Chest Heart Journal
Vol. 41, No. 2, July 2017

ORIGINAL ARTICLE

Effects of Spirulina on Oxidative Stress in Patients with Stable Chronic Obstructive Pulmonary Disease (COPD)

Mohammad Touhiduzzaman[1], Md.Ali Hossain[2], SK.Shahinur Hossain[3], Md.Masudur Rahman[4], Md.Abdur Rouf[5], Md. Zakir Hossain Sarker[3], Kazi Saifuddin Bennoor[3], Md.Habib uddin ahmad[3], Nirmal Kanti Sarker[6] Pulok Kumar Dey[4]

Abstract:

Background & objective: *Chronic obstructive pulmonary disease (COPD), is characterized by airflow limitation which is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases. Lungs are continuously exposed to oxidants and oxidative stress is associated with pulmonary airway narrowing due to enhanced inflammation. This study was designed to assess the effect of dietary supplementation of spirulina on oxidative stress in patients with stable Chronic Obstructive Pulmonary Disease.*

Patients & Methods: *This was a single-blind, randomized, prospective, placebo-controlled trial. Sixty COPD Patients were recruited from the indoor and outpatients department of National Institute of Diseases of Chest and Hospital (NIDCH), Mohakhali, Dhaka. Patients then subjected to randomize into 'Group-A' and 'Group-B'. Group-A was treated with spirulina and Group-B was given placebo in associations with conventional treatment.*

Results: *Spirulina intake for consecutive two months at 500 mg twice daily has significantly reduced serum content of MDA (pÂ0.05) and increased antioxidant parameters compared to their baseline levels (pÂ0.05 for GSH, Vit C level, activity of SOD and GST) while no difference occurred for lipid hydroperoxide and catalase (pÃ0.05). The magnitude of changes in MDA (pÂ0.05), GSH (pÂ0.05), GST (pÂ0.05), SOD(pÂ0.05) and Vit C (pÂ0.05) were all significantly greater in the Spirulina vs. the control group except lipid hydroperoxide (pÃ0.05) while significant decrease in catalase occurred in control group (pÂ0.05).*

Conclusion: *Spirulina reduces oxidative stress in COPD patients.*

Key words: *COPD, Spirulina, Oxidative stress.*

[Chest Heart Journal 2017; 41(2) : 102-108]

Introduction:

Chronic obstructive pulmonary disease (COPD), is defined as "a preventable and treatable disease with some significant extra pulmonary effects that may contribute to the severity in individual patients. The pulmonary component is characterized by airflow limitation which is usually progressive and associated with an

1. Assistant Registrar, NIDCH, Dhaka.
2. Professor, Respiratory Medicine, NIDCH, Mohakhali, Dhaka.
3. Assistant Professor, Respiratory Medicine, NIDCH, Mohakhali, Dhaka.
4. Medical Officer, Respiratory Medicine, NIDCH, Mohakhali, Dhaka.
5. Associate Professor, Respiratory Medicine, NIDCH, Mohakhali, Dhaka.
6. Jr. Consultant, Respiratory Medicine, NIDCH, Mohakhali, Dhaka.

Correspondence to : Dr. Mohammad Touhiduzzaman, Assistant Registrar, NIDCH, Dhaka. Mobile: 01712082646
E-mail: drmtzaman74@yahoo.com

Submission on: 28 May 2017 Accepted for Publication: 28 June 2017
Available at http.//www.chabjournal.org

abnormal inflammatory response of the lung to noxious particles or gases".[1]

Lungs are continuously exposed to oxidants, either generated endogenously by metabolic reactions or exogenously, such as air pollutants or cigarette smoke.[2] The lung has heavy reliance on superoxide dismutase (SOD) (EC 1.15.1.1), catalase (EC 1.11.1.6), and glutathione peroxidase (GSH-Px) (EC 1.11.1.9) as they are the major enzymatic antioxidants of the lungs.[3] Glutathione-S-transferase (GST) (EC 2.5.1.18), another antioxidant enzyme family, inactivates secondary metabolites, such as unsaturated aldehydes, epoxides, and hydroperoxides.[4] Vitamin C reduces oxidative stress and improves vascular function and structure.[5] Glutathione (GSH) provides antioxidant defenses by their ability to exist in reversible oxidized and reduced forms.[6] Reactive oxygen species are highly short lived and therefore the best way to estimate oxidative stress is to quantify the products of their reaction with lipids, proteins, and nucleic acids.[7] Thiobarbituric acid reactive substance measure is one such test which quantifies malondialdehyde (MDA), a product of lipid peroxidation.[7]

When the balance between oxidants and antioxidants shifts in favor of the former, from either an excess of oxidants and/or depletion of antioxidants, oxidative stress occurs.[8] *Spirulina* sp. (*Arthrospira* sp.) is a photosynthetic, filamentous nondifferentiated, spiral-shaped, multicellular, and blue-green microalgae that grows naturally in warm climates.[9] Spirulina contains several active ingredients, notably phycocyanin and ²-carotene that have potent antioxidant and anti-inflammatory activities.[10]

There is a current worldwide interest in finding new and safe antioxidants from natural sources such as plant material to prevent oxidative deterioration of food and to minimize oxidative damage to living cells.[11] The use of synthetic antioxidants has decreased due to their suspected activity as promoters of carcinogenesis as well as a general consumer rejection of synthetic food additives.[12]

Spirulina with its good content of antioxidant nutrients such as ²-carotene, vitamin E, selenium could possibly play a role in alleviating the pulmonary function abnormalities by scavenging endogenous and/or environmental oxidant sources.[13]

Patients & Methods:

This was a single-blind, randomized, prospective, placebo-controlled trial with initial screening of patients that included 4-weeks intensive investigation and management phase (run in period), followed by a baseline, monthly for 2 months follow-up phase to determine the serum level of Malondialdehyde (MDA), Lipid hydroperoxide, catalase, Superoxide Dismutase (SOD), Glutathione-S-transferase (GST), Glutathione (GSH) and Vitamin C of stage-III COPD patients.

The study was carried out in the Department of Respiratory Medicine in National Institute of Diseases of the Chest and Hospital (NIDCH), Mohakhali, Dhaka during April 2014 to March 2015. Samples were collected from both indoor and outpatients department (OPD). Spirometry was performed in respiratory laboratory of NIDCH and serum oxidative stress markers Malondialdehyde (MDA), Lipid hydroperoxide, catalase, Superoxide Dismutase (SOD), Glutathione-S-transferase (GST), Glutathione (GSH), Vitamin C were measured in the Department of Biochemistry of Dhaka University.

60 patients with COPD (defined by specific criteria) were reviewed and if inclusion and exclusion criteria fulfilled, written consent was taken and were registered for the study and data were collected.

Each case was included in this study by purposive sampling method. Sample patients were divided in to two groups by simple randomization.

- One group was given Cap.Spirulina 500 mg twice daily for oral intake after breakfast and after dinner in addition of their standard management (Inhaled Tiotropium- 18 mic.gm, Salmeterol- 50 mic.gm and Fluticasone- 500mic.gm, Tablet Theophylline 400 mg) for consecutive 60 days. (Group-A)
- Another group was given placebo in addition of their standard management (Inhaled Tiotropium- 18 mic.gm, Salmeterol- 50 mic.gm and Fluticasone- 500mic.gm,Tablet Theophylline 400 mg) for consecutive 60 days. (Group-B)

A well structured questionnaire was administered among the patients. Changes in symptomatology (by CAT score), spirometry result, serum oxidative stress enzyme, oxidant and antioxidant level were recorded in specific format.

Data analysis :

All the data were recorded systematically. Collected data were compiled and tabulated on a master sheet. Data was analyzed by using SPSS version 19 and was expressed as Mean ± SD. Appropriate test (Chi-square and both paired and unpaired t' test) was applied to find the significance of difference. Level of significance was set at p-value dd0.05.

Result:

A total number of 60 samples were collected from both inpatient department and outpatient department of Respiratory medicine at National Institute of Diseases of the Chest and Hospital (NIDCH), Mohakhali, Dhaka. Among them 21 patients in group-A and 20 patients in group-B came to final follow-up. Serum level of MDA, Lipid hydroperoxide, catalase, SOD, GST, GSH, Vitamin C level were performed in each case at the beginning and monthly for consecutive 2 months. In the study mean age 56.81 (± 9.19) years were in group-A and 57.66 (± 9.17) years were in group-B. Male were predominant, 27 (90%) were in group-A and 28 (93.3%) were in group-B. Male and female ratio was 11:1. Mean BMI in group A were 23.45 (±4.21) and 22.95 (±4.54) in group B. Majority 29 (96.66%) smoker in group A and 28 (93.33%) smoker in group B (p Ã 0.05) that was not statistically significant and results are shown in Table-I. Spirulina intake for consecutive two months at 500 mg twice daily has significantly reduced serum content of MDA (pÂ0.05) and increased antioxidant parameters compared to their baseline levels (pÂ0.05 for GSH, Vit C level, activity of SOD and GST) while no difference occurred for lipid hydroperoxide and catalase (pÃ0.05). The magnitude of changes in MDA (pÂ0.05), GSH (pÂ0.05), GST (pÂ0.05), SOD(pÂ0.05) and Vit C (pÂ0.05) were all significantly greater in the Spirulina vs. the control group except lipid hydroperoxide (pÃ0.05) while significant decrease in catalase occurred in control group (p<0.05)

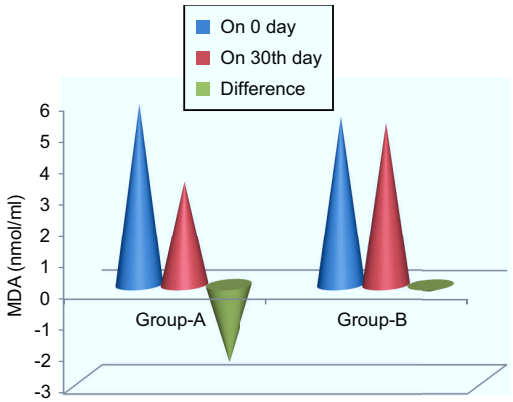
Table-I

Study population according to base line characteristics

Characteristics	Group-A (Case)	Group-B (Control)	P- value
Age in years (Mean±SD)	56.81 (± 9.19)	57.66 (± 9.17)	0.72
Gender			
Male (%)	27 (90%)	28 (93.3%)	0.55
Female (%)	03 (10%)	02 (6.7%)	
Smoking status			
Smoker (%)	29 (96.6%)	28 (93.33%)	0.65
Nonsmoker (%)	01 (3.4%)	02 (6.67%)	
BMI (Mean±SD)	23.45 (±4.21)	22.95 (±4.54)	0.89

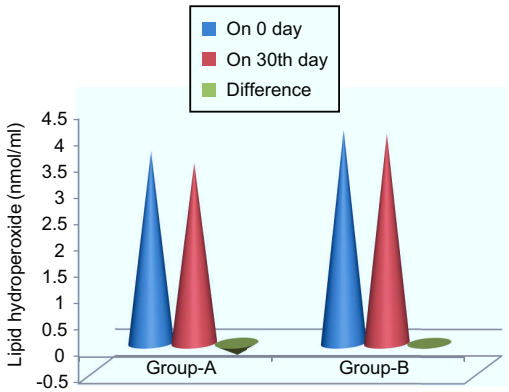
Group-A : Spirulina, Group-B : Placebo, p value reach from t-test and Chi square test.

Group-A: Spirulina Group-B: Placebo



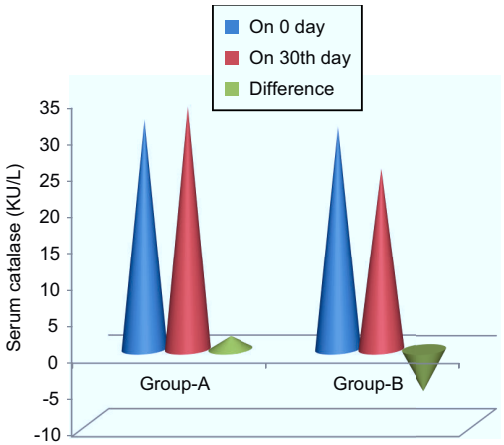
Group-A: Spirulina Group-B: Placebo

Fig.-1: *Serum MDA in two groups at the end of 30th day*



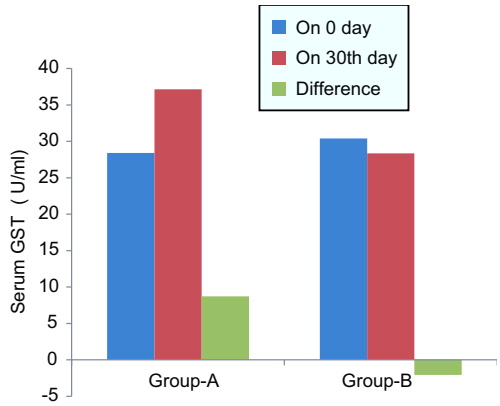
Group-A: Spirulina Group-B: Placebo

Fig.-2: *Serum lipid hydroperoxide in two groups at the end of 30th day*



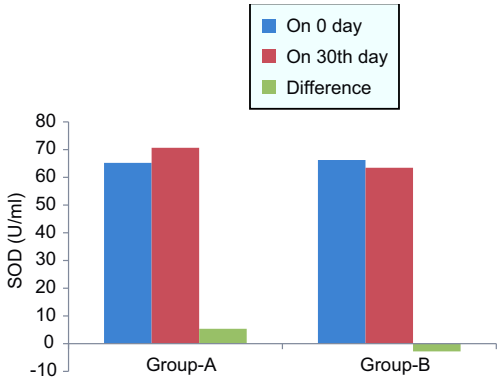
Group-A: Spirulina Group-B: Placebo

Fig.-3: *Serum catalase in two groups at end of 30th day*



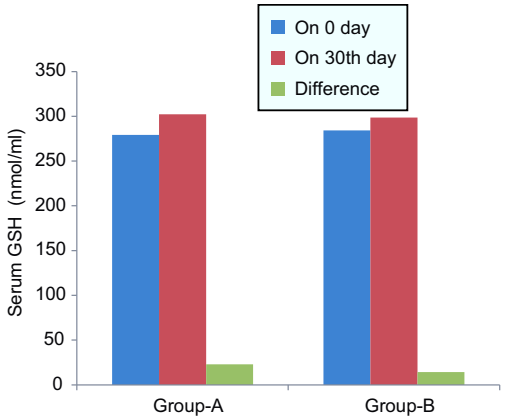
Group-A: Spirulina Group-B: Placebo

Fig.-5: *Serum gst in two groups at the end of 30th day.*



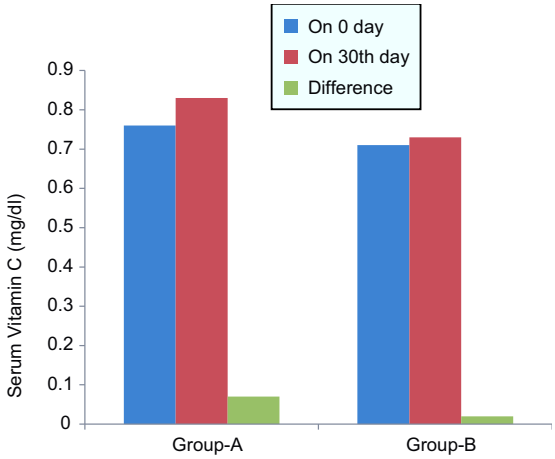
Group-A: Spirulina Group-B: Placebo

Fig.-4: *serum sod in two groups at the end of 30th day.*



Group-A: Spirulina Group-B: Placebo

Fig.-6: *serum glutathion (gsh) in two groups at end of 30th day*



Group-A: Spirulina Group-B: Placebo

Fig.-7: *serum vitamin c in two groups at the end of 30th day.*

Table-II

oxidative stress biomarkers in two groups at the end of 60th day

Oxidative stress biomarker	Group-A (Spirulina) n= 21 On 0 day Mean(±SD)	On 60th day Mean(±SD)	p-Value	Group-B (Placebo) n= 20 On 0 day Mean(±SD)	On 60th day Mean(±SD)	p-Value
MDA (nmol/ml)	5.58± 0.34	2.99±0.22	0.01s	5.78± 0.24	5.28± 0.27	0.28ns
Lipid Hydroperoxide (nmol/ml)	3.23±0.12	3.09±0.22	0.12ns	3.78±0.29	3.82±0.17	0.58ns
Catalase(kU/L)	28.53±7.42	30.05±8.08	0.33ns	31.03±8.41	24.83±5.37	0.03s
SOD(U/ml)	67.25±6.43	75.89±5.34	0.008s	65.21±8.43	60.15±7.63	0.07ns
GST (U/ml)	29.21±4.02	39.95±3.50	0.02s	28.41±4.02	25.35±3.82	0.41ns
GSH (nmol/ml)	281.32±21.24	332.61±13.61	0.01s	285.31±22.23	301.85±21.27	0.02s
Vitamin C (mg/dl)	0.78±0.07	0.92±0.05	0.02s	0.74±0.09	0.71±0.09	0.76ns

ns : not significant; S : significant; Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione-S-transferase (GST), Glutathione (GSH), Vitamin C

Table-III

Magnitude of changes in oxidative stress biomarkers in two Groups at the end of 60th day:

Parameter change	Group-A(Spirulina)	Group-B (Placebo)	p-Value
MDA (nmol/ml)	-2.59±0.25	-0.50±0.21	0.02^{s}
Lipid hydroperoxide (nmol/ml)	-0.14±0.17	0.04±0.21	0.66^{ns}
Catalase(kU/L)	1.52±4.70	-6.20±3.85	0.02^{s}
SOD(U/ml)	8.64±2.01	-5.06±2.65	0.03^{s}
GST (U/ml)	10.74±0.44	-3.06±0.25	0.01^{s}
GSH (nmol/ml)	51.30±5.04	16.54±4.35	0.01^{s}
Vitamin C (mg/dl)	0.44±0.02	-0.03±0.05	0.02^{s}

ns : not significant; S : significant; Malondialdehyde (MDA), Superoxide Dismutase (SOD), Glutathione-S-transferase (GST), Glutathione (GSH).

Discussion:

The inflammation in the respiratory tract of COPD patients appears to be a modification of the inflammatory response of the respiratory tract to chronic irritants such as cigarette smoke. The mechanisms for this amplified inflammation are not yet understood but may be genetically determined. Patients can clearly develop COPD without smoking, but the nature of the inflammatory response in these patients is unknown. Oxidative stress and an excess of proteases in the lung further modify lung inflammation. Together, these mechanisms lead to the characteristic pathological changes in COPD.[14]

Oxidative stress may be an important amplifying mechanism in COPD.[15] Oxidative stress not only produces direct injurious effects in the lungs, but also activates the molecular mechanisms that initiate lung inflammation and may have a role in many of the processes thought to be involved in the complex pathological events that result in COPD.[16]

The patients were predominantly male in the whole study population, male were predominant, 27 (90%) were in group-A and 28 (93.3%) were in group-B. Male and female ratio was 11:1 which indicate that the disease incidence was higher in male patients. Baseline and demographic

characteristics of the patients were similar between two groups.(pÃ 0.05)

No significant difference was observed between the groups regarding their base line oxidative stress biomarkers including MDA, lipid hydroperoxide, Vit C, GSH, SOD, GST,catalase (PÃ0.05). In the placebo group only serum GSH (p Â 0.05) concentrations was significantly increased by the end of first month and end of trial but catalase activity had significantly decreased (p Â 0.05). No difference occurred in placebo group for serum vit C, GST, SOD, lipid hydroperoxide and MDA (p Ã 0.05). In contrast, all assessed antioxidant parameters except catalase had remarkably increased in the spirulina group after first month and end of trial compared to their baseline levels (p Â 0.05 for vitC, GSH, SOD and GST). We found no difference in spirulina group for serum catalase and lipid hydroperoxide (p Ã 0.05) which differs from previous study.[17] The results of the present analysis clearly highlight the efficacy of spirulina as an MDA lowering agent. MDA levels were reduced in spirulina group by the end of first month and end of trial (pÂ0.05). This outcome is similar to the finding of another study.[18] The magnitude of changes in MDA (pÂ0.05), GSH (pÂ0.05), GST (pÂ0.05), SOD(pÂ0.05) and Vit C (pÂ0.05) were all significantly greater in the Spirulina vs. the control group except lipid hydroperoxide (pÃ0.05) while significant decrease in catalase occurred in control group (pÂ0.05) both in 30th and 60th day of trial. The above findings clearly in favour of ameliorating antioxidant status in COPD patients with spirulina supplementation. These findings are consistent with that reported in previous study.[19]

Conclusion:

COPD patients who received Spirulina as adjunctive therapy with conventional medication experienced improvement in serum antioxidant status.

Acknowledgement:

Department of Biochemistry, Dhaka University.

Referrences:

1. Rabe K, Hurd S, Anzueto A, et al. Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2007;176:532–55.
2. Rahman I., Adcock M. Oxidative stress and redox regulation of lung inflammation in COPD. *Eur Respir J*. 2006;28:.219–42.
3. Kinnula V , Crapo J. Superoxide dismutases in the lung and human lung diseases. *American Journal of Respiratory and Critical Care Medicine*.2003;167(12):1600–19.
4. Ladner J, Parsons J, Rife C, Gilliland G, Armstrong R. Parallel evolutionary pathways for glutathione transferases: structure and mechanism of the mitochondrial class kappa enzyme rGSTK1-1. *Biochemistry.2004;*43(2):.352–61.
5. Vojdani A., Bazargan M., Vojdani E., Wright J. New evidence for antioxidant properties of vitamin C. *Cancer Detection and Prevention.2000;*24(6):508–23.
6. Attia M., Sayed A., Ibrahim A, Mohammed S. Effects of antioxidant vitamins on the oxidant/antioxidant status and liver function in homozygous beta-thalassemia. *Romanian Journal of Biophysics*.2011; 21:.93–106.
7. Arja C , Surapaneni KM , Raya P, Adimoolam C, Balisetty B, Kanala KR. Oxidative stress and antioxidant enzyme activity in South Indian male smokers with chronic obstructive pulmonary disease. *Respirology*.2013;18(7):1069–75.
8. Schunemann HJ, Muti P, Freudenheim JL, et al. Oxidative stress and lung function. *Am J Epidemiol*. 1997;146:.939–48.
9. Sánchez M. *Spirulina* (*Arthrospira*): an edible microorganism- A review. *Univer Scient.2003;* 8:7.
10. Romay C, Armesto J, Remirez D, González R., Ledon N, García I..Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae. *Inflamm Res*.1998; 47:36–41.
11. Pratt E. Natural antioxidants from plant material. In: Huang MT, Ho T & Lee Y. (Editors), *Phenolic Compounds in Food and their Effects on Health. II*. American Chemical Society, Washington. 1992; 54-71 (ACS Symposium Series, 507).

12. Namiki M. Antioxidants/antimutagenics in food. *CRC Critical Reviews in Food Science and Nutrition. 1990;*29: 273-300.

13. Sakata K, Yamamoto K & Watanable N. Antioxidative compounds from marine organisms. In: Ho CT, Osawa T, Huang MT & Rose RT (Editors), *Food Phytochemicals for Cancer Prevention. II.* American Chemical Society, Washington.1992; 165-182 (ACS Symposium Series 547).

14. Cosio MG, Saetta M, Agusti A. Immunologic aspects of chronic obstructive pulmonary disease. *N Engl J Med.*2009;360:2445-54.

15. Rahman I. Oxidative stress and gene transcription in asthma and chronic obstructive pulmonary disease: antioxidant therapeutic targets. *Curr Drug Targets Inflamm Allergy.*2002; 1:291–315.

16. Rahman I, MacNee W. Role of transcription factors in inflammatory lung diseases. *Thorax. 1998;*53:601–12.

17. Panahi Y, Tavana S, Sahebkar A, Masoudi H, Madanchi N. Impact of adjunctive therapy with Cholera vulgaris extract on Antioxidant status, Pulmonary function and Clinical symptoms of Patients with Obstructive Pulmonary Diseases. *Sci Pharm.*2012; 80:719-30.

18. Lu HK, Hsieh CC, Hsu JJ, Yang YK, Chou HN. Preventive effects of *Spirulina platensis* on skeletal muscle damage under exercise-induced oxidative stress. *Eur J Appl Physiol.*2006; 98:220–26.

19. Bermejo-Bescós P, Piñero-Estrada E , Villar del Fresno AM. Neuroprotection by *Spirulina* platensis protean extract and phycocyanin against iron-induced toxicity in SH-SY5Y neuroblastoma cells. *Toxicol In Vitro.* 2008;22:1496–1502.