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Apocynin ameliorates endotoxin-induced acute lung injury in rats

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ABSTRACT

Acute lung injury (ALI) is a serious clinical syndrome with a high rate of mortality. In this study, the effects of apocynin, a NADPH-oxidase (NOX) inhibitor on lipopolysaccharide (LPS)-induced ALI in rats were investigated. Male Sprague–Dawley rats were treated with apocynin (10 mg/kg) intraperitoneally (i.p.) 1 h before LPS injection (10 mg/kg, i.p.). The results revealed that apocynin attenuated LPS-induced ALI as it decreased total protein content, lactate dehydrogenase (LDH) activity and the accumulation of the inflammatory cells in the bronchoalveolar lavage fluid (BALF), In addition, apocynin significantly increased superoxide dismutase (SOD) and reduced glutathione (GSH) activities with significant decrease in the lung malondialdehyde (MDA) content as compared to LPS group in lung tissue and decreased pulmonary artery contraction induced by LPS. It also up-regulated mRNA expression of inhibitory protein kappaB-alpha (NFκBia) and downregulated mRNA expression of Toll-Like receptor 4 (TLR4) and decreased inflammation observed in lung tissues.
Collectively, these results demonstrate the protective effects of apocynin against the LPS-induced ALI in rats through its antioxidant and antiinflammatory effect that may be attributed to the decrease in mRNA expression of TLR4 and increasing that of NFκBia.

1. Introduction

Acute lung injury (ALI) is a frequent complication following sepsis in critically ill patients and is associated with high rates of morbidity and mortality [1]. It is characterized by inflammation, increase in pulmonary vascular permeability, loss of alveolar-capillary barrier in the lungs and flooding of air space with protein-rich pulmonary edema that leads to impaired respiratory function of the lungs [2] in addition to both epithelial and endothelial cell death [3].

Endotoxin or lipopolysaccharide (LPS) is one of the most powerful proinflammatory factors and considered the most important cause of ALI [4]. LPS is a glycolipid that constructs the outer membrane of Gram-negative bacteria and is well-known as an important mediator of sepsis [5]. It can cause experimental ALI *in vivo* that closely resembles ALI in humans [6] as LPS exposure displays major features of microvascular lung injury including lung inflammation, pulmonary edema and leukocyte accumulation in lung tissue in rats and mice [7,8].

Inflammation is the major mechanism through which LPS can induce ALI [9]. LPS activates mainly its membrane Toll-Like receptor 4 (TLR4), in human monocytes, inducing signal transduction pathways leading to activation of various transcription factors such as nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1) [10]. The degree of inflammatory mediator expression in response to LPS is principally regulated by NF-κB [11]. In the unstimulated cells, NF-κB is constitutively localized as a heterodimer in the cytosol by physical association with the inhibitory protein kappaB-alpha (NFκBia or IKB-α) [12]. LPS activates NF-κB by phosphorylation and proteolytic degradation of IκB-α protein upon which NF-κB migrates to the nucleus which result in activation of a variety of target genes that involved in inflammation [13,14].

Another mechanism of LPS-induced ALI is oxidative stress [15,16]. LPS-induced ALI is associated with an exaggerated production of cell-damaging reactive oxygen species (ROS) by neutrophils sequestered within the lung vasculature and by stimulated macrophages [17]. ROS may play a role in enhancing inflammation, either directly by activation of pro-inflammatory mediators activated by NF-κB transcription or *via* the formation of lipid peroxidation products, through the activation of stress kinases and AP-1 that lead to cell death [13].

Apocynin (4-hydroxy-3-methoxy-acetophenone) is a naturally occurring methoxy-substituted catechol that inhibits the NADPH-oxidase (NOX) which is the major enzyme responsible for generating the initial ROS molecule superoxide in activated leukocytes [18]. Apocynin is a pro-drug that is oxidized by peroxidases in the cell and converted into more active metabolites [19]. Several *in vivo* studies have demonstrated that apocynin can prevent neutrophil oxidative burst and chemotaxis, therefore reduce neutrophil-mediated cell injury [20]. It has been studied as a possible remedy for inflammation-mediated diseases, including asthma, arthritis, and cardiovascular diseases [21]. A previous study reported the protective effect of derivatives of apocynin, but not apocynin itself, on intratracheal LPS-induced ALI in rats through inhibition of NADPH oxidase [22].

Since LPS-induced ALI involves inflammation and oxidative stress, therefore we investigated the protective effect of apocynin in a model of LPS-induced ALI in rats.

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Table 1
Effect of apocynin on LPS-induced changes on total and differential cell count in BALF of rats:

Group	Total cell (cell/lung) $\times 10^6$	Lymphocyte (cell/lung) $\times 10^6$	Neutrophil (cell/lung) $\times 10^6$	Monocyte (cell/lung) $\times 10^6$
Control	0.30 ± 0.05	0.20 ± 0.05	0.07 ± 0.01	0.03 ± 0.01
LPS	2.29 ± 0.26*	1.40 ± 0.36*	0.49 ± 0.01*	0.37 ± 0.04*
Apocynin	0.38 ± 0.06	0.23 ± 0.04	0.08 ± 0.01	0.07 ± 0.01
Apocynin + LPS	0.77 ± 0.17$	0.55 ± 0.13$	0.08 ± 0.03$	0.12 ± 0.06$

Rats were treated with apocynin (10 mg/kg, i.p.) 1 h before LPS injection (10 mg/kg, i.p.) and BALF was collected after 24 h to determine total and differential cell count. Data are expressed as mean ± SEM, n = 6.
*, $Significantly different from control or LPS group respectively, using one-way ANOVA test with Tukey–Kramer multiple comparisons *post hoc* test at $p < 0.05$.
LPS: lipopolysaccharide; ANOVA: analysis of variance.

2. Materials and methods

2.1. Drugs and chemicals

Lipopolysacchride (LPS, *Escherichia coli* serotype 0111:B4), apocynin (Acetovanillone; purity ≥98%), 1,1′, 3,3′-tetramethoxypropane, acetylcholine hydrochloride (ACh), phenylephrine hydrochloride (PE), sodium nitroprusside (SNP), pentobarbital sodium, Ellman's reagent [5,5′-dithio-bis(2-nitrobenzoic acid)], pyrogallol, reduced glutathione, thiobarbituric acid (TBA), Tris (hydroxymethyl) aminomethane, and trichloroacetic acid (TCA) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Experimental animals

Male Sprague–Dawley rats, with average age of 6–8 weeks were purchased from "Egyptian Organization for Biological Products and Vaccines", Giza, Egypt.

This study protocol was approved by the "Research Ethics Committee" of Faculty of Pharmacy, Mansoura University, Egypt in accordance with "The Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985).

2.3. Experimental protocol

The rats were allocated into 4 groups each consists of 6 rats. Group (1): Control group; rats receiving normal saline i.p. Group (2): LPS group: rats receiving LPS (10 mg/kg, i.p.) [23,24]. Group (3): Apocynin group: Rats receiving apocynin (10 mg/kg, i.p.) [25–27]}. Group (4): (Apocynin + LPS group): rats receiving LPS (10 mg/kg i.p.) 1 h after apocynin (10 mg/kg, i.p.) injection.

Normal saline was used for dissolving LPS, 95% ethyl alcohol was used for dissolving apocynin then diluted with saline so that the final volume of the organic vehicle is negligible and not affecting the parameters measured.

Rats were anesthetized with pentobarbital sodium (40 mg/kg intravenously) after 24 h, then bronchoalveolar lavage fluid (BALF), pulmonary arterial (PA) rings were isolated for *in-vitro* vascular reactivity and lung tissue was used for quantitative RT-PCR.

Another set of experimental animals that undergo the same experimental protocol ($n = 4$) was served for measuring the oxidative biomarkers and histopathological examination. Samples were analyzed freshly on the same day.

2.3.1. BALF preparation

BALF preparation was carried out by cannulating the trachea and infusing the lung 3 times with 6 ml sterile of 0.9% saline, with 50–70% fractions recovered of the initial volume of saline. BALF fractions were spun using cooling centrifuge (Sigma D-37520, Germany) at 2000 *g* for 10 min and 4 °C.

The cell-free supernatant was used to measure total protein content and lactate dehydrogenase (LDH) activity. The cell pellets of BALF fractions were pooled and resuspended with 500 μl of sterile saline and aliquots of the cell suspensions were used to determine total and differential cell count.

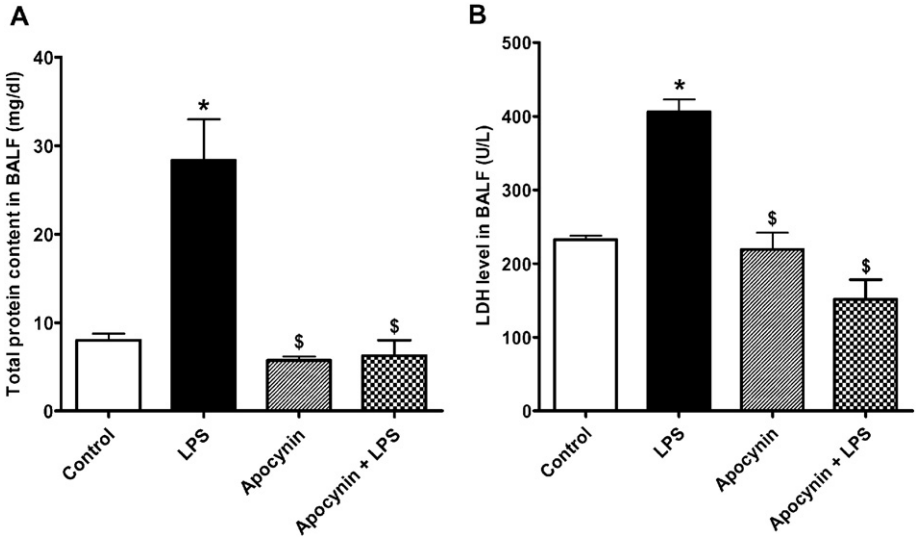


Fig. 1. Effect of apocynin on LPS-induced changes in total protein content and LDH activity in BALF of rats. Rats were treated with apocynin (10 mg/kg, i.p.) 1 h before LPS injection (10 mg/kg, i.p.) and BALF was collected after 24 h to determine total protein content (A) and LDH (B). Data are expressed as mean ± SEM, n = 6. *, $Significantly different from control or LPS group respectively, using one-way ANOVA test with Tukey–Kramer multiple comparisons *post hoc* test at $p < 0.05$. LPS: lipopolysaccharide; LDH: lactate dehydrogenase; BALF: Bronchoalveolar lavage fluid: ANOVA: analysis of variance.

2.3.2. Total and differential cell count

After centrifugation, the cell pellets obtained of the BALF were counted by Auto hematology analyzer (Diagon Ltd. D-cell 60, Hungary) and expressed as number of cells per lung.

2.3.3. Determination of total protein content in the BALF

Total protein content was determined according to the method of Smith et al. using a commercial kit (Biomed Diagnostics, Badr City, Egypt). Proteins react with copper(II) ions to produce a blue violet color compound in alkaline medium, then the absorbance was measured at 546 nm against blank reagent [28]. Total protein content was expressed as mg/dl.

2.3.4. Determination of LDH activity in the BALF

LDH activity was determined using a commercial kit (Human diagnostics, Wiesbaden, Germany). The experimental procedure works by monitoring the disappearance of $NADH^+$ which absorbs at 340 nm. By time, $NADH^+$ was consumed and serum LDH concentration was directly proportional to its consumption [29]. LDH activity was expressed as a unit per liter (U/L).

2.3.5. In vitro vascular reactivity

The first branches of the main pulmonary arteries were separated quickly and placed in a cold oxygenated physiological salt solution (PSS) which is composed of (mmol/L): glucose 11.1, $NaHCO_3$, $CaCl_2$ 2.5, NaCl 118, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2 and KH_2PO_4 1.2, 25; pH 7.4. The vessels were dissected free of fats and connective tissue and cut into rings of 2–4 mm thickness.

The PA rings were mounted in a 10 ml organ bath and aerated with a mixture of 95% O_2 and 5% CO_2 at 37 °C and. Rings were allowed to equilibrate for 60 min under 0.8 g of force. Isometric tension generated by the vascular PA rings was measured using Riegestab K30 force transducer (Hugo Sachs Electronik, D7806 March, Germany), and recorded with a Powerlab unit/400 linked to a PC running Chart v4.2 software (ADInstruments Pty Ltd., Australia). The vascular responsiveness was assessed by evaluating their contractile response to 80 mmol/L of KCl after equilibration.

Vascular reactivity to PE (10^{-8}–10^{-5} M) was determined (to measure α-adrenoceptor mediated contraction) by construction of cumulative concentration response curve. Additionally vascular relaxation was measured to ACh (10^{-8}–10^{-5} M) (to measure endothelium-dependent vasorelaxation) and SNP (10^{-8}–10^{-5} M) (to measure endothelium-

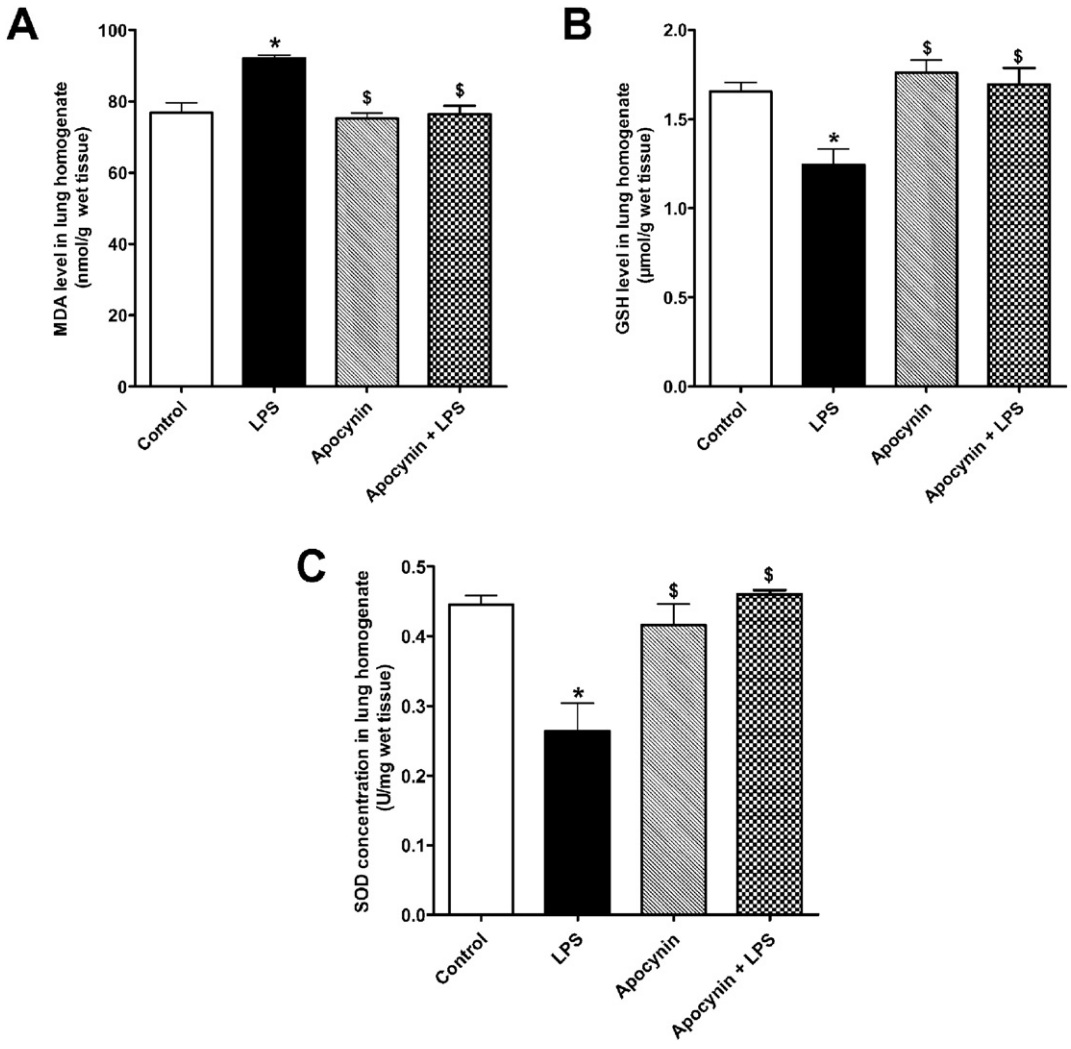
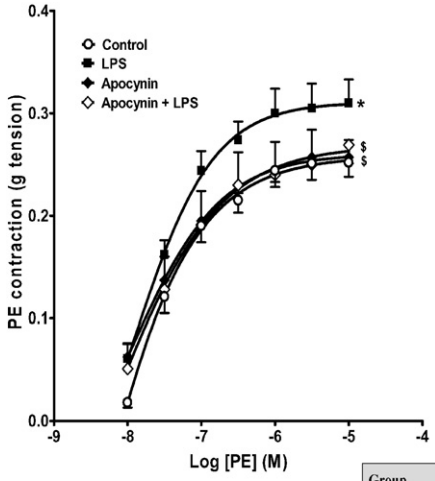


Fig. 2. Effect of apocynin on LPS-induced changes in oxidative biomarkers in lung tissue of rats. Rats were treated with apocynin (10 mg/kg, i.p.) 1 h before LPS injection (10 mg/kg, i.p.) and lung tissue was collected after 24 h to determine MDA (A), GSH (B), SOD (C). Data are expressed as mean ± SEM, n = 4. *, $Significantly different from control or LPS group respectively, using one-way ANOVA test with Tukey–Kramer multiple comparisons *post hoc* test at $p < 0.05$. LPS: lipopolysaccharide; MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase; ANOVA: analysis of variance.



Group	E_{max} (g tension)	pD_2
Control	0.25 ± 0.006	8.69 ± 1.1
LPS	0.31± 0.004*	7.91 ± 0.3
Apocynin	0.26± 0.005$	7.98 ± 0.3
Apocynin+LPS	0.27 ± 0.005$	8.25 ± 1.0

Fig. 3. Effect of apocynin on LPS-induced changes in vascular reactivity in isolated rat PA rings. Rats were treated with apocynin (10 mg/kg, i.p.) 1 h before LPS injection (10 mg/kg, i.p.) and rat pulmonary arteries were isolated after 24 h to measure vascular contraction to PE. Data are expressed as mean ± SEM, n = 6. *, $Maximal contraction is significantly different from control or LPS group respectively, using one-way ANOVA with Tukey–Kramer multiple comparisons *post hoc* test at $p < 0.05$. LPS: lipopolysaccharide; PA: pulmonary arterial; PE: Phenylephrine hydrochloride; ANOVA: analysis of variance.

independent vasorelaxation) after precontraction of PA rings with PE (1 μM).

2.3.6. Preparation of lung homogenate

The upper right pulmonary lobes were excised from all rats, weighed, and homogenized in phosphate buffered saline (PBS) as 10% (w/v) using Omni-125 hand held homogenizer (Omni International, USA). The homogenates were spun at 2000 g, 4 °C for 15 min, and the supernatant was used for assay of oxidative biomarkers malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD) on the same day.

2.3.7. Determination of MDA, GSH content and SOD activity in lung homogenate

MDA, the end product of lipid peroxidation was determined by measuring thiobarbituric acid reactive substances. The absorbance was determined at 532 nm and expressed as nmol/g tissue [30].

For determination of GSH, TCA-deproteinized tissue supernatant was used to measure non-protein sulfhydryl compound by the method of Ellman which is based on the reaction of GSH with Ellman's reagent to give a yellow compound that absorbs at 412 nm and expressed μmol/g tissue [31].

In addition, SOD activity was measured by the degree of inhibition of the auto-oxidation of pyrogallol at an alkaline pH by SOD in which the change in absorbance was recorded at 420 nm and activity was expressed as U/mg tissue [32].

2.3.8. Quantitative real-time polymerase chain reaction (RT-PCR)

The lung right posterior lobe was isolated, weighed and preserved in RNA Later (Qiagen, Germany) (50–100 mg tissue/1 ml RNA later). One microgram from each sample was reverse transcribed using revert aid first strand cDNA synthesis kit (Thermo Scientific Rockford, IL, USA) into complementary DNA (cDNA) according to the manufacturer's procedures. RT-PCR was performed using HOT Firepol Evagreen qPCR mix plus kit (Solis BioDyne, Tartu, Estonia), with a thermocycler Rotor Gene Q (Qiagen, Hilden, Germany).

The mRNA levels of TLR4 and NFκBia were normalized relative to 18S ribosomal RNA (Rn18S) in the same sample. Primers for the TLR4, NFκBia and Rn18S were as the following: TLR4 (forward: TGTTCCTTTC CTGCCTGAGA, reverse: GGTTCTTGGTTGAATAAGGGATG, amplicon size = 129), NFκBia (forward: GTGACTTTGGGTGCTGATGT, reverse: ACACTTCAACAGGAGCGAGA, amplicon size = 111), and Rn18S (forward: AGTTGGTGGAGCGATTTGTC, reverse: GAACGCCACTTGTCCC TCTA, amplicon size = 121).

The results were expressed using ΔΔCt method as an n-fold change of the relative expression levels of target genes from control group.

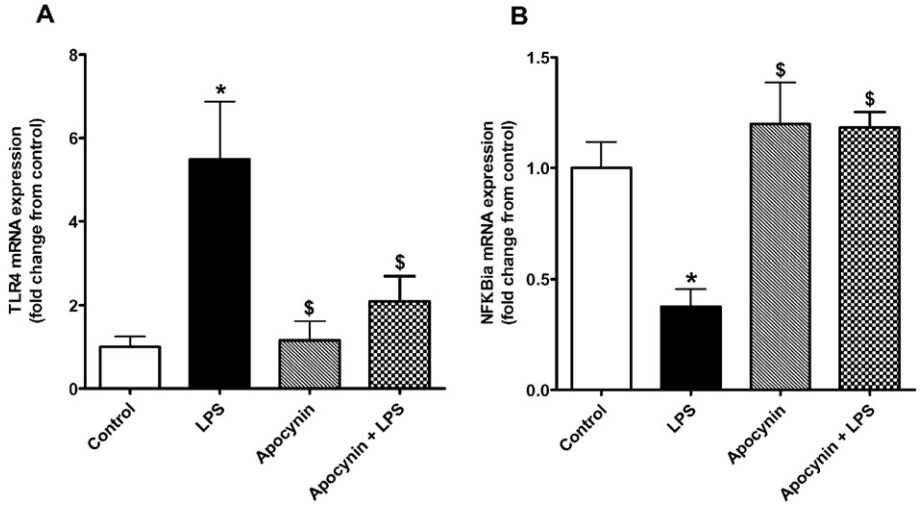


Fig. 4. Effect of apocynin on LPS-induced changes of mRNA expression of TLR4 and NFκBia in lung tissue of rats. Rats were treated with apocynin (10 mg/kg, i.p.) 1 h before LPS injection (10 mg/kg, i.p.) and lung tissue was isolated after 24 h to measure mRNA expression of TLR4 (A) and mRNA expression of NFκBia (B). Data are expressed as mean ± SEM, n = 6. *, $Significantly different from control or LPS group respectively, using one-way ANOVA test with Tukey–Kramer multiple comparisons *post hoc* test at $p < 0.05$. LPS: lipopolysaccharide; TLR4: Toll-Like receptor 4; NFκBia: the inhibitory protein kappaB-alpha; ANOVA: analysis of variance.

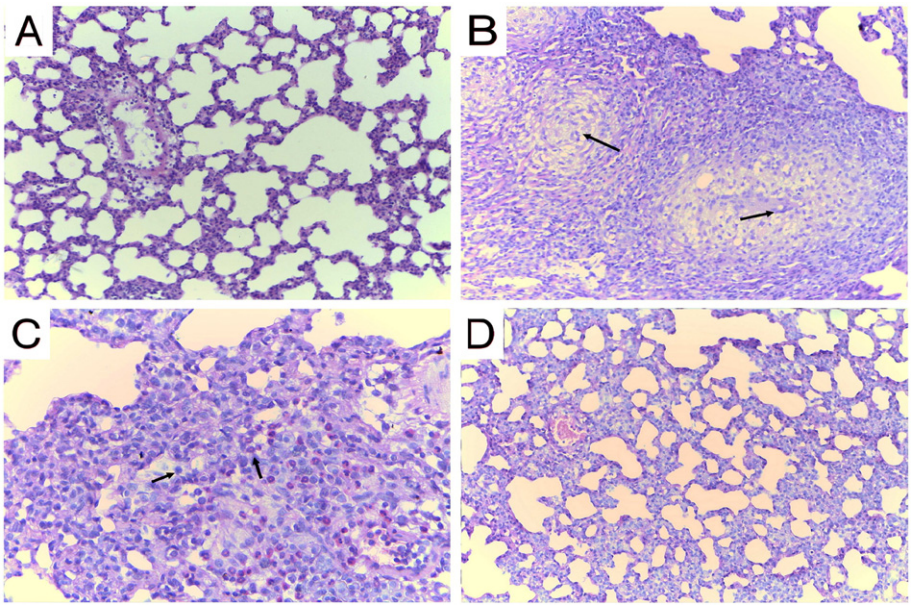


Fig. 5. Effect of apocynin on LPS-induced changes in histopathological examination of lung tissue of rats. Rats were treated with apocynin (10 mg/kg, i.p.) 1 h before LPS injection (10 mg/kg, i.p.) and rat left lungs were collected after 24 h. Group A: Control group showing normal lung tissue with no evidence of inflammation or other abnormalities; Group B: LPS group showing a marked inflammation in the lung tissue of rats with tubercular granuloma indicated by arrow; Group C: LPS group showing numerous neutrophils; Group D: Apocynin + LPS group showing moderate inflammation with no granuloma and decreased number of neutrophils. LPS: lipopolysaccharide; H&E: hematoxylin and eosin; A, B, D (×200); C (×400).

2.3.9. Histopathological examination

The left lungs of rats were harvested, fixed in 10% neutral-buffered formalin for 24 h, embedded in paraffin wax, sectioned (6 μm) and stained with hematoxylin–eosin (H&E) then it was assessed for the presence of inflammation. The analyses were performed microscopically (Leica Imaging Systems, Cambridge, UK). The scores of lung injury for each animal were histopathologically evaluated using a semi-quantitative scoring. Lung injury was graded from 0 (normal) to 3 (severe) in the category of neutrophil infiltration. The pathologist performing histopathological evaluation was blinded to the study treatment assignment.

2.4. Statistical analysis

Data are expressed as mean ± standard error of mean (SEM). Vascular contraction was calculated as gram tension. The highest response obtained was considered as the maximum response (Emax). pD2 (negative log the concentration producing 50% of maximal response) was determined from nonlinear regression analysis (four-parameter curve fit). Statistical analysis and graphing were carried out using Graphpad software Prism V 5 (GraphPad Software Inc., San Diego, CA, USA), significant differences between groups was measured by one-way analysis of variance (ANOVA) followed by Tukey–Kramer's multiple comparisons post-hoc test. Kruskal–Wallis test was used followed by Dunn test as a nonparametric measure. Statistical significance was considered at $p < 0.05$.

Table 2
Average score of lung injury.

Treatment	Neutrophil infiltration				
	Scores				Average score
	0	1	2	3	
Control	4	0	0	0	0 ± 0.0
LPS	0	0	1	3	2.75 ± 0.50*
Apocynin	4	0	0	0	0 ± 0.0#
Apocynin + LPS	0	2	2	0	1.50 ± 0.58

Values represent the mean ± SD of 4 rats/group.
Mean values were compared using Kruskal–Wallis followed by Dunn test ($p < 0.05$).
* Significantly different from the mean value of the control group.
\# Significantly different from the mean value of LPS group.

3. Results

3.1. Effect of apocynin on LPS-induced changes on total and differential cell count in BALF of rats

The injection of LPS caused a significant elevation in the total cell count, lymphocyte, neutrophil and monocyte differential count in the BALF of rats by 7.6, 7.0, 7.0 and 12.3 folds respectively in comparison to control group, ($p < 0.05$, n = 6) (Table 1). Injection of apocynin before LPS significantly decreased LPS-induced elevation in the total cell, lymphocyte and neutrophil count by 2.8, 2.5, 6.1 and 3.1 folds respectively when compared to LPS group, ($p < 0.05$, n = 6) (Table 1).

3.2. Effect of apocynin on LPS-induced changes in total protein content and LDH activity in BALF of rats

The injection of LPS caused a significant increase in the total protein content (Fig. 1A) and LDH activity (Fig. 1B) by 3.5 and 1.7 folds respectively when compared to control group ($p < 0.05$, n = 6). Injection of apocynin before LPS significantly reduced LPS-induced elevation in the total protein content and LDH level in BALF of rats where both parameters returned to baseline ($p < 0.05$, n = 6).

3.3. Effect of apocynin on LPS-induced changes in oxidative biomarkers in lung tissue of rats

The injection of LPS significantly increased MDA content (Fig. 2A), decreased GSH content (Fig. 2B) and decreased SOD activity (Fig. 2C) by 1.2, 1.3 and 1.7 folds respectively compared to control group ($p < 0.05$, n = 4). Injection of apocynin before LPS significantly reduced LPS-induced elevation in MDA content. Additionally, it prevented LPS-induced decrease in GSH content and SOD activity ($p < 0.05$, n = 4).

3.4. Effect of apocynin on LPS-induced changes in vascular reactivity in isolated rat PA rings

LPS caused no significant effect on KCl-induced contraction or ACh or SNP-induced relaxation in PA rings (data not shown) when compared to control group. Conversely, LPS caused a significant increase in PE-induced maximal contraction (Fig. 3) by 1.2 fold when compared to control group ($p < 0.05$, n = 6). Preinjection with apocynin significantly decreased LPS-induced elevation of maximal contraction to PE ($p < 0.05$, n = 6).

3.5. Effect of apocynin on LPS-induced changes of mRNA expression of TLR4 and NFκBia in lung tissue of rats

The injection of LPS caused a significant elevation in mRNA expression of TLR4 (Fig. 4A) and reduction of mRNA expression of NFκBia (Fig. 4B) by 5.5 and 2.7 folds respectively in comparison to control group ($p < 0.05$, n = 6). Pre injection of apocynin significantly decreased LPS-induced elevation in mRNA expression of TLR4 by 2.6 folds and prevented LPS-induced decrease in mRNA expression of NFκBia when compared to control group ($p < 0.05$, n = 6).

3.6. Effect of apocynin on LPS-induced changes in histopathological examination of lung tissue of rats

Histopathological examination of the lung using hematoxylin and eosin (H&E) stain in control rats revealed normal lung tissue with no evidence of inflammation or other abnormalities (Fig. 5A). LPS caused a marked inflammation in the lung tissue of rats with tubercular granuloma (Fig. 5B) and numerous neutrophils (Fig. 5C). Injection of rats with apocynin before LPS showed moderate inflammation (Fig. 5D). Injection of rats with apocynin showed normal lung tissue with no inflammation (not shown). Semi-quantitative analysis of lung injury scores is demonstrated in Table 2.

4. Discussion

ALI is a frequent complication following sepsis, which is caused mainly by LPS [3]. Szarka et al. showed that LPS can cause marked acute injury that maximizes in 24 h. Therefore, in our model, BALF and lung tissues were collected 24 h after LPS exposure [33].

Previous studies have shown that ALI induced by LPS is associated with increased inflammatory cell count such as neutrophils and lymphocytes, total protein content and LDH activity in BALF of rats [34–36] and a marked lung injury that is characterized by histopathological changes [37,38]. Our results showed that LPS caused a marked increase in total and differential cell count, total protein level and LDH activity in BALF of rats. Additionally, LPS caused a marked inflammation in the lung tissue of rats with accumulation of numerous neutrophils and tubercular granuloma which confirms the presence of ALI. Pretreatment with apocynin resulted in a marked protection against LPS-induced ALI as shown by the decrease of the elevated total and differential cell counts, total protein content and LDH activity and improved the tissue inflammation. These observations are in agreement with the previous study which showed that apocynin reduced the augmented total cell, neutrophils and macrophage count in rats that exposed to bleomycin [39]. Therefore, apocynin may prevent LPS-induced ALI in our model.

Inflammation is a major pathway involved in ALI induced by LPS [9]. TLR4 is the major receptor through which LPS activates the inflammatory pathway [40]. Our results showed that LPS increased TLR4 mRNA expression and apocynin decreased this increase in TLR4 mRNA expression. These observations are in agreement with the previous studies that showed the increased mRNA expression levels of TLR4 after injection of LPS in rats [41,42]. Lin et al. showed that stimulation of human aortic smooth muscle cells with LPS significantly increased TLR4 expression which is regulated by NADPH oxidase and treatment with apocynin significantly decreased LPS-induced TLR4 expression [43].

Activation of NF-κB is responsible for expression of different inflammatory mediators induced by LPS and it is increased during inflammation [44]. Since NFκBia is a major regulator of NF-κB activation [45] therefore, we investigated the effect of LPS on mRNA expression of NFκBia. In this study, LPS decreased mRNA expression of NFκBia after 24 h of LPS injection. LPS markedly decreased the NFκBia protein level by increasing its degradation and phosphorylation in murine microglial cells [46]. additionally, It has been shown that NFκBia mRNA expression is dependent on the concentration and the exposure time of LPS, where NFκBia mRNA expression increases in the first 5 h then returns back to normal or even decreases at 20 h after LPS administration in the RAW264.7 a murine macrophage cell line [47]. IL-10 and dexamethasone have been shown to inhibit NF-κB activation and increase NFκBia mRNA expression [48,49].

Administration of apocynin prevented LPS-induced decrease in NFκBia mRNA which may explain its protective role in this model through its antiinflammatory effect. Our results are in agreement with the antiinflammatory effect of apocynin which is demonstrated via reduction of NF-κB p65 activation in mice colitis induced by dextran sulfate sodium [50]. A previous study has shown that IκB degradation and nuclear translocation of NF-κB p65 induced by advanced oxidation protein products were significantly blocked by apocynin in fibroblast-like synoviocytes from knee of rats [51].

Another mechanism of LPS-induced ALI is oxidative stress [16] LPS increases oxidative biomarkers such as MDA, decreases GSH content and SOD activity in lung tissue of rats [52]. In this study, LPS-induced ALI was manifested by increased lung content of MDA, decreased SOD activity and decreased GSH content. Pretreatment with apocynin reduced the decreased contents of SOD and GSH and also reduced the increase in MDA level. These observations are in agreement with the previous study in which apocynin is shown to decrease MDA level and increase SOD activity in bleomycin-induced lung fibrosis in rats [39]. Sener et al. show that apocynin reverses MDA elevation and prevents GSH reduction induced by testicular ischemia–reperfusion injury in rats [53].

ALI induced by LPS is associated with vascular dysfunction [54,55]. Zhang et al. reported that pulmonary arteries of LPS-treated rats have an increase in the contractile response to PE [56]. In addition, LPS-treated rabbits showed a decrease in both endothelium-dependent and -independent vasorelaxation [57].Conversely, the relaxation response to SNP in PA rings of LPS-treated rats has been shown to be unaffected [58].

In this study, LPS-treated rats showed an increase in the contractile response of PA rings to PE without affecting either endothelium-dependent or -independent vasorelaxation. Additionally, the contraction responses to KCl were not affected. The mechanism by which LPS increases the contractile response of PA rings to PE is not clear and requires further investigations.

Apocynin reduced the enhanced contractile response of PA rings to PE in our model. This effect could be related to the antiinflammatory and antioxidant effects of apocynin. Moreover apocynin has been shown to activate ATP-sensitive K^+ channels in rat pulmonary artery *in vitro* [59] that may have a role in preventing excessive PE-induced contraction in our model. These results confirm that apocynin can protect against pulmonary artery dysfunction in LPS-induced acute lung injury.

In conclusion, this study showed that apocynin has a protective effect against LPS-induced ALI in rats through its antioxidant and antiinflammatory effect which may be due to the decrease in the mRNA expression of TLR4 and increasing the mRNA expression of NFκBia. Therefore, apocynin may have a beneficial effect in prevention of ALI associating sepsis.

Conflict of interest statement

None declared.

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