Oxidative stress in lichen planus

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ABSTRACT

Background. Increased reactive oxygen species (ROS) and lipid peroxides have been implicated in the pathogenesis of atopic dermatitis, psoriasis, vitiligo, and lichen planus (LP). We therefore evaluated the status of oxidative stress and the antioxidant defense system in Egyptian patients with LP.

Methods. This study included 45 Egyptian LP patients and 45 healthy volunteers as controls that were age- and sex-matched with the patients. Serum levels of nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD), and erythrocyte catalase (CAT) were measured.

Results. There was an increase in the serum levels of NO, SOD and the lipid peroxidation product MDA ($p = 0$, $p = 0.009$ and $p = 0.005$, respectively) and a decrease in CAT levels in LP patients compared to controls ($p = 0$), leading to an imbalance in the antioxidant defense system in our study. Oxidative stress was greater in men than in women because MDA levels were increased ($p = 0.045$) and erythrocyte CAT levels were decreased ($p = 0$). In addition, there was also a positive correlation between NO, MDA, and SOD and a negative correlation between erythrocyte CAT and the duration of LP. No relation between the four parameters and the clinical types of LP was noted.

Conclusion. Our findings point to an increase in oxidative stress and an imbalance in the antioxidant defense mechanisms in LP. This may play a role in the pathogenesis of LP.

KEY WORDS

catalase (CAT), lichen planus (LP), malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD)

Introduction

Lichen planus (LP) is defined as a subacute, chronic dermatosis characterized by small, flat-topped, shiny, polygonal violaceous papules that may coalesce into plaques. It involves the skin, mucous membranes, genitalia, nails, and scalp. The clinical presentation of LP has several forms, including the actinic, hypertrophic, annular, erosive, follicular, linear, pigmented, and bullous types. It affects all races equally and presents mainly in the range from 30 to 70 years of age (1).

The exact pathogenesis of the disease remains unclear, but both antibodies and T-cell mediation

have been implicated. Activated T cells release cytokines leading to the attraction of inflammatory cells and the destruction of keratinocyte by cell-mediated cytotoxicity (2). Recently it has been suggested that increased reactive oxygen species (ROS) and lipid peroxides may play a part in the pathogenesis of various skin diseases, such as atopic dermatitis (3), psoriasis (4), vitiligo (5), and LP (7).

Anshumalee et al. (6) reported that oxidative stress may play a role in oral LP. Moreover, Sezer et al. (7) reported that there was increased oxidative stress and lipid peroxidation together with an imbalance in the antioxidant defense system in 40 patients with cutaneous LP suggesting that ROS may be involved in the pathogenesis of LP.

The aim of this study was to evaluate the status of oxidative stress and antioxidant defense systems in Egyptian patients with LP by measuring their serum levels of nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD), and erythrocyte catalase (CAT).

Patients and methods

This study included 45 Egyptian patients (26 females and 19 males). Their age ranged from 23 to 65 years. They had different types of LP (classic, actinic, hypertrophic, and lichen planopilaris). In addition, 45 healthy volunteers that were age- and sex-matched with the patients served as controls. All subjects were retrieved from the dermatology outpatient clinic of the National Research Center, Cairo, Egypt.

Exclusion criteria included subjects that had received any systemic treatment suppressing the immune system such as systemic steroids or other immunosuppressive drugs, as well as NSAIDs, for the last 4 weeks, and topical medications for the last 2 weeks prior to sample collection. Also, patients with a history of trauma or any surgery 4 weeks prior to sampling, those suffering from any systemic or dermatological disease affecting the immune system or any malignancy, and subjects with specific habits such as smoking were excluded.

Sample collection

All of the assays were performed on cases and controls in a blind fashion on coded samples by an investigator that was not informed of the patients' clinical status after the collection of all samples had been completed.

Methods

Blood samples were obtained from patients with LP and healthy controls after 12 hrs of fasting. The blood samples were centrifuged at 3,000 g for 5 min at 4 °C. Erythrocyte suspension was prepared by removing the buffy coat from the erythrocyte and diluting the remainder of the erythrocytes with 10 mL of 0.9% NaCl. The resuspended erythrocytes were then centrifuged at 3,000 g for 5 min and the upper layer was removed again. This was repeated 3 times and then diluted 4 times with water and mixed by vortex. Samples were stored at −40 °C until assayed.

Measurement of superoxide dismutase levels

SOD activity was determined in samples using reagents supplied by Randox Laboratories Ltd. (Crumlin, UK). This method employs xanthine-xanthine oxidase (XOD) to generate superoxide radicals, which react with 2 iodophenyl-3-(4-nitrophenyl)-5-phenyltetrazolium (INT) chloride, to form a red formazan dye. The SOD activity in the sample hemolysate was then measured by the degree of inhibition of this reaction. The final color was measured at 505 nm and the results were expressed as SOD unit/g Hb.

Measurement of serum nitric oxide levels

Serum NO levels were assayed using the sandwich ELISA technique employing a kit from R&D systems (R&D Systems Inc. Minneapolis, MN). The assay determines NO concentrations based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Griess Reaction. This reaction is based on the two-step diazotization reaction in which acidified NO_2 produces a nitrosating agent, which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to N-(1-naphthyl) ethylenediamine to form the chromophoric azo-derivative, which absorbs light as 540 nm.

Measurement of serum malondialdehyde levels

MDA was assayed spectrophotometrically by a kit supplied by OxisResearch (Portland, OR). The method is based on the reaction of the chromogenic reagent N-methyl-2-phenylindole (NMPI) with MDA at 45 °C. One molecule of MDA reacts with

2 molecules of NMPI to yield a stable carbocyanine dye, the absorbance of which is measured at 586 nm.

Measurement of serum catalase levels

Catalase is assayed spectrophotometrically by a kit supplied by OxisResearch. It is a two-step procedure. The rate of dismutation of hydrogen peroxide (H_2O_2) to water and molecular oxygen is proportional to the concentration of catalase (reaction 1). The sample containing catalase is incubated in the presence of a known concentration of H_2O_2. After incubation for exactly 1 min, the reaction is quenched with sodium azide. The amount of H_2O_2 remaining in the reaction mixture is then determined by the oxidative coupling reaction of 4-aminophenazone (4-aminoantipyrene, AAP) and 3,5,-dichloro-2-hydroxylbenzenesulfonic acid (DHBS) in the presence of H_20_2 and catalyzed by horseradish peroxidase (HRP) in reaction 2. The resulting quinone-imine dye is measured at 520 nm (N-(4-antipyrl)-3-chloro-5-sulfonate-p-benzoquinonemonoimine).

Statistical analysis

Data were analyzed using the standard SPSS program (Echo Soft Corp., USA). They were expressed as mean ± standard deviation (*M* ± *SD*). Comparison between groups was done using Student's *t*-test with significance defined as $p \leq 0.05$. Pearson's correlation coefficient *(r)* was used to determine the relationship between different variables. The prognostic of the studied markers was assessed applying one-way analysis of variance (ANOVA) test.

Results

Forty-five Egyptian LP patients were included in the study, 26 females (57.7%) and 19 males (42.2%). Their ages ranged from 23 to 65 years with a mean ± SD of 45.32 ± 11.69 years. They were age- and sex-matched with 45 healthy individuals that served as controls with a mean ± SD of 45.32 ± 11.69 years. The duration of the disease ranged from 4 months to 5 years with a mean ± SD of 1.362 ± 1.18 years (Table 1).

Cutaneous lesions were present in all 45 cases. Twenty-two of the patients (48.8%) presented with classic LP, 14 patients (31.1%) had the actinic type, and 9 patients (20%) had the hypertrophic type. Mucosal lesions were present in 26 of our patients (57.7%) in the form of white reticular streaks on the buccal mucosa. None of the patients had erosive oral LP. Nail involvement was also present in 30 patients (66.6%) in the form of ridging, grooving, and longitudinal striations. Pterygium was observed in one of the patients.

Serum levels of NO, MDA, and SOD were higher in LP patients with means ± SD of 75.86 ± 11.95, 18.08 ± 3.02, and 17.33 ± 2.05, respectively, when compared to controls. Conversely, the serum

Table 1. Descriptive statistics.

Patients	***n***	***Min.***	***Max.***	***M***	***SD***
Age	45	23	65	45.32	11.69658
Duration (yr)	45	0.33	5	1.362	1.18995
NO (umol/L)	45	52.16	92.01	75.8688	11.95458
MDA (uM)	45	13.03	23	18.086	3.02205
SOD (U/mL)	45	14.01	20.22	17.3396	2.05529
CAT (U/mL)	45	10,412.12	16,712.31	13,306.06	2,246.347
Controls	***n***	***Min.***	***Max.***	***M***	***SD***
NO (umol/L)	45	47.06	80.19	60.6472	11.19309
MDA (uM)	45	10.01	20.01	15.4648	3.31923
SOD (U/mL)	45	11.01	19.48	15.4408	2.80876
CAT (U/mL)	45	8,366.14	29,123.07	19,514.88	7,284.516

NO = nitric oxide, MDA = malondialdehyde, SOD = superoxide dismutase, CAT = catalase

levels of erythrocyte CAT in the patients were 13,306.06 ± 2,246.34, which was lower than the controls (Table 1).

A highly statistically significant increase in the serum levels of NO (t = 4.64, p = 0), MDA (t = 2.92, p = 0.005), and SOD (t = 2.72, p = 0.009) in LP patients was present compared to the controls. In contrast, erythrocyte CAT levels showed a high significant decrease in the patient group compared to the control group (t = 4.07, p = 0) (Table 2).

There was a statistically significant increase in the serum levels of MDA (t = 2.14, p = 0.045) and a highly statistically significant decrease in the serum levels of erythrocyte CAT levels (t = 4.91, p = 0) in the male patients when compared to the females. However, the serum levels of NO and SOD showed no statistical difference between the sexes (Table 3).

There was also a statistically significant increase in the serum levels of NO (t = 1.79, $p \leq 0.05$), MDA (t = 2.15, $p \leq 0.05$), and SOD (t = 2.18, $p \leq 0.05$) and a highly statistically significant decrease in the serum level of erythrocyte CAT (t = 12.63, $p \leq 0.001$) in patients that had oral involvement with their skin lesions (Table 4).

Moreover, a statistically significant positive correlation between NO (r = 0.65, p = 0.001), MDA (r = 0.54, p = 0.005), SOD (r = 0.57, p = 0.003), and the duration of illness, and a statistically negative correlation between erythrocyte CAT (r = −0.48, p = 0.009) and the duration of the disease was found. In addition, there was a significant positive correlation between NO and SOD and erythrocyte CAT and MDA (Table 5).

However, there was a statistically insignificant relation between the four parameters and the different clinical types of LP (Table 6).

Discussion

Oxidative stress is caused by an imbalance between the production of reactive oxygen and the ability of the biological system to readily detoxify the reactive intermediates or easily repair the resulting damage. This usually results in the production of free radicals that can damage cell membranes through the production of lipid peroxides as well as numerous cellular molecules such as proteins, nucleic acids, amino acids, carbohydrates, and vitamins (8, 9).

NO is a gaseous free radical that is released by the family of NO synthetase enzymes. It is a potent vasodilator, thus contributing considerably to the cardinal signs of inflammation. It is also known to exhibit cytotoxic effects in human skin (10). In this study, the serum levels of NO were higher in patients with LP than in the control group, suggesting that oxidative stress resulting in the generation of ROS may play a role in the pathogenesis of LP.

One of the major presentations of oxidative stress is lipid peroxidation (11). Peroxidation of lipid-rich membranes alters their fluidity and signaling

Table 2. Student's t-test.

Group	*n*	*M*	*SD*	*t*	*p*	Significance
NO(umol/L)						
Controls	45	60.6472	11.19309			
Patients	45	75.8688	11.95458	4.647	0	HS
MDA(uM)						
Controls	45	15.4648	3.31923			
Patients	45	18.086	3.02205	2.92	.005	HS
SOD(U/mL)						
Controls	45	15.4408	2.80876			
Patients	45	17.3396	2.05529	2.728	.009	HS
CAT(U/mL)						
Controls	45	19,514.88	7,284.516			
Patients	45	13,306.06	2,246.347	4.072	0	HS

HS = highly significant

Table 3. Student's t-test.

Sex	*n*	*M*	*SD*	*t*	*p*	Significance
NO(umol/L)						
Female	26	79.6264	11.10267			
Male	19	72.9164	12.1524	1.439	.164	NS
MDA(uM)						
Female	26	16.6982	3.06486			
Male	19	19.1764	2.59319	2.145	.045	S
SOD(U/mL)						
Female	26	17.2927	2.29366			
Male	19	17.3764	1.93607	0.097	.924	NS
CAT(U/mL)						
Female	26	11,565.57	1,309.364			
Male	19	14,673.6	1,849.257	4.913	0	HS

NS = non significant, S = significant, HS = highly significant

Table 4. Student's t-test.

Oral lesion	*n*	*M*	*SD*	*t*	*p*	Significance
NO(umol/L)						
0	14	70.597	11.15765	1.79	< .05	S
1	26	79.4	12.60224			
MDA(uM)						
0	14	19.3	2.83304	2.15	< .05	S
1	26	16.6	3.23141			
SOD(U/mL)						
0	14	17.653	2.17651	2.18	< .05	S
1	26	15.8	2.01971			
CAT(U/mL)						
0	14	13,160.03	1,730.676	12.63	< .001	HS
1	26	1347	2,588.343			

S = significant, HS = highly significant

efficiency, leading to inflammatory changes and to aberrant cell proliferation responses (13–15). MDA, the end product of lipid peroxidation, is considered a good marker of free radical–mediated damage and oxidative stress (14). In this study we recorded higher serum levels of MDA in the patient group than in healthy subjects. We believe that our findings indicate that there was a disturbance in the antioxidant defense mechanism leading to increased production of ROS, thus resulting in increased lipid peroxidation and its product MDA. Our findings are in accordance with several studies. Sander et al. (15) demonstrated a significant increase in lipid peroxidation products MDA and 4-hydroxynonenale (4-HNE) in lichen sclerosis vulvae tissue specimens, particularly within the basal cell layers, which also showed increased oxidative DNA damage, increased protein oxidation, and disturbed enzymatic

Table 5. Spearman's ranked correlation coefficient.

Patients	Duration	MDA	SOD	CAT
NO(umol/L)				
r	0.651	−0.037	0.391	−0.049
p	.001	.859	.053	.815
Significance	HS	NS	S	NS
MDA(um)				
r	0.549			
p	.005			
Significance	HS			
SOD(U/mL)				
r	0.572	0.027		
p	.003	.899		
Significance	HS	NS		
CAT(U/mL)				
r	−0.482	−0.542	−0.037	
p	.009	.005	.861	
Significance	HS	HS	NS	

The critical value for r at n = 45 is 0.29359; i.e., r > 0.29359 is significant and r < 0.29359 is insignificant.

Table 6. Analysis of variance (ANOVA).

Type	*n*	*M*	*SD*	*F*	*p*	Significance
NO(umol/L)						
Classic	22	76.0217	13.76424			
Actinic	14	76.0075	11.87442			
Hypertrophic	9	75.28	9.44334			
Total	45	75.8688	11.95458	0.007	.993	NS
MDA(uM)						
Classic	22	17.7242	2.82245			
Actinic	14	17.6363	3.02436			
Hypertrophic.	9	19.674	3.59628			
Total	45	18.086	3.02205	0.854	.439	NS
SOD(U/mL)						
Classic	22	18.0167	1.91746			
Actinic	14	16.9375	2.21832			
Hypertrophic	9	16.358	1.92867			
Total	45	17.3396	2.05529	1.423	.262	NS
CAT(U/mL)						
Classic	22	13,128	2,317.303			
Actinic	14	12,603.3	2,129.15			
Hypertrophic	9	14,857.84	1,866.315			
Total	45	13,306.06	2,246.347	1.719	.202	NS

NS = nonsignificant

antioxidant defenses. In addition, Sezer et al. (7) reported that the serum levels of MDA were also higher in their LP patient group than the control group. Moreover, Rai et al. (8) reported high MDA levels in LP, leukoplakia, and cancer.

It has been proposed that the radical NO interacts with ROS and contributes to inflammatory responses. The inflammatory cellular infiltrate in LP, which consists mainly of CD4+ lymphocytes, is a well-known source of ROS (7, 15). ROS in toxic concentrations have been shown to cause damage to endothelial cells with further upregulation and expression of intercellular adhesion molecule (ICAM)1. ICAM-1 expression is important in facilitating the recruitment of T lymphocytes at the site of inflammation; this is an interaction that may result in the dermal perivascular T-cell infiltration and lymphocyte exocytosis observed in LP (7).

Moreover, free radicals have also been implicated in the activation of nuclear factor ϰB, an important transcription factor in inflammatory systems controlling the transcription of a number of cytokine genes including IL-2 and tumor necrosis factor alpha (TNF-α), as well as MHC class 1 gene and IL-2 receptor gene. H_2O_2 and superoxide anions (O_2–) may be produced in epidermal keratinocytes by TNF-α (16, 17).

In our opinion, these data support our hypothesis that increased ROS and lipid peroxidation in LP may enhance the inflammatory response by immunological mechanisms.

The skin possesses an array of defense mechanisms that interact with ROS to obviate their deleterious effect. SOD is considered the first-line defense against oxygen-derived free radicals, converting the superoxide anion (O_2^-) into H_20_2. H_2O_2 is dangerous in the cell because it can be easily converted into hydroxyl radicals, one of the most destructive free radicals (18). There are also other enzymes such as CAT, which decomposes peroxides. CAT is considered the main enzyme involved in removing H_20_2 (6). We found a highly significant increase in the serum levels of SOD and a significant decrease in the serum levels of CAT in the patient group compared to the controls in our study. Any significant reduction in CAT enzyme activity can increase the production of highly deleterious H_2O_2. Similar results to ours were also reported by other writers, who suggested that the imbalance in the antioxidant status may result in the accumulation of H_2O_2, thus leading to the vacuolization of the basal layer seen in LP (7).

Furthermore, we tested the hypothesis that oxidative stress is greater in men than in women (19). We found a significantly high increase in the serum levels of MDA and a highly significant decrease in the serum levels of erythrocyte CAT levels in the male patients when compared to the females. However, NO and SOD showed an insignificant difference between the sexes. The mechanism by which females are thought to be more protected from the damaging effects of oxidative stress may be related to the antioxidant properties of estrogens. Moreover, estradiol has been documented as having antioxidant effects (20).

In some studies, a negative correlation was reported between SOD activity and the duration of other diseases for which ROS is thought to be involved in the pathogenesis (21, 22). We could not establish this in our study. However, we found a positive correlation between NO, MDA, SOD, and the duration of illness, and a negative correlation between erythrocyte CAT and the duration of the disease.

We also investigated the possible relation between the measured oxidative stress parameters and the clinical manifestations of LP. A highly significant increase in the serum levels of NO, MDA, and SOD and a highly significant decrease in the serum level of erythrocyte CAT in patients that had oral involvement with their skin lesions were found. However, we found no correlation between the parameters measured and the different types of LP. To our knowledge, there are few reported studies investigating the involvement of oxidative stress and antioxidant enzyme expression on oral LP patients. Anshumalee et al. (6) reported that oxidative stress may play a role in oral LP. Meanwhile, in another study, the potent antioxidant lycopene was found effective in the management of oral LP. This therapeutic effect indirectly points to the role of oxidative stress in the pathogenesis of LP (23).

Whether oxidative stress is the initial event in the pathogenesis of LP or not, we think that a rational strengthening of the antioxidant defenses should be part of an optimal treatment strategy. We suggest that antioxidant drugs may also reverse the increased oxidative status in LP, thus theoretically resulting in clinical improvement. The problem of oxidative stress should also be further investigated and studies should be extended to include a larger number of patients suffering from different forms of this disease to confirm this opinion (7).

In summary, we conclude that there was an increase in the NO serum levels together with the increased lipid peroxidation product MDA. Oxidative stress was greater in men than in women. There was also an increase in SOD together with decreased CAT levels leading to an imbalance in the

antioxidant defense system in our study. In addition, there was also a positive correlation between NO, MDA, SOD, and the duration of illness, and a negative correlation between erythrocyte CAT and the duration of LP. In our opinion, all of this plays a role in the pathogenesis of LP.

REFERENCES

1. Kumar V, Nangia A, Logani KB, Sharma RC. Lichen planus: a clinico-histopathological correlation. Indian J Dermatol Venereol Leprol. 2000;66(4):193–5.

2. Middel P, Lippert U, Hummel KM, et al. Expression of lymphotoxin-alpha by keratinocytes: a further mediator for the lichenoid reaction. Pathobiology. 2000;68:291–300.

3. Omata N, Tsukahara H, Ito S, et al. Increased oxidative stress in childhood atopic dermatitis. Life Sci. 2001;69:223–8.

4. Relhan V, Gupta SK, Dayal S, et al. Blood thiols and malondialdehyde levels in psoriasis. J Dermatol. 2002;29:399–403.

5. Yildirim M, Baysal V, Inaloz HS, et al. The role of oxidants and antioxidants in generalized vitiligo. J Dermatol. 2003;30:104–8.

6. Anshumalee N, Shashikanth MC, Sharma S. Oxidative stress and oral lichen planus: A possible association? Cusp. 2007;4(2):31–4.

7. Sezer E, Ozugurlu F, Ozyurt H, Sahin S, Etikan I. Lipid peroxidation and antioxidant status in lichen planus. Clin Exp Dermatol. 2007;32:430–4.

8. Rai B, Kharb S, Jain R, Anand SC: Salivary lipid peroxidation product malonaldehyde in pre-cancer and cancer. Adv Med Dent Sci. 2008;2(1):7–8.

9. Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med. 2001;30(11):1191–212.

10. Ormerod AD, Copeland P, Hay I, et al. The inflammatory and cytotoxic effects of a nitric oxide releasing cream on normal skin. J Invest Dermatol. 1999;113:392–7.

11. Haliwell B, Gutteridge JMC. Free radicals in biology and medicine. New York: Oxford University Press; 2002. p. 701–7.

12. F'Guyer S, Afaq F, Mukhtar H. Photochemoprevention of skin cancer by botanical agents. Photodermatol Photoimmunol Photomed. 2003;19:56–72.

13. Briganti S, Picardo M. Antioxidant activity, lipid peroxidation and skin diseases. What's new? J Eur Acad Dermatol Venereol. 2003;17:663–9.

14. Kasperska-Zajac A, Brzoza Z, Rogala B, Polaniak R, Birkner E. Antioxidant enzyme activity and malondialdehyde concentration in the plasma and erythrocytes of patients with urticaria induced by nonsteroidal anti-inflammatory drugs. J Investig Allergol Clin Immunol. 2008;18(5):372–5.

15. Sander CS, Ali I, Dean D, Thiele JJ, Wojanarowska F. Oxidative stress is implicated in the pathogenesis of lichen sclerosis. Br J Dermatol. 2004;151:627–35.

16. Goebeler M, Roth J, Brocker EB, et al. Activation of nuclear factor-kappa B and gene expression in human endothelial cells by the common haptens nickel and cobalt. J Immunol. 1995;155:2459–67.

17. Fuchs J, Zollner TM, Kaufmann R, Podda M. Redox modulated pathways in inflammatory skin diseases. Free Radic Biol Med. 2001;30:337–53.

18. Han D, Williams E, Cadenas E. Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space. Biochem J. January 2001;353 (Pt 2):411–6.

19. Ide T, Tsutsui H, Noriko Ohashi N, Hayashidani S, Nobuhiro Suematsu N, Tsuchihashi M, Tamai H, Takeshita A. Greater oxidative stress in healthy young men compared with premenopausal women. Arterioscler Thromb Vasc Biol. 2002;22:438.

20. Bureau I, Gueux E, Mazur A, Rock E, Roussel AM, Rayssiguier FY. Female rats are protected against oxidative stress during copper deficiency. J Am Coll Nutr. 2003;22(3):239–46.

21. Urakami K, Sano K, Matsushima E, Okada A, Saito H, Takahashi K, et al. Decreased superoxide dismutase activity in erythrocyte in Parkinson's disease. Jpn J Psychiatry Neurol. 1992;46:933–6.

22. Bostantjopoulou S, Kyriazis G, Katsarou Z, Kiosseoglou G, Kazis A, Mentenopoulos G. Superoxide dismutase activity in early and advanced Parkinson's disease. Funct Neurol. 1997;12:63–8.

23. Anshumalee N and Shashikanth MC. Efficacy of oral lycopene in management of lichen planus [dissertation]. [Bangalore]: Rajv Ghandi University of Health Sciences, Bangalore. April 2007; 91-119.

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