



Research article

## Effects of elevated body temperature on structure and function of cerebrum and cerebellum of Wistar male albino rats

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<p>Article history Received: 20 Mar, 2015 Revised: 24 Apr, 2015 Accepted: 27 Apr, 2015</p>	<p><b>Abstract</b> Elevated body temperature above normal level during infection, environmental factors, and drug-treatment interfere with body function especially nervous tissues. The present study illustrates the effects of experimental hyperthermia in the brain of rat especially cerebrum and cerebellar region. Eighty fertile male Wistar rats were arranged into four groups; control, and hyperthermic-treatment groups for 14, 28, and 42 days. The body temperatures were elevated 4°C above normal body temperature by placement in a dry-air incubator at 42-43°C for 1 hour daily. At the end of treatment, the animals were sacrificed and subjected for investigations of light and transmission electron microscopy, neurotransmitters, antioxidant enzymes, malondialdehydes, caspase-7 and DNA fragmentation. The present findings revealed marked increase of oedematous lesions associated with neuronal cell damage in both brain regions. Cerebellar cortex exhibited massive degeneration of Purkinje cells and demyelination. Atrophy of mitochondria, vesiculation of rough endoplasmic reticulum and apparent loss of ribosomes. The cytopathological alterations coincides with marked depletion of neurotransmitters and antioxidant enzymes glutathione peroxidase, catalase and superoxide dismutase and increase of apoptic markers including malondialdehyde and caspase-7 and confirmed DNA fragmentation. The authors finally concluded that elevated body temperature interfered with depletion of neuronal cell function assessed by depletion of neurotransmitters and decreased antioxidant enzymes and increase cell damage. <b>Keywords:</b> Body temperature; brain; oxidative stress; electron microscopy</p>
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### Introduction

Thermoregulation is the ability of an organism to keep its body temperature within certain boundaries, even when the surrounding temperature is very different. This is a dynamic state of the stability between an animal's internal and external environment. On the other hand, hyperthermia is an abnormal elevation of body temperature, usually as a result of a

pathophysiological process, have various origins of unusual interventions (viruses, bacteria or other toxins, incompatible proteins, extended necrosis, inflammation, etc.), or might be induced by failures in the temperature-regulating system (Latchman, 1999; Milton, 1994). Eleven thousand hyperthermic stress-related deaths were reported in China and 1841 in Liverpool (Sharma, 2005). Similarly, in the Netherlands, approximately 1000 deaths due to hot

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weather in 1996 occurred in nursing homes of Rotterdam (Kunst et al., 1993; Garssen et al., 2005). Approximately 700 persons died in 1995 due to hot weather conditions in Chicago during summer months (CDC, 2003). Hyperthermia was found to induce systemic inflammatory response that leads to multi-organ dysfunction is largely dominated by severe encephalopathy (Knöchel and Reed, 1994; Bouchama and Knöchel, 1994). Hyperthermic heat deaths were found to result from cardiovascular or mental diseases, liver damage or stroke (Mastrangelo et al., 2006; Medina-Ramon et al., 2006). Heat-intense occupations such as bakers, cooks, fire-fighters, welders, ceramists and foundry workers are potentially exposed to elevated radiant heat as environmental stress factors (Figà-Talamanca et al., 1992; Tas et al., 1996). The present work aimed to illustrate the elevated body temperature on the structure and function of cerebrum and cerebellum of Wistar male albino rats.

## Materials and Methods

All experiments were conducted in accordance with the national laws for the use of animals in research and approved by the local ethical committee.

### Experimental induction of hyperthermia

The body temperatures of male Wistar rats were elevated 4°C above normal body temperature (~37.8±0.6°C and ~37.3±1.0°C, respectively) by placing in a dry-air incubator at 42-43°C for 1 hour daily for 42 days. Body temperature was monitored using a rat rectal thermistor probe. Rats were then removed from the incubator and allowed to recover at room temperature (RT). Rats were sacrificed at 14, 28, and 42 days post-treatment.

### Experimental work

Eighty fertile male albino rats of Wistar strain with average 100-110 g body weight were obtained from the Hellwan Breeding Farm (Ministry of Health, Cairo, Egypt). Free access of food and water were allowed *ad-libitum*. They were housed in good ventilation with 12 hour light and dark cycle. Rats were arranged into four groups (n=20 per each) control (G0), hyperthermic-treatment for 14 (G1), 28 (G2) and 42 days (G3). At the end of treatment, the animals were sacrificed with diethyl ether, dissected and the brain were separated and processed for the following investigations.

### Light microscopic investigations

Cerebral hemisphere and cerebellum of control and experimental groups were incised immediately, fixed in 10% phosphate-buffered formalin (pH 7.4) and processed for histological investigations. Serial 5 µm thick sections were cut and stained with hematoxylin

and eosin, examined under bright field light microscope and photographed.

### Transmission electron microscopy

Extra cerebellum specimens of both control and experimental hyperthermic-treated groups were separated and immediately fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). After rinsing in 0.1M cacodylate buffer, the specimens were postfixed in a buffered solution of 1% osmium tetroxide at 4°C for 1.5 h, dehydrated in ascending grades of ethyl alcohol, and embedded in epoxy resin. Ultrathin sections were cut with a LKB Ultratome IV (LKB Instruments, Bromma, Sweden) and mounted on grids, stained with uranyl acetate and lead citrate, and examined on a Joel 100CX1 transmission electron microscope (Musashino 3-chome; Akishima, Tokyo, Japan).

### Determination of brain neurotransmitters

Cerebrum and cerebellum of both control and experimental groups were quickly removed, rinsed in cold tris buffer and immediately homogenized in 10% ice-cold 2.5 mM-tris buffer adjusted to pH 7.5 with 1 M HCl, containing 1.0 mM EDTA and centrifuged at 14000 x g for 60 min for 5 min at 4°C. The supernatant was separated and stored at refrigerator. Catecholamines (adrenaline and nor adrenaline), serotonin (5-HT) and dopamine (DA) were determined fluorometrically as described by Schlumpf et al. (1973). GABA (γ-amino-butyric acid) was determined by high performance liquid chromatography (HPLC) using the precolumn PTC derivatization technique according to the method of Henrikson and Meredith (1980).

### Biochemical assessments of catalase, glutathione peroxidase, and superoxide dismutase

Known weights of cerebrum and cerebellum of control and experimental groups were homogenate and kept in refrigerator at -20°C. Catalase was determined according to the method of Bock et al. (1980).

### Determination of malondialdehyde and caspase-7

End product of lipid peroxidation, malondialdehyde, was determined according to the procedure of Ohkawa et al. (1979). Two hundred microliters of tissue homogenate supernatant was added to 100 µl of sodium dodecyl sulfate (SDS), 750 µl of 20% acetic acid (pH 3.5), 750 µl of 0.6% thiobarbituric acid, and 300 µl of distilled water and were incubated at 95°C for 60 min. After addition of 2.5 ml of butanol: pyridine (15:1) and 500 µl of distilled water, the solution was vortexed and then centrifuged at 2000 g for 15 min. A reddish-pink colour developed and was analyzed at 532 nm.

Caspase-7 is a member of the caspase (cysteine aspartate protease) family of proteins and was

determined by using an ELISA kit of (Uscn Life Science Inc., Wuhan, China, Cat. No.: E0449Ra). The procedure given in the brochure of the kit was followed.

### Single cell gel electrophoresis (Comet assay)

Fresh cerebral and cerebellum specimens of both control and experimental hyperthermic groups were separated and immediately stored at  $-80^{\circ}\text{C}$ . Homogenization was processed in chilled homogenizer buffer, pH 7.5, containing 75 mM NaCl 306 and 24 mM Na<sub>2</sub>EDTA to obtain a 10% tissue solution. Six microliters of homogenate was suspended on 0.5% low-melting agarose and sandwiched between a layer of 0.6% normal-melting agarose and a top layer of 0.5% low melting agarose on fully frosted slides. The slides were kept on ice during the polymerization of each gel layer. After the solidification of the 0.6% agarose layer, the slides were immersed in a lyses solution (1% sodium surcosinate, 2.5 m NaCl, 100 mM Na<sub>2</sub>EDTA, 10 mm Tris-HCl, 1% tritonX-100, and 10% DMSO) at  $4^{\circ}\text{C}$ . After 1 h, the slides were placed in the electrophoresis buffer (0.3 M NaOH, 1 mM Na<sub>2</sub>EDTA, pH 13) for 10 min at  $4^{\circ}\text{C}$  to allow the DNA to unwind. Electrophoresis was performed for 10 min at 300 mA and 1 V/cm. The slides were neutralized with Tris-HCl buffer, pH 7.5, and stained with 20  $\mu\text{g}/\text{ml}$  ethidium bromide for 10 min. Each slide was analyzed and photographed using a Leitz Orthoplan (Wetzlar, Germany) epifluorescence microscope (Sasaki et al., 1997; Robbiano et al., 2004).

### Statistical analysis

Data were presented as means  $\pm$  standard error (SE). The statistical analysis was performed with multi-variant analysis of variance (MANOVA) using the SPSS (version 13) software package for Windows. The F-test was calculated and considered statistically significant at  $P < 0.05$ .

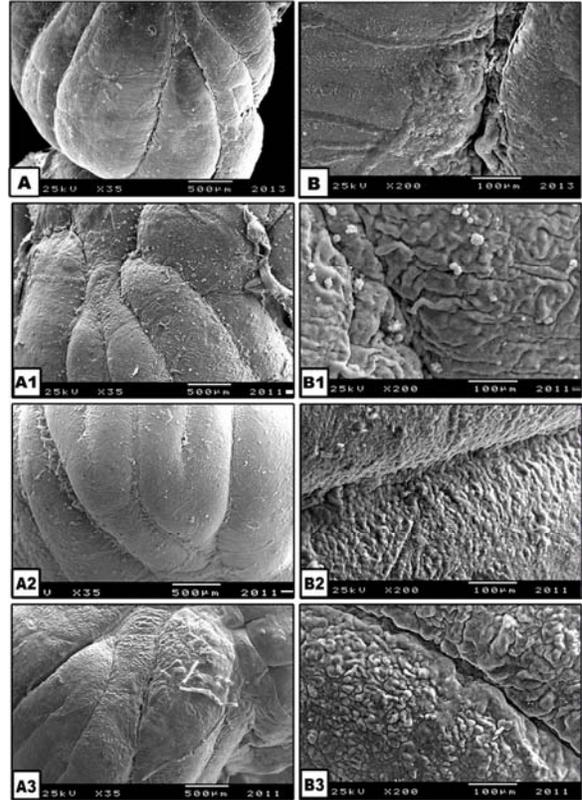
## Results

### Neurotransmitters

Adrenaline and nor-adrenaline contents of cerebrum and cerebellum markedly increased post-hyperthermic stress at 14, 28, and 42 day of treatment. However, GABA, dopamine, and 5-HT showed markedly depleted in post-hyperthermic stress. Both brain regions were equally affected by hyperthermic stress (Table 1).

### Antioxidant enzymes, caspase-7 and malonadialdehyde

Table 2 illustrates the antioxidant enzymes catalase, glutathione peroxidase and superoxide-dismutase activities as well as malonadialdehyde and caspase-7 of cerebrum post-hyperthermic stress.



**Fig. 2: Scanning electron micrographs of cerebellum of rats. A&B.**

Control. A1&B1. 14 d post-hyper-thermic stress. A2&B2. 28 d post-hyperthermic stress. A3&A4. 42 d post-hyperthermic stress. Note decreased folding of cerebellar surfaces compared with control.

Hyperthermic stress led to marked decline of the assayed enzymes activities and increased the markers of apoptic cell death including malonadialdehydes and caspase-7.

### Histological and transmission electron microscopy Cerebral hemisphere

The control cerebral cortex is complex containing different types of nerve cells including glial (GIC) and pyramidal cells (PYC). The cerebral cortex is composed of 6 cell layers, including molecular, outer granular, outer pyramidal, inner granular, inner pyramidal and multiform layer (Fig. 1A).

In experimental group subjected to hyperthermic stress for 14 days, intensive cell damaged was detected throughout the cerebral cortex. Oedema, congestion and degenerative changes in the neurons were more observed in the frontal cortex comparing with other cerebral regions. In the outer granular cell layer, a spongiform change and vacuolation of varying size in the neuroplasm was observed. Many of the neuroglial cells showed either clumping nuclear chromatin (Pyknosis) or chromatolysis (Karyolysis). In outer

**Table 1: Neurotransmitter content of cerebral and cerebellum of male rats subjected to hyperthermic stress**

	AD (ng/g)		ND (ng/g)		GABA (ng/g)		DA (ng/g)		HST (ng/g)	
	C	CE	C	CE	C	CE	C	CE	C	CE
Control	4.1±0.2	3.4±0.4	3.1±0.2	3.1±0.4	4.2±0.3	4.1±0.6	17.8±2.3	19.9±0.3	5.8±0.8	5.8±0.71
14 days	4.3±0.7**	4.2±0.6**	4.7±0.2*	4.5±0.5*	3.3±0.5*	2.9±0.2*	15.3±2.2*	13.5±0.6*	5.1±0.7	4.1±0.5*
28 days	4.6±0.4**	4.6±0.6**	4.2±0.9*	4.2±0.4*	2.9±0.7*	3.1±0.3*	13.2±1.1*	13.9±0.6*	4.1±0.6*	4.2±0.7
42 days	4.9±0.9*	5.4±0.7*	3.9±0.5*	3.7±0.5*	2.6±0.5*	3.3±0.4*	12.3±2.3*	14.2±0.5*	3.7±0.7*	4.4±0.5*

\*significance at P<0.05; \*\*significance at P<0.01; C, cerebrum; CE, cerebellum; AD, adrenalin; ND, noradrenalin; GABA, amino-butyric acid; DA, dopamine; HST, histamine.

**Table 2: Antioxidant enzymes, malondialdehyde and Caspase-7 in cerebrum and cerebellum of male rats subjected to hyperthermic stress**

	CAT (nmol/min/100mg)		GPase (nmol/min/100mg)		SOD (μmol/100mg)		MD (μmol/100mg)		Casp-7 (μg/100mg)	
	C	CE	C	CE	C	CE	C	CE	C	CE
Control	5.2±0.5	8.3±1.1	4.9±0.3	5.4±0.5	68.3±3.5	69.3±3.4	20.9±2.3	14.8±1.3	1.9±0.3	1.7±0.2
14 days	5.3±0.2	7.3±0.8	3.9±0.2**	5.1±0.6	61.2±3.8**	62.3±3.9	22.6±2.8*	17.1±1.4	2.9±0.2	2.9±0.3
28 days	3.9±0.7*	4.4±0.6	3.6±0.2**	3.4±0.3	50.9±2.3**	51.8±3.3	30.8±3.2**	24.8±2.2	3.3±0.2	3.1±0.4
42 days	2.4±0.3	2.7±0.44	3.3±0.2**	3.3±0.4	45.1±3.5**	42.8±3.3	34.6±3.2**	27.3±0.3	3.7±0.2	3.4±0.7

\*significance at P<0.05; \*\*significance at P<0.01 C, cerebrum; CE, cerebellum; CAT, catalase; GPase, glutathione peroxidase; SOD, superoxide dismutase; MD, malondialdehyde.

pyramidal cell layer, altered pyramidal cells surrounded by a necrotic zone were observed. The glial cells attained considerable atrophy. In inner pyramidal cell layer, there was marked spongiform damage and pyknotic pyramidal cells (Fig. 2 B1-B3).

At 28 days post hyperthermia exposure, a focal collection of glial cells in the outer granular cell layer was seen. In addition, in outer pyramidal cell layer, a widespread spongiform degeneration of neuropil and pyknotic pyramidal cells was found. In inner pyramidal cell layer, pyramidal cells attained considerable swelling enclosed within necrotic zone associated with glial cells (Fig. 2 C1-C3).

At 42 days exposure to hyperthermia, the outer molecular cell layer exhibited parenchymal gliosis with leukocyte infiltration. The pial arterioles exhibited arteriosclerosis characterized by thickening of the arteriole walls. The inner pyramidal cell layer revealed severe vacuolation in neuropil, and abundant pyknosis of pyramidal cells (Fig. 1 D1-D3).

### Cerebellum

Histologically, the control cerebellum consists of deeply convoluted folds called Folia which are supported with branches of central medulla of white matter. Each folium consists of an innermost white matter (cerebral medulla). The cerebellar cortex is formed of three cell layers from outwards to inwards. The first molecular cell layer forms the outermost and contains two types of nerve cells; basket cells and outer stellate cells. The axons and dendrites form the ground structural components. The second, Purkinje cells which are composed of large flask-shaped cells impregnated in fibrillar network of axons and situated between the granule and the molecular layer. Their nuclei occupied almost of their cell bodies with prominent nucleoli and very thin coat of basophilic

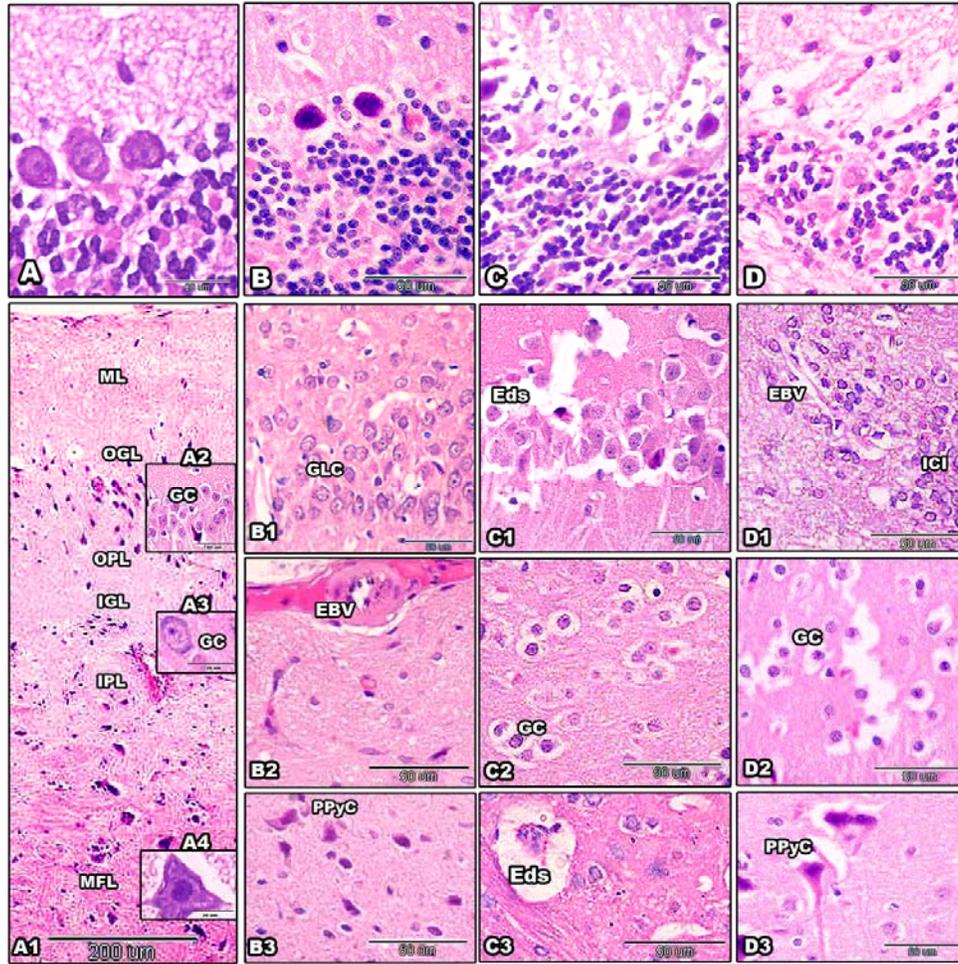
cytoplasm were clearly evident. The third is the granular cell layer which forms tightly packed small Golgi neuronal cells (Fig. 1 A).

In experimental group subjected to hyperthermia (14 days), changes were observed in spongiform in the molecular layer with characteristic darkly stained basket cells and stellate cells. The hyperthermic stress led to marked numerical reduction of the Purkinje cells. Most of them appeared abnormally shrunken and had apparent characteristic of cell death. The granular cells possessed massive reduction of their neuronal cells and glomerular spaces were markedly widened. Vacuolation and reticular degeneration of nerve fiber of white matter were also observed in between granular cell layer (Fig. 1 B).

Experimental group subjected for 28 days showed increased cerebellar cell damage. There was a massive Purkinje cell loss associated with cytological and nuclear damage. The granular cells showed apparent pyknotic cell death and increased glomerular spaces (Fig. 1 C).

In experimental group subjected for 42 days, there was a massive deterioration of the neuronal tissues. Purkinje cells were markedly altered and their deterioration was characterized by an increase of necrotic zone around them. Widened glomerular spaces aroused from necrotic areas in granular cell layer. There was a significant increase of cell damaged correlated with the intervals of the experimental work (Fig. 1 C).

At SEM level, in control, the cerebellar surface showed deep convolution with apparent blood vessel and cerebellar cortical nerves. When the cerebellar cortical surface of rats was subjected to hyperthermic stress showed comparatively decreased cerebellar folds manifested by decreased invagination and the differentiation of superficially emerging cerebellar cortical nerves (Fig. 2).



**Fig. 1: Photomicrograph of sagittal section of cerebral and cerebellar cortex of rat.**

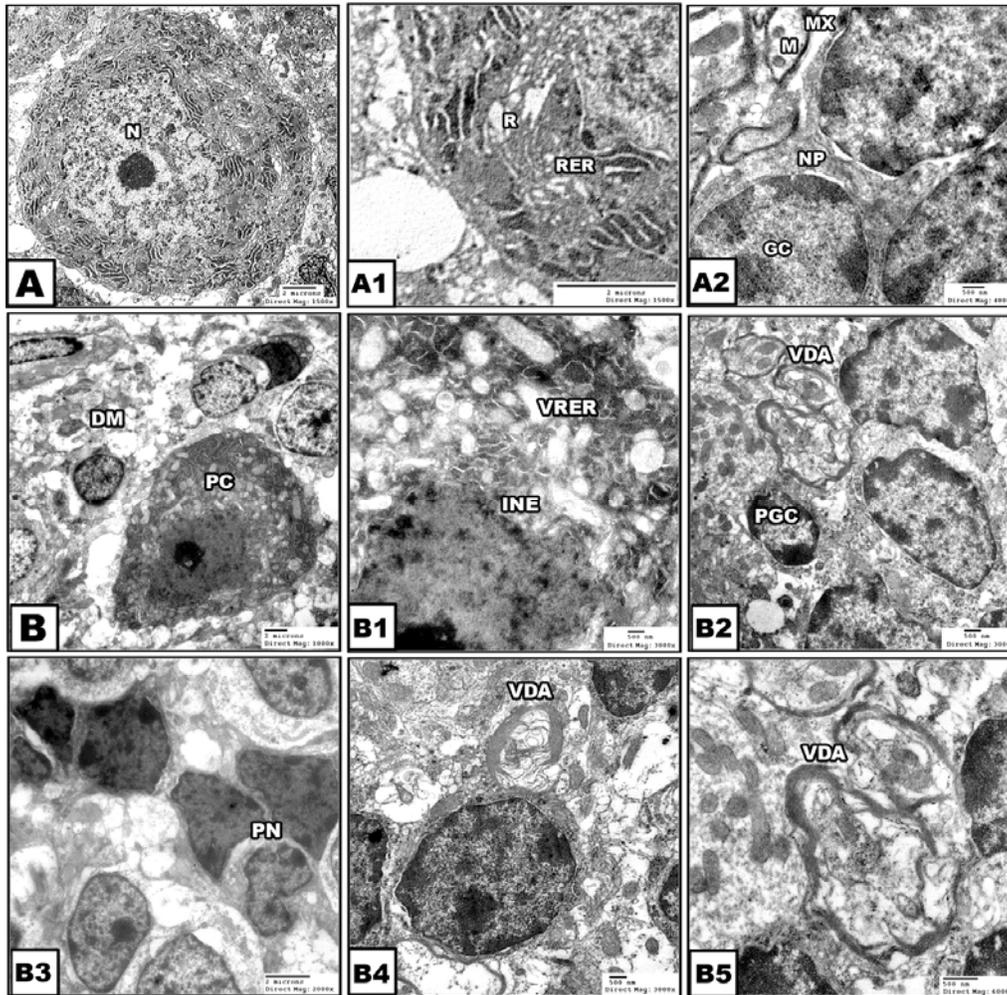
A: Control cerebellum showing normal molecular, Purkinje cells and granular layer. B: Cerebellum 14 d post-hyperthermic stress showing damaged Purkinje cells and widened spaces between degenerated granular cells. C: Cerebellum 28 d post hyperthermic stress showing widespread damage of Purkinje cells and granular cells. D: Cerebellum 42 d post hyperthermic stress showing apparent Purkinje cell loss and eosinophilic granular cells. A1-A4: Control cerebral showing normal arrangement of cell layers. B1-B3: 14 d post-hyperthermic stress showing edematous neural tissue (B1), congested blood vessel with fibrotic tunica adventitia (B2) and pyknotic pyramidal cells (B3). C1-C3: 28 d post hyperthermic stress showing vacuolar degenerated granular cells (C1-C2) and oedematous of neural tissue (C3). D-D3: 42 d post hyperthermic stress showing spongiform neural tissue and focal collection of glial cells (D1) with apparent vacuolar degeneration (D2) and pyknotic pyramidal cells (D3).

Abbreviations; ML, Molecular cell layer; OGL, outer granular cell layer; OPL, outer pyramidal cell layer; OPL, outer pyramidal cell layer; IGL, inner granular cell layer; IPL, inner pyramidal cell layer; IPL, Inner pyramidal cell layer ;GL, granular layer; PC, Purkinje cells; PPC, Pyknotic Purkinje cells; MFL, Multiform cell layer ; IGL, Inner granular cell layer; GLC, glial cells ; PYC, Pyramidal cells.

At TEM level, the Purkinje cells showed normal morphological characteristic features. Their cytoplasm appeared rich in dense regularly arranged rough endoplasmic reticulum, filamentous mitochondria with well developed cristae and Golgi apparatus. The nucleus occupied the main part of the cell body and bounded by double walled nuclear membrane and formed mainly of nuclear membrane with centrally situated nucleolus. Small tufts of heterochromatin were regularly arranged on nuclear membrane. The neuronal axon exhibited the presence of dense myelinated sheath.

The granular cells are densely packed with centrally located nuclei occupied almost total of the cell body (Fig. 3 A-A2).

In experimental hyperthermic stress, especially post 42 days, a massive deterioration of cerebellar cortex cells was detected. The Purkinje cells become shrunk and possessed abnormal ultrastructural features. The cytoplasmic organelles were disorganized. The rough endoplasmic reticulum appeared either fragmented or vesiculated and irregularly distributed throughout the cytoplasm. The mitochondria were



**Fig. 3: Transmission electron micrographs of cerebellar cortex.**

A.A1: Control showing normal Purkinje cells (A), with regular arrangement of rough endoplasmic reticulum, mitochondria and ribosomes (A1). A2: Normal ganglion cells spread in between myelinated axons. B-B5: 42 d post hyperthermic stress showing damaged Purkinje cells with degenerated mitochondria and vesiculated rough endoplasmic reticulum. Granular cells appeared with either pleomorphic (B2) or pyknotic nuclei (B2&3). Demyelination of axons is seen (B5).

Abbreviations; PN, pyknotic nuclei; VDA, Vacuolar degenerated axons; DM, degenerated mitochondria; PC, Purkinje cells; INE, irregular nuclear envelope; N, nucleus; M, mitochondria, MA, Myelinated axons.

swollen and lacked differentiation of their internal compartments. The synaptic processes and cell membrane were degenerated. The Golgi apparatus was abnormal elongated and hypertrophied. The neuronal axons showed a marked depletion of their myelinated coat. Some of the neuronal axons showed complete demyelination (Fig. 3 B-B5).

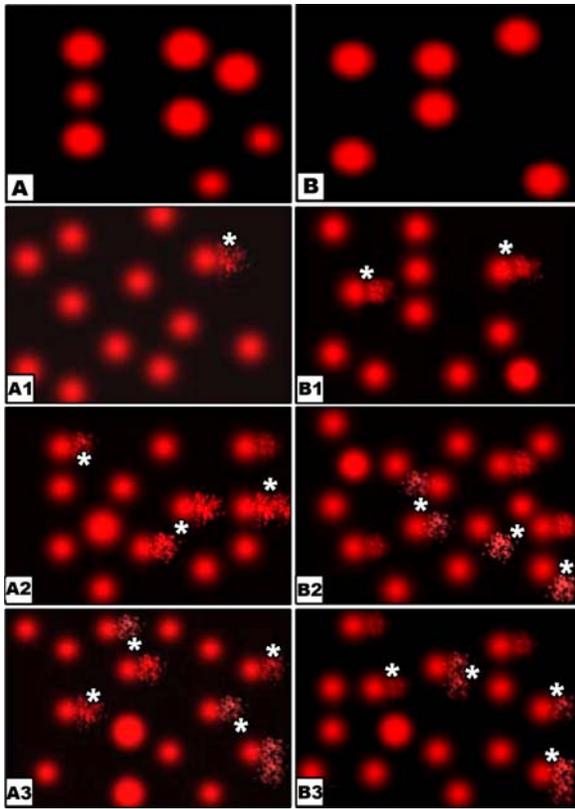
#### Comet assay

Figure 4 illustrates the cerebral and cerebellar cell DNA damage post-hyperthermic stress. The genomic expression of the degree of laddering increased during the intervals of hyperthermic stress. The single strand nucleotide each brain cells were detached with increased tail length and DNA

concentration compared with normal pattern structure of control.

#### Discussion

Hyperthermia is a state of thermoregulatory failure resulting from the inability to dissipate heat at a sufficient or excessive heat production with a normal rate of heat loss. Dehydration, the most common cause of hyperthermia, leads to vasoconstriction and decreased sweating, which impair heat dissipation causing a rise of body temperature. Hyperthermia may coexist with fever: hyperthermia, caused by dehydration, may occur on top of fever due to infection, or a febrile seizure caused by infection may lead to



**Fig. 4: Comet assay of cerebrum (A-A3) and cerebellum (B-B3).**

A&B. Control. A1&B1. 14 d post-hyperthermic stress. A2 &B2. 28 d post-hyperthermic stress. A3&A4. 42 d post-hyperthermic stress. Note detached neuronal cells (\*) manifested damage post-hyperthermic stress. X 400

hyperthermia from intense muscular contractions (Peterka et al., 1994), sauna (Hamilton, 1985) and hot tub paths (Chambers, 2006) represent the modern uses causing elevation of body temperature.

Cerebrum and cerebellum of rats subjected to hyperthermic stress for 14, 28, and 42 days led to marked increase of adrenaline and nor adrenaline. Similar findings were reported by Williams et al. (1985) who reported elevation of norepinephrine levels above epinephrine by a 4:1 ratio early in the pig subjected to inhalational anesthetics or the administration of depolarizing muscle relaxants such as succinylcholine.

On the other hand, the observed neurotransmitters GABA, DA, and 5-HT showed marked depletion in post-hyperthermic stress condition. Similar core temperature was significantly elevated ( $>$  or  $= 2^{\circ}\text{C}$ ) in mice treated with doses of methamphetamine which produced  $>$  or  $= 90\%$  losses in striatal dopamine but not in mice less severely affected (Albers and Sonsalla, 1995).

Gong et al. (2012) found that mitochondrial malondialdehyde level significantly increased in the cerebral cortex in mild hypothermia. Moreover, mild hypothermia attenuated the reduction in Complex I and Complex III (i.e., major sites of reactive oxygen species production) activities of the mitochondrial respiratory chain.

From the present findings, hyperthermic stress was found to decrease the activities of catalase, glutathione peroxidase and superoxide-dismutase and increased the markers of apoptic cell death including malondialdehyde and caspase-7. Mitochondrial dysfunction and oxidative stress are considered to be key determinants with respect to the extent of injury during cerebral ischemia (Chan, 2005). Impairment of mitochondrial function leads to reduced ATP production, impaired calcium buffering and, in particular, the overproduction of reactive oxygen species (ROS). Under physiological conditions, ROS do not cause injury because they are quickly scavenged by the intramitochondrial antioxidant system which are regulated by nuclear factor erythroid 2-related factor 2 (Nrf2), a key nuclear transcription factor in maintaining redox balance (Jaiswal, 2004). Under pathological conditions, ROS are excessively produced, and react with nitric oxide (NO) to produce reactive nitrogen species (RNS). The products exceed the scavenging capacity of the endogenous antioxidant system in mitochondria, and consequently oxidative stress occurs. As a result, the excess production of mitochondrial ROS and RNS can damage mitochondria by initiating peroxidation of intramitochondrial lipids and proteins, inhibiting the activity of mitochondrial respiratory enzymes and breaking mitochondrial DNA (Szabo, 2003), which induces cell apoptosis or necrosis (Chan, 2005).

From our findings, rats subjected to hyperthermic stress for 14, 28, and 42 days, caused oedematous lesions, congestion and degenerative changes in the neurons of the frontal cortex compared with other cerebral regions of cerbrum. Further, in the outer granular cell layer, a spongiform change and vacuolation of varying size in the neuroplasm was observed. Many of the neuroglial cells showed either clumping nuclear chromatin (Pyknosis) or chromatolysis (Karyolysis). There were focal collections of glial cells in the outer granular cell layer and a widespread spongiform degeneration of neuroplasm and pyknotic pyramidal cells with the advancement of hyperthermic stress. Hyperthermia-induced neuronal death depends on duration of exposure. Severe hyperthermia can produce necrotic neuronal death. For a window of less severe heat stresses, cultured neurons was found to exhibit a delayed death with apoptotic characteristics including cytochrome c release and caspase activation (White et al., 2007).

From the present findings, the hyperthermic stress caused cerebral and cerebellar cell death coincided with increased malondialdehyde and caspase-7. In the cell death program, cells induce signalling pathways involving the coordinate action of multiple kinases and cysteine proteases, known as caspases, which cleave various target substrates, bringing about the cell's own demise (Dorstyn et al., 1998; Earnshaw et al., 1999; Wolf et al., 1999). One of the characteristic morphological changes associated with apoptosis is the cleavage of DNA, which can be detected in situ by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) method (Gavrieli et al., 1992). Apoptosis is distinct from necrosis, which is a pathological form of cell death in response to extreme trauma or environmental disruption. Morphological alterations that are associated with necrosis include cell swelling, degeneration of organelles, membrane disruption, and cell lyses, causing inflammation (Buja et al., 1993).

Finally the authors concluded that treatment of elevated body temperature should be done immediately to prevent neuronal cell function and damage.

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