تأثيرات بعض مضادات الاكسدة على المؤشرات الحيوية في التهاب المفاصل المحدثة تجريباً عند الجرذان

صبحي البحري*

الملخص

خلفية البحث: إن حقن الجرذان بالكولاجين -2 يؤدي الى إحداث التهاب مفاصل، وهذا الالتهاب يتميز بالتبدلات الكيميائية المرضية والفيسيولوجية المرضية نفسها للداء الرثواني عند الإنسان. قمنا في هذا البحث بدراسة التبدلات الكيميائية والمرضية لكل من الفيتامين ه والالوبورينول على الجرذان التي أحدث عندها نموذج التهاب مفاصل رثواني. تمت دُرِسَتْ مؤشرات ضغط الأكسدة والبروستاغلاندين ي-2 عند الجرذان جميعها.

الطرائق: أُجْرِيَتِ الدراسة على40 جرذاً ذكراً قسمت إلى4 مجموعات (10جرذان في كل مجموعة): مجموعة الشاهد، مجموعة الكولاجين -2، مجموعة الكولاجين-2 مع الألوبورينول،مجموعة الكولاجين -2 مع الفيتامين ه. قِيسَتْ مستويات البلازما لكل من وأكسيد النيتريك، الأ كسدة الفائقه للدسم، سيرولوبلازمين، سوبر وأكسيد ديزموتاز،حمض البول،الغلوتاثيون بعد 6 أسابيع من إعطاء مضادات الأكسدة باستخدام طريقة القياس الضوئي.أيضاً قِيسَتْ مستويات البروستاغلاندين ى2 باستخدام طريقة الاليزا، وقِيسَ مستوى النحاس والتوتياء باستخدام الامتصاص الذري.

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النتائج: في مجموعة الكولاجين -2 كانت مستويات الأكسدة الفائقة للدسم ووأكسيد النيتريك والبروستاغلاندين وحمض البول والسيرولوبلازمين والنحاس مرتفعة بدلالة إحصائية، لكن مستويات سوبر اوكسيد ديزموتاز وغلوتاثيون و التوتياء كانت منخفضة بشكل له دلالة إحصائية عن الشاهد. في المجموعة التي أعطيت الألوبورينول بعد الكولاجين -2 كانت مستويات الغلوتاثيون وسوبر وأكسيد ديزموتاز مرتفعة بمدلول إحصائي لكن البروستاغلاندين والأكسدة الفائقة للدسم ووأكسيد النتريك وحمض البول والنحاس والسيرولوبلازمين كانت منحفضة بمدلول إحصائي مقارنة بالمجموعة التي أعطيت الكولاجين -2.مستويات سوبر أوكسيد ديزموتاز، الغلوتاثيون والتوتياء كانو مزدادين بمدلول إحصائي لكن البروستاغلاندين والسيرولوبلازمين وأوكسيد النيتريك كانت منخفضة بمدلول إحصائي في مجموعة الفيتامين ه.

فضلاً عن ذلك كانت هناك تبدلات نسيجية بين مختلف المجموعات.

الخلاصة: يمكن الاستنتاج بأنَّ إعطاء كمية من مضادات الأكسدة يمكن أن تقلل من تشكيل الجذور الحرة ويحسن التهاب المفاصل الرثواني. الالوبورينول والفيتامين ه يمكن أن يؤثر بدرجات مختلف في إعادة اضطراب جهاز الاكسدة /مضاد الاكسدة الي الحالة الطبيعية ويمكن أن تكون مفيدة في تأخير مضاعفات الداء الرثواني. فضلاً عن ذلك فإنَّ مضادات الأكسدة هذه أدّت دوراً كمضادات للالتهاب بالتقليل من الوذمة في القدم وإنقاص مستويات البروستاغلاندين ى2 في الداء الرثواني.

Effect of Some Antioxidants on Bio-Indices in Arthritis of Experimentally Induced in Rats

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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 and histopathological effects of vitamin E, allopurinol (Allo), on RA of rats were investigated. The oxidative stress indices and prostaglandin E were evaluated.

Methods and Material: Forty male rats were divided into four groups (10 rats each): control group, collagen II -induced RA group (C II group), CII group treated with allopurinol (C II+ Allo), CII group treated with vitamin E (C II + Vit E) . After 6 weeks of antioxidants treatment, the plasma levels of lipid peroxides (LPO), nitric oxide (NO), ceruloplasmin (PC), superoxide dismutase (SOD), uric acid (UA) and glutathione (GSH) were detected using colorimetric methods. The plasma levels of prostaglandins E_2 (PGE_2) were measured using ELISA. The plasma levels of copper (Cu) and zinc (Zn) were determined using atomic absorption/flame emission spectrometer.

Results: In C II group, the levels of LPO, NO, PGE_2, UA, CP and Cu were significantly higher but the levels of SOD, GSH and Zn were significantly lower than controls. In C II + Allo treated group, the levels of SOD and GSH were significantly increased but the levels of PGE_2, LPO, NO, UA, Cu and CP were significantly decreased in comparison with C II group. The levels of SOD, GSH and Zn were significantly increased but the levels of PGE_2, NO and CP were significantly decreased in the vitamin E treated group. The histological changes were comparable among different groups.

In conclusion, our study suggests that antioxidant treatment may reduce generation of free radicals and improve RA. allopurinal and vitamin E may effectively normalize in different degrees the impaired oxidant/ antioxidant system and may be useful in delaying the complication of RA. Moreover, these antioxidants display anti-inflammatory action by alleviating foot swelling and decreasing PGE2 level in RA

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Introduction:

Rheumatoid arthritis (RA) is a polyarticular disease affecting about 1 % of the population of the word. It is an autoimmune disease characterized by chronic inflammation, progressive joint destruction, physical impairment, work disability and early mortality [(1)]. The process of disease progression is characterized by hyperplasia of synoviocytes, mainly of synovial fibroblasts, resulting in bone and joint destruction [(2).] However, the proliferation of synovial cells is not limitless and spontaneous suppression of synovial proliferation has been observed [(3).]

Immunization of mice with collagen II (CII) leads to the development of arthritis. 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[(4,5).]

Inflammation is known to result in increased production of nitric oxide (NO) and prostaglandins [(6)]. NO is an important mediator of diverse physiologic and pathologic processes, including arthritis [(7)]. 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 [(8).]

Prostaglandins are well known as proinflammatory mediators, and inhibition of cyclooxygenase (COX) has long been 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 [(9).]

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 results of the pro-oxidant/antioxidant imbalance in RA [(10)]. It was demonstrated that the level of free radical-induced damage to proteins in the synovial fluid was twice as high in RA [(11)]. 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 [12]. The two most often suggested mechanisms for the increased incidence and activity of free radicals in RA joints are: (i) the production of various free radicals, such as superoxide, hydroxyl and hypochlorus by the invading phagocytes [13]; and (ii) an increase in the intra-articular pressure above the synovial capillary perfusion pressure, causing intra-articular hypoxia.On cessation of exercise of the RA-inflamed joint, an injurious reperfusion mechanism occurs, resulting in oxidative damage to lipids and immunoglobulin within the joint [14].

Zinc (Zn) is a crucial element in a series of cellular functions as normal growth, protein metabolism, membrane stability, and metalloenzyme functions [15]. In addition, Zn has several other effects on immune response, complement system, lysozomal enzyme release, and macrophage functions [16]. Zn is also indispensible in many steps of the inflammatory reactions. Among these are prostaglandin biosynthesis, stimulation of lymphocytes and immune response, and scavenging of toxic free oxygen radicals. Zn is likewise an important element in collagen tissue formation and bone metabolism [16].

Copper (Cu) is abundant in the human body and nature[17]. Cu is incorporated into the structure of a great many enzymes and proteins [16]. It is reported that 30 to 50% increases in serum Cu level during an acute phase response triggered by interleukin -1 (IL-1) release largely depend upon 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 [18].

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. Most notable among these are; metallothioneins, CP, and superoxide dismutase (SOD) [19]. Intracytoplasmic SOD includes both Cu and Zn, while CP is a powerful antioxidant in serum carries only Cu [17]. Substantial alterations in metabolisms of Cu and Zn occur through some physiological control mechanisms over an inflammatory reaction [15]

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 prove beneficial in several pathological conditions[20]. 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 [21].

Some functions of CP can be inactivated by exposure to the free oxygen radical (FR) flux generated by the hypoxantine/xantine oxidase system[21]. A significant inactivation of CP occurs during oxidative stress, this phenomenon being associated with the uncoupling of copper atoms from the CP molecule. It is likely that this is why an alteration of the CP can lead to the extension of FR-mediated dysfunction to other molecules also exposed to oxidative stress. The antioxidant activity of CP has been reported in several studies, and there are reasons to believe that this is one of the most important functions of CP during inflammatory and acute-phase reactions [21].

In view of the recent 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 [22]. Various forms of antioxidant therapy have demonstrated promising results in experimental RA models [23,24&25].

Aim of work:

This work aims to the effect of antioxidants as vitamin E, allopurino on rat model of RA was investigated. So, the plasma levels of lipid peroxides, NO, PGE_2, glutathione (GSH), SOD, CP, uric acid, Cu and Zn were determined. Moreover, the histopathological examination was associated with biochemical evaluation.

Materials and Methods

1-Chemicals

Allopurinol,alpha tocopherol, thiobarbituric acid, reduced glutathione, naphthylenediamine dihydrochloride, sulphanilamide, sodium nitrite, sodium azide, 5,5-dithio bis (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.

2-Animals and experimental design

Forty healthy male albino rats (Rattus norvegicus) with average body weight 150–170 gm were utilized for this study. They were obtained from King Saud University college of medicine (Ryadh). All animals were conditioned at room temperature at natural photoperiod for 1 week before the start of the experiment. A commercial balanced diet and tap water ad libitum were provided. The duration of experiment was 40 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 1mg/ml heat-killed Mycobacterium tuberculosis (Sigma-Aldrich). The initial immunization with100µg/100µl emulsion induced rheumatoid arthritis 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 100µg/100µl of bovine CII emulsified in incomplete Freund's adjuvant (IFA) [26].

(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.) [27] beginning with the day of adjuvant injection for 45days.

(4) CII + Allopurinol – treated group (CII+ Allo group) was also injected with CII, and received allopurinol (50 mg /kg /day/ I.P.) [28&29] of beginning with the day of adjuvant injection and was continued until the 45th day of the experiment.

All the tested antioxidants (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 min for plasma separation. The plasma sample was divided into aliquots and kept at -26 ^{o}C until biochemical analyses. The joints were excised immediately after scarification and washed in ice-cold isotonic saline for histological examination.

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 [30]. 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 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 550nm against reagent blank using sodium nitrite as standard [31]. The plasma GSH levels were determined chemically as described by some authors [32]. The plasma SOD activity was determined according to its ability to inhibit the autooxidation of epinephrine at alkaline medium[33]. The plasma CP activity was determined using a para-phenylenediamine dihydrochloride method [34]. The plasma uric acid level was determined by enzymatic colorimetric method [35].

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 specific cathode lamps were used. Three determinations were made fro each solution. The accuracy and precision of the analytical methods were tested with standard reference materials.

Histopathological studies:

The red, edematous and swollen knee joint were excised and fixed in 10% neutral buffer formalin and processed for histopathological examination. Section of 4 microns thickness were stained routinely with HάE-stain [36]

Statistical analysis

The results were 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. Fig. (1) shows the plasma levels of: (A) PGE_2, (B) LPO, (C) NO, (D) Glutathione, (E) SOD and (F) Uric acid in different rat groups. Fig. (2) shows the plasma levels of: (A) Copper, (B) Ceruloplasmin and (C) Zinc in different rat groups.

Rat group with rheumatoid arthritis: In the C II 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 C II + Allo group**,** the levels of LPO, NO, uric acid, CP and Cu were significantly increased and Zn levels were significantly decreased while levels of PGE_2, SOD and GSH did not show significantly 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 C II –treated group.

Vitamin E effect: In the C II + Vit. C group and C II + Vit. E group**,** the levels of LPO, NO, 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 significantly changes in comparison with controls. The levels of PGE2 were significantly increased in C II + Vit. E group.

In comparison with C II –treated group, the levels of SOD, GSH and Zn were significantly increased but the levels of PGE2, NO and CP were significantly decreased in C II + Vit. E group. In addition, the levels of LPO and uric acid were insignificantly decreased in both antioxidant vitamin treated group. The levels of Cu were insignificantly decreased in C II + Vit. E group.

Table (1) Comparison of plasma levels of bioindices among different treated rat groups and control groups .

Parameters	(A) Controls	(B) CII-treated Group	(C) CII + Allo. Treated group	(D) CII + vitamin E-treated Group	**A vs B**	**A vs C**	**A vs D**	**B vs C**	**B vs D**
PGE2 (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
LPO (nmol/ml)	3.218 ± 0.441	8.000 ± 1.193	4.491 ± 0.355	5.520 ± 0.289	<0.01	<0.05	<0.001	<0.05	>0.05 NS
NO (ng/ml)	3.619 ± 0.215	8.520 ± 1.248	5.721 ± 0.366	4.758 ± 0.3466	<0.001	<0.001	<0.05	<0.05	<0.01
GSH (nmol/ml)	4.265 ± 0.249	2.749 ± 0.306	4.692 ± 0.396	4.354 ± 0.258	<0.01	>0.05 NS	>0.05 NS	<0.01	<0.001
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
Uric acid (mg/ml)	4.319 ± 0.139	8.422 ± 0.853	5.594 ± 0.298	6.593 ± 0.534	<0.001	<0.01	<0.001	<0.01	>0.05 NS
Copper (g/ml)	2.374 ± 0.098	3.645 ± 0.166	2.923 ± 0.139	3.557 ± 0.188	<0.001	<0.01	<0.001	<0. 01	>0.05 NS
Ceruloplasmin (mg/dl)	95.860 ± 11.320	225.500 ± 13.090	135.400 ± 12.980	166.700 ± 13.070	<0.001	<0.05	<0.001	<0.001	<0.01
Zinc (g/ml)	3.662 ± 0.311	0.616 ± 0.078	0.6832 ± 0.088	1.334 ± 0.199	<0.001	<0.001	<0.001	>0.05 NS	<0.01

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

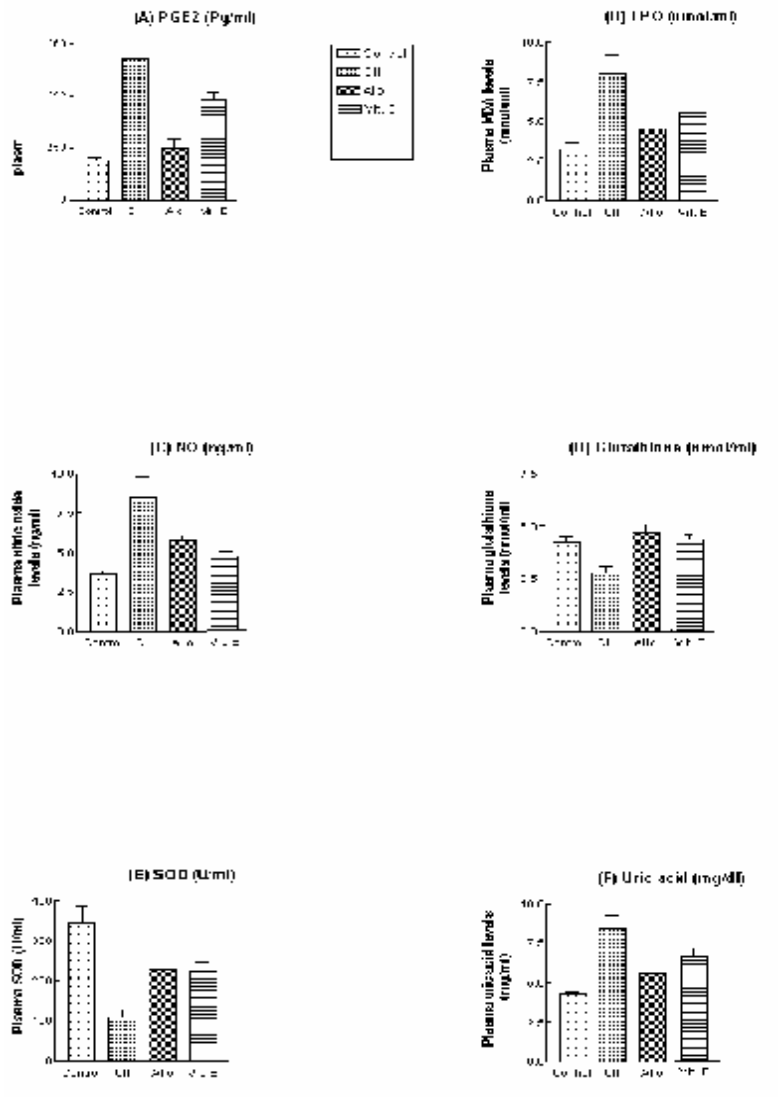


Fig. (1) shows the plasma levels of: (A) PGE2, (B) LPO, (C) NO, (D) Glutathione, (E) SOD and (F) Uric acid in different rat groups

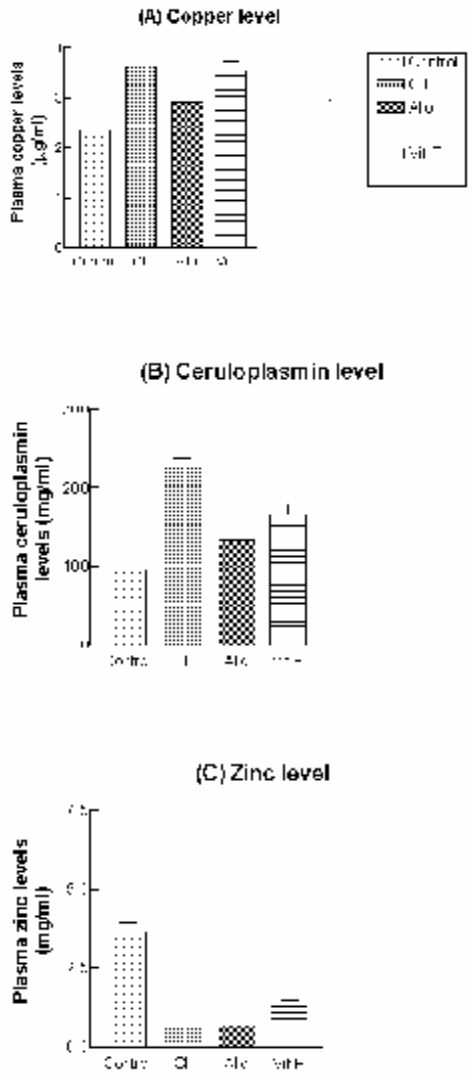


Fig.(2)shows the plasma levels of: (A) Copper, (B) Ceruloplasmin and (C) Zinc in Different rat groups

Histological Results

The Figs. (3-7) show the clinical changes of joints in lower limbs (edema, swelling, and erythmatous) in different rat groups.

The classical histological alterations due to RA showed in the present study as follows:

In cases treated with CII alone the following histological changes were observed:

1.Superficial striated (skeletal) muscles trophy with focal necrosis and mononuclear leukocytic infiltrations.

2. The synovial membrane lining the joint showed the following:

a.Numerous villi and frond-like projections that fill the peripheral recesses of the joint.

b. Edema, and fibrinous exudation.

c. Accumulation of plasma cells, macrophages and giant cells.

d. Eosinophilic infiltration of the subsynovial stroma.

e.The cells lining the synovium showed hypertrophy, hyperplasia, necrosis and lymphocytic aggregations (Fig.8-16).

3. Concerning the articular cartilage the following changes were seen:

a. Severe degeneration, erosion and necrosis,

b. Subchondral congestion and haemorrhages.

c.Eburnation (complete destruction of articular cartilage that replaced by the bony surface prominence) also observed,

d. Development of subchondral cysts (Fig. 17-23).

4.Regarding the osseous structural changes the following were observed:

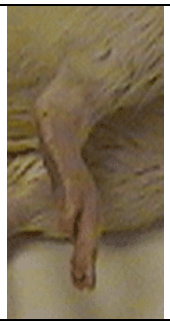
a. Dgeneration, formation of empty lacunae (dead osteocytes),

b. Thinning of periosteum

c. Increased osteoclastic activity in the underlying bone.

d.The bone marrow showed advanced fatty change with little lymphoid elements.

In cases treated with CII and vitamin E or CII and allopurinol a less destructive histological change were seen compared to changes seen in cases treated with CII alone. These changes were represented by mild to moderate degree of inflammatory cells infiltrations (plasma cells, macrophages, giant cells and eosinophils)in the subsynovium stroma. There was increase in the number of lymphoid cells of bone marrow, decrease in osteoclastic activity, less fibrin deposits, little necrosis, no subchondral cyst observed (Fig.24,25).



Fig(3):Normal joints

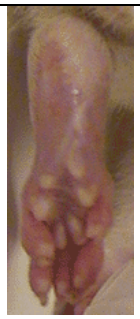


Fig. 4 (CII) Red,edematous,swelling. hyperplastic and transformation of the smooth contour of the joint surface

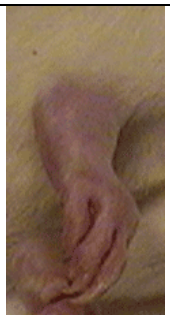


Fig.5(CII) Red,edematous,swelling.hyperplastic and transformation of the smooth contour of the joint surface

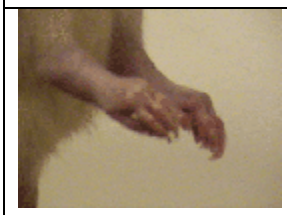


Fig.6(CII+vitaminE)

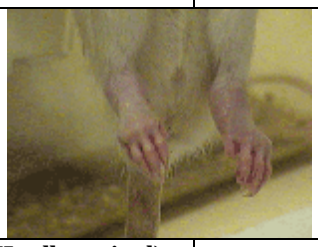


Fig.7(CII+allopurinol)

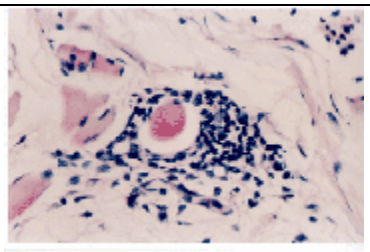
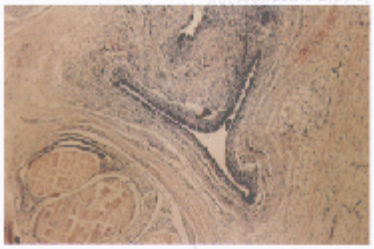
	

Fig. (8) Skeletal muscle of rats were injected with CII,showing:atrophy an necrotic myositis. Hα Ex. 400.	**Fig. (9)Synovial joint membrane of rats were injected with CII,showing:numerous villi and folds Hα Ex. 400..**

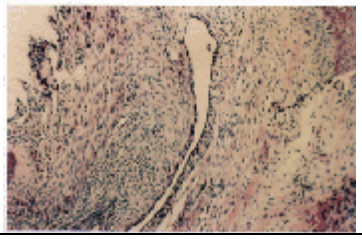
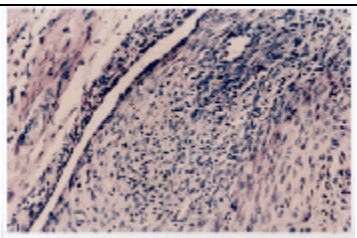

	

Fig. (10) Synovial joint membrane of rats were injected with CII,showing:edema, fibrin exudation, necrosis with inflammatory cells infiltration Hα Ex. 100.	**Fig. (11) A high power of Fig.(10) Hα Ex. 200.**

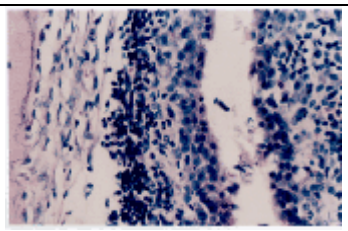
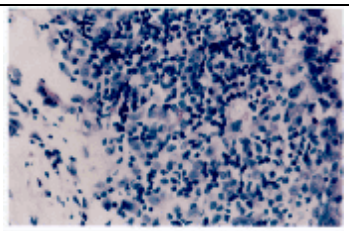
	

Fig. (12) Joint of rats ,were injected with CII showing hyperplasia,hypertrophy, Mitotic figure,necrosis of the synovial lining cells besides lymphocytic aggregation Hα Ex. 400.	**Fig. (13) Joint of rats ,were injected with CII showing mononuclear cells infiltration and edema in the subsynovial stroma Hα Ex. 400.**

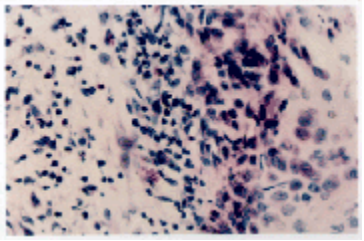
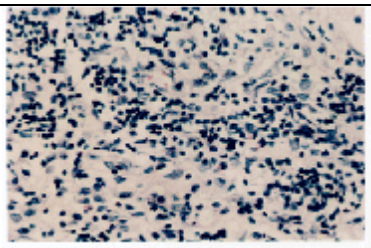
	

Fig. (14) Joint of rats ,were injected with CII showing macrophages, giant cells and eosinophils infiltration in the subsynovial stroma(Allergic synovitis).Hα Ex.400.	**Fig. (15) Joint of rats ,were injected with CII showing lymphocytic cells infiltration and edema in the underlying stroma Hα Ex. 400**

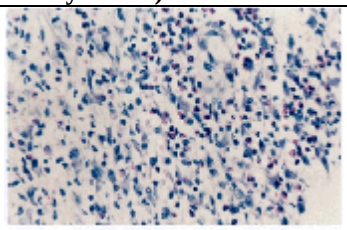
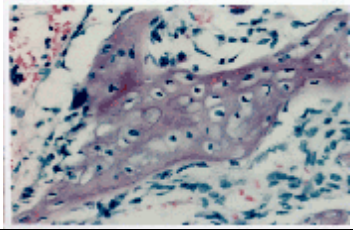

	

Fig. (16) Joint of rats ,were injected with CII showing extensive eosinophils infiltration in subsynovial stroma . Hα Ex. 400.	**Fig. (17) Articular cartilage,(hyaline C)of rats were injected with CIIshowing degeneration,necrosis ,congestion and haemorrhages(subchondral) Hα Ex. 400.**

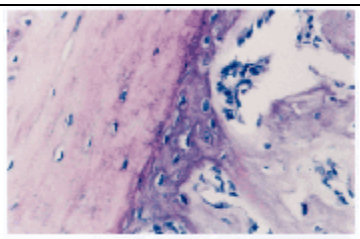
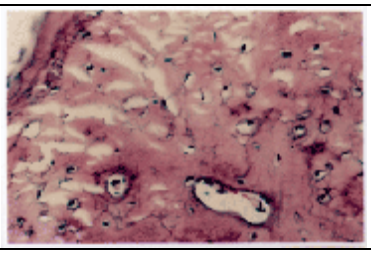
	

Fig. (18) Articular cartilage,(hyaline C)of rats were injected with CII showing:Eburnation. Hα Ex. 400.	**Fig. (19) Subchondral bone of rats were injected with CII,showing destructive bone changes"degeneration and dead osteocytes". Hα Ex. 400**

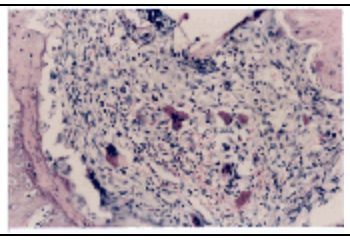
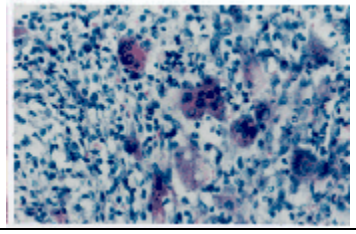

	

Fig. (20) Subchondral bone of rats were injected with CII,showing osteoclastic hyperactivity, increaseing thevascularity with leukocytic cells infiltration (pannus formation). Hα Ex. 200.	**Fig. (21)A high power of Fig.20 Hα Ex. 400.**

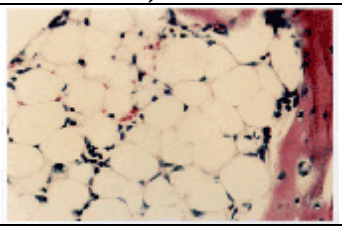
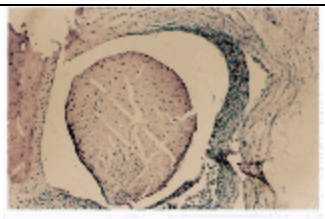

	

Fig. (22) Subchondral bone of rats were injected with CII, showing fatty bon marrow with little lymphoid alls. Hα Ex. 400.	**Fig. (23) Subchondral bone of rats were injected with CII,showing subchondral bone cyst . Hα Ex. 100.**

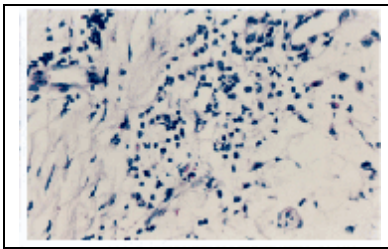


Fig. (24) Synovial stromal membrane, of rats were injected with CII and vitamin E,showing mild to moderate leukocytic cells infiltration. Hα Ex. 400

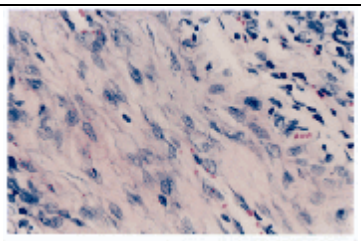


Fig. (25) Subsynovium of rats were treated with CII and allopurinol, showing moderate degree of infiltrated of inflammatory cells besides edema. Hα Ex. 400.

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 (Bauerova and Bezek, 1999)(36). The pathogenesis of this disease is linked predominantly with the formation of free radicals at the site of inflammation. Heliovaara et al., (1994)(12) reported that a low antioxidant level is a risk factor for RA.

The present study was performed to evaluate the effect antioxidants on RA rats and to assess oxidative stress markers in the blood. Recent investigations have consistently indicated that the inflammatory process produces oxygen radicals and decreased antioxidant levels, which may worsen the symptoms of the RA (Darlington and Stone, 2001)(38).

Lipid peroxidation has been implicated in the pathogenesis of degenerative diseases, and inflammatory arthritis. 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 (Tiku et al., 2000)(39).

Oxidative injury and inflammatory status in various rheumatic diseases was confirmed by increased levels of prostaglandins in serum and

synovial fluid compared to controls (Bae et al., 2003)(40). 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 all antioxidants (Allo -, and, Vit E) - treated groups. Moreover, levels of LPO were significantly reduced in Allo- ,treated group. Similarly, the levels of plasma LPO were found to be significantly higher in RA than controls in many previous studies (Kiziltunc et al., 1998 and Ozturk et al., 1999)(41&42). Fermor et al. (2007)(43) suggested that many factors such as inflammation and mechanical loading in RA can lead to increased production of inflammatory mediators such as NO and PGE2. Mahajan and Tandon (2004)(21) indicated the increased NO and LPO levels in RA. They proposed antioxidant therapy strategies for the prevention and treatment of RA. Jaswal et al., (2003)(44) and Rennie et al., (2003)(45) found that vitamin E and supplementation increase significantly the levels of antioxidants and decrease the concentration of LPO along with improved symptoms of RA.

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 (Gambhir et al., 1997)(46).

The levels of SOD activity in RA rat group were significantly lower than controls but they were significantly higher in antioxidants (Allo -, and, Vit E) - treated groups. This finding is in agreement with previous reports (Cimen et al., 2000 and Hassan et al., 2001)(47&48). Similarly, Bae et al. (2003)(40) found the SOD activity was significantly lower in RA than controls. Edmonds et al. (1997)(49) showed vitamin E supplementation improved clinical symptoms of RA patients. A possible mechanism by which a vitamin E alleviated RA symptoms is reduced formation of prostaglandins, major molecules produced during the inflammation process. DiSilvestro et al. (1992)(50) showed that the administration of anti-inflammatory drugs increases plasma SOD activity, indicating the inflammation process produces free radicals, thereby decreasing SOD activity. Disease itself may inhibit the activity of SOD and reduce the synthesis of SOD (Puscas et al., 1999)(51). Also, Thabrew et al. (2001)(52) indicated increases in serum SOD activity in RA treated with antioxidant herbal preparations resulted either from transcriptional activation of these enzymes or removal of oxidative stress.

Ceruloplasmin (CP) is considered the principle plasma and synovial antioxidant in RA, being responsible for up to 70% of the protective capacity against superoxide free radicals (Biemond et al., 1984)(53), which have been shown to be directly related to the pathogenesis of the inflamed joint in RA and the related increases in lipid peroxidation, ascorbate depletion and hyaluronate degradation (Halliwell, 1995)(54). The functions of CP include copper transport, iron metabolism, antioxidant defense, and involvement in angiogenesis and coagulation. It has been shown that CP catalyzes the oxidation of Fe^{2+} to Fe^{3+}, with a catalytic cycle that involves four of the six atoms of copper associated to CP, and uses dioxygen as an electron acceptor without the mediation of an incompletely reduced reactive oxygen species such as $O_2^{-\bullet}$ or H_2O_2 (Kang et al. 2001)(55). This activity as ferroxidase is increased during inflammation, infections, and other conditions, and these observations seem to suggest that there is a possibility that CP acts both as an antioxidant and an acute-phase reactant (Kang et al. 2001)(55).

In the present study, the levels of CP and Cu were significantly higher in C II group than control group. The levels of CP and Cu were significantly reduced in all groups treated with Allo-and , Vit E- groups except Vit E for Cu levels in comparison with C II group. Similarly, many investigators found the plasma levels of CP were significantly higher in RA than in controls (Kiziltunc et al., 1998; Ozturk et al., 1999 and Vijayakumar et al., 2006)(56,57&58). Moreover, Amancio et al., (2003) (59)found the 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 (Zoli et al., 1998)(60). 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 (Percival, 1998)(61). 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 development and maintenance of the immune system (Percival, 1998)(61). Zoli et al., (1998)(60) revealed that IL-1β and TNF-α 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 inhibition of mononuclear cell infiltration, and pannus formation (Omoto et al., 2005)(62). Also, Brewer (2005)(63) reported that anticopper therapy such as penicillamine has efficacy in RA.

Zinc is metal antioxidant and it is required in over 200 enzymes and so deficiency is likely to affect a number of different systems. Zinc, like copper, is a component of CuZn-superoxide dismutase, an important antioxidant enzyme. Zinc has a stabilising effect on membranes possibly by displacing bound transition metal ions and thereby preventing peroxidation of membrane lipids (Evans, and Halliwell, 2001)(64).

In the present study, the levels of Zn and GSH were significantly lower in C II group than control group. Moreover, the levels of Zn and GSH were significantly elevated in all groups treated with Vit E in comparison with C II groups. Previously, Tuncer et al., (1999)(65) found plasma zinc levels are decreased significantly in RA. The authors suggested that low plasma Zn levels in RA is one of the nonspecific features of inflammation. It has been postulated that low serum zinc may be caused by elevated IL-1 associated with RA (Honkanen et al., 1991)(66). With acute inflammation, the acute phase response may move Zn into the liver and the reduced plasma concentration may not be indicative of overall deficiency (Shenkin, 1995)

(6 7). 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 detoxication of xenibiotics, free radicals, peroxides and regulation of immune function (Ganesan et al., 2003)(6 8). The authors reported that low levels of GSH are implicated in RA. In addition, it was found that Zn-deficient rats have lowered GSH concentrations (Kraus et al. 1997)(6 9). This finding may explain the reduction of plasma level of GSH in RA in our study. Moreover, Miesel

and Zuber (1993)(7 0) suggested that the participation of xanthin oxidase in the depletion of serum GSH in RA. Also, Hassan et al., (2001)(48) found that RA was associated with significant depletion in GSH levels.
Uric acid is considered as one of non enzymatic antioxidants, but increased production of uric acid means increased free radical production due to activation of the xanthine oxidase enzyme system (Nemeth et al., 2002)(71). The levels of uric acid were significantly higher in our RA rats. Moreover, the levels of uric acid were insignificantly reduced in group treated with Vit E except for Allo-treated group was significantly reduced in comparison with RA rats. Smolenska et al., (1999)(72) found high levels of uric acid in RA. Forrest et al., (2004)(73) suggested that hyperuricemia may enhance some aspects of rheumatoid inflammation, and uric acid may modulate an important component of rheumatoid autoimmunity. Hagfors et al., (2003)(74) reported that the inverse correlation between the thrombocyte count and uric acid indicates to the association of uric acid levels with 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 (Edmonds et al., 1997)(49). In a separate study of patients with RA, supplementation with antioxidants vitamin A, and E, increased plasma antioxidant levels with a corresponding decrease in LPO, a marker of oxidative stress; however, a clinical response was not reported (Jaswal et al., 2003)(44). Miesel et al., (1994)(75) suggested that antioxidants like allopurinol, which simultaneously modify the oxidative brust of phagocytes, inhibit xanthine oxidase, and display immunosuppressively effects may well be suited to control the consequences of chronic phagocytic hyperreactivity in RA. Namazi (2004)(76) reported that Allo, a competitive inhibitor of xanthine oxidase , decrease serum levels of uric acid in autoimmune disorders such as RA. Reaction of uric acid with free radicals, such as hydroxyl radical and hypochlorous acid (HOCl) results in allantoin production, suggested that uric acid acts as a free radical scavenger and thus is converted to allantoin. Increased allantoin levels suggested the possible involvement of free radicals in rheumatoid arthritis (Yardim-Akaydin et al., 2004)(77).

In the present investigation, the prominent gross picture of the effected synovial joints were edematous, swellen reddened, hyperplastic, hypertrophic with thickneded. The injury to the articular cartilage of the joint is very common and many involved in many ways, with several different cells and substances participating. It may be consequences of direct trauma, joint instability, lubrication failure associated with changes synovial fluid and the synovial membrane, or enzymes, All the previous mentioned gross changes agree with those reported by some authors (Ostergoard et al., 2005 ; Kumar et al., 2006 and Carol and Richard 2007)(78,79 ,&80).

In addition to joints of rats that were treated with either CII + allopurinol or CII + vitamin E were significantly less destructive changes as represented mild to moderate swelling, less edema, little congestion. Microscopically, the changes classified into two main categories as follow: (1) Severe destructive changes (as in case of CII group), (2) Partial (mild to moderate changes (as in CII + Allo and CII + Vit E groups). The histological changes showed skeletal muscle necrosis and atrophy. Synovial membrane villi, stromal synovial membrane edema with inflammatory cells infiltration (lymphocytes, plasma cells, macrophages, giant cells, and eosinophils), fibrin exudation, necrosis, synovial cell lining hypertrophy and hyperplasia were also noticed.

Synovial membrane enzymes as well as lysosomal enzymes of inflammatory cells(collagenase, cathepsins, clastase and arylsulfatase), are capable of degrading proteoglycan or collagen of articular cartilage, that derived from the inflammatory cells, synovial lining cells, or chondrocytes themselves. Also, in generative and inflammatory joint disease the levels of PGE2 in joints are increased and these substances inhibit also proteoglycan synthesis and mediate loss of articular cartilage of the joint. Also, the inflammatory cells that infiltrated the synovial membrane may impair fluid drainage from the joint and joint fluid may lose some of its lubricating properties, because hyaluronic acid may be degenerated by superoxide generating system of the inflammatory cells. All the previous mentioned explained by many investigators (Ostergoard et al., 2005 ; Kumar et al., 2006 and Carol and Richard 2007)(78,79&80). Partial protection showed in case of CII+Vit E was due to vitamin E is the primary fat soluble antioxidant, which may have an important role in scavenging free radicals and stabilizing the cell

membrane (Navaro et al., 1999)(81). In addition, some partial protection against CII intoxication in rats showed in CII + Allo group may be due to antioxidant effect.

In conclusion, this study suggests that proper management antioxidant intake management may reduce free radical generation and improve antioxidant status in RA. allopurinal 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 alleviating foot swelling and decreasing PGE2 level in RA rat model.

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