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Original Research

Effects of silymarin, N-acetylcysteine and selenium in the treatment of papulopustular acne

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Acne vulgaris; Inflammation; N-acetylcysteine; Oxidative stress; Selenium; Silymarin

Abstract
There has been an increasing focus on the extent to which oxidative stress is involved in the pathophysiology of acne. The aim of this study is to investigate the effect of silymarin, N-acetylcysteine (NAC) and selenium in the treatment of acne vulgaris. A randomized prospective clinical trial was carried out on 56 patients of both sexes with age range of 14-35 years who attend to outpatient clinic in Al-Hussein Teaching Hospital, Karbalaa, Iraq over a period from December 2011 to May 2012, all patients examined clinically by dermatologist and classified according to disease severity. Serum levels of glutathione (GSH), malondialdehyde (MDA) and interleukine-8 (IL-8) in the acne patients were measured by using ready-for-use Elisa kits, and compared to that of 28 healthy volunteers. The clinical follow up was done every two weeks in order to assess the changes in the number of inflammatory lesions. Administration of antioxidants silymarin, NAC and selenium (but not placebo) to patients with acne vulgaris significantly reduced serum MDA and increased serum GSH levels after 8 weeks compared to pre-treatment value; also significantly reduced serum IL-8 levels and the number of inflammatory lesions in patients with acne compared to placebo. The results obtained in this study clearly showed the beneficial effect of using silymarin, NAC and selenium to patients with acne vulgaris as indicated by the clinical improvement and biochemical findings, and confirmed the role of new strategy in the targeting of pathophysiological changes accompanied with acne by using antioxidant agents.

INTRODUCTION

Acne is an inflammatory disease, and it has been reported that oxidative stress induced by reactive oxygen species (ROS) plays a major role in inflammation [1]. Another hypothesis focuses on the importance of ROS as inflammatory mediators released by phagocytes such as neutrophils, which produce these mediators for lysis of invading microorganisms. Excessive generation of ROS by the immune system could result in inflammatory responses; this can also to be induced by skin irritants [2]. 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 antioxidant system in acne pathophysiology have been performed [3].

Propionibacterium acnes cause the release of some chemotactic factors leading to neutrophils accumulation, and this causes damage to follicular epithelia after the release of some inflammatory factors such as lysosome enzymes as a result of phagocytosis. ROS are released from the active neutrophils in the inflammatory tissue. These oxidants attack DNA and/or membrane lipids and cause chemical damage to them [4]. The indicator of oxidative stress in the cell is the level of lipid peroxidation and its final product is malondialdehyde (MDA).

Milk thistle (*Silybum marianum*), or silymarin, is a flavonoid herb that has long been used to support liver health [5]. This flavonoid 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 [6]. The key mechanism that ensures hepatoprotection appears to be free radical scavenging. In addition, it protects the cell from oxidative damage through its membrane stabilizing properties [7].

N-Acetylcysteine is a cell-permeable compound acting as a source of cysteine for the synthesis of the intracellular antioxidant glutathione (GSH) [8]. Its antioxidant action is believed to originate from its being a precursor of GSH providing the cysteine moiety that is involved in GSH synthesis so acting as an indirect antioxidant through this way [9]. It also possesses a direct free radical scavenging activity against ROS [10].

Selenium is an essential trace element, used particularly in the antioxidant system which protects intracellular structures against oxidative damage. Selenium participates in the antioxidant defense mechanism as an integral constituent of glutathione peroxidase (GSH-Px), the antioxidant enzyme, by 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 aims of this study were to study the oxidative status of Iraqi patients with acne vulgaris; and the effect of different antioxidants, namely silymarin, NAC and selenium in the treatment of acne vulgaris; the papulopustular form. Also to investigate the effect of used antioxidants on the inflammatory markers in patients with acne vulgaris and to find out the possible correlation between oxidative stress markers GSH and MDA as well as the inflammatory marker interleukine-8 (IL-8) with the clinical features of acne.

MATERIALS AND METHODS

This randomized, single-blind, prospective, placebo controlled trial 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.

Patients, criteria and grouping

Fifty-six patients of both sexes (age range of 14-30 years old) have been enrolled in this study, divided into 4 groups of 14 patients each. Complete history was taken from each patient regarding age, gender, marital state, duration of the disease, previous treatment and past medical history. Their consent to be involved in the study was obtained.

Clinical examination was done by the dermatologist. The selected patients were complaining of papulo-pustular 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. All of the included subjects study had similar dietary habits.

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 as well as patients with known hypersensitivity to any of the drugs that were used in the study were also excluded. The duration required for each individual patient to complete the course of the treatment was 8 weeks; clinical and laboratory assessment would be carried out at baseline, during and by the end of this period.

In addition, 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.

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

-*Group 1*: includes 14 patients, 8 males and 6 females, treated with silymarin 210 mg/day orally. One tablet of silymarin 70 mg (Legalon®; Madaus Company) was given three times daily after meals along with a topical moisturizing cream (Aqua Rosa) once daily at bed time.

-*Group 2*: includes 14 patients, 7 males and 7 females, treated with NAC 1200 mg/day orally. One effervescent tablet of NAC 600 mg (Fluimucil®; Zambon Company) was given twice daily together with the same topical moisturizing cream once daily at bed time.

-*Group 3*: includes 14 patients, 8 males and 6 females, treated with selenium 200 µg/day orally. One tablet of selenium 100 µg (Selenium; Jamieson Company) was given twice daily plus the same topical moisturizing protocol.

-*Group 4*: includes 14 patients, 7 males and 7 females, treated with placebo capsule (500 mg glucose powder) orally once daily in addition to Aqua Rose cream at bed time.

The clinical follow up was done every two weeks in order to assess the changes in the number of inflammatory lesions (the papules and the pustules) and to monitor any side effects that might appear systemically or topically.

There are two commonly used measures which are

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grading and lesion counting [12]. In 2008, Hayashi *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 [13].

The patients were educated neither to squeeze the lesions nor dig them, as this can aggravate 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 on every visit every two weeks for each patient till the end of the eight weeks; the percentages of changes in the lesions were analyzed statistically. The percent reduction in lesion count was calculated through the following equation:

$$\frac{\text{Baseline lesion counts} - (x)\ \text{week's lesion counts}}{\text{Baseline lesion counts} \times 100\%}$$

Analysis of the laboratory parameters

Using ready-made Elisa kit obtained from Cusabio Biotech, China, for analysis of serum GSH [14]; serum MDA [15]; and serum IL-8 [16].

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, and whether this effect is systemic or topical. Side effects would be registered and then be searched about in the references and literatures.

Statistics

Results represented as the mean ± standard deviation (SD), using Statistical Package for Social Science SPSS version 18.0. Paired Student t-test would be used to compare between pre- and post-treatment values within the same group, while independent t-test was used to compare means between two study groups. One-way analysis of variance (ANOVA) test would be used to compare between the different groups concerning pre- and post-treatment values. $P < 0.05$ was considered as significant, and $P < 0.01$ as highly significant changes.

RESULTS

Serum levels of GSH, MDA and IL-8 for patients with acne were measured and compared to that of healthy subjects. Results showed that serum GSH levels were highly significantly lower in patients with acne (0.65 ± 0.22 mcg/ml) compared to those of healthy subjects (1.61 ± 0.99 mcg/ml) (Fig.1). On the other hand, results showed that serum MDA levels were highly significantly increased in acne patients (8.68 ± 1.55 mcg/ml) compared to healthy subjects (5.46 ± 2.82 mcg/ml) (Fig.1), indicating the existence of oxidative stress in patients with acne. Furthermore, serum levels of IL-8 found to be significantly higher in patients with acne (61.17 ± 39.92 pg/ml) compared to healthy subjects (36.65+25.73 pg/ml) (Fig.2).

Administration of silymarin, NAC or selenium to patients with acne for eight weeks resulted in highly significant elevation in the serum GSH levels compared to pre-treatment values; the elevation percents were 271%, 205% and 201%, respectively (Fig.3).

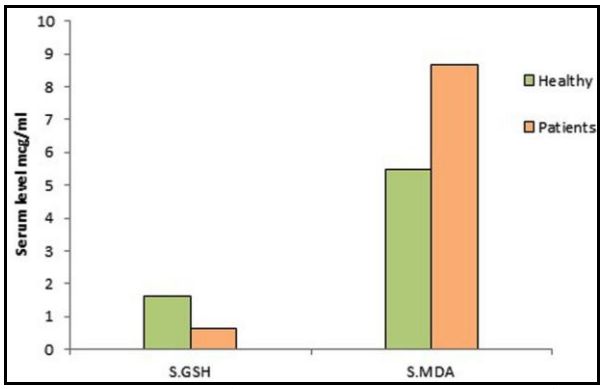

Figure 1. Serum levels of glutathione and malondialdehyde of acne patients compared to healthy subjects.

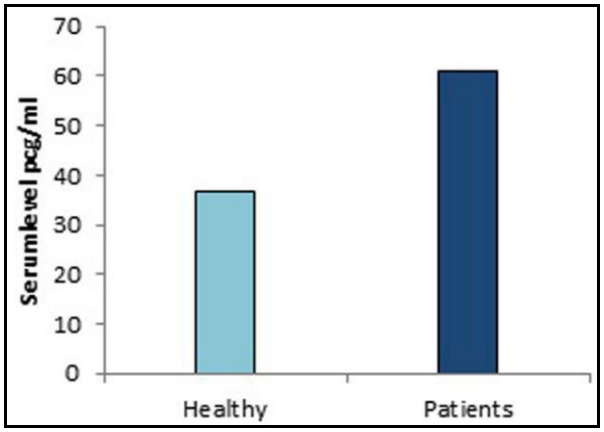

Figure 2. Serum level of interleukin-8 in acne patients compared to healthy subjects.

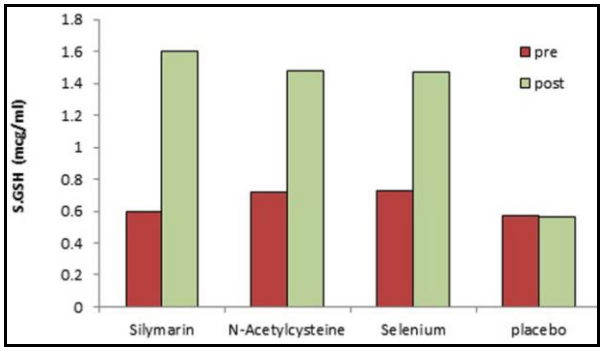

Figure 3. Effects of silymarin, NAC and selenium on serum glutathione in acne patients compared to placebo.

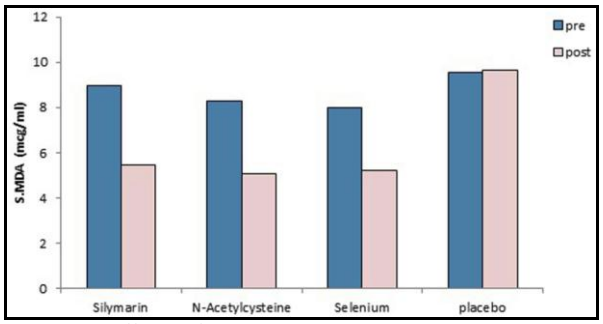

Figure 4. Effects of silymarin, NAC and selenium on serum malondialdehyde in acne patients compared to placebo.

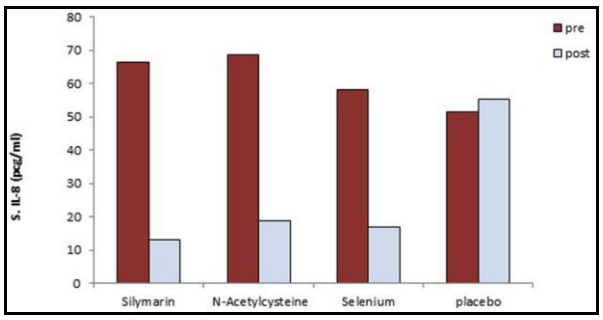

Figure 5. Effects of silymarin, NAC and selenium on serum interleukine-8 levels in acne patients compared to placebo.

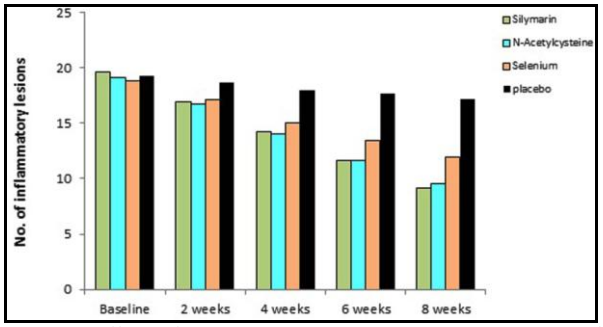

Figure 6. Effects of silymarin, NAC and selenium on the number of inflammatory lesions on half-face of patients with acne vulgaris compared to placebo.

On the other hand, Fig.4 showed that administration of silymarin, NAC or selenium for eight weeks to patients with acne resulted in highly significant reduction of the serum MDA level compared to pre-treatment values; the reduction percents were 39.2%, 38.8% and 35%, respectively.

At the same time, administration of silymarin, NAC and selenium to patients with acne for eight weeks resulted in significant reduction of serum IL-8 levels; the reduction percents were 80%, 72% and 71%, respectively (Fig.5).

Concerning the effect of silymarin, NAC or selenium on the clinical feature (score) results showed that the number of inflammatory lesions was reduced every two weeks during the use of silymarin; the reduction was significant at the sixth and the eighth week compared to the baseline, after eight weeks of treatment, the reduction percent of lesion count was 53%. No significant reduction in number of inflammatory lesions happened with placebo (Fig.6).

In addition, there was no significant difference at the baseline for the number of inflammatory lesions between the patients treated with NAC and the patients treated with placebo. The number of inflammatory lesions was reduced every two weeks during the use of NAC, and the reduction was significant at the sixth and the eighth week compared to the baseline. After eight weeks of treatment, the percentage of reduction in the total count of inflammatory lesions was 50% (Fig.6).

Furthermore, there was no significant difference at the baseline for the number of inflammatory lesions between the patients treated with selenium and the patients treated with placebo. Although there was a reduction in the number of inflammatory lesions by using selenium every two weeks compared to placebo, this reduction was not significant compared to the baseline. A reduction percent in the number of inflammatory lesions achieved by selenium was 37%; yet, this reduction was statistically non-significant (Fig.6).

Statistical analysis between these three different groups was carried out utilizing one way ANOVA test; the results showed that there was no significant difference among silymarin, NAC and selenium groups concerning their effects on serum levels of GSH, MDA and IL-8, and the number of inflammatory lesions after eight weeks period.

DISCUSSION

In recent years there has been an increasing focus on the extent to which oxidative stress is involved in the pathophysiology of acne. Emerging 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 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 evaluate the effects of silymarin, NAC and selenium in patients with acne.

Silymarin acts by many mechanisms that may include antioxidative, anti-lipid-peroxidative, anticarcinogenic, antifibrotic, anti-inflammatory, membrane stabilizing, immune-modulatory and liver regenerating mechanisms [19]. The anti-inflammatory effects of silymarin are based on multiple activities including

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mast cell stabilization, inhibition of neutrophil migration, Kuppfer cell function inhibition and inhibition of leukotriene and prostaglandin formation [20]. Endogenous prostaglandins (PGs) are shown to enhance keratinocyte proliferation; cycloxygenase (COX)-2 inhibition may lead to down-regualtion of an existing inflammatory reaction [21]. The flavonoids of *Silibum marianum* may also exert their anti-inflammatory action through inhibition of lipooxygenase and COX-2 activity [22]. Leukotriene B4 (LTB4) induces recruitment and activation of neutrophils, monocytes and eosinophils; it also stimulates the production of a number of pro-inflammatory cytokines and mediators that augment and prolong tissue inflammation [23]. Leukotrienes B4 and C4 are shown to have a mitogenic effect on keratinocytes and may therefore participate in mediating hyperproliferation in the pilosebaceous unit [24]. A study done by Zouboulis found that the 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 nuclear factor kappa B (NF-κB), which regulates and coordinates the expression of various genes involved in the inflammatory process [26]. It was postulated that there are other pharmacological properties for silymarin. It possesses an antibacterial activity against Gram-positive bacteria as its ingredient silybin inhibits ribonucleic acid (RNA) and protein synthesis on Gram-positive bacteria [27].

Various scientific investigations have been carried out to explore the fact that androgens are also involved in the pathogenesis of acne vulgaris. Androgens may be produced by gonads and adrenal gland or locally within the sebaceous gland. Testosterone and dihydrotestosterone are the major androgens that are responsible for acne vulgaris [28]. The androgenic stimulation, potentiated by synergistic growth factors, nuclear proteins and IL-1α, leads to abnormal ductal and infundibular hyperkeratinization. *Silybum marianum* (silibinin) has an antiandrogenic activity and was able to down-regulate 5α-dihydrotestosterone; thus, milk thistle may be beneficial to prostate health [29].

Over all, the powerful antioxidant, anti-inflammatory effect of silymarin in combination with its other pharmacological effects, has been clearly shown above, which may represent a rational explanation for the results obtained in this study when silymarin was used for 8 weeks, and was very effective to treat acne patients.

N-Acetylcysteine, has been studied and utilized as a source of cysteine, which is a precursor in the synthesis of reduced GSH and as a direct free radical scavenger that protects cells from oxidant damage [30]. It has also been suggested that the drug has anti-inflammatory properties by suppressing the activation of NF-kB [31]. N-Acetylcysteine has been shown to be a potent inhibitor of NF-kB activation. This may be the mechanism how NAC can inhibit the production of tumor necrosis factor-α (TNF-α). *In vivo*, it has been reported that NAC prevented lipopolysaccharide-induced production of TNF-α, IL-1β, or monocyte chemo-attractive protein (MCP) in rats [32]. In addition, it was found that NAC inhibited the production of TNF-α, IL-6 and IL-8 in GSH-depleted human *in vitro* [33].

N-acetylcysteine has anti-bacterial properties. NAC also decreases biofilm formation by a variety of bacteria and reduces the production of an extracellular polysaccharide matrix, while promoting the disruption of mature biofilms [34]. The pronounced antibacterial effect of NAC against *Staphylococcus epidermidis* indicates the use of NAC as a potential therapeutic agent as alternative to antibiotics [35]. 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.

The essential trace element selenium acts as an integral constituent of the antioxidant GSH-Px, which detoxifies hydrogen peroxide (H_2O_2) and organic lipid peroxides at the expense of GSH. The selenium-dependent enzyme GSH-Px reduces lipid peroxidation by catalyzing the reduction of peroxides, including H_2O_2; this represents a crucial component of antioxidant potential in humans [36].

Several *in vivo* studies have shown that serum selenium levels are inversely correlated with serum concentrations of IL-8. Two possible explanations have been suggested: first, that the increased oxidative stress is caused by elevated IL-8 levels, which exhausts the available selenium that protects the cells exposed to inflammatory stress; and second, as shown by *in vitro* studies, where selenium (in the GSH-Px system) can inhibit IL-8 release [37]. Another study has reported the molecular basis of the anti-inflammatory property of selenium to be driven by modulation of 15-Deoxy-12,14-prostaglandin J2 metabolism [38]. 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, NAC and selenium) resulted in elevating the serum levels of GSH significantly and reducing the serum levels of MDA and IL-8 and in the acne patients. All the three antioxidants reduced the number of the inflammatory lesions; however the level of this reduction was not the same for all the agents.

The results of the present study are compatible with many other studies regarding the presence of (and targeting) oxidative stress and inflammation in acne. In a study done by Khurana *et al*, 100 patients with untreated mild to moderate acne vulgaris and 30 age- and sex-matched controls were included and three parameters, *i.e*. MDA, ferric reducing ability of plasma (FRAP) or total antioxidant capacity of body and total thiol content (SH), were measured in plasma. Lipid peroxidation (MDA) was significantly increased in patients implying significant oxidative membrane damage, while antioxidant capacity measured in terms of FRAP and total thiols was significantly low [39]. In another study, Arican *et al* examined the role of oxidative stress in forty-three acne patients (age range 13-35 years). The parameters of oxidative stress such as catalase (CAT), glucose-6-phosphate dehydrogenase (G-6-PD), superoxide dismutase (SOD), and MDA in venous blood samples of the cases were measured spectrophotometrically. As result, CAT and G-6-PD levels in patients were found to be statistically decreased, and MDA levels were found to be statistically increased [40]. These findings clearly indicate that oxidative stress exists in acne and may play an important role in its pathogenesis. Thus, antioxidant oral supplementation or topical application may be an effective approach in improving the efficacy or avoiding the potentially damaging effects of the therapeutic agents.

Rubin *et al* discovered that the self-administration of an omega-3 fish oil-based nutrient combination for two months did appear to have some influence on the acne process, and perhaps more importantly, on mental outlook. The average total lesion count among the group dropped from 62.8 to 40.4. It was in the area of inflammatory lesions where the intervention seemed to make a more significant difference. All patients had a reduction in inflammatory papules. The average inflammatory lesion count at baseline was 20.8 which decreased to 6.8 after two months [41]. Another prospective, non-comparative clinical trial was conducted with a polyherbal formulation containing *Lenus culinaris*, *Aloe barbadensis*, *Vitex negundo* and *Andrographis paniculata* with a total of 26 acne patients. This study observed significant reduction in number of blackheads, whiteheads, inflamed pustules and overall inflammation. The results obtained might be due to the antioxidant, anti-inflammatory, antiandrogenic and antimicrobial properties of the ingredients of the polyherbal formulation [42].

Depending on the results obtained by this study, it can be concluded that oxidative stress exists in patients with acne vulgaris and may be considered as one of the contributing factors involved in the etiopathogenesis of the disease, the use of antioxidant agents might be one of the original strategies in the management of acne vulgaris. Besides that, there is interplay between inflammation and oxidative stress in acne patients, and targeting of these changes strongly improve the clinical outcome. Administration of silymarin, NAC and selenium caused significant correction of the disturbed antioxidant 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.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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