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RESEARCH ARTICLE

EFFECT OF YOGA ON SERUM ADRENALINE, SERUM CORTISOL LEVELS, PLASMA MELATONIN AND CARDIOVASCULAR PARAMETERS IN HYPER-REACTORS TO COLD PRESSOR TEST IN YOUNG HEALTHY VOLUNTEERS

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ABSTRACT

Background: Now days, stress is dangerous & significant problems of World & affect physical, mental, behavioral, and emotional health. Yoga has been reported to control stress, to be beneficial in treating stress related disorders, improving autonomic functions, lower blood pressure, increase strength and flexibility of muscles, improve the sense of wellbeing, slowed ageing process, control breathing, reducing signs of oxidative stress and improving spiritual growth. (Chanda Rajak *et al*., 2012)

Objective: The aim of present study was to investigate whether regular practice of Yoga for six months can reduce the level of adrenaline, serum cortisol, plasma melatonin and cardiovascular reactivity induced by cold pressor test.

Material and Method: The study group comprises 50 healthy subjects of 18-25 years age group. Initially there were 30 hyper-reactors to cold pressor test. The hyper-reactivity of 25 volunteers converted to hypo-reactivity after the yoga therapy of six months (83.3%). The parameters like level of serumadrenaline, serum cortisol, plasma melatonin, basal blood pressure, rise in blood pressure and pulse rate were also significantly reduced statistically by using student't' test.

Result: Regular practice of yoga significantly reduce the level of Adrenaline, serum cortisol, plasma melatonin and cardiovascular hyper-reactivity to cold pressor test, in basal blood pressure, rise in blood pressure and pulse rate after six month of yoga practice.

Conclusion: We concluded that regular practice of yoga for six months reduced the possibly by inducing parasympathetic predominance and cortico-hypothalamo-medullary inhibition, who was hyper–reactive to cold stress, This shows that yoga provides significant improvement in aging to reduce the morbidity and mortality from stress induced disorders. Yogic practices can be used as psychophysiologic stimuli to increase endogenous secretion of melatonin, which, in turn, might be responsible for improved sense of well-being.

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INTRODUCTION

Stress is an extremely adaptive phenomenon in human, contributing to his/her survival, activities, and performance (ArnljotFlaa *et al*., 2008). Physical and psychological stresses can induce a wide range of immunological alteration in the cell mediated and humoral immunity (Reddy, 2004). Stressors may influence the immune function via their impact on neuroendocrine, autonomic and central nervous system. (Gaziano, 2007) Psychological stresses may influence the immunological functions both indirectly through hormonal changes, and directly through nervous regulation during brief but acute stressful periods. (http://kukaimikkyo.wordpress.com) Stress is described as a state of anxiety, strain, nervousness, tension, constant worry or pressure. It is an accepted fact that psychosocial factors operate through mental processes, consciously or unconsciously, to produce hypertension and other cardiovascular disorders. (Iyengar, 2002) Cardiovascular disease has become a major cause of mortality in developing nations in the age group of 30- 69 years, the cardiovascular mortality due to hypertension is seen more in developing nations. (Nagarathna and Nagendra, 2006; Walter Cannon, 1911) Independent research has shown that

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significantly reduce levels of cortisol reduce the level of stress, relieve anxiety, depression, increase anti – oxidant production, enhance brain function, enhance health well – being and peace of mind. Yoga is an ancient philosophical and religious tradition thought to have originated in India in 5000 BC. It has been incorporated into modern medicine during the few decades because of increasing incidence of diseases of modern civilization such as obesity, hypertension, coronary artery diseases, and diabetes mellitus, which are rooted in faulty lifestyle and psychological stress. Yoga is the best lifestyle modification, which aims to attain the unity of mind, body and spirit through yoga. (Nagendra and Nagaratna, 1994) Meditation is relaxation technique to be used for treating stress and stress related illnesses. (Udupa *et al*., 1975) *Walter Cannon (1911) et al was* the first person to propose that emotional stress causes excess of adrenaline secretion from adrenal medulla leading to tachycardia, high blood pressure etc. Later it was found that all these manifestations occur not only from adrenaline secretion but also from over activity of the sympathetic nervous system which liberates nor-adrenaline at its nerve endings. (Selvamurthy *et al*., 1983a) Psychosocial stresses of our modern life precipitates various cardiovascular and other disorders by distorting basic neuroendocrine mechanism. The psychosocial stresses activate limbic system and hypothalamus which stimulate autonomic nervous system, increase in output of both adrenaline and nor-adrenaline, both from sympathetic nerve fibers as well as from adrenal medulla causing increase in heart rate, systolic and diastolic blood pressures and an increased secretion of glucocorticoid & aldosterone from adrenal cortex causing salt and fluid retention which increases blood volume and blood pressure imposing severe strain on the heart. (Selvamurthy *et al*., 1983b)

The stress hormone, cortisol, is public health enemy number one. Scientists have known for years that elevated cortisol levels: interfere with learning and memory, lower immune function and bone density, increase weight gain, blood pressure, cholesterol, Heart diseases and elevated cortisol levels act as a potential trigger for mental illness and decreased resilience—especially in adolescence. (Ray *et al*., 2001) In the study of hypertension, cold pressor test, introduced by Hines and Brown (Murugesan *et al*., 2000) was employed to measure the cardiovascular reactivity. The persons hyper-reactive to cold pressor test are susceptible for early onset of hypertension. (Sathyaprabha *et al*., 2001; Telles *et al*., 1997; Manchanda *et al*., 2000; Ornish *et al*., 1998) We tested whether regular practice of Yoga for 3 months can reduce the serum cortisol level and cardiovascular hyper-reactivity, for reducing the morbidity and mortality from cardiovascular disorders. "Yogic" postures are now, one of the non-pharmacological therapies against stress and strain."Yoga" practice has been shown to be effective in improving mood and decreasing stress and depression. (Nayar *et al*., 1975) A state of mental tranquility is achieved by the practice of yoga as revealed by increase in alpha index of electroencephalogram (James *et al*., 1987; Waldhauser *et al*., 1990) and development of good physique, strong ethical values and good stress tolerance. (Waldhauser *et al*., 1990) Yoga can protect the individual by bringing harmony between mind and body, modulating stress responses and one's attitude to stress as also improving mental faculties such as attention, sharpen memory, concentration, decrease anxiety levels, learning efficiency and positive attitude to life (Hoyos *et al*., 2000; Kornblihtt *et al*., 1993; Akbulut *et al*., 2001; Gitto *et al*., 2001; Kitajima *et al*., 2001) and bestows a proportionate, flexible, normally relaxed body with an ability to withstand stress efficiently (Kitajima *et al*., 2001) Yoga is the best lifestyle modification, which aims to attain the unity of mind, body and spirit through asanas, pranayama, and meditation (Hines and Brown, 1936) At spiritual level yoga creates an awareness to look for happiness from within oneself and to be at peace with oneself.

Objective

The aim of present study was to investigate whether regular practice of Yoga for 6 month can reduce the serum Adrenaline. Serum Cortisol level & cardiovascular hyper-reactivity induced by cold pressor test.

MATERIALS AND METHODS

Study group comprised 50 male healthy subjects of 18-25 years. They were subjected to cold pressor test according to Hines & Brown (1936) Out of 50 volunteers, 30 turned out to be hyper-reactive to this provocative test. The hyper-reactivity of 25 volunteers converted to hypo-reactivity after the yoga therapy of three months (83.3%). The parameters like level of serum adrenaline, serum cortisol, plasma melatonin, basal blood pressure, rise in blood pressure and pulse rate were also significantly reduced statistically by using student 't' test. The study protocol was explained to the subjects and written consent obtained. Approval by ethical committee of S.S. Medical College, Rewa, M. P., was obtained. All the volunteers were clinically examined to rule outany systemic diseases. All subjects were non-alcoholic and non-smokers. They were not taking any drugs, and they had similar dietary habits as well as physical and mental activities at work and home. They were not practicing any known stress relieving or relaxation technique previously

- All the 30 volunteers of **study group** were trained under theguidance of a certified "yoga" teacher for 15 days in the Department Of Physiology. They carriedout "Yogasanas, Pranayama and Meditation" 60 minutes, twice a day for three months, under supervision, in aprescribed manner. The schedule consisted of-
- Yogasanas -10 minutes
- Pranayama- 10 minutes
- Meditation- 40 minutes
- ***The asanas practiced were*:** Ardhachakrasana, Tadasana, Paschimottasana, Utthita Trikonasana, Vajrasana, Salamba Sarvangasana, and Halasana.
- ***The Pranayama performed was****: Anolom-vilomi*
- The volunteers practiced these exercises early in themorning and in evening, in a quiet, well ventilated room or in open airspace sitting in a comfortable posture.
- ***The Meditation performed was****: the same, as was told by Lord Krishna to Arjun in Kuruchhetra (Method is available in BhagvatGeeta. 9th to 16 slokes of Dhana Yoga chapter)*

- BP was measured in supine posture by Sphygmomanometer, [39]tworeading were taken five minutes apart and the mean of two was taken as the BP.

For cold pressor test, a thick walled thermocol box measuring 38 cm × 26 cm × 18 cm, closed from all sides, was used. A hole was made in the centre of the top of the box to allow entry to one hand of the subject. Another small hole was made at the corner of the top of the box for laboratory thermometer. Before starting the experiment the box was filled a mixture of ice and water and the laboratory thermometer was placed such that its mercury bulb was immersed in the mixture of ice and water. Temperature inside the box was measured about 3^0-4^0C. The hand was immersed in cold water up to the wrist for one minute (cold stress). An elevation above the basal level of more than 20 mm of Hg in systolic or of more than 15 mm in diastolic was considered as hyper-reactive response. (Hines and Brown, 1936)

Collection of blood sample for Serum cortisol, Serum Adrenaline and plasma melatonin levels

All of the subjects of study group were asked to report at 9 am. Taking all aseptic precautions, 5 ml venous blood sample was drawn from the antecubital vein of each subject. Second blood sample was taken in study group after 6 month of yoga practice from the start of study. The serum separated after centrifugation was divided into aliquots and batch analyzed by Enzyme-Linked Immunosorbent Assay (ELISA), using a commercial ELISA kit (IBL-Hamburg GmbH) for serum cortisol. After every collection. Plasma adrenaline analysis was done by ELISA (Adrenaline EIA kit. Circulatory levels of melatonin were estimated in 1mL of plasma using double-antibody radio-immunoassay based on the Kennaway G280 antimelatonin antibody kits.

Statistics

The data was analyzed statistically by using statistical software Graph Pad in Stat vs. 3.10 and MS Excell (2003).

Statistical analysis of serum cortisol level, plasma adrenaline, plasma melatonin, BP and pulse rate were done usingstudent 't' test and $p < 0.01$ was considered as significant.

RESULTS

Our results showed that "Yoga" causes significant reduction in serum cortisol level, plasma adrenaline, plasma melatonin ,the cardiovascular hyper-reactivity. A total of 50 male volunteers were included in the study. Out of which 30 were hyper-reactor to cold pressor test. These hyper-reactors practiced yoga regularly for three months and after this period 25 volunteers become hypo-reactors. The statistical analysis was carried out using student t' test. It was observed that the serum cortisol level,, plasma adrenaline, plasma melatonin, basal blood pressure, rise in BP due to cold stress and pulse rate were statistically more significantly altered. (Table-1)

Blood Pressure

The mean basal systolic blood pressure was123.8 ±3.08 mm Hg, mean diastolic blood pressure 81.92 ± 3.39mm Hg, mean basal Pulse rate77.52 ±5.33 / min and basal Serum cortisol level10.05 ±.631 microgram / dl, plasma adrenaline level 9.67 ± .93picogram/Land, plasma melatonin84.58 ±7.75pg/ ml.

Due to effect of cold pressor test before yoga

The mean rise in systolic blood pressure, was 145.4 ±2.85 mm Hg (p<0.000). While the rise in diastolic blood pressure was 98.16 ±3.73 mm Hg, (p<0.000). Rise in Pulse rate85.84 ±5.28 / min (p<0.000). Rise in serum cortisol level12.62 ±.582 microgram / dl (p<0.000),rise in plasma adrenaline was375.5 ±50.18picogram/L and fall in plasmamelatonin38 ±2.85pg/ ml.

The effect of 06 months of yoga only in study group

The mean systolic blood pressure decreased from 123.8 ±3.08mmHg to 119.8 ±2.107 mm Hg (p<0.000), mean diastolic Blood pressure was decreased from81.92 ± 3.39 to77.44 ± 4.18 mm Hg. (p<0.000), mean Pulse rate was decreased from77.52 ±5.33/ min to75.05 ±4.97 / min (p<0.000), and mean Serum cortisol level was decreased from

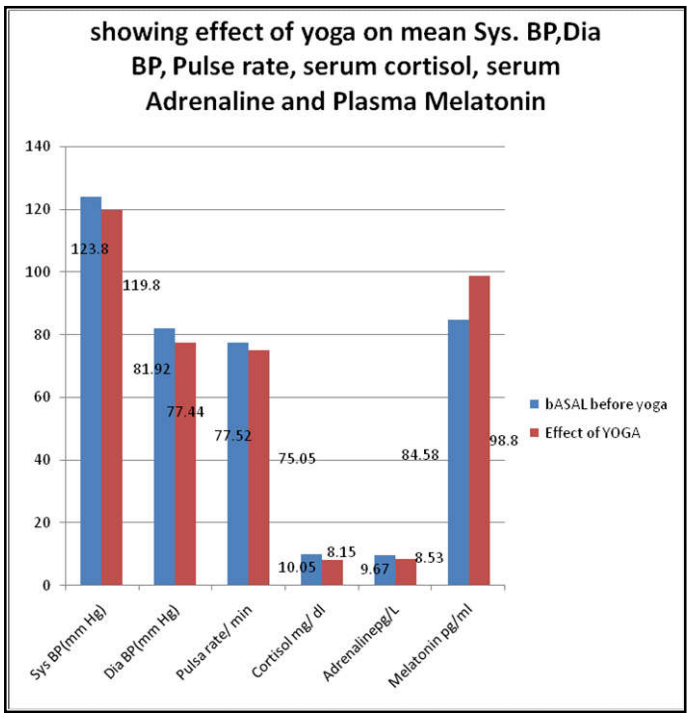
Table 1. Changes in basal SystolicB.P., basal Diastolic B.P., Pulse Rate s/min, serum Cortisol microgm/dl**serum Adrenaline ng/L and plasma melatonin**pg/ ml **before and after six months of yoga in hyper-reactors to cold pressor test**

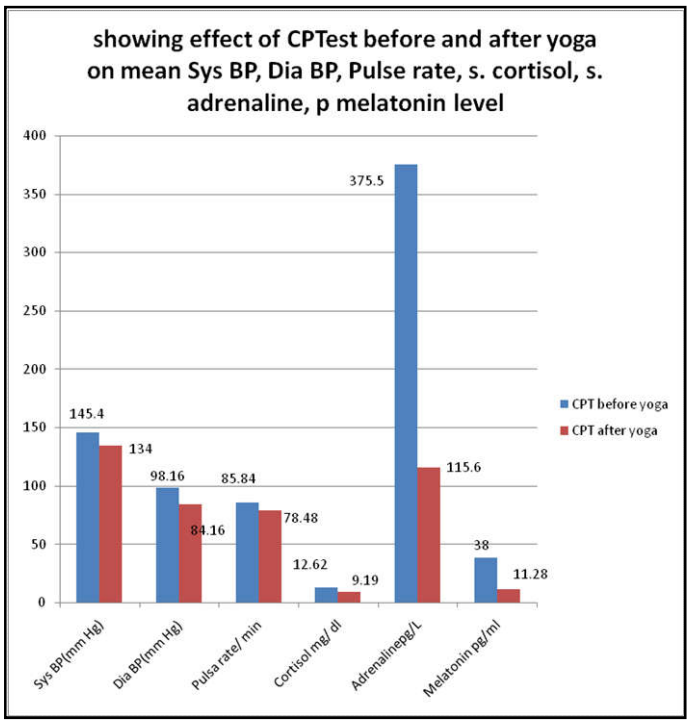
S. No.	Parameters	Before yoga.		After six months of yoga.		P Value
		Mean Value	**S. D.**	**Mean Value**	**S. D.**	
1	**Basal** Systolic B.P. **(mm Hg)**	**123.8**	**± 3.02**	**119.8**	±2.107	p<0.000
2	Diastolic B.P.**(mm Hg)**	**81.92**	**3.39**	77.44	**± 4.18**	p<0.000
3	**Pulse Rate/min**	**77.52**	**± 5.33**	**75.05**	**± 5.28**	p<0.000
4	*SerumCortisol* microgram /dl	**10.09**	**± 0.61**	**8.15**	**± 1.89**	p<0.000
5	Serum Adrenaline ng/L	**9.76**	**± 0.66**	**8.53**	±7.14	p<0.000
6	**plasma melatonin**pg/ ml.	84.58	±7.75	98.8	± 6.14	p<0.000

Table 2. Effects of cold pressor test before & after 6 month of Yoga practices on basal Systolic B. Pbasal Diastolic B. P. basal Pulse rate/ min, Serum Cortisol, serum Adrenaline &plasma melatonin level in hyper-reactors to cold pressor test

Parameters	Effect of cold pressor test before yoga Mean value & Standard deviation	Effect of cold pressor test after Six month Yoga Practices Mean value & Standard deviation	P Value
Systolic Blood Pressure (mm Hg)	**145.4 ±2. 85**	**134–±4.35**	**(p<0.000).**
Diastolic Blood Pressure (mm Hg)	98.16 ±3.73	84.16 ±3.08	**(p<0.000).**
Pulse rate/ min	85.84 ±5.28	78.48 ±.4.55	**(p<0.000)**
Serum Cortisol in microgram /dl	12.62 ±.582	9.19 ±.769	**(p<0.000).**
Serum Adrenaline ng/L	375.5 ±50.18	115.6 ± 27.36	**(p<0.000).**
plasma melatonin**pg/ ml**	38 ±2.85	11.28 ±4.35	**(p<0.000).**

10.05± 0.631microgram / dl to 8..15±1.89 microgram / dl (p<0.000), mean plasma adrenaline was decreased from9.67 ± .93picogram/L and rise in plasmamelatonin98.8± 6.14pg/ ml.





After 6 months of yogic exercises, pranayama and meditation &were statistically highly significant. (Table-2)

Due to effect of cold pressor test after yoga, the mean rise in systolic blood pressure, was 134 ±4.35mm Hg (p<0.000). While the rise in diastolic blood pressure was 84.16 ±3.08mm Hg, (p<0.000). Rise in Pulse rate78.48 ±.4.55 / min (p<0.000). Rise in serum cortisol level9.19 ±.769microgram / dl (p<0.000), rise in plasma adrenaline was115.6± 27.36 picogram/L and fall in plasmamelatonin11.28 ±4.35pg/ ml.

DISCUSSION

On analyzing the effect of yoga on hyper reactor subjects of first-year MBBS student age group 18-25 years, in our study, the basal level of serum cortisol, serum Adrenaline, plasma melatonin level and cardiovascular autonomic function tests were studied in study group before yoga and after six months of "yoga" (Asana, Pranayama and Meditation)and also were studied the effect of cold pressor test before yoga and after six months of "yoga". The study group volunteers showed the effect of cold pressor test on level, mean serum cortisol, mean serum adrenaline, mean plasma melatonin level, mean systolic blood pressure, mean diastolic blood pressure and mean pulse rate were increased (p<0.000) due to increase sympathetic activity of nervous system and were statistically highly significant before yoga and the reactivity to cold pressor test were also decreased (p<0.000) due to decrease sympathetic activity and increase parasympathetic activity of nervous system and were statistically highly significant after yoga. In the present study we observed that due to regular practices of yoga, mean serum cortisol, mean serum Adrenaline, mean systolic blood pressure, mean diastolic blood pressure and mean pulse rate were decreased (p<0.000) and due to autonomic equilibrium between sympathetic and parasympathetic nervous system and due to increase in vagal tone. (Gharote, 1973; Gopal *et al*., 1973; Vyas *et al*., 2002; Udupa *et al*., 2003) were statistically highly significant. Increase end ogenous secretion of melatonin, which, in turn, might be responsible for improved sense of well-being. On Transcendental Meditation, the cortisol levels was a significant drop in the meditation group (Michaels *et al*., 1979; Walton *et al*., 2004; MacLean *et al*., 1997; MacLean *et al*., 1994; Bullen *et al*., 2006) mainly due to decrease release of stress hormone " cortisol " from adrenal cortex and the Adrenaline levels was also significant drop in the meditation group (Infante *et al*., 2001; Eliasson *et al*., 1983; Ganong, 2007) due to decrease sympathetic discharge from Adrenal medulla and on Transcendental Meditation, the Adrenaline and cortisol levels both was a significant drop in the meditation group (Jin, 1989; BalakrishnanVandana, 2011) decrease release of Adrenaline and cortisol from adrenal gland.

The significant decrease in resting pulse rate, systolic and diastolic blood pressure after the yoga practice in the present study is in accordance with the findings of other studies on physiological effects of yoga practice in healthy individuals. (Bharshankar *et al*., 2003; Madanmohan *et al*., 2004; Murugesan *et al*., 2000; Barnes *et al*., 2004) Similar reduction in resting PR and blood pressure after yoga practice were also reported in hypertensive patients, (Santha Joseph *et al*., 1981; Damodaran *et al*., 2002) in asthmatic patients (Katiyar and Bihari, 2006) and in diabetic patients. (Singh *et al*., 2004) Can be attributed to modulation of autonomic activity with parasympathetic predominance and relatively reduced sympathetic tone. This modulation of autonomic nervous system activity might have been brought about through the conditioning effect of yoga on autonomic functions and mediated through the limbic system and higher areas of central

nervous system (Subbalakshmi *et al*., 2005) and mechanical and hemodynamic adjustments causing both tonic and phasic changes in cardiovascular functioning. (Raghuraj *et al*., 1998) and increases the baro-reflex sensitivity and decreases the sympathetic tone, thereby restoring blood pressure in patients of essential hypertension. (Vyas and Dikshit, 2002) The practice of "asanas" relaxes the muscles and joints which influences the hemodynamic mechanism, thereby improving blood circulation to vital organs. This may also activate the neuro-endocrine axis which is important in facing physical and mental stress. Restoring equilibrium, thereby avoiding intervention of inhibitory parasympathetic system. (Vatve *et al*., 2010) Combined practice of physical posture, breathing exercises, and meditation, needs of society, thus yoga to stop the stress response. (Herur Anita *et al*., 2010) The increase in melatonin secretion after the yogic practices may either be caused by increased secretion of hormone by the pineal gland or decreased clearance from the circulation. Walton *et al*. (1995), have reported that yogic practices increase serotonin, which in turn might be acting as a precursor for increasing melatonin synthesis during yogic practices. (Walton *et al*., 1995) Our observations on increase in nighttime melatonin after yoga and meditation are in consonance with the findings of Tooley *et al*. (2000) who have also reported an increase in melatonin following meditation. (Tooley *et al*., 2000) The exact significance and mechanisms responsible for increase in melatonin levels after yoga and meditation remains speculative. The higher melatonin levels during night after yoga and meditation showed a positive correlation with well-being. (Kasiganesanharinath *et al*., 2004) Yoga with physical, emotional, mental, personality developmental and holistic understanding offers to cope with stressful states. To meet the modern lifestyle full of challenges, stress and tensions an all-round personality development has become mandatory for the student. The aspect of relaxation and detachment is lacking in our education process and it is this new dimension that needs to be added to the curriculum. Thus yoga can be beneficial in achieving a tranquil state of mind during routine activities and yet providing then concentration and arousal essential in demanding or stressful situations like examinations. (Malathi and Damodaran, 1999)

Conclusion

Non pharmacological methods like yogic asanas, pranayama, and meditation should be encouraged to control the modifiable risk factors by increasing parasympathetic activity and decreasing sympathetic activity and provides significant improvements in cardiovascular parameters and respiratory functions. It can thus be concluded that these results would justify the incorporation of yoga as part of our life style in prevention of hyper-reactivity to stress related disorders and age–related cardiovascular complications. "In a tension-filled society, yoga, pranayama, and meditation alone will bring solace from problems and hence they are essence of the life".

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