Effect of housing rats within a pyramid on stress parameters

Surekha Bhat*, Guruprasad Rao, K Dilip Murthy & P Gopalakrishna Bhat

Departments of *Biochemistry and ^Physiology, International Centre for Health Sciences and ^Department of Biochemistry, Kasturba Medical College, Manipal 576119, India

Received 26 March 2003; revised 18 June 2003

The Giza pyramids of Egypt have been the subject of much research. Pyramid models with the same base to height ratio as that of the Great Pyramid of Giza, when aligned on a true north-south axis, are believed to generate, transform and transmit energy. Research done with such pyramid models has shown that they induced greater relaxation in human subjects, promoted better wound healing in rats and afforded protection against stress-induced neurodegenerative changes in mice. The present study was done to assess the effects of housing Wistar rats within the pyramid on the status of oxidative damage and antioxidant defense in their erythrocytes and cortisol levels in their plasma. Rats were housed in cages under standard laboratory conditions. Cages were kept in the open (normal control), under a wooden pyramid model (experimental rats) or in a cubical box of comparable dimensions (6 hr/day for 14 days). Erythrocyte malondialdehyde and plasma cortisol levels were significantly decreased in rats kept within the pyramid as compared to the normal control and those within the square box. Erythrocyte reduced glutathione levels, erythrocyte glutathione peroxidase and superoxide dismutase activities were significantly increased in the rats kept in the pyramid as compared to the other two groups. There was no significant difference in any of the parameters between the normal control and rats kept in the square box. The results showed that exposure of adult female Wistar rats to pyramid environment reduces stress, oxidative stress and increases antioxidant defense in them.

Keywords: Cortisol, Glutathione, Glutathione peroxidase, Malondialdehyde, Pyramid model, Stress, Superoxide dismutase

Civilization and modernization have made stressful life inevitable. In the competitive world of modern technology, mental and emotional stress have become an unavoidable part of life. However, stress is known to have deleterious effects on physical and mental well-being. Emotional stress is known to increase the plasma levels of malondialdehyde (MDA), which has been reported to directly correlate with the severity of emotional stress in human beings. Stress is also known to cause an increase in glucocorticoid levels. High levels of glucocorticoids have been reported to decrease blood glutathione and superoxide dismutase activity in erythrocytes of rats.

Reactive oxygen species (ROS) produced during oxidative metabolism are known to cause oxidative damage to macromolecules and this is also referred to as oxidative stress. MDA is produced during the ROS mediated damage to the polyunsaturated fatty acids. Reduced glutathione, glutathione peroxidase and superoxide dismutase form part of the antioxidant defence systems produced by the body to protect the cellular constituents from the damages caused by ROS$. A disturbance in this ROS-antioxidant equilibrium results in increased oxidative stress. Thus, increased stress is also accompanied by an increase in oxidative stress.

The Giza pyramids are one of the wonders of the ancient world. Pyramid models with the precise ratios of the Great Pyramid of Giza centered on the true north-south axis like the great pyramid, are believed to generate, transform and transmit energy. Research using pyramid models has shown that they promote greater relaxation and improved tranquility in human subjects, better wound healing in rats, and protection against stress-induced neurodegenerative changes in mice. Studies with pyramid models have also shown that pyramid environment enhances learning and memory. It is also reported that the maximum beneficial effects of pyramid energy is directly below the apex of the pyramid at one-third the height of the pyramid from the base.

Therefore, the environment within the pyramid is useful in coping up with stress which in turn may be useful in coping with oxidative stress. Our experiments were performed with an attempt to study the effects of placing adult female Wistar rats within...
the pyramid, on the status of oxidative damage, antioxidant profile and cortisol level in them.

Materials and Methods

Animals—Female Wistar albino rats, Rattus norvegicus (Henry H Donaldson), 4-5 months old, weighing 150-225 g were used in this study. Rats were maintained under standard laboratory conditions at 25°C. 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 rats were divided into three groups, viz. Normal Control group, (NC; n=20), with rats maintained in their home cage under standard laboratory conditions for 14 days; Pyramid exposed group, (PE; n=20) wherein rats in their cages were housed in a wooden pyramid model for 6 hours/day for 14 days and Square box Exposed group, (SE; n=12) wherein rats in their cages were kept in a wooden square box of comparable dimensions as that of the pyramid for 6 hr/day for 14 days. Blood was obtained from all the groups of rats in oxalate coated tubes between 9.00 and 10.00 a.m. on 15\textsuperscript{th} day. The blood obtained was centrifuged to separate the plasma and cells. Packed red cells were washed three times in ice-cold phosphate buffered saline (PBS-phosphate buffer, pH 7.4, containing 0.15 M NaCl) and used for the estimation of malondialdehyde levels and reduced glutathione levels. An equal volume suspension of erythrocytes in PBS was prepared and used for assay of glutathione peroxidase activity and superoxide dismutase activity. Plasma was used for the estimation of cortisol.

Dimensions of the pyramid and square box\textsuperscript{12} (Fig. 1)—A wooden pyramid of 75 cm height, 112.5 cm base and 78.75 cm side fabricated locally, was used. Holes were provided for ventilation and a glass window was provided for observation, entry of light and for placing or removing the cages.

A wooden square box of 75 cm height and 112.5 cm base fabricated locally, with all other provisions as in the case of the pyramid mentioned above, was used. The square box was designed and used in this study taking into account any alterations that may result from housing the rats in closed space.

Exposure within the pyramid and square box—Each of the four faces of the pyramid/square box was made to point to the four cardinal points: north, south, east and west with the help of a standard compass. Each cage was oriented in such a way that their long axis was in the north-south plane. They were placed directly below the apex of the pyramid/square box at one-third the total height of the pyramid from the base, on a wooden stool of 25 cm height. All high voltage-producing devices were avoided in the vicinity as the process is based on cosmic, magnetic and other natural radiation energies.

Estimation of malondialdehyde (MDA)—Packed cells (0.2 ml) were used for the estimation of MDA as thiobarbituric acid reactive substances (TBARS)\textsuperscript{13}. The absorbance at 600 nm was subtracted from absorbance at 532 nm. MDA values were expressed as nanomoles/gram hemoglobin. Hemoglobin concentration was measured by cyanmethemoglobin method of Drabkin\textsuperscript{14}.

Estimation of reduced glutathione (GSH)—Reduced glutathione level in rat erythrocytes was measured by the colorimetric method of Beutler et al.\textsuperscript{15}. Packed cells (0.2 ml) were used in the assay. The glutathione was made to react with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) which reacts with sulphhydryl groups, to develop a stable color. The absorbance was measured at 412 nm. Glutathione content was expressed as milligrams/gram hemoglobin.

Assay of glutathione peroxidase activity (GSH-Px)—The erythrocyte suspension in PBS was used for the assay. The erythrocytes were hemolysed with water and all the hemoglobin in the lysate was converted to stable cyanmethemoglobin by adding Drabkin’s reagent. This hemolysate was used for the assay\textsuperscript{16}. The
rate of oxidation of GSH by H$_2$O$_2$, catalyzed by GSH-Px was measured. The rate of formation of GSSG was measured by following the decrease in absorbance of the reaction mixture at 340 nm as NADPH is converted to NADP$^+$. Non-enzymatic oxidation of GSH was measured in a simultaneous assay system, without the hemolysate. The difference between the two systems gave the enzyme activity, which was expressed as units/gram of hemoglobin where units represented the number of micromoles of NADPH oxidized per minute in the reaction mixture.

Assay of superoxide dismutase (SOD) activity — Inhibition of the reduction of nitro blue tetrazolium (NBT) by superoxide radicals, generated by the illumination of riboflavin in the presence of oxygen and the electron donor methionine was used as the basis for the assay of superoxide dismutase$^{17}$. A chloroform ethanol extract was prepared$^{17}$ from the hemolysates and the supernatant obtained was used for the assay. The solution was illuminated for 10 min. The absorbance was then read at 560 nm. Control with and without NBT were always included in the assay. One unit of SOD activity was taken as that producing 50% inhibition of NBT reduction. Values were expressed as units of enzyme activity/gram hemoglobin.

Estimation of plasma cortisol levels — Plasma (2 ml) was mixed with 15 ml methylene dichloride and centrifuged to separate plasma and methylene dichloride layer. The lower methylene dichloride layer was used for cortisol estimation$^{18}$. A blank containing 2 ml water and a standard containing 2 ml of standard cortisol were included in the assay. Ten ml of the extract was then mixed with 5 ml fluorescence reagent containing 70% conc. H$_2$SO$_4$ and 30% absolute ethanol. The fluorescence of the lower layer of acid extract was measured at 530 nm at exactly 12 min of adding the fluorescence reagent, with excitation wavelength 470 nm. Cortisol concentration was expressed as nanomoles/litre of plasma.

Statistical analysis — All statistical analyses were done using one-way analysis of variance (ANOVA) in the Graph Pad Instat (GPIS) package.

Results and Discussion

The results for the various measurements are shown in Figs 2 to 6.

An attempt was made to examine whether placing the rats within the pyramid in a particular optimum “position” had an influence on the status of oxidative stress and neuroendocrine stress in female rats. We used erythrocyte GSH, GSH-Px and SOD as indicators of antioxidant defense, erythrocyte MDA as an indicator of lipid peroxidation and plasma cortisol as an indicator of neuroendocrine stress.

Widely read articles in the popular press have claimed that pyramids influence energy fields within them. Consequently, placing living objects within the pyramids (Giza pyramid or its smaller replicas) results in perceptible effects on living objects, namely longer preservation of fruits and vegetables, faster growth of plants, greater relaxation and improved tranquility in human subjects$^{6,8}$ and better wound healing in surgically operated horses$^{20}$. Attempts to assess these claims scientifically with appropriate control and tests are being carried out around the world. Pyramid exposure has been reported to promote better wound healing$^{8,9}$ and reverse the action of dexamethasone in maturation and organization of formed collagen in rats$^9$. Housing mice in wooden pyramids reportedly enhances learning with correlated increase in hippocampal dendritic arborization and also protects against stress-induced atrophy of hippocampal neurons$^{10}$.

This study is another attempt to assess the effects of pyramid exposure on living systems in a scientifically controlled manner. Scientific literature suggests the role of free radicals and the disruption of prevailing antioxidant systems in tissues in aging$^{21,22}$. A significant age-related decrease in GSH content has been demonstrated in tissues such as brain, heart, liver, erythrocytes and lymphocytes$^{23,24}$. Excessive free radical activity has been implicated in neurodegenerative diseases, chronic inflammatory diseases, cardiovascular diseases and cancer$^{25}$. It has been reported that oxygen free radicals impair wound healing in rats$^{26}$. Diabetes mellitus$^{27}$ is also being viewed as a disease where the complications are contributed largely by increased free radical production. In the light of the above scientific studies and taking into account claims by pyramidologists that pyramid “energy” has been successfully used in the treatment of cancer, diabetes mellitus, epilepsy, chronic inflammatory diseases like arthritis, clearing of choked arteries$^7$ and restoration of youthfulness$^6$, we thought it relevant to examine the effect of pyramid exposure on the status of oxidative stress.

Since civilization and modernization have made stressful life inevitable, we also measured plasma cortisol in all the groups to assess the role of pyramid shape in stress management.

Erythrocyte indicators of antioxidant defense were significantly higher in PE group in comparison with
NC (P<0.001 for GSH and GSH-Px, P<0.01 for SOD—Figs 2 to 4 respectively) and SE groups (P<0.001 for GSH and SOD, P<0.05 for GSH-Px). Antioxidants normally protect cells from the deleterious effects of ROS. However, antioxidant defenses are not completely efficient and some free radicals escape to damage cellular macromolecules such as DNA, proteins and PUFA. Pyramid exposure may have increased the efficiency of the antioxidant system which in turn results in lower levels of ROS and lesser lipid peroxidation. This is reflected by the significant decrease in the level of MDA in erythrocytes of PE group as compared to NC (P<0.01) and SE groups (P<0.01; Fig. 5).

It was also observed that plasma cortisol levels were significantly lower in PE group as compared to NC (P<0.05) and SE groups (P<0.05; Fig. 6) suggesting that even normal levels of stress are
lowered in rats exposed to pyramid. This may support earlier claims by human volunteers that sitting in a pyramid for a definite period of time results in greater relaxation\(^9\). Since increased stress is known to increase plasma MDA levels\(^1\) and decrease blood GSH and erythrocyte SOD activity\(^2\), the lowering of the normal levels of stress within the pyramid may further help in improving antioxidant defense in the PE group.

There was no significant difference in any of the parameters between NC and SE groups (\(P>0.05\)) showing that the shape of the pyramid was responsible for the difference in results between the PE and NC groups.

In conclusion, the present study shows that exposure of adult female Wistar rats to pyramid environment reduces neuroendocrine stress and increases antioxidant defense which in turn reduces oxidative stress. This observation suggests that the shape of the housing has its effects and pyramid-shaped housing appears to have beneficial effects as far as stress management is concerned. Hence, pyramid models can be used for beneficial effects in workplaces especially hospitals for management of diseases in which the role of free radicals has been implicated and also in educational institutions for improved learning and memory. Further studies are required to determine the nature of, and quantitate the ‘energy’ that is claimed to be present within the pyramid and to correlate it with the present findings.

References


19. Toth Max & Nielsen Greg, Transform yourself with pyramid energy, in Pyramid power (Inner Traditions India, One Park Street, Rochester, Vermont, USA 05767) 1985, 129.

20. Roby Jeanne, A healing space, in Inside outside (Business India Group, Mumbai) (February 2002) 155.


