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Research Article

Evaluation of Variations Antioxidant Systems in the Negative Induced-Fear in High Stress Volunteers

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ABSTRACT:

Introduction: Following theactivityof the stresssystem, reactions which are generally called fear occur in aperson. Thisfearcan causeside effectsinindividuals such as anxiety anddepression. Also one of the important mechanisms of action of stress is production of free radicals and oxidative stress in body. Theaimofthisstudy is toevaluate antioxidant system activity and hormonal changes in young male volunteers after experiencing fear and after using Migun bed.

Methods: Participants had experienced negative fear andused the Migun bed for 35 minutes. Before and after experiencing fear and using the bed, saliva samples were collected and serum samples were discreted from clot blood. Then α-amylase enzyme level and cortisol hormone were assayed by ELISA kit; moreover, superoxide dismutase (SOD) and catalase (CAT) activities, and the levels of glutathione (GSH) and malondialdehyde (MDA) were measured using biochemical methods.

Result:α-amylase enzyme level after experiencing fear increased and after using the bed also decreased in comparison with pre-fear and pretreatment state. The cortisol level in saliva of individuals after experiencing fear didn't increase and after using the bed decreased significantly in comparison with pre-fear and pretreatment state. Fear significantly increased SOD and CAT activities and MDA level, while GSH level decreased in comparison with pre fear in individuals.

Conclusion: Migun Thermal Bed System may also improve the functioning of chief components of the stress axis, and experiencing fear and chronic stress by individuals probably leads to free radical production.

Key Words: fear,antioxidant system, Migun bed.

1.INTRODUCTION

Stress is a state of threatened homeostasis provoked by a psychological, environmental, or physiologic stressor and the ability to cope with such stressful stimuli is a crucial determinant of health and disease. Response to various stresses is regulated through interactions between physiological and neurochemical factors. Different types of stress can affect body in different axes. Studies have shown that there are three physiological pathways for stress response: the neural axis, the neuroendocrine axis, and the endocrine axis (Cannon,1963).The autonomic nervous system (ANS) is the first and the most important physiological axis embedded in the stress-induced responses. Primary ANS monitors general stress-induced responses

including control of heart rate, respiratory rate, blood pressure, heart rate variability, cardiac output, etc (Sherwood,2010). Activation of hypothalamic–pituitary–adrenal (HPA) axis is the second major neuroendocrine response to stress. Stress induces changes in HPA axis which culminates with glucocorticoids and chemical mediators release including adrenocorticotropic hormone (ACTH), norepinephrine (NE), serotonin, dopamine, and acetylcholine (Nadeem et al., 2006;Chakraborti, Gulati, & Ray,2008; Goncalves,Dafre,&GoncalvesCarobrez,2008). In humans, cortisol is the main glucocorticoid (hormone of the adrenal cortex) that can be used as a peripheral indicator of hypothalamic neural activity. The studies have proposed that measurement of salivary cortisol takes advantage of a simple, painless, and non-invasive sampling procedure (Kalman& Ruth, 2004). On the other hand, studies have shown that the sympathetic adrenal medullar system (SAM) activation, as a part of the stress response, is monitored bymeasurement of salivary alpha amylase (sAA) levels in several studies (Rohleder, Wolf ,&Maldonado, 2006)Studies have shown that sAA levels increase in response to stressful tasks or procedures, such as a parachute jump (Chattertonet, Vogelsong,& Hudgens,1997), or a stressful video game(Skosnik, Chatterton,& Swisher, 2000), as well as other types of psychological (e.g. pre examination) stress-inductions (Bosch et al., 1996; Bosch,Geus,&Veerman,2003). Eventually, pharmacological manipulation of the SAM system underscored the role of sAA amylase as an indicator of sympathetic activity.In addition, researches have shown thatsAA amylase enzyme isa valuable toolforstudying thefear that iscaused bystressbecausethose levels ofthe enzymein response toacute and chronic stress have changed. Level elevation of alpha amylase enzyme occursby adrenergic systemactivityin the salivary glands (Nater&Rohleder, 2009)

On the other hand, studies have shown that glucocorticoids may increase the basal level of reactive oxygen species (ROS) in cells and also increase the toxicity of oxygen radical generators(Uysal et al.,2005), in addition, understanding the molecular and cellular pathways activated in response to stress exposure is important for the development of pharmacological intervention to stress induced diseases. One of the most important mechanisms of action of stress is production of ROS, which can react with biological macromolecules such as DNA, proteins, carbohydrates and lipids. ROS neutralization occurs in the cells through antioxidant defense system including superoxide dismutase (SOD), catalase (CAT), glutathione S -transferase (GST) and glutathione (GSH). The balance of oxidants and antioxidants in the body is important; if this balance is disturbed, oxidative stress will occur (Nadeem,Masood,&Masood, 2006;Kamper et al.,2009). Studies have shown that the alteration of antioxidant enzyme activities in different kinds of stress was associated with a depletion of GSH and an increase in lipid peroxidation, all of which can lead to oxidative stress and finally cell death (Pajovic et al.,2006;Depke et al.,2009; Lucca et al.,2009; Ahmad et al.,2012).

Today, different ways have been proposed to reduce and control stress in people with stress-related complaints such as drugs (chemical (Selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and pregabalin), herbal supplements, alternative and complementary therapies, etc (Keegan, 2003). furthermore, as mentioned earlier, stress can affect the body's antioxidant system and cause free radicals. Studies have shown that the use of antioxidants may help to reduce free radicals and boost the body's antioxidant system. Today in addition to using antioxidant products such as various vitamins (E, C, vitamins), alternative and complementary therapies are employed to reduce oxidative stress induced by different stresses. Previous researches have shown that alternative and complementary therapieshave been considered more due to their fewer side effects than other treatments(Keegan, 2003). The most frequently used therapies include relaxation techniques, massage therapy, Yogatherapy, practice of Tai Chi, music therapyetc(Qian et al.,2012;Epel,Daubenmier ,&Moskowitz, 2009).

Several studies have shown that Hathayoga exercise can likely prevent oxidative stress by influencing the activity of antioxidant enzymes; moreover, Tai Chi and massage therapy can probably reinforce activity of body antioxidant system and prevent production of free radicals (Qian et al., 2012;Epel, Daubenmier ,&Moskowitz, 2009).

In addition to complementary therapies mentioned above, Migun Thermal Bed System is another type of treatment considered in today's world. The principles behind the Migun bed are acupuncture, energy healing, the principles of Chiropractic, massage therapy and therapeutic pressure. Studies have proposed that the main mechanism of this bed is based on the effect that it has on the spinal cord (www.berkeleywellnessalerts.com). Several researches have shown this bed to be effective in dealing with many diseases such as diabetes, hypertension and inflammatory diseases related to the immune system as well as digestive system problems, sleep problems and muscle pain (Chang Sok So et al., 2003). Therefore, theaimofthisstudy is toevaluate antioxidant system activity and hormonal changes in young male volunteers after experiencing fear and after using Migun bed.

2.MATERIALS AND METHODS

2.1. Chemicals

Reduced glutathione (GSH), Nitrobluetetrazolium (NBT), 1-chloro-2, 4-dinitrobenzene(CDNB) and 5, 5'-dithiobis 2-nitrobenzoic acid (DTNB) were obtained from Sigma Chemical Company. All other chemicals used were of extra pure grade and obtained from Sigma and Merck.

2.2. Subjects:

Twenty healthy men, aged 20-25, weighing 50-70 kg were entered into the study. Participants were recruited by DASS questionnaire (Depression, Anxiety and stress Scales) (25> score). Exclusion criteria included a history of systemic diseases such as diabetes and rheumatoid arthritis, substance abuse/dependence, smoking, a history of chronic low back pain over the past year and a history of fracture and surgery in areas of the spine.

2.3. Experimental design:

Participants had a free fall from a platform at a height of 30 cm from the ground and experienced negative fear after the opening stage, when those subjectsused the Migun bed in "ON" manner for 35 minutes to decrease acute stress (fear experience). Before and after experiencing fear and using the bed, saliva and blood samples were collected from all the participants then blood serum was prepared and then all samples were stored at -80 ° C until Biochemical analysis

2.4. Migun Thermal Bed System:

The bed hy-5000 model was manufactured by Migun Company. The Migun Thermal Massage Bed design applies heat (Helium lamp, Infrared and jadestone) and pressure to the muscles along the spine causing massaging of the muscles and tendons around the spine to relieve tension, relax nerves, and facilitate blood flow.

2.5. Data acquisition:

On the day of experiment, first the saliva samples were melted at room temperature and after centrifuging with round 3000 for 5 minutes, 20 microliters of each sample was separated for testing. Then, Cortisol ELISA kit (Cortisol ELISA KIT, Diagnostics IBL Germany) and α-amylase kit (α-amylase kit Pars azmun Company (Tehran, Iran)) were employed for measurement of human salivary cortisol and α-amylase enzyme**.** Then, on the day of experiment, serum samples were melted at room temperatureand used for enzyme activities assays and protein determination.

2.5.1. SOD activity assay

The activity of SOD was determined using the method described by Winterbourn et al (Winterbourn, Hawkins,& Brian,1975) based on the ability of SOD to inhibit the reduction of NBT by superoxide. The absorbance of samples was read on a Genesys 10 UV spectrophotometer at 560 nm for 5 min. The amount of enzyme required to produce 50 % inhibition was taken as 1 U and the results were expressed as U/mg protein.

2.5.2. CAT activity assay

CAT activity in tissue homogenates was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H2O2 as

the substrate of the enzyme using the method of Aebi (Aebi, 1984). A molar absorption of 43.6 Mcm−1 was used to determine CAT activity. Enzymatic activity was expressed as U/mg protein, one unit (U) of which was equal to 1 mole of H2O2 degraded/ min/mg of protein.

2.5.3. Determination of GSH level

GSH level was measured using the method of Tietz (Tietz ,1969). GSH in the supernatant was assayed at 412 nm by monitoring the absorbance of DTNB for 5 min. GSH levels were determined from a standard curve and expressed as nmol/mg protein.

2.5.4. Determination of MDA level

Liver MDA level as an indicator of lipid peroxidation was determined at 532 nm using 2-thiobarbituric acid according to the method of Satoh (Satoh, 1978). MDA concentrations were determined using 1,1, 3,3-tetraethoxypropane as standard and expressed as nmol/mg protein.

2.5.5. Protein level assay

The total protein contents of the samples were measured by Bradford's method (Bradford ,1976) using bovine serum albumin as Standard.

3. RESULT

3.1. The impact of fear experience and Migun Thermal Bed System on salivary cortisol

To determine the magnitude of change in salivary cortisol concentration (before and after), fear experience following the Migun use in individuals was examined by ELISA kit. The results showed that the cortisol level in saliva of individuals after experiencing fear has not increased in comparison with the state before experiencing fear, moreover, cortisol level in saliva of individuals after using the bed has decreased significantly in comparison with the pretreatment state ($P<0.05$) (table 1). The impact ofexperiencing fear and Migun Thermal Bed System on salivary cortisol concentration in individuals is shown in table1.

Table1: Salivary cortisol concentration changes: Saliva cortisol concentration changes in the participants before and after experiencing fear and using bed show cortisol concentration does not have a significant change after experiencing fear but cortisol concentration decreased after using the bed in comparison with pretreatment(*$P<0.05$).

Testing trail	Salivary Cortisol(ng/ml) (Mean)	(SD)
Pre fear	79.4	11.12
Post fear	75.3	6.81
Post treatment	70.6*	4.23

3.2. The impact of fear experience and Migun Thermal Bed System on α-amylase enzyme

In order to test the effects of fear experience and Migun bed on the level of saliva α-amylase enzyme, we collected the subjects' saliva before and after fear experience and after using Migun bed, and checked them by ELISA kit. Our results indicated that the α-amylase enzyme level in saliva of individuals after experiencing fear has increased significantly in comparison with the state before experiencing fear, moreover, α-amylase enzyme level in saliva of individuals after using the bed has decreased significantly in comparison with the pretreatment state ($P<0.05$) (table. 2)

Table2: Salivary alpha amylase level changes: Salivary alpha amylase level changes in the participants before and after experiencing fear and using Migun Thermal Bed System show a significant increase in Salivary alpha amylase after experiencing fear and a significant decrease after using the bed in comparison with pre-fear state and pretreatment (*$P<0.05$).

Testing trail	Salivary alpha amylase (U/L) (Mean)	(SD)
Pre fear	4.668	66.36
Post fear	7.79*	127.41
Posttreatment	4.36*	74.7

3.3. The impact of fear experience and Migun Thermal Bed Systemon antioxidant enzyme activities

Our results indicated that the SOD enzyme activity in individuals after experiencing fear has increased significantly in comparison with the state before experiencing fear, moreover, SOD enzyme activity after using the bed has decreased significantly in comparison with the pretreatment state ($P<0.05$), furthermore, CAT enzyme activity in individuals after experiencing fear has increased significantly in comparison with the state before experiencing fear, moreover, CAT enzyme activity after using the bed has decreased significantly in comparison with the pretreatment state ($P<0.05$) (table. 3)

Table3:SOD and CAT enzyme activity changes in the participants before and after experiencing fear and using bed Migun Thermal Bed System (*$p<0.05$)

Testing trail	SOD(U/mg)	CAT(U/mg)
Pre fear	1.72±72.70	3.04±1.00
Post fear	2.13±74.38*	4.39±1.86*
Posttreatment	1.61±42.28*	3.09±0.87*

3.4. The impact of fear experience and Migun Thermal Bed Systemon MDA and GSH levels

Our results indicated that the GSH level in individuals after experiencing fear has decreased significantly in comparison with the state before experiencing fear, moreover, GSH level after using the bed has increased significantly in comparison with the pretreatment state ($P<0.05$), additionally, MDA level in individuals after experiencing fear has increased significantly in comparison with the state before experiencing fear, moreover,MDA level after using the bed has decreased significantly in comparison with the pretreatment state ($P<0.05$) (table 4)

Table4:MDA and GSH levelschanges in the participants before and after experiencing fear and using Migun Thermal Bed System. (*$p<0.05$)

Testing trail	MDA (nmol/mg protein)	GSH (nmol/mg protein)
Pre fear	0.118±0.208	0.421±0.127
Post fear	1.039±1.52*	0.34±0.095*
Posttreatment	0.220±0.189*	0.474±0.063*

4. DISCUSSION

Stress produces both psychological and physical responses. Together, these reactions lead to a biochemical cascade by the body. Limbic system—the brain's alarm center activates once a change (noise, sound, smell, pain, etc.) is observed and verified as a threat by the parts. The limbic system then guides the sympathetic nervous system (SNS) to alert the body. The SNS stimulates the adrenal medulla to release the adrenaline-like compounds, epinephrine and norepinephrine, into the bloodstream. The limbic system also stimulates the HPA axis to produce other chemical signals to assist further activate the body; as a result, the adrenal cortex releases cortisol, an important stress hormone that changes body's ability such as increasing energy, heart rate and blood sugar, increasing arousal and pain relief (Charney,2004).In the absence of a continued threat, the body relaxes and returns to its normal state of tension. The acute stress is part of life and the body is adapted to handle them. In times of extreme stress, however, this stress response can become "turned on" at all times, with no relaxation. This can have serious physical and psychological consequences (Charney, 2004).

There are ways to turn off the stress response. There are various ways to reduce psychological and physical stress. One way is the use of alternative and complementary therapies. The treatments have fewer side effects than chemical drugs. Theaimofthisstudy is toevaluate antioxidant system activity and hormonal changes in young male volunteers after experiencing fear and after using Migun bed.

In the present study, a comparison of the level of cortisol before and after using the bed in subjects with high stress showed a statistically significant decrease in cortisol levels after using

the bed in individuals in comparison with pretreatment state (Table1).

The studies have proposed that Mindfulness-based therapy as an alternative and complementary therapy is one such therapy that has been proven to reduce stress-related medical conditions. It has also been approved by Health Canada as a first-line alternative and complementary therapy. Result of this study showed that Mindfulness-based therapy can decrease cortisol level in individuals (www.healthmomentum.ca). Another complementary therapy is massage therapy. The studies have shown that massage benefits the body both physically and mentally, also massage therapy can reduce stress and cortisol levels in volunteers.Getting a massage regularly is a great way to lower anxiety levels and maintain a healthy stress response(www.healthmomentum.ca). So, the study results show that Migun Thermal Bed System with 5 of its important functions including Heat Therapy, Acupuncture Inspired, Chiropractic Inspired, Acupressure Inspired and Massage can reduce cortisol level, showing that the effect of these functions are centered on HPA axis.

In the present study,a comparison of the level of α-amylase before and after using the bed in subjects with high stress showed a significant decrease in α-amylase levels after using the bed in individuals in comparison with pretreatment state (Table2). Studies have also shown that sympathetic (or parasympathetic) responses are activated in the individuals due to stress system (Feinstein,Adolphs,&Damasio ,2011;Nater,&Rohleder, 2009). Secretion of saliva (salivary alpha-amylase) enzyme raises rapidly after stress (within a few minutes). Hence, measurement of this factor is known as a non-invasive biological index for the activity of the sympathetic nervous system. This enzyme is also an important device for the study of stress. This increase occurs through an increase of the adrenergic system activity in the salivary glands (Davis ,&Granger,2009; Lupien,&Lepage,2001).

On the other hand, Oxidative stress plays a crucial role in the initiation and progression of differentdiseases such as hepatocyte diseases (Depke, et al,2009; Ahmad, et al, 2015). Differentkinds of stress can increase the formationof ROS and oxidative stress conditions(Nadeem,Masood, &Masood2006;Kamper, et al,2009). Neutralization of ROS is created by the antioxidant defense mechanisms. SOD and CAT enzymes are the first line of cellular defense againstoxidative injury. SOD enzyme catalyzes convertsthe superoxide anion into H2O2 and CATdegrades H2O2 to water (Sarumathi&Saravanan, 2012).

In the present study, the SOD and CAT activities increased afterexperiencing fear**,** in addition, using the bed somewhat reduced their activitiesin comparison with pretreatment state (Table3).

Stress can induce the production of ROS and oxidative stress, which is associated with a depletion of GSH, alteration of antioxidant enzyme activities and increased lipid peroxidation (Abdou&Mzoudy, 2010; Lukaszewicz-Hussain, 2010; Jafari, et al, 2012).There are several antioxidant enzymes in cells that prevent ROS formation and oxidative stress induction and limit their damaging effects. SOD is the first line of defense against superoxide anion radicals, which converts it into H2O2. H2O2 is converted into H2O through CAT (Astiz, de Alaniz,&Marra, 2009). The SOD activity elevation increases H2O2 level and decreases superoxide anion radical level; additionally, CAT activity elevation decreases H2O2 level and these biology systems will prevent damage to the cell.

Activity elevation of these enzymes is related to defense mechanism against oxidative stress.

Animal studies have shown that exposure to acute physical and psychological stressors can produce reactive oxygen species and oxidative stress due to increased CAT and SOD activities or decreased CAT and increased SOD (Ahmad, et al, 2012; Sarumathi&Saravanan, 2012).

Activity elevation of SOD and CAT is likely due to an increase in ROS by acute stress (experiencing fear), and a decrease in SOD and CAT activity after using the bed is probably related to the ability of this treatment in direct removal of ROS(Handy, et al,2009;Saitoet al,2004)

The study performed by Gordon et al. shows that the *Hatha* yoga exercise decreases SOD and CAT activities in control group in comparison with post-treatment state but SOD and CAT activities increased in end-stage renal disease (ESRD) patients after 4 months of treatment (Gordon,Pena Yeiny,&Cabrera Lawrence-Wright Marilyn, 2013). Other studies have shown that Hatha yoga exercise increases SOD and CAT activities and Total Antioxidant in obese subjects in comparison with post-treatment state (Cheong & Lim,2012; Karabulut, Kafkas,&Sahin , 2013) .

The study conducted by Rosado-Pérezet al. shows that practice of Tai Chi reduces oxidative stressthrough affectingSOD andglutathione peroxidase (GPx) activities, and total antioxidant status (TAS) in older adultscompared to the walking exercise (Leung, Chan Tsang,&Tsang,2011; Bassey ,2000).

Other study about the effect of massage therapy on antioxidant system has proposed that massage therapy can decrease oxidative stress through affecting SOD activity and other antioxidant factors in sedentary women (Karabulut,Kafkas,&Sahin2013).

The difference in the results obtained in studies is due to various treatments, different subjects under treatment, duration of treatment and other factors.

GSH is an important non-enzymatic antioxidant that plays a crucial role in the detoxification of ROS (Sahinet al.,2006; Ghizoni et al.,2006; Karabulut,Kafkas,&Sahin 2013). GSH protects essential thiol groups from oxidation and serves as a substrate for glutathione peroxidase and GST (Glutathione-S- transfers). In addition, GSH is involved in protection of other antioxidants, such as ascorbate and α-tocopherol. Glutathione is synthesized in the cytoplasm of the liver cells and then distributed into different organs (Ghizoni et al., 2006; Jafariet al.,2012).

In the present study, the GSH level after experiencing fear has decreased significantly in comparison with the state before experiencing fear, moreover, GSH level after using the bed has increased significantly in comparison with the pretreatment state (Table4).

The decreased GSH may be due to the presence of ROS produced by acute stress (experiencing fear). The studies have shown that depletion of GSH leads to oxidized GSH (GSSG) production and finally decreases the GSH/GSSG ratio in different tissues in stressed rats, which is an index of tissue oxidative stress (Samson,Sheeladevi,&Ravindran, 2007; Jafariet al,2012).

Animal studies have shown that exposure to acute physical and psychological stressors can produce reactive oxygen species and oxidative stress due to decreased level of GSH in various tissues (Samson,Sheeladevi,&Ravindran, 2007;Atif,Yousuf ,&Agrawal,2008;Sarumathi&Saravanan,2012; Ahmad et al.,2012).

The study conducted by Cheonget al. shows thatHatha yoga exercise increased GSH level and decreased oxidative stress in students after treatment (Cheong& Lim, 2012).

Other study about the effect of massage therapy on antioxidant system has proposed that massage therapy can decrease oxidative stress through affecting theincrease in GSH level and other antioxidant factors in sedentary women(Çakir-Atabek et al,2010; Aslan et al,1998).Lipid peroxidation (LPO) is an oxidative degeneration of polyunsaturated fatty acids, which impairs membrane structure and functions. MDA level, as an important index of LPO, indirectly reflects the extent of cellular injury in vivo(Chakraborti et al.,2008; Jafari et al.,2012).

In the present study, MDA level after experiencing fear has increased significantly in comparison with the state before experiencing fear, moreover,MDA level after using the bed has decreased significantly in comparison with the pretreatment state (Table4).

The increase in MDA level can be due to increased free radicals and oxidative stress after acute stress (experiencing fear), on the other hand, reduction in MDA level can be directly related to the ability of this treatment (Migun bed) to remove free radicals and inhibit lipid peroxidation by ROS.

Former studies have shown that GSH depletion may lead to an increased lipid peroxidation, possibly due to the lowering of the cellular

defense system against endogenous toxic intermediates (jafariet al., 2014). Numerous studies have shown that exposure to a variety of acute stress models (e.g., restraint, immobilization, cold and psychological stressors) significantly increased MDA content in various tissues of animals (Zaidi, Al-Qirim,&Banu, ,2005; Sahin et al.,2006; Atif,Yousuf ,&Agrawal, 2008; Ahmad et al.,2012).

The studies have shown that massage therapy can decrease MDA level, lipid peroxidation and oxidative stress in sedentary women (Leafet al.,1997; Miyazakiet al,2001). Rosado-Pérez study and associateshave shown that the practice of Tai Chi can decrease levels of lipoperoxides (LPO), in older adults in comparison with walking(Leung,Chan Tsang,Tsang,& 2011). Other studies have showed that Hatha yoga exercise can decrease MDA level and oxidative stress in Students after treatment (Karabulut, Kafkas,Sahin,&2013), as wellas, Kumari N study shown that *Hatha* yoga can decrease MDA level and oxidative stress in exercise obese male and femaleSubjects (SuchethaKumari, DamodaraGowda,&Sukesh, 2011).

5. CONCLUSION

The present study showed that stress caused by fear can activate the first stress system namely the sympathetic nervous system and show the sympathetic nervous system symptoms. On the other hand, Migun Thermal Bed System may also improve the functioning of chief components of the stress axis (the hypothalamic-pituitary-adrenal or HPA axis) in individuals who experienced fear; moreover, acute stress (experiencing fear) induces oxidative stress by production of free radicals and increases antioxidant enzymes activities and membrane lipid peroxidation and reduces glutathione level, from another perspective, using Migun bed may reduce oxidative stress of acute stress through clearing free radicals.

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REFERENCES:

1. Abdou, H.M., &ElMzoudy, R.H. (2010). Oxidative damage, hyperlipidemia and histological alterations of cardiac and skeletal muscles induced by different doses of diazinon in female rats. J Hazard Mater,182(1-3), 273-278.
2. Aebi, H. (1984). Catalase in vitro. Methods Enzymol, 105, 121-6.
3. Ahmad, A., Rasheed, N., Chand, K., Maury, R., Banuc, N., &Palita, G. (2012). Restraint stress-induced central monoaminergic and oxidative changes in rats and their prevention by novel Ocimum sanctum compounds. Indian J Med Res, 135,548-54
4. Aslan, R., Sekeroglu, M.R., Tarakçioglu, M.,Bayiroglu, F., &Meral, I. (1998). Effect of acute and regular exercise on antioxidative enzymes, tissue damage markers and membrane lipid peroxidation of erythrocytes in sedentary students. Age (years). 21, 19–25
5. Astiz, M., de Alaniz, M.J.,&Marra, C.A. (2009). Antioxidant defense system in rats simultaneously intoxicated with Agrochemicals. Environ ToxicolPharmacol, 28(3), 465-473.
6. Atif, F., Yousuf, S.,&Agrawal, S.K. (2008). Restraint stressinduced oxidative damage and its amelioration with selenium, Eur J Pharmacol, 600,59-63
7. Bassey, E. J. (2000). The benefits of exercise for the health of older people," Reviews in Clinical Gerontology, 10(1), 17–31.
8. Bosch, J.A., Brand, H.S., Ligtenberg, T.J.,Bermond, B., Hoogstraten, J.,&NieuwAmerongen, A.V.(1996). Psychological stress as a determinant of protein levels and salivary induced aggregation of Streptococcus gordonii in human whole saliva. Psychosom Med, 58 (4), 374–382
9. Bosch, J.A., de Geus, E.J., Veerman, E.C., Hoogstraten, J.,&NieuwAmerongen, A.V.(2003). Innate secretory immunity in

response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. Psychosom Med, 65 (2), 245–258.
10. Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem,72, 248-54.
11. Çakir-Atabek, H., Demir, S., Pinarbasili, R.D.,&Gündüz N. (2010).Effects of different resistance training intensity on indices of oxidative stress. J Strength Cond Res. 24(9), 2491–2497
12. Cannon, W.B. (1963).The Wisdom ofthe Body.
13. Chakraborti, A., Gulati, K., & Ray, A. (2008). Age related differences in stress-induced neurobehavioral responses in rats: Modulation by antioxidants and nitrergic agents, Behav Brain Res, 194, 86–91
14. Chang, Sok So OMD., Roland, A., Giolli, Maritza Jauregui., Tonya, L., Schuster, Haiou .,&et all. (2003). Thermomechanical Massage Devices used in China and South Korea: A Preliminary Report of Health Outcomes and Side Effects. J. Vertebral Subluxation Res, 30, 1-9
15. Charney, D.S.(2004). Psychobiological mechanisms of resilience and vulnerability:
16. Implications for sucessufl adaptation to extreme stress. American Journal of Psychiatry,161, 195-216
17. Chatterton, J.r., R.T., Vogelsong, K.M., Lu, Y.C., &Hudgens, G.A. (1997). Hormonal responses to psychological stress inmen preparing for skydiving. J, Clin. Endocrinol. Metab, 82 (8), 2503–2509
18. Cheong, K.J.o., & Lim, S Ah. (2012). Antioxidant Effects of Regular Yoga Training on the Healthy University Students Controlled Clinical Trial, Cheong and Lim, J Yoga PhysTher, 2(6), 1-6
19. Davis, E P., &Granger, D A. (2009). Developmental differences in infant Salivary
20. alphaamylase and cortisol responses to stress. Psychoneuroendocrinology, 34(6), 795-804.
21. Depke, M., Steil,L.,Domanska, G., Volker, U., Schutt, C., &Kiank, C.(2009). Altered hepatic mRNA expression of immune response and apoptosis-associated genes after acute and chronic psychological stress in mice, MolImmunol, 46, 3018–28.
22. Epel, E., Daubenmier, J., Moskowitz, J., Folkman, T S., & Blackburn, E. (2009). “Can meditation slow rate of cellular aging? Cognitive stress, mindfulness, and telomeres,” Annals of the New York Academy of Sciences, 1172, 34–53.
23. Feinstein, J S., Adolphs, R., Damasio, A R., and Tranel, D. (2011). The human amygdala and the induction and experience of fear. Current biology, 21(1), 34-38.
24. Ghizoni,DM., Pavanati,KC., Arent, AM., Machado, C., Faria, MS., Pinto, CM., & et al. (2006). Alterations in glutathione levels of brain structures caused by acute restraint stress and by nitric oxide synthase inhibition but not by intraspecific agonistic interaction,Behav Brain Res, 166, 71-7.
25. Goncalves, L., Dafre, AL., GoncalvesCarobrez, S., &CezarGasparotto, O. (2008). A temporal analysis of the relationships between social stress, humoral immune response and glutathione-related antioxidant defenses. Behav Brain Res, 192,226–31
26. Gordon McGrowder, D A., Pena Yeiny, T., & Cabrera Lawrence-Wright Marilyn, B. (2013). Effect of yoga exercise therapy on oxidative stress indicators with end-stage renal disease on hemodialysis, Int J Yoga, 6(1), 31–38H
27. Handy, RD., Abd-El Samei, HA.,Bayomy MFF., Mahran, AM., Abdeen, AM.,& El-Elaimy, E.A. (2002). Chronic diazinon exposure: Pathologies of spleen, thymus, blood cells, and lymph nodes are modulated by dietary protein or lipid in the mouse, Toxicology, 172(1), 13-34.
28. Jafari, M., Salehi, M., Asgari, A., Ahmadi, S., Abbasnezhad, M., Hajihoosani, R.,&Hajigholamali, M. (2012). Effects of paraoxon on serum biochemical parameters and oxidative stress induction in various tissues of Wistar and Norway rats, Environ ToxicolPharmacol. 34(3), 876-887.
29. Jafari M, Salehi M, Zardooz H, &Rostamkhani F. (2014). Response of liver antioxidant defense system to acute and

chronic physical and psychological stress in mail rat, EXCLI Journal, 13,161-171

30. Kalman,BA.,& Ruth, EG. (2004). Measuring Salivary Cortisol in the Behavioral Neuroscience Laboratory, The Journal of Undergraduate Neuroscience Education, 2(2), A41-A49
31. Kamper, EF.,Chatzigeorgiou, A., Tsimpoukidi, O., Kamper, M., Dalla, C., Pitychoutis, PM&et al. (2009). Sex differences in oxidant/antioxidant balance under a chronic mild stress regime. PhysiolBehav, 98, 215–22.
32. Karabulut, A.B., Kafkas, M.E ., Sahin, K.A., Önal, Y., &Rabia, K T.(2013) . The effect of regular exercise and massage on oxidant and antioxidant parameters, Indian J PhysiolPharmacol , 57(4):378–383
33. Keegan, L. (2003). Alternative and Complementary Modalities for Managing Stress and Anxiety. Critical Care Nurse, 23 (3), 55-60
34. Leaf, D.A., Kleinman, M T., Hamilton, M., &Barstow, T.J. (1997). The effect of exercise intensity on lipid peroxidation, Med Sci Sports Exerc, 29(8), 1036–1039
35. Leung, D. P. K., C. K. L., Chan, Tsang H. W. H., Tsang W. W. N.,&Jones A. Y. M. (2011). "Tai chi as an intervention to improve balance and reduce falls in older adults: a systematic and meta analytical review," AlternativeTherapies in Health andMedicine, 17(1), 40–48.
36. Lucca, G., Comim, CM.,Valvassori, SS., Reus, GZ., Vuolo, F., Petronilho, F.,&et al. (2009). Effects of chronic mild stress on the oxidative parameters in the rat brain NeurochemInt, 54,358–62.
37. Lupien, S J., &Lepage, M. (2001). Stress, memory, and the hippocampus: can't live with
38. it, can't live without it. Behavioural brain research,127(1), 137-158.
39. Lukaszewicz-Hussain,A. (2010). Role of oxidative stress in organophosphate insecticide toxicity-short review. Pest BiochemPhysiol, 98(2), 145-150
40. Miyazaki, H., Oh-ishi, S., Ookawara, T., Kizaki, T., Toshinai K., Ha, S., Haga, S.,&etal(2001). Strenuous endurance training in humans reduces oxidative stress following exhausting exercise,Eur J ApplPhysiol, 84(1-2), 1–6.
41. Nadeem, A., Masood, A., Masood, N., AfzalGilani, R., & Ahmad Shah, Z. (2006). Immobilization stress causes extracellular oxidant-antioxidant imbalance in rats: Restoration by L-NAME and vitamin E, EurNeuropsychopharmacol, 16, 260-7
42. Nater, U. M., &Rohleder, N. (2009). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research,Psychoneuroendocrinology. (34), 486-96.
43. Pajovic, SB., Pejic, S., Stojiljkovic, V., Gavrilovic, Lj.,Dronjak, S., &Kanazir, DT. (2006). Alterations in hippocampal antioxidant enzyme activities and sympatho-adrenomedullary system of rats in response to different stress models, Physiol Res, 55, 453-60.
44. Qian, G., Xue, K., Tang, L., Wang, F., Song, X., Chyu, MC., &et al.(2012). "Mitigation of oxidative damage by green teapolyphenols and tai chi exercise in postmenopausal women with osteopenia," PLoS ONE, 7(10), 1-9(e48090).
45. Rohleder, N., Wolf, J.M., Maldonado, E.F., &Kirschbaum, C. (2006). The psychosocial stress induced increase in salivary alpha-amylase is independent of saliva flow rate. Psychophysiology, 43 (6), 645–652.
46. Saito, K., Sakai, N., Kim, HS.,Ishizuka, M., Kazusaka, A.,&Fujita, S. (2004). Strain differences in diazepam metabolism at its three metabolic sites in Sprague Dawley, brown Norway, dark Agouti, and Wistar strain rats. Drug MetabDispos. 32(9), 959-965.
47. Sahin, M., Sagdıc, G., Elmas, O., Akpınar, D., Derin, N., Aslan, M, & et al. (2006). Effect of chronic restraint stress and alpha-lipoic acid on lipid peroxidation and antioxidant enzyme activities in rat peripheral organs, Pharmacol Res, 54,247–52.
48. Samson, J., Sheeladevi, R., &Ravindran, R. (2007). Oxidative stress in brain and

antioxidant activity of Ocimum sanctum in noise exposure, Neurotoxicology,28,679–85
49. Sarumathi, A., &Saravanan, N. (2012). Antioxidant status in kidney and liver of rats during immobilization stress and treated with Centellaasiatica (Linn.), Int J Res BiolSci, 2, 165-9.
50. Satoh, K. (1978). Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method, ClinChimActa, 90, 37–43.
51. Sherwood, L. (2010). Human physiology: From cell to system. London, England: Cengage Learning.
52. Skosnik, P.D., Chatterton, Jr, R.T., Swisher,T., & Park, S. (2000). Modulation of attentional inhibition by norepinephrine and cortisol after psychological stress. Int. J, Psychophysiol, 36 (1), 59–68
53. SuchethaKumari, N., DamodaraGowda, K M., Sukesh, N., &MadhuKathyayani, L. N. (2011). Effect of yoga therapy on body mass index and oxidative status, NUJHS, 1, 1-3.
54. Tietz, F. (1969). Enzymic method for quantitative determination of nanogram amount of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem. 27, 502-22.
55. Uysal, N., Acikgoz, O., Gonenc, S., KayatekinBM,nKiray, M., Sonmez, A., & et al., (2005). Effects of acute footshock stress on antioxidant enzyme activities in the adolescent rat brain. EffectsPhysiol Res. 54, 437-42.
56. www.healthmomentum.ca
57. http://www.berkeleywellnessalerts.com
58. Winterbourn, C., Hawkins, R., Brian, M., &Carrell, R., (1975). The estimation of red cell superoxide dismutase activity, J Lab Clin Med, 85, 337-41.
59. Zaidi, S M., Al-Qirim, T M., &Banu, N. (2005). Effect of antioxidant vitamins on glutathione depletion and lipid peroxidation induced by restraint stress in the rat liver, Drugs RD, 6, 157-65