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ORIGINAL ARTICLE

Effects of methyl palmitate and lutein on LPS-induced acute lung injury in rats

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Abstract

AIM: To investigate the effects of methyl palmitate and lutein on lipopolysaccharide (LPS)-induced acute lung injury (ALI) in rats and explore the possible mechanisms.

METHODS: Male Sprague-Dawley rats were divided into 4 groups: (1) control; (2) LPS; (3) Methyl palmitate; and (4) Lutein groups. Methyl palmitate (300 mg/kg, *ip*) was administered 3 times per week on alternating days while lutein (100 mg/kg, oral) was given once daily. After 1 wk of vehicle/methyl palmitate/lutein treatment, ALI was induced by a single dose of LPS (7.5 mg/kg, *iv*). After 24 h of LPS injection, animals were sacrificed then biochemical parameters and histopathology were assessed.

RESULTS: Treatment with methyl palmitate attenuated ALI, as it significantly decreased the lung wet/dry weight (W/D) ratio, the accumulation of the inflammatory cells in the bronchoalveolar lavage fluid (BALF) and histopathological damage. However, methyl palmitate failed to decrease lactate dehydrogenase (LDH) activity in BALF. On the other hand, lutein treatment produced significant anti-inflammatory effects as revealed by significant decrease in accumulation of inflammatory cells in lung, LDH level in BALF and histopathological damage. Methyl palmitate and lutein significantly increased superoxide dismutase (SOD) and reduced glutathione (GSH) activities with significant decrease in the lung malondialdehyde (MDA) content. Importantly, methyl palmitate and lutein decreased the level of the inflammatory cytokine tumor necrosis factor-α (TNF-α) in the lung. Lutein also reduced LPS-mediated overproduction of pulmonary nitrite/nitrate (NO_2^-/NO_3^-), which was not affected by methyl palmitate pretreatment.

CONCLUSION: These results demonstrate the potent protective effects of both methyl palmitate and lutein against LPS-induced ALI in rats. These effects can be attributed to potent antioxidant activities of these agents, which suppress inflammatory cell infiltration and regulated cytokine effects.

Key words: Acute lung injury; Lipopolysaccharide; Methyl palmitate; Lutein; Rats

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INTRODUCTION

Acute lung injury (ALI) is an acute inflammatory process in the air spaces and lung parenchyma with increased vascular permeability leading to impairment of respiratory

function. It is caused by an imbalance of pro-inflammatory and anti-inflammatory mediators. Pro-inflammatory cytokines, such as interleukin (IL)-8, IL-1, and tumor necrosis factor-alpha (TNF-α) cause neutrophils to adhere to pulmonary capillaries, extravasate into the alveolar space and undergo activation[1-3]. Activated neutrophils propagate inflammation and injury through the release a variety of factors, such as reactive oxygen species (ROS) and proteolytic enzymes, which contribute to local tissue damage, accumulation of edema fluid in the airspaces, and surfactant inactivation, thereby making the alveolar unit unable to expand. These destructive forces released by neutrophils can be counteracted by endogenous anti-proteases, antioxidants, and anti-inflammatory cytokines (*e.g.,* IL-10). The balance between the destructive and protective factors determines the degree of tissue injury and clinical severity of ALI[3,4].

Lipopolysaccharide (LPS) is a main constituent of the cell wall of the gram-negative bacteria[5]. When delivered into animals and humans, LPS displays major features of microvascular lung injury, including leukocyte accumulation in lung tissue, pulmonary edema, profound lung inflammation and mortality. It is a common cause of both direct lung injury (pneumonia) and indirect lung injury (sepsis)[6]. LPS produces acute injury to the endothelial and epithelial barriers in the lungs and acute inflammatory response in the air spaces either by inhalation or systemic (intravenous and intraperitoneal) administration[7]. LPS-induced injury is a very useful experimental *in vivo* model, closely resembling ALI in humans[8].

Methyl palmitate is an endogenous naturally occurring fatty acid methyl ester. Recently, methyl palmitate has been shown to possess potent anti-inflammatory and anti-fibrotic effects through multiple pathways, inhibiting the activation of isolated macrophages of alveolar origin (RAW cells)[9]. It can also attenuate the severity of oxidative stress and inflammatory response[10]. Methyl palmitate decreases secretion of IL-10, TNF-α, NO, and prostaglandin E_2. This effect is thought to occur by the inhibition of nuclear factor kappa-B (NF-κB)[11]. In the liver, methyl palmitate is reported to play an important role in regulating the inflammatory process as it inhibits the secretion of TNF-α and NO by its ability to inhibit Kupffer cells[12,13].

Lutein is a xanthophyll carotenoid which is usually consumed in the diet by humans and has good biovailability. Lutein is found in fruits and vegetables like spinach, broccoli, kale, egg yolk, zucchini, corn, peas and kiwi[14,15]. It has a strong antioxidant activity due to its chemical structure. Lutein not only has conjugated double bonds but also has hydroxyl groups at both ends making it a stronger antioxidant then other carotenoids[16]. It was reported that lutein can reduce the concentrations of NO, TNF-α in the aqueous humor. Lutein also suppressed the expression of inducible nitric oxide synthase (iNOS) and COX-2 in RAW cells[17]. The present study aimed to test the potential protective effects of methyl palmitate and lutein treatments against LPS-induced ALI and to investigate their possible mechanism(s).

MATERIALS AND METHODS

Drugs and chemicals

LPS (Escherichia coli serotype O111:B4), was purchased from Sigma-Aldrich, St. Louis, MO. LPS was dissolved in normal saline and prepared fresh on the day of the experiment. Methyl palmitate (Sigma-Aldrich, St. Louis, MO) was kindly provided by Dr. El-Demerdash E, and was dissolved in corn oil by vortex. Lutein capsules were manufactured by United States Nutrition Inc. (Bohemia, NY). All other chemicals and bio-chemicals used in this study were of high analytical grade.

Experimental animals

Male Sprauge Dawely rats (150-200 g) were purchased from the "Egyptian Organization for Biological Products and Vaccines", Giza, Egypt. The animals were housed (4/cage) in an air-conditioned room maintained at 25 ± 2 ℃ with regular 12 h light/12 h dark cycle. All procedures involving animals were conducted in accordance with the protocol approved by the committee of animal experimentation of the Faculty of Pharmacy, Mansoura University.

Experimental design

Animal groups: Male Sprague-Dawley rats were randomly divided into 4 groups each containing six animals (n = 6). These groups were (1) control; (2) LPS, (3) Methyl palmitate; and (4) Lutein groups. Methyl palmitate (300 mg/kg, *ip*) was administered three times per week on alternating days[9,18] while lutein (100 mg/kg, oral) was given once daily[17]. Rats of the control and LPS-treated groups received 1% (w/v) CMC orally as vehicle, once daily for one week. After one week of vehicle/methyl palmitate/lutein treatment, ALI was induced by a single dose of LPS (7.5 mg/kg) intravenously[19,20] in LPS, methyl palmitate and lutein groups while rats of the control group were treated with saline. Twenty-four hours after LPS injection, animals were killed under diethyl ether anesthesia. The lungs were lavaged, collected, perfused with ice-cold saline and taken for analysis. The remaining lung was flash frozen in liquid nitrogen and stored at -80 ℃ for further analysis.

Measurement of lung wet/dry weight ratio: The wet upper left lung was excised, blotted dry and weighed to calculate "wet" weight, and then placed in an oven at 80 ℃ for 24 h to obtain the "dry" weight. The lung wet/dry weight (W/D) ratio was calculated to evaluate tissue edema.

Bronchoalveolar lavage fluid: Bronchoalveolar lavage (BAL) was performed by cannulating the trachea and infusing the right lung with sterile 0.9% saline. The volume of saline used for BAL was 6 mL. Bronchoalveolar lavage fluid (BALF) fractions were centrifuged (4000 rpm,

10 min, 4 ℃) using a cooling centrifuge (Damon/IEC Division, Model: CRU-5000, Needham, MA) to collect the cell pellet for the total cell count determination. The supernatants of the BALF were stored at -80 ℃ until required for determination of protein content and lactate dehydrogenase (LDH) activity.

Measurement of lung injury in BALF: (1) Total cell count: The cell pellets obtained after centrifugation of the BALF were resuspended in 100 μL of saline, centrifuged onto slides and stained for 8 min with Wright-Giemsa staining. Differential cell count was determined through quantification of the slides for neutrophils and lymphocytes by counting a total of 200 cells/slide at 40 × magnification; (2) Protein content: The total protein concentration was measured in BALF using a commercial kit (Thermo Scientific, Rockford); and (3) LDH: LDH activity was assessed in BALF using a commercial kit (Human Gesllschaft fur Biochemica und Diagnostica, Germany). In brief, the reaction mixture consisting of NADH (0.8 mmol/L), sodium pyruvate (1.5 mmol/L) and TRIS buffer (50 mmol/L, pH 7.4) was added to the sample. The changes in absorbance at 340 nm were recorded and enzyme activity was calculated and expressed in U/L.

Histopathological examination of lung: The lower left pulmonary lobe was harvested and flushed with phosphate buffered saline then fixed in 10% neutral-buffered formalin for 24 h, embedded in paraffin wax, sectioned (6 μm) and stained with hematoxylin-eosin (HE). The tissues were examined under a microscope in a random order and without knowledge of animal or group. The structural alterations of tissue were assessed based on the degree of cellular proliferation, alveolar wall thickening, and inflammatory lesions. Such changes were graded in terms of severity and distribution. The grading system adopted was as follows and was utilized for each group of animals[21]. For severity of lesions: 0 = nothing/zero, 1 = marginal, 2 = slight, 3 = moderate, 4 = severe, 5 = very severe. For distribution of lesion over the tissue: 0 = absent, 1 = rare/occasional (10% of the lung area), 2 = sparse/limited (10%-25% of the lung area), 3 = moderate (25%-50% of the lung area), 4 = extensive/widespread (50%-75% of the lung area), 5 = very extensive/predominant (over 75% of the lung area).

Measurement of oxidative stress: (1) Measurement of malondialdehyde: malondialdehyde (MDA) concentration was determined as an indicator of lipid peroxidation in the lung tissue. The tissue samples of lung were weighed and homogenized (1:10, w/v) in 0.1 mol/L phosphate buffer (pH 7.4) in an ice bath. The homogenate was centrifuged at 3000 *g* for 20 min at 4 ℃. Subsequently, MDA content in the supernatants was measured according to the method described by Satoh[22] using a commercial kit purchased from Bio-diagnostic Company (Giza, Egypt); (2) Measurement of superoxide dismutase: The superoxide dismutase (SOD) activity was estimated according to the method described by Nishikimi *et al*[23] using a commercial kit purchased from Bio-diagnostic Company (Giza, Egypt); and (3) Measurement of reduced glutathione: The concentration of reduced glutathione (GSH) in the lung homogenate was measured according to the method described by Beutler *et al*[24] using a commercial kit purchased from Bio-diagnostic Company (Giza, Egypt).

Measurement of NO_2^-/NO_3^- concentration: NO_2^-/NO_3^- concentration was determined according to the method described by Green *et al*[25]. Briefly, 6 μL of lung homogenate were mixed with 44 μL of double distilled water, 20 μL 0.31 mol/L phosphate buffer (pH 7.5), 10 μL of 0.86 mmol/L NADPH, 10 μL of 0.11 mmol/L FAD and 10 μL of 1 U/mL nitrate reductase in individual wells of a 96-well plate. Samples, standards and controls were incubated for 1.5 h at room temperature. 200 μL of Griess reagent were added to each well. Absorbance at 540 nm was measured after 10 min of incubation at room temperature. Nitrite concentration (μmol/L) was determined for each sample based on a standard curve.

Assay of TNF-α: The level of TNF-α in the supernatant of the lung homogenate was determined by using a commercial enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's instructions (Bender Med. systems Gmbh, Vienna, Austria). TNF-α was determined from a standard curve.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's Kramer multiple comparisons Test. The values are means ± SE. for six rats in each group. P value < 0.05 was considered as significant.

RESULTS

Effect of methyl palmitate and lutein on lung W/D ratio and protein content in LPS-induced ALI rats

As shown in Figure 1A, there was a significant increase (P < 0.05) in lung W/D ratio in the LPS group as compared to the control group. Methyl palmitate pretreatment resulted in a significant decrease (P < 0.001) in lung W/D ratio as compared to the LPS group, while, lutein groups produced no significant change as compared to the LPS group. As shown in Figure 1B, LPS injection caused a significant increase (P < 0.01) in the level of total protein as compared to the control group. There was no significant change in the level of total protein between methyl palmitate and lutein groups and that of the LPS group.

Effect of methyl palmitate and lutein on total and differential cell counts in the BALF of LPS-induced ALI rats

The injection of LPS caused a significant elevation (P < 0.001) in the total and differential cell counts in com-

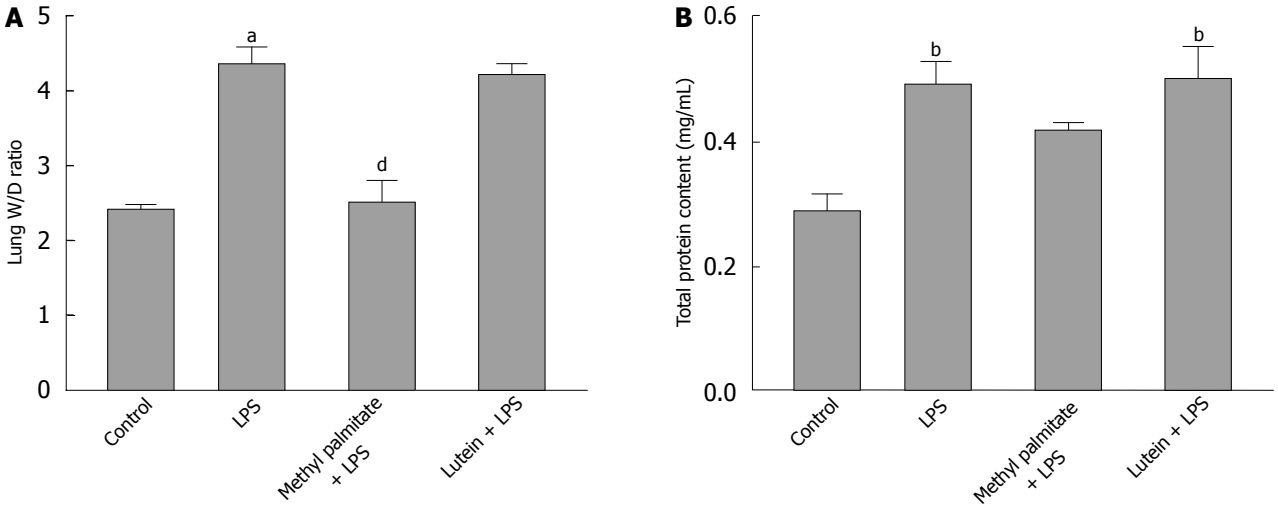

Figure 1 Effect of methyl palmitate and lutein on wet/dry ratio of the lung weight and total protein content in the bronchoalveolar lavage fluid of lipopolysaccharide-induced acute lung injury in rats. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* the control group; $^{d}P < 0.01$ *vs* the lipopolysaccharide (LPS) group (one way ANOVA followed by Tukey-Kramer's multiple comparisons test). W/D: Wet/dry.

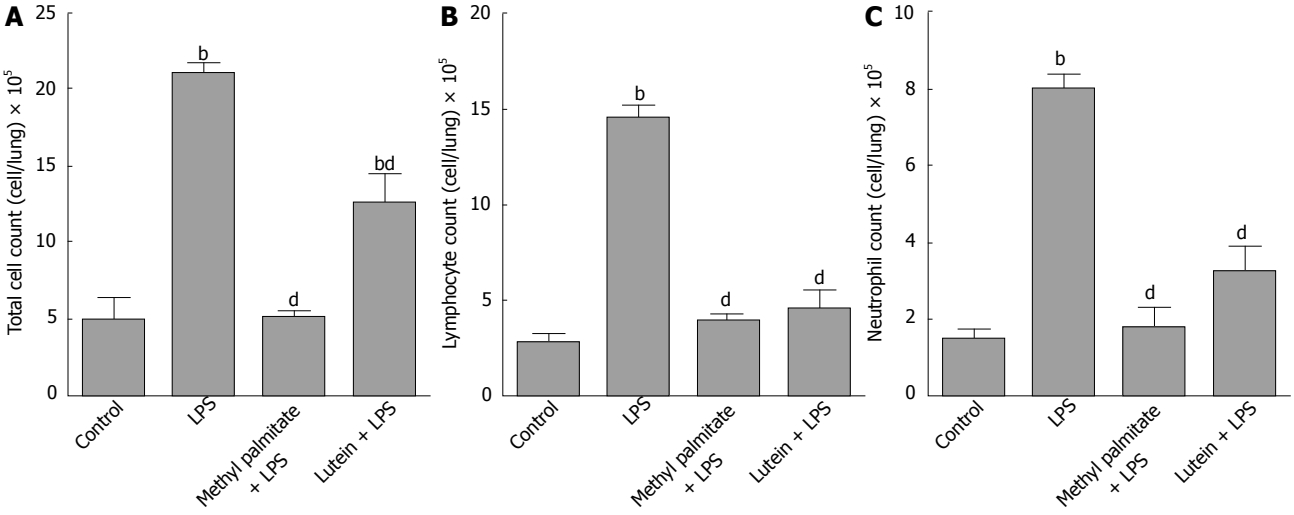

Figure 2 Effect of methyl palmitate and lutein on total and differential cell counts in the bronchoalveolar lavage fluid of lipopolysaccharide-induced acute lung injury in rats. $^{b}P < 0.01$ *vs* the control group; $^{d}P < 0.01$ *vs* the lipopolysaccharide (LPS) group (one way ANOVA followed by Tukey-Kramer's multiple comparisons test).

parison to those of the control group (Figure 2). The administration of methyl palmitate and lutein resulted in a significant decrease in the total ($P < 0.001$, $P < 0.01$ respectively) and differential ($P < 0.001$) cell counts as compared to the LPS group.

Effect of methyl palmitate and lutein on LDH activity in the BALF in LPS-induced ALI rats

LPS injection produced a significant increase ($P < 0.001$) in LDH activity as compared to the control group (Figure 3). The lutein group showed a significant decrease ($P < 0.001$) in LDH activity as compared to the LPS group. The methyl palmitate group did not show any significant decrease in the elevated LDH activity as compared to the LPS group.

Effects of methyl palmitate and lutein on lung histology of rats with LPS-induced ALI

As shown in Figure 4, rats of the control group showed normal histology while the lungs of rats of the LPS group showed severe inflammatory reaction, marked alveolar wall thickness with oedema and haemorrhage, decreased alveolar spaces and extensive (widespread) distribution affecting 50%-75% of the lung area (Figure 4B). As shown in Table 1, LPS injection caused a significant increase ($P < 0.001$) in the severity and the distribution grade of the lesions, as compared to the control group. Methyl palmitate pretreatment resulted in a significant reduction ($P < 0.001$) in the severity and the distribution grade of the lesions as compared to the LPS group. The specimen collected from the methyl palmitate group showed mild inflammatory reaction with thickened alveolar wall edema and hemorrhage, less alveolar space and the distribution of the lesions was sparse (limited), affecting 10%-25% of the lung area (Figure 4C).

The distribution as well as the severity of the lesions were significantly decreased ($P < 0.001$, $P < 0.01$) in the

Table 1 Effect of methyl palmitate and lutein on lung histopathology of the lung in lipopolysaccharide -induced acute lung injury in rats

Groups	Severity of lesion grade						Average severity grade	Distribution of lesion grade						Average distribution grade
	0	1	2	3	4	5		0	1	2	3	4	5	
Control	3	3	0	0	0	0	0.5 ± 0.2	3	3	0	0	0	0	0.500 ± 0.224
LPS	0	0	0	3	3	0	3.5 ± 0.2[b]	0	0	0	3	3	0	3.5 ± 0.2[b,d]
Methyl palmitate + LPS	0	1	5	0	0	0	1.83 ± 0.17[b,d]	0	0	6	0	0	0	2.0 ± 0.0[b,d]
Lutein + LPS	0	0	3	3	0	0	2.5 ± 0.2[b,d]	0	0	5	1	0	0	2.17 ± 0.17[b,d]

Values represent the mean ± SE of 6 rats/group. [b]$P < 0.01$ *vs* the control group; [d]$P < 0.01$ *vs* the lipopolysaccharide (LPS) group (one way ANOVA followed by Tukey-Kramer's multiple comparisons test).

Table 2 Effect of methyl palmitate and lutein on oxidative stress in the lung homogenate in lipopolysaccharide induced cute lung injury in rats

	Parameters		
	MDA (nmol/g tissue)	SOD (units/mg protein)	GSH (μmol/g tissue)
Control	114.036 ± 10.2	25.750 ± 0.629	6.559 ± 0.26
LPS	214.799 ± 9.932[b]	9.667 ± 0.882[b]	3.049 ± 0.309[a]
Methyl palmitate +LPS	164.200 ± 11.707[a,c]	22.108 ± 1.080[d]	7.352 ± 0.738[d]
Lutein + LPS	155.649 ± 6.852[d]	25.333 ± 1.333[d]	6.051 ± 0.315[a,c]

Values represent the mean ± SE of 6 rats/group. [a]$P < 0.05$, [b]$P < 0.01$ *vs* the control group; [c]$P < 0.05$, [d]$P < 0.01$ *vs* the lipopolysaccharide (LPS) group (one way ANOVA followed by Tukey-Kramer's multiple comparisons test). MDA: Malondialdehyde; SOD: Superoxide dismutase; GSH: Glutathione.

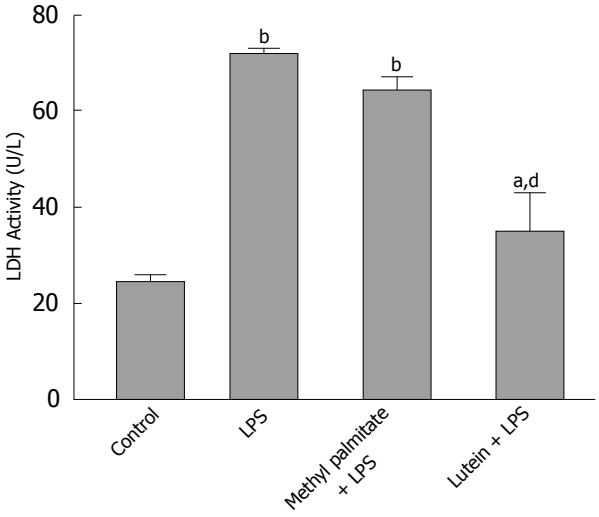

Figure 3 Effect of methyl palmitate and lutein on lactate dehydrogenase activity in the bronchoalveolar lavage fluid of lipopolysaccharide-induced acute lung injury in rats. [a]$P < 0.05$, [b]$P < 0.01$ *vs* the control group; [d]$P < 0.01$ *vs* the lipopolysaccharide (LPS) group (one way ANOVA followed by Tukey-Kramer's multiple comparisons test). LDH: Lactate dehydrogenase.

lutein group as compared to the LPS group. The specimen collected from the lutein group showed moderate inflammatory reaction, moderate alveolar wall thickness, oedema and hemorrhage, decreased alveolar spaces and the distribution of the lesions was moderate, affecting 25%-50% of the lung area (Figure 4D).

Effect of methyl palmitate and lutein on oxidative stress in lung homogenate in LPS-induced ALI rats

LPS injection resulted in a significant increase ($P < 0.001$) in MDA content in lung while GSH and SOD activities were markedly decreased ($P < 0.001$, $P < 0.05$ respectively), as compared to the control group. These changes were all blocked by methyl palmitate or lutein treatment for one week prior to LPS challenge (Table 2).

Effect of methyl palmitate and lutein on total NO_2^-/NO_3^- content in lung homogenate in LPS-induced ALI rats

As demonstrated in Table 3, the level of total NO_2^-/NO_3^- in the LPS group was significantly higher ($P < 0.001$) than that of the control group. Lutein group showed a significant decrease ($P < 0.05$) in total NO_2^-/NO_3^- content, as compared to the LPS group. Methyl palmitate failed to significantly decrease the elevated NO_2^-/NO_3^- level, as compared to the LPS group.

Effect of methyl palmitate and lutein on TNF-α level in lung homogenate in LPS-induced ALI rats

As shown in Figure 5, the level of TNF-α in the LPS group was significantly higher ($P < 0.001$) than that of the control group. Methyl palmitate and lutein groups showed a significant reduction ($P < 0.001$, $P < 0.05$ respectively) in TNF-α level, as compared to the LPS group.

DISCUSSION

The results of the present study indicate that pretreatment with methyl palmitate or lutein exerts potent anti-inflammatory effects against ALI induced by LPS in rats. Therefore, they may represent potential new therapeutic agents against lung inflammation. Previously, LPS was shown to induce the production of several inflammatory cytokines, tissue edema and injury[5,6] starting after 2-4 h and reaching a maximum at 24-48 h[3,26]. Therefore, in this

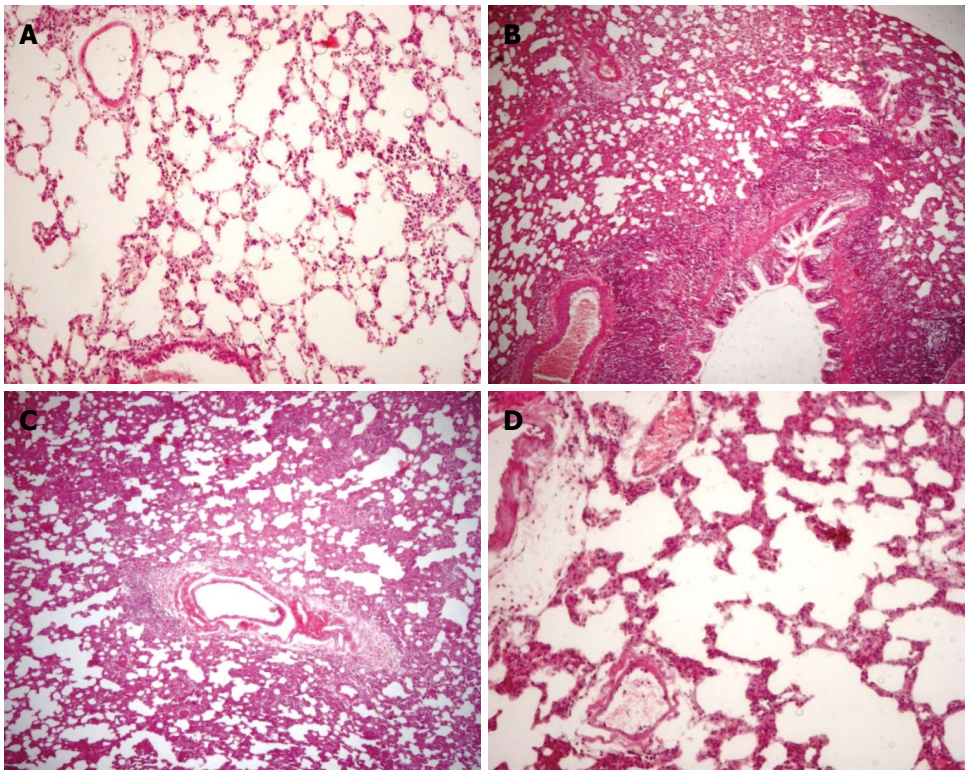

Figure 4 Effect of methyl palmitate and lutein on lung histopathological examination in lipopolysaccharide -induced acute lung injury in rats (HE, × 200). A: Control, no inflammatory infiltrate, edema or fibrosis (score 0); B: Lipopolysaccharide (LPS), severe interstitial inflammation, peribronchial inflammation, fibrosis and edema (score 4); C: Methyl palmitate, mild interstitial inflammation, edema and peribronchial inflammation (score 2); D: Lutein, mild interstitial inflammation, edema and peribronchial inflammation (score 1).

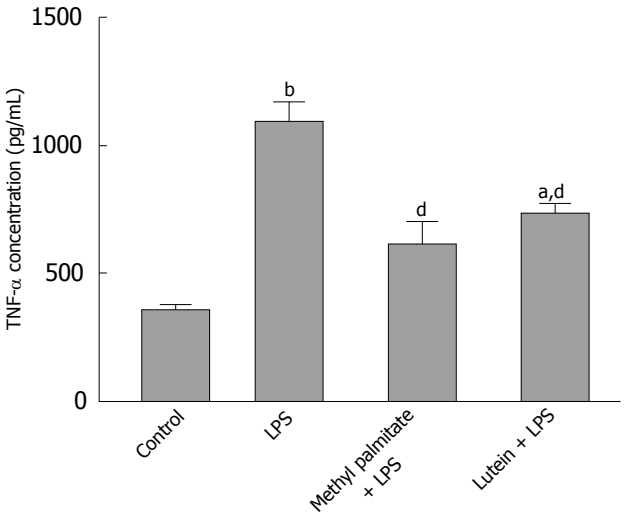

Figure 5 Effect of agmatine, methyl palmitate and lutein on tumor necrosis factor-α level in lung homogenate in lipopolysaccharide -induced acute lung injury in rats. $^{a}P < 0.05$, $^{b}P < 0.01$, *vs* the control group; $^{d}P < 0.01$ *vs* the lipopolysaccharide (LPS) group (one way ANOVA followed by Tukey-Kramer's multiple comparisons test). TNF-α: Tumor necrosis factor-α.

Table 3 Effect of methyl palmitate and lutein on total nitrite/nitrate content in lung homogenate in lipopolysaccharide induced cute lung injury in rats

Treatments	Total nitrite/nitrate (μmol/L)
Control	26.459 ± 0.665
LPS	69.129 ± 0.612[b]
Methyl palmitate + LPS	65.723 ± 2.4[b]
Lutein + LPS	58.018 ± 3.56[b,c]

$^{b}P < 0.01$ *vs* the control group; $^{c}P < 0.05$ *vs* the lipopolysaccharide (LPS) group (one way ANOVA followed by Tukey-Kramer's multiple comparisons test).

study BALF and tissue samples were collected 24 h after LPS exposure.

Initially, both W/D ratio of lung weight and total protein content were measured to estimate the integrity of the lung air/blood barrier and the extent of lung injury. LPS elevated the lung W/D ratio and total protein content in BALF, indicating pulmonary edema and an increase in the leakage of serous fluids into lung tissue, in agreement with previous reports[3,27]. Also, LDH activity was markedly elevated after LPS injection, indicating cell damage or cell death[28], in agreement with Shen *et al*[29] and Kung *et al*[30]. The inflammatory cells, which play a central role in the pathogenesis of ALI, were estimated in the BALF. Activated neutrophils adhered to lung endothelium, transmigrated across endothelial surfaces and into the tissues, where they exerted their toxic effects through the release of ROS, resulting in microvascular dysfunction and local inflammatory response[31,32]. Consistent with previous studies[27,33,34], rats exposed to LPS presented a massive infiltration of inflammatory cells in the lungs. These results were supported by the histopathological examination, which showed an increase in the alveolar wall thickness, severe edema and inflammatory reaction in lung specimens.

Pretreatment with methyl palmitate or lutein resulted, to a varying degree, in marked protection against LPS-induced lung injury. Methyl palmitate reduced lung W/D ratio, total and differential cell count, and histopathological damage (mainly the severity and distribution scores

of the lesions), while it failed to attenuate the elevated total protein level and LDH activity. These results are in accordance with the previous studies[35,36] that proved the anti-inflammatory effects of methyl palmitate both in the suppression of inflammatory cell infiltration and in the management of paw edema. On the other hand, the histopathological results showed that lutein decreased LDH activity and inflammatory cell counts as well as the severity and the distribution of the inflammatory lesions. However, lutein failed to attenuate the increased W/D ratio of lung weight and the total protein level, indicating its inability to counteract pulmonary edema.

To explore the possible mechanism(s) of the protective action of methyl palmitate and lutein against LPS-induced ALI, oxidative stress and inflammatory factors were evaluated in the lung tissue.

One of the earliest manifestations of ALI is the activation of free radical generation by the pulmonary endothelium and neutrophils[37]. During the inflammatory response, neutrophils undergo a respiratory burst and produce superoxide. Overproduction of ROS is highly toxic to host tissues, and their interactions with various cellular macromolecules can have severe pathophysiological consequences[38,39]. GSH acts as a major cellular antioxidant defense system by scavenging free radicals and other ROS. LPS-induced oxidative stress can lead to GSH depletion[40] which aggravates LPS toxicity, probably *via* diminution of the antioxidant defense. SOD is the only antioxidant enzyme that can scavenge superoxide and it has been reported to be markedly decreased in LPS-induced ALI[41]. Certain antioxidants and some synthetic drugs can control the LPS-induced inflammation either by directly scavenging free radicals or by enhancing the endogenous antioxidant defense system[42,43].

Results of the present study showed that LPS injection increased oxidative stress and inflammatory cytokines in the lungs, as seen from the significant increase in MDA content, which is commonly regarded as a marker of oxidative stress and antioxidant status[44]. This was accompanied by a significant decrease in SOD and GSH activities. These results are in accordance with the previous study of Bhavsar *et al*[40]. Pretreatment with methyl palmitate or lutein controlled the levels of SOD and GSH to near control values with resultant decrease in MDA content. Previous investigations have shown the ability of methyl palmitate to attenuate liver fibrosis by decreasing oxidative stress and hence the inflammatory response that leads to fibrosis[10]. Lutein showed a decrease in MDA level and an increase in GSH and SOD levels[45]. Furthermore, Miki[16] stated that lutein enhances the antioxidant enzyme system in blood and liver tissue, proving that lutein has a profound effect on the antioxidant defense system. Therefore, the present study showed that both methyl palmitate and lutein may effectively reduce oxidative burden during the inflammatory response to LPS.

Additionally, it was essential to evaluate the effect of methyl palmitate and lutein on LPS-induced production of inflammatory cytokines which induce, enlarge and facilitate the entire or focal inflammatory reaction.

LPS is known to stimulate various cell types within the lung, including bronchial epithelia, pulmonary artery smooth muscle cells, macrophages, and neutrophils, to over-express iNOS[46]. Results presented here revealed an increased level of NO_2^-/NO_3^- in lung after LPS injection, which is consistent with previous studies that showed the overproduction of NO after LPS injection due to activation of iNOS[47,48]. Excessive production of NO by iNOS mediates increased protein leakage as well as hemodynamic and vascular permeability changes[49]. Furthermore, NO up-regulates inflammatory cytokines, such as TNF-α, and amplifies the inflammatory response during inflammation[50]. TNF-α is considered the first multifunctional cytokine produced from LPS-stimulated monocytes and macrophages. It elicits the inflammatory cascade and contributes to the severity of lung injury[51]. As expected, LPS caused significant increase in TNF-α production in lung. This result is in agreement with previous studies which reported a significant increase in TNF-α after LPS exposure[3,8,52].

Results for methyl palmitate are in agreement with a previous study[36] which reported that methyl palmitate decreased TNF-α level in the systemic LPS injection model. However methyl palmitate failed to reduce elevated NO level indicating that it protective effect against LPS-induced ALI is mainly mediated through its antioxidant activity and it ability to suppress TNF-α production. In addition, lutein has shown ability to suppress elevated NO level and TNF-α production. This is in accordance with the previous investigation of Sasaki *et al*[53] which showed the effect of lutein in decreasing TNF-α level and quenching ROS in LPS-induced retinal inflammation. Furthermore, the previous study of Jin *et al*[17] showed that the expression of iNOS in LPS-stimulated RAW cells was inhibited by lutein. Thus, it appears that lutein suppresses NO production by blocking iNOS protein expression. However, the intracellular mechanisms by which lutein exerts its effects on NO expression are still to be explored.

In conclusion, the present study shows that methyl palmitate and lutein can protect against LPS-induced ALI. Pretreatment with these agents reduced inflammatory changes as well as histological damage during LPS-induced ALI. The protective effect of these agents may be related to their ability to depress ROS generation, enhance antioxidant status and regulate proinflammatory cytokine production. Therefore, methyl palmitate and lutein may possibly represent a novel therapeutic strategy for lung inflammatory diseases.

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COMMENTS

Background

The lack of effective pharmacological interventions remains a major impediment in the treatment of inflammatory diseases of the lung. Lipopolysaccharide (LPS), a bacterial cell wall component, is known to induce the production of several inflammatory cytokines, tissue edema and injury. It is considered to be the most important pathogen that leads to the development of acute lung injury (ALI) in rats.

Research frontiers

Methyl palmitate and lutein are natural compounds that have been shown to possess different beneficial activities against induced organ damage. The research issue is how to use these compounds against ALI. The observed protective effects of these agents suggest their possible medical uses in different inflammatory lung diseases.

Innovations and breakthroughs

The present study demonstrates the protective effects of methyl palmitate and lutein against LPS-induced ALI. Pretreatment with these agents reduced inflammatory changes as well as histological damage during LPS-induced ALI. Furthermore, the present study found that the protective effect of these agents may be related to their ability to depress reactive oxygen species generation, enhance antioxidant status and regulate proinflammatory cytokine production.

Applications

The present study reveals that use of methyl palmitate and lutein possibly represent a novel therapeutic strategy for lung inflammatory diseases.

Terminology

ALI is characterized by the abrupt onset of significant hypoxemia and diffuse pulmonary infiltrates in the absence of cardiac failure. There is also an inflammation-associated increase in pulmonary vascular permeability, and epithelial and endothelial cell death.

Peer review

The study explored the effects of methyl palmitate and lutein on ALI in rats. The results are interesting and comprehensive. The authors should be congratulated on their comprehensive assessment of ALI parameters *i.e.*, wet/dry weight, cellular infiltration, histological scoring and a cytokine of choice, in this case tumor necrosis factor-α.

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