Protective effect of curcumin treatment on some organs in collagen-induced arthritis in rats.

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Abstract: Objective: To evaluate the protective effect of curcumin administration on various internal organs in collagen-induced arthritis in rats. **Material and methods:** Animals were divided into 4 groups, 10 rats each. *Group I:* Normal control, *Group II* was injected with DMSO, *Group III* arthritic rats, *Group IV* arthritic rats received curcumin (100 mg/ Kg, i.p) thrice a week for 7 weeks prior to induction of arthritis. Rats were sacrificed and thymus, spleen, liver, lung and kidney were collected for histopathological evaluation. **Results:** Mild improvement in examined organs from rats injected with curcumin prior to induction of arthritis as compared with arthritic group. Curcumin minimized the complications caused by arthritis on the examined internal organs. **Conclusion:**The study showed that the administration of curcumin prior to induction of arthritis, to a certain extent minimized the complications caused by the disease on the internal organs under study.
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Key words: curcumin**,** arthritis, histopathological evaluation.

1.Introduction:

Autoimmune diseases are a major cause of morbidity and mortality in the industrialized world, affecting 3–8% of the population. In principle, autoimmunity develops after breaking self-tolerance of the immune system, a process that involves many different molecules and yet poorly understood processes **(Kunz and Ibrahim, 2009)**. It remains an open question whether bacterial or viral pathogens contribute to the initiation of these diseases as major causative agents **(Carroll, 2001; Christen and von Herrath, 2004)**.

About 5% of the population, however, suffers from a chronic inflammatory rheumatic disease [e.g., RA, spondyloarthritis (SpA), and vasculitis] **(Schirmer et al., 2012).** During the last decade, knowledge about the pathophysiological background of rheumatic diseases has significantly increased, especially of the immune-mediated inflammatory diseases **(Schirmer et al., 2012).**

Patients with RA have a two-fold increased mortality risk. When RA involves other organs, its morbidity and severity are higher, and life expectancy can be reduced by 5–10 years **(Weissmann, 2006; Wiens et al., 2012).** With disease progression, patients develop inability to perform their daily and professional activities.

Pharmacotherapy for rheumatoid arthritis generally involves treatment regimens including non-steroidal anti-inflammatory drugs (NSAIDs) for the pain management, low-dose therapy using oral or intra-articular gluco-corticoids, disease-modifying anti-rheumatic drugs (DMARD) and the newer biological treatments **(Rindfleisch and Muller, 2005; Marks, 2011; Chandran and Goel, 2012).** Unfortunately, the majority of these drugs typically associate with severe side effects including gastrointestinal bleeding, increased blood pressure, accelerated osteoporosis, myelo-suppression, hepato-toxicity, ocular toxicity, hypersensitivity and allergic reactions, as well as, increased risk of infections **(Lipsky et al., 2000; Newsome, 2002; Rahme and Bernatsky, 2010; Chandran and Goel, 2012).**

Agents derived from plants that can modulate the expression of pro-inflammatory signals clearly have potential against arthritis. These include flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins and anthoxanthins, all of which are known to have anti-inflammatory effects. **(Khanna *et al.*, 2007).**

Turmeric (the common name for *Curcuma longa,* known as *haldi* in Hindi) (Fig. 7) is an Indian spice derived from the rhizomes of the plant that belongs to the ginger family Zingiberaceae **(Aggarwal and Sung, 2009; Jurenka, 2009, Zhou *et al.*, 2011)**. It is cultivated in India and other parts of Southeast Asia **(Ammon and Wahl, 1991).** Besides the use as a spice, food preservative and coloring agent, turmeric has been traditionally used in Ayurveda, an Indian system of medicine, for the treatment of various ailments such as arthritis, ulcers, jaundice, wounds, fever, trauma as well as skin diseases like psoriasis **(Singh, 2007; Zhou *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 **(Funk *et al.*, 2006).** Its diverse array of molecular targets affords it great potential as a therapeutic agent for a variety of inflammatory conditions and cancer types. Thus, curcumin could be a potential anti-arthritic drug **(Jurenka, 2009)**. The present study attempts to investigate the protective effect of curcumin on CIA.

2.Material and Methods

2.1.Materials:

2.1.1.Animals

Male albino rats (Sprague-Dawely 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 20ºC 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 4 groups, 10 rats each:

Group I: Normal rats

Group II: Rats were injected with DMSO

***Group III*:** Rats were immunized with Lyophilized bovine type II collagen

Group IV: Rats were injected 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.

***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).**

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 ºC. 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 4 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*:** Rats were injected with curcumin in a dose of 100 mg/kg b.w thrice a week for 7 weeks by i.p route prior to induction of arthritis.

2.2.2.Histopathological Examination:

Rats were sacrificed on the 69th day. Thymus, spleen (lymphoid organs), lung, liver, 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:

Thymus from normal control rats was normal in size. Histological investigation of thymus sections revealed no histopathological changes in cortex and medulla (Fig. 1. a). Also, thymus sections from rats injected with DMSO showed no histopathological alterations (Fig. 1. b). Thymus section of rats from non-treated arthritic group revealed dilatation and congestion of blood vessels accompanied by hemorrhage and thymic inflammatory cells infiltration. Demarcation between cortex and medulla was lost denoting severe loss of lymphocytes in cortex which was evident (Fig. 1.c).Thymus sections of rats injected with curcumin before the induction of arthritis revealed lymphocytic depletion in thymic medulla leading to the loss of marked segregation between thymic cortex and medullary areas. Congestion of thymic blood vessels was evident (Fig. 1. d).

Curcumin treatment before RA induction showed no positive effect on thymic architecture. Since thymic depletion was evident, this could be explained by the fact that thymus is a primary lymphoid organ thus; it comprises more immature lymphocytes than mature ones. It is well known that immature lymphocytes were much more sensitive than mature thymocytes **(Smith et al., 1977).**

Spleen

It was observed that spleen size was normal in rats of the normal control group. Red and white pulps were well demarked, white pulp being loaded with lymphocytes in spleen sections of the same group (Fig. 2. a).

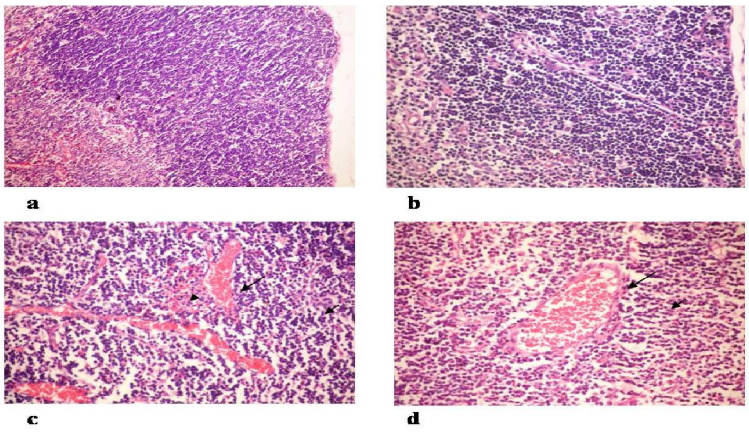

Fig. 1.
(a) Thymus of normal control rat showing no histopathological changes (H&E x200).
(b) Thymus of rat injected with DMSO showing no histopathological changes (H&E x400).
(c) Thymus of arthritic non-treated rat showing lymphocytic depletion in the medulla (small arrow), congestion (large arrow) and haemorrhage (arrow head) (H&E x400).
(d) Thymus of rat injected with curcumin before induction of arthritis showing lymphocytic depletion in thymic medulla (small arrow) and congestion of thymic blood vessels (large arrow) (H&E x400).

Spleen from of rat injected with DMSO revealed no histopathological changes, also white pulp showed healthy condensation of lymphocytes (Fig. 2. b). Spleen sections of rats from non-treated arthritic group showed no defined segregation between white and red pulps due to lymphopenia of white pulp. The occurrence of unusually large numbers of macrocytes in the circulating blood (megalocytosis) was apparent (Fig. 2. c). No histopathological changes were noticed in spleen sections from rats injected with curcumin before the induction of arthritis (Fig. 2. d) demonstrating its protective effect.

Thus, in case of spleen (secondary lymphoid organ), the protective effect of curcumin was evident, as the splenic structure was well saved from RA destructive effects. This observation supports the previous phenomenon as the spleen hold mature lymphocytes which are responding more effectively to curcumin than immature thymic lymphocytes **(Smith et al., 1977). Liver**

Curcumin, a hydrophobic polyphenol exerts its anti-arthritic action by suppressesing synthesis of proinflammatory prostaglandins and leukotrienes **(Haung et al., 1994).** It interferes with the conversion of linoleic acid to arachidonic acid and its uptake by macrophages. Curcumin suppresses the activation of NF-κB, leading to poor expression of cyclooxygenase-2 **(Singh and Aggarwal, 1995).** Curcumin also down regulates the expression of tumor necrosis factor and proinflammatory interleukins which play a pivotal role in arthritis.

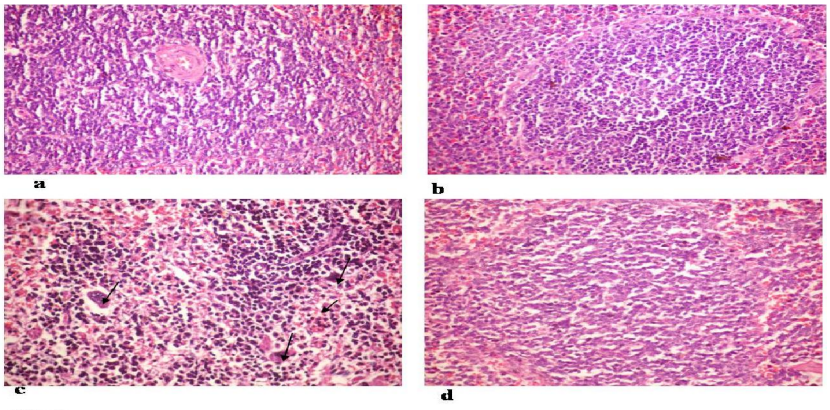

Fig. 2.
(a) Spleen of normal control rat showing normal lymphoid follicle (H&E x400).
(b) Spleen of rat injected with DMSO showing no histopathological changes (H&E x400).
(c) Spleen of arthritic non-treated rat showing lymphocytic depletion (small arrow) and megalocytosis (large arrow) (H&E x400).
(d) Spleen of rat injected with curcumin before induction of arthritis and treated with curcumin after induction showing no histopathological changes (H&E x400).

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). No histopathological changes were found in the liver of rat injected with DMSO (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). Liver of rats injected with curcumin before the induction of arthritis showed no histopathological changes except Kupffer cells activation (Fig. 3. d). The improvement in the liver structure of curcumin treated rats could be attributed to its effect on the level of various interleukins **(Singh and Aggarwal, 1995)**.

In case of the liver it was profoundly evident that RA induction caused some vascular defects. These defects were neutralized by curcumin treatment before RA induction suggesting its protective effect. Although, Kupffer cells were activated by curcumin treatment indicating liver malfunction which may be attributed to RA induction.

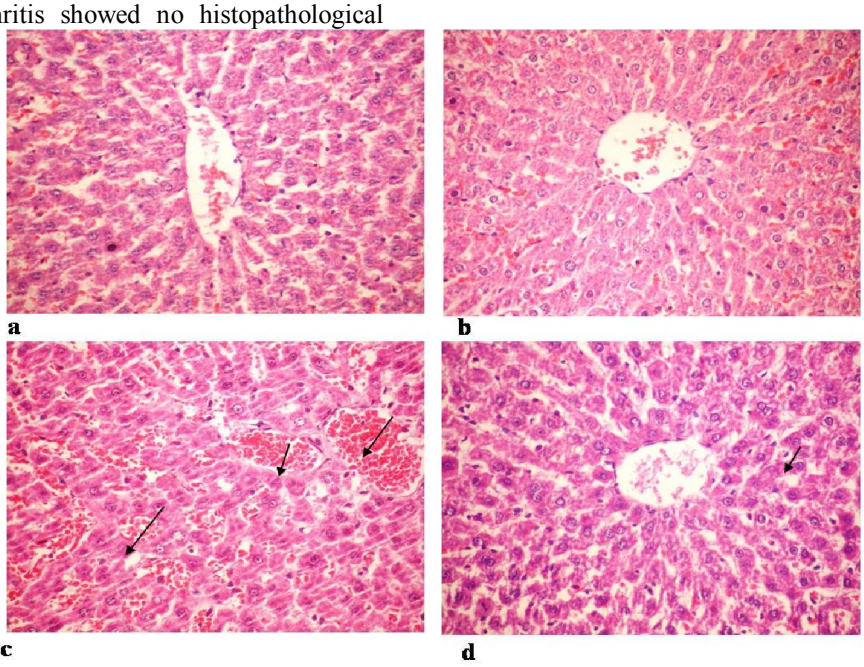

Fig. 3.
(a) Liver of normal control rat showing the normal histological structure of hepatic lobule (H&E x400).
(b) Liver of rat injected with DMSO showing no histopathological changes (H&E x400).
(c) Liver of arthritic non-treated rat showing marked dilatation and congestion of central veins (small arrow) and hepatic sinusoids (large arrow) (H&E x400).
(d) Liver of rat injected with curcumin before induction of arthritis showing kupffer cells activation (arrow) (H&E x400).

Lung:

Histological screening revealed that lung sections from normal control rats were normal in architecture with thin wall with no inflammatory cells (lymphocytes, plasma cells and eosinophils) (Fig. 4. a). Also, lung of rat injected with DMSO showed no 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). Lung of rats injected with curcumin followed by the induction of arthritis revealed perivasculitis and slight thickening of interstitial tissue (Fig. 4. d). Our results are in concordance with Schurgers and co-workers who demonstrated that macroscopic, microscopic and functional characteristics of pulmonary inflammation in the mice resembled those seen in human RA. The pleura and the airways, as well as the interstitium and vasculature of the lung can be affected **(Young et al., 2007; Sokka et al., 2008).** Pleuritis is the most common manifestation of lung disease associated with RA, but this condition often remains asymptomatic **(Young et al., 2007; Matsuoka et al.,**

2008). Furthermore, administration of curcumin before induction of CIA resulted in reduction of pulmonary pathology

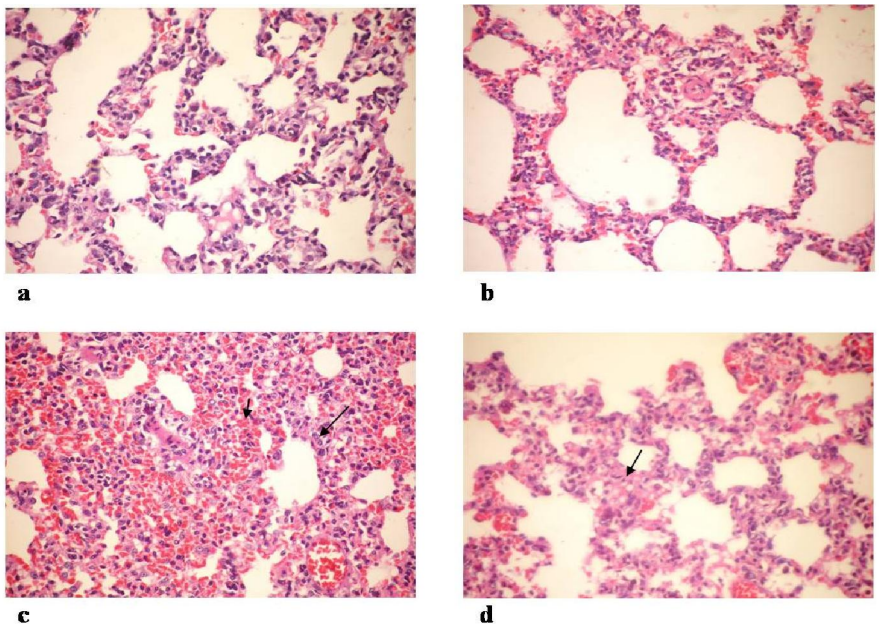

Fig. 4.
(a) Lung of normal control rat showing no histopathological changes (H&E x400).
(b) Lung of rat injected with DMSO showing no histopathological changes (H&E x400).
(c) Lung of arthritic non-treated rat section showing pulmonary haemorrhage (small arrow) associated with inflammatory cells infiltration (large arrow) (H&E x400).
(d) Lung of rat injected with curcumin before induction of arthritis section showing slight thickening of interstitial tissue (arrow) (H&E x400).

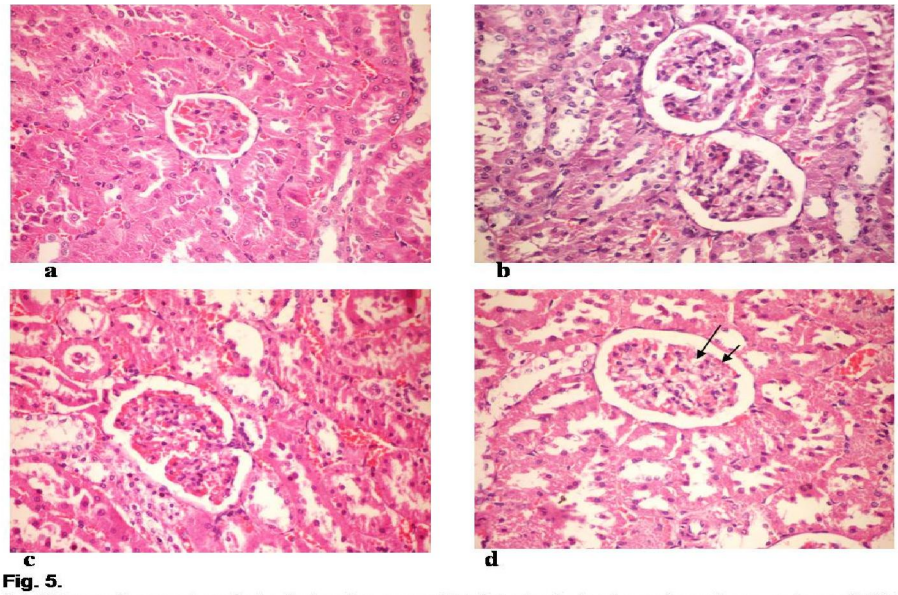

Fig. 5.
(a) Kidney of normal control rat showing normal histological structure of renal parenchyma (H&E x400).
(b) Kidney of rat injected with DMSO showing no histopathological changes (H&E x400).
(c) Kidney of arthritic non-treated rat showing congestion of glomerular tuft (small arrow) and necrobiosis of epithelial lining renal tubule (large arrow) (H&E x400).
(d) Kidney of rat injected with curcumin before induction of arthritis showing congestion (small arrow) and vaculations of the glomerular tuft (large arrow) (H&E x400).

Kidney:

Kidneys of normal control rat showed intact glomerular architecture with the normal histological structure of renal parenchyma (Fig. 5. a). Examined sections of rats injected with DMSO revealed no histopathological changes (Fig. 5. b). 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. 5. c). RA may affect the kidney glomerulus directly through a vasculopathy or a mesangial infiltrate but this is less well documented (though this is not surprising, considering immune complex-mediated hypersensitivities are known for pathogenic deposition of immune complexes in organs where blood is filtered at high pressure to form other fluids, such as urine and synovial fluid) **(Robbins et al., 2010).** Kidneys of the group injected with curcumin before the induction of arthritis revealed congestion and vaculations of the glomerular tuft (Fig. 5. d). Thus, it was observed that curcumin treatment minimize the destructive effects of RA to some extent. Further studies are deeply warranted using other curcumin regimes that may ameliorate the kidney conditions.

It could be deduced that administration of curcumin before induction of arthritis, to a certain extent minimized the complications caused by the disease on the internal organs under study.

4.References:

1-Aggarwal, B.B. and Sung, B. (2009): Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. *Trends Pharmacol. Sci.,* 30(2):85-94.

2-Ammon, H.P. and Wahl, M.A. (1991): Pharmacology of *Curcuma longa. Planta Med.,* 57(1):1-7.

3-Banji, D.; Pinnapureddy, J.; Banji, O.J.; Saidulu, A. and Hayath, M.S. (2011): Synergistic activity of curcumin with methotrexate in ameliorating Freund's Complete Adjuvant induced arthritis with reduced hepatotoxicity in experimental animals. *Eur. J. Pharmacol.,* 668(1-2):293-8.

4-Carroll, M. (2001): Innate immunity in the **etiopathology** of autoimmunity. *Nat Immunol.,* 2(12):1089-90.

5-Chandran, B, and Goel, A. (2012): A **Randomized**, **Pilot Study** to **Assess** the **Efficacy** and Safety of Curcumin in Patients with Active Rheumatoid Arthritis. *Phytother. Res.,* doi: 10.1002/ptr.4639.

6-Christen, U. and von Herrath, M.G. (2004): Initiation of autoimmunity. *Curr. Opin. Immunol.,* 16(6):759-67.

7-Funk, J.L.; Oyarzo, J.N.; Frye, J.B.; Chen, G.; Lantz, R.C.; Jolad, S.D.; Sólyom, A.M. and Timmermann, B.N. (2006): Turmeric extracts containing curcuminoids prevent experimental rheumatoid arthritis. *J. Nat. Prod.,* 69(3):351-5.

8-Haung, M.T.; Lou, Y.R. and Ma, W. (1994): Inhibitory effects of dietary curcumin on fore stomach, duodenal and colon carcinogenesis in mice. *Cancer Res.,* 54, 5841–5848.

9-Hemeida, R.A. and Mohafez, O.M. (2008): Curcumin attenuates methotraxate-induced hepatic oxidative damage in rats. *J. Egypt. Natl. Canc. Inst.,* 20(2):141-8.

10-Jurenka, J.S. (2009): Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research. *Altern. Med. Rev.,* 14(2):141-53.

11-Khanna, D.; Sethi, G.; Ahn, K.S.; Pandey, M.K.; Kunnumakkara, A.B.; Sung, B.; Aggarwal, A. and Aggarwal B.B. (2007): Natural products as a gold mine for arthritis treatment. *Curr. Opin. Pharmacol.*, 7:344–351.

12-Kunz, M. and Ibrahim, S.M. (2009): Cytokines and cytokine profiles in human autoimmune diseases and animal models of autoimmunity. *Mediators Inflamm.,* **2009**:979258.

13-Lipsky, P.E.; van der Heijde, D.M.; St Clair, E.W.; Furst, D.E.; Breedveld, F.C.; Kalden, J.R.; Smolen, J.S.; Weisman, M.; Emery, P.; Feldmann, M.; Harriman, G.R.; Maini, R.N. and Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group (2000): Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study group. *N. Engl. J. Med.,* 343: 1594–1602.

14-Marks, W.H. (2011): Tripterygium wilfordii Hook F. versus Sulfasalazine in the treatment of rheumatoid arthritis: a well-designed clinical trial of a botanical demonstrating effectiveness. *Fitoterapia,* 82: 85–87.

15-Matsuoka, T.; Jin, E.; Tachihara, A.; Ghazizadeh, M.; Nakajima, A.; Yoshino, S.; Katayama, Y. and Kawanami, O. (2008): Induction of pulmonary thromboembolism by neutrophil elastase in collagen-induced arthritis mice and effect of recombinant human soluble thrombomodulin. *Pathobiology,* 75: 295-305.

16-Newsome, G. (2002): Guidelines for the management of rheumatoid arthritis: 2002 update. J. *Am. Acad. Nurse. Pract.,* 14: 432–437.

17-Rahme, E. and Bernatsky, S. (2010): NSAIDs and risk of lower gastrointestinal bleeding. *Lancet* 376: 146–148.

18-Rindfleisch J.A. and Muller, D. (2005): Diagnosis and Management of Rheumatoid Arthritis. *Am. Fam. Physician,* 72:1037-47.

19-Robbins, Stanley Leonard; Kumar, Vinay; Abbas, Abdul K.; Cotran, Ramzi S.; Fausto, Nelson (2010): Vinay Kumar, Abul K. Abbas, Nelson Fausto. ed. *Robbins and Cotran pathologic basis of disease*. Elsevier. pp. 205.

20-Schirmer, M.; Dejaco, C. and Duftner, C. (2012): Advances in the evaluation and classification of chronic inflammatory rheumatic diseases. *Discov Med.,* 13(71):299-304.

21-Singh, S. (2007): From exotic spice to modern drug? *Cell,* 130:765–8.

22-Singh, S. and Aggarwal, B.B. (1995): Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. *J. Biol. Chem.,* 270:24995–5000.

23-Smith, K.A.; Crabtree, G.R.; Kennedy, S.J. and Munck, A.U. (1977): Glucocorticoid receptors and glucocorticoid sensitivity of mitogen stimulated and unstimulated human lymphocytes. *Nature,* 267(5611):523-6.

24-Sokka, T.; Abelson, B. and Pincus, T. (2008): Mortality in rheumatoid arthritis: 2008 update. *Clin. Exp. Rheumatol.,* 26: S35-S61.

25-Weissmann, G. (2006): The pathogenesis of rheumatoid arthritis. *Bull. NYU Hosp. Jt. Dis.,* 64(1–2):12–5.

26-Wiens, A.; Grochocki, M.C.; Pontarolli, D.R.; Venson, R.; Correr, C.J. and Pontarolo, R. (2012): Profile of users of anticytokines offered by the health care system in the state of Paraná for the treatment of rheumatoid arthritis. *Rev. Bras. Reumatol.,* 52(2):208-13.

27-Young, A.; Koduri, G.; Batley, M.; Kulinskaya, E.; Gough, A.; Norton, S. and Dixey, J. (2007): Mortality in rheumatoid arthritis. Increased in the early course of disease, in ischaemic heart disease and in pulmonary fibrosis. *Rheumatology*, 46: 350-357.

28-Zhou, H.; Beevers, C.S. and Huang, S. (2011): The targets of curcumin. *Curr. Drug Targets,* 12(3):332-47.

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