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Didox ameliorates sepsis induced acute lung injury via down regulation of IL-33 signaling pathway in mice

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ABSTRACT

Background: Sepsis very well define as life-threatening medical condition and may be characterize as dangerous condition described by dysregulated inflammation. The objective of this study is investigating the effects of pre-treatment with didox in attenuation of acute lung injury caused by sepsis through down regulation of IL-33 pathway.

Method and materials: A total of 30 Adult male of Swiss Albino mice were used for the study, with weights ranged between 30 g - 35 g. The experimental animal subjected to cecal ligation and puncture (CLP) and pretreated with 200mg /Kg i.p. of didox 1 hour before sepsis induced. The lung tissue for histopathology examination and proinflammatory markers level (IL-1β, IL-33 and MCP-1) beside serum samples were analyzed by enzyme-linked immunosorbent assay (ELISA). ApHOX analyzer (Nova Biomedical, Waltham, USA) used for the determination of blood gases as indication to lung function in mice groups.

Results: When compared the data of different mice sets with the sham collection, levels of serum and lung tissues IL-1β and MCP-1 are showed significant ($p<0.001$) increased within control and vehicle groups. Sepsis induction set also shows significant ($p<0.001$) increase in serum IL-33 which cause impairment of lung functions expressed as a significant reduction in partial pressure values for oxygen (pO2) and as well as a significant elevation in partial pressure values for carbon dioxide (pCO2). These changes are consistent with histopathologic examination which shows severe pulmonary damage as compared to sham set. All these changes were counteracted by administration of didox.

Conclusions: Our results revealed didox have been shown to decrease sepsis-induced acute lung injury through interfering with inflammatory mediators (IL-1β, IL-33 and MCP-1) and down regulation of IL-33 signaling pathway.

Keywords: Didox, Sepsis, Lung injury, IL-33

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INTRODUCTION

Sepsis very well define as life-threatening medical condition and may be characterize as dangerous conditions described by dysregulated inflammation, disarranged blood coagulation, and different organ injuries. Acute Lung Injury (ALI) subaltern to septic condition may reported as the provenance of significant death-rate within grown-up and pediatric populaces and is a considerable contributor of emergency unit costs (1-4). The commonness of sepsis as general worldwide is evaluated at 19 million (5). Regardless of present-day antimicrobials, extreme sepsis-related mortality stays high at 20- 30%. New remedial targets are earnestly expected to enhance the survival results of septic patients. Sepsis has a biphasic inflammatory process; an early stage portrayed by individual cytokines which have pro-inflammatory action, for example, tumor necrosis factor (TNF) and interleukins, and a late stage intervened byinflammatory cytokines (6).IL-33, is an individual from the IL-1 group of cytokines that incorporates IL-1, IL-18 and many other, actuates Th2-type immune reactions such eosinophil-rich inflammation in the digestive organs, pulmonary system (7-10). IL-33 is vital for Th2 cytokine-related host guard against infection through signals from IL-33 receptor (IL-33R) (11). As a Th2-activating cytokine, IL-33 is thought to associated with initiation the hypersensitive infections, for example, atopic asthma (12-14). IL-33 is likewise known to upgrade the advancement of Th1 cytokine-related atherosclerosis in trial animals (15) and quicken cell-interceded murine joint pain (16). In this manner, it is presently imagined that IL-33 work as a Th2-actuating cytokine, as well as a proinflammatory cytokine, like IL-1 and IL-18,in different immune reactions (17) and LPS-instigated endotoxin shock (18). ProIL-33 has natural action and is proposed to work as an alarmin, an endogenous risk signal that cautions immune cells, in putrefaction, as opposed to apoptosis, related tissue damage amid injury or potentially disease (19-21). In condition of homeostatic state, endogenous IL-33 is constitutively expressed in the nucleus of cells, however its nuclear actions not completely discover (22). If there arise an occurrence of tissue harm, for example, necrotic cell death, cell stress as well as mechanical damage, IL-33 articulation increments and it is discharged into the extracellular space also(23-25).Didox (3,4-Dihydroxy-Benzohydroxamic Acid), is an synthetic ribonucleotide reductase (RR) inhibitor,it is fundamentally created as substitutional to another medication which is hydroxyurea, an intense, however more toxic side effects, ribonucelotide reductase inhibitor used to treat sickle-cell infection, mastocytosis, and different cases (26). Didox for the most part consider as a simple, manufactured cancer prevention agent that has been found in numerous investigations that bringing down the levels of oxidative damage markers in the brains of HIV patients with dementia and accordingly may have strong anti- oxidative properties (27). The multifunction impact of

didox notwithstanding antineoplastic operator might be appear by trials about that demonstrated the utilization of didox may hinder NF-κβ enactment (28) one of the real players in inflammation that including oxidative injury (29). Moreover, didox additionally repress T- cell expansion in murine model of organ dismissal and graft versus host malady, with accompanying effects for both pro-inflammatory and effector cytokines (3-32). Didox could lessen ROS generation to close standard levels, proposing either a capacity to restrain NADPH-oxidase exercises that prompt ROS creation, or by repressing the exhaustion of glutathione that clarified the cell antioxidant impact of didox (33). For the most part the principle component for didox activity is the restraint of ribonucleotide reductase. The aims of the study, to examine the effectiveness of didox in attenuating acute lung injury following sepsis in adult male mice.

METHODS

Experimental animals

Adult (4–6 months) male Albino-Webster mice and their weights ranged from 25 to 30 g obtained from the College of Science, Babylon University. Mice were acclimated for 14 days in a 12:12-h light-dark cycle with free access to water and regular chow diet before the experiments in animal house of Medical College, Kufa University, and this investigation conforms to the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 1996).

Cecal ligation and puncture procedure in mice

Cecal ligation and puncture (CLP) was performed to induce sepsis in mice as previously described.[12] Briefly, mice were anesthetized by i.p., injection of ketamine (Ketamin; DeltaSelect, Dreieich, Germany) and xylazine (Rompun; Bayer, Leverkusen, Germany). The cecum was exposed through a 2-cm abdominal midline incision, and about two-thirds of the cecum was ligated. The ligated part of the cecum was punctured through and through with a 21-gauge needle. After repositioning the bowel, the abdomen was closed in layers, using a 5.0 surgical suture (Ethicon, Norderstedt, Germany). Mice were monitored for various signs of sickness every 4 h for 24 h. Sham surgical operated mice (anesthesia and laparotomy) served as the surgical control group.

Experimental protocol

Mice were assigned to one of the following experimental groups (n = 8 in each group): Sham group, vehicle (LPS) group, CLP group, and CLP + Didox(Sigma-aldrich, Germany), 200mg/Kg/I. Pas previously described (Thabe et al., 2015, 1 hour before CLP. Mice were morning and followed for survival for 24 h. After analysis thelung function, the lung tissue and blood were collected and prepared for analysis.

Lung function measurements

A 24 hours after cecal ligation and puncture technique, anesthetic procedure to mice via mixing the two chemical solutions (2 parts of ketamine to 1 part of xylaxine) and blood test from the intra cardiac were gathered in 0.5 ml heparinized Eppendorf cups as depicted beforehand (34). The collected blood sample were infused either from the Eppendorf container or directly from capillary into in to apHOX analyzer (Nova Biomedical, Waltham, USA) for the assurance of blood gases. The partial pressure values for carbon dioxide (pCO2) and oxygen (pO2) were then decided electrochemically. By this technique we can obtain the blood gases analysis as mark for lung capability.

Enzyme-linked immunosorbent assay

The samples of blood from mice were centrifuged (in 10,000 RPM, for 10 min), and myocardial tissue was homogenized and treated in phosphate-buffered saline containing 0.5% Triton X100 with a protease inhibitor cocktail. Commercial enzyme-linked immunosorbent assay (ELISA) kits (R and D Systems) were utilized to quantify monocyte chemoattractant protein-1 (IL-1β, IL-33 and MCP1) in plasma and lung tissue. Samples and standards were prepared according to manufacturer's instructions. Absorbance of standards and samples were determined spectrophotometrically at 450 nm, by a microplate reader (Bio-Rad Laboratories, CA, USA). Obtained data were plotted against the linear portion of a standard curve

Histological examination

The lung tissue samples were fixed in 4% paraformaldehyde for 24 h. Sections 5 μm in thickness were paraffin embedded according to the standard procedure. Then, the samples were stained with the hematoxylin and eosin (H and E). The degree of lung damage and photographs were obtained from each lung section (n = 3 sections per lung) under optical microscopy.

Statistical Analysis

Statistical analysis of the information was accomplished by utilizing Stat View software (Abacus Concepts, USA). ANOVA and Fisher Post Hoc test was connected to analyze contrasts and compare between experimental animal (mice). In all results of tests, P ≤0.001 was tallied that is Statistically Significant results.

RESULTS

Role of cytokineIL-1βafter cecal ligation and puncture

Sepsis leads to upstream release of both cytokines and chemokines, we investigated the importance effects of didox on the local and systemic proinflammatory responses during CLP. At the end of the experiment (24 h after CLP), the levels of inflammatory modulators IL-1β in plasma and lung tissue are measured by ELISA according to manufacture protocol. The resulted data showed that (IL-1β) are increased after CLP and vehicle treatment compared with sham group (P < 0.001) in both plasma and myocardial tissue. While, pretreated mice group by didox in dose of 200mg/kg I.P showed attenuates IL-1β in both plasma and lung tissue (P < 0.001) as in Figures 1

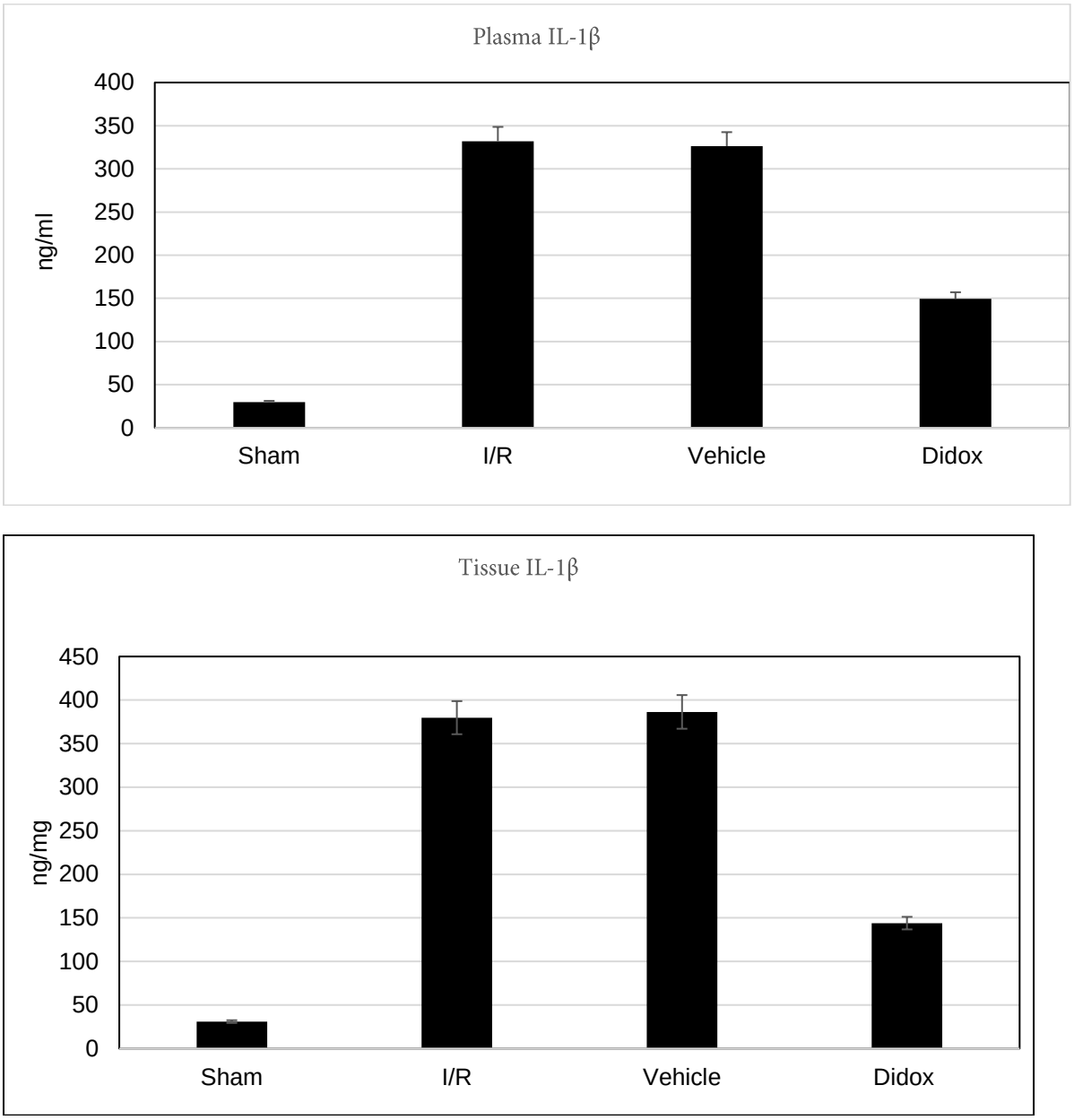

Figure 1.

The mean of plasma proinflammatory cytokine (ng/ml) in the four experimental groups 24 h after cecal ligation and puncture. Data are expressed as mean ± standard error, n = 8 in each group; *P < 0.001 versus corresponding sham, **P < 0.001 versus untreated and vehicle.

Effect of sepsis and the studied drug on serum and lung Tissues MCP-1

Theserum and pulmonary MCP-1 levels are expanded in significant way (p<0.001) within control and vehicle mice versus sham gathering. There was insignificant distinction (p>0.001) between vehicle and CLP mice when the value was (843.4 ng/ml and 844.0 ng/ml) respectively. It was important to remember that the mean value of sham group was (128.6 ng/ml). The MCP-1 levels in didox pre-treated gatherings is lower in significant way (p<0.001) than which seen in control and vehicle gatherings and its level in the pretreated erythropoietin mice was insignificantly (p>0.001) lower than that of pretreated didox gathering. The measurable value for didox was (632.8 ng/ml). However, the means of measuring values of MCP-1 in lung tissues was as following; sham mice (70.8 ng/ml), CLP mice (567.2 ng/ml), vehicle mice (563.2 ng/ml), didox pre-treated mice (329.6 ng/ml), The adjustments in serum and lung tissues MCP-1 are abridged in Figure 2.

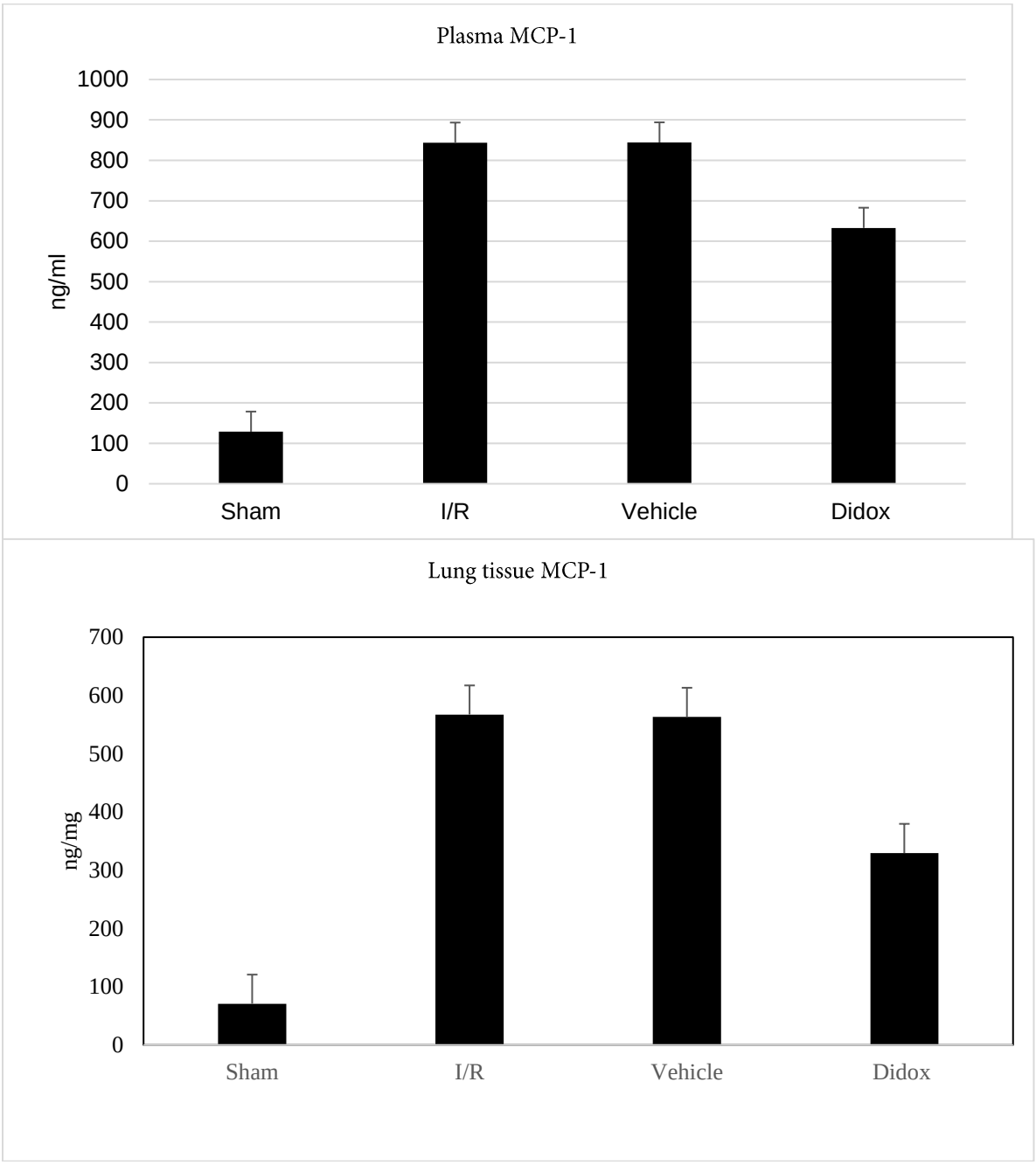

Figure 2.

The mean of plasma and lung tissue MCP-1 in the four experimental groups 24 h after cecal ligation and puncture. Data are expressed as mean ± standard error, n = 8 in each group; *P < 0.001 versus corresponding sham, **P < 0.001 versus untreated and vehicle.

Effect of sepsis and studies drugs on serumIL-33.

The level of IL-33 in the serum of mice was significantly ($p<0.001$) rise in CLP mice (86.8 ng/ml)and vehicle mice (90.4 ng/ml) as compared with mice sham gathering (11.0 ng/ml). There was insignificant difference ($p>0.001$) amongst vehicle and CLP mice. The IL-33 level of pre-treated didox(45.8 ng/ml) is in significant way ($p<0.001$) lower from that in control group. **Figure (3)**.

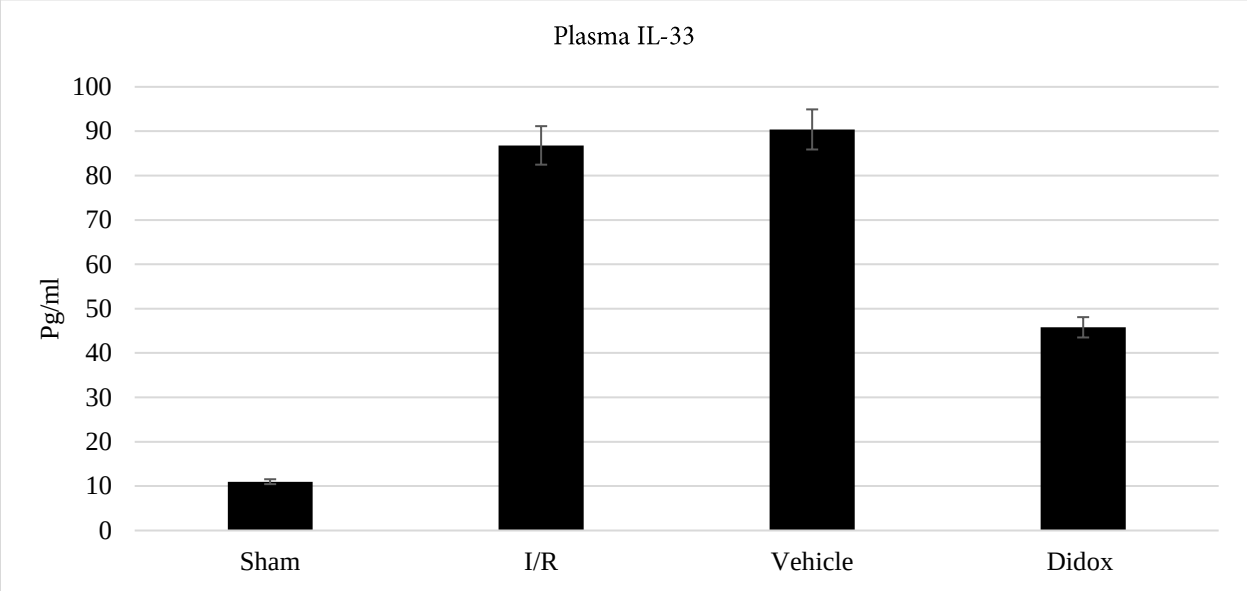

Figure 3.
The mean of serum IL-33(pg/ml) for five sets of mice exoposed to cecal ligation and puncture

Effect of Sepsis and Studies Drugs on Blood Gases

24 hours after cecal ligation and puncture technique, partial pressure values for oxygen (pO2) was significantly (p<0.001) diminish in CLP mice (32.9 mg/ml) and vehicle mice (34.2 mg/ml) as contrasted mice sham gathering (96.5 mg/ml). There was insignificant difference (p>0.001) amongst vehicle and CLP mice. Partial pressure for oxygen (pO2) of pre-treated didox (83.7 mg/ml) and erythropoietin group (84.9mg/ml) was significantly (p<0.001) higher from that found in control group. While partial pressure for carbon dioxide (pCO2) was showing significant (p<0.001) increment in control mice (65.7 mg/ml) and vehicle gatherings of mice (67.5 mg/ml) as compared with mice sham gathering (25.6 mg/ml). There was insignificant difference (p>0.001) amongst vehicle and CLP groups. In any case, pCO2 was significantly (p<0.001) diminish in pretreated didox mice (45.4 mg/ml) and erythropoietin mice group (47.5 mg/ml). The partial pressure estimations of blood gases are summarized in **Figure (4)**.

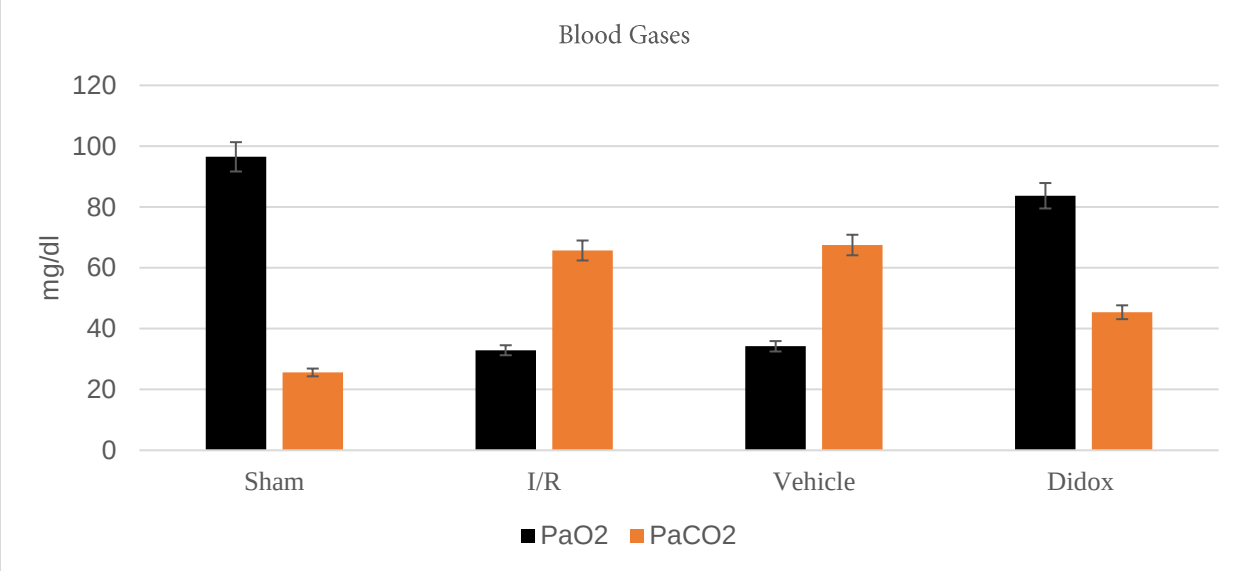

Figure 4.
The mean of partial pressure values of blood gases (mg/ml) for five mice sets exposed to cecal ligation and puncture.

Histological changes of lung tissue in response to sepsis

Pulmonary injury was estimated in the mouse's lung tissues of the 4 group. To gauge the distinction in lungs damage, histological segments from mice were analyzed and scored rely on the past investigation (Zhu et al., 2012). The mice

assessed after finishing of the examination and the outcomes are:

Sham Group:

A cross section of sham mouse s' lung demonstrated the typical tissues structure: no interstitial edema, no thickening of the alveolar septae, no formation of nodules or zones of pneumonitis that mutilated the ordinary architecture, no neutrophil invasion, no vessels compacting and no hemorrhage. All mice in this group indicated typical lung tissues as appeared in Figure (5)

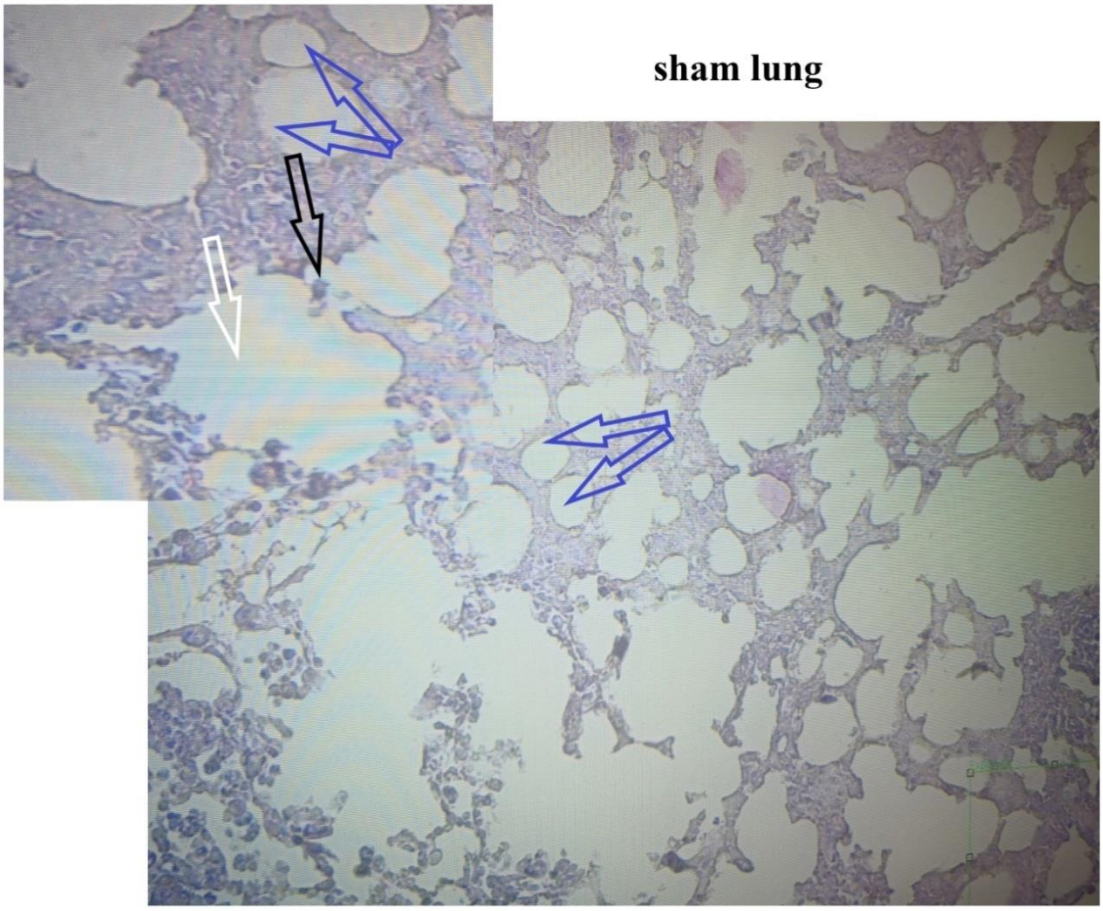

Figure 5

Representative H and E staining at 40X and 100X magnification of adult male mice lung. Mice without induced sepsis as sham, bronchioles tissues were normal (white raw), intact blood vessels (black raw) and normal alveoli (blue raws).

Control Group

Following 24 long periods of enlistment of sepsis, lungs tissue from CLP mice histologically demonstrate marked damage with the advancement of leukocytes infiltration and deposition of fibrins noticeable in the parts of airways was characteristic of edema with formation of nodules or areas of pneumonitis that distorted the normal architecture; and total obliteration of lung tissue. The changes appeared in Figure (6).

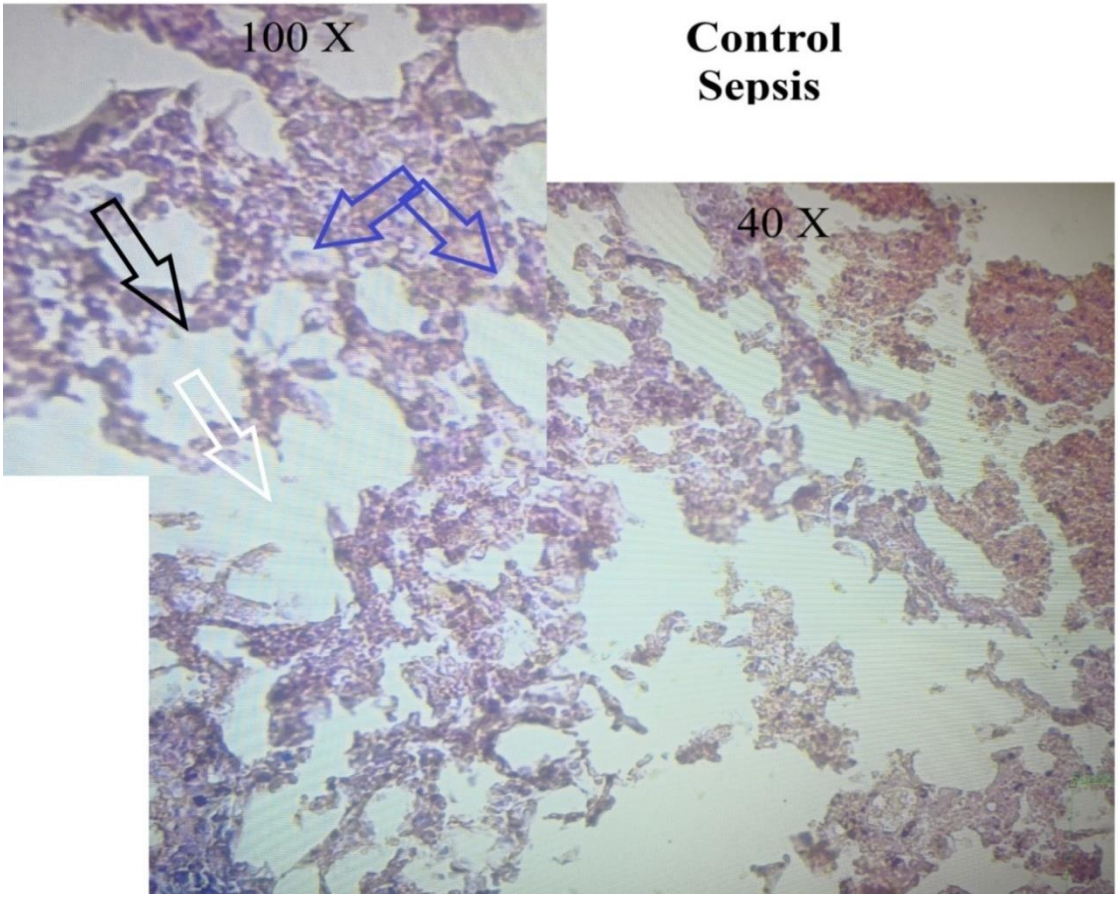

Figure 6

Representative H and E staining at 40X and 100X magnification of adult male mice lung. Mice induced sepsis as control, bronchioles tissues (white raw) and alveoli (blue raw) were indicate the infiltration of cells. Furthermore, deposition of fibrins in parts of airways is indication for edema with formation of nodules or areas of pneumonitis that distorted the normal architecture; and total obliteration of lung tissue.

Vehicle Group

This group revealed similar description to that found in control group which display unmistakable infiltration process for cells, deposition of fibrinous substance in the air ways passage was indication for edema in alveoli with formation of nodules or areas of pneumonitis that distorted the normal architecture; and total obliteration of lung tissue .The progressions appeared in Figure 7.

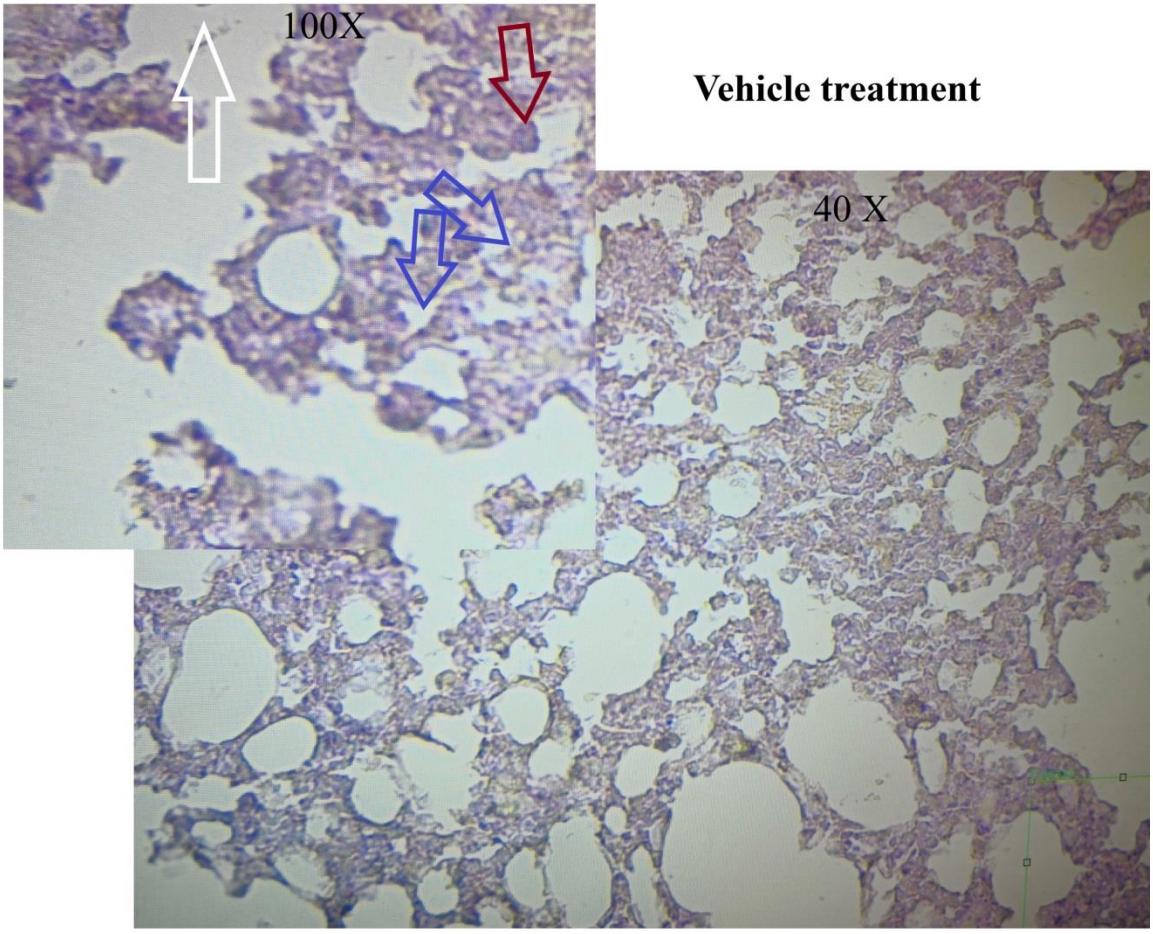

Figure 7

Representative H and E staining at 40X and 100X magnification of adult male mice lung. Mice induced sepsis and treated with normal saline as Vehicle group, bronchioles tissues (white raw) and alveoli (blue raw) were remarked infiltration process for cells, deposition of fibrinous substance in the air ways passage was indication for edema in alveoli with formation of nodules of pneumonitis that distorted the normal tissues; and total obliteration of lung tissue.

Didox Group

Treatment of mice with didox enhanced lung damage as contrasted with CLP and vehicle group and this this improvement including less prominent cell infiltration, no alveolar edema and greater alveolar openness. The changes shown in Figure 8.

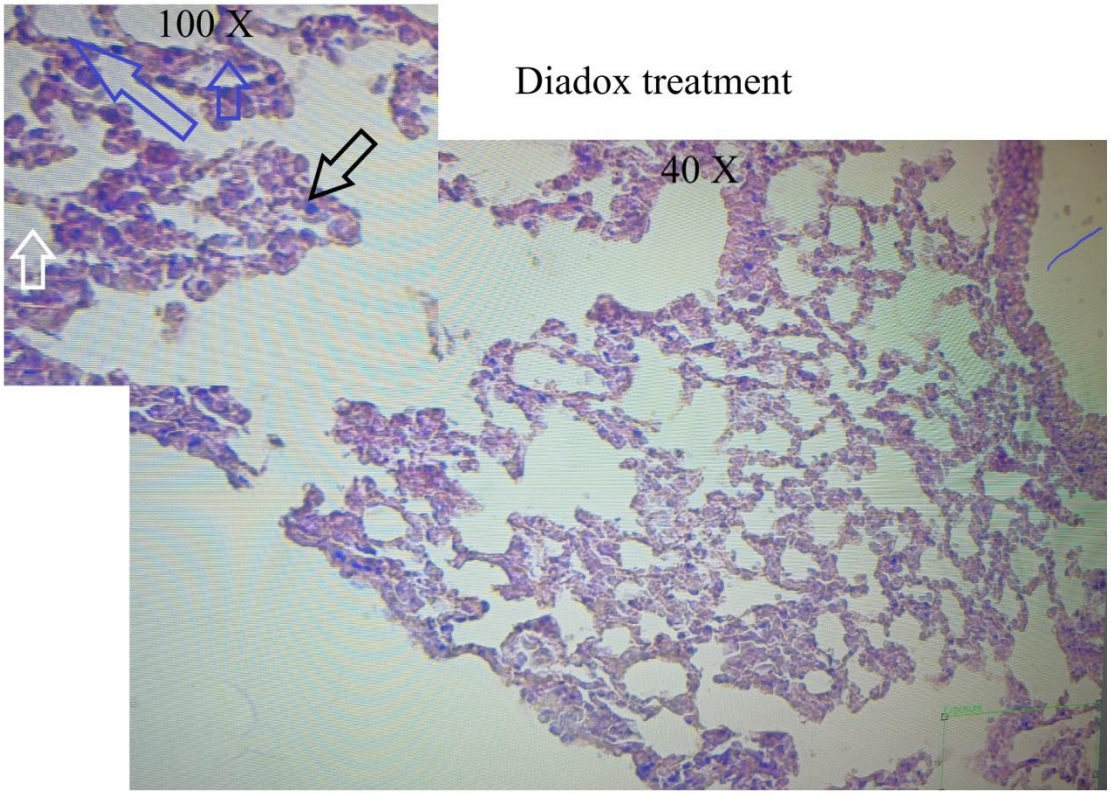

Figure 8

Representative H and E staining at 40X and 100X magnification of adult male mice lung. Mice induced sepsis and pretreated with Diadox, bronchioles tissues (white raw) and alveoli (blue raw) were exhibit more less prominent cell infiltration, no alveolar edema and greater alveolar openness.

DISCUSSION

Acute lung damage is a noteworthy complexity of septic shock that outcomes in the high death rate with sepsis patients in the concentrated care units (35). In the present study, CLP procedure is used in mice model to initiate sepsis which is consider as the cornerstone for modeling of poly-microbial sepsis. This model of technique has numerous advantages since it give rise the conspicuous changes in lung function like that found in people with sepsis. Alongside that, CLP technique prompt major liberation of pro inflammatory cytokines (36). Additionally; CLP model has numerous aspects that resemble the entangled clinical course of sepsis, these incorporate tissue injuries caused by a laparotomy, necrosis caused by a cecum ligation, infection caused by the infiltration of peritoneal microbial flora into the peritoneum, and consequence release of inflammatory mediator (37). In this experiment, we illustrated that sepsis augmented the generation of generation of inflammatory markers (IL-33, MCP-1, and IL-1β) in both serum with lung tissue of CLP and vehicle mice group as contrasted with sham gathering, which related with changing in pulmonary function. This finding is in concurrence with reviews done by (38) and can be clarified by that the incitement of neutrophils, macrophages, monocytes and endothelial cells potentially happens through TLR-4 that starts downstream signaling pathway and results in the initiation of NF-κB, which prompts the transcription of an extraordinary number of pro-inflammatory cytokines (39).

MCP-1 (monocyte chemoattractant protein-1) one individual from chemokine group, presented by numerous cell kinds and up-controls the infiltration and relocation of monocytes and neutrophils. MCP-1 was rapidly up-regulated by many stimuli for example oxidative stress, inflammation and shear forces, prompting the induction for more mediators of inflammation, especially macrophages with neutrophils, penetrating inside harmed tissues. Several examinations affirmed that MCP-1 played a key part in the pathogenesis of ALI, particularly at the underlying stage (40). Barricade of MCP-1 in a few models of malady has enhance the prognosis of the inflammatory disease, proposing that restraint of MCP-1 is a promising and legitimate system to treat patients with inflammatory disease (41). Patients with higher MCP-1 levels had more regrettable results, as demonstrated by a more extended emergency unit and more prerequisite for mechanical ventilation (42). In the present information, we notice that levels of marker within serum, tissues of lung were showed significant s raising within control mice and vehicle gathering of mice than that seen in sham gathering of mice. MCP-1 cause neutrophil collection and up regulate cytokine/chemokine presentation in the lungs (43) and this prompting for the weakness of lung capacity which revealed as bringing down in partial pressure of oxygen with increasing in the value of partial pressure of carbon dioxide together with the change for histological appearance in tissues of lung (3).

Our examination exhibited that the expression IL-33 is up-regulated or it is steadily presented in CLP and vehicle mice

group as contrasted and sham mice. This finding is likewise proving by (28). Our data propose that IL-33 action is exceptionally up-regulated in serum in light of increase the creation of pro-inflammatory cytokines that happen amid tissue damage as seen by (37). IL-33production outcomes activation numerous of inflammatory cascade which end with neutrophils and another cell penetration in the lung. Besides, fibrinous deposition in the air compartment with alveolar edema which inversely decrease lung function (2).

In this investigation, sepsis unfavorably rises the level of IL-1β in CLP and vehicle mice group as contrasted sham gathering. This outcome is in concurrence with that revealed by (5). Prior examinations have demonstrated that IL-1β is a standout amongst the most biologically dynamic cytokines found in the lung tissues of ALI patients (7). IL-1β has been appeared to cause an elevated in protein penetrability over the lung alveolar capillary barrier (9) repress liquid transport in distal lung epithelium and cause surfactant variations. In this manner, the expansion of IL-1β level may act as mirror to indicate the hidden pulmonary dysfunction since its increment related to lung damage so the watched rise may show that there is alveolar damage because of the induction of sepsis (11). Correspondingly, the consequences of present examination are supporting the past investigations that showed the lifting levels of IL-1β in the blood are correlates to tissues injury, change in wall layers' porousness, and diffuse alveolar septal thickening which saw in CLP and vehicle gatherings of mice (12).

In this investigation, histological examination for the grown-up male mice lung is seen under a light microscope instrument (x40 and x100 amplification). In the sham mice, without sepsis case, structures are unmistakable, bronchioles tissues were clear ordinary, intact blood vessels and typical alveoli. Then again, lungs from CLP and vehicle mice revealed marked damage with the advancement numerous cell penetration in the bronchiole's tissues and alveoli. Furthermore, the lung sections indicated thickened alveolar septa with the invasion of mononuclear cells and disturbed alveolar film, deposition of fibrinous substance in airways passage are marker on edema in alveoli with formation of pneumonitis areas that distorted the normal architecture; aggregate obliteration of lung tissue; and destruction of lung tissue. Comparable finding was accounted for by (29).

In present experiment, sepsis initiated lung damage brings about worsening in lung work within surgical group and vehicle mice in contract to sham gathering showed as noteworthy decrease in partial pressure values for oxygen (pO2) joined by significant increment in partial pressure values for carbon dioxide (pCO2) and in the long run decrease in oxygenation and lung consistence. This observation is in concurrence with investigations of (33). Since the levels of partial pressure for oxygen and carbon dioxide are powerful pointers and clear landmark of respiratory action that is at present use in clinical application (21). So we rely upon its qualities greatly to assess the impact of sepsis on the lungs. Taken together, alveolar wall disturbance by inflammatory mediators, alveolar edema initiated by sepsis and loss of the benignity of alveolar structure as identified by IL-33 over presentation which might contribute factors, taking an interest in debilitated lung consistence which results in pulmonary dysfunction as recognized by an adjustment in partial pressure of blood gases. This recognition consent to the actualities which specify in numerous investigations (44).

In current examination, didox is found to deliver a evident decrease in both serum and lung tissue levels of nominated cytokines and chemokine (IL-1β, IL-33 and MCP-1). Comparative findings are acquired by (21). In opposition to (6) suggested that the levels of chemokine MCP-1 were unaltered by didox treatment. The watched decrease in pro-inflammatory cytokines might be imputed to anti-inflammatory impact of didox likely through hindrance of NF-κB (43). Our numerical information clears up that didox constricts cytokine creation following IL-33 activation in mouse lung. These impacts relate with loss of NF-κB transcriptional action and can be magnified by cellular antioxidant activity and that practically equivalent to that said in (37). Concerning IL-33 expression, this investigation demonstrates that treatment with didox significantly down-managed the level of cytokine presentation in lung cells. This perception is likewise seen by (9). Moreover, in the present investigation our numerical information exhibits that didox administration enhances lung damage as evidenced by significant reduction in seriousness of lung damage in mice got the medication before the initiation of sepsis. This finding is in concurrence with (18). A reasonable clarification is that didox expand cellular antioxidant capacity of lung tissues and reestablishes glutathione status in the tissue (31). At long last, we found the treatment with didox enhances pulmonary capacity as recognized by significant increment in partial pressure of oxygen associated with significant decreased in partial pressure of carbon dioxide. The clarifications of these outcomes are may because of the multifunction work of didox which show up anti-inflammatory and anti-oxidative effects. These observations are predictable with that detailed by (32).

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