ABSTRACT
If wrinkles must be written upon our brows let them not be written upon the heart; the spirit should not grow old

- James A. Garfield

The advancement of science has given birth to modernization and urbanization due to which, on one hand, man has been thinking to settle on moon while on the other hand, the whole of the environment has been polluted. This has produced many health hazards. Different types of stresses are present in our daily life and they affect the different systems of the body at different rates. Nervous system is the most vulnerable component of the body. In the present study, an attempt has been made to delineate the age-related and stress-induced alterations in the central nervous system of rat biochemically, histochemically and ultrastructurally. Effects of N-acetylcysteine thiolactone (citiolone) alone and following restraint stress have also been studied in various regions of aged rat CNS particularly related to some of the biochemical parameters.

For the present study, five different age groups of albino rats (Charles Foster, 3, 6, 12, 18 and 24 months old) were used. They were divided into four groups: (i) Control (ii) Experimental rats which were kept in specially designed cages in which their movement was restricted for 24 hours, (iii) Experimental rats which were given N-acetylcysteine thiolactone (8.0 mg/kg body weight for 7 days, i.p. daily), and (iv) Experimental rats which were administered N-acetylcysteine thiolactone as mentioned above and restraint stress for 24 hours prior
to their sacrifice for biochemical estimation. However, for histochemical and electron microscopic studies, only 3 and 24 months rats were used. They were exposed to 24 hours restraint stress and thereafter sacrificed and their brain and spinal cord were removed rapidly and dissected out into different regions - hippocampus, hypothalamus, cerebrum, cerebellum, brain stem and spinal cord. Finally, various parts of CNS were processed and neurobiochemical, histochemical and ultrastructural studies were made following standard methods.

Results concerning the effects of restraint stress and N-acetylhomocysteine thiolactone as well as N-acetylhomocysteine thiolactone plus restraint stress in different regions of CNS of aged rats are summarized as follows:

- Restraint stress produces modifications of the gastric mucosa leading to focal areas of partial surface erosions, followed by a detachment of the superficial cells resulting into gastric ulcers of variable shapes and sizes. Restraint stress-induced elevation of cortisol was also observed in the present study.

- Brain is rich in lipids. The data show that total lipid, phospholipids, cholesterol, triglycerides increased with the passage of time (3 to 24 months), whereas, gangliosides elevated from 3 to 18 months while, depleted in 24 months old rats. However, significant decrease was found in all the parameters after 24 hours restraint stress.

- The major consequences of reactive oxygen species damage in a tissue are lipid peroxidation and their products. In the present study the levels of lipid peroxides, lipid hydroperoxides, conjugated dienes and lipofuscin elevated with senescence as well as following 24 hours restraint stress in various regions of rat CNS.
Antioxidant defence system - Glutathione is one of the major aqueous antioxidant of cellular systems and its content is correlated with life-span of organisms. The present study shows extensive loss of reduced glutathione (GSH), total -SH (T-SH) and protein -SH levels in hippocampus, hypothalamus, cerebrum, cerebellum, brain stem and spinal cord of aged rats after restraint stress. Interestingly, oxidized glutathione (GSSG) contents increased with age and restraint stress. From these observations it can be inferred that there is an increase in oxidative stress with age and a concomitant loss of antioxidant defenses.

SOD is an enzyme which participates to control the damage of tissue produced by free radicals. The data show a progressive loss of SOD enzyme activity with age and restraint stress in various regions of CNS. This may be the result of proliferation of glial cells due to neuronal degeneration associated with age.

Catalase activity is very low in brain tissue. It was observed that catalase activity declined in various regions of brain (hypothalamus, hippocampus, cerebrum, cerebellum, brain stem) and spinal cord with age following continuous 24 hours restraint stress.

Glutathione reductase, glutathione peroxidase and glutathione-S-transferase were found to reduce with age following restraint stress. The decline in GSH pool may be limiting the activity of these enzymes with age and stress.

The present study show age-related increase in MAO activity in different brain regions and spinal cord following restraint stress. The increase in MAO further confirms the observations made with protective enzymes. The age-related and stress-induced increase in MAO may contribute to oxyradical fluxes in brain as one of its products. \( \text{H}_2\text{O}_2 \) can generate highly reactive oxiradical species - hydroxyl radical (\( \text{OH}^+ \)). Increase in lipid peroxidation products also confirmed elevation of oxygen-centered species.
- Inhibition of DNA with passage of time and restraint stress in various regions of CNS was observed in the present investigation. However, RNA levels were decreased in different regions of brain with ageing. Interestingly, RNA levels were elevated after restraint stress. On the other hand, DNase activity enhanced with senescence following restraint stress, whereas, RNase activity depleted following restraint stress.

- Protein levels were decreased in different regions of brain in aged rats following restraint stress. This establishes the role of intracellular proteolysis in aged animals.

- N-acetylhomocysteine thiolactone (citiolone) administration in rats of different ages produced inhibition of lipid peroxidation products and elevation of antioxidant - glutathione and antioxidative enzymes such as SOD, CAT, GSHPx, GR and GST in various regions of brain and spinal cord. Interestingly, when citiolone was administered for 7 days following restraint stress for 24 hours, the lipid peroxidation products were decreased. They came nearer to control values. Glutathione, SOD, catalase, and glutathione related enzymes were also reduced.

- Total lipids, phospholipids, lipofuscin and nucleic acids (DNA and RNA) contents after restraint stress were also studied histochemically. The histochemical observations were in concurrence with the biochemical findings.

- Light microscopic study showed the degeneration of pyramidal cells of hippocampus following restraint stress. Purkinje cells of cerebellum also showed degeneration and appearance of vacuoles in white matter. Neuroglia cells increased in number after restraint stress.
The main findings of the transmission electron microscopic study of the hypothalamus, hippocampus and cerebellum following restraint stress were increase of lipofuscin pigment, electron dense bodies, clumping of chromatin, irregular nuclear profile, vacuolar spaces in axonal profiles and mitochondria with dense matrix. An increase in GERL-like profiles in hypothalamus and cerebellum was also seen.

The results of the present study clearly indicate that restraint stress induces biochemical and histological changes in the CNS. Regional heterogeneity observed after restraint stress, suggests a selective vulnerability of the nervous system.