دراسة مقارنة لتأثير بعض مضادات الأكسدة في المؤشرات الحيوية في التهاب المفاصل الرثواني المحدثة تجريبياً عند الجرذان

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الملخص

المقدمة: يؤدي حقن الجرذان بالكولاجين–2 إلى إحداث التهاب مفاصل وهو نموذج لالتهاب مفاصل مزمن محدث بالمتفطرات الدرنية والكولاجين–2. وهذا الالتهاب يتميز بالتبدلات الكيميائية المرضية والفيسيولوجية المرضية نفسها للداء الرثواني عند الإنسان. قمنا في هذا البحث بدراسة التبدلات الكيميائية والمرضية لكل من الفيتامين سي وخلاصة الشاي الأخضر على الجرذان التي أحدث عندها نموذج التهاب مفاصل رثواني. تمت دراسة مؤشرات ضغط الأكسدة و البروستاغلاندين ي–2 عند جميع الجرذان.

الطرائق: أُجْرِيَت الدراسة على 40 جرذاً ذكراً وقسمت إلى 4 مجموعات (10 جرذان في كل مجموعة): مجموعة الشاهد،مجموعة الكولاجين–2، مجموعة الكولاجين–2 مع الفيتامين سي، ومجموعة الكولاجين -2 مع خلاصة الشاي الأخضر. تم قياس مستويات البلازما لكل من أوكسيد النيتريك، الأكسدة الفائق للدسم، سسوبر أوكسيد ديزموتاز، حمض البول، الغلوتاثيون بعد 6 أسابيع من إعطاء مضادات الأكسدة باستخدام طريقة القياس الضوئي. أيضاً تم قياس مستويات البروستاغلاندين ى2 باستخدام طريقة الاليزا.

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النتائج: في مجموعة الكولاجين -2 كانت مستويات الاكسدة الفائقة للدسم وأوكسيد النيتريك والبروستاغلاندين وحمض البول مرتفعة بدلالة إحصائية لكن مستويات سوبر أوكسيد ديزموتاز وغلوتاثيون كانت منخفضة بدلالة إحصائية عن الشاهد. مستويات سوبر أوكسيد ديزموتاز، الغلوتاثيون كانوا زائدين بمدلول إحصائي لكن البروستاغلاندين والسيرولوبلازمين و اوكسيد النيتريك كانت منخفضة بمدلول احصائي في مجموعة الفيتامين سي. في المجموعة التي أعطيت الكولاجين -2 مع خلاصة الشاي الأخضر كانت مستويات البروستاغلاندين ي2، الأكسدة الفائقة للدسم، أوكسيد النيتريك، كانت منخفضة لكن مستويات الغلوتاثيون والسوبر أوكسيد ديزموتاز كانت مرتفعة بالمقارنة مع مجموعة الكولاجين -2. بالإضافة كانت هناك تبدلات نسيجية بين مختلف المجموعات.

الخلاصة: تقترح دراستنا أنَّ إعطاء كمية مناسبة من مضادات الأكسدة يمكن أن تنقص تشكيل الجذور الحرة في حالة التهاب المفاصل الرثواني. خلاصة الشاي الأخضر والفيتامين سي ويمكن أن يؤثر بدرجات مختلف في إعادة اضطراب جهاز الاكسدة /مضاد الاكسدة إلى الحالة الطبيعية، ويمكن أن تكون مفيدة في تاخير مضاعفات الداء الرثواني. فضلاً عن ذلك فإنَّ مضادات الأكسدة هذه أدَّت دورا كمضاد للالتهاب بالتقليل من الوذمة وإنقاص مستويات البروستاغلاندين ى2 في الداء الرثواني.

Comparative Study Between Some Antioxidants on Bio Indices in Rheumatoid Arthritis 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 vitamins C, and aqueous extract of green tea (GTE) on RA of rats were investigated. The oxidative stress indices and prostaglandin E were evaluated.

Methods: 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 vitamin C (C II + Vit. C), and CII group treated with green tea extract (GTE) (C II + GTE). After 6 weeks of antioxidants treatment, the plasma levels of lipid peroxides (LPO), nitric oxide (NO),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 assay.

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

Conclusion: our study suggests proper antioxidant intake management may reduce free radical generation and improve antioxidant status in RA. GTE, and vitamins C may effectively normalize in different degrees the impaired the 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 has an autoimmune disease characterized by chronic inflammation, progress\sive 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, 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 [(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 [11]. 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 [12]; 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 [13].

In view of the recent animal studies strongly suggesting anti-inflammatory role of antioxidants like SOD and in experimentally induced arthritis, antioxidant therapy strategies have been proposed for the prevention and treatment of RA[14].Various forms of antioxidant therapy have demonstrated promising results in experimental RA models [15-17].

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease [18]. More attention has been paid to the protective effects of natural antioxidants against compounds-induced free radical generation [19]. Flavonoids have been found to play important roles in the non-enzymatic protection against oxidative stress [20].Flavonoids are group of polyphenolic compounds that occur widely in fruit, vegetables and tea [21]. Fresh tea leaves are rich in flavanol monomers known as catechins such as epicatechins, which are 13.6 g/100 g in green tea and 4.2 g/100 gm dry weight in black tea [22], they found the supplementation of green tea extract (GTE) attenuates cyclosporine A-induced oxidative stress in rats. Moreover, GTE can be reduced the risk of colorectal inflammatory disease and muscle necrosis [23].

Aim of work:

In the present study, the effect of antioxidants as vitamin C, and GTE on rat model of RA was investigated. So, the plasma levels of lipid peroxides, NO, PGE_2, glutathione (GSH), SOD, CP, uric acid, were determined. Moreover, the histopathological examination was associated with biochemical evaluation.

Materials and Methods

1-Chemicals

L-ascorbic acid, 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 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 1mg/ml heat-killed Mycobacterium tuberculosis (Sigma-Aldrich). Rheumatoid arthritis was induced by the initial immunization with100µg/100µl 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 100µg/100µl of bovine CII emulsified in incomplete Freund's adjuvant (IFA) [24].

(3) CII + vitamin C – treated group (CII+ Vit. C group) was injected by CII, and received vitamin C daily via oral rout (30 mg/kg/day/oral) [25] of beginning with the first day of adjuvant injection.

(4) CII + GTE – treated group (CII+ GTE group) was also injected with CII, and received GTE of beginning for 45 days. The GTE was made by soaking 15 g of instant green tea powder in 1 L of boiling distilled water for 5 minutes. The solution was filtered to make 1.5% GTE. This solution was provided to rats as their sole source of drinking water[11].

All the tested antioxidants (vitamin C, GTE) 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 [26]. 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 [27]. The plasma GSH levels were determined chemically [28]. The plasma SOD activity was determined according to its ability to inhibit the autooxidation of epinephrine at alkaline medium [29]. The plasma uric acid level was determined by enzymatic colorimetric method [30].

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.

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 .

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. 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.

Rat group with rheumatoid arthritis: In the C II group, the levels of LPO, NO, PGE_2, uric acid, were significantly higher than controls. Contrarily, the levels of SOD, and GSH were significantly lower than controls.

Vitamin C effect: In the C II + Vit. C group the levels of LPO, NO, uric acid, were significantly increased but the levels of SOD were significantly decreased and the levels of GSH did not show significantly changes in comparison with controls. The levels of PGE2 were insignificantly increased in C II + Vit. C group in comparison with control group.

In comparison with C II –treated group, the levels of SOD,and GSH were significantly increased but the levels of PGE_2,and NO were significantly decreased in both the C II + Vit. C . Also, the levels of LPO and uric acid were insignificantly decreased in antioxidant vitamin treated group.

GTE effect: In the C II + GTE group, the levels of LPO, NO, uric acid and were significantly increased but the levels of SOD were significantly decreased and the levels of PGE_2, and GSH, did not show significantly changes in comparison with controls.

In comparison with C II –treated group, the levels of PGE_2, LPO,and NO were significantly decreased but the levels of GSH,and SOD were significantly increased and the levels of uric acid did not show significantly changes in the C II + GTE 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 + vitamin C-treated group	(D) CII + GTE-treated group	A vs B	A vsC	A vs D	B vs C	B vs D
PGE2 (pg/ml)	192.500 ± 12.200	683.400 ± 43.190	329.400 ± 74.380	305.400 ± 57.210	<0.001	>0.05 NS	>0.05 NS	<0.001	<0.001
LPO (nmol/ml)	3.218 ± 0.441	8.000 ± 1.193	5.626 ± 0.526	5.182 ± 0.485	<0.01	<0.01	<0.01	>0.05 NS	<0.05
NO (ng/ml)	3.619 ± 0.215	8.520 ± 1.248	4.629 ± 0.091	5.275 ± 0.151	<0.001	<0.001	<0.001	<0.01	<0.05
GSH (nmol/ml)	4.265 ± 0.249	2.749 ± 0.306	4.330 ± 0.366	4.242 ± 0.184	<0.01	>0.05 NS	>0.05 NS	<0.01	<0.001
SOD (U/ml)	344.700 ± 41.220	109.800 ± 18.720	197.800 ± 14.380	195.900 ± 30.450	<0.001	<0.01	<0.01	<0.01	<0.05
Uric acid (mg/ml)	4.319 ± 0.139	8.422 ± 0.853	6.948 ± 0.755	6.417 ± 0.600	<0.001	<0.01	<0.01	>0.05 NS	>0.05 NS

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

Histological Results

The Figs. (2-7) showed 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 aggregates (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. 17-21).
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 GIT of less destructive histological change when 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, macropgages, gaint 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.22,23).
In cases were with CII and vitamin C showed an extremely degree of protection against CII toxicity in comparing to GTE.The joints were nearly normal subsynovial stroma (free from WBCs), normal cartillagenous and osseous structure, normal bone marrow lymphoid elements, no osteoclastic hyperactivity and no hyperplasia of the cells lining the joint synovial membranes (Fig.24-25). No markable changes showed in the control group in the present work.

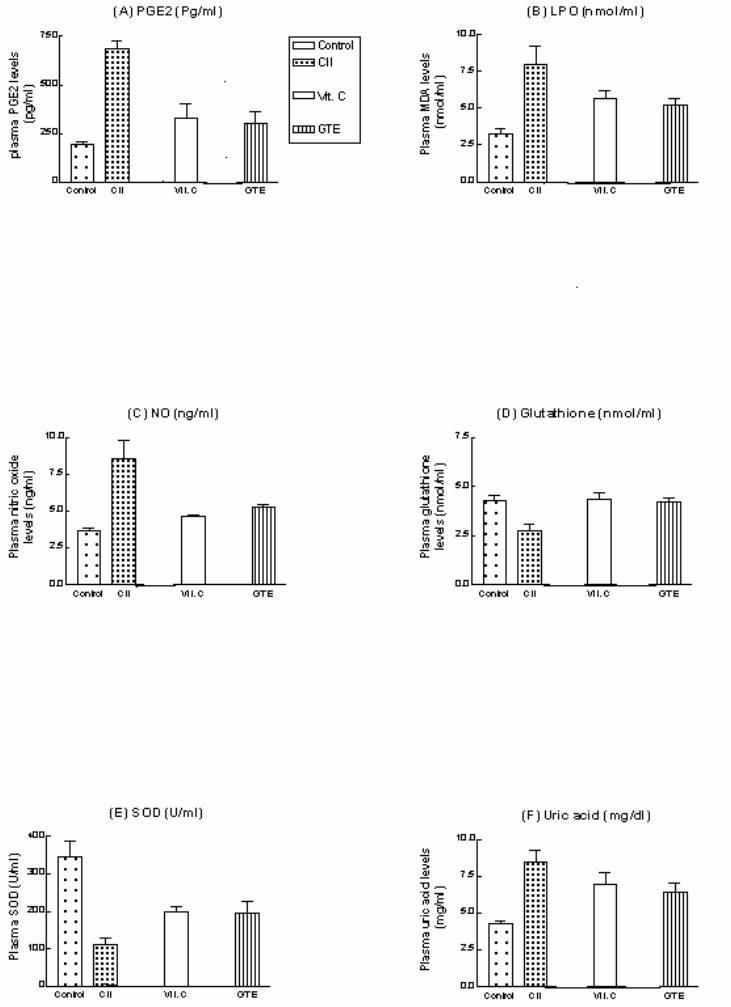
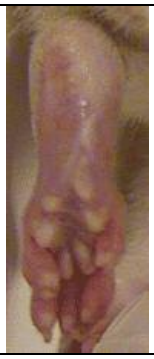
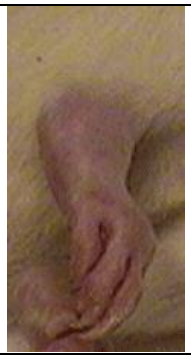


Fig. (1) shows the plasma levels of: (A) PGE2, (B) LPO, (C) NO, (D) Glutathione, (E) SOD and (F) Uric acid in different rat groups.

		
Fig(2):Normal joints	**Fig.3 (CII) Red,edematous,swelling.hyperplastic and transformation of the smooth contour of the joint surface**	**Fig.4 (CII) Red,edematous,swelling.hyperplastic and transformation of the smooth contour of the joint surface**
		
Fig.5 (CII+vitaminC)	**Fig.6 (CII+GTE)**	**Fig.7 (CII+GTE)**

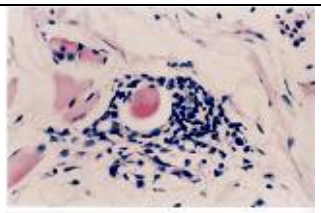
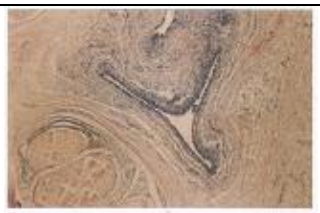
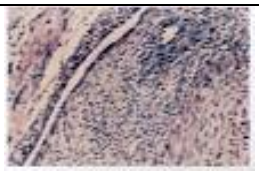
	

Fig. (8) Skeletal muscle of rats were injected with CII,showing:atrophy an necrotic myositis. Hα Ex. 400.	**Fig. (9)Synovial joint membrane of rats were injected with CII,showing:numerous villi and folds Hα Ex. 400..**
	
Fig. (10)Synovial joint membrane of rats were injected with CII,showing:edema, fibrin exudation, necrosis with inflammatory cells infiltration Hα Ex. 100.	**Fig. (11) A high power of Fig.(11) Hα Ex. 200.**

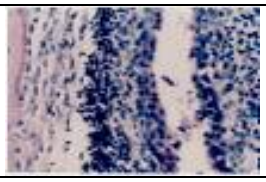
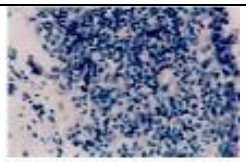
	

Fig. (12) Joint of rats ,were injected with CII showing hyperplasia,hypertrophy, Mitotic figure,necrosis of the synivial lining cells bosides lymphocytic aggregates Hα Ex. 400.	**Fig. (13) Joint of rats ,were injected with CII showing mononuclear cells infiltration and edema in the subsynovial stroma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

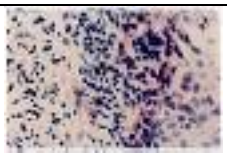
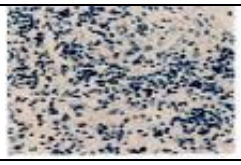

	

Fig. (14) Joint of rats ,were injected with CII showing macrophages,gaint cells and eosinophils infiltration in the subsynovial stroma(Allergic synovitis).Hα Ex.400.	**Fig. (15) Joint of rats ,were injected with CII showing lymphocytic cells infiltration and edema in the underlying stroma Hα Ex. 400**

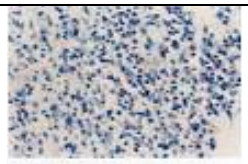
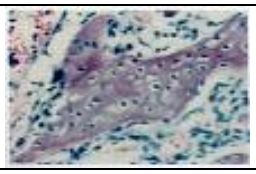
	

Fig. (16) Joint of rats ,were injected with CII showing extensive eosinophils infiltration in subsynovium stroma . Hα Ex. 400.	**Fig. (17) Articular cartilage,(hyaline C)of rats were injected with CIIshowing degeneration,necrosis ,congestion and haemorrhages(subchondral) Hα Ex. 400.**

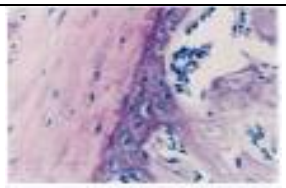



Fig. (18) Articular cartilage,(hyaline C)of rats were injected with CII showing:Eburnation. Hα Ex. 400.	**Fig. (19) Subchondral bone of rats were injected with CII,showing destructive bone changes"degeneration and dead osteocytes". Hα Ex. 400**

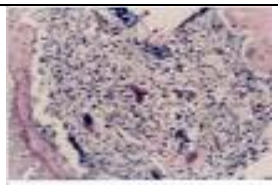
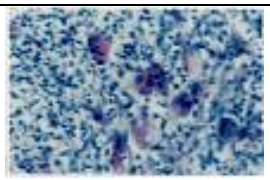
	

Fig. (20) Subchondral bone of rats were injected with CII,showing osteoclastic hyperactivity,increase thevascularity with leukocytic cells infiltration (pannus formation). Hα Ex. 200.	**Fig. (21)A high power of Fig.24 Hα Ex. 400.**

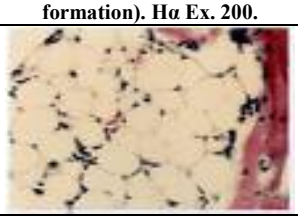
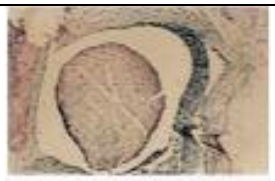

	

Fig. (22) Subchondral bone of rats were injected with CII,showing fatty bon marrow with little lymphoid structure . Hα Ex. 400.	**Fig. (23) Subchondral bone of rats were injected with CII,showing subchondral bone cyst . Hα Ex. 100.**

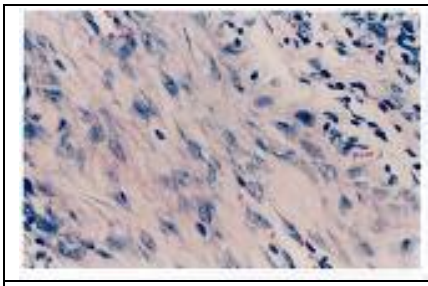
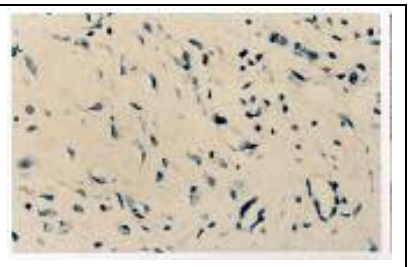
	

Fig. (24) Subsynovium of rats were treated with CII and Green Tea Extract, showing moderate degree of infiltrated inflammatory cells besides edema. Hα Ex. 400.	**Fig. (25) Subsynovium of rats , were treated with CII andGTE, showing moderate degree of infiltrated inflammatory cells besides edema. Hα Ex. 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 [31]. The pathogenesis of this disease is linked predominantly with the formation of free radicals at the site of inflammation. Some authors[32] 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 [33].

Lipid peroxidation has been implicated in the pathogenesis, 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 [34].

Oxidative injury and inflammatory status in various rheumatic diseases was confirmed by increased levels of prostaglandins in serum and synovial fluid compared to controls [35]. 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 antioxidants (Vit C -, and GTE) treated groups. Moreover, levels of LPO were significantly reduced in GTE-treated group. Similarly, the levels of plasma LPO were found to be significantly higher in RA than controls in many previous studies [36,37]. Another study [38] 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. Some investigators [14] indicated the increased NO and LPO levels in RA. They proposed antioxidant therapy strategies for the prevention and treatment of RA. Another study [39,40] found that vitamins E and C supplementation increase significantly the levels of antioxidants and decrease the concentration of LPO along with improved symptoms of RA.

Another study [41] reported that an antioxidant rich polyphenolic fraction isolated from green tea possesses anti-inflammatory effects in experimental animals. In addition, many investigators suggested that GTE has antioxidants and scavenge free radicals actions [42]. Moreover, recent studies have demonstrated that green tea possess both anti-inflammatory properties in normal human cells [43]. On the other hand, Some authors[43] noticed that the green tea inhibits production of NO in human chondrocytes. Furthermore, green tea is shown to reduce inflammation in arthritis and is rich in antioxidants which may be useful in the prevention of onset and severity of RA [41,43]. Moreover, the reduction of NO and PGE_2 by GTE in RA were also shown in some studies [44,45].

Some investigators[41] found that the potential disease-modifying effect of green tea on arthritis came to light when it was shown that collagen type II-induced RA (C II RA) in mice, was ameliorated by prophylactic administration of green tea polyphenols in drinking water. The reduced C II RA incidence and severity was reflected in a marked inhibition of the inflammatory mediators COX2, interferon-gamma and tumor necrosis factor - alpha (TNF-α) in arthritic joints of green tea-fed mice. Since increasing PGE_2 can cause inflammatory reactions, such as local congestion, edema, and pain in rheumatoid arthritis, the significant decrease of PGE_2 in rheumatoid rats may be due to inhibition of COX2 activity following the administration of GTE [46,47].

An increase the vivo generation of oxidants and lipid peroxidation products in the plasma of RA was found to be negative correlated with the antioxidant levels [48].

The levels of SOD activity in RA rat group were significantly lower than controls but they were significantly higher in antioxidants (Vit C, and GTE) treated groups. This finding is in agreement with previous reports [49]. Similarly, other [35] found the SOD activity was significantly lower in RA than controls. Another study[39] 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 [51]. Also, they 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[51].

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 [52]. The levels of uric acid were significantly higher in our RA rats. Moreover, the levels of uric acid were insignificantly reduced in all groups treated with antioxidants Vit C- , and GTE-groups. Another's [53] found high levels of uric acid in RA. Some studies [54] suggested that hyperuricemia may enhance some aspects of rheumatoid inflammation, and uric acid may modulate an important component of rheumatoid autoimmunity. Another [55] reported that the inverse correlation between the thrombocyte count and uric acid indicates to the association of uric acid levels with degree of inflammation. Tariq et al [41] reported that an antioxidant rich polyphenolic fraction isolated from green tea possesses anti-inflammatory effects in experimental animals. 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 [41]. In a separate study of patients with RA, supplementation with antioxidants vitamin A, E, and C increased plasma antioxidant levels with a corresponding decrease in LPO, a marker of oxidative stress, 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. suggest 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 [56].

In the present investigation, the prominent gross picture of the effected synovial joints were edematous, swelling reddened, hyperplastic, hypertrophic with thickneded. The injury of 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 pictures were coincide with those reported by some authors [57-59].

In addition to joints of rats that treated with CII + GTE were significantly less destructive changes as represented mild to moderate swelling, less edema, little congestion, while the joints of rats treated with CII + vitamin C were nearly similar to the control group. Microscopically, the changes classified into three main categories as follow: (1) Severe destructive changes (as in case of CII group), (2) Partial (mild to moderate changes (as in CII + GTE), and (3) Nearly normal state (complete protection) as in CII + Vit C. The histological changes showed skeletal muscle necrosis and atrophy. Synovial membrane villi, stromal synovial membrane edema with inflammatory cells infiltration (lymphocytes, plasma cells, macrophages, gaint 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 [57-59].

Mild to moderate histological changes partial protection reported in the present study in case of CII + GTE group was protective due to green tea (camellia sinenesis) contains high levels of polyphenols including catechin, epicatechin, gallocatechin, epigallocatechin, and gallocatechin gallate. Polyphenols from green tea are efficient free radical and singlet oxygen scavengers, and inhibits lipid peroxidation [60].

Finally, nearly complete protection showed in case of CII + Vit C because of vitamin C is a potent scavenger of free oxygen radicals and it has been shown also that marginal vitamin C deficiency results in intracellular oxidative damage in experimental animals [61]. All the previous mentioned results were coincide with those reported by some authors [60,61].

In conclusion, our study suggests proper antioxidant intake management may reduce free radical generation and improve antioxidant status in RA. GTE, and vitamin C 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. The anti-inflammatory activity of GTE was most likely comparable to other antioxidants. We recommend to give these antioxidants as a part of drug course of RA treatment.

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