



Assessment of different stressed conditions associated with blood group and WBCs among Medical Students and their management with anti-oxidant therapy and yoga.

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Abstract

Background

Human body responds to stress through the activation of a complex mechanism involving behavioral and physiologic responses. The health effects of stress involve - autonomic, cardiovascular, and immune systems. There are several factors causing stress among medical students, like examination, smoking, poverty, which affects white blood cells count. This research work was taken to find out different stressed situations associated changes and to combat with orange antioxidant therapy and yoga.

Methods

Printed questionnaires were distributed firstly and STAI values were calculated. 117 students were selected based on stressed situations like examination, smoking and poverty (experimental 54, and 63 control) were divided into into high, medium and low stress. Each group again divided into orange consuming group, sudarshan kriya performing group and combined therapy group. Before and after the experiment, STAI values and differential count was performed.

Results

O was the most common blood group for high stress, followed by A group. Almost all groups state and trait values of STAI, significantly decreased after consuming orange and combined therapy. Most of the high, medium and low stress students, neutrophil count significantly decreased by consuming orange, performing yoga or both.

Conclusion

Orange antioxidants and SKY decreased the stress level, so these modes of therapeutic approach should be adopted among medical students, in different stressed situations.

Key words

Differential count, medical students, orange, stress, sudarshan kriya yoga, white blood cells.



Background

Human body responds to stress through the activation of a complex mechanism involving behavioral and physiologic responses. The health effects of stress involve a number of systems - autonomic, cardiovascular, and immune systems [1]. Several aspects such as curriculum with overload of information, loss of control over time and difficulties resulting in managing leisure activities are other significant source of stress among medical students [2]. Stress is associated with poor performance and emotional distress, may lead to serious problems in social life in subsequent years [3]. Psychiatric symptoms like depression are also associated with stress [4]. Immune system also suppressed by stress, which have been described by lot of studies in medical students. These changes reflected in blood cell parameters and cell mediated immune response [5, 6]. There are several factors causing stress among medical students namely examination, smoking, poverty, which are reflective in the stress questionnaire results and white blood cells count. Examination stress is considered to be a major cause of stress [7]. Fear of failure causes anxiety and depression, at the beginning of the career [8]. Tobacco smoke is also well-known risk factors for oxidative stress. Teenagers with a smoking habit vulnerable to oxidative stress and weaker oxidant defense system [9]. Smoking causes chronic obstructive pulmonary diseases (COPD) [10]. Apart from this, poor socioeconomic background sometimes plays a vital role for stress. Parent's education, family size, siblings, influences this. Orange juice is a great source of vitamin C and flavonoids, has antioxidant activity towards free radicals to inhibit low density lipoprotein oxidation and scavenging intermediary peroxy and alkoxy radicals which reduces oxidative stress [11, 12]. Yoga is another easy way of improvement of psychological status, improving depression, and anxiety [13, 14]. Sudarshan kriya Yoga (SKY), designed by Sri Sri Ravi Shankar, is a rhythmic breathing exercise, is very effective in these contexts. SKY is composed of Ujjayi Pranayama, Bhastrika Pranayama and brief chanting, which is done in sitting posture and closed eye [15]. Benefits includes reduction in stress, anxiety and depression, post-traumatic stress disorder, substance abuse, improve antioxidant status *etc.* [16, 17]. Till date the majority of the studies are focused on stress in medical education correlating it with a variety of outcomes. Surprisingly these studies not approached to categorize different types of stress and neither its management. So herbal antioxidant research regarding health effects of compounds which is derived from fruits on modulation of Reactive oxygen species (ROS) and yogic approach have recently received a great deal of attention. This research work was taken to find out different stressed situations and to combat with orange antioxidants and yoga.

Material and Methods

Study Period

The present study was undertaken at Manipal College of Medical Sciences, Nepal, during September 2013 to May 2014. Experiments were performed in the Hematology and Physiology laboratories.

Study design, participants and the collection of data

First year medical students of basic sciences, experiencing different types of stresses caused by examination, smoking and poverty. Further the role of antioxidants and yoga under stressed conditions are evaluated. Printed questionnaires were distributed firstly and STAI values were calculated. Then, by personal interviewing, 117 students were selected based on stressed situations like examination, smoking and poverty (54 study group, and remaining 63 control group). Study group students were divided into high, medium and low stress, and each group students were again separated into orange consuming group, sudarshan kriya performing group and combined therapy group (both orange consuming and Sudarshan kriya). All subjects were instructed not to take any medication, antioxidant supplements or any other citrus fruits, which could interfere with study results. Orange consuming group students were instructed to take 200 ml of orange juice (freshly prepared), after breakfast and Sudarshan kriya performing group students were instructed to perform the yoga regularly for one month in the morning time for 30 minutes [15, 18]. Before and after the experiment, STAI values (Spielbergers STAI questionnaire) calculated and differential count (Leishman's staining) was done [19].

Data collection

One of the study investigator collected data personally by distributing the questionnaires to the adult males of (26-49) years of age. The participants were asked to tick one response for each item. The identity of the subjects was kept confidential to avoid bias in this study.

Response Rate

Initially the questionnaires were distributed among 217 medical students out of which a total of 212 responded correctly giving an overall response rate of 97.69%.

Questionnaire design

A multigraded questionnaire, which was printed and distributed by the principal investigator, covered personal details and general health status. State Trait Anxiety Inventory (STAI) questionnaires - both the A-State and A-Trait (to measure the level of stress) were also distributed separately. Students were instructed to choose the correct option and fill the Information properly [20].



Inclusion criteria

All the healthy students (both mentally and physically) studying in the 1st year, 18 years of age or older, who were interested to participate voluntarily, were included in the study.

Exclusion criteria

Incomplete questionnaires, students below 18 years, ill, undergoing treatment were excluded. Subjects screened for needle phobia and bronchial asthma or respiratory disorders, excluded accordingly.

Ethical committee approval

This research study was conducted in accordance with the latest version of the declaration of Helsinki. Ethical committee approval was taken from college authority and a written informed consent was obtained from the participants prior to the research work.

Outcome variable

Stress scores, differential count of white blood cells were set up as outcome variables.

Explanatory variables

The demographic factors were defined at individual level. Factors at individual level were age, gender, area of residence (rural, urban) etc.

Data management and statistical analysis

The data collected was analyzed using Statistical Package for the Social Sciences (SPSS) for Windows Version 16.0 (SPSS Inc; Chicago, IL, USA). The associations between the different variables were analyzed by using the Student's t-test (paired sample t test), where different control and experimental groups were compared and the level of significance ($p < 0.05$) was considered as statistically significant).

Results

Table – 1: Distribution of gender, blood group and Rh factor n(%)

Gender	Male	72(61.5)
	Female	45(38.5)
Blood Group	A	37(31.6)
	B	26(22.2)
	AB	9(7.7)
	O	45(38.5)
Rh factor	Negative	8(6.8)
	Positive	109(93.2)

Table-1 explains, majority of the participants were male. Among the students, O blood group was most common followed by A, B and AB. Predominance of Rh positive blood group was also observed among the subjects.

Table – 2: Correlation of blood group and stress level n(%)

	A	B	AB	O	Total
High	12(30.80)	2(5.10)	3(7.70)	22(56.4)	39(100)
Medium	13(33.30)	11(28.20)	2(5.10)	13(33.3)	39(100)
Low	12(30.80)	13(33.30)	4(10.30)	10(25.6)	39(100)
Total	37(31.60)	26(22.20)	9(7.70)	45(38.5)	117(100)
P value			0.031		

$P < 0.05$, statistically significant

Table-2 elucidates, O was the most common blood group for high stress, followed by A group. Among the medium stressed students, predominance of O and A blood group followed by B groups were observed. In case of low stress study populations, prevalence of B and A followed by O blood group was evident.

Table- 3 explains the STAI score values (STAI State and STAI Trait) of the subjects. In the high and medium stress group, state and trait values significantly decreased after consuming orange and combined therapy. Almost similar results found in the state values of yoga performing students, except smoking associated medium stress group and all participants of low stress group. Among yoga performers, trait values were significant in examination and smoking associated high stress group and poverty induced medium stress group. In the combined therapy, all state & trait values were statistically significant except exam and poverty induced low stress group. There were no apparent alterations observed in the pre and post results among the controls.

Table-4a and 4b clarifies, initially there was increase in the number of neutrophils and eosinophils and decrease in lymphocytes, almost all stress groups. Afterwards, most of the high, medium and low stress students, neutrophil count significantly decreased by consuming orange, performing yoga or both. Students, who had taken orange as an antioxidant, among them, high and medium stressed poverty group, medium stressed smoking group and low stressed examination group showed significant increase in the number of lymphocytes. Same pattern was observed in the yoga performing high stress poverty group. Almost all the cases (high, medium and low) after the combined therapy, (except medium and low stress poverty group; high and medium smoking group) lymphocytes number increased significantly. Although there was no statistically significant changes observed in all cases of eosinophilic count, but mean value of the count decreased in all experimental groups except yoga performing low stress poverty group. There was no noticeable changes occurred in the control group subjects.



Table – 3 Spielbergers State Trait Anxiety Inventory (STAI) Self – evaluation scores (mean±SD) in different stress groups

	Stress Level	Cause of stress	STAI State			STAI Trait			
			Pre	Post	P value	Pre	Post	P value	
Control Group	High	Exam	52.42±1.39	52.71±3.86	0.812 ^x	52.85±2.47	54.42±3.55	0.130 ^x	
		Smoking	59.71±2.62	60.28±2.87	0.321 ^x	60.28±4.53	60.00±6.11	0.772 ^x	
		Poverty	52.57±1.71	51.00±2.51	0.139 ^x	53.14±1.86	53.57±2.82	0.573 ^x	
	Medium	Exam	36.28±3.45	35.42±4.72	0.395 ^x	38.14±5.89	39.00±5.22	0.289 ^x	
		Smoking	43.85±4.29	44.28±6.77	0.718 ^x	43.14±6.44	43.71±5.63	0.578 ^x	
		Poverty	39.85±2.91	40.00±4.76	0.877 ^x	41.57±2.37	43.14±1.46	0.081 ^x	
	Low	Exam	25.57±2.57	26.28±2.62	0.094 ^x	26.57±2.87	27.14±1.06	0.578 ^x	
		Smoking	24.00±2.58	24.85±3.62	0.111 ^x	25.85±1.77	26.28±2.13	0.482 ^x	
		Poverty	25.42±2.57	25.85±3.53	0.573 ^x	20.57±2.76	21.14±2.79	0.140 ^x	
EXPERIMENTAL GROUP	Orange consuming group	Exam	53.50±0.70	43.50±0.70	0.037 [*]	54.50±3.53	42.00±4.24	0.025 [*]	
		Smoking	61.50±2.12	50.00±1.41	0.028 [*]	57.00±1.41	45.00±2.82	0.053 [*]	
		Poverty	56.50±2.12	45.00±1.41	0.028 [*]	58.00±1.41	46.00±2.82	0.053 [*]	
	Medium	Exam	47.50±3.53	38.00±2.82	0.033 [*]	45.00±2.82	34.50±3.53	0.030 [*]	
		Smoking	46.00±4.24	37.50±4.94	0.037 [*]	43.50±2.12	35.00±2.82	0.037 [*]	
		Poverty	41.50±3.53	33.00±2.82	0.037 [*]	43.00±2.82	33.50±2.12	0.033 [*]	
	Low	Exam	27.00±1.41	22.00±0.00	0.126 ^x	27.50±2.12	22.00±1.41	0.058 ^x	
		Smoking	26.50±2.12	21.50±0.07	0.126 ^x	26.00±0.00	20.50±0.70	0.058 ^x	
		Poverty	25.00±1.41	20.50±0.07	0.070 ^x	26.00±1.41	21.50±2.12	0.070 ^x	
	Yoga performing group	High	Exam	56.50±3.53	46.00±4.24	0.030 [*]	55.00±1.41	44.50±0.70	0.030 [*]
			Smoking	60.00±2.82	49.50±3.53	0.030 [*]	58.00±5.65	49.50±0.94	0.037 [*]
			Poverty	57.50±3.53	48.00±4.24	0.033 [*]	52.00±1.41	43.00±2.83	0.070 ^x
Medium		Exam	38.00±2.82	31.50±2.12	0.049 [*]	36.50±6.36	31.00±5.65	0.058 ^x	
		Smoking	34.00±2.82	28.50±3.53	0.058 ^x	38.50±0.70	35.00±1.41	0.258 ^x	
		Poverty	44.50±4.94	38.00±4.24	0.049 [*]	41.50±0.70	35.00±1.41	0.049 [*]	
Low	Exam	26.50±3.53	23.50±4.94	0.205 ^x	25.50±2.12	21.00±0.41	0.070 ^x		
	Smoking	25.00±1.41	21.00±0.00	0.156 ^x	26.00±2.82	21.50±0.70	0.205 ^x		
	Poverty	27.00±1.41	22.50±0.70	0.070 ^x	28.00±1.41	22.50±0.70	0.058 ^x		
Both Orange consuming and Yoga performing group	High	Exam	56.50±2.12	42.00±1.41	0.022 [*]	56.00±2.82	43.50±2.12	0.025 [*]	
		Smoking	61.50±2.12	46.50±0.70	0.042 [*]	58.50±2.12	43.00±2.82	0.021 [*]	
		Poverty	52.50±2.12	39.00±1.41	0.024 [*]	50.00±0.00	37.25±0.35	0.012 [†]	
	Medium	Exam	43.50±2.12	32.75±1.76	0.015 [*]	41.00±1.41	30.50±0.70	0.030 [*]	
		Smoking	45.50±2.12	34.00±1.41	0.028 [*]	47.00±2.28	34.50±3.53	0.025 [*]	
		Poverty	35.00±1.41	26.50±2.12	0.037 [*]	39.50±2.12	29.00±1.41	0.030 [*]	
	Low	Exam	25.50±0.70	20.50±0.70	0.126 ^x	26.00±1.41	21.50±0.70	0.707 ^x	
		Smoking	28.50±0.70	22.00±1.41	0.049 [*]	25.50±2.12	20.50±0.70	0.126 ^x	
		Poverty	25.50±0.70	21.00±0.00	0.700 ^x	25.50±0.70	20.50±0.70	0.126 ^x	

^xP>0.05, statistically not significant, ^{*}P<0.05, statistically significant, [†]P<0.01, statistically significant

Discussion

Gender, blood group, Rh factor distribution in the subjects

In earlier studies from Nepal by Roy and coworkers showed that O blood group was most common among Nepalese population followed by A and B, same pattern followed in this research. AB blood group was relatively rare, 5.5% in the earlier study, here also almost same result 6.3% observed. 93.7% students of this research shows Rh positivity which is also in accordance with the study of Roy and coworkers [21]. Majority of the participants were male, because there were no female smokers.

Blood group and the relation between stress level among the subjects

There are relatively less scientific reports stating the correlation between blood group and stress. Neumann *et al.* found strong association between 'A' and 'O' blood group with stress response, similar to our findings. He also stated that the 'A' blood group had higher initial levels of low density lipoprotein toxicity-preventing activity (TxPA) and cortisol as well as quicker stress recovery rates than the type 'O' group [22].



Table – 4a : White blood cell parameters (Differential count) Control group

Stress Level	Cause of stress	Neutrophil		Eosinophil		Basophil		Monocyte		Lymphocyte		
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
HIGH	Exam	72.57±2.2	72.00±2.	3.85±1.3	3.00±0.8	0.28±0.4	0.42±0.	3.00±0.8	2.85±0.3	20.28±2.	21.71±2.21	
		2	08	4	16	8	53	1	7	42		
			0.587 ^x		0.078 ^x		0.604 ^x		0.736 ^x		0.082 ^x	
	Smoking	52.0±4.35	52.85±4.	6.57±1.1	5.71±0.9	1.00±0.5	0.71±0.	3.00±1.1	3.57±0.9	37.42±3.	37.14±2.96	
		5	18	3	5	7	48	5	7	95		
			0.325 ^x		0.143 ^x		0.172 ^x		0.172 ^x		0.631 ^x	
Poverty	72.00±2.3	70.42±4.	4.42±0.9	4.57±1.2	0.57±0.5	0.42±0.	3.42±0.9	3.42±0.9	19.14±3.	21.14±5.39		
	8	85	7	7	3	53	7	7	13			
		0.256 ^x		0.736 ^x		0.356 ^x		1.000 ^x		0.270 ^x		
MEDIUM	Exam	69.14±2.6	68.57±3.	3.71±0.9	3.28±0.9	0.42±0.5	0.57±0.	3.85±0.8	3.42±0.9	22.85±3.	24.14±3.89	
		0	90	5	5	3	53	9	7	23		
			0.596 ^x		0.356 ^x		0.356 ^x		0.289 ^x		0.314 ^x	
	Smoking	54.28±4.1	56.14±4.	5.71±0.7	5.71±1.3	0.42±0.5	0.42±0.	3.85±0.8	3.85±0.8	35.71±3.	33.85±5.52	
		5	45	5	8	3	78	9	9	40		
			0.180 ^x		1.000 ^x		1.000 ^x		1.000 ^x		0.239 ^x	
Poverty	70.00±1.9	69.85±4.	3.85±0.6	3.42±0.5	0.57±0.7	0.85±0.	2.85±0.6	3.42±0.9	22.71±0.	22.42±3.95		
	1	25	9	3	8	69	9	7	75			
		0.903 ^x		0.200 ^x		0.356 ^x		0.172 ^x		0.842 ^x		
LOW	Exam	61.42±3.1	61.71±3.	4.14±0.8	3.85±0.8	0.42±0.5	0.71±0.	3.0±1.41	2.71±0.7	31.0±2.8	31.0±4.54	
		0	98	9	9	3	48		5	2		
			0.689 ^x		0.522 ^x		0.172 ^x		0.569 ^x		1.000 ^x	
	Smoking	56.00±4.0	56.00±3.	4.57±1.2	3.85±1.3	0.28±0.4	0.57±0.	3.00±1.1	2.71±0.7	36.14±3.	36.85±3.48	
		8	69	7	1	8	78	5	5	76		
			1.000 ^x		0.140 ^x		0.172 ^x		0.356 ^x		0.526 ^x	
Poverty	59.42±4.8	59.28±4.	3.71±0.7	3.42±0.9	0.71±0.7	0.42±0.	2.28±0.9	2.42±0.9	35.14±4.	34.42±4.19		
	9	07	5	7	5	53	5	7	81			
		0.818 ^x		0.356 ^x		0.172 ^x		0.604 ^x		0.564 ^x		

^xP>0.05, statistically not significant, *P<0.05, statistically significant, †P<0.01, statistically significant

Sherrington *et al.* reported that although blood group 'A' individuals respond to stress by releasing profuse adrenaline, but elimination is also very fast. On contrast, 'O' blood group appears to have more difficulties with catecholamines; once it is produced, difficult to eliminate them and their effects. This event may be connected with the 'O' genotype and its influence via gene linkage on the actions of the enzyme dopamine beta hydroxylase [23]. Another striking research by Rinieris *et al.* established significant associations of different types of neurological disorders and blood group association. Obsessive-compulsive neurosis and blood type 'A'; phobic neurosis and 'O'; hysteria and 'A' were mentioned in their research [24]. So all these findings correlate with the high stressed situations associating with 'A' and 'O' blood group.

Stress related alteration in STAI scores and role of different therapies

From the initial values (before treatment) of STAI, it is evident that students were in stress before examination, similar to others [25]. When the stress level evaluated in contrast with normal, Zunhammer *et al.* found that, STAI score was 4 units higher. Amazingly, when authors compared with low stress group, high stress students were more than two times stressed [26]. All of these findings

support this research outcome. Smoking is associated with high level of oxidative stress, which was reflected in the state and trait values of STAI, corroborate present findings. Depression is common in nicotine addicts [27 - 29]. Poverty is associated with weaker immune response. A higher STAI score was positively correlated with a weaker immune response, when administering the same antigen dose to the subjects [30, 31] which can be linked with poverty associated high stress values.

Vitamin C decreased stress level among students, reflected in STAI scores, more in high stressed group, followed by medium and low [11, 12]. Apart from vitamin C, other antioxidants present in orange may be responsible for the stress reduction and it was reflected in the STAI scores of post treatment group. Earlier scientists reported, STAI values were significantly reduced in State anxiety level after performing yoga. It also improved the sleep quality, an indicative of less stress [16]. All these findings supportive to present work. The probable reason of this calm and quite state was sequence of breath practices which provides a neurophysiological “workout” leads to greater flexibility and plasticity in the nervous system [32]. In the combined therapy group, more changes observed in STAI scores which may be the collective effect of Orange antioxidants and yoga.



Table – 4b : White blood cell parameters (Differential count) – Experimental group (mean±SD)

Stress Level	Cause of stress	Neutrophil		Eosinophil		Basophil		Monocyte		Lymphocyte					
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post				
O	H	E	71.50±0.70	63.50±0.70	2.00±0.00	4.50±0.70	0.00±0.00	0.70±0.50	5.50±2.12	4.00±0.00	21.00±1.41	27.50±0.70			
				0.079 ^x		0.126 ^x		0.500 ^x		0.500 ^x		0.049 [*]			
		S	50.5±2.12	59.50±4.94	8.00±1.41	2.5±0.70	1.00±0.00	0.50±0.70	3.50±2.12	3.50±0.70	37.00±2.82	34.00±5.65			
	M	H	P	74.00±1.41	65.50±0.707	7.00±1.41	3.50±0.70	1.00±0.00	0.50±0.70	3.50±0.70	3.50±0.70	14.00±0.00	27.50±0.70		
					0.037 [*]		0.090 ^x		0.500 ^x		1.000 ^x		0.024 [*]		
			E	70.50±0.70	65.50±2.12	4.00±0.41	2.50±0.70	0.00±0.00	0.50±0.70	1.50±0.70	1.00±0.00	24.00±1.41	30.50±0.70		
		L	H	S	53.50±2.12	62.50±3.53	6.00±1.41	3.50±0.70	1.50±0.70	1.00±0.00	2.00±1.41	3.50±2.12	37.00±1.41	26.50±0.70	
						0.070 ^x		0.126 ^x		0.500 ^x		0.205 ^x		0.030 [*]	
				P	69.50±2.12	63.00±1.41	3.50±0.70	1.50±0.70	1.00±0.00	0.50±0.70	2.00±0.00	3.50±0.70	24.00±1.41	31.50±2.12	
			Y	H	P	64.50±0.70	58.00±1.41	3.00±0.00	2.50±0.70	0.00±0.00	0.50±0.70	3.50±0.70	3.00±0.00	28.50±0.70	36.0±0.00
							0.049 [*]		0.500 ^x		0.500 ^x		0.500 ^x		0.042 [*]
					E	56.50±2.12	63.50±0.70	3.5±0.70	2.0±0.00	1.00±0.00	0.50±0.70	2.00±0.00	4.00±1.41	37.00±2.82	30.00±1.41
O+Y				H	S	59.50±6.36	57.00±1.41	2.50±0.70	2.00±0.00	1.50±0.70	1.00±0.00	1.50±0.70	3.00±1.41	35.00±5.65	36.50±0.70
							0.605 ^x		0.500 ^x		0.500 ^x		0.205 ^x		0.742 ^x
					P	73.00±1.41	66.50±0.70	6.5±5.00	5.0±0.00	1.00±0.00	0.50±0.70	3.50±0.70	4.50±0.70	16.00±0.00	23.50±0.70
	O+Y			H	E	50.00±2.82	59.00±4.24	8.00±1.41	3.50±0.70	2.00±0.00	0.50±0.70	4.00±1.41	3.50±0.70	36.00±5.65	33.50±4.94
							0.070 ^x		0.070 ^x		0.205 ^x		0.795 ^x		0.126 ^x
					S	74.50±2.12	67.00±1.41	8.50±0.70	8.00±1.41	0.50±1.50	1.50±0.70	4.50±2.12	3.00±1.41	12.00±0.00	21.50±0.70
		O+Y		M	P	63.50±6.36	57.00±5.65	5.50±0.70	4.00±1.41	0.50±0.70	0.50±0.70	3.00±1.41	3.50±0.70	27.50±4.94	35.00±8.48
							0.049 [*]		0.205 ^x		1.000 ^x		0.795 ^x		0.205 ^x
					E	52.50±3.53	61.00±2.82	6.50±3.53	3.50±2.12	0.50±0.70	1.00±0.00	2.50±0.70	3.00±1.41	38.00±1.41	31.50±0.70
			O+Y	M	S	68.50±2.12	62.00±2.82	5.00±1.41	3.50±2.12	1.00±0.00	0.50±0.70	2.50±0.70	4.00±0.00	23.50±3.53	29.00±5.65
							0.037 [*]		0.205 ^x		0.500 ^x		0.795 ^x		0.144 ^x
					P	61.00±7.07	54.50±6.35	3.00±1.41	3.50±0.70	0.50±0.70	1.00±0.00	2.50±0.70	2.00±0.00	33.00±8.48	39.00±7.07
O+Y				L	P	61.00±7.07	54.50±6.35	3.00±1.41	3.50±0.70	0.50±0.70	1.00±0.00	2.50±0.70	2.00±0.00	33.00±8.48	39.00±7.07
							0.049 [*]		0.500 ^x		0.500 ^x		0.500 ^x		0.105 ^x
					E	55.50±2.12	61.50±3.53	4.50±3.53	3.50±0.70	1.50±0.70	1.00±0.00	1.50±0.70	3.00±1.41	37.00±5.65	31.00±1.41
	O+Y			L	S	56.00±1.41	52.00±0.00	3.00±0.00	4.50±0.70	0.50±0.70	0.50±0.70	2.50±0.70	2.50±0.70	39±2.82	40.50±0.70
							0.156 ^x		0.205 ^x		1.000 ^x		1.00 ^x		0.656 ^x
					P	72.50±2.12	59.50±0.70	8.00±1.14	2.50±0.70	1.00±1.41	1.00±0.00	5.00±0.00	3.50±0.70	13.00±4.24	33.50±2.12
		O+Y		H	E	49.50±2.12	58.00±1.41	5.5±0.707	2.00±1.41	0.500±0.70	1.00±0.00	4.50±2.12	3.00±1.41	40.50±0.70	36.00±1.41
							0.049 [*]		0.058 ^x		1.00 ^x		0.205 ^x		0.046 [*]
					S	68.00±2.82	56.00±1.41	8.00±1.41	3.50±0.70	0.50±0.70	1.00±0.00	3.50±0.70	3.00±0.00	20.00±1.41	37.00±0.00
			O+Y	M	P	68.00±2.82	56.00±1.41	8.00±1.41	3.50±0.70	0.50±0.70	1.00±0.00	3.50±0.70	3.00±0.00	20.00±1.41	37.00±0.00
							0.053 [*]		0.070 ^x		0.500 ^x		0.500 ^x		0.037 [*]
					E	68.00±1.41	54.50±0.70	4.00±0.00	2.50±0.70	1.00±0.00	0.50±0.70	5.50±0.70	5.00±1.41	21.50±0.70	37.50±0.50
O+Y				L	P	53.00±2.82	60.00±4.24	4.00±1.41	2.00±0.00	1.00±1.41	0.50±0.70	4.50±0.70	3.50±2.12	37.50±6.36	34.00±7.07
							0.024 [*]		0.205 ^x		0.500 ^x		0.500 ^x		0.040 [*]
					S	67.00±4.24	56.00±2.82	2.50±0.70	2.50±0.70	0.50±0.70	0.00±0.00	3.00±1.41	3.50±0.70	27.00±5.65	38.00±2.82
	O+Y			L	P	63.00±0.00	53.50±0.70	3.50±0.70	2.00±0.00	1.5±0.70	2.00±0.00	2.50±0.70	3.00±1.41	29.50±2.12	40.00±1.41
							0.033 [*]		0.205 ^x		0.500 ^x		0.500 ^x		0.030 [*]
					E	53.50±2.12	61.00±2.82	3.50±2.12	2.5±0.70	0.00±0.00	0.50±0.70	1.00±0.00	2.50±0.70	42.00±4.24	33.50±3.53
		O+Y		L	S	64.00±5.65	52.50±3.53	3.500±0.70	2.00±0.00	1.50±0.70	2.00±1.41	2.50±0.70	2.50±0.70	28.50±4.94	41.00±1.41
							0.042 [*]		0.500 ^x		0.500 ^x		0.205 ^x		0.037 [*]
					P	64.00±5.65	52.50±3.53	3.500±0.70	2.00±0.00	1.50±0.70	2.00±1.41	2.50±0.70	2.50±0.70	28.50±4.94	41.00±1.41

[O - Orange consuming group, Y - Yoga performing group, O+Y - Both Orange consumes and Yoga performing group. H – High stress, M – Medium stress, L – Low stress. E – Examination induced stress group, S – Smoking induced stress group, P – Poverty induced stress group]



Stress associated changes in WBCs and the effect of different types of therapies

Examinations in medical colleges are always stressful. A study by Qureshi *et al.* showed that examination stress significantly increases the neutrophil count and decreases the lymphocyte count supportive of our result [6, 33]. A decrease in the number of eosinophils was observed in the control and experimental group supports other workers [6]. Although few reports mentioned decrease in basophil count and increase in monocytes, but no such changes were observed [6, 34]. Lower number of lymphocytes reduces performance in their key functions indicating stress associated changes our study [35, 36]. A recent report showed that lymphocytes numbers increased in smokers [37], similar to this work. Eosinophils are particularly evident in chronic lung inflammation where their products appear to play roles to compromise lung function [38]. Mediators like major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO) takes active role in smokers [39]. Taylor and coworkers also reported the same [40]. Psychological stress and grief decrease the lymphocyte count [41]. Poverty induced stress group, showed the same. Epidemiological cohort studies, found strong relations between psychosocial stress, preterm birth and low birth weight [42] which predicts poorer vaccine responses in adolescence, also higher cortisol responses to acute psychosocial stress in adulthood, and increased risk of cardiovascular and metabolic disorders including diabetes later in life [43, 44]. Neutrophils and eosinophils were more in the "pre" values compared with the normal available ranges and low stress group. The probable reason either by stress hormones or the factors stated above.

Vitamin C accumulates in activated human neutrophils. In higher stressed groups relatively lower concentrations of vitamin C in serum indicated oxidative stress and neutrophils were more active and accumulated maximum amount of vitamin C inside them, along with increase in number [45]. Earlier study showed, the concentration of ascorbic acid in the WBCs was found to be much higher. So change in the neutrophilic population in our stressed group students was observed [46]. In this study, most of the student after practicing SKY, showed significant decrease in neutrophil count and increase in lymphocyte count corroborates other research, mentioned three and six weeks practice of SKY. In our study, smoking associated stressed groups lymphocyte count decreased initially but came into normal range after the therapy [36]. There are no statistically significant changes observed in most of the cases of eosinophilic count in SKY practicing group, but mean value decreased in all, supported by a randomized control study [47]. Authors also found, Monocytes decreased significantly where as no reportable changes observed in basophil and monocyte counts same like our study. Raised free radicals in stressed

situations, increased eosinophil count, but as stress was reduced by SKY, count also decreased in the post treatment group [36, 47]

In the combined therapy group, there is also same pattern of changes observed. As compared to other groups, changes were more prominent and show statistical significance.

Conclusion

In summary, it can be concluded that there are different types of stressed situations which affects hematological, biochemical parameters and psychological status of medical students. Findings highlight the need to augment the efforts of smoking-cessation programs. The most positive and promising aspect of this study was treating with Orange juice and yoga. As orange antioxidants and SKY decreased the stress level, so these modes of therapeutic approach should be adopted. Apart from these interventions, proper counseling is strongly recommended. Government education system needs to develop proper interventional strategies which cause less stress among medical students, and imply better support programmes for students struggling for their well being.

Limitations & future scope of the study

The sample size of the study was a limitation. The study experienced difficulty in finding enough volunteers as the study subjects had to be available to donate blood sample at the two consecutive periods (pre and post experimentation), thus leading to relatively smaller number of subjects. The clinical significance of the findings of present research awaits comprehensive clinical trials on larger population groups.

Abbreviations

Chronic obstructive pulmonary diseases (COPD), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), low density lipoprotein toxicity-preventing activity (TxPA), lung carcinoma (LC), major basic protein (MBP), Reactive oxygen species (ROS), Sudarshan kriya Yoga (SKY), White blood cell (WBC).

Competing interests

Authors declare that they do not have any competing interests.



Authors' contribution

BR, VA and IAK designed the study, prepared questionnaire, interpreted the data, drafted the manuscript, and revised it. BR conceived of the study with IAK, IB and BS, acquired, analyzed and interpreted the data, revised the manuscript. BR and BS participated in data analysis and revised the manuscript. Final manuscript was approved by all authors.

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