

# The Histological effects of Methotrexate on the lungs of albino rats: An experimental study

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## **ABSTRACT**

**Introduction:** *Despite its potentially pulmonary complications, Methotrexate (MTX) is widely utilized in the treatment of arthritis. Several studies reported development of various forms of lung disease in patients treated with MTX. The aim of this study is to investigate the adverse effects of MTX on the lung structure and the likelihood of recovery following MTX withdrawal.*

**Materials and Methods:** *A total of 30 albino rats were studied in 3 different groups of ten rats. Group I was considered as control whereas group II (MTX treated group), received 5 mg/ kg subcutaneous MTX (SC) for 5 days. Lastly, group III (withdrawal group) received similar doses of MTX for the same duration but sacrificed 5 days after MTX withdrawal for lung dissection and histological examination.*

**Results:** *The lungs of MTX treated rats demonstrated a significant increase in the inter-alveolar thickness with concomitant mononuclear cell infiltration linked with alveolar collapse and damage. After MTX withdrawal, the changes in pulmonary structure exhibited partial recovery. This in turns suggests that MTX-induced lung toxicity was to some extent reversible.*

**Conclusion:** *MTX could result in pulmonary inflammation and alveoli damages, therefore, this study recommends regular examinations for MTX patients designed for early detection of interstitial pneumonitis.*

**Key Words:** *methotrexate, lung, interstitial pneumonia, rat.*

## **INTRODUCTION**

Methotrexate (MTX) is an anti proliferative chemotherapeutic that interrupts folate metabolism by inhibition of dihydrofolate reductase. It is one of the most widely used broad spectrum immunomodulatory drugs in the medical management of arthritis and

other rheumatic conditions(1). Initially, it was utilized as an antineoplastic drug in the treatment of acute lymphoblastic leukemia, lymphoma, breast cancer and several solid organ tumors(2). Generally, low doses of MTX is used in patients with rheumatoid arthritis or psoriasis while high doses are reserved for cancer patients as chemotherapy, nevertheless, its use was limited due to various side effects(3). Pulmonary toxicity has been reported as one of the serious consequences of MTX treatment occurring in more than 5% of treated patients(4). These effects were first detected in 1969 during the treatment of leukaemia and later in malignancy and psoriasis(5). Zisman *et al.* observed acute pulmonary toxicity at any time during MTX even with low doses which can progress rapidly into a potentially fatal condition(3).

Recently, there has been an increasing number of studies reporting MTX-induced lung pathologies including infectious and noninfectious diseases, especially with low doses used in the treatment of rheumatic and non rheumatic conditions(3). On that basis, the aim of this experimental study was to investigate the histological changes of MTX treatment on the lung tissue and the likelihood of pulmonary healing after its withdrawal in vivo.

## MATERIALS AND METHODS

A total of 30 adult male albino rats weighing from 200 to 220gm were enrolled. All animals were accommodated under similar environmental conditions in clean, well-ventilated cages with free access to water and nutrition. A period of one week was planned prior to intervention to allow for specimens adaptation to the laboratory conditions. The eligible sample was categorized into three main groups of ten animals:

**Group I** was considered as the control group which was further divided into n=5 cases treated with 1ml of distilled water SC once per day for 5 days whereas the remainder (n=5) were injected with distilled water similar to the first subgroup but were deprived of treatment for another 5 days to assess withdrawal.

**Group II** were administered MTX at a dose of 5 mg/kg SC once daily for 5 days. The doses of MTX in our experiment were focused in the previous studies(6).

**Group III** received the same dose of MTX as the second group but the treatment was paused for additional 5 days for recovery. Five days reversibility period was considered sufficient for MTX clearance from the rodent's blood stream(7).

Animals of all groups were sacrificed with thoracotomy followed by lungs dissection. All specimens were treated with 10% neutral-buffered formalin for 24 hours and the final blocks were cut into 5 µm sections, stained with hematoxylin and eosin (H&E) and examined under light microscope.

These sections were scored from 0 to 4 as minimal/ negligible, mild, moderate, or severe lung damage according to the presence or absence of alveolar congestion, bleeding, inflammation or infiltration of neutrophils and lymphocytes in the alveoli or vascular walls(8).

Data analysis was carried out using SPSS software with the analysis of variance (ANOVA) performed on tissue damage scores to evaluate the difference between the three groups in addition to U test. P-value less than 0.05 was considered as significant and the effect measures were expressed as mean $\pm$ SD.

## RESULTS

The congestion scores (CS) and inflammation scores (IS) of the three groups were evaluated and displayed in Table 1

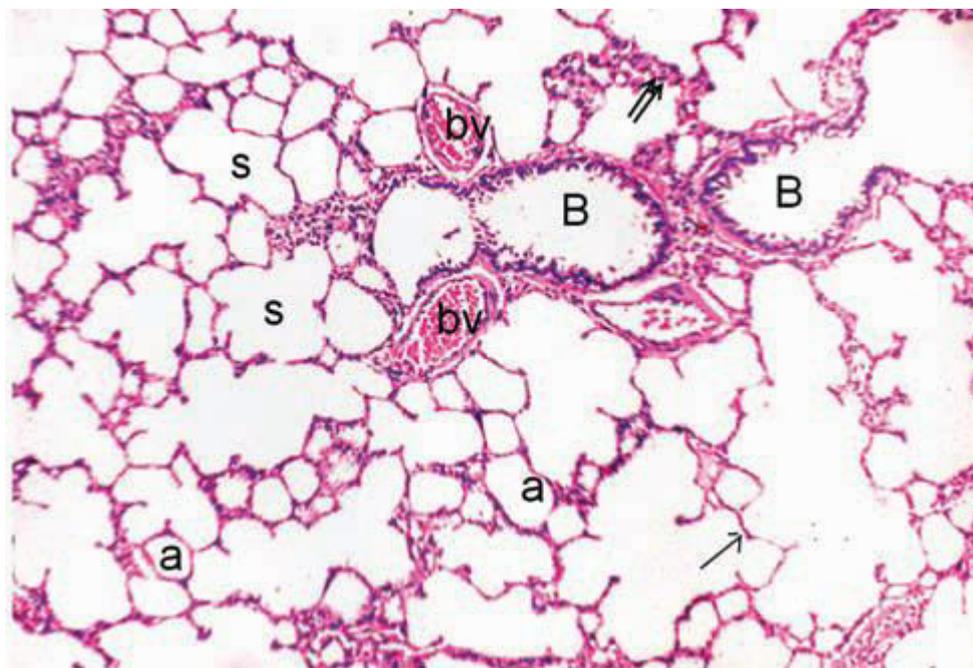
CS and IS were significantly higher in group II & III ( $p < 0.05$ ) (Figure 1,2,3). Additionally, CS in MTX group was significantly higher than those in withdrawal groups ( $p < 0.05$ ) (Figure, 2-B,3).

With regards to IS, all groups exhibited an inflammatory cell infiltration (Figure 2-A, 3) with a higher tendency for IS detected in group II

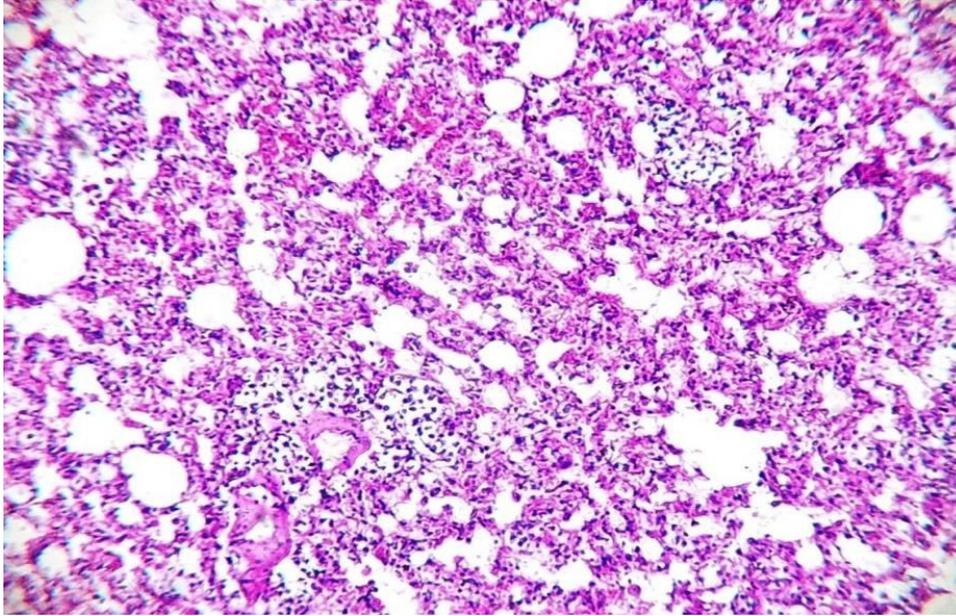
**Table 1.** pulmonary tissue damage scores in all study groups

	Group I		Group II	Group III
	Control	withdrawal	MTX	Withdrawa
Congestion Score (CS)	0.03 $\pm$ 0.60*	0.02 $\pm$ 0.01	3.98 $\pm$ 0.65 $\dagger$ , $\ddagger$	1.50 $\pm$ 0.48
Inflammation Score (IS)	0.01 $\pm$ 0.01	0.08 $\pm$ 0.02	3.83 $\pm$ 0.75	2.45 $\pm$ 1.45

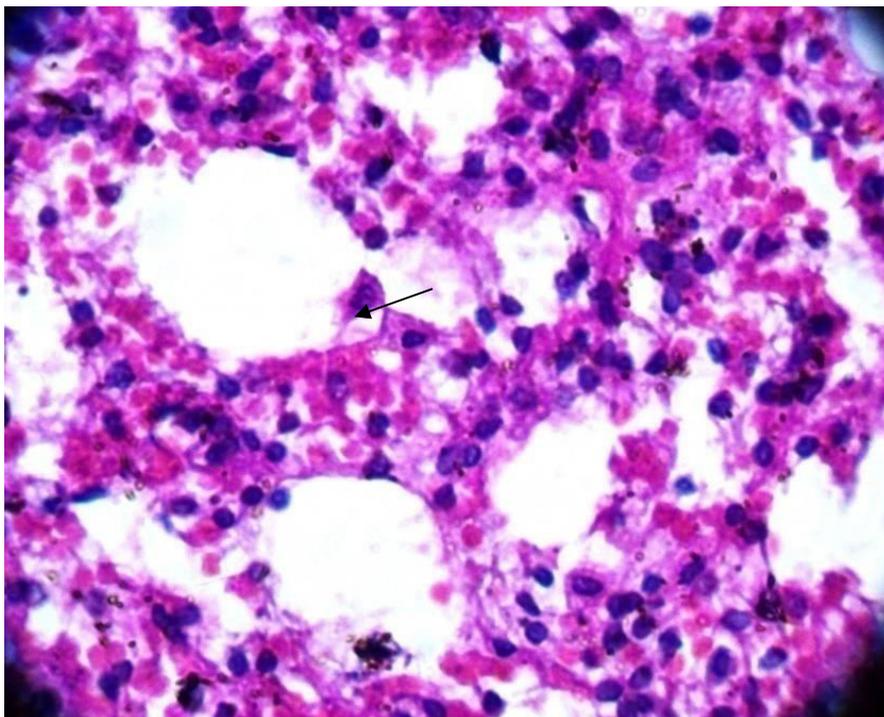
\*Data are presented as mean $\pm$



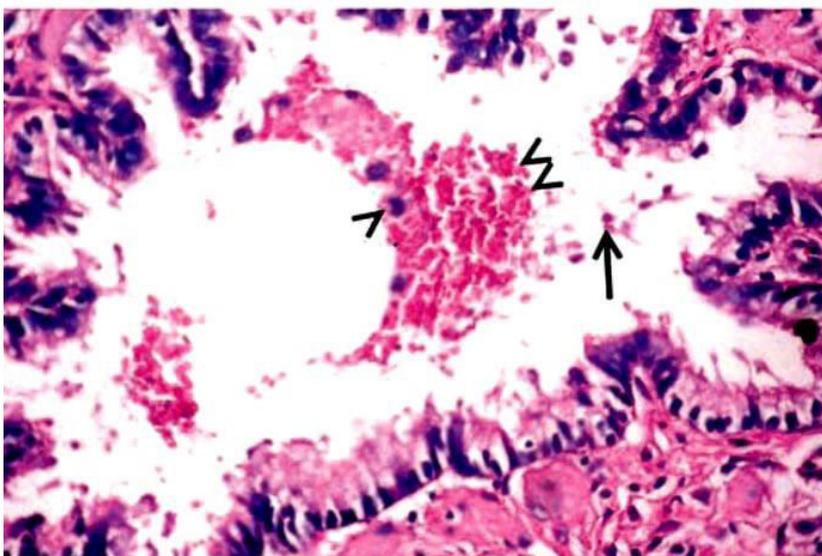
**Fig (1):** H&E(X100) section demonstrating a control rat lung (group I) with normal architecture including alveoli (a), alveolar sacs (s), bronchioles (B), and blood vessels (bv), thin $\uparrow$  and thick $\uparrow\uparrow$  interalveolar septa.



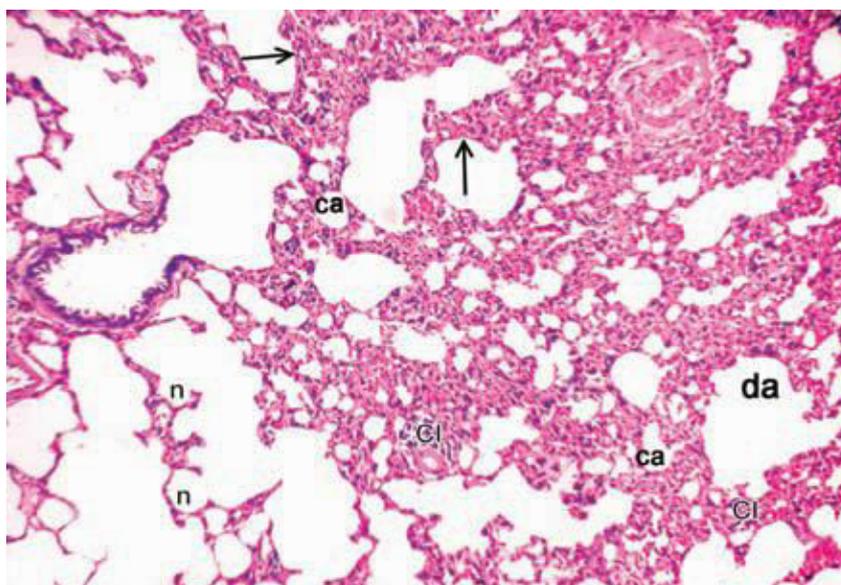
**Fig 2-A :** H&E(X100) section of group II (MTX treated) lungs displaying a marked thickening of interalveolar septa along with intercellular inflammatory infiltration.



**Fig 2-B:** H&E(X400) section showing congested blood capillaries with extravasation of red blood cells within alveolar lumen.



**Fig 2 –C:** H&E(X400) section displaying RBCs (w) and alveolar macrophages, hemosiderin laden macrophages (>) as intrabronchial cellular debris.



**Fig 3 :** H&E(X100) section of Group III Withdrawal group) lung demonstrating foci of collapsed alveolar tissue (ca) and compensatory dilatation of adjacent alveoli (da) with the intervening thickened septa, normal alveoli (n) and moderate intercellular infiltration (CI).

## DISCUSSION.

Methotrexate as an anti neoplastic drug has been used increasingly to treat chronic inflammatory disease and malignancies(1). Given its high efficacy, low-dose MTX is now first-line therapy for the treatment of rheumatoid arthritis, and has been well tolerated, although toxicities were encountered. Jakubovic *et al.* reported lung toxicity in 2-7% of patients receiving low-dose MTX therapy(4). Our study demonstrated that early and low dose of MTX can result in a histopathological injury to the pulmonary tissue presented as disorganized of alveolar architecture, marked thickening of the

inter-alveolar septa with mononuclear cellular infiltration and increased congestion in multiple areas in the lung tissue. These changes were suggested by Fragoulis *et al.* to indicate interstitial pneumonia(9).

Additionally, Jakubovic *et al.* in 2013, reported hypersensitivity pneumonitis as the most common pulmonary toxicity accompanying MTX therapy(4). These effects were explained by experimental models suggesting that oxidative stress constitute a vital role in MTX-induced pulmonary destruction(6). Moreover, our findings of cellular infiltration and vascular congestion can be attributed to disruption of the endothelial barrier affecting the vessel integrity and increasing its permeability inducing an inflammatory response through activation of oxidative stress sensitive signaling pathways(10). In the meantime, the toxic effects of MTX on the bronchiolar lining were directly related to with the intrabronchial cell debris(11). The results from our study were correspondent with earlier researches studying drug-induced pulmonary disease(9,12). In fact, in 2018, Thanian *et al.* reported a human case of MTX-related lung condition in which the hisopathological section demonstrated elements of interstitial pneumonitis with mononuclear cell infiltration, bronchiolitis and granuloma formation(13). Other pulmonary toxicities were also confirmed including acute lung injury and fibrosis presenting in 1% of the patients and necessitates termination of the drug(12).

The pathophysiology of MTX-induced pneumonitis remains unclear. One could argue that these reactions were related to folate deficiency resulting in cellular toxicity. However, Kim *et al.* (2009) suggested that damaged pulmonary cells release an increased levels of inflammatory cytokines triggering hypersensitivity and autoimmune reactions. Furthermore, the extravasation of red blood cells can induce inflammation by attracting mononuclear cells to the site of congestion. These theories were additionally supported by our findings.

Finally, our study suggested a partial recovery of the histological injuries of lung tissue after MTX withdrawal. This finding could be associated with the precipitation of this medication in the renal tissues resulting in nephrotoxicity and in worst scenarios could lead into renal failure. This could ultimately delay the drug clearance resulting in residual circulatory levels of MTX with subsequent rebound injuries(14).

## Conclusion

This study confirms that administration MTX could induce interstitial pneumonia and alveolar damage even if used in low dose and its withdrawal causes partial recovery. Therefore, regular examinations for patients treated with MTX should be carried out to allow early detection of interstitial pneumonitis. Furthermore, more studies should be designed to investigate and introduce protective agents against MTX- related pulmonary injury.

Ethics: This study meets the appropriate ethical standards of the responsible committee

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