Modulatory Effects of Curcumin and Pirfenidone on Hematological, Oxidative, Immunohistochemical evaluation of iNOS in Responses to Potassium Dichromate in Male Wistar Rats.

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Abstract

This study was aimed to evaluate the protective role of curcumin and pirfenidone alone or in combination against potassium dichromate induced toxicity in adult male Wistar rats. Rats (n = 40) were randomly assigned to five groups (n= 8/groups) and treated for 28 days. Animals were administered intranasally potassium dichromate at (2 mg/kg) on days 1, 14 and 28 and orally curcumin (150 mg/kg/day) and pirfenidone (125 mg/kg/day).

Potassium dichromate caused significant hematological changes ($p < 0.05$) with increased WBC count and decreased RBC, hemoglobin and platelet counts. Exposure to $K_2 Cr_2 O_7$ also significantly increased NO levels and markedly up-regulated iNOS immunoreactivity suggesting increased inflammatory and nitrosative responses.

Treatment with curcumin or pirfenidone significantly ameliorated the hematological alterations, NO levels and iNOS expression compared with the potassium dichromate group ($p < 0.05$. Pirfenidone also dropped to reduce the inflammatory and nitrosative responses provoked by potassium dichromate, while curcumin had a more

pronounced effect on the hematological parameters and NO levels.

KEY WORD:

Potassium dichromate**,** Curcumin, Pirfenidone, Hematotoxicity, pulmonary toxicity, Nitric oxide, iNOS.

1. Introduction

The pulmonary system is continuously exposed to the external environment, making it highly vulnerable to airborne pollutants and industrial toxicants. Persistent exposure to these harmful agents can induce inflammation and tissue injury and contribute to the development of chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD) [1].

Among environmental toxicants, potassium dichromate ($K_2 Cr_2 O_7$), a hexavalent chromium compound Cr(VI), is widely recognized for its potent oxidizing properties and severe toxicity, Cr(VI) is highly bioavailable and associated with respiratory disorders, tissue injury, and lung carcinogenesis [2].

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Alterations in oxidative stress biomarkers such as glutathione (GSH) and malondialdehyde (MDA) have been linked to inflammatory and fibrotic pulmonary disorders, including idiopathic pulmonary fibrosis (IPF) and cystic fibrosis [3].

Curcumin, a natural lipophilic polyphenol, has demonstrated potent antioxidant, anti-inflammatory, and anti-apoptotic properties, protecting against pulmonary injury by reducing interstitial edema, improving arterial blood gases (ABG), and suppressing inflammatory responses [4]. Experimental studies have shown that curcumin attenuates cigarette smoke-induced inflammation via modulation of the PPARy/NF- κB pathway, and alleviates emphysematous changes and cytokine overexpression in COPD models [5].

In parallel, antifibrotic agents such as pirfenidone (5-methyl-1-phenyl2[1H]-pyridine) have been widely investigated for their ability to suppress fibroblast proliferation through inhibition of transforming growth factor *B*-l signaling [6].

Therefore, the present study was designed to investigate the modulatory effects of curcumin and pirfenidone alone or in combination on the hematological parameters, NO levels and iNOS expression in male Wistar rats exposed to potassium dichromate.

2. Materials and Methods

2.1 Chemicals and reagents

Potassium dichromate ($K_2 Cr_2 O_7$) was purchased from Loba Chemie (India). Curcumin awas obtained from Solarbio (China) and dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) immediately before administration. Pirfenidone was supplied by Care Formulation Labs (India). Normal saline (0.9% w/v sodium chloride; Al-Mustaqbal Pharmaceutical Co. Ltd., Baghdad, Iraq) was used as the vehicle for intranasal administration, were dissolved in appropriate vehicles, and administration volumes were individually calculated and adjusted according to each animal's body weight to deliver the exact specified dose for each group.

2.2 Animals

40 male Wistar rats, aged (10 to 12) weeks and weighing between (200 and 250) grams, were chosen for this study. All animals were sourced from a facility in animal house College of Veterinary Medicine, Al-Qasim Green University. Animals were kept in ventilated cages in a well-ventilated room with temperatures between 21 and 23 °C at standardized conditions without stress. They were maintained under a 12/12-h light-dark cycle, with free access to humidity and drinking water (tap water), as well as standard rodent chow continuously.

Experiments Design

G1 = Rats received intranasal drops normal saline on days 1, 14, 28 of the experiment + oral vehicle (distilled water) by gavage daily (Days 1–28).

G2 = Rats received intranasal drops $K_2 Cr_2 O_7$ at a dose of 2 mg/kg on days 1, 14, 28.

G3 = Rats received intranasal drops $K_2 Cr_2 O_7$ (2 mg/kg) on days 1, 14, 28 + oral gavage of curcumin (150 mg/kg) daily (Days 1–28).

G4 = Rats received intranasal drops $K_2 Cr_2 O_7$ (2 mg/kg) on days 1, 14, 28 + oral gavage of pirfenidone (125 mg/kg) daily (Days 1–28).

G5= Rats received intranasal drops $K_2 Cr_2 O_7$ (2 mg/kg) on days 1, 14, 28 + oral gavage of curcumin (150 mg/kg) daily (Days 1–28) + oral gavage of

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pirfenidone (125 mg/kg) daily (Days 1–28).

All groups were administered for 28 days. The doses in $K_2 Cr_2 O_7$, Curcumin, and Pirfenidone were decided based on previous studies evaluating their effects in rodents

[7] ,[8] ,[9].

2.3 Animal Sacrifice and Sample Collection :

Twenty-four hours following the last dose, the animals were anesthetized and blood was collected by cardiac puncture. Blood was collected in Gel tubes for serum separation and EDTA tubes for complete blood count (CBC) analysis. Centrifuged (2,000-3,000 rpm for 20 min) and stored at -20°C until biochemical analysis of NO. After blood collection, lung tissues were collected, washed with normal saline and fixed in 10% neutral buffered formalin for histopathological and immunohistochemical examination. All procedures were performed in accordance with the ethical approval given by the Animal Ethics Committee (qgcc/5/2025), College of Veterinary Medicine, Al-Qasim Green University.

2.4 Hematological Analysis: -

Complete Blood Count (CBC) was performed using the Diagon Automated Hematology Analyzer (Diagon, Hungary). All parameters including WBC, RBC, Hemoglobin, and platelets were measured depending to the fixed manufacturer's information.

2.5Immunohistochemical Examination :-

For immunohistochemical analysis,na rabbit polyclonal anti-inducible nitric oxide synthase (iNOS) antibody was obtained from Elabscience (China) Immunohistochemical staining was performed according to the manufacturer's instructions [10].

2.7. Statistical AnalysisResults:-

Statistical analysis was performed using one-way Analysis of Variance (ANOVA) in SPSS (version 22) to determine significant differences among the five experimental groups (G1–G5, n=8 animals per group) for serum biochemical parameter NO). Prior to conducting ANOVA, the underlying assumptions of the test, including data normality and homogeneity of variance, were formally assessed and verified. Subsequently, the Least Significant Difference (LSD) post-hoc test was applied for pairwise comparisons. Differences were considered statistically significant at ($p \leq 0.05$) [11].

3. Results and discussion

3.1 Hematological Parameters Complete Blood Count (CBC)

Statistical analysis revealed that the (G2;6.95 ± 0.20 10^3/µL) group exhibited a highly significant ($P < 0.05$) elevation in WBC count compared with all other experimental groups (G1;4.75 ± 0.25 10^3/µL, G3;5.15 ± 0.37 10^3/µL, G4 ;5.40 ± 0.11 10^3/µL, and G5;5.05 ± 0.43 10^3/µL), which were found non-significant different statistically by more than 0.05 value among each others and recorded the lowest WBC levels. Conversely, for RBC count, HGB levels, and PLT count (G2 ;373.5± 40.7 10^3/µL) group recorded a highly significant less than 0.05 reduction in compare among all other groups. Meanwhile, no significant differences ($P > 0.05$) were detected (G1;537.5 ± 7.21 10^3/µL, G3;523 ± 9.38 10^9/I, G4;515 ± 16.07 10^3/µL, and G5 ;524.7 ± 1.43 10^3/µL) groups, which consistently maintained the highest values for RBC, HGB, and PLT, sharing the

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same statistical letters within each parameter.

Table (1-1): complete blood count (CBC) levels

Groups	WBC $10^3/\mu L$	RBC $10^6/\mu L$	HGB g/Dl	PLT $10^3/\mu L$
G1	4.75±0.25b	5.795±0.02a	13.55±0.02a	537.5±7.21a
G2	6.95±0.20a	4.22±0.50b	11.35±1.21b	373.5±40.7b
G3	5.15±0.37b	5.735±0.14a	13.3±0.40a	523±9.38a
G4	5.40±0.11b	5.425±0.003a	13.55±0.14a	515±16.07a
G5	5.05±0.43b	5.742±0.08a	14.35±0.02a	524.7±1.43a
LSD(P<0.05)	0.906	0.713	1.771	61.14

Means with different letters are significantly different.

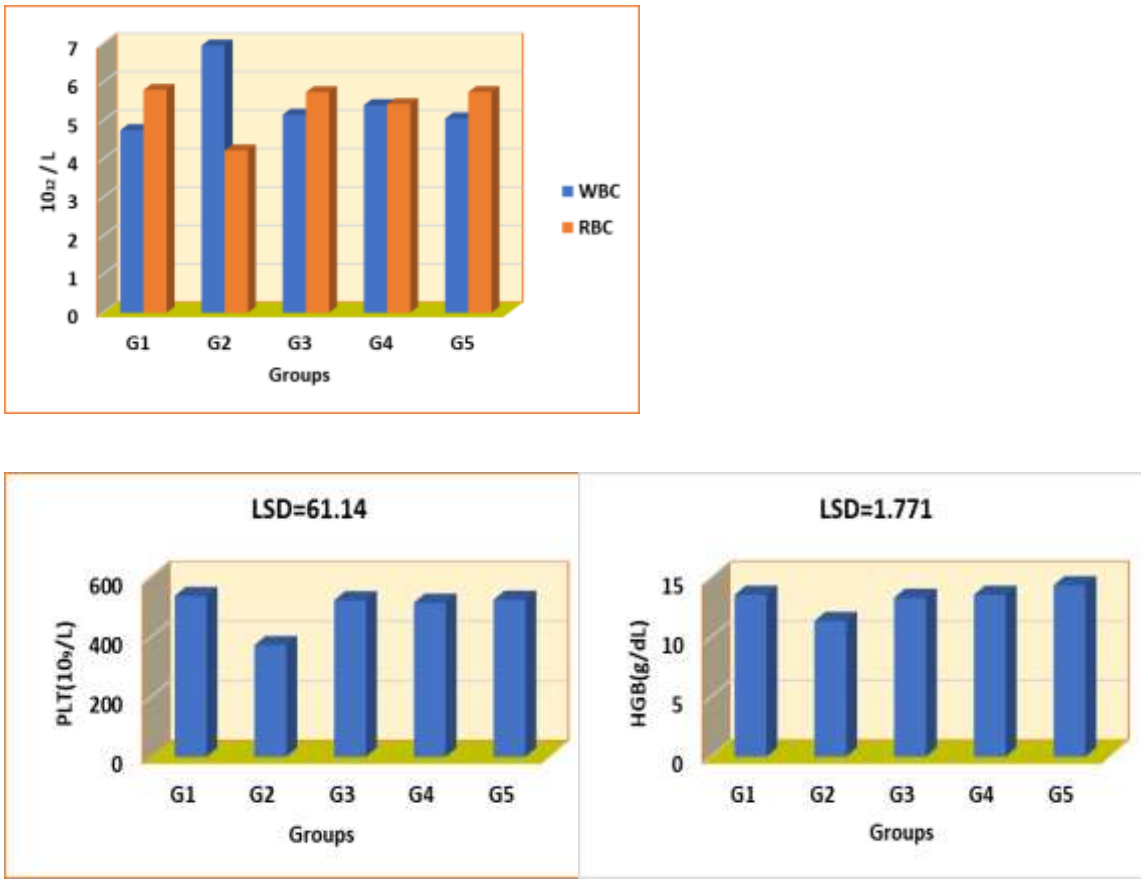


Figure (1.1): Effects of curcumin and pirfenidone on complete blood count (CBC) parameters (WBCs, RBCs, HG, and PLT) in male rats exposed to potassium dichromate for 28 days.

The CBC is a sensitive indicator of systemic toxicity caused by xenobiotic exposure. In the present study, K_2 Cr_2 O_7 exposure produced marked hematological alterations, characterized by increased WBC count and decreased RBC, HGB,

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and PLT counts compared with the control group. These findings indicate the hematotoxic and pro-oxidant effects of hexavalent chromium.

The increased WBC count in G2 may be attributed to the inflammatory response induced by $K_2 Cr_2 O_7$ exposure. Hexavalent chromium can promote excessive ROS generation and inflammatory activation, leading to leukocyte disturbances. Similar hematological alterations following potassium dichromate exposure have been reported previously [12].

The curcumin-treated group (G3) showed a marked improvement in WBC count compared with G2. This finding is consistent with the reported antioxidant and anti-inflammatory effects of curcumin, which may reduce oxidative stress and inflammatory responses induced by toxicant exposure [13]. The improvement in WBC count observed in G3 may therefore reflect the protective effects of curcumin against $K_2 Cr_2 O_7$ -induced hematological injury.

Pirfenidone treatment (G4) also improved the hematological profile, particularly by reducing the elevated WBC count observed in G2. While pirfenidone is essentially an antifibrotic agent approved for idiopathic pulmonary fibrosis (IPF), increasing experimental evidence shows that its effects are mostly attributable to antioxidant and anti-inflammatory properties rather than solely its antifibrotic action. Pirfenidone has been reported to reduce inflammatory signaling, particularly through inhibition of NF-κB pathway activation [14] , an US FDA approved drug that alleviated oxidative stress, inflammatory cytokine release and apoptosis in smoke-inhalation lung injury.

The combined (G5) groups, indicates the anti-inflammatory action of both active components is via inhibition of NF-κB mediated cytokine expression by curcumin and inhibition of pro-inflammatory mediator secretions, including tumor necrosis factor-alpha TNF-α and IL-6, by pirfenidone [15], [16]. The ability of curcumin to modulate neutrophil activity and restore WBCs may be the contribute considerably to the improvement of the inflammatory response observed in the treated [17].

These findings are consistent with the erythrotoxic action of chromium (VI). G2 has the most signature reductions in RBC number and HGB concentration**.** [18] commented on decreased numbers of erythrocytes and hemoglobin levels with increasing potassium dichromate exposure potency as dependent-dose manner. The mechanism is based on the intracellular progeny of probabilistic hexavalent chromium to reactive trivalent and pentavalent intermediates that produce hydroxyl and superoxide radicals.

As observed in similar study, CUR utilized in the present study shows significant improvement of RBC and HGB values being co-administered with G3. Curcumin significantly enhanced the erythrogram similar to where curcumin was able to increase of erythropoiesis and maintain stabilized cell membranes that decreased cellular damage [19],

Showed a very significant increase in RBC and Hb levels via decreased pro-inflame-cytokine release that is also correlated with the inhibitory effect of pirfenidone on TGF-β1 expression. In addition, this cancellation of these pro-inflammatory pathways protects the hemato-poietic stem cells in the BM, from damage caused by inflammation and micro-fibrosis due to chromium toxicity thereby aiding the survival of normal erythropoiesis and preventing functional suppression of marrow red cell production thus precluding anemia of chronic disease through modulation iron homeostasis

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responsiveness to EPO, the potential therapeutic effects of pirfenidone likely depend on the attenuation of inflammatory burden and preservation of bone [20].

Compared with G2, the treatment groups treated with curcumin, pirfenidone and combined regimen showed significant recovery of RBC and HB values toward those of the healthy control group. However, no significant differences were observed among G1, G3, G4, and G5 ($P > 0.05$) The almost sustained revival of RBC and HGB values are decisively due to the rather potent membrane-stabilizing antioxidant effects of curcumin that effectively protects the structural integrity and deformability of erythrocytes against oxidative insult by eliciting nuclear factor erythroid two-related factor two, antioxidant signaling pathway stimulation and inhibition of glycation caused by stress or chromium, lipid peroxidation pertaining to the meltdown of red cell membrane [21]. Because of its natural ability to scavenge free radicals, some additional protection with pirfenidone occurs by reducing NADPH-free radical-induced lipid peroxidation and normalizing systemic glutathione levels [15].

In group G2 ($K_2 Cr_2 O_7$ -exposed), the platelet (PLT) count significantly decreased compared with control (G1), confirming chromium-induced thrombocytopenia. This result is in agreement with the already established role of Cr(VI) as an inhibitor of thrombopoiesis by direct cytotoxicity to megakaryocytes, the platelet progenitor cells found in the bone marrow and through oxidative damage to circulating platelets resulting in accelerated clearance from circulation [22]. On the other hand, all treated groups recovered by a large margin and administration of curcumin (G3) resulted in an improved platelet count returned to similar levels of normal control group. Curcumin directly inhibits the proinflammatory cytokines expressed to inhibit hematopoiesis [23].

Overall, platelet counts in G3, G4, and G5 were significantly higher than in G2 and were comparable to the control group ($P > 0.05$). These findings indicate that curcumin and pirfenidone attenuated the platelet alterations induced by $K_2 Cr_2 O_7$.

In all treated groups, the platelet counts were restored significantly when compared to G2 with values close to that of the healthy control ($p > 0.05$ vs. G1). Curcumin indirectly protects the function of megakaryocytes and thrombopoiesis by improving the systemic oxidative and inflammatory milieu induced by Cr(VI). Through similar mechanisms, pirfenidone may enhance platelet recovery. Pirfenidone acts through its anti-inflammatory properties, which may reduce the consumption of platelets brought about by cytokines and suppression from the bone marrow [24]. In the combined treatment group, showed values of RBC, HGB, PLT, and WBC that approached those observed in the healthy control group, consistent with the potential protective effects of the combined treatment [25]. The equalizing effect of the recovery that was seen in G3, G4 and G5 groups hints at both agents therapeutic significance as hepatoprotective adjuncts in chromium-associated toxicity models. Together, the current CBC findings provide evidence that curcumin and pirfenidone antagonize $K_2 Cr_2 O_7$ -induced exposure toxicity (haemotoxicity), potentially offering therapeutic advantages in oxidative-stress-mediated hematological disorders.

Nitric Oxide (NO): - µmol/L

Results of nitric oxide (NO) levels were clarified in figure (1.2), Table (1-2), analysis statistically found high levels

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significantly under 0.05 elevation in (G2; 6.14±0.31 µmol/L) group compared with all other experimental groups. While, G4 group showed a moderate significant increase compared with (G1; 2.64±0.33 µmol/L, G3; 3.65±0.30 µmol/L, and G5; 2.02±0.25 µmol/L) groups. On the other hand, G3 group exhibited intermediate values that were significantly higher than G1 and G5 but lower than G2 and (G4; 4.25± 0.47 µmol/L). Both G1 and G5 groups recorded the lowest NO levels with non-significant differences between them.

Table (1.2): Serum nitric oxide levels (mol/L)

Groups	NO (µmol/L)
G1	2.64±0.33d
G2	6.14±0.31a
G3	3.65±0.30c
G4	4.25±0.47b
G5	2.02±0.25d
LSD(P<0.05)	1.009

Means with similar letters are non-significantly different.

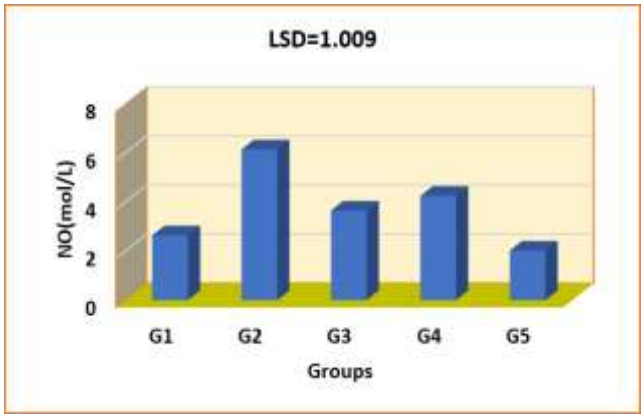


Figure (1.2): Effects of curcumin and pirfenidone on serum nitric oxide (NO) levels of male rats exposed to potassium dichromate for 28 days

Small varied letters refer to present significant statistical different among groups under probability value less than 0.05.

Nitric oxide (NO) is an important signaling molecule produced from L-arginine by nitric oxide synthase (NOS). isoforms, with constitutively (eNOS) and (nNOS) expressed under normal conditions, provide basal NO levels for the maintenance of vascular tone, bronchodilation, immune regulation whilst iNOS only being osmotically active when intricated causing sustained NO overproduction leading to nitrosative tissue damage under pathological situations [26].

The results of the current study for the negative control group G1 (control data which consisted of untreated and unstressed rats). In particular, G5 had the lowest observed NO level and non-statistical significant difference from G1 (LSD; P more than 0.05) , LPS for (G5; P

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> 0.05) showed an equivalent but non-significantly lower level of NO than the other groups after treatment was completed with G4 and G3 also showing trends towards reduced levels.

In this regard, pulmonary NO was found to be significantly higher in the stressed untreated group (G2) following potassium dichromate exposure compare to all other experimental treatment groups (LSD, P less than 0.05). This sizeable increase is mechanistically due to the reduced species of hexavalent chromium.

. Excess NO may react with superoxide to form peroxynitrite, contributing to oxidative and nitrosative tissue damage. [27] Curcumin treatment (G3) significantly reduced NO levels compared with G2, although the values remained higher than the control group. This reduction may be related to the anti-inflammatory and antioxidant effects of curcumin, including inhibition of inflammatory signaling and iNOS-associated NO production [28]
Pirfenidone treatment (G4) significantly reduced NO levels compared with G2, although the reduction was less pronounced than that observed with curcumin. This effect may be related to the anti-inflammatory and antioxidant properties of pirfenidone, including modulation of NF-κB signaling and reduction of iNOS-associated NO production [29]**.**

A simple and important comparative observation derived from these data is that curcumin significantly outperforms pirfenidone in lowering dichromate-induced serum NO elevation; importantly, we propose that combination may result through multiple mechanisms working together. Balance between NO/ROS are critical to homeostasis in the vascular system and vital during inflammation and immune responses. The curcumin found to decrease nitric oxide synthase enzyme activity and regulate surfactant expression in lung tissues [30]. Exposure to oxidative stress as indicated by increased ROS species, nitric oxide (NO), levels of malondialdehyde; and low in (GSH) glutathione and glutathione peroxidase (GPx) activities [31].

3.3 Immunohistochemical Examination: -

The immunohistochemistry findings of rat lungs in group G1 indicated low expression of iNOS, with weak immunoreactivity dispersed allover the pulmonary tissue as depicted in (Figures 1.3- A). However, group G2 showed strong positive immunostaining with extensive expression of iNOS in bronchiolar epithelial lining, alveolar walls and inflammatory cells (Figures 1.3- B, C). The G3 sections exhibited moderate staining (iNOS limited to alveolar walls and perivascular areas) (Figure 1.3- D). In group G4, the lung demonstrated diffused strong immunopositivity, which means iNOS was widely distributed in pulmonary structures (a), figure 1.3- E). There was also slight variation in 3 of the lesions from group G5, which showed low iNOS (Figure 1.3- F).

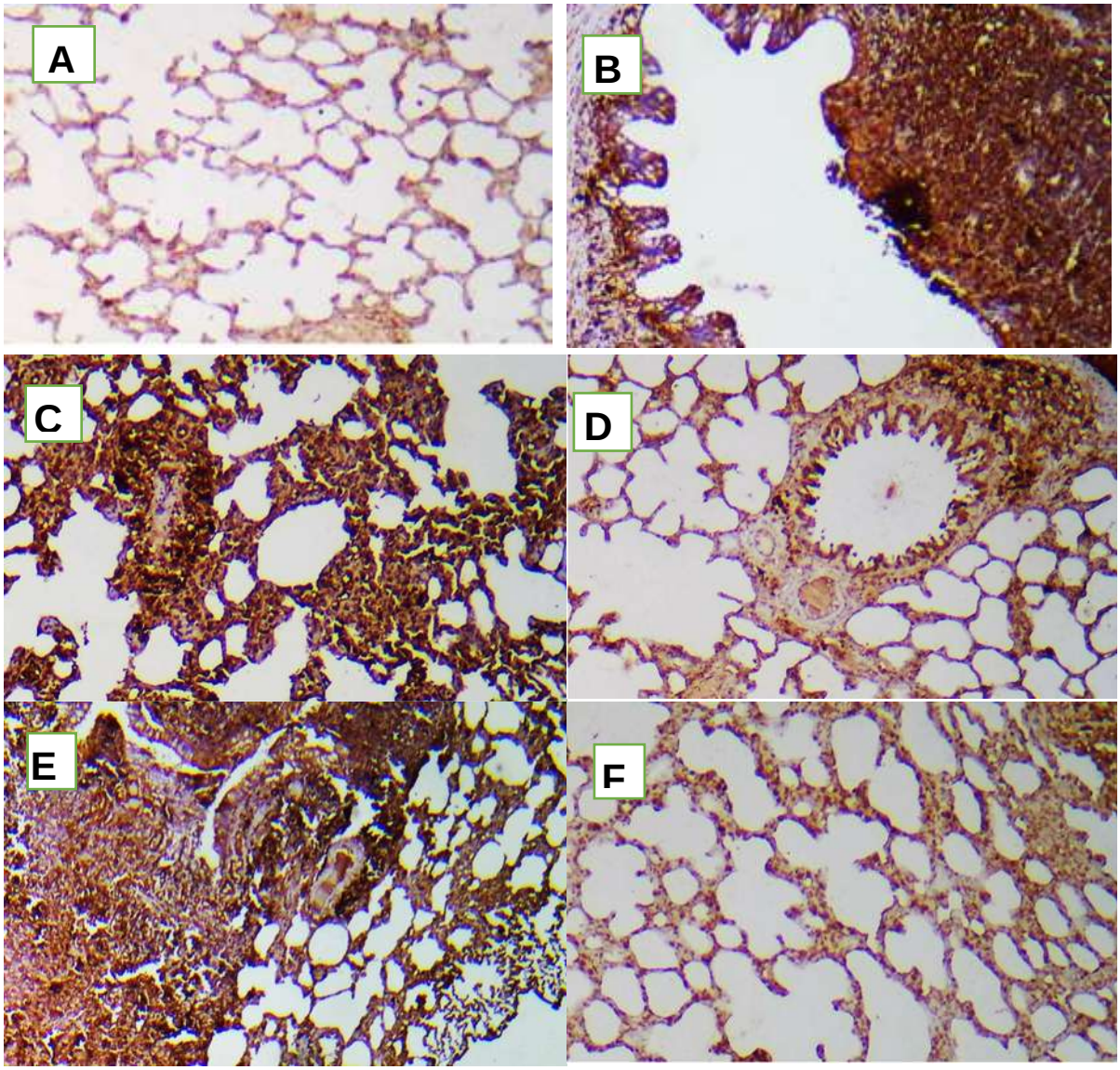


Figure (1.3): A/ Immunohistochemical of paraffin embedded rat lung section for (G1) showing low expression of iNOS polyclonal antibody (DAB chromogen and Hematoxylin stains,10X). B/ Immunohistochemical of paraffin embedded rat lung section for G2 showing highly expression of iNOS polyclonal antibody in the epithelial cell lining the bronchioles and inflammatory cells (DAB chromogen and Hematoxylin stains,10X). C/ Immunohistochemical of paraffin embedded rat lung section for G2 showing highly expression of iNOS polyclonal antibody in the alveolar wall and around.b.v (DAB chromogen and Hematoxylin stains,10X). D/ Immunohistochemical of paraffin embedded rat lung section for G3 showing moderate expression of iNOS polyclonal antibody in the alveolar wall and around.b.v. (DAB chromogen and Hematoxylin stains,10X). E/ Immunohistochemical of paraffin embedded rat lung section for G4 showing highly expression of iNOS polyclonal antibody in the lung tissue (DAB chromogen and Hematoxylin stains,10X). F/Immunohistochemical of paraffin embedded rat lung section for G5 showing low expression of iNOS polyclonal antibody in the lung tissue (DAB chromogen and Hematoxylin stains,10X).

NO generated by the inducible isoform of iNOS has both positive and negative effects [32] and is, in fact, endowed with antimicrobial and antitumor properties;

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however, an excess NO appears to be strongly implicated in the sustainment of chronic inflammation and can generate tissue damage promoting reactive nitrogen species (RNOS), such as peroxynitrites [26].

Immunohistochemical profiling indicated a upregulation of (iNOS) immunoreactivity in lung tissues after potassium dichromate $K_2 Cr_2 O_7$ exposure, and the expression level was differentially modulated by curcumin or pirfenidone or their combination. In the healthy control group (G1), iNOS expression showed a weak baseline pattern indicative of normal unprovoked pulmonary parenchyma with this sparse signal limited exclusively to isolated alveolar macrophages and a thin continuous rim along the bronchiolar epithelium figure (1.3-A).

In contrast, hexavalent chromium in toxification of group G2 resulted in a fulminant, tissue-wide blast of immunopositively iNOS use drastic diffuse brown diaminobenzidine (DAB) signals far beyond their localized compartments and highly penetrating the alveolar walls astoundingly expanding into the perivascular connective tissue filling the bronchiolar epithelial linings and surrounded inflammatory cell populations figure (1.3-B). Pulmonary exposure to hexa-valent chromium (Cr VI) triggers intracellular redox cycling between Cr (VI) and Cr (III), yields to excessive created of (ROS) and rapid depletion of glutathione. This oxidative imbalance promotes inflammatory infiltration and the upregulation of iNOS and angiogenic markers, thereby aggravating the severity of lung tissue lesions [33].

The curcumin-treated group (G3) easily demonstrated mitigation of iNOS immunostaining, and the remaining signals were localized almost exclusively to the alveolar walls and a few perivascular areas figure (1.3-D). The nuclear translocation of the transcription factor is inhibited, and with it there are downstream pro-inflammatory targets including (iNOS and COX-2) that will be repressed via the parallel activation of the cytoprotective Nrf2/HO-1 pathway [34]**.** Comparable anti-iNOS efficacy and consequent reductions in alveolar wall thickening have been reported with a variety of acute lung injury models.

Interestingly, during these studies, the pathway which was most upregulated across treatment groups was that of the Inducible Nitric Oxide Synthase (iNOS) immunoreactivity through the lung parenchyma figure (1.3-E). Considering the narrow experimental window of the current study, it is likely that the immediate therapeutic effect of pirfenidone was suffocated by its direct TGF-β1 antifibrotic axis, so that a time separation existed where its antifibrotic effects outstepped its ability to dampened iNOS-mediated inflammation [16].

Perhaps most importantly, G5 yielded the greatest effect with iNOS immunoreactivity almost completely erased following combined treatment. Staining patterns reverted to baseline values, resembling the architecture of healthy controls (G1), with only faint, isolated signals evident in rare macrophages.

This morphological rescue is consistent with extensive pharmacological literature documenting curcumin as a privileged Nrf2 agonist that preserves the oxidant reserve of the tissue following acute, large-scale xenobiotic challenge, thereby allowing for a more favorable baseline setting whereby co-administered therapeutic agents such as pirfenidone [35]**.**

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The immunohistochemical distribution of iNOS across the cohorts follows a relatively clear, tightly constrained trajectory: little in G1, maximal with diffuse pattern to G2 and optimal normalization to G5 in tackling this complex nitrosative injury. These dynamics confirm iNOS to be a highly sensitive biomarker of chromium-induced respiratory injury.

References :-

[1] L. Zuo *et al.*, "Interrelated role of cigarette smoking, oxidative stress, and immune response in COPD and corresponding treatments," *Am. J. Physiol. Cell. Mol. Physiol.*, 2014.

[2] M. A. Hamza, M. S. Hassan, R. F. Mousa, and M. S. Majed, "Evaluation of the antioxidant properties of ginseng green nanoparticles in protecting male rats against potassium dichromate toxicity," *J. Kerbala Agric. Sci.*, vol. 11, no. 2, pp. 42–51, 2024.

[3] M. Zhang *et al.*, "Curcumin ameliorates alveolar epithelial injury in a rat model of chronic obstructive pulmonary disease," *Life Sci.*, vol. 164, pp. 1–8, 2016.

[4] R. Zhong *et al.*, "Physalin B ameliorates inflammatory responses in lipopolysaccharide-induced acute lung injury mice by inhibiting NF-κB and NLRP3 via the activation of the PI3K/Akt pathway," *J. Ethnopharmacol.*, vol. 284, p. 114777, 2022.

[5] Q. Li, J. Sun, N. Mohammadtursun, J. Wu, J. Dong, and L. Li, "Curcumin inhibits cigarette smoke-induced inflammation via modulating the PPARγ-NF-κB signaling pathway," *Food Funct.*, vol. 10, no. 12, pp. 7983–7994, 2019.

[6] S. M. Ruwanpura, B. J. Thomas, and P. G. Bardin, "Pirfenidone: molecular mechanisms and potential clinical applications in lung disease," *Am. J. Respir. Cell Mol. Biol.*, vol. 62, no. 4, pp. 413–422, 2020.

[7] S. Z. Ghasemi, A. Memarzia, S. Behrouz, Z. Gholamnezhad, and M. H. Boskabady, "Comparative effects of Curcuma longa and curcumin on paraquat-induced systemic and lung oxidative stress and inflammation in rats," *Avicenna J. Phytomedicine*, vol. 12, no. 4, p. 414, 2022.

[8] S. S. Paik *et al.*, "Pirfenidone alleviates inflammation and fibrosis of acute respiratory distress syndrome by modulating the transforming growth factor-β/smad signaling pathway," *Int. J. Mol. Sci.*, vol. 25, no. 15, p. 8014, 2024.

[9] A. Salama, H. M. Fayed, and R. Elgohary, "L-carnitine alleviated acute lung injuries induced by potassium dichromate in rats: involvement of Nrf2/HO-1 signaling pathway," *Heliyon*, vol. 7, no. 6, 2021.

[10] J. A. Ramos-Vara and Ma. Miller, "When tissue antigens and antibodies get along: revisiting the technical aspects of immunohistochemistry—the red, brown, and blue technique," *Vet. Pathol.*, vol. 51, no. 1, pp. 42–87, 2014.

[11] B. Baarda and C. van Dijkum,

ISSN 2072-3857

Introduction to Statistics with SPSS. Routledge, 2019.

[12] S. A. Ejoh, F. N. Iheagwam, and A. O. Olusola, "Potassium dichromate-induced hepato-and hematotoxicity in rats: Nutritive composition and ameliorative role of Acacia nilotica L. leaf," 2021.

[13] M. A. Emam, S. M. Farouk, A. Aljazzar, A. A. Abdelhameed, A. A. Eldeeb, and F. A. Gad, "Curcumin and cinnamon mitigates lead acetate-induced oxidative damage in the spleen of rats," *Front. Pharmacol.*, vol. 13, p. 1072760, 2023.

[14] T. Lv, K. Yang, and J. Wang, "Pirfenidone alleviates smoke inhalation lung injury of rats via the NF- κB signaling pathway," *Immunity, Inflamm. Dis.*, vol. 12, no. 11, p. e70014, 2024.

[15] A. G. Fois *et al.*, "Effects of pirfenidone and nintedanib on markers of systemic oxidative stress and inflammation in patients with idiopathic pulmonary fibrosis: a preliminary report," *Antioxidants*, vol. 9, no. 11, p. 1064, 2020.

[16] A. Torre, F. D. Martínez- Sánchez, S. M. Narvaez- Chávez, M. A. Herrera- Islas, C. A. Aguilar- Salinas, and J. Córdova- Gallardo, "Pirfenidone use in fibrotic diseases: What do we know so far?," *Immunity, Inflamm. Dis.*, vol. 12, no. 7, p. e1335, 2024.

[17] D. Banji, J. Pinnapureddy, O. J. F. Banji, A. R. Kumar, and K. N. Reddy, "Evaluation of the concomitant use of methotrexate and curcumin on Freund′ s complete adjuvant-induced arthritis and hematological indices in rats," *Indian J. Pharmacol.*, vol. 43, no. 5, pp. 546–550, 2011.

[18] C. M. Mary Momo *et al.*, "Oxidative effects of potassium dichromate on biochemical, hematological characteristics, and hormonal levels in rabbit doe (Oryctolagus cuniculus)," *Vet. Sci.*, vol. 6, no. 1, p. 30, 2019.

[19] Z. K. El-Maddawy and Y. S. El-Sayed, "Comparative analysis of the protective effects of curcumin and N-acetyl cysteine against paracetamol-induced hepatic, renal, and testicular toxicity in Wistar rats," *Environ. Sci. Pollut. Res.*, vol. 25, no. 4, pp. 3468–3479, 2018.

[20] C. J. Schaefer, D. W. Ruhrmund, L. Pan, S. D. Seiwert, and K. Kossen, "Antifibrotic activities of pirfenidone in animal models," *Eur. Respir. Rev.*, vol. 20, no. 120, pp. 85–97, 2011.

[21] Y. M. Abd-Elhakim *et al.*, "Protective effect of curcumin against sodium salicylate-induced oxidative kidney damage, nuclear factor-kappa dysregulation, and apoptotic consequences in rats," *Antioxidants*, vol. 10, no. 6, p. 826, 2021.

[22] K. P. Nickens, S. R. Patierno, and S. Ceryak, "Chromium genotoxicity: a double-edged sword," *Chem. Biol. Interact.*, vol. 188, no. 2, pp. 276–288, 2010.

[23] X. Dong *et al.*, "Emodin: a review of its pharmacology, toxicity and pharmacokinetics," *Phyther. Res.*, vol. 30, no. 8, pp. 1207–1218, 2016.

[24] T. E. King Jr *et al.*, "A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis," *N. Engl. J. Med.*, vol. 370, no. 22, pp.

ISSN 2072-3857

2083–2092, 2014.

[25] A. Amirkhosravi *et al.*, "Combination of losartan with pirfenidone: a protective anti-fibrotic against pulmonary fibrosis induced by bleomycin in rats," *Sci. Rep.*, vol. 14, no. 1, p. 8729, 2024.

[26] C. Bogdan, "Nitric oxide synthase in innate and adaptive immunity: an update," *Trends Immunol.*, vol. 36, no. 3, pp. 161–178, 2015.

[27] R. Chakraborty *et al.*, "Mechanism of chromium-induced toxicity in lungs, liver, and kidney and their ameliorative agents," *Biomed. Pharmacother.*, vol. 151, p. 113119, 2022.

[28] X. Yang *et al.*, "Curcumin-mediated enhancement of lung barrier function in rats with high-altitude-associated acute lung injury via inhibition of inflammatory response," *Respir. Res.*, vol. 25, no. 1, p. 354, 2024.

[29] H. Nakanishi *et al.*, "Pirfenidone inhibits the induction of iNOS stimulated by interleukin-1β at a step of NF-κB DNA binding in hepatocytes," *J. Hepatol.*, vol. 41, no. 5, pp. 730–736, 2004.

[30] M. M. Gouda and Y. P. Bhandary, "Acute lung injury: IL-17A-mediated inflammatory pathway and its regulation by curcumin," *Inflammation*, vol. 42, no. 4, pp. 1160–1169, 2019.

[31] D. Sharma, V. Soni, S. Singh, P. K. Chauhan, A. K. Singh, and R. Singh, "Protective effects of Curcumin on Short and Long-term Particulate matter (PM10/2.5) induced lung damage severity via modulation of Neutrophil Extracellular traps (NETs) Formation in mouse model," *Tissue Cell*, p. 103575, 2026.

[32] Q. Xue, Y. Yan, R. Zhang, and H. Xiong, "Regulation of iNOS on immune cells and its role in diseases," *Int. J. Mol. Sci.*, vol. 19, no. 12, p. 3805, 2018.

[33] C. Long *et al.*, "Potential mechanisms of lung injury and repair after hexavalent chromium [Cr (VI)] aerosol whole-body dynamic exposure," *Chemosphere*, vol. 349, p. 140918, 2024.

[34] M. K. Lee, H. D. Kim, S. H. Lee, and J. H. Lee, "Curcumin ameliorates particulate matter-induced pulmonary injury through bimodal regulation of macrophage inflammation via NF-κB and Nrf2," *Int. J. Mol. Sci.*, vol. 24, no. 3, p. 1858, 2023.

[35] V. Singh *et al.*, "Hexavalent-chromium-induced oxidative stress and the protective role of antioxidants against cellular toxicity," *Antioxidants*, vol. 11, no. 12, p. 2375, 2022.

ISSN 2072-3857