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ALTERATIONS IN STRESS PARAMETERS IN RATS HOUSED IN A PYRAMID MODEL - SEASONAL VARIATIONS

***#SUREKHA BHAT, #GURUPRASAD RAO, ##K.DILIP MURTHY AND $P.GOPALAKRISHNA BHAT**

Department of #Biochemistry, Melaka Manipal Medical College, Manipal University, Manipal, India;
##Department of Physiology, School of Medicine Universiti of Malaysia Sabah, Sabah, Malaysia.
$Department of Biochemistry, Kasturba Medical College, Manipal 576 104, INDIA

Corresponding author surekhakka@gmail.com

ABSTRACT

The space within the Great pyramid and its smaller replicas are believed to have healing powers due to the presence of cosmic and earth's radiations which are influenced by seasons. The present study was done to determine if seasonal variations influence pyramid-mediated decrease in neuroendocrine and oxidative stress in rats. The experiment was conducted in Manipal, India (74º53'E, 12º55'N), through two seasons - hot (March-May, average temperature 33-35ºC , average humidity 60-70%) and cool (June-October, average temperature 26-29ºC, average humidity 80-90%). Wistar rats were divided into 4 groups, normal controls (NCH) and pyramid exposed in the hot season (PEH) and normal controls (NCC) and pyramid exposed in the cool season (PEC). Plasma cortisol was significantly lower in PEC as compared to NCC but not in PEH as compared to NCH rats. Erythrocyte TBARS was significantly lower and reduced glutathione and glutathione peroxidase activity were significantly higher in PEH and PEC than those in normal controls, statistical significance being more in the cool season for TBARS. SOD activity was significantly higher in PEH than in NCH, but not in PEC when compared to NCC. This shows that the effect of seasons and hence geographic location is a variable factor to be considered during pyramid therapy.

KEY WORDS

pyramid, cortisol, oxidative stress, seasonal variations, antioxidants

INTRODUCTION

The intriguing geometry of the pyramids and the accurate precision with which the Great pyramid of Giza was constructed, have always puzzled scientists. Pyramid models with the same base to height ratio as of the Great Pyramid of Giza, when aligned on a true north-south axis, are believed to generate, transform and transmit energy[1]. Pyramidologists have reported that this pyramid energy promotes rapid growth of plants, faster healing of bruises and burns, longer preservation of milk and increased vitalization and better relaxation in human subjects[1]. Pyramid therapists all over the world claim to have treated many diseases/disorders of social concern like rheumatoid arthritis, diabetes mellitus, anxiety neurosis etc by exposing their patients to this so called pyramid energy[2,3,4]. Although these claims

ALTERATIONS IN STRESS PARAMETERS IN RATS HOUSED IN A PYRAMID MODEL - SEASONAL VARIATIONS

expressed in the popular press are merely based on observation and experience, some of these claims have been assessed and validated scientifically through studies carried out with appropriate control and tests. Exposure of adult female Wistar rats to pyramid environment reduced neuroendocrine and oxidative stress and increased antioxidant defense in them[5]. Pyramid exposure also counteracted chronic restraint stress-induced neuroendocrine and oxidative stress in Wistar rats[6]. Both these studies suggest that sitting inside a pyramid shaped structure can be used as an effective technique for stress management and for non invasive treatment of diseases in which the role of free radicals and reactive oxygen species has been implicated. Research using pyramid models has shown that they promote better wound healing in rats[7,8] and protect against stress-induced neurodegenerative changes in mice[9]. Research has also shown that the alignment of the pyramid in the North-South plane is crucial for the beneficial outcome[10]. Prof. S. M. Klimenko and Dr. D. N. Nosik have reported that the antiviral effect of venoglobin kept in the pyramid was maintained even at concentrations as low as 0.005 and 0.00005 micrograms/ml[11]. Prof. A. G. Antonov's team investigated the influence of pyramid treated distilled water on newborns with high-gravity pathologies and reported that indices of instantaneous state (IIS) essentially increased practically up to normal values even for patients with very low initial values of IIS, close to zero point[11]. Most of the above studies indicate that pyramid shaped rooms can be built in residences, hospitals and recreation centres for therapy and management of diseases. Since pyramidologists claim that pyramids concentrate cosmic as well as earth's radiations[12], and since it is known that the nature of cosmic radiations and earth's radiations vary with seasons, the objective of the present study was to find out if seasonal variations influence the decrease in neuroendocrine & oxidative stress produced in rats by pyramid exposure

MATERIALS AND METHODS

Animals

Adult female Wistar albino rats (3-4 month old) weighing 130-220g were used. Standard pelleted food and water was provided ad libitum to all the groups. Proper ventilation was provided. Standard hygienic conditions were maintained in the animal house and the rats were exposed to proper light and dark cycle (12 hr each of light and darkness). The experimental protocol was approved by the Institutional Animal Ethics Committee.

Experimental Design

The experiment was carried out from the beginning of March till the end of September in Manipal, a coastal town in the southern part of India (74º53' East, 12º55' North). Manipal is recorded to have a hot and humid climate between March and May with average temperatures ranging from 33-35ºC and average humidity ranging from 60-70%. Wet and cool climate is recorded between June and October with average temperatures ranging from 26-29ºC and average humidity ranging from 80-90%. There is no clear-cut winter in this region but southwest monsoons are heavy during the months of June to September with an average rainfall of 260-300 inches/year. The experiment was carried out in both the seasons mentioned above, that is, the hot season and the cool season.

Study groups

In each season, there were two group of rats, the normal controls **(NC)** that were maintained under

ALTERATIONS IN STRESS PARAMETERS IN RATS HOUSED IN A PYRAMID MODEL - SEASONAL VARIATIONS

standard laboratory conditions in their home cages and the pyramid exposed rats **(PE)** where the rats were housed under a wooden pyramid for 6 hours/day for 2 weeks[5,6]

The first batch of rats was sampled for estimation of stress parameters between the months of March and May and were categorized as **normal controls in the hot season (NCH, n=9)** and **pyramid exposed group in the hot season (PEH, n=9)**. The average temperature of the well ventilated non air-conditioned room in which the animals were kept, matched with the atmospheric temperature and ranged from 32.8 - 34.9^{0}C. Likewise, the average humidity of the room matched with that of the atmosphere and was found to range from 60 - 70%.

The second batch of rats sampled between the months of June and September were similarly divided into **normal controls in the cool season (NCC, n=11)** and **pyramid exposed group in the cool season (PEC, n=11)**. The average temperature of the room matched with the atmospheric temperature and ranged from 26 - 29^{0}C and the average humidity of the room matched with that of the atmosphere and was found to range from 83 - 91%.

On the 15th day, in both the seasons, blood was obtained from all rats in oxalate coated tubes between 9.00 a.m. and 10.00 a.m. The blood obtained was centrifuged to separate the plasma and cells. Plasma was used for the estimation of cortisol[13]. Packed red cells were washed three times in ice-cold phosphate-buffered saline (PBS-phosphate buffer 0.01 M, pH 7.4, containing 0.15 M NaCl) and used for the estimation of thiobarbituric acid reactive substances (TBARS)[14] and reduced glutathione levels[15]. An equal volume suspension of erythrocytes in PBS was prepared and used for assay of glutathione peroxidase activity[16] and SOD activity[17]. All parameters measured in the erythrocytes were expressed as the corresponding units per gram hemoglobin. Hemoglobin concentration was measured by cyanmethemoglobin method of Drabkin[18]. All statistical analyses were carried out using students T-test in the Graph Pad Instat (GPIS) package.

RESULTS

Results are shown in Tables I and II. Higher levels of erythrocyte TBARS and plasma cortisol (p<0.01 for both) and lower levels/activities of all parameters of antioxidant defence (p<0.05 for all) were observed in the NCH rats as compared to those in the NCC rats. These results reflect increased neuroendocrine and oxidative stress even within the normal controls in the hot season **(Table I)**. Hence, to assess the influence of seasons on the beneficial effects of pyramid exposure, we compared the mean values of the different parameters of PE rats with NC rats of the same season.

Plasma cortisol level was significantly lower (25% decrease, p<0.001) in PEC group of rats as compared to NCC group of rats. However, there was no significant difference in the means between NCH and PEH group of rats although there was a 15% decrease in the steroid upon pyramid exposure **(Table II)**

Erythrocyte TBARS level in the pyramid exposed rats was significantly lower than that in normal controls in both seasons. However, statistical significance between the two groups was more in the cool season with pyramid exposure showing a decrease of 17% in TBARS in the cool season (p<0.01) and 13% in the hot season (p<0.05) **(Table II)**

Erythrocyte reduced glutathione level and glutathione peroxidase activity were significantly greater in the pyramid exposed rats than in the normal control rats and the extent of this increase was similar in both seasons **(Table II)**

SOD activity was significantly higher in the PEH rats compared to NCH rats (25% more, p<0.05). There was no significant difference in the enzyme activity of the PEC and NCC groups in the cool season although PEC rats had 15% more activity than NCC rats **(Table II)**

Table I.

Parameters of neuroendocrine stress, oxidative stress and antioxidant defence in the normal control rats in the hot (NCH) and cool seasons (NCC) (values: Mean ± SD)

	NCH (n=9)	**NCC (n=11)**	p value
Plasma Cortisol (nmoles/l)	4473.96±696.36	3663.53±379.69	<0.01
Erythrocyte TBARS (nmoles/g Hb)	6.84±0.68	6.05±0.52	<0.01
Erythrocyte GSH (mg/g Hb)	1.78±0.26	2.18±0.39	<0.05
Erythrocyte GSH-Px (U/g Hb)	67.23±7.66	76.91±7.71	<0.05
Erythrocyte SOD (U/g Hb)	1789.58±450.54	2598.36±500.21	<0.05

Table II.

Parameters of neuroendocrine stress and oxidative stress in pyramid exposed adult female rats in comparison with their normal controls in hot and cool seasons (values: Mean ± SD)

		Hot season (n=9)	**Cool season (n=11)**
Plasma Cortisol (nmoles/l)	Normal controls (NC)	4473.96±696.36	3663.53±379.69
	Pyramid exposed (PE)	3911.45±1113.72	2742.13±586.58
	p for PE vs NC	**>0.05**	**<0.001**
	% change from NC	**15% decrease**	**25% decrease**
Erythrocyte TBARS (nmoles/g Hb)	Normal controls (NC)	6.84±0.68	6.05±0.52
	Pyramid exposed (PE)	5.93±0.78	5.02±0.76
	p for PE vs NC	**<0.05**	**<0.01**
	% change from NC	**13% decrease**	**17% decrease**

Erythrocyte GSH (mg/g Hb)	Normal controls (NC)	1.78±0.26	2.18±0.39
	Pyramid exposed (PE)	2.5±0.38	2.96±0.47
	p for PE vs NC	**<0.001**	**<0.001**
	% change from NC	**40% increase**	**35% increase**
Erythrocyte GSH-Px (U/g Hb)	Normal controls (NC)	67.23±7.66	76.91±7.71
	Pyramid exposed (PE)	87.05±14.76	98.94±18.22
	p for PE vs NC	**<0.01**	**<0.01**
	% change from NC	**29% increase**	**29% increase**
Erythrocyte SOD (U/g Hb)	Normal controls (NC)	1789.58±450.54	2598.36±500.21
	Pyramid exposed (PE)	2244.05±307.79	2997.99±726.17
	p for PE vs NC	**<0.05**	**>0.05**
	% change from NC	**25% increase**	**15% increase**

DISCUSSION

The seasonal variations in erythrocyte TBARS and antioxidant levels, and plasma cortisol levels even in normal controls, agree with previous studies that have reported similar effects on other animal species exposed to varying conditions of the environment[19,20,21,22,23,24,25]. A detailed discussion about the probable reasons for these variations has been presented by the authors in their earlier studies[26]. Thus it was decided to analyse the seasonal variations in the benefits of pyramid exposure, by comparing the parameters studied in the pyramid exposed rats with those in the normal controls of the same season.

Results of this study indicated that pyramid exposure proved beneficial in terms of decreasing neuroendocrine and oxidative stress in both the seasons. However, the lowering effect of pyramid exposure on plasma cortisol and erythrocyte TBARS was more in the cooler months of June to October than in the hot season of March to May. On the contrary, pyramid-mediated increase in erythrocyte SOD activity was more in the hot season. This shows that the pyramid is more effective in lowering stress and oxidative processes in the cool season whereas more effective in increasing the antioxidant enzyme activity in the hot season. Overall, in terms of decreasing neuroendocrine and oxidative stress, the pyramid seems more effective in the cool season in our part of the world.

Some pyramidologists like Schul B and Pettit E[27] have reported seasonal variations in the effects of pyramid on plants, solids and liquids in their pyramid model. At times they found milk kept in pyramid changing to yogurt and at times, the milk simply stratified, separating its components into layers. They hypothesized that this change could be due to variations in sunspots or solar flares. Pyramidologists

ALTERATIONS IN STRESS PARAMETERS IN RATS HOUSED IN A PYRAMID MODEL - SEASONAL VARIATIONS

believe that variations in temperature, humidity, seasons, phases of the moon and varying degrees of cosmic radiation influence the pyramid effect in their home experiments. The energy fields within the pyramid vacillate from time to time in home experiments[28]. In the present study also, differential effects of pyramid exposure on stress and oxidative stress in different seasons were observed. Professor Giorgie Piccardi, director of the Institute for Physical Chemistry in Florence, studying the erratic behaviour of water in a pyramid has reported that the water was influenced by cosmic forces and that chemical reaction times coincided precisely with sunspot activity, reactions being rapid when sunspot activity was at a peak and slow when sunspot activity was ebbing[28]. Based on the research of Piccardi on water, home experiments of pyramidologists with pyramid models and the results of the present scientific study, it is apparent that cosmic forces are concentrated in the cavity of the pyramid and because of this, seasonal variations of the environment are reflected in the results obtained during pyramid research. The authors of the present work have, in their earlier studies, suggested that pyramid shaped rooms could be used in hospitals and recreation areas for stress management and treatment of stress related disorders and of oxidative stress implicated diseases. Pyramidologists have always claimed that the type of pyramid to be constructed and used for therapeutic purposes are to be decided based on the geographical location chosen. The present study gives some validation to this claim since weather conditions are known to vary from one geographic location to another. Hence, it is advisable to consult geologists and related experts before choosing a location for construction of pyramids for therapeutic purposes. Further studies could be conducted to determine what other variable factors related to geographic location influence the behaviour of the 'energy fields' and cosmic forces in the pyramidal cavity and hence form a variable factor during pyramid therapy.

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ALTERATIONS IN STRESS PARAMETERS IN RATS HOUSED IN A PYRAMID MODEL - SEASONAL VARIATIONS

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