Indian J Physiol Pharmacol 2002; 46 (3) : 349–354

IMPROVEMENT IN OXIDATIVE STATUS WITH YOGIC BREATHING IN YOUNG HEALTHY MALES

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(Received on July 5, 2001)

Abstract : The modern living lifestyle is known to produce various physical and psychological stresses and subject the individual to produce oxidative stresses as well. The aim of this study has been to assess the effect of yogic breathing exercises (pranayama) on the oxidatives stress. The study group consisted of 30 young male volunteers, trained for the purpose of this study and an equal number of controls were used. The free radicals and Super oxide dismutase levels were measured before the study and at the end of the study. The free radicals were decreased significantly in the study group but the SOD was increased insignificantly as compared to the control group. Yogic breathing exercises not only help in relieving the stresses of life but also improve the antioxidant status of the individual. An improvement in the antioxidant status is helpful in preventing many pathological processes that are known with impaired antioxidant system of body.

Key words : SOD free radicals Pranayama Yoga

INTRODUCTION

The paradox of aerobic life is that aerobic organisms cannot exist without oxygen, yet oxygen also happens to be inherently dangerous to their very existence. The reductive environment of the cellular milieu provides ample opportunities for oxygen to undergo unscheduled univalent reduction. Thus the superoxide anion radical, hydrogen peroxide and the extremely reactive hydroxyl radical are common products of life in an aerobic environment, and these agents are responsible for oxygen toxicity (1). To survive in such an unfriendly oxygen environment, living organisms generate or garner from their surroundings a variety of water and lipid-soluble antioxidant compounds. Additionally, a series of antioxidant enzymes, whose role is to intercept and inactivate reactive oxygen intermediates, are synthesized by all known aerobic organisms. Although extremely important, sometimes the antioxidant enzymes and compounds are not completely

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effective in preventing oxidative damage. To deal with the damage that does still occur, a series of damage removal/repair enzymes, for proteins, lipids and DNA, are synthesized. Since oxidative stress may vary from time to time, organisms are able to adapt to such fluctuating stressors by inducing the synthesis of antioxidants and damage removal/repair enzymes (2).

Since time immemorial the ancient culture of India has professed the virtues of Yoga, a system of integral education. Education not only of the body and mind or intellect, but also of the inner spirit. It is believed by Yogis that practice of Yoga brings about a decrease in the stress level of the individual. The aim of the present study is to assess this effect on the antioxidant status of the body.

METHODS

The present study was conducted in the Department of Physiology in collaboration with the Department of Biochemistry King George's Medical College, Lucknow. The cases selected for this study were 30 male volunteers between 18–21 years who were enthusiastic for yoga and the design of the study was of prospective type. The control group was a batch of 30 age, sex matched sedentary volunteers. The methodology of the study was explained to them and informed consent was taken. Only those volunteers were chosen who did not have history or clinical symptoms of any disease and did not indulge into any addiction like alcohol, smoking or tobacco in any form. The subject were given training in yogic breathing exercises and relaxation techniques. They exercised daily for 30 min under supervision for 10 weeks in the morning hours. At the start of the training schedule blood samples were collected of the subjects of both experimental as well as control group for estimation of SOD activity and Lipid peroxide levels. Similarly a sample of blood was taken at the end of 10 weeks of training.

The objective of exercise was to improve the stress adaptability of the subjects. Various exercise were so chosen so as to achieve the stated objectives. Exercises carried out were Pranayama, which included – Deep inspiration/Breath Retention/Expiration known as Puraka/ Kumbhaka/Rechaka in yogic literature, in a ratio of 1:4:2 (3). At the end of the pranayama session, subjects took up practice of meditation. They were made to sit in a comfortable asana - sukhasana or padmasana. They were asked to concentrate on a point between their two eyebrows and try to 'visualise' the point with their eyes closed. The session closed daily after doing 10 min of shavasana in which the subjects lay still with their eyes open.

Biochemical test

9 ml blood was drawn in disposable plastic syringe which was previously rinsed with and contained 1 ml. of 3.8% sodium citrate solution. Samples were transferred to plastic tubes and were sent to the Department of Biochemistry, King George's Medical College, Lucknow, for biochemical estimations.

The lipid peroxide (MDA) contents were estimated according to the modified method by Ohkawa et al (4). 1 ml of plasma was mixed with 1.0 ml of 20% acetic acid. Subsequently 0.5 ml of 8.0% aqueous Sodium Dodecyl Sulphate was mixed in the above reaction mixture, the pH of the mixture was adjusted at 4.0 using concentrated NaOH solution if needed 1.5 ml of 0.8% TBA solution and sufficient amount of distilled water were added to a final volume of 4 ml, after adjusting the pH of the reaction mixture, the reaction was kept in a boiling water bath for one hour, 3.0 ml of n-Butanol was mixed, after cooling to room temperature, the reaction mixture was then centrifuged at 10,000 rpm for 15 minutes. A clear butanol fraction obtained after centrifugation was used for measuring the absorbance at 532 nm in DU-6 Beckman spectrophotometer. An appropriate standard made up of malondialdehyde (MDA) 2.5 n mole was run simultaneously.

Calculation : Standard absorbance of malondialdehyde (2.5 n mole) was used to calculate the amount of lipid peroxide in the samples and results were expressed as a n mole of MDA/ml plasma.

SOD was measured by the modified method of McCord and Fridovich (5) 2 ml of RBC were hemolysed with distilled water and dispensed in centrifuge tubes. The tubes were placed in a refrigerated centrifuge at 10000 rpm. 313 mg/ml Ammonium sulphate was added to the supernatant from each sample thoroughly and kept for 4 hours in cold (4°C). Thereafter, the tubes were centrifuged at 14000 rpm for 30 minutes at 4°C. The supernatant sample was dialyzed against cold triple distilled water with three changes, each change after three hour interval. The contents of the dialysis bags were subsequently used as enzyme source. The two reaction setups were run later in parallel. The tubes in first set up (experimental) received 0.3 ml nitro blue tetrazolium, 0.2 ml phenazine methosulphate, 1.0 ml pyrophosphate buffer, 1.0 ml triple distilled water and 0.2 ml enzyme source. The second set up (reference) tubes received all the above reagents except the enzyme source. The reaction started simultaneously in the two sets by then addition of 0.2 ml NADH. 1.0 ml of glacial acetic acid was added to each tube after an interval of 90 seconds, for checking the reaction. 0.2 ml enzyme sources was added in reference tubes. The absorbance in these tubes was read at 560 nm on a spectrophotometer against a blank (NBT+PMS+Buffer+TDW).

Calculation : The unit of enzyme activity was defined as the amount of enzyme required to inhibit the optical density at 560 nm of NBT reduction by 50% in one minute under the assay conditions. The results were expressed as units/ml/mg protein.

Statistical analysis of the work was carried out using Student's paired t-test on a software in a Pentium 133 computer. The P value, mean and standard deviation of the data were determined.

RESULTS

TABLE I: MDA and SOD levels in the study group.

Yoga group (n=30)	*Before training (Mean ± S.D.)*	*After training (Mean ± S.D.)*	*P value*
M.D.A $x10^{-6}$ mole/ml	9.57±0.56	8.21±0.76	<0.01 Very significant
SOD Units/mg protein	11.60±3.14	13.04±2.66	>0.05 Not significant

TABLE II: MDA and SOD levels in the control group.

Control group (n=30)	*At the start of study (Mean ± S.D.)*	*At the end of study (Mean ± S.D.)*	*P value*
M.D.A $x10^{-6}$ mole/ml	9.32±0.63	9.06±0.54	>0.05 Not significant
SOD Units/mg protein	11.95±3.14	12.96±2.66	>0.05 Not significant

DISCUSSION

Pranayama is a Yogic breathing practice, which is known experimentally to produce a profound calming effect on the mind. They are well known for their effect on relieving mental stress (6, 7, 8). In the present study emphasis has been given on the breathing exercises and meditation. Both of them are considered to be relaxation methods, which helps a person to de-stress. Pranayama breathing exercises appear to alter autonomic responses to breath holding probably by increasing vagal tone and decreasing sympathetic discharges (9). These results suggest that breathing selectively through either nostril could have a marked relaxing effect on the sympathetic nervous system. The therapeutic implications, being able to alter metabolism by changing the breathing pattern (10). Practice of such technique not only helped the subjects to de-stress but also improved their response to further stressful stimulus.

The plasma lipid peroxide level showed a significant change from a pre training level of 9.57 nm/ml to a post training mean value of 8.21 nm/ml (P value <0.01) in the exercising group as compared to a non-significant change in the control group. This implies that there was a decrease in the production of free radicals following yogic practice. Lipid peroxidation is a free radical mediated phenomenon and plasma lipid peroxidation level may reflect the degree of free radical mediated stress (11).

Moreover, the activity of SOD was found to be a increased from a pre training level of 11.60 units/mg protein to a post training mean value of 13.04 units/mg protein. However, this change was not found to be

statistically significant, but it does show a tendency to increase in value. In the control group there was a similar increase from 11.95 to 12.96 Units/mg protein.

This biochemical study implies that the products of lipid peroxidation decrease with yogic exercises viz. pranayama and meditation. Free radicals are produced secondary to various stimuli - Biological, Chemical and Environment (12). These stimuli include any form of stress, either physical or mental to the biological system.

The relaxation technique taken up for this study involved meditative processes and pranayama. Pranayama is an art of control of breathing. A practitioner of pranayama not only tries to breath but at the same time tries to keep his attention on the act of breathing, leading to concentration. This act of concentration removes his attention from worldly worries and 'de-stresses' him. This stress free individual may be able to adapt better to the daily emotional, physical and mental stresses. Various animal experiments have shown that physical as well as emotional stress increases free radical production (13, 14, 15). Apart from the concentration and meditative aspect, pranayama involves taking in breath, retaining it and then exhaling it. The time period for this process has been defined to be 1:4:2 for inhalation/retention/exhalation respectively in yogic literature. This type of breathing emphasizes a lot on the retention aspect of pranayama, this is called 'kumbhaka' in Yoga. In Yogic terminology it is said that in such a way by preserving 'prana' i.e. breath one can increase the life span and also live a better life (16).

The results of the present study demonstrating decrease in lipid peroxide level and increase in the activity of the enzyme SOD following training. Experiments conducted on nematodes showed that elevated oxygen decreased and subnormal oxygen increased the mean and maximum life spans of nematodes (17). Moreover an increase in SOD of such nematodes has been reported by another study (18). Studies on housefly showed reduced mean and maximum life spans in an atmosphere of 100% oxygen (19, 20). Unfortunately, similarly designed experimental study is inapplicable to mammalian systems and elevations or decreases in ambient oxygen in mammals are likely to be confounded by the overt pathology of hypo- or hyperoxia. However interspecies comparisons of oxidative damage, antioxidant defenses and oxidant generation provide some of the most compelling evidence that oxidants are one of the most significant determinants of life span (21). Studies on mammalian species showed that SOD level co-related positively with increase in mean life span (22, 23). Perhaps control of breathing by decreased oxygen utilization causes increase in SOD. This is comparable with experimental models on nematodes and housefly and to some extent on some animal models (21).

In conclusion it may be stated that practice of pranayama by regulating the oxygen intake down regulates the lipid peroxide production and increases the activity of SOD. Yogic exercises improve the free radical status, moreover it may also aid in checking oxidative stress induced damages.

ACKNOWLEDGEMENTS

All the samples were analyzed in the Department of Biochemistry, K.G. Medical College, Lucknow. We are thankful for the co-operation extended by Dr. A. A. Mahdi, Department of Biochemistry, K.G. Medical College, Lucknow.

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