



RESEARCH ARTICLE

EFFECT OF ACUTE AND CHRONIC STRESS ON BRAIN TISSUE MDA LEVEL  
IN NORMAL WISTAR ALBINO RATS

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ABSTRACT

Stressful stimuli can disrupt the physiological homeostasis. Acute and chronic stressors influence brain function and it is known to be the key factor for the genesis of various psychological disorders. The present study was designed to elucidate the influence of acute and chronic stress on different brain tissues of wistar albino rats. The animals were divided into two major groups as non stressed group (n =10) and stressed group (n =10). The stressed groups were divided into acute (one day) stress groups and chronic (30 days) groups. The animals of the stressed groups were subjected to acute and chronic types of swimming stress and immobilization stress. Lipid peroxidation was estimated in cerebral cortex, hypothalamus and cerebellum. Acute swimming stress and chronic immobilization stress significantly (P<0.001) increased the lipid peroxidation level in the cerebral cortex and hypothalamus. Whereas, acute immobilization stress and chronic swimming stress increased (P<0.001) the cerebellar lipid peroxidation. The observed regional specific alterations in lipid peroxidation levels show that the nature of the stressors are probably capable of generating a different degree of cellular imbalance in between pro-oxidants and antioxidants which may provide some approach on variety of neurological and psychological processes as well as their treatment.

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INTRODUCTION

Stressful situations can lead to many physiological and psychological alterations. In human beings, a series of physical illness ranging from asthma to ulcerative colitis and hypertension can be linked to stressful life (Vanitallie, 2002). Similarly, impaired physiological responses to stress have been associated with major depressive illness (Tennant, 2001) and post-traumatic stress disorders (Heim *et al.*, 1997; Kathol *et al.*, 1989). Organisms are constantly subjected to stimuli that can be construed as stressors. In terms of duration, stressors may be divided into two main categories as acute (single, intermittent and time limited exposure) and chronic (repeated and prolonged continuous-exposure) stressors. The duration and the frequency of the stress period are important determinants for the induction of the cascade of stress - triggered neurobiological processes. Stress regulation is a highly integrated process controlled largely by the brain. The brain is capable of translating a wide range of stress related inputs into a general class of neuroendocrine/autonomic responses designed to enable the organism to efficiently cope with environmental change elicited by variety of stressors. Stress may also impair antioxidant defenses leading to oxidative damage by changing the balance between oxidants and antioxidant factors (Herman *et al.*, 1996). Oxidative stress can be considered as another type of stressor that participates in degeneration of brain cells (Halliwell and Gutteridge, 1999). In the central nervous system, the membrane lipids of neurons have high content of polyunsaturated fatty acids

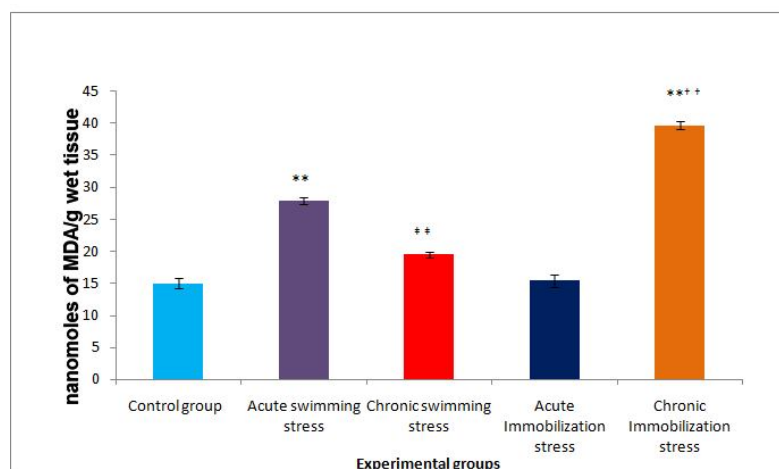
which is the main substrate for lipid peroxidation (Morgan *et al.*, 1999). The products of lipid peroxidation are themselves reactive species and lead to extensive membrane, organellar and cellular damage (Abuja and Albertoni, 2001). Elevated levels of malondialdehyde (MDA) one of the by-products of lipid peroxidation has been reported in the cardiovascular, neurological and in other diseases (Cotran *et al.*, 1999). There is a lack of information available in literature on the stress induced oxidative damage that may occur in various brain regions depending on time variant exposure to stressors. Hence the present study was designed to address the issue of stress induced formation of free radicals in acute and chronic condition on various brain tissues in wistar rats

MATERIALS AND METHODS

All experimental procedures and animal maintenance confirmed to the strict guidelines of Institutional Ethics Committee and that of Federal laws for the use of animals in the experiment. Adult albino rats (150 to 250 g) of Wistar strain were used in the present study. The rats were procured from the central animal breeding center at our university. Animals were housed individually in polypropylene cages (29cms x 22cms x 14cms) during the experimental period at 28±2° C temperature and 50±5% humidity. The rats were maintained under standard laboratory conditions with 12h light: 12h dark cycle. Animals were fed on laboratory chow (Gold Mohur; Lipton India, Ltd) and tap water in drinking bottles were made available *ad libitum*. The animals were divided into two major groups as non stressed group (n =10) and stressed group (n =10). The stressed groups of rats were subjected to swimming stress and immobilization stress.

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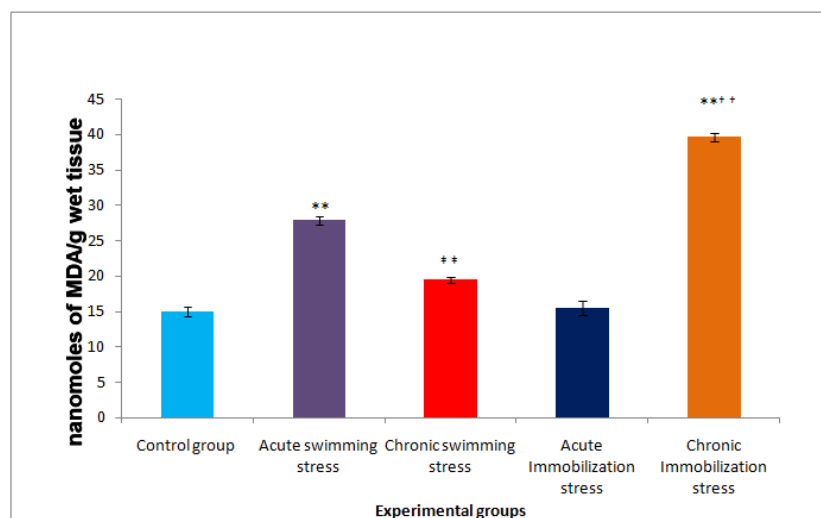


**Fig. 1. Acute and chronic stress induced changes in the MDA level of the cerebral cortex in normal wistar rats. Values are expressed as mean  $\pm$ SEM; nanomoles of MDA/g wet tissue. In each group ten animals were used.**

\*\* P< 0.001; versus control group

†† P< 0.001 versus acute swimming stress group

††† P< 0.001 versus acute immobilization stress group

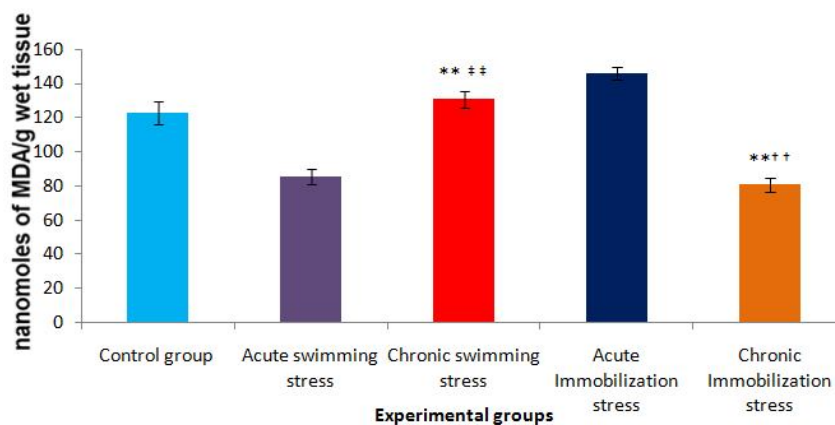


**Fig. 2. Acute and chronic stress induced changes in the MDA level of the hypothalamus in normal wistar rats. Values are expressed as mean  $\pm$ SEM; nanomoles of MDA/g wet tissue. In each group ten animals were used.**

\*\* P< 0.001; versus control group

†† P< 0.001 versus acute swimming stress group

††† P< 0.001 versus chronic immobilization stress group



**Fig. 3. Acute and chronic stress induced changes in the MDA level of the Cerebellum in normal wistar rats. Values are expressed as mean  $\pm$ SEM; nanomoles of MDA/g wet tissue. In each group ten animals were used.**

†† P< 0.001 versus acute swimming stress group

††† P< 0.001 versus acute immobilization stress group

The stressed groups were grouped into acute stress groups and chronic stress groups. All the stress experiments were done between 10AM to 12 Noon to minimize circadian variability.

### Swimming stress

Rats exposed to swimming stress (Yan Hu *et al.*, 2000) were allowed to swim in the plastic tubs containing tap water maintained at room temperature. The water level in the plastic tub was always kept at 30cms from the bottom. Rats were forced to swim in this tub until exhaustion. The point at which the animals became unable to stay at surface and showed signs of sinking was considered to be the point of exhaustion. After the stress session, the rats were towel dried and then placed back in their respective cages where water and food were available *ad libitum*. Duration of stress in acute swimming stress group was 60 minutes or till exhaustion (whichever is earlier) and the stress period is only for one day. In chronic swimming stress group, the total duration of stress was 7 days where rats were forced to swim daily for 60 minutes or till exhaustion (whichever is earlier).

### Immobilization stress

The immobilization chambers (Nagaraja and Jeganathan, 1999; Nayanatara *et al.*, 2012) used in this study were plastic tubes of varying sizes to accommodate all sizes of rats (15cms long and 4cms diameter, 16cms long and 5cms diameter, 17cms long with 6cms diameter). The tubes had a conical head at one end. The conical head area contained numerous perforations which served as breathing holes. The rat was placed inside the tube with head in the conical end. The rats were totally restrained by packing the rear end of the tube and closing it firmly with a stopper. This immobilization procedure minimized the space around the rat and prevented it from turning and moving and thus provided a rather strong stressful condition without causing any injury to the animal. Acute immobilization group of rats were immobilized for 1 hour only, for one day. Chronic immobilization group of rats were exposed to immobilization for 1 hour per day for a period of 30 days. At the end of experiment, all rats were anaesthetized with sodium pentobarbital (40 mg/kg body weight). The whole brain was removed from the cranial cavity. Cerebral cortex, cerebellum, and hypothalamus were dissected out according to the method of Glowinsky and Inversion (Glowinski and Iversen, 1966). The specimens were stored at -80° C until assay. The collected tissues were homogenized with a motor driven glass homogenizer in ice-cold phosphate buffer at 0°C for lipid peroxidation analysis. Lipid peroxidation in the different brain was estimated spectrophotometrically by thiobarbituric reactive substance (TBARS) as previously described by Kartha and Krishnamurthy (Kartha R and Krishnamurthy S, 1978). The data obtained was analyzed statistically using SPSS (Statistical Package for Social Sciences) version 11.5. The data was summarized using mean  $\pm$  SEM, one way ANOVA was used with a significant criteria of  $P < 0.05$ .

## RESULTS

Acute swimming stress significantly ( $P < 0.001$ ) increased the lipid peroxidation level in the cerebral cortex and hypothalamus (Fig 1 and Fig 2) when compared to the control group and chronic swimming stress groups. In the cerebellum (Fig 3) chronic swimming stress increased the level of lipid peroxidation when compared to acute swimming stress and control groups. Exposure to chronic immobilization stress significantly increased ( $P < 0.001$ ) the amount of MDA level in the cerebral cortex and the hypothalamus (Fig 1 and Fig 2). Further, in the cerebellum (Fig 3) acute immobilization stress significantly increased ( $P < 0.001$ ) the level of lipid peroxidation when compared to chronic immobilization stress and control groups.

## DISCUSSION

Stress is becoming a prominent integral part of life in the society, understanding the mechanisms by which it manifests itself in the body and potential treatment for these manifestations is imperative in the development of effective treatment strategies. In the present study, both acute and chronic stress effects were studied by using two

different stressors namely swimming which is more of a physical stress and immobilization stress which is a psychological type of stress. Stress induced oxidative metabolism on the body depends upon the involvement of various brain tissues. Hence, the estimation of these oxidative metabolic products on certain tissues was included in this study. The central nervous system has anatomic and metabolic features that make it sensitive to oxidative stress (Gupta *et al.*, 2003). The brain, spinal cord and peripheral nerves are rich in both unsaturated fatty acids and iron (Skaper *et al.*, 1999). The brain, spinal cord and peripheral nerves are rich in both unsaturated fatty acids and iron (Skaper *et al.*, 1999). The high lipid content of the nervous tissue coupled with its high aerobic metabolic activity make it particularly susceptible to oxidative damage. Some selective areas of brain have high iron content (Gilman *et al.*, 1993). A number of pathological conditions of the brain have been implicated due to free radical formation (Sayre *et al.*, 2001; Klein JA and Ackerman SL, 2003). Thus stress conditions with high production of free radicals make the brain particularly vulnerable to oxidative damage. Therefore, lipid peroxidation was estimated in different brain tissues in order to evaluate their relative role in stress induced regulatory mechanisms. The present results showed that, different models of acute and chronic stressors induce the formation of free radicals in different brain tissues. In conclusion we suggest that, different models of stressors might induce different degree of lipid peroxidation in various brain tissues. Recent studies have shown the response of antioxidant defense system in stress differs in different brain tissues (Ahmed, 2005). The present study might provide some insights on region specific type of stress induced formation of free radical in brain regions as well as their treatment. The assessment of the different antioxidants during acute and chronic stress on different brain tissues will be an interesting step to identify the mechanisms involved.

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