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Research Article

EFFECT OF SUBACUTE HEAT STRESS ON NEUROBEHAVIOUR AND BRAIN HISTOLOGY IN WISTAR RATS

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ABSTRACT

The study aimed to determine the effect of subacute heat stress on neurobehaviour and brain histology in wistar rats. A total 40 rats (20 male and 20 female) were divided into 2 groups (N=10 rats/sex/group). Group I, (Control group) maintained at comfortable: 22 ± 3˚C temperature, Group II (Heat stressed group) rats were subjected to heat stress @ 42 ±1°C for 2 hrs daily for the period of 30 days. The neurobehavioral observation was done on day 30 by open field test. At the end of the experiment, rats were humanely sacrificed and the brain was collected for histopathology. Group II male rats revealed higher anxiety level as significant increase ($P<0.05$) in no. of defecation and urination was noted. A significant decrease in no. of rearing, boxes crossed, central arena crossed and a significant increase in latency was observed compared to control rats pointing towards decreased activity. In females (Group II), no significant difference was noted in any of the parameters across the groups compared to control. Histopathological observation revealed congestion, vacuolation, neuronal degeneration. Heat stress has negatively impacted the locomotor function and caused increased anxiety in male rats.

Keywords: Brain, Heat stress, Histopathology, Neurobehavioural observation, Open field test.

INTRODUCTION

Climate change has become one of the most serious problems in last few years. The major driver involved in this climate crisis is global warming. The rise in average surface temperature due to increased concentration of greenhouse gases resulted in high frequency and intensity of heat waves (Rossati, 2017). Global warming is drastically contributing to frequent episodes of heat stress in animals as well as in humans. Heat stress can significantly affect neurobehavioural responses such as locomotor responses, activity level etc. Thus, it is important to investigate the changes which result due to thermoregulation, brain function, and anxiety-like behavior in heat stress environments (Nakagawa *et al*., 2020). Animals' body, in response to exposure to high environmental temperature, alter and adjust various physiological processes to maintain normal body temperature and the homeostasis. Heat stress causes physiological and psychological stress to the body, resulting in activation of the hypothalamic-pituitary-adrenocortical (HPA) axis. Various studies have focused on the adverse effects of high environmental temperatures (Haveman *et al*., 2005). However, few studies have been performed on high environment heat-induced changes in behavioural disorders.

Hyperthermia disturbs the permeability of the blood brain barrier (BBB) which results in the leakage of serum proteins in the brain consequently leading to brain oedema. Heat-related neuronal degeneration has also been reported in the previous studies. As per the previous reports, hyperthermia causes increased apoptosis. Thus, increased brain hyperthermia may cause neurotoxicity either directly or through disruption of BBB (Mete *et al*., 2012). After chronic exposure to heat stress, animals adapt to their environmental conditions by changing physiological function which is known as heat acclimation. Rats acclimate to higher environmental temperatures to inhibit

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increased body temperature through various responses, such as spreading of saliva over their bodies (Horowitz *et al*., 1983). Furthermore, during heat acclimation, core body temperature increases but returns to the basal level two weeks after exposure to heat (Nakagawa *et al*., 2016). Continuous heat stress leads to physiological and metabolic alteration to compensate for changes in thermal environment which impacts the health of animals as well as man. Heat stress causes the damaging effect by enormous generation of free radicals (FRs) and other reactive oxygen species (ROS) resulting in abrupt degenerative changes in the various tissues of the system leading to oxidative stress (Altan *et al*., 2003). In this study, we have examined the impact of heat stress on neurobehavioral pattern and histopathology of the brain in both male and female wistar rats.

MATERIALS AND METHODS

Animal and experimental design

The study was conducted in albino wistar male and female rats **(***Rattus norvegicus*) of 6-8 weeks of age. Rats were provided with *ad-libitum* commercial pelleted feed (Nutrivet life sciences, Pune) and R.O. water. The rats were maintained as per the guidelines of Committee for Control and Supervision of Experiments On Animals (CCSEA) where environmental conditions like 22±3°C temperature and 12 hours light and dark cycle were provided. A total of 20 male and 20 female rats were randomly segregated into two groups (N=10 rats/sex/group). Rats of group I served as control (CN) were maintained at a temperature 22±3 ˚C. Group II rats were subjected to heat stress (HS) at 42 ± 1˚C temperature for 2 hrs duration, daily for 30 days. Heat stress was provided by a halogen room heater (1200 watt) set at a temperature of 42±1 ºC, controlled by a safety thermostat connected to the heater to prevent overheating. A ceiling fan provided ventilation and a humidifier maintained the relative humidity (RH) at 40-45 %.

Clinical observation

During the course of the experimentation all rats belonging to various groups were closely observed for behavioral pattern, development of any clinical signs and activity level on daily basis during and after exposure to heat stress (2 hrs duration) throughout the experimental period for 30 days.

Neurobehavioral examination (Open Field Test)

Neurobehavioral observation was done on day 30 of experiment by open field test as per the method described by Odaci *et al*. (2013) with suitable modification. The open field apparatus was made of white wood, where the floor was divided into 16 equal squares with an open top and dimensions of 100 cm x 100 cm x 30 cm. Rats were placed in the apparatus from a fixed corner and observed for 5 min for following parameters: to assess motor activity; number of squares crossed, numbers of rearings (inquisitive investigation of surroundings by rising onto its hind legs), number of central arena crossed and latency period were recorded. To assess rats' anxiety; number of defecations and urinations were also recorded. To avoid the previous animal's odor influencing the subsequent one's behaviour, the apparatus was cleaned with 70% alcohol. Mobile camera was used to record the data.

Histopathology

At the end of 30th day of experimental period, rats from each group were humanely euthanized and brain was collected in 10% formalin for histopathology. Collected tissue samples were washed overnight in running tap water then dehydrated and cleared in acetone - benzene series respectively. Then tissues were embedded in paraffin wax (58-60 ºC). Section cutting was done at 5 µm thickness and Hematoxylin & Eosin staining was performed. The entire procedure was carried out as per the method described by Gridley (1960).

Statistical Analysis

The quantitative data obtained in the study is presented in mean ± SE of the group in tabular format and subjected for one way Anova for between-group analysis.

RESULTS AND DISCUSSION

In males of heat stressed group, it was apparent from behavioral observations that all rats were restless. There was excessive running in the cage as the rats were trying to escape. Hiding behavior was exhibited as the rats were trying to cover the side of the cage with bedding material to obstruct the heat waves coming from heater. In the later phase of heat stress, rats were exhibiting dullness and depression, lying down in a supine position along-with decreased reaction upon provocation. Female rats of heat stressed group were restless and hyperactive as evident by frequent hanging on cage grill during initial period of heat stress followed by declined activity. However, they remained alert throughout the heat stress period of 2 hrs.

In open field observation, male rats exposed to heat stress (group II) revealed a significant increase in latency period along with a significant decrease in the number of boxes crossed, central arena crossed and number of rearing was noticed as compared to group I (control). The frequency of defecation (Figure 1) and urination was significantly increased in group II rats comparative to group I. Table 1 presents data of various parameters recorded during open field test in male rats on day 30 of experiment. On day 30 of experimental period, female rats did not show any significant difference in the neurobehavioural observations like latency period, number of rearings (Figure 2) number of boxes crossed, frequency of defecation and urination during open field test across the groups except there was a significant increase in number of central arenas crossed in

group II. Data of various parameters observed during open field test in female rats on day 30 of the experiment are presented in table 2.

Table 1. Neurobehavioural observation in male rats of different experimental groups on day 30 (Mean ± SE)

Parameter	Group I	Group II
No. of Defecation	00.93^{b} ± 0.40	02.73^{a} ± 0.62
No. of urination	01.28^{b}± 0.47	02.91^{a} ± 0.21
No. of rearing	07.64^{a} ± 0.84	02.06^{b} ± 0.51
No. of boxes crossed	23.00^{a} ±5.79	05.66^{b} ± 1.30
Centre arena crossed	05.00^{a} ±0.68	00.16^{b} ± 0.16
Latency (Sec)	12.33^{b} ± 0.91	46.33^{a} ± 2.96

Mean value with different superscript in row differ significantly (P<0.05)

Table 2. Neurobehavioural observation in female rats of different experimental groups on day 30 (Mean ± SE)

Parameter	Group I	Group II
No. of Defecation	00.16^{a} ± 0.16	01.00^{a} ± 0.81
No. of urination	00.76^{a} ± 0.73	01.58^{a} ± 0.61
No. of rearing	02.00^{a} ± 0.57	03.00^{a} ± 0.36
No. of boxes crossed	20.00^{a} ± 5.65	10.00^{a} ± 0.73
Centre arena crossed	05.00^{a} ± 0.68	03.16^{b} ± 0.30
Latency (Sec)	12.33^{a} ± 0.91	16.16^{a} ± 3.87

Mean value with different superscripts in row differ significantly (P<0.05)

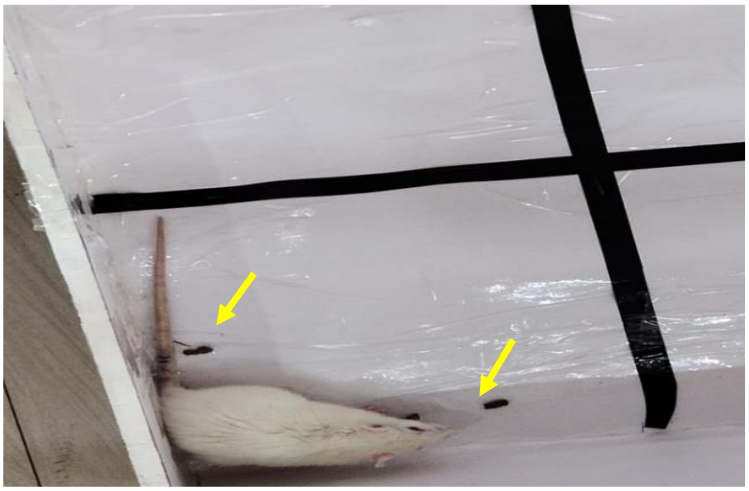

Figure 1. Anxiety in male rat (Heat-stressed group) indicated by increased defecation (arrow).

Figure 2. Rearing activity in female (Heat stressed group).

Histological examination of the brain was performed in the cerebrum and cerebellum region. The rats (male and female) of control group which were kept at 22±3°C temperature; the Cerebrum was comprised of normal cerebral hemisphere with grey and white matter. In white matter there were long radiating nerve fiber. Cerebellar cortex was organized into three layers outer molecular layer, inner granular layer and a row of piriform cells of Purkinje cell layer in-between. Group II rats subjected to heat stress (male and female) revealed several degenerative changes both in cerebellum and cerebrum. Congestion was frequently evident in choroid plexus along with exudation of fluid in the surrounding tissue (Figure 3). In the cerebrum darkening and shrinkage of neurons were observed (Figure 4). Vacuolation in white matter was clearly evident in majority of the cases. Meningeal fold was found in one of the sections. In cerebellum, reduction, darkening and shrinkage of Purkinje cells was evident (Figure 5) along with spongiosis and chromatolysis in nerve fibres (Figure 6).

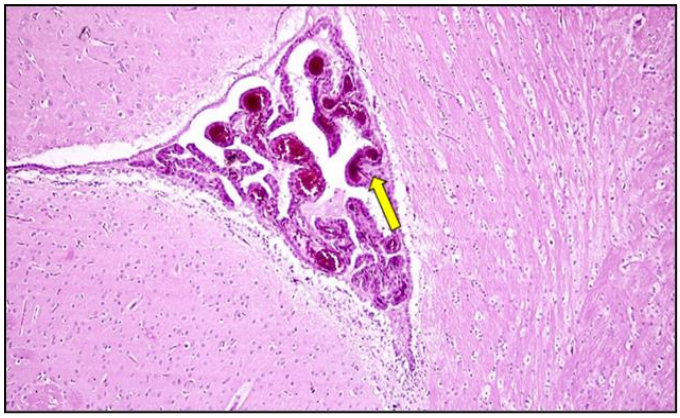

Figure 3. Microscopic section of rat brain (Heat stressed male) showing congested choroid plexus (arrow). H&E X 100.

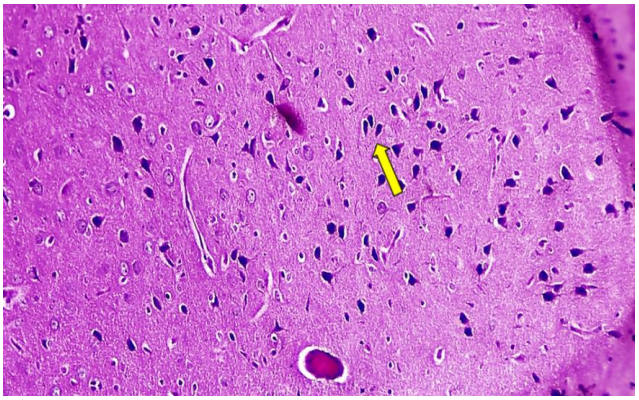

Figure 4. Microscopic section of rat cerebrum (Heat stressed male) showing darkening and shrinkage of neurons of moderate degree (arrow). H&EX 200.

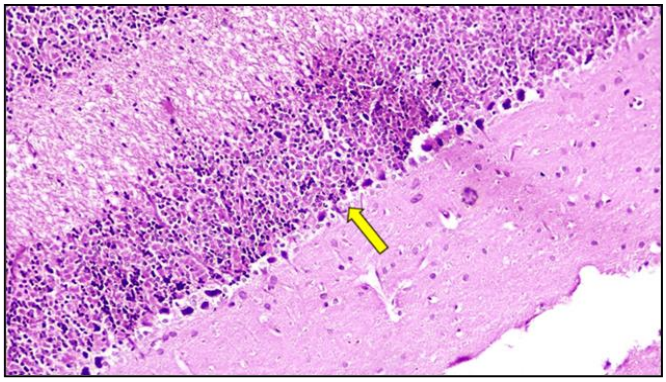

Figure 5. Microscopic section of rat cerebellum (Heat stressed male) showing dark and reduced no. of Purkinje cells (arrow). H&E X200.

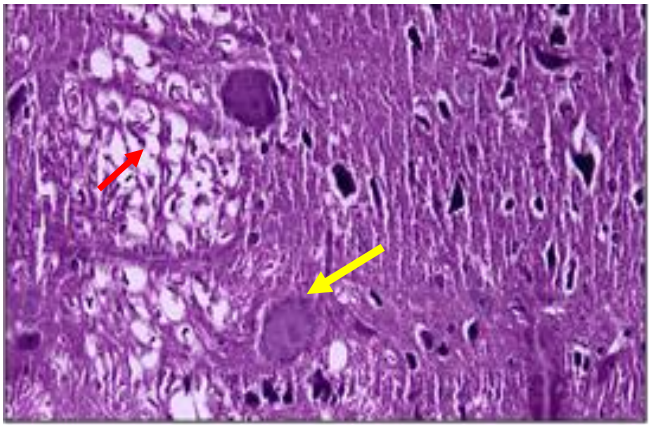

Figure 6. Microscopic section of rat cerebellum (Heat stressed female) showing spongiosis (red arrow) and chromatolysis (yellow arrow). H&E X400.

Any sort of distress in animals can be assessed through their behavioural changes. Heat stress is one of the major cause of discomfort which can be estimated through their behaviour and activity level. Rats subjected to 42 ± 1°C temperature displayed discomfort and escaping behaviour which could be due to increased core body temperature leading to hyperthermia and psychological stress. (Sinha and Ray, 2004; Agarwal and Gupta 2013 and Nakagawa *et al*., 2020). Open field test is used to observe behaviour activities, cognitive function, locomotor activities and anxiety level in rodents (Alhassan *et al*., 2016). Rats normally move at the periphery; central arena crossing and rearing mention about their exploratory behaviour. In our study heat stress has produced decrease in locomotor function, cognitive and exploratory behaviour and increased anxiety level significantly in male rats which could be due to degenerative changes in the cerebrum, hippocampus and cerebellum along with alteration in neurotransmitters (Sharma, 2006 and Nakagawa *et al*. 2020). Talking about female rats where changes are not appreciated in the parameters of open field test when compared to male rats which might be attributed to sex-specific psychological and endocrinal responses. However, further detailed hormonal and molecular-level of investigations are required to be done in larger population to confirm this perspective.

The histopathological picture reveals several degenerative changes in the brain picture. The degeneration in the brain could be due to the increased permeability of blood brain barrier and oxidative stress-induced neuronal changes (Sharma and Cervós-Navarro 1990). As a result of the increased permeability of blood brain barrier there is increased chances of neuroinflammation and cerebral oedema causing various level of brain damage (Yoneda *et al*., 2024).

CONCLUSION

In conclusion, our findings indicate that exposure of 42 ± 1°C temperature in rats has produced a significantly high level of stress, discomfort and anxiety specifically in males which was reflected in various criteria of open field test. However, females did not get impacted much as the readings of different parameters were in line with that of the control group. Comprehensive molecular and hormonal investigations are required to be done on large population to determine the cause.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ETHICS APPROVAL

The rats were maintained according to the guidelines of CCSEA where environmental conditions like 22 ± 3°C temperature and 12 hours light and dark cycle were provided. The experimental protocol was approved by institutional animal ethics committee (58/IAEC/Vety. /2023, Dated: 21.09.2023).

REFERENCES

Agarwal, S and Gupta, D. (2013). Assessment of Liver Damage in Male Albino Rats after Repetitive Heat Stress of Moderate Level. National Journal of Physiology, *Pharmacy and Pharmacology*, 3(2), 147-152.

Alhassan, A. W., El-Khashab, M. M., Saleh, M. I. A., Yarube, I. U., and Lawan, I. (2016). Effect of Ascorbic Acid on Behaviour of Wistar Rats Exposed to Environmental Heat Stress. *Bayero Journal of Medical Laboratory Science*, 1(1), 144-151.

Altan, O., Pubucuogin, A., Altan, A., Konyaliogl, S. and Bayraktar. (2003). Effects of heat stress on oxidative

stress, lipid peroxidation and some stress parameter in broilers. British Poultry Science, 44, 545-550.

Gridley, M.F. (1960). Manual of histological and special staining technique. 2nd Edn., McGraw-hill Book Company, New York.

Haveman, J., Sminia, P., Wondergem, J. and van der Zee, J., and Hulshof, M. C. C. M. (2005). Effects of hyperthermia on the central nervous system: What was learnt from animal studies? *International Journal of Hyperthermia*, 21(5), 473-487.

Horowitz, M., Argov, D. and Mizrahi, R. (1983). Interrelationships between heat acclimation and salivary cooling mechanism in conscious rats. *Comparative Biochemistry and Physiology*, 74A, 945-949.

Mete, F., Kilic, E., Somay, A. and Yilmaz, B. (2012). Effects of heat stress on endocrine functions & behaviour in the pre-pubertal rat. *Indian Journal of Medical Research*, 135(2), 233-239.

Nakagawa, H., Matsumura, T., Suzuki, K., Ninomiya, C. and Ishiwata, T. (2016). Changes of brain monoamine levels and physiological indexes during heat acclimation in rats. *Journal of Thermal Biology*, 58, 15–22.

Nakagawa, H., Matsunaga, D. and Ishiwata, T. (2020). Effect of heat acclimation on anxiety-like behavior of rats in an open field. *Journal of Thermal Biology*, 87, 102458.

Odaci, E., Ikinci, A., Yildirim, M., Kaya, H., Akça, M., Hanci, H. and Bas, O. (2013). The effects of 900-megahertz electromagnetic field applied in the prenatal period on spinal cord morphology and motor behavior in female rat pups. *Neuro Quantology*, 11(4), 573-581.

Rossati, A., (2017). Global warming and its health impact. *International Journal of Occupational and Environmental Medicine*, 8 (1), 7–20.

Sharma, H. S. (2006). Hyperthermia influences excitatory and inhibitory amino acid neurotransmitters in the central nervous system. An experimental study in the rat using behavioural, biochemical, pharmacological, and morphological approaches. *Journal of Neural Transmission*, 113, 497-519.

Sharma, H. S. and Cervós-Navarro, J. (1990). Brain oedema and cellular changes induced by acute heat stress in young rats. In Brain Edema VIII: Proceedings of the Eighth International Symposium, Bern, June 17-20, 383-386).

Sinha, R. K. and Ray, A. K. (2004). An assessment of changes in open-field and elevated plus-maze behaviour following heat stress in rats. *Iranian Biomedical Journal*, 1,127-133.

Yoneda, K., Hosomi, S., Ito, H., Togami, Y., Oda, S., Matsumoto, H. and Oda, J. (2024). How can heatstroke damage the brain? A mini review. *Frontiers in Neuroscience, 18*, 1437216.