Effect of Allopurinol and Vitamin E on Rat Model of Rheumatoid Arthritis

Mohamad Alorainy

*Department of Pharmacology and Therapeutics, College of Medicine,
Qassim University, Saudi Arabia*

Abstract:

Objectives: Rat collagen II–induced arthritis is a model of chronic inflammation induced by mycobacterium butyricum and collagen II. It is characterized by similar pathophysiological and pathobiochemical changes as rheumatoid arthritis (RA) in humans. In the present study, the biochemical effects of vitamin E and allopurinol (Allo) on RA of rats were investigated.

Methods: Forty male rats were divided into four groups (10 rats each): control group, collagen II-induced RA group (CII group), CII group treated with allopurinol (CII+ Allo), and CII group treated with vitamin E (CII + Vit E). After 6 weeks of treatment, the plasma levels of lipid peroxides (LPO), nitric oxide (NO), ceruloplasmin (CP), superoxide dismutase (SOD), uric acid (UA) and glutathione (GSH) were detected using colorimetric methods. The plasma levels of PGE2 were measured using ELISA assay. The plasma levels of copper (Cu) and zinc (Zn) were determined using atomic absorption spectrometer.

Results: In CII treated group, the levels of LPO, NO, PGE2, UA, CP and Cu were significantly higher, but the levels of SOD, GSH and Zn were significantly lower than controls. In CII + Allo treated group, the levels of SOD and GSH were significantly increased, but the levels of PGE2, LPO, NO, UA, Cu and CP were significantly decreased in comparison with CII–treated group. The levels of SOD, GSH and Zn were significantly increased, but the levels of PGE2, NO and CP were significantly decreased in the vitamin E treated group in comparison with CII–treated group. The levels of PGE2, LPO, Cu and Zn were significantly lower in vitamin E treated group than Allo-treated group. In conclusion, the study suggests that proper antioxidant intake management may reduce free radical generation and improve antioxidant status in RA. Allopurinol and vitamins E may effectively normalize in different degrees the impaired the oxidant/antioxidant system and may be useful in delaying the complication of RA. Moreover, they display anti-inflammatory action by decreasing PGE2 level in RA.

Keywords: Rat, Rheumatoid arthritis model, Antioxidants, Allopurinol, Vitamin E.

Running title: Effect of antioxidants on RA rats.

Correspondence:
Mohamad S. Alorainy
Department of Pharmacology and Therapeutics
College of Medicine
Qassim University
Saudi Arabia
E-mail: msorainy@gmail.com

Introduction

Rheumatoid arthritis (RA) is a polyarticular disease affecting about 1% of the population worldwide. It can be an autoimmune disease characterized by chronic inflammation, progressive joint destruction, physical impairment, work disability and early morbidity and mortality. The process of disease progression is characterized by the hyperplasia of synoviocytes, mainly of synovial fibroblasts, resulting in bone and joint destruction [1].

The immunization of mice with collagen II (CII) leads to the development of arthritis, the collagen-induced arthritis model for RA. CII-specific activation of both T and B cells is critical for the development of arthritis, and the transfer of both rodent and human serum with CII-specific antibodies induces arthritis in mice [2].

Inflammation is known to result in increased production of nitric oxide (NO) and prostaglandins. NO is an important mediator of diverse physiologic and pathologic processes, including arthritis [3]. Joint inflammation in autoimmune adjuvant-induced arthritis is dependent on the enhanced production of NO. NO is ideally suited as a potent inflammatory mediator because of its strong reactivity with oxygen, superoxide, and iron-containing compounds [4].

Prostaglandins are well known as proinflammatory mediators. The inhibition of cyclooxygenase (COX) has been widely used in the management of joint inflammation, with more recent strategies selectively targeting the proinflammatory inducible form of the enzyme COX-2. Levels of prostaglandin E_2 (PGE$_2$), the key prostaglandin mediating the cardinal signs of inflammation, are increased in various states of inflammation [5].

Several lines of evidence suggest that oxidative stress has a role in the pathology of RA. This oxidative stress, associated with the generation of free radicals, is a major contributor to joint damage in RA. The insufficiency of antioxidant defense systems and the acceleration of the oxidative reactions can be the results of the pro-oxidant/antioxidant imbalance in RA [6]. It was demonstrated that the level of free radical-induced damage to proteins in the synovial fluid was twice as high in RA [7]. Moreover, it was also found that individuals with innately low levels of protecting antioxidants in their plasma, such as vitamins A and E, carotene and selenium, are also at greater risk of developing RA [8].

Zinc (Zn) is a crucial element in a series of cellular functions as normal growth, protein metabolism, membrane stability, and metalloenzyme functions. In addition, Zn has several other effects on immune response, complement system, lysozomal enzyme release, and macrophage functions [9]. Zn is also indispensible in many steps of the inflammatory reactions. Among these are prostaglandin biosynthesis, stimulation of lymphocytes and immune response, and the scavenging of toxic free oxygen radicals. Zn is likewise an important element in collagen tissue formation and bone metabolism [9].

Copper (Cu) is abundance in the human body and nature [10]. Cu is incorporated into the structure of many enzymes and proteins [9]. It is reported that 30-50% increase in serum Cu level during acute phase response triggered by interleukin -1 (IL-1) release largely depend on the increased synthesis of ceruloplasmin (CP). It is also demonstrated that CP increases during acute phase reactions in order to scavenge toxic free oxygen radicals [11].

Inflammation within tissues induces a series of anti-inflammatory responses in which a number of proteins and enzymes carrying Zn and Cu elements are involved. The most notable among these are: metallothioneins, CP and superoxide dismutase (SOD). Intracytoplasmic SOD includes both Cu and Zn, while CP is a powerful antioxidant in serum carries only Cu [10]. Substantial alterations in the metabolisms of Cu and Zn occur through some physiological control mechanisms over inflammatory reaction [12].

CP is a major protein that circulates in the plasma and functions as a copper transporter that is able to couple and transport 90-95% of serum copper. It has been shown that this protein has also antioxidant functions, which can be proven beneficial in several pathological conditions [13]. CP is an acute-phase protein with a moderate reaction, up to 2- or 3-fold increase, in inflammatory conditions. CP is mainly synthesized in hepatocytes and is secreted in plasma with six copper atoms strongly coupled to the molecule. CP and the copper are modified in parallel during inflammatory disease. This seems to indicate a linked mediation or a coordinated regulation of CP and serum copper [14].

In view of the animal studies strongly suggesting anti-inflammatory role of antioxidants like SOD and vitamin E in experimentally induced arthritis, antioxidant therapy strategies have been proposed for the prevention and treatment of RA [15]. Various forms of antioxidant therapy have demonstrated promising results in experimental RA models [16].

Materials and Methods
Chemicals

Allopurinol, alpha tocopherol, thiobarbituric acid, reduced glutathione, naphthylenediamine dihydrochloride, sulphanilamide, sodium nitrite, sodium azide, 5,5-Dithiobis (2-Nitrobenzoic acid), epinephrine and p-phenylene diamine dihydrochloride, complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) were fine grade and obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Animals and experimental design

Forty healthy male albino rats (Rattus norvegicus) with average body weight 150–170 g were utilized for this study. All animals were conditioned at room temperature at a natural photoperiod for 1 week before experiment excution. A commercial balanced diet and tap water ad libitum were provided. The duration of experiment was 45 days. They were randomly divided into 4 groups (10 rats each) as the following:

1. Control group (Normal group) served as a negative control.
2. Adjuvant arthritic group (CII group) served as positive control. Bovine collagen type II (CII) was dissolved in 0.01 N acetic acid and emulsified in an equal volume of complete Freund's adjuvant (CFA) containing 1 mg/ml heat-killed mycobacterium tuberculosis (Sigma-Aldrich). Rheumatoid arthritis was induced by the initial immunization with 100 μg/100 μl emulsion by an intradermal injection in the base of the tail. Twenty one days later after the initial immunization, the rats received a boost intradermal injection (base of the tail) of 100 μg/100 μl of bovine CII emulsified in incomplete Freund's adjuvant (IFA) [17].

3. CII + vitamin E–treated group (CII+ Vit. E group) was injected with CII, and received concomitant vitamin E (100 mg/kg/day/I.M.) [18] beginning with the day of adjuvant injection for 45 days.
4. CII+ Allopurinol–treated group (CII+ Allo group) was also injected with CII, and received allopurinol (50 mg/kg/day/I.P.) [1, 19] beginning with the day of adjuvant injection and was continued until the 45th day of the experiment.

All tested substances (vitamin E; allopurinol) were administrated daily for 45 days (experiment duration). The animals of different groups were sacrificed under light anesthesia 1 day after the end of the treatment. The blood samples from all groups were collected from the orbital vein in heparinized tubes and were centrifuged at 5000 rpm for 10 minutes for plasma separation. The plasma sample was divided into aliquots and kept at -26°C until biochemical analyses.

Biochemical Analysis

The plasma levels of lipid peroxides (LPO) were measured as thiobarbituric acid reactivity (TBARS). The product of the reaction between malondialdehyde and thiobarbituric acid was measured as described elsewhere [20]. The plasma levels of nitric oxide (NO) was determined as total nitrite after deproteinzation with $ZnSO_4$ (30%), nitrate reduction with cadmium (activated by 2% HCL) and the color developed by the reaction with Griess reagent (1% sulfanilamide/ 0.1% naphthyelthylene diamine diHCL, w/v in 2.5% H_3PO_4) was recorded at 550 nm against reagent blank using sodium nitrite as standard [21]. The plasma GSH levels were determined chemically as described by Ellman [22]. The plasma SOD activity was determined according to its ability to inhibit the autooxidation of epinephrine at alkaline medium [23]. The plasma CP activity was determined using a para-phenylenediamine dihydrochloride method [25]. The plasma uric acid level was determined by enzymatic colorimetric method [15].

The plasma level of PGE_2 was detected using ELISA kit (Cat No. KGE004, R&D System GmbH, Germany). The minimum detectable plasma levels of PGE2 was 27.5 pg/ml.

The plasma levels of zinc and copper were determined by employing flame atomic absorption spectrometry. The concentrations of zinc and copper were determined by using concentrated H_2SO_4 and 1% H_2NO_3 mixture. Standard solutions used for calibration for Zn and Cu were 987 mg/ml (in 1% HNO3) and 1005 mg/mL (in 1% HCl) (Sigma-Aldrich, St. Louis, MO, USA) respectively. Metal measurement was performed with a Perkin-Elmer model 400 Atomic Absorption Spectrometer (PerkinElmer, Shelton, CT, USA), double beam and deuterium background correction. Hollow cathode lamps of Zn, and Cu were used at 213.86 and 249.22 respectively.

Statistical Analysis

The results are expressed as mean ± standard error (SE). Differences between groups were assessed by one-way analysis of variance using the Prism version 4 software package for Windows. The level of significance was accepted with $P < 0.05$.

Results

Table 1 shows the measured bioindices in different treated rat groups compared with control group.

Table (1) Comparison of the effect of allopurinol and vitamin E on the plasma levels of bio-indices among different treated rat groups and control group.

Parameters	A) Controls)	(B) CII-treated Group	(C) CII+ Allo. Treated group	(D) CII + vitamin E- treated Group	P- Value					
					A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
PGE$_2$ (pg/ml)	± 192.500 12.200	± 683.400 43.190	± 249.300 48.080	± 484.800 34.860	0.001>	0.05< NS	0.001>	0.001>	0.01>	0.001>
(LPO (nmol/ml	0.441 ± 3.218	1.193 ± 8.000	0.355 ± 4.491	0.289 ± 5.520	0.01>	0.05>	0.001>	0.05>	0.05< NS	0.05>
(NO (ng/ml	0.215 ± 3.619	1.248 ± 8.520	0.366 ± 5.721	0.3466 ± 4.758	0.001>	0.001>	0.05>	0.05>	0.01>	0.05< NS
(GSH (nmol/ml	0.249 ± 4.265	0.306 ± 2.749	0.396 ± 4.692	0.258 ± 4.354	0.01>	0.05< NS	0.05< NS	0.01>	0.001>	0.05< NS
(SOD (U/ml	± 344.700 41.220	± 109.800 18.720	± 227.700 44.980	± 226.000 20.460	0.001>	0.05< NS	0.05>	0.05>	0.001>	0.05< NS
Uric acid (mg/ (ml	0.139 ± 4.319	0.853 ± 8.422	0.298 ± 5.594	0.534 ± 6.593	0.001>	0.01>	0.001>	0.01>	0.05< NS	0.05< NS
Copper (µg/ml)	0.098 ± 2.374	0.166 ± 3.645	0.139 ± 2.923	0.188 ± 3.557	0.001>	0.01>	0.001>	01 .0>	0.05< NS	0.05>
Ceruloplasmin (mg/dl)	11.320 ± 95.860	± 225.500 13.090	± 135.400 12.980	± 166.700 13.070	0.001>	0.05>	0.001>	0.001>	0.01>	0.05< NS
Zinc (µg/ml)	0.311 ± 3.662	0.078 ± 0.616	0.088 ± 0.6832	0.199 ± 1.334	0.001>	0.001>	0.001>	0.05< NS	0.01>	0.01>

Values are means ±SE for 10 rats (N= 10 for each group). Other details are given in materials and methods section.

Rat group with rheumatoid arthritis

In the CII group, the levels of LPO, NO, PGE_2, uric acid, CP and Cu were significantly higher than controls. Contrarily, the levels of SOD, GSH and Zn were significantly lower than controls.

Allopurinol effect

In the CII+ Allo group, the levels of LPO, NO, uric acid, CP and Cu were significantly increased and Zn levels were significantly decreased, while the levels of PGE_2, SOD and GSH did not show significant changes in comparison with controls. On the other hand, the levels of SOD and GSH were significantly increased, but the levels of PGE_2, LPO, NO, uric acid, Cu and CP were significantly decreased and Zn levels were insignificantly increased in comparison with CII–treated group.

Vitamin E effect

In the CII+ Vitamin-E group, the levels of LPO, NO, PGE_2, uric acid, CP and Cu were significantly increased, but the levels of SOD and Zn were significantly decreased and the levels of GSH did not show significant changes in comparison with controls.

In comparison with CII–treated group, the levels of SOD, GSH and Zn were significantly increased, but the levels of PGE_2, NO and CP were significantly decreased in CII+ Vit. E group. Also, the levels of LPO, Cu and uric acid were insignificantly decreased in vitamin-E treated group.

The levels of PGE2, LPO, Cu and Zn were significantly lower in vitamin E treated group than Allo-treated group.

Discussion

Rheumatoid arthritis is an autoimmune disease that causes chronic inflammation of the joints and tissue around the joints with infiltration of macrophages and activated T cells [26]. The pathogenesis of this disease is linked predominantly with the formation of free radicals at the site of inflammation. Heliovaara et al. [8] reported that a low antioxidant level is a risk factor for RA. Some investigations have consistently indicated that the inflammatory process produces oxygen radicals and decreased antioxidant levels, which may worsen the symptoms of the RA [27].

During lipid peroxidation, polyunsaturated fatty acids are oxidized to produce lipid peroxyl radicals that in turn lead to further oxidation of polyunsaturated fatty acid in a perpetuating chain reaction that can lead to cell membrane damage. Matrix degradation arising from cytokine-stimulated chondrocytes was shown to be primarily due to lipid peroxidation, and to be preventable by vitamin E, the primary antioxidant for lipids [28].

Oxidative injury and inflammatory status in various rheumatic diseases were confirmed by the increased levels of prostaglandins in serum and synovial fluid compared to controls [29]. In the current work, the levels of LPO, NO and PGE_2 in RA rat group were significantly higher than controls, but NO and PGE_2 levels were significantly reduced in Allo-, and vitamin E-treated groups. Moreover, the levels of LPO were significantly reduced in Allo-treated groups. Similarly, the levels of plasma LPO were found to be significantly higher in RA than controls in many previous studies [30]. Fermor et al. [31] suggested that many factors such as inflammation and mechanical loading in RA can lead to the increased production of inflammatory mediators such as NO and PGE2. Mahajan and Tandon [15] indicated the increased NO and LPO levels in RA. They proposed antioxidant therapy strategies for the prevention and treatment of RA. Rennie et al. [32] found that vitamin E supplementation increase significantly the levels of antioxidants and decrease the concentration of LPO along with improved symptoms of RA.

Previously, Gambhir et al. [33] reported that an increase in the in vivo generation of oxidants and lipid peroxidation products in the plasma of RA was found to be negatively correlated with the antioxidant levels. The levels of SOD activity in RA rat group in the current study were significantly lower than controls, but they were significantly higher in Allo- and vitamin E-treated groups. This finding is in agreement with previous reports [34]. Similarly, Bae et al. [29] found the SOD activity was significantly lower in RA than controls. Edmonds et al. [35] showed vitamin E supplementation improved clinical symptoms of RA patients. A possible mechanism by which a vitamin E alleviated RA symptoms is the reduction of prostaglandins formation, major molecules produced during the inflammation process. DiSilvestro et al. [36] showed that the administration of anti-inflammatory drugs increases plasma SOD activity, indicating the inflammation process produces free radicals, thereby decreasing SOD

activity. The disease itself may inhibit the activity of SOD and reduce the synthesis of SOD [37]. Also, Thabrew et al. [38] indicated increases in serum SOD activity in RA treated with antioxidant herbal preparations resulted either from the transcriptional activation of these enzymes or the removal of oxidative stress.

In the present study, the levels of CP and Cu were significantly higher in CII group than control group. The levels of CP and Cu were significantly reduced in the groups treated with Allo and vitamin E, except vitamin E for Cu levels in comparison with CII group. Similarly, many investigators found the plasma levels of CP were significantly higher in RA than in controls [21]. Moreover, Amancio et al. [39] found a significant increase of plasma Cu in RA. However, the increase in the antioxidant capacity produced by CP seems unable to cope with the RA-induced oxidative stress, and thus the induced lipid peroxidation is not fully prevented (as indicated by the increase in LPO values). The finding of raised Cu levels in the plasma is to be expected because of a concomitant rise of CP, which is an acute phase reactant [40]. The increased levels of CP observed in the present study may be related to its scavenging action of superoxide radicals that are generated during the inflammatory process of RA.

Acute or chronic inflammatory processes cause an accumulation of copper in many organs, particularly in the inflamed areas. Additionally, a number of biologically active extracellular polypeptides, including cytokines and angiogenic factors, which participate in the pathogenesis and development of inflammatory processes, are known to be involved in trace metal metabolism. Copper plays an important role in the development and maintenance of the immune system [41]. Zoli et al. [40] revealed that IL-1β and tumor necrosis factor-alpha levels significantly correlate with serum copper concentrations. In the recent study, in vivo, copper chelation with tetrathiomolybdate strongly repressed acute inflammation and onset of RA model through the inhibition of mononuclear cell infiltration, and pannus formation [42]. Also, Brewer [43] reported that anticopper therapy such as penicillamine has efficacy in RA.

In the present study, the levels of Zn and GSH were significantly lower in CII group than control group. Moreover, the levels of Zn and GSH were significantly elevated in the vitamin E and Allo-treated groups in comparison with CII group. Previously, Tuncer et al. [44] found plasma zinc levels are decreased significantly in RA. The authors suggested that low plasma Zn levels in RA is one of the non-specific features of inflammation. It has been postulated that low serum zinc may be caused by elevated IL-1 associated with RA [10]. With acute inflammation, the acute phase response may move Zn into the liver and the reduced plasma concentration may not be an indicative of overall deficiency [45]. It is unclear whether chronic cytokine release, as is seen in RA, causes a shift of Zn from one compartment to another or if there is a true Zn depletion.

GSH plays an important role in the protection of cells and tissue structures. Its role includes the detoxication of xenibiotics, free radicals, peroxides and the regulation of immune function [46]. The authors reported that low levels of GSH are implicated in RA. In addition, it is found that Zn-deficient rats have lowered GSH concentrations. This finding may explain the reduction of plasma level of GSH in RA in our study. Moreover, Miesel and Zuber [47] suggested that the participation of xanthin oxidase in the depletion of serum GSH in RA. Also, Hassan et al. [34] found that RA was associated with significant depletion in GSH levels. The effect of GSH and Zn on each other is still controversial according to the available literature.

Uric acid is considered one of the non-enzymatic antioxidants, but increased production of uric acid means increased free radical production due to the activation of the xanthine oxidase enzyme system [48]. The levels of uric acid were significantly higher in our RA rats than control. Moreover, the levels of uric acid were significantly reduced in the groups treated with vitamin E and allopurinol in comparison with RA rats. Smolenska et al. [49] found high levels of uric acid in RA. Forrest et al. [50] suggested that hyperuricemia may enhance some aspects of rheumatoid inflammation, and uric acid may modulate an important component of rheumatoid autoimmunity. Hagfors et al. [51] reported that the inverse correlation between the thrombocyte count and uric acid indicates to the association of uric acid levels with a degree of inflammation. Specific supplementation of oral vitamin E, the major lipid-soluble antioxidant in human plasma, erythrocytes, and tissue, had no effect on RA

disease activity or indices of inflammation, but did improve pain, suggesting a role in central analgesia mechanisms [35]. In a separate study of patients with RA, supplementation with vitamin E increased plasma antioxidant levels with a corresponding decrease in LPO, a marker of oxidative stress; however, a clinical response was not reported [52]. Miesel et al. [53] suggested that antioxidants like allopurinol, which simultaneously modify the oxidative burst of phagocytes, inhibit xanthine oxidase, and display immunosuppressively effects may well be suited to control the consequences of chronic phagocytic hyperreactivity in RA. Namazi [54] reported that Allo, a competitive inhibitor of xanthine oxidase, decreases serum levels of uric acid in autoimmune disorders such as RA. The reaction of uric acid with free radicals, such as hydroxyl radical and hypochlorous acid, results in allantoin production, which suggests that uric acid acts as a free radical scavenger and thus is converted to allantoin. Increased allantoin levels suggest the possible involvement of free radicals in rheumatoid arthritis [55].

In conclusion, this study suggests a proper management of antioxidant intake may reduce free radical generation and improve antioxidant status in RA. Allopurinol and vitamin E may effectively normalize in different degrees the impaired the oxidant/antioxidant system and may be useful in delaying the complication of RA. These antioxidants display considerable potency in anti-inflammatory action and have prominent effects on RA by decreasing PGE_2 level in RA rat model. The anti-inflammatory activity of Allopurinol was most likely comparable to other antioxidants. We recommend that they may be considered in drug therapy of RA.

Acknowledgment

References
[1]   Yossif AM, Ibrahim TM, Salem HA, Gamil NM, El-Sayed LM. Effect of high lipid diet and allopurinol on the development of experimentally induced arthritis in rats. Pharmacology. 1995; 51(3): 160-4.

[2]   Hietala MA, Nandakumar KS, Persson L, Fahlen S, Holmdahl R, Pekna M. Complement activation by both classical and alternative pathways is critical for the effector phase of arthritis. Eur J Immunol. 2004; 34: 1208-1216.

[3]   Wallace JL. Nitric oxide as a regulator of inflammatory processes. Mem Inst Oswaldo Cruz. 2005; 100: 5-9.

[4]   McCartney-Francis NL, Song XY, Mizel DE, Wahl CL, Wahl SM. Hemoglobin protects from streptococcal cell wall-induced arthritis. Arthritis Rheum. 1999; 42: 1119-1127.

[5]   Emery P. Clinical implications of selective cyclooxygenase-2 inhibition. Scand J Rheumatol. 1996; Suppl 102: 23-8.

[6]   Ozkan Y, Yardym-Akaydyn S, Sepici A, Keskin E, Sepici V, Simsek B. Oxidative status in rheumatoid arthritis. Clin Rheumatol. 2007; 26(1): 64-68.

[7]   Mantle D, Falkous G, Walker D. Quantification of protease activities in synovial fluid from rheumatoid and osteoarthritis cases: Comparison with antioxidant and free radical damage markers. Clin Chim Acta. 1999; 284: 45-58.

[8]   Heliovaara M, Knekt P, Aho K, Aaran RK, Alfthan G, Aromaa A. Serum antioxidants and risk of rheumatoid arthritis. Ann Rheum Dis. 1994; 53: 51-3.

[9]   **Milanino R, Frigo A, Bambara LM, Marrella M, Moretti U, Pasqualicchio M, Biasi D, Gasperini R, Mainenti L, Velo GP. Copper and zinc status in rheumatoid arthritis: Studies of plasma, erythrocytes and urine, and their relationship to disease activity markers and pharmacological treatment. Clin Exp Rheumatol. 1993; 11: 271-281.**

[10] Honkanen V, Konttinen YT, Sorsa T, Hukkanen M, Kemppinen P, Santavirta S, Saari H, Westermarck T. Serum zinc, copper and selenium in rheumatoid arthritis. J Trace Elem Electrolytes Health Dis. 1991; 5(4): 261-3.

[11] Kremer JM, Bigaoutte J. Nutrient intake of patients with rheumatoid arthritis is deficient in pyridoxine, zinc, copper, and magnesium. J Rheumatol. 1996; 23: 990-994.

[12] Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. Physiol Rev. 1993; 73(1): 79-118.

[13] Halliwell B, Gutteridge JM. The antioxidants of human extracellular fluids. Arch Biochem Biophys. 1990; 280: 1-8.

[14] Giurgea N, Constantinescu MI, Stanciu R, Suciu S, Muresan A. Ceruloplasmin: Acute-phase reactant or endogenous antioxidant? The case of cardiovascular disease. Med Sci Monit. 2005; 11(2): RA48-51.

[15] Mahajan A, Tandon VR. Antioxidants and rheumatoid arthritis. J Indian Rheumatol Assoc. 2004; 12: 139-142.

[16] Bandt MD, Grossin M, Driss F, Pincemail J, Babin-Chevaye C, Pasquier C. Vitamin E uncouples joint destruction and clinical inflammation in a transgenic mouse model of rheumatoid arthritis. Arthritis Rheum. 2002; 46: 522-532.

[17] Hegen M, Gaeste M, Telliez J-B. MAPKAP Kinase 2-deficiencent mice are resistant to collagen-induced arthritis. J Immunol. 2006; 177: 1913-1917.

[18] Can C, Cinar MG, Koşay S, Evinç A. Vascular endothelial dysfunction associated with elevated serum homocysteine levels in rat adjuvant arthritis: Effect of vitamin E administration. Life Sci. 2002; 14, 71(4): 401-10.

[19] Klocke R, Mani AR, Moore KP, Blake DR, Mapp PI. Inactivation of xanthine oxidoreductase is associated with increased joint swelling and nitrotyrosine formation in acute antigen-induced arthritis. Clin Exp Rheumatol. 2005; 23(3): 345-50.

[20] Thayer WS. Serum lipid peroxides in rats treated chronically with adiramycin. Biochem Pharmacol. 1985; 33(14): 2259-2263.

[21] Van Beezooijen RL, Que I, Ederveen AG, Kloosterbor HJ. Plasma nitrate + nitrite level are regulated by ovarian steroids but do not correlate with trabecular bone mineral density in rats. J Endocrinol. 1988; 159: 27-34.

[22] Ellman GL. Tissue sulfhydryl groups. Archives Biochem Biophys. 1959; 82: 70-77.

[23] Misra HP, Fridovich I. The generation of superoxide radical during the autoxidation of hemoglobin. J Biol Chem. 1972; 247(21): 6960-2.

[24] Houchin OB. Clin Chem. 1959; 4: 519-523. Quoted in: Practical Clinical Enzymology. Ed. King J. Van Nostrand Company, London. 1959; pp. –1177.

[25] Fossati P, Prencipe L, Berti G. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem. 1980; 26(2): 227-31.

[26] Bauerova K, Bezek A. Role of reactive oxygen and nitrogen species in etiopathogenesis of rheumatoid arthritis. Gen Physiol Biophys. 1999; 18 Spec No: 15-20.

[27] Darlington LG, Stone TW. Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. Br J Nutr. 2001; 85: 251-269.

[28] Tiku ML, Shah R, Allison GT. Evidence linking chondrocyte lipid peroxidation to cartilage matrix protein degradation: Possible role in cartilage aging and the pathogenesis of osteoarthritis. J Biol Chem. 2000; 275: 20069-20076.

[29] Bae SC, Kim SJ, Sung MK. Inadequate antioxidant nutrient intake and altered plasma antioxidant status of rheumatoid arthritis patients. J Am Coll Nutr. 2003; 22(4): 311-5.

[30] Ozturk HS, Cimen MY, Cimen OB, Kacmaz M, Durak I. Oxidant/antioxidant status of plasma samples from patients with rheumatoid arthritis. Rheumatol Int. 1999; 19: 35-7.

[31] Fermor B, Christensen SE, Youn I, Cernanec JM, Davies CM, Weinberg JB. Oxygen, nitric oxide and articular cartilage. Eur Cell Mater. 2007; 13: 56-65.

[32] Rennie KL, Hughes J, Lang R, Jebb SA. Nutritional management of rheumatoid arthritis: A review of the evidence. J Hum Nutr Diet. 2003; 16(2): 97-109.

[33] Gambhir JK, Lali P, Jain AK. Correlation between blood antioxidant levels and lipid peroxidation. Clin Biochem. 1997; 30: 351-5.

[34] Hassan MQ, Hadi RA, Al-Rawi ZS, Padron VA, Stohs SJ. The glutathione defense system in the pathogenesis of rheumatoid arthritis. J Appl Toxicol. 2001; 21: 69-73.

[35] Edmonds SE, Winyard PG, Guo R, Kidd B, Merry P, Lnagrish-Smith A, Hansen C, Ramm S, Blake DR. Putative analgesic activity of repeated oral doses of vitamin E in the treatment of rheumatoid arthritis: Results of a prospective placebo controlled double blind trial. Ann Rheum Dis. 1997; 56: 649-655.

[36] DiSilvestro RA, Marten J, Skehan M. Effects of copper supplementation on ceruloplasmin and Cu/Zn superoxide dismutase in free-living rheumatoid arthritis patients. J Am Coll Nutr. 1992; 11: 177-180.

[37] Puscas I, Coltau M, Baican M, Domuta G. Omeprazole has a dual mechanism of action: It inhibits both H(+)K(+)ATPase and gastric mucosa carbonic anhydrase enzyme in humans (in vitro and in vivo experiments). J Pharmacol Exp Ther. 1999; 290: 530-534.

[38] Thabrew MI, Senaratna L, Samarawickrema N, Munasinghe C. Antioxidant potential of two polyherbal preparations used in Ayurveda for the treatment of rheumatoid arthritis. J Ethnopharmacol. 2001; 76: 285-291.

[39] Amancio SOM, Chaud ADM, Yanaguibashi G, Esteves Hilario MO. Copper and zinc intake and serum levels in patients with juvenile rheumatoid arthritis. Eur J Clin Nutr. 2003; 57(5): 706-12.

[40] Zoli A, Altomonte L, Caricchio R, Galossi A, Mirone L, Ruffini MP, Magaro M. Serum zinc and copper in active rheumatoid arthritis: Correlation with interleukin 1 beta and tumour necrosis factor alpha. Clin Rheumatol. 1998; 17: 378-382.

[41] Percival SS. Copper and immunity. Am J Clin Nutr. 1998; 67: 1064-1068.

[42] Omoto IA, Kawahito Y, Prudovsky I, Tubouchi Y, Kimura M, Ishino H. Copper chelation with tetrathiomolybdate suppresses adjuvant-induced arthritis and inflammation-associated cachexia in rats. Arthritis Research & Therapy. 2005; 7: R1174-R1182.

[43] Brewer GJ. Anticopper therapy against cancer and diseases of inflammation and fibrosis. Drug Discovery Today. 2005; 10: 1103-1109.

[44] Tuncer S, Kamanli A, Akcil E, Kavas GO, Seckin B, Atay MB. Trace element and magnesium levels and superoxide dismutase activity in rheumatoid arthritis. Biol Trace Elem Res. 1999; 68(2): 137-42.

[45] Shenkin A. Trace elements and inflammatory response: Implications for nutritional support. Nutrition. 1995; 11: 110-15.

[46] Ganesan N, Chegu H, Chandrasekaran AN. Effect of type II collagen treatment on the antioxidant status in immune tissues of adjuvant induced arthritic rats. Indian J Clin Biochem. 2003; 18(2): 216-222.

[47] Miesel R, Zuber M. Elevated levels of xanthine oxidase in serum of patients with inflammatory and autoimmune rheumatic diseases. Inflammation. 1993; 17(5): 551-61.

[48] Nemeth I, Talosi G, Papp A, Boda D. Xanthine oxidase activation in mild gestational hypertension. Hypertens Pregnancy. 2002; 21(1): 1-11.

[49] Smolenska Z, Kaznowska Z, Zarowny D, Simmonds HA, Smolenski RT. Effect of methotrexate on blood purine and pyrimidine levels in patients with rheumatoid arthritis. Rheumatology. 1999; 38(10): 997-1002.

[50] Forrest CM, Harman G, McMillan RB, Rana C, Shaw S, Stone TW, Stoy N, Darlington LG. Purine modulation of cytokine release during diuretic therapy of rheumatoid arthritis. Nucleosides Nucleotides Nucleic Acids. 2004; 23(8-9): 1107-10.

[51] Hagfors L, Leanderson P, Skoldstam L, Andersson J, Johansson G. Antioxidant intake, plasma antioxidants and oxidative stress in a randomized, controlled, parallel, Mediterranean dietary intervention study on patients with rheumatoid arthritis. Nutr J. 2003; 30, 2(5): 1-11.

[52] Jaswal S, Mehta HC, Sood AK, Kaur J. Antioxidant status in rheumatoid arthritis and role of antioxidant therapy. Clin Chim Acta. 2003; 338: 123-129.

[53] Miesel R, Zuber M, Sanocka D, Graetz R, Kroeger H. Effects of allopurinol on in vivo suppression of arthritis in mice and ex vivo modulation of phagocytic production of oxygen radicals in whole human blood. Inflammation. 1994; 18(6): 597-612.

[54] Namazi MR. Cetirizine and allopurinol as novel weapons against cellular autoimmune disorders. Int Immunopharmacol. 2004; 4(3): 349-53.

[55] Yardim-Akaydin S, Sepici A, Ozkan Y, Torun M, Simşek B, Sepici V. Oxidation of uric acid in rheumatoid arthritis: Is allantoin a marker of oxidative stress? Free Radic Res. 2004; 38(6): 623-8.