Effect of *Nigella Sativa* and Vitamin E on Some Oxidative / Nitrosative Biomarkers in Systemic Lupus Erythematosus Patients

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Abstract: Introduction: Although the cause of SLE is likely multifactorial, it has been suggested that the increased production of reactive oxygen species or/and reactive nitrogen species may favor the development of SLE. Thymohydroquinone is the most abundant active ingredients of *Nigella sativa*. The free radical scavenging capability of TQ is as effective as superoxide dismutase. Vitamin E is the most powerful biological antioxidant. **Aim of the work**: To assess the co-therapeutic efficacy of supplementation with *Nigella Sativa* and vitamin E. **Patients and methods:** 50 subjects were included; 25 patients diagnosed with mild to moderate SLE, and 25 healthy individuals served as control. SLE patients were supplemented with antioxidants (Vitamin E and *Nigella Sativa*) for three months to assess the impact on some markers of oxidative and nitrosative stress. **Results:** We noticed a significant increase in the levels of SOD and GSH in SLE patients following treatment with Nigella sativa and vitamin E in comparison to pre-treatment levels (P<0.001 for both). While the levels of IL-10, MDA, NO, iNOS decreased significantly following treatment (P<0.001 for all). **Conclusion:** The improvement of selected oxidative and nitrosative biomarkers and SLEDAI score favors antioxidant therapy in SLE.
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1. Introduction:

Systemic lupus erythematosus (SLE) is one of the most diverse autoimmune diseases as it may affect any organ in the body and it displays a broad spectrum of clinical and immunological manifestations [1].

Oxidative stress is a situation whereby cellular levels of reactive oxygen species (ROS) or/and reactive nitrogen species (RNS) overwhelm the cellular antioxidant capacities [2].

Although the cause of SLE is likely multifactorial, it has been suggested that the increased production of (ROS) and (RNS), as an important arm of the innate immune response, may favor the development of SLE. Oxidative stress may contribute to immune-cell dysfunction, autoantigen production and autoantibody reactivity in SLE [3].

Anti-oxidant enzymes, such as reduced glutathione (GSH), super oxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and glutathione peroxidase (GSH-Px) constitute the main pool of the anti-oxidant system of most cells. It is well known that anti-oxidant enzymes are responsible for neutralizing the free radical-induced oxidative damage [4].

Nigella sativa (NS) is a herbal plant which belongs to Ranunculaceae family. It is also known as black cumin or habatus sauda, and has a rich historical and religious background [5]. Furthermore, the Prophet Muhammad (PBUH) advised: “Hold on to use the black cumin, because it can heal every disease except death [6].

The main active ingredients of NS are thymoquinone, dithymoquinone, thymohydroquinone, and thymol. Among the compounds identified, thymoquinone (TQ) is the most abundant, which makes up 30–48% of the total compounds [7]. It is interesting to find that the most significant property of TQ is its antioxidative activities. It has been reported that the free radical scavenging capability of TQ is as effective as superoxide dismutase [8].

Plant-based anti-oxidants have recently gained popularity due to their role as dietary supplements with minimal side effects [9].

Vitamin E (α-tocopherol) is fat-soluble vitamin. It is the most powerful biological antioxidant [10]. A large number of clinical studies in healthy individuals, populations at risk for certain diseases, and patients undergoing disease therapy indicate that supplementation with vitamin E may result in changes in either oxidative status, disease risk, or disease outcome [11].

Aim of the work:

To assess - alongside conventional therapy to SLE – the co-therapeutic efficacy of supplementation with Nigella sativa and vitamin E.

2. Patients and methods:

Twenty five patients fulfilling the American College of Rheumatology revised criteria for SLE [12] were recruited at the wards and outpatient clinic of Internal Medicine department of Tanta University Hospital, Egypt, during a period from 1/4/2014 to 1/4/2015, they formed group I. Twenty five healthy volunteers of matching age and sex were also included as a control group (group II).

SLE patients (group I) were divided into 2 subgroups: Ia: before supplementation and Ib: after supplementation. Patients older than 18 years with mild to moderate systemic lupus erythematosus disease activity index (SLEDAI) score (5.52±1.92) were included in the study, while pregnant females and patients with any chronic illness like diabetes, hepatic or renal insufficiency or malignancies were excluded. All the patients completed the study period and no patient was dropped.

All participants provided a written informed consent and were subjected to: full history taking, thorough clinical examination, laboratory investigations in the form of: Complete blood count(CBC), erythrocyte sedimentation rate (ESR) by Westergren method, c reactive protein (CRP) by latex agglutination, antinuclear antibody (ANA) by ELISA technique(the ELISA kit was supplied by Sigma scientific service company (USA), anti-double strand DNA(Anti ds DNA) by ELISA technique(the ELISA kit was supplied by Sigma scientific service company (USA), interleukin-10 (IL-10) by ELISA technique (the ELISA kit was purchased from Boster Biological Technology Co., Inc.), oxidative / nitrosative biomarkers: super oxide dismutase (SOD) by spectrophotometric method, using commercial kit supplied by (Biodiagnostic, Egypt), reduced glutathione (GSH) by spectrophotometric method using commercial kit supplied by (Biodiagnostic, Egypt), malondialdehyde (MDA) by spectrophotometric method using commercial kit supplied by (Biodiagnostic, Egypt), nitric oxide (NO) by spectrophotometric method using commercial kit supplied by (Biodiagnostic, Egypt), inducible nitric oxide synthase (iNOS) by ELISA technique (the ELISA kit was purchased from Glory Science Co., Ltd.USA).

For SLE patients only, the co-therapeutic strategy entailed the daily oral intake of the following drugs for 3 successive months: Nigella sativa (Baraka; 450 mg capsules, Pharco pharmaceutical company, Egypt) and vitamin E (Vitamin E; 400 mg capsules, Pharco pharmaceutical company, Egypt) both recommended once daily. No supplementations were given to the control group.

The given drugs may have minimal side effects- which were explained to the patients- in the form of: gastrointestinal upset, nausea, vomiting and diarrhea.

Blood samples of total 25 patients were collected as baseline at the start of the study. After 12 weeks of supplementation, blood samples were drawn again for the analysis.

Statistical analysis:

Comparisons between two groups were performed using a two-tailed unpaired Student t test. Data were presented as mean ± standard deviation and range. Correlations were assessed by using the Pearson correlation test. P value less than 0.05 was considered statistically significant. All analyses were performed using SPSS statistical software (SPSS V.16, Inc., Chicago, IL).

3. Results:

In this work, group I included 25 patients with SLE. All of them were females. Twenty five healthy females of matching age and sex served as control group (group II). Table (1) shows age, sex, duration of diagnosis, SLEDAI score, and treatment received in the studied groups.

In our study we found that SOD and GSH levels of SLE patients prior to supplementation (group I a) were significantly lower than healthy subjects (P<0.001 for both). While the levels of IL-10, MDA, NO, iNOS were significantly higher in SLE patients prior to supplementation in comparison to controls (P<0.001 for all) table (2).

Table (1): Demographic data of SLE participants:

	Patients(25)	**Control (25)**
Age in years	22-40 (38.2±3.1)	20-39 (36.8± 3.11)
Sex	30 females (100%)	30 females (100%)
Disease duration	6-50 (17.8±10.34)	NA
SLEDAI score	3-9 (5.52±1.92)	NA
Treatment received: • **Steroids** • **Azathioprin** • **hydroxychloroquin**	 25/25 18/25 23/25	NA

Table (2): Comparison of some laboratory investigations in SLE patients pre-supplementation and controls.

	25 SLE Pre-supplementation (mean±SD)	**25 Control (mean±SD)**
ANAIU/ml	57.46±18.1	- ve
Anti ds DNA IU/ml	166.28±66.98	- ve
CRP mg/L	4.32±2.75	-ve
IL-10 pg/ml	116.85±8.94	12.77±1.51
MDA nmol/ml	12.98±0.64	5.75±0.53
NO umol/L	302.35±12.76	170.54±7.08
iNOS IU/ml	29.84±2.78	6.59±0.91
GSH mg/dl	19.8±2.93	44.66±2.45
SOD U/g Hb	357.74±49.97	765.9±25.84

Table (3): Comparison of some laboratory investigations in SLE patients pre and post supplementation.

		SLE patients			
		Pre-supplementation	Post- supplementation	Difference mean±SD	*P*-value
ANA IU/ml	Range	34.2±95.3	21.4-73.1	20.82±4.42	<0.001
	mean±SD	57.46±18.1	36.64±16.34		
Anti ds DNA IU/ml	Range	69-287	40-150	81.36±49.02	<0.001
	mean±SD	166.28±66.98	84.92±33.55		
CRP mg/L	Range	0-6	0-6	1.2±2.45	0.151
	mean±SD	4.32±2.75	3.12±3.06		
SLEDAI score	Range	2-9	0-7	1.44±1.45	0.0112
	mean±SD	5.52±1.92	4.08±1.98		
IL-10 pg/ml	Range	100.78-130	60.87-79.98	45.79±3.2	<0.001
	mean±SD	116.85±8.94	71.06±5.86		
MDA nmol/ml	Range	12-14	8-10	4.07±0.26	<0.001
	mean±SD	12.98±0.64	8.91±0.66		
NO umol/L	Range	280.98-320	200-219.98	91.73±8.18	<0.001
	mean±SD	302.35±12.76	210.62±6.16		
iNOS IU/ml	Range	25.09-35	15.01-20	12.77±1.52	<0.001
	mean±SD	29.84±2.78	17.07±1.47		
GSH mg/dl	Range	15.76-25	35.03-44.87	19.81±0.47	<0.001
	mean±SD	19.8±2.93	39.62±3.05		
SOD U/g Hb	Range	300.67-445.28	500.76-600	192.29±19.79	<0.001
	mean±SD	357.74±49.97	550±32.27		

Table (4): Percent of change in SLE patients before & after co-therapy regarding ANA, anti ds DNA antibodies, SLEDAI score, IL-10, MDA, NO, iNOS, GSH and SOD.

	% of change Mean±SD
ANA IU/ml	37.98±7.85
Anti-ds DNA IU/ml	46.19±14.99
SLEDAI score	23±2.05
IL-10 pg/ml	39.21±0.7
MDA nmol/ml	31.45±2.32
NO umol/L	30.29±1.64
iNOS umol/L	42.72±2.11
GSH mg/dl	102.05±14.38
SOD	55.39±12.37

We also noticed that the levels of SOD and GSH in SLE patients increased significantly following treatment with nigella sativa and vitamin E in comparison to pre-treatment levels (P<0.001 for both). While the levels of ANA, anti ds DNA, SLEDAI score, IL-10, MDA, NO, INOS decreased significantly following treatment (P<0.001 for all) as shown in table (3).

We also found marked improvement of of ANA, anti-ds-DNA and SLEDAI score, IL-10, MDA, NO, INOS, SOD and GSH in SLE patients after co-therapy as noticed by the percent of change before & after co-therapy as shown in table (4).

4. Discussion:

Disease activity and progression of (SLE) including cell signaling, differentiation, proliferation and apoptosis contribute to functional oxidative modification [13]. Consistently, the ability/inability of the antioxidant defense system to cope with oxidative stress is one of the possible causes in the pathogenesis of SLE [14-15].

In this study we tried to assess the co-therapeutic role of nigella sativa and vitamin E on some laboratory, clinical and oxidative / nitrosative biomarkers in SLE patients. The results of present study indicate that SLE patients are under oxidative stress, demonstrated by decreased levels of the antioxidant enzymes.

In our study the relative magnitude of oxidative stress in SLE patients before treatment had contributed to the monitored increments of NO, iNOS and MDA.

In parallel, the assessed increments in NO, iNOS and MDA versus the decrements SOD, GSH was co-linked with cellular dysfunction and to deranged bioenergetics via defective redox capacity in T lymphocytes and neutrophils which was in agreement with Li *et al.* [16].

In our study the assessed decrements of GSH in group Ia via the increased thiol oxidation by ROS coordinates with associated increments of nitric oxide (NO) the product of inducible nitric oxide synthase (iNOS) whose expression is usually accompanied by inflammatory lesions. It agrees with the notation that in SLE there are likely to be multiple tissue sites of increased iNOS expression due to the systemic circulation of a variety of immune stimuli [17].

Also, the reported findings documentation that the endothelium and keratinocytes in SLE models overexpress iNOS , provide unexpected insights into the inflammatory processes characteristic of SLE [18]. This results in the conversion of NO to various reactive nitrogen species (RNS) including the peroxynitrite whose formation can be a basis for antibody modification [19].

It is verified by the paralleled increments of ANA and anti ds DNA antibodies in group Ia SLE patients with increased NO leading to overproduction of peroxynitrite which furthermore generate hydroxyl radicals the most damaging ROS species [19]. McKnight *et al.* [20] as well reported that Th1 cells upon activation secrete proinflammatory cytokines (as IL-12 and also interferon γ) which mainly activates macrophages to produce ROS and NO in order to mediate innate immunity.

Notably, the assessed increments of IL-10 detected in our study coordinated with Yuan et al [21] who reported that a defect in IL-10 homeostatic function via down regulation of immune complex induces inflammatory cytokine production by IL-10 that was contributed to SLE pathogenesis.

IL-10 - being an important immunoregulator cytokine produced by various immune cell populations including all leucocytes - can directly result in autoantibody production [22]. Administration of IL-10 monoclonal antibodies was noted to lead to long-lasting reduction in clinical symptoms and disease activity in SLE [23].

In view of the management strategy applied herein the current findings in group Ib displayed a synergestic response to the immunomodulatory and antioxidative role of both Nigella sativa and vitamin E co-therapeutic supplementation to SLE cases. It elaborated the role of coordination between reductions of SLEDAI score, ANA, anti ds DNA antibodies, with respective intervention of ROS and RNS induced apoptotic stimuli via multiple signaling mechanisms.

Accordingly, the measurement of the selected biomarkers in the present study, were intended to determine the outcome of Nigella sativa and vitamin E co-therapeutic intervention via assessment of antibody production versus apoptotic mechanisms influenced by the replenished redox balance that was confirmed by increase in SOD and GSH accomplished via reshifting of GSH/GSSG ratio which plays a major role in the modulation of Th1/Th2 balance mediating autoimmune inflammation.

Hence, reduced glutathione (GSH) was significantly higher in group Ib patients than in the group Ia. These findings could be explained by the ability of vitamin E to increase glutathione synthesis that is brought about by the stimulation of glutathione synthetase activity. An alternative possibility is a reduced utilization of glutathione for the detoxification of free radicals as explained by Costagliola *et al.* [24]. Also Suwannaroj *et al.* [25] reported that replenishment of the intracellular glutathione has been associated with diminution of autoantibody levels. Perl A [26] also reported that it

has been associated with improved disease activity and fatigue that may attenuate disease complications.

Furthermore, the significant increase in SOD in treated group can be explained by the fact that Thimoquinones TQ and dithymoquinone DTQ showed superoxide dismutase (SOD)-like activity [27]. Via protective action against superoxide anion radical either generated photochemically, biochemically, indicating its clear potent superoxide radical scavenger activity [28].

In this study the complementary of NS and vitamin E potentiate the decrease in levels of MDA, NO and iNOS. This change coordinated with diminution of immune-inflammatory status which altogether minimized the initiation and execution phases of apoptosis with subsequent reduction of autoantibody production of ANA and anti dsDNA antibodies.

These results agree with reports of Kashiwagi *et al.* [29] reflecting the role of vitamin E which suppress signal transducers and activator of transcription 3 (STAT3) signaling pathways. Moreover, anti-proliferative effect of the vitamin was noted to occur via inhibition of mitogen activated protein kinase (MAPK) and Janus Kinase (JNK) As well, vitamin E inhibits expression of cyclin D which affects SLE pathogencity [30].

Confirmatively, NS and vitamin E augmented antioxidant defenses proved as beneficial adjunctive therapy in the treatment and attenuation of various oxidative stress induced apoptotic manifestations verified by reducing increments of in NO, iNOS and IL-10. Referencewize, vitamin E modulates cell signaling and gene expression besides several enzymes and transcription factors [31], [32].

In addition, (TQ) in a dose- and time-dependently manner, reduce nitrite production, a parameter for NO synthesis [33].

Oral administration of TQ in Wistar rats at 5 mg/kg body weight for 21 days resulted in a significant reduction of the levels of different antioxidant parameters (myeloperoxidase MPO, LPO, GSH, catalase (CAT), SOD and NO) in collagen induced arthritis (CIA) [34].

Khader and Eckl [35] reported that thymoquinone, has a wide spectrum of favorable effects. Two of them are anti-inflammatory, antioxidant effects.

Perl A [26] reported that the imbalance between oxidant and antioxidant enzymes in favor of the former contributes to the pathogenesis of SLE. The restoration of the redox balance using antioxidant agents or diminishing effect of oxidative stress by intake of antioxidant nutrients including vitamins E may attenuate various oxidative stress induced complication in SLE.

Costenbader *et al.* [36] reported that studies with MRL/lpr mice have shown that treatment with vitamin E supplementation modulates the levels of inflammatory cytokines, delays the appearance of autoimmunity, and increases survival.

Minami *et al.* [37] also documented that the adequate consumption of E is inversely related to the SLE activity.

There has been great progress in the development of oxidative stress biomarkers, but due to the complex nature of disease, there is an extremely low possibility that a single biomarker can reflect the whole body of oxidative damage and its role in the pathophysiology of disease [19].

Conclusion:

The positive relationships between selected oxidative and nitrosative biomarkers together with the improvement of SLEDAI score, reinforced the contribution of antioxidant therapy in SLE. Thus, we recommend that, this co-therapy should be taken in consideration in the management of SLE disease.
However, further studies with larger number of patients are needed in elaborating the role of antioxidant enzymes in SLE. Also, randomized placebo controlled trials may explore the exact therapeutic role of vitamin E, and N. sativa in SLE patients.

Conflict of interest:

The authors have no conflict of interest to declare.

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