Antioxidant Effects of Regular Yoga Training on the Healthy University Students Controlled Clinical Trial

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Abstract

Background: To assess the properties of regular yoga training on oxidative stress and antioxidant components, including total glutathione (GSH) and its redox systems (GSH-peroxidase and reductase), total antioxidant capacity (TAC), and activities of superoxide dismutase (SOD) and catalase in healthy university students.

Study design and methods: Current study involved total 25 healthy participants who were divided into two groups as control and regularly yoga group. All subjects undertook the regularly control or yoga program for 90 minutes, once every week, for 12 weeks under the care of yoga and health professional instructor. Before the start and end of each program, the serum level of oxidative stress and antioxidant components were estimated.

Results: The serum MDA level considerably decreased in yoga training group and significantly increased TAC levels, total GSH contents and SOD activities the serum level (P < 0.05 or P < 0.01) as compared with the changes value of control care program.

Conclusions: From the results, present study supports the utility of yogic exercises as a remedy to control or check oxidative stress in the healthy volunteers.

Keywords: Yoga; Antioxidant; Complementary and alternative medicine

Materials and Methods

Subjects

Subjects who undertook in the intervention were students from different universities on Daejeon, Rep. of Korea. (Table 1) shows the base line of characters for all participants. Total 25 university students undertook the yoga (n=13) and control program (n=12) for one semester, from September to December in 2010.

Study design

Yoga group was a weekly 90 min yoga training class for 12 weeks. All of participants were instructed by a professional yoga instructor. The yoga program is shown in table 2. The yoga class was composed up of 3 steps, ‘introduction (mainly stretching)-development (mainly

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breathing and meditations)-meditation (mainly relaxing)" and the program was changed to different programs in each 4-week. All participants practiced yoga program for 30 min, and 5 times a week by themselves. After regularly yoga class, the participants reported their yoga training on the diary. Every yoga class, the instructor observed the yoga diary and considered the contents. Control program was consisted a weekly 90 min aerobic exercise program for 12 weeks, the same duration with yoga program. Participants were instructed by a professional exercise trainer, and the program also consisted of 3 steps, 'breathing-stretching-running' (Table 3). To examine the yoga or control care program affected on the oxidative stress/antioxidant components were determined by comparing serum samples. Whole blood was collected from the arm veins at the day before each class the first and final experiment under condition of 8 hrs fasting. To obtain the serum samples from participants, whole blood samples were placed at room temperature (25 ± 2°C) for 40 minutes to remove protein such as fibrinogen. All serum samples were stored at -70°C for further experiments. Before starting this study, we received the consents from all of the participants. Each one who has a health problem was excluded before starting present study and all of the participants completely finished.

Chemicals and materials

The following reactants were purchased from Sigma (St. Louis, MO, USA): 1,1,3,3-tetraethoxypropane (TEP), N,N-diethyl-para-phenylenediamine (DEPPD), ferrous sulfate, trichloroacetic acid (TCA), 5,5-dihydroxy-(2-nitro benzoic acid) (DTNB), potassium phosphate, reduced glutathione (GSH), myoglobin, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), glutathione reductase (GSH-reductase), L-glutathione oxidized disodium salt (GSSG), potassium phosphate reduced form of β-nicotinamide adenine dinucleotide phosphate (β–NADPH), and tert-butyl hydroperoxide. Thiobarbituric acid (TBA) was obtained from Lancaster Co. (Lancashire, England). Hydrogen peroxide was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). n-butanol was purchased from J.T. Baker (Mexico City, Mexico). Tris–hydrochloric acid (Tris-HCl, pH 7.4, 1 M) and ethylenediaminetetraacetic acid (EDTA, 500 mM, pH 8.0) were purchased from Bioneer (Daejeon, Republic of Korea).

Determination of lipid peroxide as malondialdehyde (MDA)

Serum lipid peroxide levels were determined using thiobarbituric acid reactive substances (TBARS) as described by Kamal et al. [14]. TBARS concentration was expressed as µM malondialdehyde (MDA) in serum. Briefly, 250 µL of serum or standard solution were added to 2.5 ml of 20% trichloroacetic acid (TCA). This was then mixed with 1 ml of 0.67% thiobarbituric acid (TBA) and heated at 100°C for 30 min, followed by cooling on ice and vigorous vortexing with 4 ml n-butanol. After centrifugation at 3000 x g for 20 min, the absorbance of the upper organic layer was measured at 535 nm with a spectrophotometer and compared with a 1,1,3,3-tetraethoxypropane (TEP) standard curve.

Determination of total antioxidant capacity

Total antioxidant capacity (TAC) was determined according to Kambysahi et al. [15]. Ninety micro liters of 10 mM phosphate-buffered saline (pH 7.2), 50 µL of myoglobin solution (18 µM), 20 µL of 3 mM ABTS solution, 20 µL of diluted serum sample, and various

Table 1: Comparison of body character changes on the participants.

<table>
<thead>
<tr>
<th>Introduction</th>
<th>Time</th>
<th>Development</th>
<th>Time</th>
<th>Arrangement</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 4 week</td>
<td></td>
<td>5 - 8 week</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stretching for the wrist, arms, knees, neck and shoulders</td>
<td>10 min</td>
<td>Sun salutation pose A, B</td>
<td></td>
<td>Relaxing set</td>
<td>15 min</td>
</tr>
<tr>
<td>9 - 12 week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stretching for the wrist, arms, knees, neck and shoulders</td>
<td>15 min</td>
<td>Sun Salutation pose A, B</td>
<td>30 min</td>
<td>Relaxing set and savasana pose</td>
<td>10 min</td>
</tr>
</tbody>
</table>

Table 2: Programs of yoga treatment.

<table>
<thead>
<tr>
<th>1-4 week</th>
<th>Time</th>
<th>5-8 week</th>
<th>Time</th>
<th>9-12 week</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stretching for wrist, arms, knees, neck and shoulders</td>
<td>25 min</td>
<td>Stretching for wrist, arms, knees, neck and shoulders</td>
<td>15 min</td>
<td>Stretching for wrist, arms, knees, neck and shoulders</td>
<td>10 min</td>
</tr>
<tr>
<td>Running and slow walking, jumping</td>
<td>50 min</td>
<td>Running and fast walking</td>
<td>15 min</td>
<td>Running and jumping</td>
<td>10 min</td>
</tr>
<tr>
<td>20 min</td>
<td>Lessening waist, pelvis and hip</td>
<td>15 min</td>
<td>Lessening waist, pelvis and hip</td>
<td>10 min</td>
<td></td>
</tr>
<tr>
<td>Breathing</td>
<td>5 min</td>
<td>Breathing</td>
<td>45 min</td>
<td>Breathing</td>
<td>50 min</td>
</tr>
</tbody>
</table>

The body characters were completed before starting control treatment or yoga training class after 12 weeks to each group. Data are expressed as means ± SD (n = 12 control group, n = 13 yoga group, respectively)

Table 3: Program of control treatment.
concentrations of gallic acid were added to a 96-well microplate and mixed well at 25°C for 3 min. Then, 20 µL of H2O2 were added to each well and incubated for 5 min. The absorbance was measured using a plate reader (Molecular Device Corp., Sunnyvale, CA, USA) at 600 nm. TAC was expressed as gallic acid equivalent antioxidant capacity (GEAC).

Determination of catalase and superoxide dismutase activity

Catalase activity in sera was determined according to the method described by previous study by Wheeler et al. [16]. Briefly, 150 µL of phosphate buffer (250 mM, pH 7.0), 150 µL of 12 mM methanol and 30 µL of 44 mM hydrogen peroxide were mixed with 300 µL of serum samples or standard solutions in the 13×100 mm test tube. The reaction was allowed to proceed for 10 to 20 minutes and finished by the addition of 450 µL of purpald solution (22.8 mM of purpald in a 2 N potassium hydroxide). The mixture was left for 20 min at 25°C, and then added by 150 µL of potassium periodate (65.2 mM in 0.5 N potassium hydrate) at the same tube. The absorbance of the purple formaldehyde adduct was measured at 550 nm using spectrophotometer (Molecular Devices, Sunnyvale, CA).

Serum superoxide dismutase (SOD) activity was determined using a SOD assay kit (Dojin Laboratories, Kumamoto, Japan) according to the manufacturer’s protocol. Bovine erythrocyte SOD (Sigma-aldrich) was used as a standard.

Determination of total glutathione content and glutathione peroxidase and glutathione reductase activities

Total glutathione content was determined according to Ellman’s [17]. Briefly, 50 µL of diluted serum (in PBS 10 mM, pH 7.2) or total glutathione (GSH) standard were combined with 80 µL of DTNB/NADPH mixture (10 µL of 4 mM DTNB and 70 µL of 0.3 mM NADPH) in a 96-well microplate. Next, 20 µL (0.06 U) of GSH-reductase (GSH-Rd) solution were added to each well and the absorbance was measured using an enzyme reader at 405 nm (Molecular Devices, Sunnyvale, CA).

G-glutathione peroxidase (GSH-Px) activity was determined according to the method of Paglia and Valentine [18]. Briefly, 50 µL of NADPH reagent (5 mM NADPH, 42 mM GSH, 10 units/mL of GSH-reductase in 1.25 mL of distilled water) were added to 890 µL of GSH-Px buffer (50 mM Tris HCl, pH 8.0, 0.5 mM EDTA). Then, 50 µL of serum sample and 10 µL of 30 mM tert-butyl hydroperoxide solution were added to the mixture. The final absorbance was measured at 340 nm using a UV-visible spectrophotometer (Varian, Agilent Technologies, Santa Clara, CA, USA). Enzyme activity was represented as a Unit (U, mmol/mL/min), and enzyme concentration was calculated according to the reference formula: \( \text{Enzyme activity} (\text{units}) = \frac{\Delta \text{A}_{340} \times (\text{dilution factor})}{(6.22 \times \text{sample volume in mL})} \).

G-glutathione reductase (GSH-Rd) activity was determined according to the slightly modified method of Worthington [19]. Briefly, 150 µL of GSSG with 30 µL of GSH-reductase assay buffer (100 mM potassium phosphate buffer, pH 7.5, with 1 mM EDTA) were added to 30 µL of serum sample and diluted with GSH-reductase dilution buffer (100 mM potassium phosphate buffer, pH 7.5, with 1 mM EDTA and 1 mg/ml bovine serum albumin). Then, 75 µL of DTNB and 2 mM NADPH were added and the absorbance was read at 412 nm. Enzyme activity was represented as a Unit (U, mmol/mL/min) using the following formula: \( \text{Enzyme activity} (\text{units}) = \frac{\Delta \text{A}_{412} - \Delta \text{A}_{412 \text{blank}}}{(\text{dilution factor})} \times (\text{volume of sample in mL}), \) where \( e_{340} \) is equal to 14.15 mM-1cm-1.

**Statistical analysis**

Statistical comparisons of the changed values between the control and yoga groups were analyzed using paired Student’s t-test with the PASW Statistics 17 program (SPSS, Inc., Chicago, IL). Statistical significance was fixed at \( P<0.05 \) or \( P<0.01 \).

**Results**

**Effects of yoga on MDA levels**

The initial points to each group, the mean levels of serum MDA 5.42 ± 1.29 µM was for control group, and 4.55 ± 1.96 µM for yoga group, respectively. After 12 weeks, the MDA level was slightly increased in control group (7.69 ± 1.68 µM), whereas yoga group showed significant decrease of MDA contents (2.66 ± 1.90 µM) in the serum levels. The change values of MDA in control program was 2.28 ± 1.89 µM and the yoga program showed -1.89 ± 3.04 µM, respectively (Figure 1A). \( P<0.01 \) on compared with change values of control group.

**Effect of yoga training on TAC levels**

The initial values of TAC in yoga and control care programs were 276.85 ± 78.47 µM and 276.39 ± 58.68 µM in the serum, respectively.

![Figure 1: Changes of MDA and TAC concentration in the serum levels.](image1)

![Figure 2: Changes of SOD and catalase activities in the serum levels.](image2)
Effect of yoga training on SOD and catalase activities

After 12 week intervention, the TAC level was slightly decreased in control group (274.32 ± 51.18 μM), while the yoga care program higher than that of the initial values (344.03 ± 71.14 μM). The change values of TAC in control program was -6.88 ± 92.13 (Figure 2B), (P<0.05, compared with changes in level of control group).

The catalase activities in the serum level of control group were not altered by 12 week intervention (359.45 ± 50.77 to 352.56 ± 88.13), whereas serum level of catalase activities in the yoga group were significantly increased (359.20 ± 53.98 to 420.35 ± 61.00). The change values of catalase activities in the serum level were significantly increased in yoga group (61.15 ± 82.64) compared with control group (-6.88 ± 92.13) (Figure 2B), (P<0.05, compared with changes in level of control group).

**Effect of Yoga training on GSH-Redox system**

The initial time point of total GSH content in the serum levels were 62.9 ± 5.9 μM for control group and 66.9 ± 8.63 μM for yoga group, respectively. Control group didn’t alter total GSH in the serum level after 12 weeks intervention (69.38 ± 7.67 μM), while the total GSH contents notably increased in the yoga training group (80.45 ± 21.89 μM, (Figure 3A), P<0.01 compared with change values of control group). There were no remarkable changes in the serum levels of GSH-Px and GSH-Rd activities to each group within 12-week intervention (Figure 3B and 3C).

**Discussion**

The oxidative stress is closely linked to the progress of pathogenesis and it leads to impairment of homeostasis in human body. Excessive oxidative stress is related to the initiation and development of various disorders including neurodegenerative diseases [20], chronic fatigue syndrome, cardiac dysfunction [21], metabolic syndromes and cancer occurrences [22].

As a free radical, the reactive oxygen species (ROS) are main donor of accelerating the harmful effects under the oxidative stress through cellular damages such as oxidation of proteins and lipids [23,24]. MDA, the end product of lipid peroxidation in cells or tissues, is formed by ROS [25,26]. To defend from oxidative stress most of the mammals well equipped of the antioxidant systems in enzymatic and non-enzymatic components. The antioxidant systems including total glutathione (GSH) and its related enzymes, superoxide radical (SOD) and catalase efficiently work to prevent from these harmful oxidative stressors [27-29].

Yoga is one of the most popular and efficient exercises for maintaining well-being personal life in the world. Recently, many studies have reported the advantages of yoga in physiological and pathological status [1,30-36]. The accumulated evidences demonstrated that yoga have benefits on the schizophrenia [32], back pain [35] and cardiac disorders [30,33] as well as gastro tract disorders [31]. Although many studies have tried to demonstrate the antioxidant properties of yoga in the case of pathological status, there are insufficient of reports about the antioxidant effects of yoga in young healthy people. Previously Sinha et al. [37] revealed the antioxidant effect of yoga by healthy male navy soldiers in India. This study investigated the properties of yoga in improvement of GSH and GSH related enzymes. However, above study didn’t provide the change of oxidative stress biomarkers.
The purpose of current study was designed to acquire scientific and clinical evidences for explaining properties of regular yoga training in healthy university populations. Herein, we confirmed results by looking for oxidative stress markers and antioxidant components in the serum level of participants. Like our thesis, 12-week yoga training significantly decreased the change values of MDA in the serum levels compared with control group (Figure 1A). Total antioxidant capacity (TAC) was expressed as gallic acid equivalence antioxidant capacity (GAEAC) which is consisted of non-enzymatic and enzymatic antioxidant components. The TAC in the serum levels were increased by 12-week yoga intervention as compared with control group (Figure 2A).

MDA is one of the main suspect that accelerated the oxidative stress to the body and it directly leads to the imbalance status of homeostasis between oxidative and antioxidant. To defend form this oxidative stress induced impairment on the body, the antioxidant system is turned on. As a representative antioxidant component, GSH has a major role in the antioxidant defense system. The central role of GSH that plays defenses specificity via antioxidant-dependent mechanisms against ROS, toxic ROS products by scavenging free radicals and reducing peroxides or by conjugation with electrophilic compounds [38,39] contents. Another antioxidant components, enzymatic-antioxidants, SOD is a crucial enzyme against to oxidative stress. It has a role of converting superoxide radicals (O2-) to hydrogen peroxide (H2O2) in body. In this study, the activity of SOD was significantly increased by yoga training but not in control group (Figure 2A). It is well-recognized in the literature that catalase activity represents enzymatic antioxidant mechanism. Catalase is one of the most important enzymatic antioxidant components which found in nearly all of living organisms. When the body has a chance to face oxidative stressors it catalyzes the decomposition of hydrogen peroxide to water and oxygen [40,41]. After regular yoga training, the serum level of catalase activity was significantly increased compared with control group (Figure 2B). It means that the yoga training keeps a maintenance of the strength of antioxidant status in the body.

The other enzymatic antioxidant components, especially GSH-Px and GSH-Rd activities, were also increased by 12-week yoga intervention (Figure 3B and 3C). Our study demonstrated that steady and regular yoga training has potent antioxidant properties in healthy young people. The yoga training not only enhanced the antioxidant systems, including serum level of TAC, total GSH contents, SOD and catalase activities, but also decreased MDA in the serum level.

One of interesting finding in this study was in the last day of yoga training that was the final examination term. So, we were anxious that all of the participants had suffered from fatigue because of studying overnight for the exam. During the last inspection of yoga and bleeding, contrary to our anxiety, most of the participants said they had felt no fatigue during examination terms. It might be the regular yoga training which affected their healthy status via increasing antioxidant status.

Conclusion

According to our results, we conclude regular yoga training efficiently help to strengthen the antioxidant systems and decreased the oxidative stress for maintaining well-being life and yoga will have protective or therapeutically effects against the oxidative stresses derived diseases.

References


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