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Effects of Oral Antioxidants on Lesion Counts Associated with Oxidative Stress and Inflammation in Patients with Papulopustular Acne

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Abstract

Background: There has been an increasing focus on the extent to which oxidative stress and inflammation are involved in the pathophysiology of acne. The aim of this study is to investigate the effect of oral Silymarin, N-acetylcysteine and Selenium in the treatment of acne vulgaris.

Methods: A randomized prospective clinical trial was carried out on 56 patients with acne vulgaris who were examined clinically by dermatologist and classified according to disease severity. Serum levels of Glutathione, Malondialdehyde and Interleukine-8 in the acne patients were measured pre- and eight weeks post-treatment with oral antioxidants and compared to that of 28 healthy volunteers. The clinical follow- up was done every two weeks to assess the changes in the number of inflammatory lesions.

Results: Administration of antioxidants to patients with acne vulgaris significantly reduce serum Malondialdehyde level; and increased serum level of Glutathione after eight weeks compared to pre-treatment value, also significantly reduce Interleukine-8 serum levels and the number of inflammatory lesions in patients with acne compared to placebo.

Conclusion: The results obtained in this study clearly showed the beneficial effect of Silymarin, N-acetylcysteine and Selenium to patients with acne vulgaris as indicated by the clinical improvement that strongly and positively correlated with improvement in biochemical data.

Keywords: Acne vulgaris; Papulopustular acne; Oxidative stress; Inflammation; Antioxidants; Silymarin; N-acetylcysteine; Selenium

Introduction

Acne vulgaris is an exclusively human disease and unique condition of human sebaceous follicles of the face, chest and back that begins in the prepubertal child. Spontaneous regression is common, but in about 5% of cases acne persists beyond the age of 25 and extends into the fourth and fifth decades of life [1]. Histological studies have shown that right at the start of the formation of a comedone, T-lymphocytes are present around the pilosebaceous follicle. Subsequently, *Propionibacterium acnes* (*P. acnes*) play a central role in the inflammatory phenomenon associated with acne. The classical mediators in inflammation become involved prostaglandins, leukotrienes, macrophages, and complements [2]. Current studies show that some membrane fractions of *P. acnes* could sometimes act as super antigen causing amplification of the inflammatory reaction by activation notably of keratinocytes and release of inflammatory cytokines (IL-1α, β-Defensin-2, IL-8) through the activation of Toll-like receptors [3,4].

It has been reported that oxidative stress induced by Reactive Oxygen Species (ROS) plays a major role in inflammation [5]. Excessive generation of ROS by the immune system could result in inflammatory responses; this can also to be induced by skin irritants [6]. It has been shown that neutrophil-derived reactive oxygen species are involved in the irritation and destruction of the follicular wall in acne patients .Although acne vulgaris is the most frequent disease of the young population, only few studies on antioxidative system in acne pathophysiology have been performed [7].

Keep in mind the above interrelated events; it is suitable in this study to use three well known antioxidants that acting by different mechanisms, Silymarin is believed to act in mammalian tissues as a potent free radical scavenger, as well as plasma membrane stabilizer, and thus confers protection to the cells during free radical stress generated by chemical reaction or radiation. This plant can also reduce the inflammatory mediators produced by *P. acne* in terms of free radical scavenging and cytokine reducing ability [8]. N-Acetylcystein is acting as a source of cysteine for the synthesis of the intracellular anti-oxidant glutathione [9]. Its antioxidant action is believed to originate from its being a precursor of Glutathione (GSH), provides the cysteine moiety that is involved in GSH synthesis, so acts as an indirect antioxidant through this way. It also possesses a direct free radical scavenging activity against reactive oxygen species [10]. Selenium is an essential trace element, participates in the antioxidant defends mechanism as an integral constituent of Glutathione Peroxidase (GSH-Px), the antioxidative enzyme, acting as a coenzyme. It is not a direct free radical scavenger; it enhances the conversion of the oxidized Glutathione (GSSG) into the reduced form (GSH) by GSH-Px enzyme [11].

The Aim of this Study was to investigate the effects of antioxidants namely; Silymarin, N-Acetylcysteine and Selenium on lesion counts,

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oxidative status and inflammation in patients with acne vulgaris; and to find out any possible correlation between oxidative stress markers Glutathione (GSH) and Malondialdehyde (MDA) as well as the inflammatory marker Interleukine -8 (IL-8) with the clinical features of acne.

Materials and Methods

A randomized, single-blind, prospective, placebo controlled trial which was carried out at the dermatologic outpatient clinic in Al-Hussein teaching hospital, Karbalaa health directorate-Iraq, during the period between December 2011 and May 2012. Fifty-six patients of both sexes, their age ranges from 14-30 years old have been enrolled in this study, divided into four groups, each include 14 patients. Complete history was taken from each patient about age, gender, marital state; duration of the disease, previous treatment and past medical history.

The Inclusion Criteria were**:** Clinical examination was done by the dermatologist, the selected patients were complaining of papulopustular acne, and had never taken previous acne therapy treatment, or stopped any systemic and topical treatment at least one month before starting the present study therapies. While Exclusion Criteria include**:** Patients with chronic diseases, diabetic patients, those who were taking steroids, hypercholestremic patients, and patients with hepatic and/or renal insufficiency were excluded from the study. Pregnant and lactating women were also excluded from the study, as well as, patient with known hypersensitivity to any of the drugs that were used in the study. The duration required for each individual patient to complete the course of the treatment was eight weeks, clinical and laboratory assessment would be carried out at baseline, during and by the end of this period. In addition to that, 28 age and sex matched healthy people (16 males and 12 females), were tested for their serum levels of GSH, MDA and IL-8 to compare these results with those of the patients with acne at the starting point of the study. Each volunteer was told about the study, and an oral consent was obtained from each one.

Groups of patients

The study was performed with 56 patients randomly allocated into four groups as follow:

Group (1): includes 14 patients , eight males and six females, were treated with silymarin 210 mg/day orally : one tablet of Silymarin 70 miligram (LEGALON ; Madous company) three times daily after meals and a topical moisturizing cream once daily at bed time (Aqua Rosa cream).

Group (2): includes 14 patients, seven males and seven females: were treated with N-acetylcysteine 1200 mg/day orally: one effervescent tablet of N-acetylcysteine 600 milligram (FLUIMUCIL; Zambon company) twice daily and the same topical moisturizing cream once daily at bed time.

Group (3): includes 14 patients, eight males and six females were treated with Selenium 200 mcg/day orally: one tablet of Selenium 100 microgram (SELENIUM; Jamieson company) twice daily and the same topical moisturizing cream once daily at bed time.

Group (4): includes 14 patients, seven males and seven females: were treated with placebo capsule (500 milligrams of glucose powder) once daily orally and the same topical moisturizing cream once daily at bed time.

Blood samples were collected from the patients as well as the healthy volunteers. At the starting point of the study the baseline serum levels of GSH [12], MDA [13] and IL-8 [14] in the acne patients as well as healthy volunteers were measured.

Assessment of clinical features in acne (Scoring of Acne)

The clinical follow up was done every two weeks to assess the changes in the number of inflammatory lesions (the papules and the pustules) and to check any side effects that might appears systemically or topically. There are two commonly used measures which are grading and lesion counting [15]. In 2008, Hayashi N. et al, used lesion counting to classify acne into four groups. They classified acne based on the number of inflammatory eruptions on half of the face as 0-5: mild; 6-20: moderate; 21-50: severe; and more than 50: very severe. Since the present study is dealing with patients with papulopustular type of acne, the Hayashi scoring in assessing the severity and the progress of the disease in the acne patients was followed [16]. Patients were educated neither to squeeze the lesions, nor dig them, as this can aggravates the lesions. They were also told to clean their faces several times daily using the medicated cleansers to get rid of the accumulated sebum. The number of the inflammatory lesions was registered every visit every two weeks for each patient till the end of the eight weeks, and the percentages of changes in the lesions were analyzed statistically. The percent reduction in lesion count was calculated through the following equation:

[Baseline lesion counts-(x) week's lesion counts]/Baseline lesion counts×100%

Assessment of side effects

Side effects would be looked for by asking the patients each visit about any abnormal effect that appeared throughout the whole course of treatment.

Statistics

Results represented as the mean ± standard deviation, using Statistical Package for Social Science SPSS version 18.0 under Windows seven, Microsoft Excel 2010. Student t-test and One-way Analysis of Variance (ANOVA) test would be used to compare between the different groups concerning pre- and post- treatment values. $P \leq 0.05$ considered significant change, and $P \leq 0.01$considered high significant change. Pearson's correlation and regression test would be used to compare between the clinical findings and the laboratory findings.

Results

Data in table 1 showed that there was non-significant difference between the baseline values of serum levels of GSH, MDA, and IL-8 for patients who were treated with Silymarin, N-Acetylcysteine and Selenium and the patients who were treated with placebo, in contrast, comparison with healthy subjects revealed highly significant change $P \leq 0.01$ in the baseline level of mentioned markers. On the contrary,

Parameters	Healthy (n=28)	Patients (n=56)
S. GSH (µg / ml)	1.61 ± 0.99	0.65 ± 0.22**
S. MDA (µg / ml)	5.46 ± 2.82	8.68 ± 1.55**
S. IL-8 (pg / ml)	36.65 ± 25.73	61.17 ± 39.92*

Results represent mean ± Standard deviation
**represents highly significant $P \leq 0.01$ change between healthy subjects and patients
*represents significant $P \leq 0.05$ change between healthy subjects and patients

Table 1: Baseline values of serum glutathione, malondialdehyde and interleukine-8 for patients with acne compared to healthy subjects.

there was a highly significant difference in the last (after eight weeks of treatment) values of serum level of GSH and MDA, and only significant difference in the level of IL-8 between the patients who were treated with different antioxidants and the patients who were treated with placebo (Table 2).

Results showed that silymarin caused an elevation in serum GSH level by about three folds (271%), and decreased MDA and IL-8 by 39.2% and 80% respectively in acne patients after eight weeks period of use (Table 2). Concerning the effect of silymarin on the clinical feature (score), the number of inflammatory lesions was reduced every two weeks during the use of silymarin, the reduction was significant (when compared to the baseline) at the sixth and the eighth week (P value ≤ 0.05). After eight weeks of treatment, the reduction in lesion count was 53%, this reduction was statistically significant P ≤ 0.05. While non significant reduction in number of inflammatory lesions happened with placebo (Table 3). On the other hand, N-Acetylcysteine caused an elevation in serum GSH by two folds (205%), and decreased MDA and IL-8 by 38.8% and 72% respectively after using for eight weeks, (Table 2). Clinically, there was non-significant difference between the baseline number of inflammatory lesions between the patients who were treated with N-Acetylcysteine and the patients who were treated with placebo. The number of inflammatory lesion was reduced every two weeks by using N-Acetylcysteine, and the reduction was significant (when compared to the baseline) at the sixth and the eighth week (P value ≤ 0.05). After eight weeks of treatment, the percentage of reduction in the total count of inflammatory lesions was 50%, which was statistically significant. No significant reduction in number of inflammatory lesions happened with placebo (Table 3). In case of selenium, an elevation in serum GSH by two folds (201%), and decreased MDA and IL-8 by 35% and 71% respectively after using for eight week, (Table 2). Furthermore, there was non-significant difference between the baseline number of inflammatory lesions between the patients who were treated with Selenium and the patients who were treated with placebo. Although there was a reduction in the number of inflammatory lesions by using Selenium in each visit (every two weeks) compared to placebo, but this reduction was not significant compared to the baseline (Table 3).

Correlation of the inflammatory lesion number (clinical score) with the serum level of GSH, MDA and IL-8 (laboratory parameters)

Using Pearson's correlation, results showed that base line value of serum GSH negatively correlated with number of inflammatory lesions, r=-0.753, P ≤ 0.001 (Figure 1). Another correlation was done between GSH (post-treatment with antioxidants) and inflammatory lesions number after eight weeks, significant negative correlation was produced P ≤ 0.05, r=-0.334; (Figure 2). Concerning the correlation of the inflammatory lesion number with serum MDA, highly significant positive correlation was found between MDA baseline value and inflammatory lesions number, P ≤ 0.001, r=0.749 (Figure 3). Besides that, after eight weeks use of different antioxidants, there was also highly significant positive correlation between MDA value and inflammatory lesions number P ≤ 0.001, r=0.481(Figure 4).

On the other hand, highly significant positive correlation was found between IL-8 (pre-treatment) and inflammatory lesions number at the baseline, P ≤ 0.001, r=0.811 (Figure 5); as well as, correlation was done between IL-8 and inflammatory lesions number after 8 weeks, a highly significant positive correlation was present P ≤ 0.001, r=0.828 (Figure 6).

Discussion

There has been an increasing focus on the extent to which oxidative stress is involved in the pathophysiology of acne; studies have shown that patients with acne are under increased cutaneous and systemic oxidative stress [17]. Additional researches seem to confirm that lipid peroxidation is the driving force behind the progression of comedogenesis and inflammation in acne, the marked increase in lipid peroxidation once inflammation is ongoing is to be expected. Undoubtedly Reactive Oxygen Species (ROS) can provoke the secretion of inflammatory cytokines; however, once initiated, inflammatory chemicals cause a subsequent increase in ROS production [18]. Based on this theory, the present study was carried out to check the effects of Silymarin, N-Acetylcysteine and Selenium in patients with acne. Results in the present study revealed that there is a highly significant (P ≤ 0.01) decrease in serum levels of GSH, highly significant increase

Variables	Timing	G1 Silymarin	G2 N-Acetylcysteine	G3 Selenium	G4 placebo
S. GSH (µg/ml)	Pre	0.593 ± 0.17	0.719 ± 0.24	0.73 ± 0.15	0.57 ± 0.25
	Post	1.604 ± 0.75**	1.48 ± 0.67**	1.47 ± 0.58**	0.56 ± 0.28
S. MDA (µg/ml)	Pre	8.94 ± 0.99	8.26±1.23	8.01 ± 1.1	9.53 ± 2.21
	Post	5.44 ± 1.79**	5.05 ± 1.74**	5.2 ± 1.95**	9.64 ± 2.2
S. IL-8 (pg/ml)	Pre	66.38 ± 40.35	68.53 ± 37.93	58.29 ± 46.9	51.45 ± 35.71
	Post	13.2 ± 13.2*	18.93 ± 15.13*	17 ± 11.49*	55.32 ± 41.23

Results represent mean ± Standard deviation
**represents highly significant P ≤ 0.01 change between treatment groups and placebo
*represents significant P ≤ 0.05 change between treatment groups and placebo

Table 2: Effects of Silymarin, N-Acetylcysteine and Selenium on serum glutathione, malondialdehyde and interleukine-8 for patients with acne compared to placebo after eight weeks.

Variables	Base line	2weeks	4weeks	6weeks	8weeks
G1/Silymarin	19.57+7.68	16.93+6.7	14.21+5.85	11.64+5.42*	9.14+4.75*
G2/N-Acetylcysteine	19.07+6.6	16.71+5.65	14.0+4.88	11.64+4.05*	9.5+3.59*
G3/Selenium	18.86+8.12	17.14+7.66	15.0+7.26	13.43+6.89	11.93+6.83
G4/placebo	19.28 ± 9.29	18.71 ± 9.381	18 ± 8.72	17.64 ± 8.67	17.14 ± 8.77

Results represent mean ± Standard deviation
*Represents significant change P≤0.05 between treatment groups vs. placebo

Table 3: Effects of Silymarin, N-Acetylcysteine and Selenium on the number of inflammatory lesions on half - face of patients with acne compared to placebo after eight weeks.

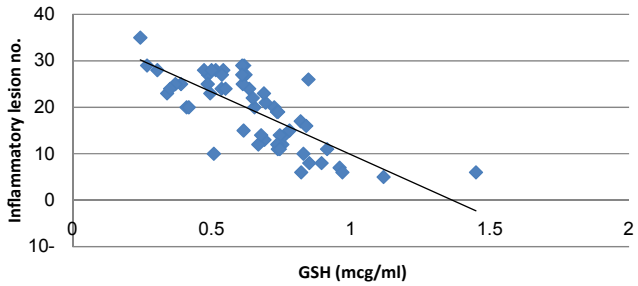

Figure 1: Correlation between serum GSH pretreatment and inflammatory lesion no. (baseline) in all patients group, r=-0.753.

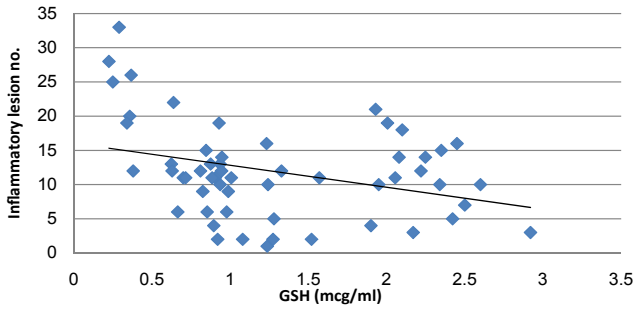

Figure 2: Correlation between serum GSH post-treatment and inflammatory lesion no. (8wk) in all patients group, r= - 0.334.

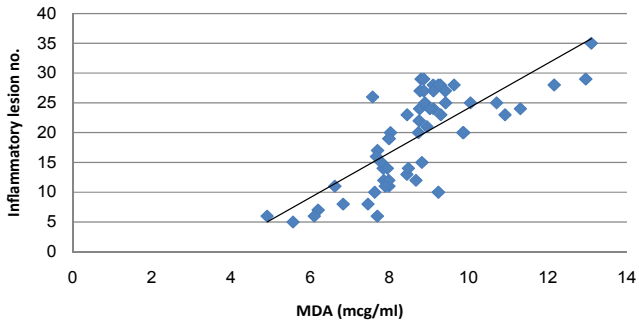

Figure 3: Correlation between serum MDA pre-treatment and inflammatory lesion no. (baseline) in all patients group, r = 0.749.

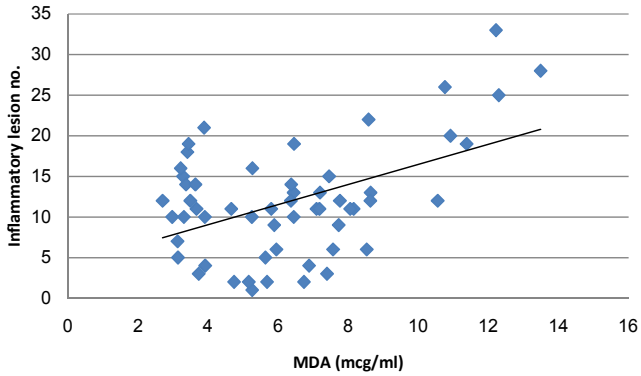

Figure 4: Correlation between serum MDA post-treatment and inflammatory lesion no. (8wk) in all patients group, r = 0.481.

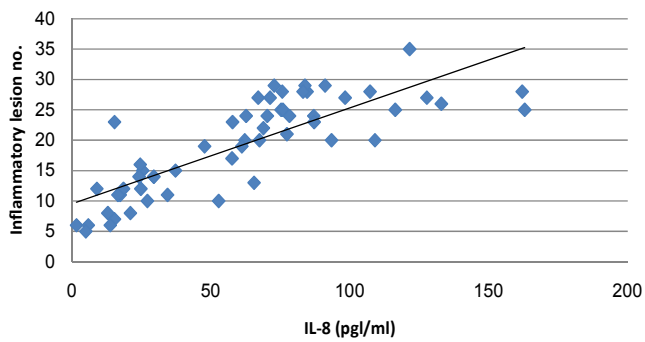

Figure 5: Correlation between serum IL-8 pretreatment and inflammatory lesion no. (baseline) in all patients group, r = 0.811.

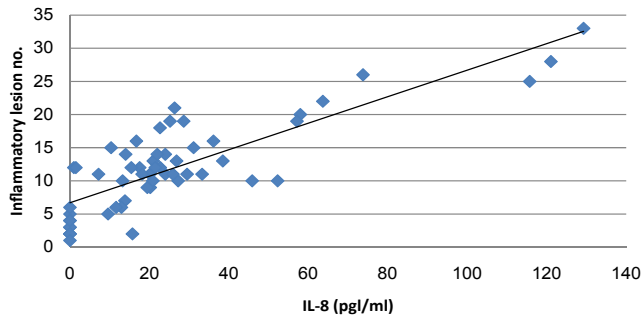

Figure 6: Correlation between serum IL-8 posttreatment and inflammatory lesion no. (8wk) in all patients group, r = 0.828.

in serum levels of MDA and a significant increase in serum levels of IL-8 were found in acne patients compared to healthy people at the baseline. Results clearly demonstrated the existence of oxidative stress and inflammation in acne patients.

Administration of silymarin caused an elevation in serum GSH by about three folds (271%), and decreased MDA and IL-8 by 39.2% and 80% respectively in acne patients, and a reduction in lesion count (percentage) by 53% after eight weeks period of use, this reduction was statistically significant. Silymarin acts by many mechanisms that may include antioxidative, anti lipid peroxidative, anticarcinogenic, antifibrotic, anti-inflammatory, membrane stabilizing, immune-modulator and liver regenerating mechanisms [19,20]. The anti-inflammatory effects of silymarin are based on multiple activities including mast cell stabilization, inhibition of neutrophil migration, Kuppfer cell function inhibition and inhibition of leukotriene and prostaglandin formation [21]. The flavounoids of *Silibum marianum* may also exert their anti-inflammatory action through inhibition of lipooxygenase and cyclooxygenase-2 activity [22]. Leukotriene B4 (LTB4) induces recruitment and activation of neutrophils, monocytes and eosinophils, it also stimulates the production of several pro-inflammatory cytokines and mediators that augment and prolong tissue inflammation [23]. Leukotrienes B4, leukotriene C4 are shown to have a mitogenic effect on keratinocytes and may therefore takes part in mediating hyperproliferation in the pilosebaceous unit [24]. A study done by Zouboulis (2009) found that 5-lipoxygenase inhibitor (Zileuton) is effective in treatment of acne vulgaris [25]. The molecular bases of the anti-inflammatory effects of silymarin might be related to inhibition of the transcription factor NF-κB, which regulates

and coordinates the expression of various genes involved in the inflammatory process [26].

Over all, the powerful antioxidant, anti-inflammatory effect of silymarin has been clearly shown above, which may represent rational explanation for the results obtained in this study when silymarin was used for eight weeks, and was very effective to treat acne patients. Administration of N-Acetylcysteine caused an elevation in serum GSH by two folds (205%), and decreased MDA and IL-8 by 38.8% and 72% respectively after using for eight weeks, and caused a reduction in the total count of inflammatory lesions, the percentage of reduction was 50%, which was statistically significant. N-Acetylcysteine (NAC), has been studied and utilized as a source of cysteine, which is a precursor in the synthesis of reduced Glutathione (GSH) and as a direct free radical scavenger that protects cells from oxidant damage [27]. It has also been suggested that the drug has anti-inflammatory properties by suppressing the activation of nuclear factor kappa (NF-kB) [28,29] . In addition, it was found that NAC inhibited the production of TNF-α, IL-6 and IL-8 in GSH-depleted human *in vitro* [30]. All these effects strongly support the results obtained in the present study, where NAC, administered in a dose of 1200 mg daily for a period of eight weeks was really effective in treating patients with acne.

Administration of Selenium to acne patients result in elevation in serum GSH by two folds (201%), and decreased MDA and IL-8 by 35% and 71% respectively after using for eight weeks. It reduced the number of inflammatory lesions, yet; this reduction was statistically non significant. The essential trace element, selenium, acts as an integral constituent of the antioxidative enzyme Glutathione Peroxidase (GSH-Px), which detoxifies Hydrogen Peroxide (H_2O_2) and organic lipid peroxides at the expense of reduced Glutathione (GSH). The selenium-dependent enzyme glutathione peroxidase (GSH-Px) recycles glutathione, reducing lipid peroxidation by catalyzing the reduction of peroxides, including hydrogen peroxide; this represents a crucial component of antioxidative potential in humans [31]. Several *in vivo* studies have shown that serum selenium levels are inversely correlated with serum concentrations of IL-8 [32]. A study has reported the molecular basis of the anti-inflammatory property of selenium to be driven by modulation of 15deoxy-Prostaglandin J2 metabolism [33]. Accordingly, the ability of selenium to increase GSH and decrease MDA and IL-8 in addition to its effectiveness to reduce the lesion number after eight weeks of using selenium as a proposed acne therapy may be explained.

The use of the well known antioxidants (Silymarin, N-Acetylcysteine and Selenium) orally, resulted in elevating the serum level of GSH significantly and reducing the serum level of MDA and IL-8 and in the acne patients. Furthermore; all the three antioxidants reduced the number of the inflammatory lesions, although the percentage and the level of significance of this reduction was not the same for all the agents. The results of the present study are compatible with other studies regarding the presence (and targeting) of oxidative stress and inflammation in acne. In 2011, a study done by Khurana et al, 100 patients with untreated mild to moderate acne vulgaris were included. Their measurement results showed that Lipid peroxidation (MDA) was significantly increased in those patients implying significant oxidative membrane damage, while antioxidative capacity measured in terms total thiol was significantly low [34]. Arican et al. studied the role of oxidative stress in 43 acne patients, they found that Catalse and G6PD levels in patients were statistically decreased, and MDA levels were found to be statistically increased [35]. Rubin et al. discovered that the self-administration of an omega-3 fish oil-based nutrient combination for two months have some influence on the acne process, and perhaps more importantly, on mental outlook. The average inflammatory lesion count at baseline was 20.8 which decreased to 6.8 after two months [36]. On the other hand, results of this study showed that correlation between the number of inflammatory lesions and serum levels of GSH, MDA and IL-8 pre- and post-treatment revealed that there was significant negative correlation between the number of inflammatory lesions and the serum levels of GSH, at the same time, there was significant positive correlation between the number of inflammatory lesions and the serum levels of MDA, beside significant positive correlation between the number of inflammatory lesions and the serum levels of IL-8. Therefore, targeting these changes, through using agents that can increase GSH level, decrease MDA and IL-8 serum levels represent a good therapeutic option for treating inflammatory acne.

Depending on the results obtained by this study, it can be concluded that oxidative stress and inflammation exist in patients with acne vulgaris and may be considered as contributing factors involved in the aetiopathogenesis of the disease. Administration of Silymarin, N-Acetylcysteine and Selenium resulted in significant correction of the disturbed antioxidative status of acne patients, and a marked reduction in the inflammation, in addition to clinical improvement represented by reduction in the number of inflammatory lesions in patients with papulopustular acne. Glutathione is negatively correlated, while malondialdehyde and interleukin-8 are positively correlated with the clinical features (score) of acne, therefore targeting these changes with oral antioxidants might represent a new therapeutic strategy in treatment of acne vulgaris.

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