Mansoura Journal of Biology, vol. 34 (1), June 2007

THE ROLE OF GREEN TEA EXTRACT AND/OR ALPHA-LIPOIC ACID IN PREVENTING SOME RISKS OF RHEUMATOID ARTHRITIS IN FEMALE RATS

EFFECT ON BONE REMODELING AND SOME INFLAMMATORY AND HEMATOLOGICAL PARAMETERS

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(Received: 28 / 4 / 2007)

ABSTRACT

The aim of the current study was to find out whether green tea extract and /or α -lipoic acid could protect against some adverse effects accompanying rheumatoid arthritis in female rats such as inflammation as well as bone remodeling and hematological disorders. Forty female rats of Fischer strain, weighing 150 ± 10 g each, were divided into tow main groups ; non arthritic and arthritic. Each of which was subdivided into four subgroups five rats each as follows : 1) control (non treated), 2) green tea (28.57mg/kg b w) treated, 3) α-lipoic acid (25mg/ kg b w)treated and 4) green tea and α -lipoic acid treated . green tea extract was administered orally while α -lipoic acid was injected intraperitoneally daily for 14 days for each.The results of the present work exhibited a significant increase in some biochemical markers of bone remodeling; a bone formation marker (alkaline phosphatase (ALP)) and a bone resorption marker (acid phosphatase (AP)) in serum and bone in experimental arthritic rats, indicating fast bone turnover rate and bone loss. Furthermore, a significant decrease in bone DNA and RNA as well as total serum protein content was recorded. On the other hand, there were significant elevation in C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Furthermore, there were significant decreases in RBCs count, HC, Hb, MCH, MCHC and MCV values in contrast to significant increases in WBCs and platelets count. However, administration of green tea and/or α-lipoic acid to arthritic rats ameliorated the mentioned

disturbances towards the normal values. Such results revealed that α-lipoic acid is an ideal antioxidant which has the ability to overcome the disturbances accompanied rheumatoid arthritis (RA) more than green tea. Moreover, the concomitant administration of green tea and α-lipoic acid was the most effective, and therefore can be recommended during treatment of patients suffering from rheumatoid arthritis.

Key words: Rheumatoid arthritis- bone remodeling- inflammation- Green tea and α-lipoic acid

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic, immune /inflammatory disease characterized by joint swelling, synovial inflammation and joint destruction that leads to significant disability. The pathology of this joint destruction is characterized by destruction of articular cartilage and marginal and subchondral bone ***(Feldmann et al., 1996)***.

The pathology of RA can be divided into two main phases, an inflammatory phase and a proliferative phase. Three cell types can be regarded as being major participants in the pathogenesis of joint inflammation and destruction in RA; synovial macrophages (type A synoviocytes), synovial fibroblasts (type B synoviocytes), and T lymphocytes. In the inflammatory phase, T lymphocytes are activated by unknown auto-antigens in the synovial tissue, which is then followed by an influx of macrophages and other immunocompetent cells into the tissue ***(Marrack et al., 2001)***. This is then followed by the proliferative phase, in which these accumulated synovial macrophages produce proinflammatory mediators, such as interleukin-1 beta (IL-1β), (IL-6) and tumor necrosis factor-alpha (TNF-α), which promote synovial fibroblasts to become undifferentiated and to proliferate. These type B synoviocytes then form the invasive front that infiltrates into cartilage, tendons and bone by synthesizing and releasing a variety of tissue degrading matrix metalloproteinases (MMPs) such as collagenases (in particular MMP-1), stromelysins (including MMP-3) and the gelatinases (MMP-2,-9) ***(Escalante and Del Rincon, 2002)***. Local osteoclasts are activated by proinflammatory cytokines as well and form a hyperplasticsynovial membrane, referred to as the pannus tissue, which damages the cartilage and bone of the affected joint in an irreversible manner ***(Katrib et al., 2002)***. Bone destruction is a critical feature of arthritis affected- joints and in severe cases of rheumatoid arthritis, marked destruction of joints

with focal erosion and juxtaarticular osteoporosis due to abnormal osteoclastic bone resorption is often observed ***(Kroger et al., 1994)***.

However, catechins, the main constituent of green tea, seems to possess two independent actions, both of which may be prophylactic for the development of arthritis and beneficial to arthritis sufferers; an anti-inflammatory and antiproteolytic chondroprotective effect ***(Ahmed et al., 2002)***. On the other hand, α-lipoic acid (ALA) seems to have a protective action related to its antioxidant effects through scavenging the free radicals ***(Gurer and Ercal, 2000)*** as well as to its anti-inflammatory action ***(Zhang and Frei, 2001)***. In addition, ALA has an inhibitory effect on the matrix metalloproteinases (MMPs) ***(Liedtke et al., 1998)***. Therefore, the present study aims to evaluate the role of green tea and/or ALA in preventing or even in decreasing some risks factors associating with rheumatoid arthritis.

MATERIALS AND METHODS

1- Experimental Animals:

This study was carried out on forty adult female Fischer rats weighing 150±10g, supplied by The Urology & Nephrology Center; Mansoura University. Rats were maintained under controlled humidity, temperature (25±2°C) and light (12h light/ 12h dark).They were maintained with water and fed *ad libitum*.

2- Experimental Protocol:

The rats were divided into two main groups:

A- Non- arthritic group **B** - Arthritic group

Each group was subdivided into 4 subgroups five animals each as follows:

1- Control group: non treated animals.

2- Green tea group: received green tea extract tablets dissolved in distilled water and given orally in a dose (28.57 mg/kg b.w /day.) by the stomach tube for 14 days. This dose is equivalent to

the human therapeutic dose and was calculated according to the body surface area ratio ***(Paget and Barnes, 1964)*** .

3- **Alpha-lipoic acid group:** injected intraperitoneally (i.p) with α-lipoic acid dissolved in saline (0.9%), alkalinized to pH 7.8 in a dose equal to 25 mg/ kg b.w./ day***(Selvakumar et al., 2004)*** for 14 days.

4- **Green tea and α-lipoic acid group**: treated with green tea + α-lipoic acid with the same doses, time and mode of treatment.

Induction of arthritis in rats:

According to ***Kleinau and Klareskog (1993),*** to induce arthritis, 8 mg of type II collagen extracted from native calf articular cartilage was dissolved in 1 ml of 0.01 M acetic acid and emulsified with an equal volume of Freund's incomplete adjuvant. Rats were injected intradermally into the base of the tail with a single dose of 0.1 ml of the emulsion. After 14 days rats received a single intradermal injection of 200µl Freund's incomplete adjuvant (FIA) into the base of the tail as an activation dose for induction of arthritis. Arthritis was developed 14 days latter. Rats were examined for RA by measuring edema in all the four paws by micrometer, as well as measuring the rheumatoid factor (R F).

3-Sampling:

At the end of the experimental period rats were sacrificed using a sharp razor blade. Blood samples were collected in clean heparinized and non heparinized centrifuge tubes. Heparinized blood samples were used for determination of complete blood picture, CRP and ESR. While the tubes containing non-heparinized blood were centrifuged for separation of the sera. Specimens from the right femur bone were cleaned from the surrounding soft tissue, crushed, weighed, homogenized in known volume of cold distilled water and then kept at -20°C until later biochemical measurements.

4- Biochemical analysis:

Serum and bone acid (A P) and alkaline phosphatase (ALP) activities as well as bone contents of DNA and RNA were determined spectrophotometrically according to the method reported by. ***Kind and***

King (1954), Dische and Schowrz (1977) and Thoresen et al. (1983) respectively. Additionally, serum total protein content was measured using kit from ABC Diagnostics, New Damietta, Egypt Company according to the method described by ***Reinhold (1953).*** Moreover, CRP was determined using CRP latex test kit optained from (Spinreact, S.A. Ctra. Santa Coloma, Spain), and ESR was calculated according to the method of ***Daice and Lewis (1984).*** The absorbance in different assays was read at Perkin- Elmer Lambda IA spectrophotometer. Furthermore, complete blood picture was analyzed using an automated cell counter (Coulter Onyx).

Statistical analysis

All data were statistically analyzed using the General Linear Models Procedure of the Statistical Analysis System ***(SAS Institute, Inc., 1982).*** The significance of the differences among treatment groups was determined by Waller-Duncan K-ratio ***(Waller and Duncan , 1969).*** All statements of significance were based on probability of $P \leq 0.05$.

RESULTS

Serum and Bone Acid Phosphatase (AP) And Alkaline Phosphatase (ALP) Activities:

As shown in table (1), there were non-significant changes in serum AP activity in all non-arthritic groups compared to that of normal control group, while for bone AP activity, significant reductions were seen. Concerning arthritic groups, serum and bone AP activities were significantly elevated compared to normal control group except (green tea + α-lipoic acid) group which exhibited a non-significant reduction in bone AP activity. On the other hand, significant reductions were seen in serum and bone AP activities in arthritic treated groups comparing to the control arthritic group, arriving the values near the normal levels.

Concerning serum and bone ALP activity, all non-arthritic groups showed non-significant changes except α-lipoic acid treated group which showed a significant increase in serum ALP level compared to normal control. On contrast, all the arthritic groups showed significant elevations except (green tea+ α-lipoic acid) group which showed a non-significant increase in serum ALP activity compared to normal control group. However, all the arthritic treated rats showed significant decreases in serum and bone ALP activity compared to arthritic control group.

Total Serum Protein Content:

As observed in table (1) non-arthritic animals showed non-significant decreases in total serum protein content compared to the normal control group. The arthritic control group showed a highly significant decrease, but all the arthritic treated groups exhibited moderate decreases in total serum protein content, such decrease was significant in green tea arthritic animals only. Treatment of arthritic rats with green tea and/or α-lipoic acid improved the decrease in total serum protein content compared to the untreated arthritic group. There were significant increases in the treated arthritic groups compared to the arthritic control one.

Bone DNA and RNA Contents:

As observed in table (1) bone DNA content showed non-significant increase in all non-arthritic rats except the green tea and α-lipoic acid treated rats which showed a significant increase compared to normal control. On the other hand, there were significant decreases in bone DNA content in all arthritic rats, such decrease was more pronounced in arthritic nontreated group compared to normal control one. Administration of green tea and/or α-lipoic acid to arthritic rats caused significant increases in bone DNA content comparing to arthritic control rats.Bone RNA contents exhibited significant increases in all the non-arthritic groups except the green tea group which exhibited non-significant increase when compared with the normal control. In contrast, there were significant decreases in bone RNA contents in arthritic control group, while the treated arthritic groups showed significant increases compared to the normal control. Administration of green tea and/or α-lipoic acid to the arthritic animals improved the decreased in bone RNA contents, compared to the arthritic control animals where there are significant increase of bone RNA as compared with either the normal control or the arthritic nontreated group.

Inflammatory Markers (CRP and ESR):

As observed in table (2) there were non-significant elevations in blood C-reactive protein content in all non-arthritic rat groups, meanwhile the arthritic rats exhibited significant elevations compared to normal control. Regarding ESR non-significant decreases were observed except non-arthritic green tea treated group and in α-lipoic acid arthritic treated group which showed significant declines compared to normal control. On the other hand, arthritic control group, showed a very highly

significant elevation in both CRP and ESR compared to control one. Treatment of arthritic rats with green tea and/or α-lipoic acid overcame the observed elevations in CRP and ESR compared to the arthritic control group.

Hematological Parameters:

a-Red (RBCs) , White Blood Cells (WBCs) and Platelets Count:

The indicated data in table (3) exhibited significant reduction in RBCs count in all arthritic treated groups compared to normal control one. Regarding WBCs count, arthritic control group showed highly significant elevation while the arthritic treated groups exhibited moderate reduction. Such reduction was significant in green tea and in ALA treated groupswhen compared to normal control. However, platelets count was significantly elevated in all arthritic rat groups compared to normal control one. Concerning non-arthritic groups, non-significant changes were seen in RBCs, WBCs and platelets count except in green tea and α-lipoic acid treated group which showed a significant increase of platelets compared to normal control group. Comparing to the arthritic control animals, all the arthritic treated animals overcame the lowering in RBCs count, and a marked elevation was observed. But for WBCs and platelets count, all the arthritic treated animals exhibited a significant reduction compared to the control arthritic animals nearly match those of the control levels.

b- HC, Hb, MCH, MCHC and MCV:

As observed in table (4), the hematocrit value showed non-significant increases in non-arthritic groups. On contrast, all the arthritic treated groups exhibited a reduction in HC, such reduction was significant in control arthritic group only compared to the normal control. Regarding Hb concentration, there were significant increases in all non-arthritic rat groups, and there were significant decreases in all arthritic ones being more pronounced in the arthritic control group compared to normal control.

Concerning MCH, non-significant increases were recorded in all non-arthritic groups and green tea and α-lipoic acid arthritic treated group

compared to normal control group. All arthritic animals, showed significant reduction in MCH except green tea and ALA treated group which showed non-significant change compared to normal control group. Furthermore, non-significant decreases were seen in MCHC in all non-arthritic groups, but all the arthritic groups exhibited a significant decrease except green tea and α-lipoic acid treated group which showed non-significant increase compared to normal control group. In addition, there were significant elevations in mean corpuscular volume in all rat groups, compared to normal control group except the control arthritic group which showed a significant decrease in MCV.

Daily administration of green tea and/or α-lipoic acid for 14 days to the experimental arthritic rats led to elevation towards the normal levels of HC, Hb, MCH, MCHC and MCV compared to control arthritic group specially in case of the combined treatment which showed normalization of the most parameters.

DISCUSSION

1-Bone remodeling

In the present investigation, some markers of bone metabolism such as AP and ALP were studied. The activities of AP and ALP in bone were elevated in arthritic rats, indicated faster bone turnover during the disease leading to bone loss. The results are in agreement with the previous studies which showed that ALP and AP activities were significantly increased in serum ***(Narayana et al., 1999 and Ramprasath et al., 2005)*** or plasma (**Silva** ***et al., 2005)*** of arthritic rat models.

However, damage to bone and cartilage caused by synovial tissue and pannus in arthritic rats is mediated by several families of enzymes including serine proteases. The most damaging enzymes are the tissue degrading matrix metalloproteinases (e.g. collagenase and gelatenase) and cathepsins ***(Escalante and Del Rincon,2002),*** which can degrade the major structural proteins in the joint. Cytokines, produced by synovial macrophages, such as IL-1 and TNF-α are potent inducers of metalloproteinases gene expression ***(Gary and Firestein, 2005)***.

Furthermore, bone loss is a common feature of various inflammatory arthritis. Localized bone loss in the form of bone erosions and periarticular osteopenia constitutes an important criterion for the diagnosis of rheumatoid arthritis (RA). This damage results from the

activation of an inflammatory response, which increases both the number and activity of osteoclasts ***(Rehman and Lane, 2001)***.

The results of the current study revealed also, the protective role of green tea and/or α-lipoic acid against bone loss resulted from RA. Similarly, some workers established the effects of catechins on the matrix-degrading enzymes. ECG and EGCG have been reported to inhibit in a dose dependent manner the actions of matrix metalloproteinases such as gelatinase A and B and elastase ***(Demeule et al., 2000)***. On the other hand, the protective action of α-lipoic acid seems to be related to its antioxidant effects through scavenging of free radicals ***(Gurer and Ercal, 2000)*** as well as its anti-inflammatory action ***(Zhang and Frei, 2001)***. The present results are in harmony with those of ***Liedtke et al. (1998)*** they explained that, ALA has an inhibitory effect on the matrix metalloproteinases (MMPs). However, the antioxidant effect of both green tea and α-lipoic acid are synergetic since combined use of them led to more enhanced ameliorating effect against adverse effects of RA on bone remodeling.

The present data indicated that, there were significant decreases in total serum protein as well as bone DNA and RNA contents in arthritic rats compared to normal ones. Such decreases were improved by the treatment with green tea and/or α-lipoic acid. These declines may be attributed to the excessive production of ROS that attack cell membranes and can lead to oxidation of amino acid residue side chains, formation of protein–protein cross-linkages, and oxidation of the protein backbone resulting in protein fragmentation ***(Berlett and Stadtman, 1997)***. Additionally, ROS-induced lipid peroxidation, in RA, involved in the disorganization of cell structure and function **(Floyd, 1990)** may consequently inhibit or damage DNA ***(Han et al., 1997)*** and hence affecting protein synthesis. Furthermore, activity of poly ADP-ribosylation associated with DNA damage caused by RA ***(Szabados et al., 1999)*** may be another explanation for depletion of total serum protein and bone DNA and RNA contents in arthritic rats.

Regarding the preventive mechanisms of green tea, it has been speculated that the antioxidative properties of green tea components may be primarily involved, because of protein changes related to the oxidative stress. However, lipid peroxidation and oxidative DNA damage are known to be eliminated by tea consumption; possibly due, in part, to scavenging the nitrogen oxide or their radical derivatives ***(Kohl et al.,1995)***. Concerning lipoate, it acts as an antioxidant in clearing

peroxyl, hydroxyl and superoxide radicals ***(Han et al.,1997)*** and thereby protects DNA. Moreover, lipoate has been demonstrated to provide protection to plasmid DNA against singlet oxygen-induced damage, through its high free radical scavenging action ***(Devasagayam et al.,1993)*** However, the concomitant use of the two antioxidants (green tea and ALA) exhibited more marked ameliorating effects than using each alone, i.e. their effects are additive.

2- Inflammatory markers:

The inflammatory process in RA is characterized by an excessive tumor-like proliferation of synovial tissue that is associated with an increase in the respective vasculature (needed to support metabolic requirements) ***(Taylor, 2005; Pap and Distler, 2005 and Strunk et al.,2006)*** and perfusion near and inside (peri and intra-articular blood vessels) in the inflamed joint capsule of patients with RA ***(Strunk et al.,2006)***. However, collagen-induced RA animals, herein, exhibited marked elevation in both the CRP and ESR compared to normal control ones reflecting severe inflammation and active phase of the disease. Such findings are consistent with those of ***Vivian et al. (2005)*** who reported that patients with RA had elevated ESR as well as elevated CRP. In addition, ***Bovin et al. (2004)*** reported that RA patients had significantly increased CRP values compared to controls.

In the present study, treatment with green tea extract and/or α-lipoic acid exhibited a marked regression of the inflammation as observed by the regression of edema and reddening features of the feet of rats as well as the reduced levels of CRP and ESR compared to nontreated arthritic rats. Similarly, some authors concluded that consumption of green tea may be prophylactic and benefit for arthritic patients by reducing inflammation and slowing cartilage breakdown ***(Haqqi et al.,1999 and Adocks et al., 2002)***. However, catechins, the main constituent of green tea, have been found to have anti-inflammatory properties which may be due to their ability to inhibit tumor-necrosis factor (TNF) synthesis ***(Yang et al., 1998)***, possibly by inhibition of Kinase (s) in signaling cascades, leading to activation of certain transcription factors ***(Mukhtar and Ahmed, 1999)***.

α-lipoic acid, in addition was suggested to be anti-inflammatory agent that interferes, at the same time, with nitric oxide released from inflammatory macrophages, thus protecting target cells from oxygen radical attack ***(Burkart et al., 1993)***. Moreover, such improvement in the

inflammatory features and markers may be attributed to the antioxidant effects of green tea *(Guo et al. 1996)* and ALA (**Sumathi *et al.*, 1993**) in addition to their anti-inflammatory properties (***Sueoka et al., 2001 and Burkart et al., 1993*** respectively) taking in consideration that their concomitant treatment was more effective.

4- Hematological Parameters:

From the present study, it is obvious that RA rats suffer from microcytic hypochromic anemia as indicated by the significant decrease in RBCs count, HC, Hb, MCH, MCHC as well as MCV compared to healthy animals. The present study exhibited also significant elevations in WBCs and platelets count in arthritic animals compared to control ones. These results are in agreement with the previous investigations illustrated that patients with RA had decreased Hb level (***Akyol et al., 2001; Vivian et al., 2005 and Vijayakumar et al., 2006***). decreased RBCs count (***Akyol et al., 2001 and Vijayakumar et al., 2006***) and suffered from thrombocytosis *(**Vivian et al., 2005**)*.

However, there is widespread lipid and protein damage in patients with RA (***McCord, 1993***). Such damage may lead to an increase of RBCs membrane fragility and rate of hemolysis. Moreover, the observed anemia in arthritic rats, in the present work can be attributed partly to the decrement in protein associated with DNA and RNA damage induced by LPO *(**Cimen et al., 2000**)*. Consequently, hemolysis will stimulate bone marrow for rapid production of immature microcytic cells liable to more hemolysis. Alternatively, congestion of the blood vessels in the inflamed joint and the escape of RBCs from these congested vessels *(**Gary and Firestein, 2005**)* may be another explanation for anemia in RA.

Concerning WBCs, the present results showed a significant elevation of WBCs count in arthritic animals compared to normal ones. These results are in accordance with previous findings revealed an elevated WBCs count in adjuvant-arthritic rats *(**Kang et al.,2002; Bovin et al., 2004; Vivian et al., 2005 and Balbir-Gurman et al.,2006**)*. However, the mean leukocyte count was closely correlated with the severity of inflammation and joint lesions in patients with arthritis *(**Aman et al., 1999**)*. In addition, RA is a disease with systemic manifestations, often affecting blood vessels and bone marrow function and it is therefore, reasonable to assume that at least some disease processes may be reflected in blood leukocytes (***Bovin et al., 2004***).

The present study exhibited also significant elevation in the platelets count in arthritic animals compared to normal ones. These findings are corroborated with the results obtained by ***Milovanovic et al. (2004); Vivian et al. (2005) and Zha et al. (2006)***. The increase of platelets count in RA was also in harmony with the results of ***Ertenli et al. (1998)*** who indicated that the stimulation of cytokines promotes the production of platelets.

On the other hand, treatment of arthritic rats with green tea and/or ALA, led to a marked improvement in all the hematological parameters including anemia (decreased RBCs count, Hb, HC, MCH, MCHC and MCV), leucocytosis as well as thrombocytosis. Such improvement may be attributed to both the antioxidant and anti-inflammatory properties of green tea and ALA and exhibited the usefulness of each alone, as well as the additive effects of the concomitant treatment .

The overall beneficial effects of green tea and/or ALA may be attributed to their high ability to scavenge ROS (***Guo et al., 1996 and Gurer and Ercal, 2000*** respectively) and to augment and repair the activity of the antioxidant system (***Saija et al., 1995; Gurer and Ercal, 2000 and Skrzydlewska et al.,2002*** respectively) as well as to their anti-inflammatory properties (***Burkart et al., 1993 and Sueoka et al., 2001***). Therefore, recommendation of their use in combination may be valuable in the management and alleviation of RA risks

In conclusion the improvement in the bone remodeling rate and the inflammatory features may be attributed to the antioxidant effects of green tea (***Guo et al. 1996***) and/or ALA (***Sumathi et al., 1993***) in addition to their anti-inflammatory properties (***Burkart et al., 1993 and Sueoka et al., 2001*** respectively),taking in consideration that their concomitant treatment was more effective. Moreover, treatment of RA rats with green tea and/or ALA, led to a marked improvement in all the hematological parameters including anemia (decreased RBCs count, Hb, HC, MCH, MCHC and MCV), leucocytosis as well as thrombocytosis. Such results exhibited the usefulness of each alone, as well as the additive effect of the concomitant treatment of both green tea extract and α- lipoic acid.

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Table 1: Serum and Bone Acid (AP) ,Alkaline phosphatase (ALP) Activities; Serum Total Protein And Bone DNA and RNA Contents in Non-arthritic and Arthritic Female Rats Treated with Green Tea and /or Alpha-Lipoic Acid .

	Non-arthritic groups				Arthritic groups			
	Normal control	Green tea (28.57mg/kgbw)	Alpha-lipoic acid (25mg/kgbw)	Green tea + Alpha-lipoic acid	Arthritic control	Green tea (28.57mg/kgbw)	Alpha-lipoic acid (25mg/kgbw)	Green tea + Alpha-lipoic acid
Serum (AP) (K.A.U / dl)	13.9 ±1.12	13.76 ±1.15	13.82 ±0.71	12.46 ±1.20	31.28 ±0.67^{a***}	22.14 ±0.06^{a***,b***,c***}	18.99 ±0.14^{a***,b***,d***}	15.10 ±0.96^{a**,b***,e***}
Bone (AP) (K.A.U. / g)	4.12 ±0.16	3.86 ±0.27^{a*}	3.83 ±0.07^{a**}	4.27 ±0.13	5.72 ±0.08^{a***}	5.18 ±0.05^{a***,b***,c***}	4.83 ±0.15^{a***,b***,d***}	4.09 ±0.01^{b***}
Serum (ALP) (KA.U./ dl)	209.09 ±0.20	209.25 ±0.43	219.26 ±3.26^{a*}	215.74 ±0.22	275.26 ±8.41^{a***}	219.66 ±9.92^{a**,b***,c*}	246.70 ±13.66^{a***,b***,d***}	212.82 ±1.78^{b***}
Bone (ALP) (K.A.U./g)	25.70 ± 0.73	27.02 ± 0.76^{a*}	29.52 ± 0.98^{a***}	31.08 ± 0.63^{a***}	42.60 ± 0.07^{a***}	27.56 ± 1.84^{a**,b***}	27.51 ±0.53^{a**,b***,d***}	28.33 ±0.23^{a***,b***,e***}
Serum total Protein (g%)	7.80 ±0.43	7.66 ±0.23	7.42 ±1.31	7.66 ±0.21	5.72 ±0.04^{a***}	6.90 ±1.20^{a*,b***}	7.66 ±0.15^{b**}	7.46 ±0.34^{b***}
Bone (DNA) (µ g / g)	20.60 ±0.89	20.80 ±1.09	20.80 ±1.09	27.20 ±0.44^{a***}	13.20 ±1.78^{a***}	15.60 ±0.54^{a***,b***,c***}	16.28 ±0.40^{a***,b***,d**}	22.8 ±1.09^{a**,b***,e***}
Bone (RNA) (µ g / g)	34.40 ±1.34	37.40 ±4.92	56.90 ±3.56^{a***}	54.74 ±7.09^{a***}	15.14 ±0.26^{a***}	31.86 ±0.94^{b***,c*}	45.70 ±2.68^{a***,b***,d***}	44.10 ±1.74^{a***,b***,e***}

Values were expressed as means ±SD of five animals in each group. * = P<0.05 (significant) , ** = P< 0.01 (high significant).
*** = P<0.001(very high significant).
a = Significant difference in comparison with normal control group.
b = Significant difference in comparison with arthritic control group .
c, d and e = Significant difference comparing each arthritic treated group with its matched non-arthritic one respectively.

Table 2: C-Reactive Protein (CRP) (mg/l) and Erythrocyte Sedimentation rate (ESR)(mm/hr) in Non-arthritic and Arthritic Female Rats Treated with Green Tea and /or Alpha-Lipoic Acid.

	Non-arthritic groups				Arthritic groups			
	Normal control	Green tea (28.57mg/kgbw)	Alpha-lipoic acid (25mg/kgbw)	Green tea + Alpha-lipoic acid	Arthritic control	Green tea (28.57mg/kgbw)	Alpha-lipoic acid (25mg/kgbw)	Green tea + Alpha-lipoic acid
CRP (mg/l)	5.00 ±0.00	5.25 ±0.00	6.15 ±0.23	6.20 ±0.45	64.40 ±9.63^{a***}	24.00 ±0.00^{a***,b***,c**}	21.60 ±3.28^{a***,b***,d***}	33.60 ±10.04^{a***,b***,e***}
ESR (mm/hr)	2.60 ±0.55	2.0 ±0.00^{a*}	2.10 ±0.18	2.20 ±0.45	5.40 ±0.55^{a***}	2.60 ±0.55^{b***,c*}	1.60 ±0.55^{a***,b***}	2.20 ±0.45^{b***}

Values were expressed as means ±SD of five animals in each group. * = P<0.05 (significant) , ** = P<0.01 (high significant), *** = P<0.001(very high significant).

a = Significant difference in comparison with normal control group.

b = Significant difference in comparison with arthritic control group .

c, d and **e** = Significant difference comparing each arthritic treated group with its matched non-arthritic one respectively.

Table 3: Red and White Blood Cells and Platelets Count in Non-arthritic and Arthritic Female Rats Treated with GreenTea and/or Alpha-Lipoic Acid .

	Non-arthritic groups				Arthritic groups			
	Normal control	Green tea (28.57mg/kgbw)	Alpha-lipoic acid (25mg/kgbw)	Green tea + Alpha-lipoic acid	Arthritic control	Green tea (28.57mg/kgbw)	Alpha-lipoic acid (25mg/kgbw)	Green tea + Alpha-lipoic acid
RBCs (millions/mm^3)	4.49 ±0.21	4.60 ±0.18	4.55 ±0.06	4.64 ±0.22	3.05 ±0.11^{a***}	3.89 ±0.09^{a***,b***,c***}	4.04 ±0.22^{a***,b***,d***}	4.04 ±0.24^{a***,b***,e***}
WBCs Thousands/mm^3	4.66 ±0.75	4.96 ±0.11	4.55 ±0.11	4.62 ±0.36	6.56 ±0.41^{a***}	4.12 ±0.07^{a***,b***,c***}	4.19 ±0.02^{a***,b***}	4.27 ±0.17^{b***}
Platelets Thousands/mm^3	212.00 ±0.00	206.00 ±8.94	205.00 ±7.07	228.12 ±1.96^{a***}	272.00 ±2.74^{a***}	220.00 ±0.00^{a***,b***,c***}	220.00 ±0.00^{a***,b***,d***}	229.60 ±12.28^{a***,b***}

Values were expressed as means ±SD of five animals in each group * = P<0.05 (significant) , ** = P<0.01 (high significant).
*** = P<0.001(very high significant)
a = Significant difference in comparison with normal control group.
b = Significant difference in comparison with arthritic control group .
c, d and **e** = Significant difference comparing each arthritic treated group with its matched non-arthritic one respectively.

Table 4: Hematocrit value (HC), Hemoglobin (Hb) Concentration, Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) in Non-arthritic and Arthritic Female Rats Treated with Green Tea and /or Alpha-Lipoic Acid .

	Non-arthritic groups				Arthritic groups			
	Normal control	Green tea (28.57mg/kgbw)	Alpha-lipoic acid (25mg/kgbw)	Green tea + Alpha-lipoic acid	Arthritic control	Green tea (28.57mg/kgbw)	Alpha-lipoic acid (25mg/kgbw)	Green tea + Alpha-lipoic acid
HC value	40.00 ±1.41	41.00 ±0.70	44.5 ±10.63	42.00 ±1.73	17.40 ±2.51^{a***}	37.00 ±1.41^{b***}	37.90 ±1.52^{b***,d*}	37.80 ±2.17^{b***}
Hb (g/ dl)	10.24 ±0.28	11.04 ±0.59^{a***}	11.21 ±0.12^{a***}	11.26 ±0.18^{a***}	6.94 ±0.47^{a***}	7.58 ±0.35^{a***,b*,c***}	7.94 ±0.26^{a***,b***,d***}	8.72 ±0.41^{a***,b***,c***}
MCH (pg/dl)	22.92 ±0.66	23.66 ±0.19	23.10 ±0.00	23.10 ±0.06	16.94 ±0.05^{a***}	19.88 ±1.38^{a***b***,c***}	21.28 ±0.44^{a***,b***,d**}	22.30 ±1.85^{b***}
MCHC (g/dl)	23.46 ±0.05	22.02 ±0.79	24.14 ±1.59	21.64 ±0.82	18.08 ±0.18^{a***}	21.22 ±1.70^{a***,b***}	20.18 ±0.56^{a***,b***,d***}	23.86 ±1.87^{b***,c**}
MCV (fl)	84.66 ±1.78	91.10 ±1.47^{a***}	92.18 ±0.85^{a***}	90.76 ±0.97^{a***}	82.78 ±0.71^{a*}	94.08 ±1.34^{a***,b***,c***}	94.52 ±1.62^{a***,b***,d**}	94.16 ±1.86^{a***,b***,c***}

Values were expressed as means ±SD of five animals in each group. * = P<0.05 (significant) , ** = P<0.01 (high significant), *** = P<0.001(very high significant).

a = Significant difference in comparison with normal control group.

b = Significant difference in comparison with arthritic control group .

c, d and e = Significant difference comparing each arthritic treated group with its matched non-arthritic one respectively

دور كل من مستخلص الشاى الأخضر و/أو حمض الليبويك للحد من بعض مخاطر الروماتويد المفصلى فى إناث الجرذان

التأثير على إعادة إحلال العظام وبعض دلالات الالتهاب والمعايير الدموية

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الملخص العربى

تهدف الدراسة إلي كشف مدي الاستفادة من استخدام بعض المواد الطبيعية المضادة للأكسدة و ذات التأثير المضاد للالتهابات مثل مستخلص الشاي الأخضر وحمض الليبويك للتقليل من حدوث وحدة مخاطر مرض الروماتويد المفصلى . وقد استخدمت في هذه الدراسة إناث الجرذان البالغة التي تزن في المتوسط 150جم حيث تم تقسيمها إلي مجموعتين رئيسيتين:

(1) مجموعة غير مصابة بالمرض (2) مجموعة أحدث بها المرض.

قسمت كل منها إلي أربعة مجموعات فرعية كما يلي :-

أ- مجموعه ضابطه (لم تتناول أي مادة) .

ب- مجموعه معامله بمستخلص الشاي الأخضر عن طريق الفم (28.75مجم/ كجم) يوميا لمدة 14 يوم.

ج- مجموعه معامله بحمض الليبويك عن طريق الحقن البريتوني (25مجم/كجم) يوميا لمدة 14يوم.

د- مجموعه معامله بكل من مستخلص الشاي الأخضر وحمض الليبويك بنفس الجرعات السابقة ولنفس المدة .

توصلت الدراسة للنتائج الآتية:

1- سجلت النتائج الآتية فى المجموعة المستحدث بها الروماتيد المفصلى مقارنة بالمجموعة الضابطة:

- ارتفاعا ملحوظا في نشاط كل من إنزيمي الفوسفاتيز الحامضي(AP) و القاعدي(ALP) في المصل والعظام.

- انخفاضا ملحوظا في محتوي المصل من البروتينات الكلية وكذلك محتوي العظام من الاحماض النووية ((DNA and (RNA)

- ارتفاعا ملحوظا في مؤشرات حدوث الالتهابات مثل (CRP) وزيادة معدل سرعة الترسيب في الدم (ESR).

- انخفاضا في عدد خلايا الدم الحمراء على العكس مما لوحظ من زيادة فى عدد خلايا الدم البيضاء وكذلك عدد الصفائح الدموية مع انخفاض في تركيز الهيموجلوبين وكذلك في كل من قيمة الهيماتوكريت والمحتوى الهيموجلوبينى ومتوسط هيموجلوبين الكرية ومتوسط تركيز الهيموجلوبين و متوسط حجم الكرية

.(HC,Hb, MCH, MCHC and MCV)

2- مع استخدام مستخلص الشاي الأخضر و حمض الليبويك لوحظ ظهور تحسن ملحوظ في معدل بناء العظام وصورة الدم. كما ارتفع محتوي البروتين الكلي والأحماض النووية في العظام بالإضافة إلي تعديل النشاط الزائد في مؤشرات الالتهاب (CRP and ESR) .

ونستنتج من هذه الدراسة أن كلا من الشاي الأخضر وحمض الليبويك له دور فعال في الحد من بعض التغيرات السلبية المصاحبة للروماتويد المفصلي وكذلك دور المعالجة المشتركة لهما حيث كان لها تأثيرا متزايدا.