

Dual effect of curcumin and methotrexate treatment on various organs in collagen-induced arthritis in rats

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Abstract: Objective: To evaluate the effect of methotrexate and curcumin administration on various internal organs in collagen-induced arthritis in rats. **Material and methods:** Animals were divided into 6 groups, 10 rats each. Group I: Normal control, Group II was injected with DMSO, Group III arthritic rats, Group IV arthritic rats received methotrexate (MTX) (1 mg/ Kg, i.p) once a week and Group V arthritic rats treated with curcumin (100 mg/ Kg, i.p) thrice a week. Group VI was treated with MTX (1 mg/ Kg, i.p) once a week and after 30 min received curcumin (100 mg/ Kg, i.p) thrice a week. Rats were sacrificed and thymus, spleen, liver, lung and kidney were collected for histopathological evaluation. **Results:** General improvement in examined organs from arthritic rats treated with both MTX and curcumin as compared with other groups. The combination of both MTX and curcumin restored the normal structure of the examined organs. **Conclusion:** The study showed an effective anti-arthritis action with the combination of MTX and curcumin.

[Elshaimaa M.Ibrahim, Abeer M. badr, Hiba Sibaii, Amany S. E.El-Wakkad, Somaya El-Deeb. **Dual effect of curcumin and methotrexate treatment on various organs in collagen-induced arthritis in rats.** *J Am Sci* 2012;8(10):447-454]. (ISSN: 1545-1003). <http://www.jofamericansscience.org>. 65

Key words: methotrexate , curcumin, arthritis, histopathological investigation.

1. Introduction:

The immune system has evolved to discriminate self from non-self-antigens, thereby protecting the host from microbial infection and cancer (**Janeway, 1992**). Nevertheless, a breakdown in the fundamental immune response often results in the development of chronic infectious diseases, malignant tumors, and organ-specific autoimmune diseases. Although the etiology of autoimmune disease is not known, it is generally believed to be mediated by autoimmune cells that are influenced by genetic, environmental, and behavioral factors.

Rheumatoid arthritis (RA) is one of the major human autoimmune diseases (**Yang *et al.*, 2011**), with unknown etiology and a chronic progressive disease (**Obayashi *et al.*, 2011**). RA is a chronic systemic inflammatory disorder that primarily affects various body joints and causes progressive destruction of articular structures, particularly, the cartilage and bone (**Chandran and Goel, 2012**).

This chronic condition of RA have a significant negative impact on patients' health-related quality of life, with many patients experiencing fatigue, decreased sleep quality, depression, and reduced work productivity. Taken together, these symptoms have a detrimental impact on the patient's physical, social, psychological, and economic well-being (**Daul and Grisanti, 2009**).

The treatment of RA has gone through many major changes in the past 100 years (**Fan and Leong, 2007**). The treatment of RA has undergone somewhat of a revolution over the last decade, with a strong

consensus emerging in favor of early, aggressive therapy (**Lee and Weinblatt, 2001; O'Dell, 2001; Goldbach-Mansky and Lipsky, 2003; Scott and Kingsley, 2006**). There is now evidence that early treatment of the disease has a beneficial impact on treatment outcome. The goals to be achieved in managing RA are prevention or control of joint damage, prevention of loss of function, and reduction of pain (**Sizova, 2008**).

Methotrexate (MTX) is an anti-metabolite and immunosuppressant (**Kumar and Marwaha, 2003**), which inhibits the synthesis of DNA, RNA, thymidine, and proteins. The anti-inflammatory effects of MTX on RA seem to be related, at least partially, to the modulation of the adenosine metabolism, and to other possible effects on the tumor necrosis factor (TNF) pathways (**Mota *et al.*, 2012**). MTX has become the predominant immunosuppressive agent used in the treatment of patients with RA (**Williams *et al.*, 1985**); it is the first-line therapy for RA (**Mota *et al.*, 2012**). MTX acts mainly on actively proliferating cells during the S-phase of proliferation, suppresses macrophage function, modulates interleukin-1 (IL-1) and superoxide anion production, and inhibits neutrophil chemotaxis (**Moreland *et al.*, 1997**).

The usage of conventional (allopathic) anti-inflammatory drugs is associated with severe adverse effects, including gastrointestinal bleeding and cardiovascular complications. Owing to the side effects and the high cost of conventionally used anti-inflammatory drugs, patients with arthritis are

increasingly using complementary and alternative medicine (CAM) modalities of treatment (**Venkatesha et al., 2011**).

Curcumin, a hydrophobic polyphenol, is a principal active constituent of turmeric (**Zhou et al., 2011**) it constitutes 3% of the total rhizome (**Aggarwal et al., 2006**). The first study on the use of curcumin in human diseases was published in 1937 (**Albert, 1937**). Its antibacterial effect and the ability to decrease blood sugar levels in human subjects were documented in 1949 and 1972, respectively (**Aggarwal and Sung, 2009**).

Although curcumin is well tolerated, and has a wide variety of beneficial activities, the *in vivo* bioavailability of curcumin is poor, which may be an important obstacle to its utility as a therapeutic agent (**Ireson et al., 2002**). Animal studies have shown that curcumin is rapidly metabolized, conjugated in the liver and excreted in the faeces, therefore having limited systemic bioavailability (**Jurenka, 2009**).

2. Material and Methods:

2.1 Materials:

2.1.1. Animals:

Male albino rats (Sprague-Dawley strains), weighing 180-200 grams, obtained from the animal house of National Research Center, Egypt were used. The environmental conditions were properly standardized with a 12- hours light cycle, a constant temperature of 20°C and humidity of 48%. The rats had free access to standard pelleted diet and tap water. Rats were fed on a standard rodent diet with water *ad libitum*. The experimental protocol was approved by the National Health and Medical Research Council guidelines and by the Institutional Animal Ethics Committee.

Animals were divided into 6 groups, 10 rats each:

Group I : Normal rats.

Group II : was injected with DMSO,

Group III : arthritic rats,

Group IV: arthritic rats received methotrexate (MTX)

Group V : arthritic rats treated with curcumin

2.1.2. Drugs and Chemicals:

Complete Freund's adjuvant (CFA), dimethyl sulfoxide (DMSO) and curcumin were purchased from Sigma, St. Louis, Mo, USA. Lyophilized bovine type II collagen was purchased from BioCol GmbH, Michendorf, Germany. MTX was purchased from Ebewe, Austria.

Dose selection:

Curcumin was dissolved in DMSO at a dose of 100 mg/kg b.w and administered i.p thrice a week. (**Banji et al., 2011**). DMSO was administered i.p at a dose of 0.2 ml/ 100 mg b.w thrice a week (**Hemeida and Mohafez, 2008**). MTX was dissolved in PBS at a

dose of 1 mg/kg body weight (b.w) once a week, administered intraperitoneally (i.p) (**Banji et al., 2011**).

2.1.3. Collagen-induced arthritis:

Lyophilized bovine type II collagen was dissolved at 4 mg/ml in 0.05M acetic acid by gentle stirring overnight at 4 °C. CFA and collagen were mixed at 1:1 to form an emulsion. 0.2 ml (200 g collagen) of the emulsion was injected subcutaneously at the base of the tail. A booster injection (0.1 ml of the emulsion) was administered on day 7 after initial immunization.

2.2. Methods:

2.2.1. Experimental Design:

Animals were divided into 6 groups, 10 rats each:

Group I: Normal rats were left without treatment as control.

Group II: Rats were injected with 0.2 ml/ 100 g b.w DMSO by i.p route thrice a week for 7 weeks.

Group III: Rats were immunized with lyophilized bovine type II collagen in CFA at the base of the tail to induce arthritis and they were injected with a booster dose on day 7.

Group IV: Arthritic rats were injected with curcumin in a dose of 100 mg/kg b.w thrice a week for 7 weeks by i.p route for 7 weeks.

Group V: Arthritic rats were injected with 1 mg/ Kg b.w MTX by i.p route once a week for 7 weeks.

Group VI: Arthritic rats were injected with 1 mg/ Kg b.w MTX by i.p route once a week for 7 weeks. After 30 mins of MTX treatment, the same rats were injected with 100 mg/kg b.w curcumin thrice a week by i.p route for 7 weeks.

2.2.2. Histopathological Examination:

Rats were sacrificed and thymus, spleen (as lymphoid organs), liver, lung and kidney were collected and fixed in 10% formalin, dehydrated in a graded series of ethanol, cleared in terpineol and embedded in tissue prep (M.P. 56.6 C, Fischer Scientific Co.). Transverse serial sections of 6 m were routinely stained in alum haematoxylin and eosin (H&E) for histopathological evaluation.

3. Results and discussion:

Thymus:

Morphological examination of the thymus, from normal control rats, showed a normal size. Normal thymic cortex and medulla revealed no histopathological changes being well demarcated (Fig. 1. a). Also, thymus sections from rats injected with DMSO showed a normal thymic pattern (Fig. 1. b). Thymus section of rats from non-treated arthritic group revealed dilatation and congestion of blood vessel leading to haemorrhage and thymic inflammatory cells infiltration. Lymphocytic

depletion in the cortex and medullary areas lead to the loss of demarcation between them (Fig. 1.c). The examined sections from the arthritic group treated with MTX revealed, focal thymic haemorrhage accompanied by severe lymphopenia especially in medullary area (Fig. 1. d). Thymus sections from arthritic rats treated with curcumin showed no histopathological changes as the cortex being loaded with lymphocytes was well defined from the medullary area (Fig. 1. e). No histopathological changes were also noticed in thymus sections from

arthritic group treated with both curcumin and MTX as the basic architecture was well kept (Fig. 1. f).

In case of the thymus, being a primary lymphoid organ, the present study profoundly showed that RA induction and MTX treatment cause drastic histopathological changes. Whereas, in case of curcumin single treatment and dual treatment with both curcumin and MTX restored the normal basic structure of the thymus to a great extent. This suggests that curcumin has an improving effect on the thymus denoting that it neutralizes the side effects of RA induction and MTX treatment.

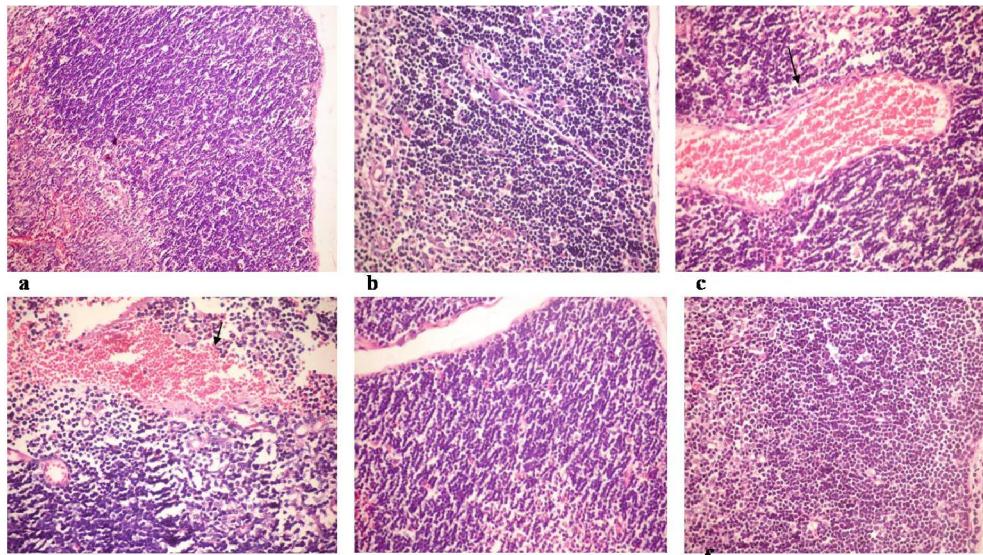


Fig. 1.

- (a) Thymus of normal control rat showing no histopathological changes (H&E x400).
- (b) Thymus of rat injected with DMSO showing no histopathological changes (H&E x400).
- (c) Thymus of arthritic non-treated rat showing dilatation and congestion of blood vessel (arrow) (H&E x400).
- (d) Thymus of arthritic rat treated with MTX showing focal thymic haemorrhage (arrow) (H&E x400).
- (e) Thymus of arthritic rat treated with curcumin showing no histopathological changes (H&E x400).
- (f) Thymus of arthritic rat treated with both curcumin and MTX showing no histopathological changes (H&E x400).

Spleen:

Spleen from normal control rats was normal in size. Sections revealed red and white pulps being well marked (Fig. 2. a). Spleen from of rat injected with DMSO revealed no histopathological changes (Fig. 2. b). Spleen sections of rats from non-treated arthritic group revealed lymphocytic depletion thus, causing a complete loss of demarcation between red and white pulps. In addition, the occurrence of unusually large numbers of macrocytes in the circulating blood (megalocytosis) (Fig. 2. c). Basic architecture of spleen was restored in spleen section from arthritic rats treated with MTX, curcumin, or both curcumin and MTX as it revealed lymphocyte loaded white pulp well segregated from the red pulp (Fig. 2. d, e and f).

Similar to the thymus, the splenic structure that was profoundly destroyed by RA induction was improved by the single treatment of curcumin or the dual treatment of curcumin and MTX. It was quite evident that the spleen (secondary lymphoid organ) was more responsive to improvement than the thymus, denoting that immature cells of the thymus are more susceptible to destruction by RA induction and more resistant to curcumin single or dual treatment with MTX than splenic mature lymphocytes.

Liver:

In spite of the efficacy of methotrexate as a disease modifying anti-rheumatic drug, the propensity to induce hepatotoxicity is quite high (**Suzuki et al., 1999**). The polyglutamated form of methotrexate will have a longer retention time in

hepatic cells, thereby enhancing the chances of hepatotoxicity. De novo synthesis of purines generally takes place in rapidly proliferating tissues like hepatocytes and bone marrow (**Baram et al., 1987**). Hence these areas are more susceptible to damage. Depletion of folates by MTX polyglutamate is a major contributing factor for hepatotoxicity (**Kremer et al., 1986**). In the present study, rats were treated with curcumin or MTX alone or combination of both after RA induction. Then, histological examination of liver sections were undertaken. Liver sections from normal control rats showed intact hepatic architecture, healthy hepatocytes, portal tracts containing bile ducts, portal veins and hepatic arteries (Fig. 3. a). Liver of rat injected with DMSO revealed no histopathological changes (Fig. 3. b). Liver sections of rats from non-treated arthritic group showed dilatation and congestion of central veins, congestion of hepatic sinusoids with leukocytes in the hepatic sinusoids, dissociation of hepatocytes, necrosis of hepatocytes, marked dilatation and congestion of central veins and hepatic sinusoids as

well as focal hepatic haemorrhage (Fig. 3. c). Examined sections of arthritic group treated with MTX revealed granularity of the cytoplasm of hepatocytes in some examined sections (Fig. 3. d). Liver sections from arthritic rats treated with curcumin revealed congestion of central vein and hepatic sinusoids (Fig. 3. e). No histopathological changes were noticed in lung sections from arthritic group treated with both curcumin and MTX (Fig. 3. f).

Our data are in agreement with Banji and colleagues who demonstrated that arthritis induced rats treated with methotrexate exhibited a toxic effect on liver with plenty of focal sinusoidal areas, portal track inflammation, proliferation of Kupffer cells, focal liver cell necrosis, fibrosis and fatty changes in hepatocytes. Treatment with curcumin along with MTX showed a significant mitigation of hepatocellular toxicity characterized by less fatty changes in hepatocytes and balloon degeneration (**Banji et al., 2011**).

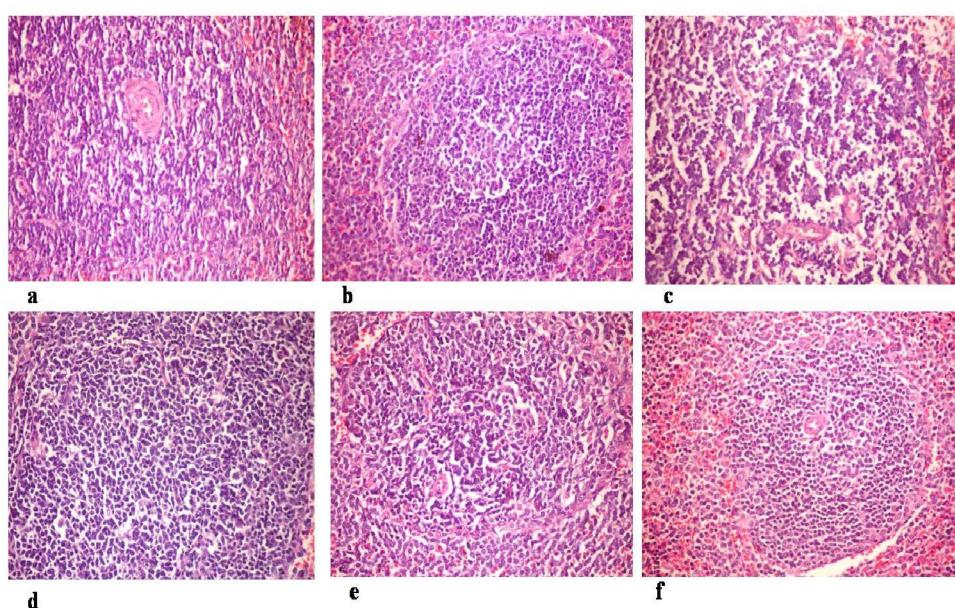


Fig. 2.

- (a) Spleen of normal control rat showing normal lymphoid follicle (H&E x400).
- (b) Spleen of rat injected with DMSO showing no histopathological changes (H&E x400).
- (c) Spleen of arthritic non-treated rat showing lymphocytic depletion (arrow) (H&E x400).
- (d) Spleen of arthritic rat treated with MTX showing no histopathological changes (H&E x400).
- (e) Spleen of arthritic rat treated with curcumin showing no histopathological changes (H&E x400).
- (f) Spleen of arthritic rat treated with both curcumin and MTX showing no histopathological changes (H&E x400).

Lung:

Lung sections from normal control rats were normal in architecture with thin wall with no inflammatory cells (lymphocytes, plasma cells and eosinophils) and revealed no histopathological

changes (Fig. 4. a). Lung of rat injected with DMSO showed no histopathological changes (Fig. 4. b). Lung sections of rats from non-treated arthritic group revealed interstitial pneumonia, marked perivasculitis, granulomatous pneumonia, pulmonary

haemorrhage associated with inflammatory cells infiltration, necrosis of epithelial lining bronchiole, peribronchiolar infiltration with massive leukocytes, hyalinosis in the wall of blood vessel and perivasculitis (Fig. 4. c). Examined sections the arthritic group treated with MTX revealed focal interstitial pneumonia, atelactasis, perivasculitis, focal pulmonary emphysema and thickening of interstitial tissue with mononuclear cells (Fig. 4. d). Lung sections from arthritic rats treated with curcumin showed perivascular massive leukocytic infiltration and congestion of perialveolar blood capillaries (Fig. 4. e). No histopathological changes were noticed in lung sections from arthritic group treated with both curcumin and MTX (Fig. 4. f).

Following immunization with collagen II in CFA mice develop arthritis of major joints (**Courtenay et al., 1980**). This experimental system, called collagen-induced arthritis (CIA), is used as a study model for RA in man (**Holmdahl et al., 2002; Asquith et al., 2009; Schurges et al., 2011**). The disease is systemic at all stages and can affect a multitude of organ systems, including mucosae (Sjögren's syndrome), pericard, pleura and lungs (**Brown, 2007; Bartels et al., 2010**). The pathogenesis of extra-articular complications in RA is incompletely understood and evidence for similar multi-organ involvement in CIA is scarce and ambiguous (**Matsuoka et al., 2008; Bongartz et al., 2010**). It

was demonstrated pulmonary inflammation accompanying CIA in mice. Pulmonary complications account for 10 to 20 % of mortality in RA patients (**Young et al., 2007; Sokka et al., 2008**).

Kidney:

Kidneys of normal control rat showed intact glomerular architecture with the normal histological structure of renal parenchyma (Fig. 5a). Examined sections of rats injected with DMSO revealed no histopathological changes (Fig. 5b). Kidney of the non-treated arthritic rats showed congestion of glomerular tufts, intertubular blood capillaries, necrobiotic changes of epithelial lining renal tubules, atrophy of glomerular tuft and pyknosis of the nuclei of epithelial lining renal tubules (Fig. 5c). Sections from arthritic group treated with MTX showed congestion of renal blood vessels, vaculations of tunica media, perivasculitis, congestion of intertubular blood vessels and renal blood vessels, as well as pyknosis of nuclei of epithelial lining renal tubules (Fig. 5. d). Kidney of rat from arthritic group treated with curcumin revealed atrophy of some glomerular tufts and pyknosis of nuclei of epithelial lining some renal tubules (Fig. 5e). No histopathological changes were noticed from arthritic group treated with both curcumin and MTX (Fig. 5. f).

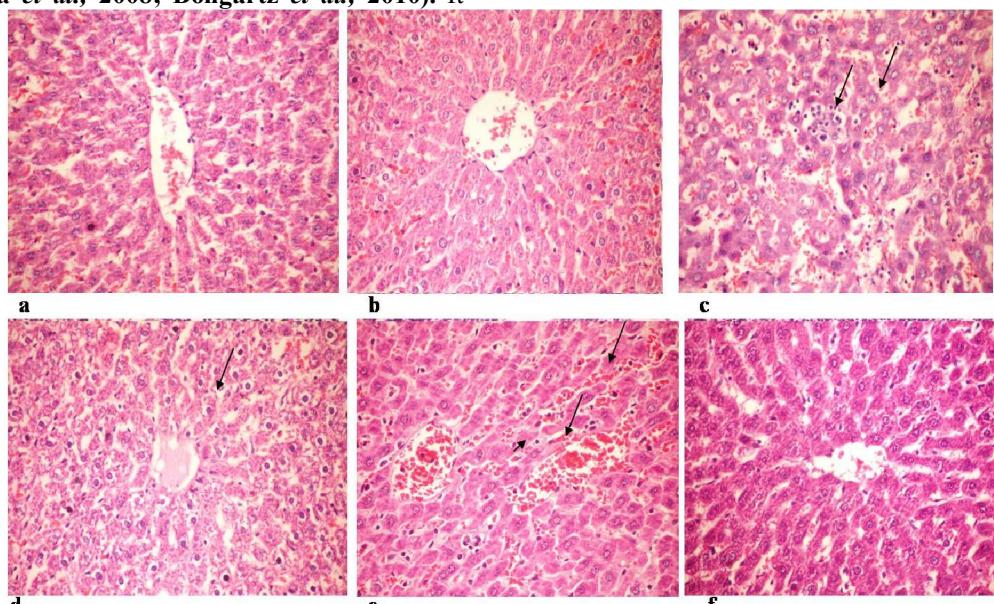


Fig. 3.

- (a) Liver of normal control rat showing the normal histological structure of hepatic lobule (H&E x400).
- (b) Liver of rat injected with DMSO showing no histopathological changes (H&E x400).
- (c) Liver of arthritic non-treated rat showing congestion of hepatic sinusoids (small arrow) with leukocytes in the hepatic sinusoids and dissociation of hepatocytes (large arrow) (H&E x400).
- (d) Liver of arthritic rat treated with MTX showing granularity of the cytoplasm of hepatocytes (arrow) (H&E x400).
- (e) Liver of arthritic rat treated with curcumin showing congestion of central vein (small arrow) and hepatic sinusoids (large arrow) as well as kupffer cell activation (arrow head) (H&E x400).
- (f) Liver of arthritic rat treated with both curcumin and MTX showing no histopathological changes (H&E x400).

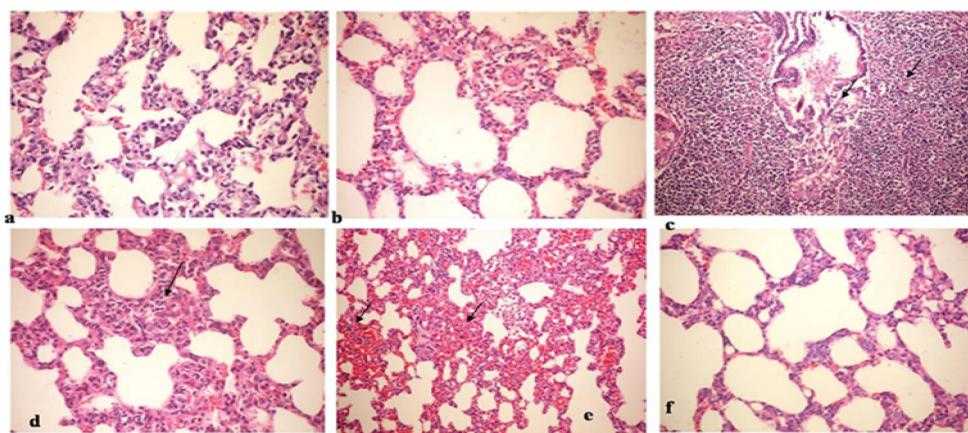


Fig. 4.

- a) Lung of normal control rat showing no histopathological changes (H&E x400).
- b) Lung of rat injected with DMSO showing no histopathological changes (H&E x400).
- c) Lung of arthritic non-treated rat showing necrosis of epithelial lining bronchiole (small arrow) and peribronchiolar infiltration with massive leukocytes (large arrow) (H&E x200).
- d) Lung of arthritic rat treated with MTX showing thickening of interstitial tissue with mononuclear cells (arrow) (H&E x400).
- e) Lung of arthritic rat treated with curcumin showing congestion of perialveolar blood capillaries (arrow) (H&E x200).
- f) Lung of arthritic rat treated with both curcumin and MTX showing no histopathological changes (H&E x400).

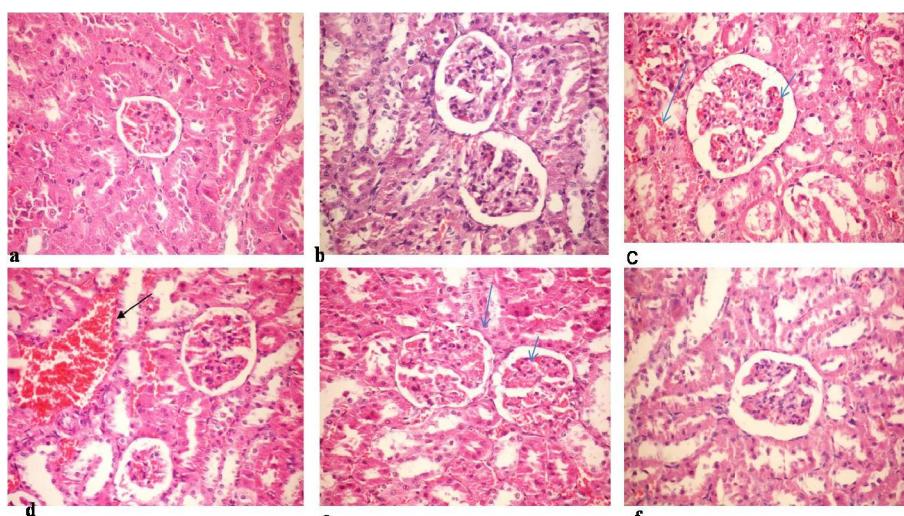


Fig. 5.

- (a) Kidney of normal control rat showing normal histological structure of renal parenchyma (H&E x400).
- (b) Kidney of rat injected with DMSO showing no histopathological changes (H&E x400).
- (c) Kidney of arthritic non-treated rat showing congestion of glomerular tuft (small arrow) and intertubular blood capillaries (large arrow) (H&E x400).
- (d) Kidney of arthritic rat treated with MTX showing congestion of renal blood vessel (arrow) (H&E x400).
- (e) Kidney of arthritic rat treated with curcumin showing congestion of glomerular tuft (arrow) (H&E x400).
- (f) Kidney of arthritic rat treated with both curcumin and MTX showing apparent normal renal parenchyma with no histopathological changes (H&E x400).

Finally, it could be deduced that curcumin alone is not able to neutralize all histopathological changes caused by MTX, however, combination of methotrexate and curcumin help to restore the normal structure of the examined organs.

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9/20/2012