

## Effect of Green Tea and Vitamin C on Rheumatoid Arthritis of Male Albino Rat Induced with Collagen II

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### ABSTRACT

*In the present study, the biochemical effects of vitamin C, and aqueous extract of green tea (GTE) on rheumatoid arthritis (RA) of rats were investigated. The oxidative stress indices and the antioxidant levels were evaluated. Forty albino 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), ceruloplasmin (PC), and glutathione (GSH) were detected using colorimetric methods. The plasma levels of copper (Cu) and zinc (Zn) were determined using atomic absorption/flame emission spectrometer. In C II treated group, the levels of LPO, NO, CP and Cu were significantly higher but the levels of; GSH and Zn were significantly lower than controls. The levels of GSH and Zn were significantly increased but the levels of NO, Cu and CP were significantly decreased in the vitamins C treated groups in comparison with C II –treated group. In the C II + GTE group, the levels of LPO, NO, CP and Cu were significantly decreased but the levels of GSH, and Zn were significantly increased in comparison with C II –treated group. The present study suggests that proper antioxidants intake may reduce free radical generation and improve antioxidant status in RA. GTE and vitamin C may effectively normalize in different degrees the impaired oxidant/ antioxidant system and may be useful in delaying the complication of RA.*

### INTRODUCTION

Rheumatoid arthritis (RA) is a polyarticular disease affecting about 1 % of the population of the world<sup>1</sup>. It is an autoimmune disease characterized by chronic inflammation, progressive joint destruction, physical impairment, work disability and early mortality<sup>2</sup>. The process of disease progression is characterized by hyperplasia of

synoviocytes, mainly of synovial fibroblasts, resulting in bone and joint destruction<sup>3,4</sup>. However, the proliferation of synovial cells is not limitless and spontaneous suppression of synovial proliferation has been observed<sup>5</sup>.

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<sup>6,7</sup>.

Inflammation is known to result in increased production of nitric oxide (NO) and prostaglandins<sup>8</sup>. NO is an important mediator of diverse physiologic and pathologic processes, including arthritis<sup>9</sup>. Joint inflammation in autoimmune adjuvant-induced arthritis is dependent on the enhanced production of NO.

McCartney-Francis et al.<sup>10</sup> (1999) reported that NO is ideally suited as a potent inflammatory mediator because of its strong reactivity with oxygen, superoxide, and iron-containing compounds.

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<sup>11</sup>. The insufficiency of antioxidant defense systems and the acceleration of the oxidative reactions can result from the pro-oxidant/antioxidant imbalance in RA<sup>12</sup>. It was demonstrated that the level of free radical-induced damage to proteins in the synovial fluid was twice as high in RA<sup>13</sup>. Moreover, it was found that individuals with initially low levels of protecting antioxidants in their plasma, such as vitamins C and selenium, are at greater risk of developing RA<sup>14</sup>.

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 hypochlorous by the invading phagocytes<sup>15</sup>; 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<sup>16</sup>.

Zinc (Zn) is a crucial element in a series of cellular functions as normal growth, protein metabolism, membrane stability, and metalloenzyme functions<sup>17</sup>. In addition, Zn has several other effects on immune response, complement system, lysosomal enzyme release, and macrophage functions<sup>18</sup>. Zn is indispensable 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<sup>18</sup>.

Copper (Cu) is abundant in the human body and nature<sup>19</sup>. Cu is incorporated into the structure of many enzymes and proteins<sup>18</sup>. 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 demonstrated that CP increases during acute phase reactions in order to scavenge toxic free oxygen radicals<sup>20</sup>.

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)<sup>21</sup>. Intracytoplasmic SOD includes both Cu and Zn, while CP is a powerful antioxidant in serum carries only Cu<sup>19</sup>. Substantial alterations in metabolisms of Cu and Zn occur through some physiological control mechanisms over an inflammatory reaction<sup>17</sup>

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 antioxidant functions, which can prove beneficial in several pathological conditions<sup>22</sup>. CP is an acute-phase protein with a moderate reaction, an 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<sup>23</sup>.

Some functions of CP can be inactivated by exposure to the free oxygen radical (FR) flux generated by the hypoxanthine/xanthine oxidase system (Giurgea et al., 2005). A significant inactivation of CP occurs during oxidative stress, this phenomenon being associated with the uncoupling of copper atoms from the CP molecule. 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<sup>23</sup>.

Recent animal studies strongly suggest anti-inflammatory role of antioxidants like SOD and vitamin C in experimentally induced arthritis. Antioxidant therapy strategies have been proposed for the prevention and treatment of RA (Mahajan and Tandon, 2004). Various forms of antioxidant therapy have demonstrated promising results in experimental RA models<sup>25-27</sup>.

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease<sup>28</sup>. More attention has been paid to the protective effects of natural antioxidants against compounds-induced free radical generation<sup>29</sup>. Flavonoids have been found to play important roles in the non-enzymatic protection against oxidative stress<sup>30</sup>. Flavonoids are group of polyphenolic compounds that occur widely in fruit, vegetables and tea<sup>31</sup>. 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<sup>32</sup>. 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<sup>33</sup>.

**Aim of work:**

The present study was aimed to investigate the effect of antioxidants as vitamin C and GTE on rat model of RA. So, the plasma levels of lipid

peroxides, NO, glutathione (GSH), CP, Cu and Zn were determined.

## MATERIAL & METHODS

### 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.

### Animal treatment

Forty healthy male albino rats (*Rattus norvegicus*) their body weight 150–170 gm were included in the present study. 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 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 with 100µg/100µl

emulsion by an intradermal injection in the base of the tail. Twenty one days later after the initial immunization, the rats received a booster intradermal injection (base of the tail) of 100µg/100µl of bovine CII emulsified in incomplete Freund's adjuvant (IFA)<sup>34</sup>.

- 3) *CII + vitamin C – treated group (CII+ Vit. C group)* was injected by CII, and received vitamin C daily via oral route (50 mg/kg/day/oral)<sup>35</sup> from the beginning of *CII injection* for 45 days.
- 4) *CII + GTE – treated group (CII+ GTE group)* was injected with CII, and received GTE from the beginning of *CII injection* for 45 days. The GTE was made according to Maity et al., (1998)<sup>36</sup>, 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.

All the tested antioxidants (vitamin C and GTE) were administered 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. Plasma samples were divided into aliquots and kept at -40 °C until biochemical analysis.

### 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<sup>37</sup>. The plasma levels of nitric oxide (NO) was determined as total nitrite after deproteinization with ZnSO<sub>4</sub> (30%), nitrate reduction with cadmium (activated by 2 % HCl) and color developed by the reaction with Griess reagent (1% sulfanilamide/ 0.1% naphthylethylene diamine diHCl, w/v in 2.5% H<sub>3</sub>PO<sub>4</sub>) was recorded at 550nm against reagent blank using sodium nitrite as standard<sup>38</sup>. The plasma GSH levels were determined chemically as described by Ellman (1959)<sup>39</sup>. The plasma CP activity was determined using a para-phenylenediamine dihydrochloride method<sup>40</sup>. 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 for each solution. The accuracy and precision of the analytical methods were tested with standard reference materials.

#### **Statistical analysis**

The results were expressed as mean  $\pm$  standard error (SE). Differences between groups were assessed by one-way analysis of variance (ANOVA) using the Prism version 4 software packages 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) Copper, (B) Ceruloplasmin in different rat groups.

#### **Rat group with rheumatoid arthritis:**

In the C II group, the levels of LPO, NO, CP and Cu were significantly higher than controls. Contrarily, the levels of, GSH and Zn were significantly lower than controls.

**Vitamins C effect:** In the C II + Vit. C group, the levels of LPO, NO, CP and Cu were significantly increased but the levels of Zn were significantly decreased in comparison with controls. GSH levels showed non significant increase when compared with that of controls.

In comparison with C II –treated group, the levels of GSH and Zn were significantly increased but the levels of NO, Cu and CP were significantly decreased in the C II + Vit.C group. Also, the levels of LPO were insignificantly decreased in vitamin C treated groups in comparison with the C II- treated group.

**GTE effect:** In the C II + GTE group, the levels of LPO, NO, and Cu were significantly increased but the levels of GSH, CP and Zn did not show significantly changes in comparison with controls.

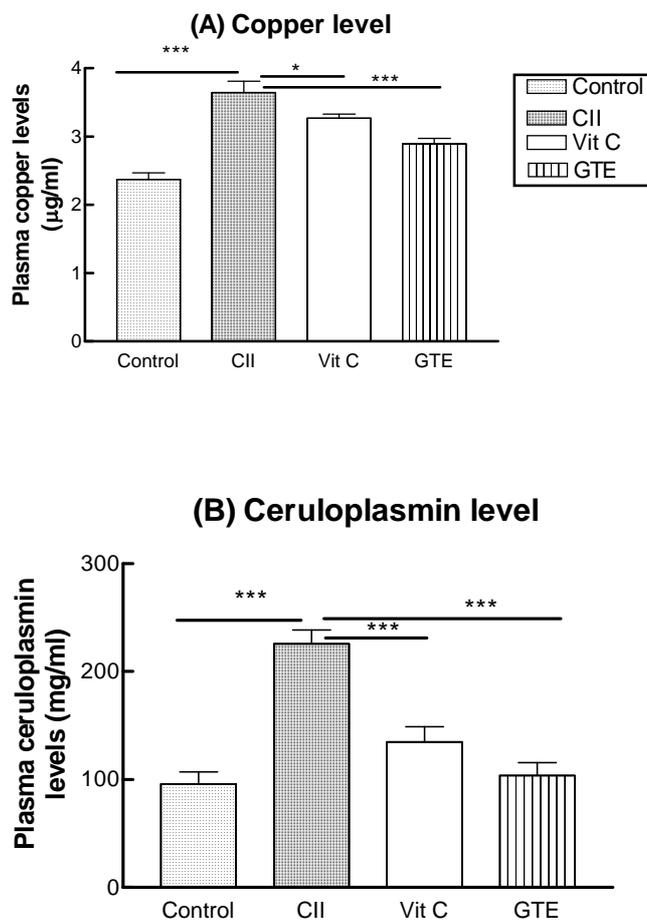
In comparison with C II –treated group, the levels of, LPO, NO, CP and Cu were significantly decreased but the levels of GSH, and Zn were significantly increased.

**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	P-Value					
					A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
<b>LPO</b> (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	>0.05 NS
<b>NO</b> (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	<0.01
<b>GSH</b> (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	>0.05 NS
<b>Copper</b> (□g/ml)	2.374 ± 0.098	3.645 ± 0.166	3.268 ± 0.064	2.892 ± 0.083	<0.001	<0.001	<0.001	<0.05	<0.001	<0.01
<b>CP</b> (mg/dl)	95.860 ± 11.320	225.500 ± 13.090	166.700 ± 13.070	103.600 ± 11.960	<0.001	<0.001	>0.05 NS	<0.001	<0.001	>0.05 NS
<b>Zinc</b> (□g/ml)	3.862 ± 0.4147	0.706 ± 0.0515	1.159 ± 0.194	4.151 ± 0.308	<0.001	<0.001	>0.05 NS	<0.05	<0.001	<0.001

Values are means ± SE for 10 rats (N= 10 for each group).

The level of significance was accepted with P <0.05.



*Fig. (1) shows the plasma levels of: (A) Copper and (B) Ceruloplasmin in different rat groups*

## 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<sup>41</sup>. The pathogenesis

of that disease is linked predominantly with the formation of free radicals at the site of inflammation. Previously, Heliovaara et al.,<sup>14</sup> reported that a low antioxidant level is a risk factor for RA.

The present study was performed to evaluate the effect of 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<sup>42</sup>).

Lipid peroxidation has been implicated in the pathogenesis of degenerative diseases and inflammatory arthritis<sup>43</sup>. 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<sup>44</sup>.

Oxidative injury and inflammatory status in various rheumatic diseases was reported<sup>45</sup>. In the current work, the levels of LPO, and NO in RA rat group were significantly higher than controls but NO levels were significantly reduced in all antioxidants (Vit C -, and GTE -) - treated groups. Moreover, levels of LPO were significantly reduced in GTE-treated groups. Similarly, the levels of plasma LPO were found to be significantly higher in RA than controls in many previous studies<sup>46,47</sup>. Fermor et al.<sup>48</sup> suggested that many factors such as inflammation and mechanical loading in RA can lead to increased production of inflammatory mediators such as NO and prostaglandins (PGE<sub>2</sub>). Mahajan and

Tandon<sup>24</sup> have demonstrated the increased NO and LPO levels in RA. They proposed antioxidant therapy strategies for the prevention and treatment of RA. Jaswal et al.,<sup>49</sup> and Rennie et al.,<sup>50</sup> 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.

Tariq et al.,<sup>51</sup> 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<sup>52</sup>. Singh et al.,<sup>53</sup> 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 (Tariq et al.,<sup>51</sup> and Adcoks et al.,<sup>54</sup>). Moreover, the reduction of NO and PGE<sub>2</sub> by GTE in RA were also shown in some studies<sup>53,55,56</sup>.

Haqqi et al.,<sup>57</sup> 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- $\alpha$ ) in arthritic joints of green tea-fed mice. Since increasing PGE<sub>2</sub>

can cause inflammatory reactions, such as local congestion, edema, and pain in rheumatoid arthritis, the significant decrease of PGE<sub>2</sub> in rheumatoid rats may be due to inhibition of COX2 activity following the administration of GTE<sup>58, 59</sup>.

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<sup>60</sup>.

Ceruloplasmin (CP) is considered the principal plasma and synovial antioxidant in RA, being responsible for up to 70% of the protective capacity against superoxide free radicals<sup>61</sup>, 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<sup>62</sup>. 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<sup>2+</sup> to Fe<sup>3+</sup>, 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<sub>2</sub><sup>-</sup> or H<sub>2</sub>O<sub>2</sub><sup>63</sup>. 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<sup>63</sup>.

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 antioxidants Vit C- and GTE-groups. Similarly; many investigators found that the plasma levels of CP were significantly higher in RA than in controls<sup>46,47</sup>. Moreover, Amancio et al.<sup>64</sup> 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<sup>65</sup>. 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<sup>66</sup>. 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<sup>66</sup>. Zoli et al.<sup>65</sup> revealed that IL-1 $\beta$  and TNF- $\alpha$  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 inhibition of mononuclear cell infiltration, and pannus formation<sup>67</sup>. Also, Brewer<sup>68</sup> reported that anticopper therapy such as penicillamine has efficacy in RA.

Zinc is a 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<sup>69</sup>.

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 antioxidants Vitamin C- and GTE-groups in comparison with C II groups. Previously, Tuncer et al.,<sup>70</sup> found plasma zinc levels were decreased significantly in RA. The authors suggested that low plasma Zn level 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<sup>19</sup>. 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<sup>71</sup>. 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 detoxification of xenobiotics, free radicals, peroxides and regulation of immune function<sup>72</sup>. 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<sup>73</sup>. This finding may explain the reduction of plasma level of GSH in RA in our study. Moreover, Miesel and Zuber<sup>74</sup> suggested participation of xanthin oxidase in the depletion of serum GSH in RA. Also, Hassan et al.<sup>75</sup> found that RA was associated with significant depletion in GSH levels.

**In conclusion**, the current study suggests proper antioxidant intake may reduce free radical generation and improve antioxidant status in RA. GTE and vitamin C may effectively normalize in different degrees the impaired oxidant/ antioxidant system and may be useful in delaying the complication of RA. These antioxidants display considerable potency in anti-inflammatory action. The anti-inflammatory activity of GTE was most likely comparable to other antioxidants. It could be recommend to give these antioxidants as a part of drug course of RA treatment.

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### تأثير فيتامين ج والشاي الأخضر على إلتهايات المفاصل الروماتزمية في ذكور الفئران البيضاء المعالجه بالكولاجين ٢

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اجريت الدراسة الحالية لمعرفة التأثيرات البيوكيميائية لفيتامين ج، والمستخلص المائي للشاي الأخضر على إلتهايات المفاصل الروماتزمية في ذكور الفئران البيضاء. وتم قياس مؤشرات اجهاد التأكسد في أربعين من ذكور الفئران وتم تقسيمهم إلى أربع مجموعات (١٠ فئران لكل مجموعة): المجموعة الأولى: مجموعة قياسية وتسمى بالمجموعة الضابطة والمجموعة الثانية تم معالجتها بمادة الكولاجين ٢ فقط والمجموعة الثالثة تم معالجتها بكل من الكولاجين ٢ وفيتامين ج والمجموعة الرابعة تم معالجتها بالكولاجين ٢ والمستخلص المائي للشاي الأخضر. وبعد ستة أسابيع من المعالجة بهذه المواد المضادة للأكسدة، تم قياس مستويات بيروكسيدات الدهون وأكسيد النترية، السيرويولازمين والجلوتاثيون المختزل في أمصال ذكور الفئران باستخدام طرق المعايرة اللونية كما تم قياس مستويات النحاس والزنك باستعمال طريقة الإمتصاص الذري. ووجد ان مستويات بيروكسيدات الدهون وأكسيد النترية، السيرويولازمين والنحاس قد زادت زيادة ذات دلالة احصائية في المجموعة الثانية عند مقارنتها بالمجموعة الضابطة وعلى العكس من ذلك وجد ان مستويات كل من الجلوتاثيون المختزل والزنك قلت وكان النقص ذا دلالة احصائية في أمصال المجموعة الثانية عند مقارنتها بنفس المجموعة. بالإضافة الى ذلك وجد ان مستويات الجلوتاثيون المختزل والزنك قد زادا بشكل ملحوظ لكن مستويات أكسيد النترية، والسيرويولازمين والنحاس قد قلت بشكل ملحوظ في المجموعة المعالجة بفيتامين ج والمجموعة المعالجة بالمستخلص المائي للشاي الأخضر عند مقارنتها بالمجموعة المعالجة بمادة الكولاجين فقط. والخلاصة: تقترح هذه الدراسة بان تناول كمية مناسبة من مضادات الأكسدة يمكن ان يكون لها دور فعال في تحسين نظام مؤشرات الأكسدة/مضادات الأكسدة الغير متزن ويمكن ان يكون لها دور في تأخير المضاعفات الناتجة عن الالتهابات الروماتزمية.