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ORIGINAL ARTICLE

Fucoxanthin Attenuates the Lipopolysaccharide-induced Sepsis and Acute Lung Injury through the Inactivation of Nuclear Factor-Kappa B Signaling Pathway

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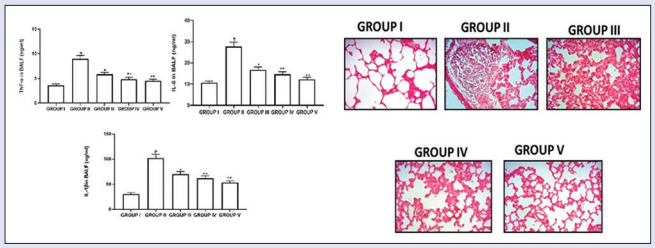
ABSTRACT

Objectives: The acute lung injury was a very severe health consequence and it was allied with the highest morbidity as well as mortality rate among the peoples. The acute lung injury can be induced through the various direct or indirect causative factors such as serious septic conditions, inflammation of the lung tissues, swelling of the lungs, injury of inhalation, pulmonary vasculitis, pancreatitis, and severe burnings which can lead to the unrestrained inflammation. **Materials and Methods:** The sepsis was a major clinical syndrome that can cause by severe infection or injury and it was distinguished by the response to the entire body inflammation. The current research work aimed to assess the curative properties of fucoxanthin against the lipopolysaccharides (LPS)-induced acute lung injury in the experimental animal model. **Results:** The fucoxanthin treatment followed by the LPS administration was exhibited the marked reduction in the wet-to-dry ratio of the lungs, reduced level of infiltration of inflammatory cells in BALF, reduced the enzymatic action of myeloperoxidase in the lungs, reduced the interleukin-6 (IL-6), IL-1 β, and tumor necrosis factor-alpha levels, possessed the notable suppression and downregulation in the expression of immunoreactivity of inflammatory markers like NF-κB p65 and decreased the oozing of mucus and the inflammatory cellular infiltration in lung histopathology which is stimulated by the LPS administration. **Conclusion:** The fucoxanthin was effectively attenuated the LPS-stimulated acute lung injury in an animal model. Hence, it can be concluded that the fucoxanthin had potent therapeutic properties against the LPS-stimulated acute lung injury.

Key words: Bronchoalveolar lavage fluid, fucoxanthin, lipopolysaccharides, lung injury and dexamethasone, myeloperoxidase, nuclear factor-kappa B, sepsis

SUMMARY

- Based on the information received from the intensive care units (ICUs) among the 50 nations, the occurrence of acute lung injury was 30% of ICU admittances recorded with the clinical mortality rate are 34.9%
- The nuclear factor-kappa B (NF-κB) was a key factor of nuclear transcription, which acts as a major factor during the inflammation. The activation of NF-κw was believed to react to the increased oxidative stress
- Fucoxanthin was excellently inhibited the NF-κB signaling cascade and prevented the lipopolysaccharides provoked acute lung injury in experimental animals.



Abbreviations used: NF-κB: Nuclear factor-kappa B; LPS: Lipopolysaccharides; BALF: Bronchoalveolar lavage fluid; MPO: Myeloperoxidase; W/D weight: Wet to dry weight; TNF-α: Tumor necrosis factor-alpha; IL-6: Interleukin-6; JNK: Jun kinase; ERK: Extracellular signal-regulated kinase.

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INTRODUCTION

The acute lung injury was a very severe health consequence and it was allied with the highest morbidity as well mortality rate among the peoples.[1,2] The acute lung injury can be induced through the various direct or indirect causative factors such as serious septic conditions, inflammation of the lung tissues, swelling of the lungs, injury of inhalation, pulmonary vasculitis, pancreatitis, and severe burnings which can lead to the unrestrained inflammation. This inflammation condition was playing as an important key factor in the pathophysiology of acute lung injury.[3-5]

The clinical condition of acute lung injury was characterized by the progression of various pathological alterations such as non-cardiovascular pulmonary edema and accretions of neutrophils which can be stimulated by the damage in the membrane of alveolar capillary. The intense infection at the lungs was known as the frequent stimulant of severe lung injury. The pathway of the development of severe lung injury syndrome was related to excessive production of regulators of inflammation such as chemokines, cytokines, and adhesive molecules.[6,7]

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The sepsis was a main clinical syndrome that can cause by severe infection or injury, and it was distinguished by the response to the entire body inflammation.[8] While the lipopolysaccharides (LPS)-induced severe lung injury, the activation of macrophages in the lungs and penetration of inflammatory cells, particularly neutrophils to the air sacs of the lungs was distinguished as an important incident to the injury lung's parenchyma tissues.[9] Predominantly, the activated neutrophils and macrophages in alveolar of lungs possibly stimulate the increased level of cytokines of proinflammation such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1 β) which can directly induce the accretion of neutrophils and additional liberation of chemokines and cytokines.[10,11] This extreme level of inflammation reaction could lead to the injury of microvascular and dispersion of damage of alveolar tissues of the lungs. Consequently, these mediators of proinflammation were thought to take a very crucial part in molecular events of acute lung injury, whereas the inhibition of these mediators production may lead to the amelioration of LPS-stimulated severe lung injury.[12]

There was a substantial concern that the few of the signaling molecules was speed up the expression and production of mediators of proinflammation at the inflammatory sites. Incidentally, the commencement of the nuclear factor-kappa B (NF-κB) took a crucial function in the pathophysiology of severe lung injury. The augmented commencement of NF-κB may stimulate the cytokines which regulating the immune responses and leads to the conscription of leukocytes at the inflammation sites which is found at the alveolar neutrophils and macrophages from the patients who suffered from acute lung injury. Moreover, the continued nuclear accretion of NF-κB may ally with an elevated rate life lost due to the increased organ failure and pulmonary damage, whereas the inhibition of NF-κB was demonstrated the defensive effects against the acute lung injury.[13-17] The NF-κB was a key factor of nuclear transcription, which acts as a major factor during the inflammation. The activation of NF-κB was believed to react to the increased oxidative stress.[18] Consequently, the inhibition of NF-κB was being as a possible way to counteract the development of acute lung injury.

Based on the topical information which is received from the intensive care units among the 50 nations of the world, the occurrence of acute lung injury was 30% of ICU admittances, with the clinical mortality rate are 34.9%.[19] To the best of the knowledge on previous literature and hospital data, presently, there were no clinically approved drugs for the effectual treatment of acute lung injury.[20] Hence, it was essential to develop the new efficient remedial drugs for the treatment and decrease the morbidity and mortality rate.

The fucoxanthin is known as the orange or brown color pigment, which naturally occurs in various seaweeds. It has belonged to the Non-Provitamin-A class of carotenoids which present on the chloroplasts of the various seaweeds.[21] On the whole, there were no scientific claims or evidence for the remedial properties of fucoxanthin against acute lung injury. Therefore, the present research work was intended to examine the therapeutic properties of fucoxanthin against the LPS-induced acute lung injury in mice model.

MATERIALS AND METHODS

Drugs

Fucoxanthin, Dexamethasone, ELISA test kits and other chemicals were commercially procured from the Sigma Aldrich Inc., USA.

Experimental animals

The Balb/c mice (male breed), weight ranging from 19 to 23 g were maintained in the polypropylene confines beneath the lab conditions (26°C ± 1°C, air moisture 60%–70%, light and dark sequence were 12 h). The animals were fed with commercially purchased food with water *ad libitum*. The animals were getting used to laboratory situations for 7 days previous to the beginning of the work. The heed and treatment of the animals were completed based on the procedure of the Committee for the Purpose of Control and Supervision of Experiments on Animals. The whole animal works in the current research were carried out in the laboratory-based on the procedures recommended by the International Animal Ethics Committee.

Experimental groups and stimulation of acute lung injury

The acute lung injury was stimulated to the experimental animals by the administration of LPS through the intratracheal route.[22] The mice were euthanized by the administration of ethers, and then the 15 μg of LPS in the 50 μl of sterile saline was administered to mice through intratracheal route by using gauge needles. After that, the mice were located in a perpendicular situation and rotated mildly for 2 min for the dispersion of instillations in the lungs. Then, the animals were separated into five groups. The first group was control; it was treated with a commercial pelleted diet. The second group was LPS (15 μg) administered group. The third group was 25 mg/kg of fucoxanthin-treated group followed by 1 h of LPS administration. The fourth group was 50 mg/kg of fucoxanthin-treated group, followed by Ihr of LPS administration. The fifth group was 50 mg/kg of the dexamethasone (standard drug)-treated group followed by 1 h of LPS administration and served as a positive control.

Determination of the wet to dry weight ratio of lungs

After the 12 h of LPS administration, the animals were anesthetized and sacrificed. Then, the lungs were expunged and washed twice with saline solution. Then, the wet weight of the lungs was weighed and noted. Then, the lungs were plotted to dry by placing it in the over at 85°C for 48 h. Then, the dry weight of the lungs was weighed and noted. Finally, the ratio of the wet and dry lung was determined to review the edema of tissues.[23]

Assay of myeloperoxidase activity

The detached tissues of lungs were mixed with the 0.1M of phosphate buffer (pH-6.5) to attain the 5% of homogenate. Then, homogenized tissues were centrifuged at the 13,000 rpm for 25 min at 4°C. The 0.1 ml of resulted supernatant was mixed with 2.9 ml of reaction medium which containing 0.16 mg/ml of o-dianisidine hydrochloride and 0.0005% of hydrogen peroxide. After 5 min of incubation, the absorbance was taken at the 460 nm by using a spectrophotometer. The obtained results depicted as units of myeloperoxidase (MPO) activity per gram of lung tissue.

Collection of serum and bronchoalveolar lavage fluid

The blood of the experimental animals was immediately collected after the animal scarification and centrifuged at 5000 rpm for 15 min for the separation of serum. After centrifugation, the serum was collected and used for the assay. The five individual aliquots of 30 ml of 0.89% sterile saline buffered solution were instilled to the right side lingual or right middle lobe. The collected BALF was instantly centrifuged at 7000 rpm for 10 min for the separation of cells and other unwanted cellular debris. After centrifugation, the cell-free supernatants were taken in polypropylene tubes and used for assays.

Determination of bronchoalveolar lavage fluid tumor necrosis factor-alpha, interleukin-1 beta, interleukin-6 and prostaglandin E2 levels by ELISA

The levels of IL-6, IL-1 β, TNF-α, and prostaglandin E2 (PGE2) in BALF of the experimental animals were estimated by using the commercially purchased ELISA kits (Sigma Aldrich, USA) based on the instructions of the manufacturer. The level of IL-6, TNF-α, IL-1 β, and PGE2 was determined by using a standard curve.

Determination of nitric oxide level

The production of nitric oxide (NO) level was circuitously measured through the levels of nitrite in BALF, which is determined by the colorimetric assay.[24] The 100 µl of the sample was taken in the well of microtiter plate after the adding of 100 µl of Griess reagent (equal volume of 1% sulfanilamide and 0.1% N-1-naphthylethylenediamine dihydrochloride in 2.5% of polyphosphoric acid). The color development was read measured by absorbance at 540 nm.

Assay of cell viability

The viability of RAW264.7 cell lines was assayed by the 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay which is already reported by the Yang *et al.* (2010).[25] The 1×10^5 cells/ml of cells were placed in the 96 well plates and maintained for 24 h, after that it was treated with the fucoxanthin (2–16 µM) and maintained for an additional 24 h. Then, the 20 µl of MTT reagent was supplemented to all the wells and stand for 4 h. Then, the growth medium was detached and 150 µl of dimethyl sulfoxide was mixed to liquefy the formazon. Finally, the absorbance was measured at 570 nm.

Western blotting assay of protein expression

For the estimation of expression of proteins, the collected lung tissues were washed twice with buffered saline. Then, cell suspensions were centrifuged 2000 rpm for 15 min. After the centrifugation, the pellet was mixed with RIPA buffer, which containing inhibitors of protease enzyme for lyses. Then, lysed cell suspension was centrifuged at 13,000 rpm for 20 min. Thereafter, the upper phase was subjected to Western blotting for determining the protein expression by the method of Cheng and Li.[26]

Histopathological analysis

After the 12 h of LPS administration, the experimental animals were sacrificed followed by ethers anesthesia. Then, the right bottom lobes of a lung of the experimental rats were collected and washed thoroughly with cold saline solution. Then, the small piece of the collected organ was fastened in 10% of formalin and then embedded with paraffin wax. Then, wax fixed lungs were cut into small slices in the size of 5 µm. Then, the slices were stained by using eosin and hematoxylin. The histopathological analysis of the lungs was performed by using an optical microscope to detect any damages and inflammation in the lung tissues.

Statistical analysis

All the results were analyzed statistically and the values were articulated as mean ± standard deviation. The one-way ANOVA and Dunnet's test was employed to the interpretation of the data and to measure the variation of significant level among the control and experimental groups. The *P* value was considered as $P < 0.05$ and $P < 0.01$, respectively.

RESULTS

Effect of Fucoxanthin on lipopolysaccharides stimulated lung wet-to-dry ratio

The significantly ($P < 0.01$) increased the level of lung wet weight and dry weight (W/D) was observed in the LPS administered animals while comparing with the control group. The increased level of W/D ratio of lungs of LPS administered animals was an indication of the development of edema in the lungs. Whereas, the treatment with the 25 mg/kg of fucoxanthin followed by the LPS administration was exhibited the significantly ($P < 0.05$) reduced W/D ratio of the lungs when compared to the LPS-treated group. The treatment with the 50 mg/kg of fucoxanthin was statistically significant ($P < 0.01$) reduction in the W/D ratio than the 25 mg/kg of fucoxanthin and LPS administration [Figure 1]. The 50 mg/kg of dexamethasone treatment also possessed a notable reduction in the lung's W/D ratio.

Effect of fucoxanthin on lipopolysaccharides stimulated inflammatory cells infiltration in bronchoalveolar lavage fluid

The LPS administered animals were exhibited significantly ($P < 0.01$) increased levels of neutrophils, macrophages, and total cell infiltration in BALF than a control group. The decreased levels of inflammatory cells infiltration of the lavage fluid of LPS administered animals were indicating the beginning of inflammation in the lungs. While the treatment with the 25 mg/kg of Fucoxanthin followed by the LPS administration was demonstrated the statistically significant ($P < 0.05$) reduction in the level of infiltration of inflammatory cells of the lavage fluid while comparing to the LPS-treated animals. The treatment with the 50 mg/kg of fucoxanthin was possessed significantly ($P < 0.01$) reduced the infiltration level of inflammatory cells than the 25 mg/kg of fucoxanthin and LPS administration [Figure 2]. The 50 mg/kg of dexamethasone treatment also exhibited a remarkable reduction in the infiltration of inflammatory cells.

Effect of fucoxanthin on myeloperoxidase activity in the lung tissue of lipopolysaccharides-induced acute lung injury

The MPO activity was exhibited a significant ($P < 0.01$) increase in the lung tissues of LPS administered experimental animals while comparing

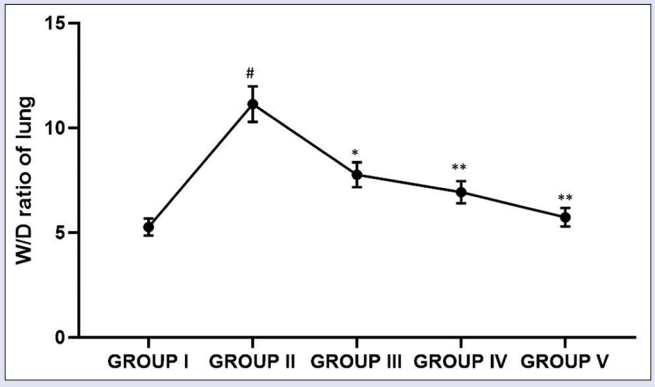

Figure 1: Effect of fucoxanthin on lipopolysaccharides stimulated lung wet and dry ratio. Results are expressed as mean ± standard deviation of six animals. #,*,** representing the statistical significance of control versus experimental groups at the $P < 0.01$ versus control, $P < 0.05$ and $P < 0.01$ versus lipopolysaccharides group, respectively

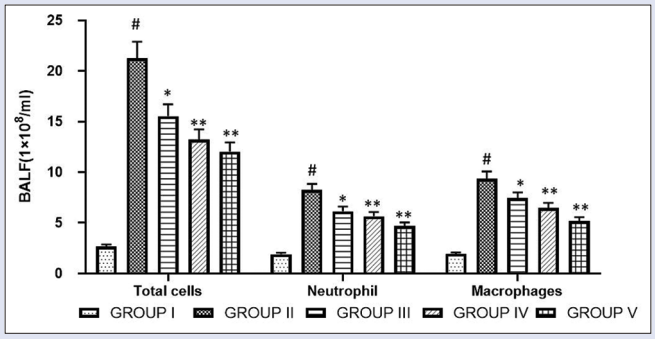

Figure 2: Effect of fucoxanthin on lipopolysaccharides stimulated inflammatory cells infiltration in Bronchoalveolar lavage fluid. Results are expressed as mean ± standard deviation of six animals. #,*,** representing the statistical significance of control versus experimental groups at the $P < 0.01$ versus control, $P < 0.05$ and $P < 0.01$ versus lipopolysaccharides group, respectively

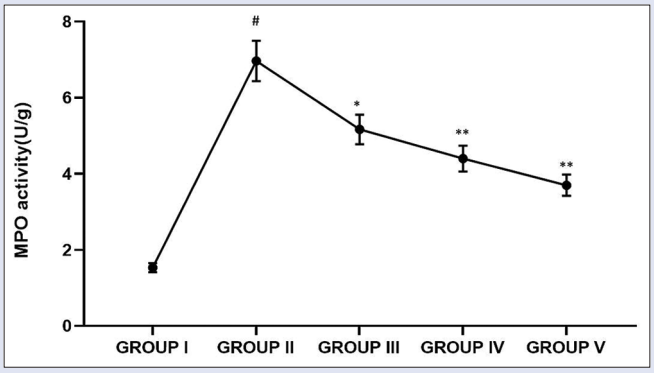

Figure 3: Effect of fucoxanthin on myeloperoxidase activity in the lung tissue of lipopolysaccharides induced acute lung injury. Results are expressed as mean ± standard deviation of six animals. #,*,** representing the statistical significance of control versus experimental groups at the $P < 0.01$ versus control, $P < 0.05$ and $P < 0.01$ versus lipopolysaccharides group, respectively

to the control. The increase in the MPO activity in LPS administered animal lungs was indicating the increased inflammation in the lungs. Whereas, the 25 mg/kg of fucoxanthintreatment after the LPS administration was demonstrated the statistically significant ($P < 0.05$) reduction in enzymatic activity of MPO in the lungs than the LPS-treated animals. The treatment with the 50 mg/kg of fucoxanthin was significantly ($P < 0.01$) reduced MPO activity than the 25 mg/kg of fucoxanthin and LPS administration [Figure 3]. The treatment with the 50 mg/kg of dexamethasone also showed an effective reduction in the activity of MPO in the lungs of experimental animals.

Effects of fucoxanthin on interleukin-6, interleukin-1 beta, and tumor necrosis factor alpha in the bronchoalveolar lavage fluid of lipopolysaccharides-stimulated acute lung injury

The administration LPS to the experimental animals was demonstrated the significantly ($P < 0.01$) elevated level of IL-6, IL-1 β, and TNF-α in the BALF which in contrast to the untreated control animals. The fucoxanthin (25 mg/kg)-treated animals was exhibited a significant ($P < 0.05$) reduction in the IL-6, IL-1 β, and TNF-α levels when compared to the LPS-treated group [Figure 4]. The treatment with the 50 mg/kg of fucoxanthin was significantly ($P < 0.01$) reduced the level of the inflammatory cytokine than the 25 mg/kg of fucoxanthin and LPS administration [Figure 4]. The treatment with the 50 mg/kg of dexamethasone also showed an effective decline in the levels of inflammatory cytokines in the BALF of experimental animals.

Effect of fucoxanthin in the activation of nuclear factor-kappa B in lipopolysaccharides-stimulated acute lung injury

The elevated immunoreactivity of NF-κB p65 was observed in the LPS stimulated acute lung injury in the experimental animals. This result was shown that the LPS administration was uplifted the inflammation in the experimental mice. Whereas, the treatment with 25 mg/kg of Fucoxanthin was possessed the significant ($P < 0.05$) suppression and downregulation in the expression of immunoreactivity of inflammatory markers such as NF-κB p65 [Figure 5]. The treatment with 50 mg/kg of fucoxanthin was also exhibited the notable suppression and down-regulation of NF-κB p65 expression. The treatment with 50 mg/kg of dexamethasone also decreased the expression of NF-κB p65 in experimental animals.

Effect of fucoxanthin on the cell viability, nitric oxide production and prostaglandin E2 level in the lipopolysaccharides stimulated RAW 264.7 cells

The viability of fucoxanthin-treated cell lines was measured by MTT assay. The results were exhibited that the fucoxanthin (2–16 μM) was showed no significant cytotoxicity to theRAW 264.7 cells while compared to control. Although the treatment with fucoxanthin (2–16 μM) was exhibited significantly ($P < 0.01$) reduced the level of PGE2 than the control group [Figure 6]. Even though the treatment with fucoxanthin (2–16 μM) was demonstrated significantly ($P < 0.01$) reduced NO level than the control group.

Effect of fucoxanthin on the histopathology of lung tissues of lipopolysaccharides stimulated acute lung injury

In this analysis, the lungs were analyzed through the histopathological staining method for detecting any injuries and cellular destructions. In the histopathological analysis of the lungs clearly showed that the presence of plentiful eosinophils in the interstitium of the airways and blood vessels. Furthermore, the contraction of the airway lumen was observed in the lungs of LPS administered mice [Figure 7]. Whereas, the treatment with the fucoxanthin at the dose of 25 mg/kg was exhibited a reduction in the secretion of mucus and inflammatory cellular infiltration while compared with the LPS administered animals. The 50 mg/kg of fucoxanthintreatment was effectively inhibited the LPS-stimulated cellular injuries in the lungs of the experimental animals. The standard drug dexamethasone was also possessed a decreased level of inflammatory cellular infiltration and tissue damages than the LPS administered group [Figure 7].

DISCUSSION

The acute lung injury is a disease condition of severe respiratory illness that was exhibited pulmonary edema and problems in respiration which can be induced by many causatives that can direct or indirectly damage the lungs. The most common clinical conditions which are linked with the progression of acute lung injury were sepsis, inflammation in lungs, trauma, and inhalation of toxic gastric contents.[27] The prevalence and pathophysiology of acute lung injury were studied scientifically, whereas the morbidity and mortality level of acute lung injury was constantly

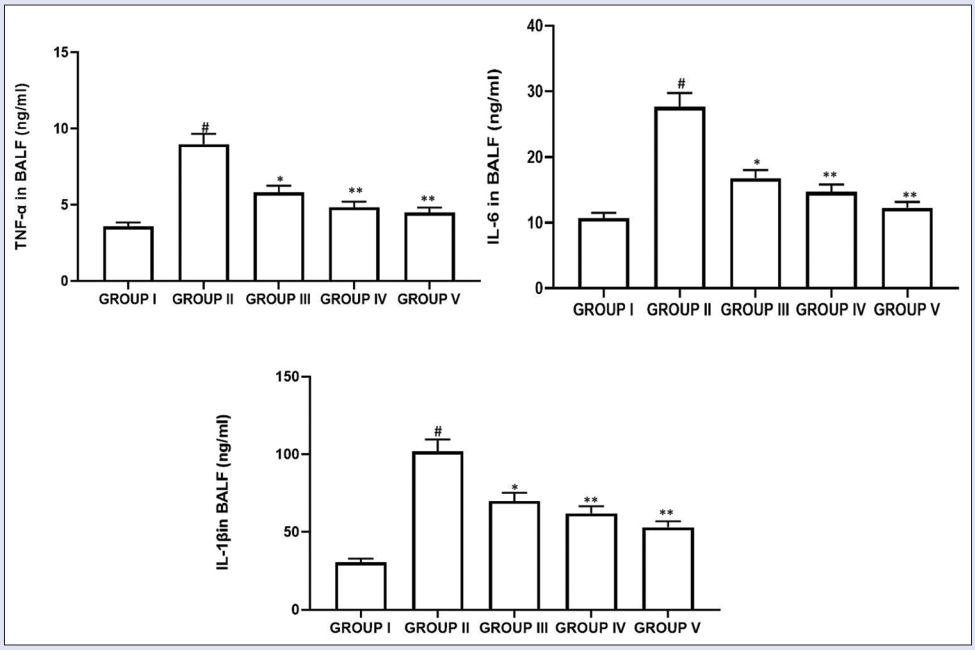

Figure 4: Effects of fucoxanthin on interleukin-6, interleukin-1 beta and tumor necrosis factor alpha in the bronchoalveolar lavage fluid of lipopolysaccharides stimulated acute lung injury. Results are expressed as mean ± standard deviation of six animals. #,*,** representing the statistical significance of control versus experimental groups at the $P < 0.01$ versus control, $P < 0.05$ and $P < 0.01$ versus lipopolysaccharides group respectively

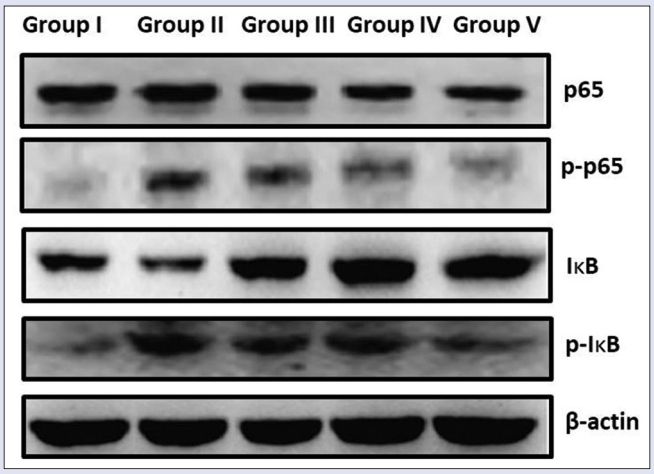

Figure 5: Effect of fucoxanthin in the activation of nuclear factor-kappa B in lipopolysaccharides stimulated acute lung injury

increased. The present scientific investigation was carried out to assess the curative efficiency of fucoxanthin against the LPS-stimulated acute lung injury in experimental animals.

The pulmonary edema was a primary feature of pathological changes in acute lung injury.[28] In the present study, the W/D ratio of the lung was studied to measure the enormity and severity of pulmonary edema. In W/D ratio analysis, the fucoxanthin was exhibited a significant ($P < 0.01$) reduction in the lung W/D ratio after the LPS challenge. This result was demonstrated the curative effects fucoxanthin against LPS stimulated acute lung injury in animals. The LPS administration to experimental animals has exhibited an increased level of W/D ratio than control. It was indicating the development of edema in the lungs which may be stimulated by the administration of LPS. The treatment with the fucoxanthin followed by the LPS administration was exhibited the reduced W/D ratio of the lungs when compared to the LPS-treated group [Figure 1]. This result clearly indicated that the fucoxanthin was effectively inhibited the LPS-induced edema in the pulmonary tissues of experimental animals.

The administration of LPS can directly stimulate the penetration of monocytes and neutrophils to the lung tissue through the steps involved by the mediators of proinflammation such as IL-1 β, IL-6 and TNF-α.[29] In the present investigation, the treatment with fucoxanthin after the LPS administration was exhibited significantly ($P < 0.01$) reduced the level of pro-inflammatory cytokines in the BALF of experimental animals [Figure 4]. This result was evidenced by the inhibitory effects of fucoxanthin against the LPS stimulated infiltration of proinflammatory cytokines.

The NF-κB p65 was an imperative key factor for the production and regulation of proinflammatory cytokines.[30] The extracellular signal-regulated kinase (ERK) pathway was an essential element of lipopolysaccharide-stimulated inflammation in the lungs.[31] The ERK pathway was categorized as a major factor in the liberation of inflammatory cytokines which is the result of LPS stimulated signal transduction pathways. These pathways are linked with the TNF-α and IL-1 β cytokines in LPS-stimulated lung damage. The pathways of MAPK, P38, Jun kinase (JNK), and ERK were concerned with the pathophysiology of LPS-stimulated acute lung injury.[32] The present investigation was exhibited that the treatment with the fucoxanthin was suppressed and downregulated the expression of NF-κB p65. Hence, this was clear that the fucoxanthin was inhibited the activation and expression of NF-κB p65, JNK, and ERK pathways which is very essential for the production of inflammatory cytokines such as TNF-α, IL-6, and IL-1 β. This result was attributed to the anti-inflammatory efficacy of fucoxanthin through inhibition of NF-κB p65 signaling pathways [Figure 5].

The intrathecal administration of lipopolysaccharide was broadly employed to stimulate the infiltration of neutrophils and induce the inflammation at pulmonary tissues in experimental animal models.[33]

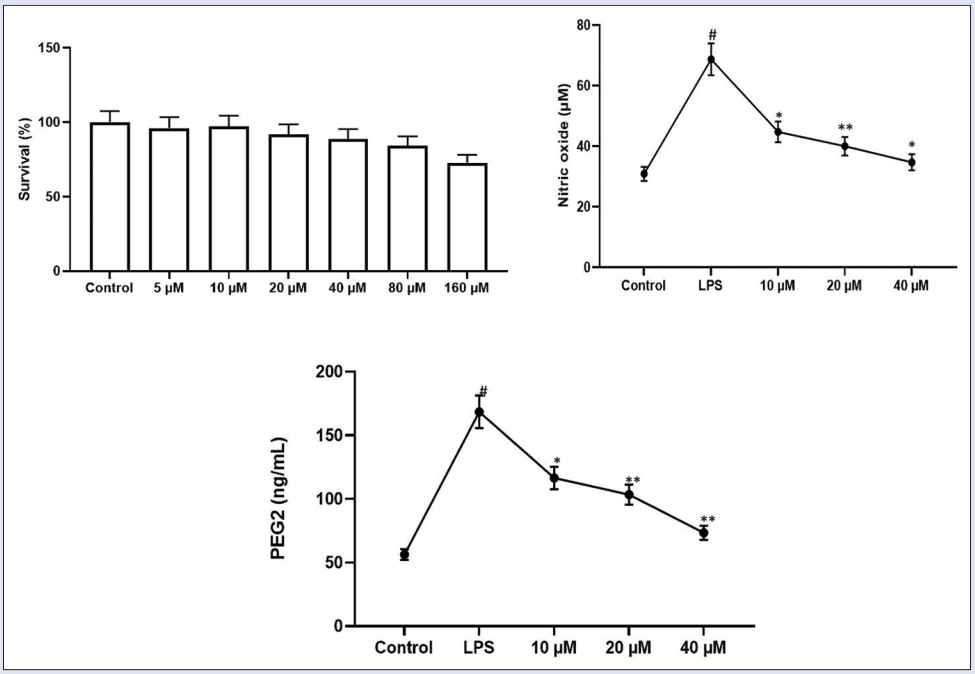

Figure 6: Effect of fucoxanthin on the cell viability, nitric oxide production and prostaglandin E2 level in the lipopolysaccharides stimulated RAW 264.7 cells. Results are expressed as mean ± standard deviation of six animals. #,*,** representing the statistical significance of control versus experimental groups at the $P < 0.01$ versus control, $P < 0.05$ and $P < 0.01$ versus lipopolysaccharides group, respectively

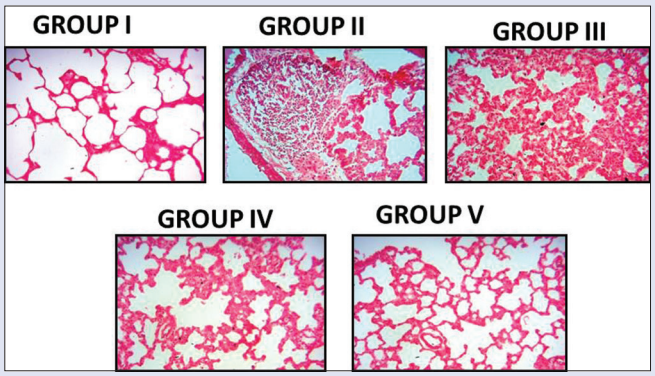

Figure 7: Effect of fucoxanthin on the histopathological changes in lung tissues of lipopolysaccharides stimulated acute lung injury. Depicted the histological changes induced by lipopolysaccharides in the lungs of the experimental animals. (Group I) Control group, (Group II) lipopolysaccharides group, (Group III) lipopolysaccharides + Fucoxanthin (25 mg/kg) group, (Group IV) lipopolysaccharides + Fucoxanthin (50 mg/kg) group, (Group V) lipopolysaccharides + Dexamethasone (50 mg/kg) group (×200)

The exposure of pulmonary tissues to the LPS can induce and upregulate cyclooxygenase-2 and NO synthase expressions. It may result in the excessive production of proinflammatory cytokines such as TNF-α and IL-6 and NO. These processes are mainly involved in the pathophysiology of acute lung injury.[34]

The inflammation is the imperative immunological process, which is essential to self-defense. The inflammation can be stimulated by the various stimuli like the external factor, infections, and injuries.[35] The progression of inflammation was interlinked with the inflammatory cytokines production by macrophages and neutrophils. The leakage of fluids and proteins may responsible for the development of edema in pulmonary tissues and the leukocyte infiltration at the inflammation site.[36,37] The acute lung injury was characterized as a common inflammatory disease that showed the characteristic features of edema of pulmonary tissue, neutrophil infiltration, damage of integrity of epithelium, and injury of lung tissues.[38] In the present investigation, the treatment with the fucoxanthin followed by the LPS challenge was demonstrated the significantly ($P < 0.05$) reduced level of infiltration of inflammatory cells of the BALF while comparing to the LPS-treated animals [Figure 2]. Hence, it was clear that the fucoxanthin was attenuated the LPS stimulated the infiltration of inflammatory cells.

The cascades of inflammation were playing a very essential function in the development of acute lung injury which may stimulate by either basic inflammatory stimuli like LPS or inflammatory disorders like sepsis.[38] In the experimental animal models of acute lung injury, the LPS were usually employed for the induction of inflammation and pulmonary tissue injury.[39] The inflammatory cells activation and the release of proinflammatory cytokines were a response to the exposure of LPS.[40]

The present study reveals that the MPO activity was significantly ($P < 0.01$) increased in lung tissues of LPS administered animals than the control. The increased level of MPO activity in the lungs of LPS administered animals was indicating the increased inflammation in the lungs. Whereas, the fucoxanthin treatment after the LPS administration ameliorated the LPS-induced enzymatic activity of MPO in the lungs than the LPS-treated animals [Figure 3]. In the histopathological examination of the lungs exhibited that the fucoxanthin was effectively attenuated the LPS induced over secretion of mucus and the inflammatory cellular infiltration while compared with the LPS administered animals [Figure 7]. Hence, the observed results were showed that the fucoxanthin was excellently inhibited the LPS-stimulated acute lung injury in experimental animals.

CONCLUSION

The findings of the present research work clearly demonstrate that the fucoxanthin was attenuated the LPS-induced acute lung injury in the experimental animal model. The fucoxanthin was effectively inhibited the LPS-induced pathological changes in animals. Hence, it can be concluded that the fucoxanthin had potent therapeutic properties against the LPS-stimulated acute lung injury.

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Conflicts of interest

There are no conflicts of interest.

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