NM_001293106.1 Gallus gallus GATA binding protein 4 (GATA4)	F1: TGAAATGGCCACCACTTGGA R1: AGTTGACACATTCTCGCCCT	60
NKX2.5	NM_205164.1 Gallus gallus NK2 homeobox 5 (NKX2-5)	F1: AGGATTCCAAGGCGGACAAG R1: TTGAGAAAAGAGGACGCGGG	60
EGFR	NM_205497.2 Gallus gallus epidermal growth factor receptor (EGFR)	F1: GTGACATTTGGGTCCAAGCCTA R1: CGGCTGTCTGCATCAATCA	60
TRIF	NM_001081506.1 Gallus gallus toll-like receptor adaptor molecule 1 (TICAM1)	F1: GTGGAAGCATCATTGGCACC R2: TCCTTGATGCGACAGGCAAT	60
NF-KB C-rel	NM_001167726.1 Gallus gallus REL proto-oncogene, NF-kB subunit (REL)	F1: ATCACAGAACCCATCACGGTC R1: CAGCGTTGATCTTTGCCTT	62
GAPDH	NM_204305.1 Gallus gallus glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	F1: GCAGCAGGAACACTATAAAGGC R1:ATTGATGGCCACCACTTGGA	60

F forward, *R* reverse

$$[TAC = Concentration\ of\ the\ S\tan dard \times \left(\Delta_A Blank - \Delta_A Sample\right) / \left(\Delta_A Blank - \Delta_A S\tan dard\right)]$$

where A_1 stands for adsorption at t_0, A_2 is the adsorption at t_1, and Δ_A is the values of difference in adsorption time between t_0 and t_1. The TAC values were presented as mM. The levels of GPx and Cat were also measured in protein lysates using Randox kit according to the manufacturer's instruction. Both Cat and GPx levels are expressed as IU/mg of protein.

Western Blotting

We performed western blotting to measure protein levels of NF-κB in chicken embryo cardiac samples exposed to LPS and LPS + Lipase. For this purpose, the protein lysates from different groups were electrophoresed on 10% SDS-PAGE and transferred onto the PVDF membrane. Thereafter, the membranes were blocked using 1% bovine serum albumin (BSA, Cat No: A7906, Sigma) and incubated with anti-NF-κB antibody (Cat No: sc-8008: Santa Cruz Biotechnology, Inc.) at 4˚C overnight. After three washes with TBS-T solution, membranes were exposed to HRP-conjugated mouse anti-rabbit secondary antibody (Cat no: sc-2357; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature followed by three-time TBS-T washes (each for 10 min). To visualize the immunoreactive bands, ECL solution (Cat no: 84785, Thermo Scientific) and X-ray films were used. The density of each immunoreactive band was calculated using ImageJ software. β-actin (Cat no: sc-47778; Santa Cruz Biotechnology, Inc.) was used as an internal housekeeping protein.

Statistical Analysis

All data are presented as mean ± SD. The statistical difference between the groups was found by One-way analysis of variance (ANOVA) with the post hoc test of LSD using

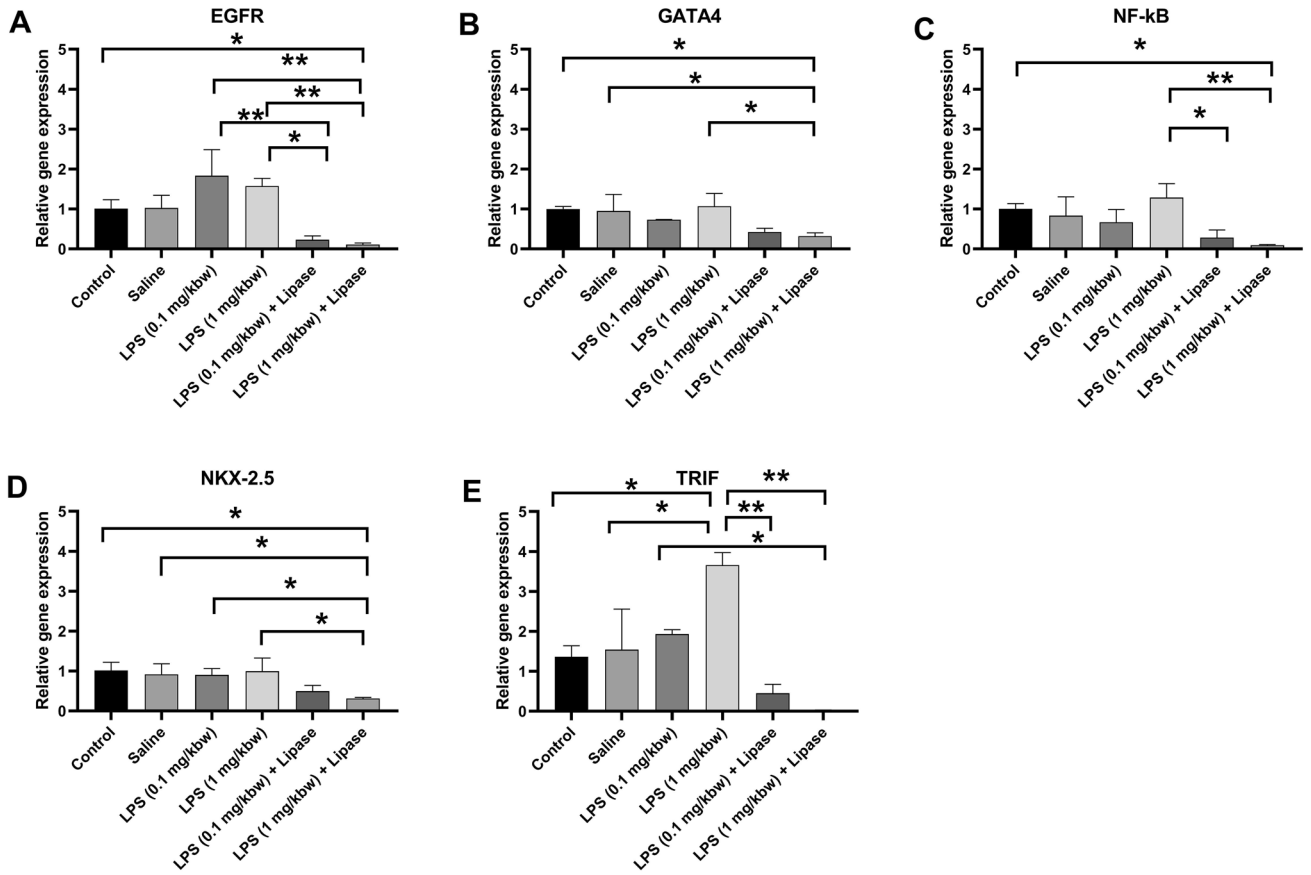

Fig.1 Real-time PCR analysis of GATA4, NKx2.5, NF-ƙB, EGFR, and TRIF genes following treatment with LPS and LPS plus Lipase. Fertilized chicken eggs were injected with different doses of LPS (0.1 and 1 mg/kbw) and LPS (0.1 and 1 mg/kbw) plus Lipase (5 mg/ml). GAPDH was used as a housekeeping gene. All data are presented as mean ± SD. One-way analysis of variance (ANOVA) with the post hoc test of LSD.*$p < 0.05$; **$p < 0.01$ ($n = 3$)

GraphPad software (Version 8.0.2). p values below 0.05 were considered statistically significant.

Results

Transcript Levels Related to Inflammation have Elevation Following LPS Treatment

The expression of genes involved in cardiac development and inflammatory response was monitored after in ovo injection of LPS and LPS plus Lipase. According to our data, the injection of different doses of LPS, 0.1 and 1 mg/kbw, did not significantly alter the expression of EGFR, GATA4, and NKx2.5 compared to the control and vehicle groups ($p > 0.05$; Fig. 1a, b, and d). Interestingly, the combination of LPS and Lipase down-regulated significantly the expression of these genes and reached below the control levels ($p < 0.05$; Fig. 1a, b, and d). We also found statistically significant differences in the expression of EGFR, GATA4, and NKx2.5 between the LPS and LPS plus Lipase ($p < 0.05$; Fig. 1a, b, and d). To assess the modulatory effect of LPS on the cardiomyocytes via the toll-like receptor signaling pathway, we monitored the expression of NF-ƙB and TRIF. Data showed that neither a higher dose of LPS 1 mg/kbw nor a low concentration of 0.1 mg/kbw can alter the expression of NF-ƙB, 10 days after in ovo injection of LPS. Again, we found that the combination of Lipase with higher doses of LPS reduced significantly NF-ƙB expression as compared with the control and control-matched LPS groups ($p < 0.05$; Fig. 1c). Based on our data, injection of LPS into fertilized eggs induced the expression of TRIF in the group that received 1 mg/kbw LPS compared to the control group ($p < 0.05$). We found that the pre-conditioning of LPS in the presence of Lipase neutralized these effects in which the expression of TRIF near-to-control levels (Fig. 1e).

Histopathological Examination Revealed Focal Tissue Injury and Inflammation

Histopathological examination revealed the adverse effects of LPS on chicken cardiac tissue 10 days after in ovo injection (Fig. 2a). We found focal to massive hemorrhagic foci in the group that received 1 mg/kbw LPS, while these effects were less in the chicken embryo that received 0.1 mg/kbw LPS. Necrotic changes and prominent interstitial edema were evident after injection of 1 mg/kbw LPS and these values were less in the 0.1 mg/kbw LPS group. Of note, overnight pre-treatment of LPS with Lipase neutralized LPS toxicity on the cardiac tissue in which all pathological indices like necrotic changes and focal hyperemia and inflammation were removed. Monitoring the microvascular density revealed a lack of statistically significant differences. Although the number of microvessels/high power field was increased in 1 mg/kbw groups these changes remained statistically non-significant (Fig. 2b).

LPS plus Lipase Reversed LPS-Induced Anterior Wall Thickness Alteration

Left ventricular anterior wall thickness of chicken embryo heart was analyzed in LPS and LPS + Lipase groups (Fig. 3a, b). According to our data, all concentrations of LPS including 0.1 and 1 mg/kbw decreased the anterior wall thickness compared to Control and vehicle groups ($p > 0.05$; Fig. 3a, b). The deactivated Lipase did not alter chicken embryo cardiac anterior wall thickness as well as compared to the Control group ($p > 0.05$; Fig. 3a, b). We noted that the combination of LPS and Lipase did not alter significantly the anterior wall thickness compared to the Control group ($p > 0.05$; Fig. 3a, b). To be specific, statistically significant differences were obtained regarding anterior wall thickness between the LPS and LPS plus Lipase groups ($p < 0.05$). Measuring left ventricle/slide section area revealed the lack of statistically significant differences between the groups ($p > 0.05$; Fig. 3c).These data showed that Lipase could blunt the detrimental effects of LPS on left ventricle wall thickness in the model of the chicken embryo.

Lipase Increased LPS-Induced TAC Reduction in Chicken Embryos

Data showed that neither Cat nor GPx activity was not altered significantly in LPS and LPS plus Lipase compared to the Control group ($p > 0.05$; Fig. 4a). Similarly, we found that 10-day incubation of chicken embryos with LPS did not alter the heart TAC, while in groups that received LPS + Lipase, a significant increase of TAC was evident compared to the Control group ($p < 0.05$; Fig. 4a). We noted that these effects were more evident in the 1 mg/kbw LPS + Lipase group rather than the 0.1 mg/kbw LPS + Lipase group.

NF-KB P65 Protein Level was Altered Following Treatment with LPS and LPS Plus Lipase

Unlike real-time PCR analysis, we found significant alteration of NF-ƙB P65 protein in groups that received LPS alone or the combination of LPS and Lipase (Fig. 4b, c). According to our data, the protein level of NF-ƙB P65 was significantly increased in groups received 0.1 and 1 mg/kbw LPS compared to the Control group ($p < 0.05$). Overnight incubation of LPS with bacterial Lipase did decrease the intracellular NF-ƙB P65 and reached near-to-normal levels (Fig. 4b, c). As a correlate, it seems that Lipase can blunt the

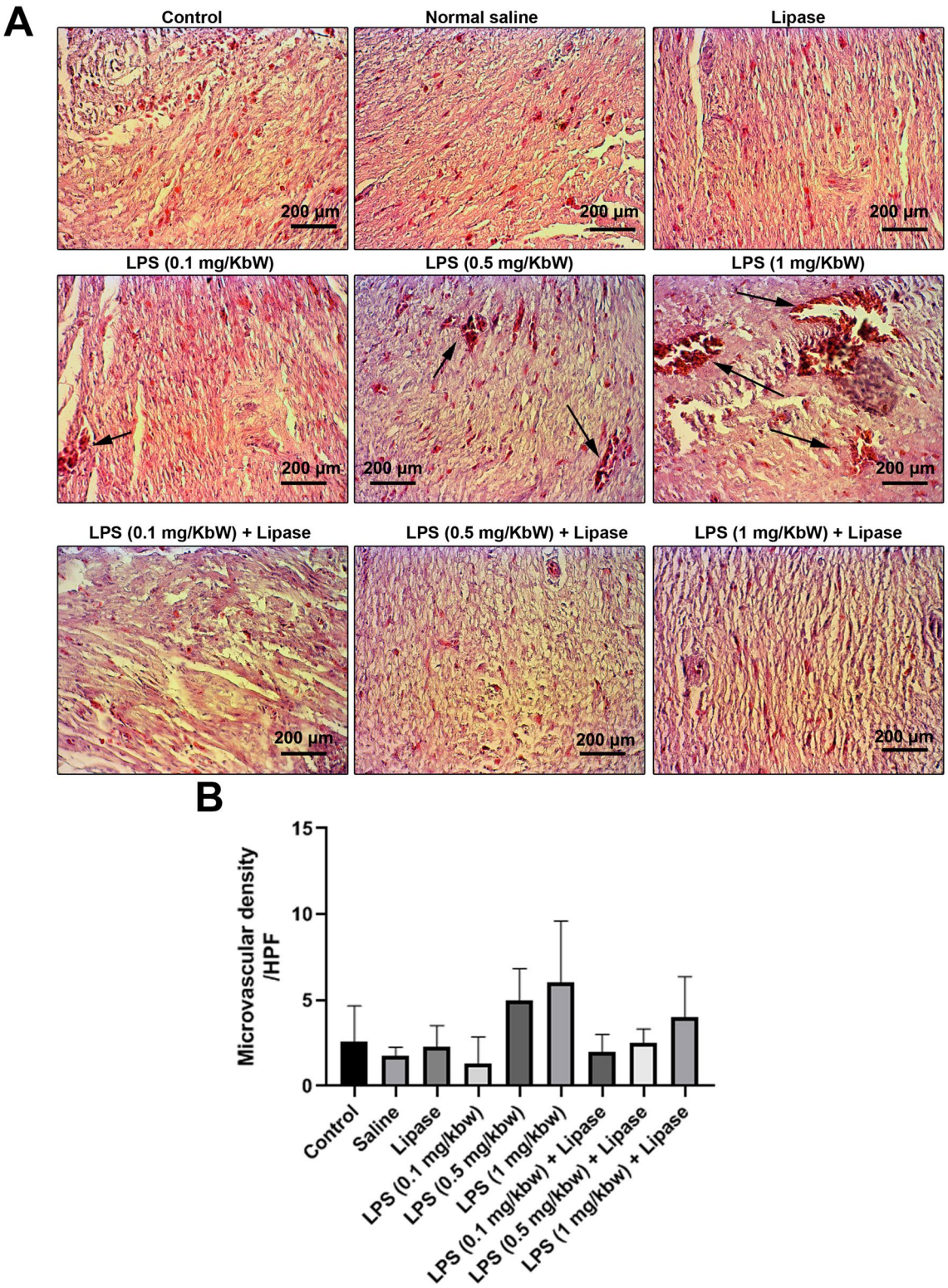

Fig. 2 Hematoxylin and Eosin (H&E) staining of the heart tissue (**a**); and microvascular density of the heart tissue (**b**; $n = 10$-high-power fields)

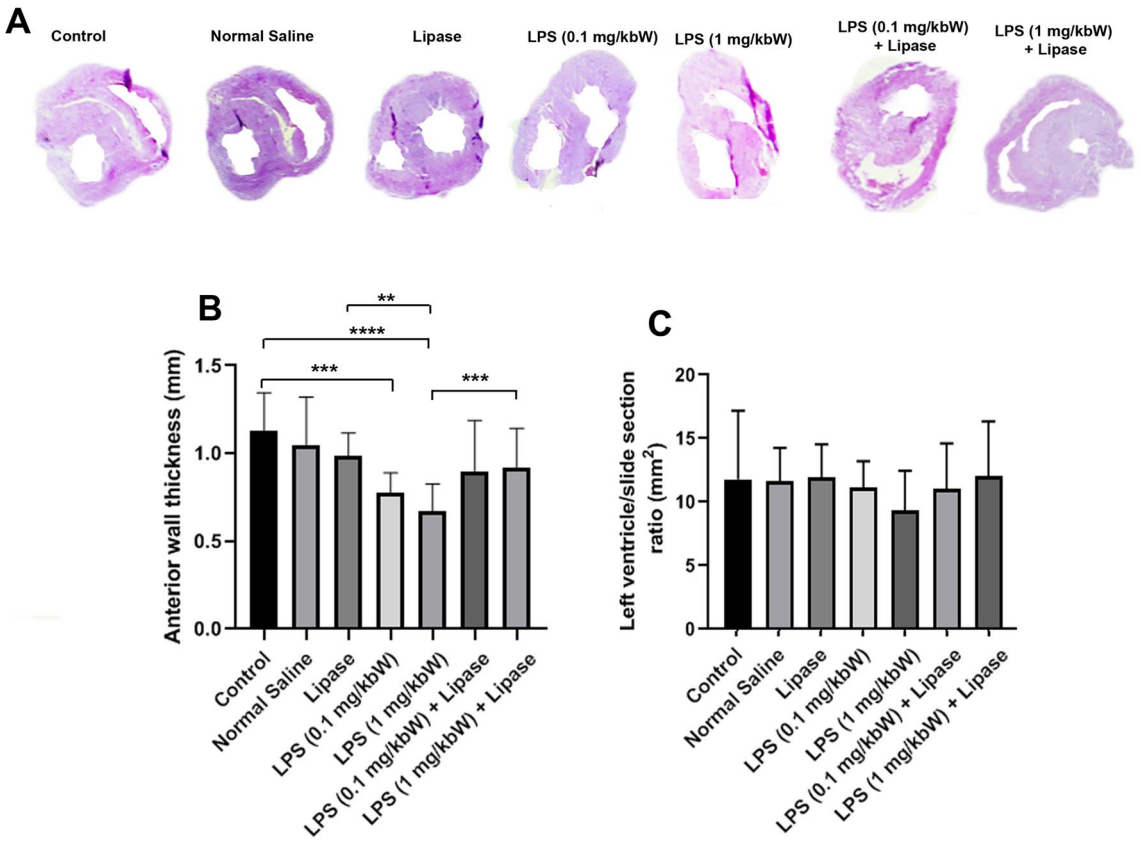

Fig. 3 Heart left ventricular anterior wall thickness (**a**); quantification of anterior wall thickness (**b; $n=6$);** and calculation of left ventricle per slide section area (mm^2). All data are presented as mean ± SD. One-way analysis of variance (ANOVA) with the post hoc test of LSD.*$p<0.05$; **$p<0.01$.; ***$p<0.001$; ****$p<0.0001$

stimulatory effect of LPS on NF-ƙB P65 in chicken embryo chicken hearts.

Discussion

In the current experiment, we tried to address the detrimental effect of bacterial LPS on the development of chicken embryo heart and inflammatory response. Whether bacterial Lipase can neutralize LPS effects was also the subject of this study. It has been shown that LPS can exert its effect on the target cells using Toll-like receptors (TLR) [14, 18]. The activation of TLR subsets like TLR4 along with co-receptor namely MD-2 triggers downstream signaling effectors mainly nuclear NF-κB [14]. It is believed that the binding of LPS to TLR4 leads to the activation of two distinct signaling cascades TRIF and MyD88 [19, 20]. These pathways end with the engaging of nuclear factors such as NF-κB which is involved in the production of pro-inflammatory cytokines, apoptosis, and cell survival factors [21]. The overactivation of TLRs receptors has been documented during sepsis and endotoxemia, contributing to cardiac dysfunction [9].

Here, we noted that in ovo injection of LPS did not alter the expression of genes such as EGFR, GATA4, and NKx2.5 in chicken embryo hearts after 10 days compared to the control embryos. Interestingly, the expression of these genes was significantly suppressed in groups that received LPS plus Lipase. Under the physiological condition, Nkx2.5, GATA4, EGFR, and BMP2 along with other factors such as Tbx20 can promote the activation of certain factors participating in cardiogenic differentiation [22]. Overactivation of GATA4 is closely associated with heart failure [23]. The absence of GATA4 activation and non-significant changes in the expression of EGFR and NKx2.5 showed that LPS could not influence cardiogenic differentiation and maturation in chicken embryos after 10 days. As expected, we found non-significant differences in the expression NF-κB. It is thought that GATA4 is an upstream factor of NF-κB [24]. Monitoring the expression of TRIF and protein levels of NF-κB showed that LPS could induce TRIF and NF-κB and these effects were reversed when the combination of LPS plus Lipase was used. Consistent with the current data, Li and co-workers confirmed that LPS increases the expression of NF-ƙB protein in rat cardiomyoblasts H9C2 cells [25]. It

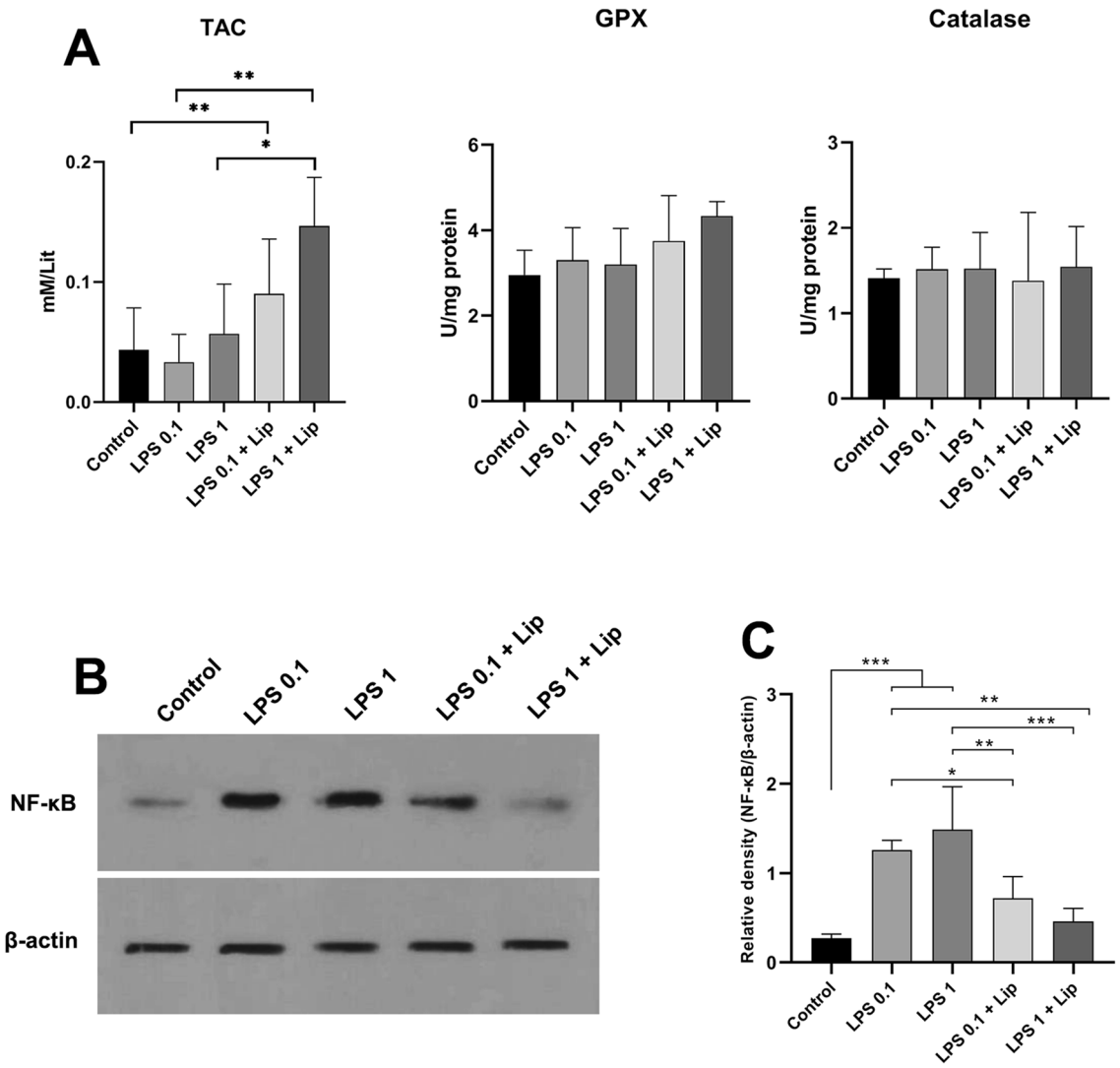

Fig. 4 Analysis of TAC, GPx, and Catalase enzyme levels (**a**; n = 3)**;** and protein levels of NF-KB and P65 (**b–c**; *n* = 3). All data are presented as mean ± SD. One-way analysis of variance (ANOVA) with the post hoc test of LSD.*$p < 0.05$; **p < 0.01.; ***p < 0.001

seems that overnight incubation of LPS with Lipase leads to structural alteration and possibly loss of LPS affinity to the TLRs. In a support of this claim, it has been shown that splenic and hepatic macrophages (Kupffer cells) can neutralize bacterial LPS by the elimination of lipid acyl chains [26]. In an experiment conducted by Mamipour et al., they found that bacterial lipase can alter the composition of fatty acids in the lipid A structure [17]. Along with these comments, one could hypothesize that the incubation of LPS with Lipase can eliminate Lipid A and decrease the LPS-TLR binding, preventing the activation of the downstream signaling pathway. How the combination of LPS and Lipase suppressed the expression of EGFR, GATA4, and NKx2.5 needs more investigations.

We also found that the injection of LPS led to pathological changes in the embryos hearts indicated with massive hemorrhagic foci and necrotic changes. Again, these effects were removed in LPS plus Lipase groups. Despite the increase of NF-ƙB, the activity of GPx and Cat did not alter. Zhen and co-workers found that LPS can induce focal coagulative necrosis, interstitial edema, and prominent fibrosis [27]. We also found that the TAC did not change significantly in LPS-treated embryos hearts. Interestingly, the co-injection of LPS and Lipase increased TAC in a dose-dependent manner compared to the control group. As expected, the production of different cytokines, promotion of oxidative and nitrosative stress, and apoptosis by LPS can lead to structural remodeling and cellular injury [28]. We also found that left ventricle anterior wall thickness decreases proportionally by increasing the dose of LPS and lipase neutralized these effects. The cardiac ventricle wall thickness is one of the main indicators in blood

pumping; therefore decreasing the ventricle anterior wall thickness reduces blood pumping. There are some limitations related to the current study. Here, we did not evaluate the endogenous lipase activity of yolk sac on the bacterial LPS. According to the previously published data, yolk sac possesses lipase activity with prominent hydrolytic activities [29]. However, the specificity of this enzyme to bacterial LPS should also be determined by conducting further investigations.

In conclusion, LPS exerts its effect on the target cells and tissues using Toll-like receptors, which the heart is more affected by LPS. Bacterial lipase Inhibits LPS by binding to lipid A component and eliminates Lipid A, resulting in the loss of LPS affinity to the TLRs, thus preventing the activation of downstream signaling related to destructive effects of LPS. In general, bacterial lipase exerts anti-inflammatory properties by modulating the TLR signaling pathway and inhibiting LPS inflammatory responses in heart tissue and can be a useful anti-inflammatory strategy. It is noteworthy to mention that some certain antibiotics such as polymyxin B can directly neutralize the LPS due to high affinity for bacterial lipopolysaccharide [30]. Therefore, the application of bacterial lipase in in vivo conditions is applicable after numerous experiments. Meanwhile, the side effects of bacterial lipase such as enzymatic activity on host tissue lipids should not be neglected.

Acknowledgements We thank the personnel of Stem Cell Research Center for help and support.

Author Contributions AB, HV, FSN, SA, AF, MF, FY, and BH performed the experiment and prepared draft. RR and MM supervised the study.

Funding This study was supported by a Grant from Tabriz University of Medical Sciences and the University of Guilan.

Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest All authors declared no conflict of interest.

References

1. Klimanskaya, I., Chung, Y., Becker, S., Lu, S.-J., & Lanza, R. (2006). Human embryonic stem cell lines derived from single blastomeres. *Nature, 444*(7118), 481–485. https://doi.org/10.1038/nature05142.
2. Hebebrand, J., & Verhulst, F. (2014). *Prenatal risk factors and postnatal central nervous system function*. . Springer.
3. Capra, L., Tezza, G., Mazzei, F., & Boner, A. L. (2013). The origins of health and disease: The influence of maternal diseases and lifestyle during gestation. *Italian Journal of Pediatrics, 39*(1), 7.
4. Koh, Y. Q., Mitchell, M. D., Almughlliq, F. B., Vaswani, K., & Peiris, H. N. (2018). Regulation of inflammatory mediator expression in bovine endometrial cells: Effects of lipopolysaccharide, interleukin 1 beta, and tumor necrosis factor alpha. *Physiological Reports, 6*(9), e13676.
5. Bononi, I., Balatti, V., Gaeta, S., & Tognon, M. (2008). Gram-negative bacterial lipopolysaccharide retention by a positively charged new-generation filter. *Applied and Environmental Microbiology, 74*(20), 6470–6472.
6. Sampath, V. (2018). Bacterial endotoxin-lipopolysaccharide; structure, function and its role in immunity in vertebrates and invertebrates. *Agriculture and Natural Resources, 52*(2), 115–120. https://doi.org/10.1016/j.anres.2018.08.002.
7. Pepels, P., Bonga, S. W., & Balm, P. (2004). Bacterial lipopolysaccharide (LPS) modulates corticotropin-releasing hormone (CRH) content and release in the brain of juvenile and adult tilapia (Oreochromis mossambicus; Teleostei). *Journal of Experimental Biology, 207*(25), 4479–4488.
8. Thieblemont, N., Thieringer, R., & Wright, S. D. (1998). Innate immune recognition of bacterial lipopolysaccharide: Dependence on interactions with membrane lipids and endocytic movement. *Immunity, 8*(6), 771–777.
9. Feng, Y., Zou, L., Zhang, M., Li, Y., Chen, C., & Chao, W. (2011). MyD88 and Trif signaling play distinct roles in cardiac dysfunction and mortality during endotoxin shock and polymicrobial sepsis. *Anesthesiology: The Journal of the American Society of Anesthesiologists, 115*(3), 555–567.
10. Zhao, P., Wang, Y., Zeng, S., Lu, J., Jiang, T.-M., & Li, Y.-M. (2015). Protective effect of astragaloside IV on lipopolysaccharide-induced cardiac dysfunction via downregulation of inflammatory signaling in mice. *Immunopharmacology and Immunotoxicology, 37*(5), 428–433.
11. Essler, M., Staddon, J. M., Weber, P. C., & Aepfelbacher, M. (2000). Cyclic AMP blocks bacterial lipopolysaccharide-induced myosin light chain phosphorylation in endothelial cells through inhibition of Rho/Rho kinase signaling. *The Journal of Immunology, 164*(12), 6543–6549.
12. Ulevitch, R. J., & Johnston, A. R. (1978). The modification of biophysical and endotoxic properties of bacterial lipopolysaccharides by serum. *The Journal of Clinical Investigation, 62*(6), 1313–1324.
13. Leskelä, J., Pietiäinen, M., Safer, A., Lehto, M., Metso, J., Malle, E., Buggle, F., Becher, H., Sundvall, J., Grau, A. J., Pussinen, P. J., & Palm, F. (2020). Serum lipopolysaccharide neutralizing capacity in ischemic stroke. *PLoS ONE, 15*(2), e0228806–e0228806. https://doi.org/10.1371/journal.pone.0228806.
14. Abreu, M. T., Vora, P., Faure, E., Thomas, L. S., Arnold, E. T., & Arditi, M. (2001). Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. *The Journal of Immunology, 167*(3), 1609–1616.
15. Du, M., Yuan, L., Tan, X., Huang, D., Wang, X., Zheng, Z., Mao, X., Li, X., Yang, L., & Huang, K. (2017). The LPS-inducible lncRNA Mirt2 is a negative regulator of inflammation. *Nature Communications, 8*(1), 1–18.
16. Thakur, S. (2012). Lipases, its sources, properties and applications: A review. *International Journal of Scientific and Engineering Research, 3*(7), 1–29.
17. Mamipour, M., Yousefi, M., Dehnad, A., Faridvand, Y., Zarezadeh, R., Khaksar, M., Pouyafar, A., & Rahbarghazi, R. (2020). Protective effect of bacterial lipase on lipopolysaccharide-induced toxicity in rat cardiomyocytes; H9C2 cell line. *Journal*

of Cardiovascular and Thoracic Research, 12(1), 35–42. https://doi.org/10.34172/jcvtr.2020.06.
18. Faure, E., Thomas, L., Xu, H., Medvedev, A. E., Equils, O., & Arditi, M. (2001). Bacterial lipopolysaccharide and IFN-γ induce Toll-like receptor 2 and Toll-like receptor 4 expression in human endothelial cells: Role of NF-κB activation. *The Journal of Immunology, 166*(3), 2018–2024.
19. Lu, Y.-C., Yeh, W.-C., & Ohashi, P. S. (2008). LPS/TLR4 signal transduction pathway. *Cytokine, 42*(2), 145–151.
20. Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Sugiyama, M., Okabe, M., & Takeda, K. (2003). Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science, 301*(5633), 640–643.
21. Wei, Z., Wang, J., Shi, M., Liu, W., Yang, Z., & Fu, Y. (2016). Saikosaponin a inhibits LPS-induced inflammatory response by inducing liver X receptor alpha activation in primary mouse macrophages. *Oncotarget, 7*(31), 48995.
22. Zhang, J., Wang, G., Liu, J., Lr, G., Liu, M., Cj, W., Chuai, M., Bao, Y., Li, G., & Rm, Li. (2018). Gut microbiota-derived endotoxin enhanced the incidence of cardia bifida during cardiogenesis. *Journal of Cellular Physiology, 233*(12), 9271–9283.
23. Octavia, Y., Tocchetti, C. G., Gabrielson, K. L., Janssens, S., Crijns, H. J., & Moens, A. L. (2012). Doxorubicin-induced cardiomyopathy: From molecular mechanisms to therapeutic strategies. *Journal of Molecular and Cellular Cardiology, 52*(6), 1213–1225.
24. Jia, W., Wu, W., Yang, D., Xiao, C., Huang, M., Long, F., Su, Z., Qin, M., Liu, X., & Zhu, Y. Z. (2018). GATA4 regulates angiogenesis and persistence of inflammation in rheumatoid arthritis. *Cell Death & Disease, 9*(5), 1–15.
25. Li, M., Gou, Y., Yu, H., Ji, T., Li, Y., Qin, L., & Sun, W. (2019). Mechanism of metformin on LPS-induced bacterial myocarditis. *Dose-Response, 17*(2), 1559325819847409.
26. Shao, B., Lu, M., Katz, S. C., Varley, A. W., Hardwick, J., Rogers, T. E., Ojogun, N., Rockey, D. C., DeMatteo, R. P., & Munford, R. S. (2007). A host lipase detoxifies bacterial lipopolysaccharides in the liver and spleen. *Journal of Biological Chemistry, 282*(18), 13726–13735.
27. Zhen, H., Wang, J., Xue, L., Rui, J., Liu, X., & Chen, Y. (2012). LPS-pretreated bone marrow stem cells as potential treatment for myocardial infraction. *Frontiers in Bioscience, 17*, 1294–1303.
28. Asgharzadeh, F., Bargi, R., Beheshti, F., Hosseini, M., Farzadnia, M., & Khazaei, M. (2018). Thymoquinone prevents myocardial and perivascular fibrosis induced by chronic lipopolysaccharide exposure in male rats:-Thymoquinone and cardiac fibrosis. *Journal of Pharmacopuncture, 21*(4), 284.
29. Shah, D. B., & Wilson, J. B. (1965). Egg yolk factor of staphylococcus aureus. II. Characterization of the lipase activity. *Journal of Bacteriology, 89*(4), 949–953. https://doi.org/10.1128/jb.89.4.949-953.1965.
30. Domingues, M. M., Inácio, R. G., Raimundo, J. M., Martins, M., Castanho, M. A., & Santos, N. C. (2012). Biophysical characterization of polymyxin B interaction with LPS aggregates and membrane model systems. *Biopolymers, 98*(4), 338–344. https://doi.org/10.1002/bip.22095.