

Effect of Hyperthermia at Different Ages and Mode of Recovery on the Chromosomal Aberrations and Biological Parameters in Female Rats.

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Abstract: The present investigate the various biological changes induced by hyperthermia (at 42°C) in female rats and the mode of recovery at 1, 6, 24 & 72 hrs at different ages (2, 6, 12 & 24 months). Biological parameters studied were red blood cells (RBCs), white blood cells (WBC's), hemoglobin (Hb), B% & T% lymphocytes. Immunoglobulin G & A (IgG & IgA) and serum activities of triiodothyronin and thyroxin (T₃ & T₄), the heat shock protein 70 (HSP70). Besides, the chromosomal aberrations test and micronucleus formation were investigated in female rats. In attempt to find out the interaction between age and hyperthermia in such parameters in normal female rats. The results revealed that Highly significant increases of WBC's, B%, IgG and HSP70 at 1 till 72hr post WBH in aged 2 and 6 months. On the other hand, WBH caused a significant decrease in each RBC's, T₃ & T₄ at 6 till 72 hr post WBH. As well as, the count of Hb decreased in age 2 month at 1 till 72 hr post the heat exposure but increased at 1 & 6 hr in 6 month aged post WBH then decrease at 24 hr & 72 hr post WBH. T% lymphocyte count significantly decreased at 1 hr post WBH and increased at 6 hr & 24 hr then decrease again at 72 hr post WBH in ages 2 & 6 month. IgA level significantly increased in 6 aged rats at 1, 6 & 24 hr post WBH then decreased at 72 hr below the control value post WBH. The results revealed that WBH caused a significant increase of B% lymphocyte, Hb and IgA at 1 & 72 hr post WBH in age 12 & 24 months, except Hb in 12 month decreased at 72 hr post heat exposure. On the other hand, T% lymphocyte, RBC's, IgG and serum T₃ & T₄ decreased at 1 & 72 hr post heat exposure except IgG level increased at 72 hr post WBH. The level of HSP70 increased significantly at 1 till 24 hr post WBH in 12 month and reached to the control value at 72 hr post WBH. On the contrary, HSP70 decreased significantly at 1hr in aged rats (24 month), then increased significantly at 6 and 24 hr post heat exposure then decreased below the control value at 72 hr post WBH. In the chromosomal aberrations test, we observed positive responses at all ages but in different frequencies and recover may occur at 72 hr for the all except young age (2 month) which needed more time to completely recover., in the micronucleus test, we observed positive responses in all ages at 24hr only, while at 72hr post heat exposure the mean frequencies of micronucleated polychromatic erythrocytes (MNPCEs) were within the vehicle control group at all ages except 2 month which increased significantly than control group. The results suggest that hyperthermia can induce both chromosomal aberrations and micronucleus formation. [Journal of American Science 2010;6(4):153-166]. (ISSN: 1545-1003).

Key words: Hyperthermia –HSP70, chromosomal aberration –micronucleus.

1. Introduction

Whole body hyperthermia (WBH), as a treatment modality, shows promising results in the management of advanced and refractory cancer in combination with chemotherapy (Ismail- Zade et al. 2002). Stress is a sudden environmental change that induces damage at the molecular, cellular and organismal level (Soti and Peter, 2007). Macromolecules such as proteins are continuously exposed to potential damaging agents that can cause loss of molecular function and depletion of cell populations over the lifetime of essential organs. One of the key homeostatic responses involved in maintaining longevity is the induction of heat shock proteins (HSPs), a conserved reaction to damaged intracellular proteins (Calderwood et al. 2009) Damage to macromolecules is characteristic of aging and degenerative diseases. Beyond DNA damage, protein

damage may not only a consequence, but also a causal factor in cellular malfunction. Damage may induce misfolding, and the aggregating oligomeric species may gain a novel toxic property, severely compromising cellular function (Dobson, 2003). The relationship between thermal resistance and expression of inducible heat shock proteins, especially Hsp70, depends on the species and temperature treatments (Bahrndorff et al. 2009). The major stress of life is oxidative stress. Thus, it is not surprising that oxidized protein level increases with aging of all animal species (Stadtman and Stadman, 2004).

The heat shock response is a highly conserved "stress response" mechanism used by cells to protect themselves from potentially damaging insults. It often involves the upregulated expression of chaperone and Hsps to prevent damage and aggregation at the proteome level (Michael et al. 2007). Induction of Hsp

expression appears to correlate with a cytoprotective effect in cultured cells and with improved healing of damaged tissues in animal models and in humans. This family of proteins can also serve as indicators of thermal stress (Rodwell et al. 2008). Area of focus has been the family of highly conserved stress proteins known as heat shock proteins (HSPs). Some of the well-studied Hsps in mammals are those with molecular masses of 70 kDa, and these ubiquitous proteins include the highly heat inducible 72 kDa protein (Hsp70). There is substantial evidence showing that Hsp70 plays a critical role in providing cellular protection against the adverse effects of a wide array of stress and toxic conditions (Kregel, 2002). Interestingly, investigators have also shown that the ability to induce Hsp70 under a variety of physiologically-relevant conditions, including hyperthermic challenge, is reduced with aging (Bonelli et al.1999; Jin et al. 2004, Kregel et al. 1995 and Vasilaki et al. 2002). Hsp70 appears to be regulated at both transcriptional and translational levels (Chandolia et al. 1999 and Vivinus et al. 2001). Zhang et al. 2003 demonstrate that the reduction in stress tolerance that accompanies aging is associated with a complex set of integrated alterations in hepatic steady-state levels of reactive oxygen species (ROS), macromolecular damage, redox buffering, and transcription factor regulation. Heat stress proteins (HSPs) are induced by a variety of stimuli. They contribute to maintaining the metabolic and structural integrity of the cell, as a protective response to external stresses. A raised body temperature raises the metabolic rate and makes the immune response more efficient (Broom, 2007). Levels of protein Hsp70 were not detectable in the control treatment and 2 h after hardening treatment. However, levels of Hsp70 gradually increased thereafter and at 4 and 6 hr after the hardening treatment expression was elevated but with large variation (Bahrndorff, 2009). Several authors reported either a decrease or no change in the number of RBC, s in animals subjected to high environmental temperature (Terajima et al. 2000).As well as these authors reported the destruction of red blood cells at 42°C. Lee and Korean (1987) observed a slight decrease in the Hb content of rabbits exposed to heat stress (37 – 80% RH) for 2- 4 hrs daily for two weeks. They also found a decrease in total leukocytes count and a significant decrease in lymphocytes without a statistically significant change in monocyte count. Whereas, Kamal et al. (1992) reported a significant increase in WBC' s count in heat stressed mature rats. Mostafa et al. (2005) reported that WBH caused significantly decrease in T3 and T4 hormones in rabbits after immediately heat exposure and after 24 hrs post WBH. Furthermore, they observed that WBH caused a significant decrease below the control values in each of RBC' s, Hb and

total WBC's counts. Following after the 24 hrs recovery period. The WBC's was significantly higher while T₃, T₄, RBC's , Hb and lymphocytes values were significantly lower than in the acute WBH group.

Hyperthermia exerts numerous effects on mammalian cells including division delay, cell killing and chromosomal aberrations when cells are heated in the S-phase (Dewey et al. 1990 and 1978). Also, Takahashi et al. (2004) suggested that heat-induced DNA double strand breaks contribute to heat-induced cell killing because heat treatment induces histone γ H2AX-containing foci. Such foci have been associated with double strand breaks induced by ionizing radiation, other agents, and other stresses. (Capetillo et al. 2004). DNA synthesis is inhibited by heat, dependent on the time and temperature of heating (Warters et al. 1984).

2. Material and Methods

Two hundred and forty female albino rats (*Rattus rattus*) at 2, 6, 12 and 24 months of age were purchased from the animal house of the National Research Center, Dokki, Egypt. Food and water were provided *ad libitum* under standard condition of light, humidity and temperature. After a period of acclimation the room environmental temperature and relative humidity (RH) will be recorded twice daily using Thermohygrometer.

Whole body hyperthermia Treatment: (WBH):

A plastic cage (45 x 25 x 20 cm), conscious and unrestrained. Rectal temperature (Tr) was continuously monitored on a digital display) was established over a 30-min control period for each rat, followed by a heating protocol. An infrared lamp was positioned 40 cm above each rat and either raised or lowered to obtain an ambient temperature of 42°C. Movement of the lamp permitted a constant heating rate (0.06 °C/min) to be attained. Heating was terminated when rectal temperature (Tr) reached 42°C, but was then commenced at an appropriate time to maintain Tr at 42°C for one hour. At the end of this period, the thermistor probe was removed and rats were allowed to passively cool in a cage at room temperature. The (Tr) of the rats will be recorded using a thermocouple (Cole Parmer type T. thermocouple thermometer) connected with a rectal probe, which will be inserted 8 cm beyond the anal sphincter. When Tr reaches (42 C), directly blood samples will be collected from **orbital venous plexus of experimental animals into fresh heparinized tubes**. The animals will be allowed recovering at room temperature and blood samples will be taken after 1,6,24 and 72 hr post WBH. Some biochemical parameters, RBC' s and WBC' s counts were determined using a haemocytometer. Hb

concentration was measured by the Cyanmethemoglobin method, B% & T% lymphocytes. IgG & IgA by ELISA kit from Life Diagnostics and plasma activities of total T₃ & T₄ were measured with radioimmunoassay kits (MP Biomedicals, Eschwege, Germany). Besides, the HSP70 by ELISA kit from Stress Biotechnologies were investigated in female rats.

Bone marrow chromosomal aberrations assay:

After heat exposure, the animals were injected intraperitoneally (i.p) with 0.5 ml colchicines (0.5% mg/kg body weight) one hour and 30 minutes before killing to block the cells in metaphase. Animals were sacrificed and chromosomes of bone marrow were prepared by using methodology of **Yosida and Amano (1965)** with slight modifications. The cells in both femurs were collected by pushing 2-3 times of 0.075M KCL into the marrow cavity, left in hypotonic KCL for 35 min. and then centrifuged at 1000 rpm for 8 min. The supernatant was removed and the pellet was resuspended and fixed in methyl: acetic acid (3:1). Centrifugation and fixation were repeated five times at 20 min. intervals. The pellet was resuspended in a small volume of fixative. Finally, the cells were dropped on a clean wet slide and stained with 5% Giemsa stain. 50 metaphases examined from each animal under 1000X magnification to determine the frequencies of chromosome damage cells. The classification of aberrations was carried out as described by **Venitt and Parry, 1984** and in the international system for Cytogenetic Nomenclature (ISCN) (**Cohen et al. 1993**).

Bone marrow micronucleus assay:

The micronucleus test was performed according to **Schmid (1975) and Heddle (1973)**. After heat exposure, animals were killed by cervical dislocation. Both femurs were dissected and bone marrow was flushed from the femoral cavity with fetal calf serum and the cells were centrifuged at 1000rpm for 5 min. The pellets were suspended and smeared on a clean dry slide. The slides were fixed by methanol and stained with Giemsa stain. The micronuclei (MN) were analyzed under a microscope with 1000X magnification using oil immersion. About 1000 polychromatic erythrocytes (PCEs.) were scored for each treated animal and control. To count the number of micronucleated polychromatic erythrocytes (MNPCEs).

Statistical analysis:

All results are expressed as mean \pm SEM. The statistical analysis was carried out with Duncan's multiple range test. A $P < 0.05$ was considered the level of statistical significance.

3. Results

Results of the present study are presented in tables (1-4) and (fig. 1-11), demonstrated the effect of WBH 42°C in young, middle and aged rats (2, 6, 12 and 24 months) on hematological indices which manifested a significant decrease of WBCs in the 6 months, rats before heat value of WBC's increased significantly after 1 hr post WBH in all groups. The highest value was showed in 2 month old rats. The count of WBC's reached to the control values at 24 & 72 hr post WBH in 12 month old rats. On the contrary, reached below the control values in 24 month old rats as shown as in fig (1). The data demonstrated in fig (2) shows the count of RBC's decreased significantly in all different ages' rats at 1 hr till 72 hr post WBH except in 2 and 24 months' rats. The count of RBC's in that both groups increased significantly at 24hr and 6hr respectively post WBH. Fig (3) shows the count of T% lymphocytes decreased significantly in all groups after 1 hr post WBH. This decrease was continues till the end of experiment 72 hr in 24 months group but in 2 & 6 month old rats, the count of T% lymphocytes increased significantly at 6 & 24 hr post WBH, then decrease at 72 hr post heat exposure (Table 1a). On the other hand, WBH caused significantly increased of B% in all experimental groups at 1hr till 72 hr post WBH. The maximum increase was recorded in 12 month old rats at 6 & 72 hr post heat exposure Fig (4).

Fig 5 & table (1a) illustrated that WBH revealed significant increase of IgG in 2 & 6 month old rats but decreased significantly in 6 & 24 month old rats after 1 hr post WBH. Then IgG values showed increase at 6 & 24 hr after heat exposure in aged rats (24 month). This parameter showed fluctuates down and up in 12 months old rats (Fig 5). WBH significantly increased of IgA in rats 6, 12 & 24 months at 1 till 72 hr post heat exposure, the only decrease showed at 72 hr post WBH in 6 month old rats. Fig 6 & table 1b shows the value of IgA in the group 2 month old rats was significantly higher than that in 6, 12 & 24 month old rats by 30.08%, 13.69% and 29.12% respectively (Table 2). The results revealed that WBH increased significantly of Hb count as compared to control values in 6, 12 & 24 months at 1 hr post heat exposure ($13.76 \pm 0.61, 12.23 \pm 1.55$ and 14.34 ± 0.40 respectively). While Hb content showed a significant decrease in 2 month old rats at 1 till 72 hr post WBH. However, the increase of Hb level in aged rats (24 months) continued at 1 till 72 hr post WBH, decreased at 72 hr in 6 & 12 months (Fig. 7). In the present study, WBH caused a significant increase of HSP70 at 1hr post WBH in 2 month old rats and this increase was higher than that of 6, 12 & 24 months by

49.71%, 20% & 78% respectively (Table 1b) . Then HSP70 decreased at 6, 24 & 72 hr post WBH in 2 month old rats group, 1.26 ± 0.43 , 2.46 ± 0.99 and 1.40 ± 0.35 respectively Table(1). This decrease was lower than that in 12 month by 139.68%, 30% & 87.14% respectively, but still higher than that control value (Fig 8). The highest decrease of HSP70 was recorded at 6 hr post WBH in aged group (24 month), this decrease was 188.89% lower than that 2 month old rats. Moreover, (Fig 9 & 10) shows WBH caused significant decreases of T_3 & T_4 in all groups at 1 hr till 72 hr post heat exposure except in 2 & 6 months showed that T_3 approached to the control values at 72 hr post WBH (Table 1b).

- Effect of WBH on chromosomal aberrations of bone marrow cells:

Our experiment was demonstrated the effect of hyperthermia on female rats at different ages (2, 6, 12 and 24 months) and different times (1, 6, 24 and 72 hrs after heat stress). These data are presented in table (3). It can be seen that heat induced various types of structural chromosomal aberrations which consisted of gaps, breaks, deletions, fragments, centromeric attenuations and endomitosis. Numerical aberrations resulted in hypoploidy and hyperploidy only. When heat was applied on rats at 2 month of age, mean values of total chromosomal aberrations were statistically increase ($P < 0.05$) in animals sacrificed 1, 6, 24 and 72 hrs after heat stress (19.0 ± 0.41 , 29.50 ± 0.29 , 27.50 ± 0.29 and 19.75 ± 0.48 respectively) than those of control (11 ± 0.41). While 72 h group statistically decreased than 6 and 24 hrs groups means that at 72 hr the aberrations begin to recover. Also, the number of abnormal metaphases significantly increased in all groups than control.

In female rats at 6 month of age, there were significant increases in the total chromosomal aberrations of animals sacrificed 1, 6, 24 and 72 hr (18.50 ± 0.50 , 30.0 ± 0.0 , 21.75 ± 0.25 and 10.75 ± 0.25 respectively) than control (9.75 ± 0.63). The value observed 72 hr after heat exposure was within the vehicle control group and thus not considered of biological significance. Abnormal metaphases significant increased than control in all groups except 72 hr post WBH in which the increasing had no significant.

The data obtained from female rats at 12 month old rats recorded that there were significant increase in the total chromosomal aberrations of rats sacrificed 1, 6 and 24 hours after heat shock (24.50 ± 0.50 , 34.0 ± 0.41 and 28.75 ± 0.48 respectively) when compared with control (13.50 ± 0.29). While, there was non-significant difference between the frequencies of the total aberrations induced by heat at

72 hr (12.0 ± 0.41) and that observed in the control. The abnormal metaphases decreased at 72 hour group and returned to the control.

In addition, heat caused significant increase in the mean values of total chromosomal aberrations of 1, 6 and 24 hrs (31.0 ± 0.41 , 32.50 ± 0.29 and 30.50 ± 0.29 respectively) than those of control (12.0 ± 0.41) at 24 month of age. While between these groups there was no significance. In contrary, 72 hr (14.50 ± 0.50) showed significant decrease in the frequencies of total aberrations than those of other groups and this value was within the vehicle control group and thus not considered of biological significance. Abnormal metaphases reached the control value at 72 hr after treatment.

At 1 hr after heat shock, there were no significance differences between 2 and 6 month of age in the total chromosomal aberrations, while between these and other two groups there were statistically significant difference. 6 h after heat stress show no significant differences between all ages. Also 24 hr after heat had no significant between all groups except 6 month of age this significantly decreased than other groups, so it is demonstrated that young age (2 month) more affected than adult (6 month) which have less frequencies than old age (12 and 24 months). 72 hr showed that there were no significant differences between all ages except 2 month old rats which increased statistically than other groups. Means that young age need more time than other groups to recover. The number of abnormal metaphases increases with increasing the age and begin to recover at 72 hr after heat stress in all ages except 2 month old rats which need more time to complete recover.

Effect of WBH on micronucleus formation of bone marrow cells:

Hyperthermia was tested for induction of micronucleus in bone marrow of female rats (table 4) and (Fig. 11). The number of MNPCEs unaffected with hyperthermia at 1 and 6 hours after heat stress and thus none statistically significant than control and this in all ages (2, 6, 12 and 24 month of age). At 24 hr after heat stress, the number of micronucleus was statistically significant increased ($P < 0.05$) for 2, 6, 12 and 24 months of age (6.75 ± 0.48 , 4.0 ± 0.41 , 9.0 ± 0.41 and 12.0 ± 1.23 respectively) than those of control (2.0 ± 0.41). Also, there were significantly decrease in the number of MNPCE's at 6 month of age than the all. At 72 hr post treatment, the mean frequencies of MNPCE's at 6, 12 and 24 months of age (2.0 ± 0.41 , 2.25 ± 0.25 and 2.75 ± 0.48 respectively) were within the vehicle control group (2.0 ± 0.41). On the other hand, there was a significant increase in 2 month old rats (4.50 ± 0.65) than control group (2.0 ± 0.41).

Table 1a: Effect of WBH at 42 °C and its late effects on various biological parameters in 2,6,12 and 24 months old rats.

Age	Time	Parameters				
		WBC's (X10 ³ /cmm)	RBC's (X10 ⁶ /cmm)	T lymphocytes (%)	B lymphocytes (%)	IgG (ng/ml)
2 month	Control	5.36±0.50 ^{efg}	3.46±0.18 ^{bc}	23.62±1.01 ^{fg}	16.59±0.51 ⁱ	826.60±21.49 ^h
	1 hr	14.51±0.50 ^a	2.01±0.15 ^g	23.15±1.06 ^{efgh}	21.31±0.92 ^{ghi}	1436.40±69.68 ^b
	6 hr	6.41±0.51 ^{defg}	2.56±0.09 ^{ef}	25.50±0.57 ^{ef}	32.54±1.20 ^b	1078.50±16.81 ^{fg}
	24 hr	8.88±0.58 ^c	3.94±0.24 ^a	26.91±0.50 ^{de}	33.48±2.81 ^b	1233.40±48.86 ^{cdef}
	72 hr	6.67±0.45 ^{defg}	2.42±0.12 ^{efg}	20.31±0.41 ^h	26.73±1.43 ^{defg}	1315.60±69.81 ^{bcd}
6 months	Control	4.99±0.07 ^g	3.88±0.16 ^{ab}	28.08±1.9 ^{bcd}	17.10±0.65 ⁱ	813.10±11.64 ^h
	1 hr	7.54±0.71 ^{cd}	2.02±0.05 ^g	20.30±0.76 ^h	27.33±2.41 ^{bcd}	942.60±19.58 ^{gh}
	6 hr	6.78±0.46 ^{def}	3.13±0.11 ^{cd}	31.08±0.69 ^{ab}	26.27±1.59 ^{efg}	957.70±32.80 ^{gh}
	24 hr	10.94±0.77 ^b	2.50±0.08 ^{ef}	30.14±1.38 ^{abc}	28.44±1.73 ^{bcd}	1382.50±53.70 ^{bc}
	72 hr	11.61±0.70 ^b	2.31±0.14 ^{fg}	27.19±1.41 ^{cde}	31.89±1.72 ^{bcd}	1717.80±49.70 ^a
12 months	Control	7.87±0.63 ^{cd}	3.85±0.16 ^{ab}	23.95±1.14 ^{fg}	23.82±1.69 ^{efgh}	1226.20±35.95 ^{cdef}
	1 hr	8.85±0.48 ^c	2.53±0.12 ^{ef}	21.44±0.80 ^{gh}	29.32±1.53 ^{bcd}	1153.40±79.47 ^{ef}
	6 hr	13.99±0.47 ^a	3.17±0.19 ^{cd}	22.85±0.53 ^{efgh}	42.69±4.33 ^a	1304.10±80.79 ^{bcd}
	24 hr	7.78±0.39 ^{cd}	3.14±0.17 ^{cd}	30.56±1.40 ^{ab}	19.78±1.09 ^{hi}	1203.50±80.74 ^{def}
	72 hr	7.58±0.51 ^{cd}	3.19±0.22 ^{cd}	27.35±0.92 ^{cde}	43.19±1.56 ^a	1308.00±47.03 ^{bcd}
24 months	Control	11.33±0.38 ^b	3.73±0.09 ^{ab}	31.67±0.39 ^a	27.12±1.55 ^{cdefg}	1179.00±44.73 ^{def}
	1 hr	5.05±0.24 ^{fg}	2.06±0.05 ^g	22.55±0.68 ^{efgh}	33.29±1.86 ^{bc}	941.50±28.37 ^{gh}
	6 hr	13.55±0.98 ^a	4.16±0.09 ^a	22.64±0.43 ^{efgh}	27.63±2.60 ^{bcd}	1350.00±70.73 ^{bcd}
	24 hr	11.35±0.79 ^b	3.49±0.14 ^{bc}	28.74±0.56 ^{abcd}	39.19±1.15 ^a	1309.00±48.79 ^{bcd}
	72 hr	6.83±0.38 ^{de}	2.78±0.11 ^{de}	28.57±1.39 ^{abcde}	30.91±1.67 ^{bcd}	978.20±41.58 ^{gh}

Data were expressed as mean ± S.E

Means with different superscript letters are significantly different (P<0.05)

Table 2: Effect of WBH at 42 °C and its late effects on various biological parameters in 2, 6, 12 and 24 months old rats (% change).

Age	Time	Parameters									
		WBC's	RBC's	T (%)	B (%)	IgG (mg/dl)	IgA (pg/ml)	Hb (g/dl)	HSP70 (ng/ml)	T3 (ng/dl)	T4 (µg/dl)
6 months	Control	6.90	-12.14	-18.88	-3.07	1.63	3.33	5.31	-54.46	6.45	7.65
	1 hr	48.04	-0.49	12.31	-28.25	34.38	-40.87	-78.0	49.71	6.92	18.06
	6 hr	-5.77	-22.27	-21.88	19.26	11.20	6.62	-24.41	30.95	-0.22	9.13
	24 hr	-23.20	36.55	-12.33	15.05	-12.09	-25.39	3.60	-13.41	1.13	-25.04
	72 hr	-74.10	4.55	-33.87	-19.30	-30.57	26.64	12.73	-65.00	4.07	-37.99
12 months	Control	-46.83	-11.27	-1.39	-43.58	-48.34	-6.09	14.47	-157.43	14.29	12.19
	1 hr	39.01	-25.87	7.39	-37.59	19.70	-5.53	-85.21	20.86	25.58	13.13
	6 hr	118.25	-23.83	11.18	-31.19	-20.92	-8.66	15.56	-139.68	19.77	24.87
	24 hr	12.39	20.30	-13.56	40.92	2.42	42.32	-8.00	-30.89	45.20	22.89
	72 hr	-13.64	-31.82	-34.66	-61.58	0.58	-16.77	-6.32	-87.14	30.56	9.22
24 months	Control	-111.38	-7.80	-34.08	-63.47	-42.63	8.89	33.96	-76.24	4.18	8.95
	1hr	65.15	-2.94	2.59	-56.22	34.45	5.78	-85.51	78.00	7.50	8.57
	6 hr	-111.39	-62.50	11.22	15.09	-25.17	30.08	13.29	-188.89	-6.00	9.75
	24 hr	-27.82	11.42	-6.80	-17.05	-6.13	13.96	5.13	3.66	19.02	-0.82
	72 hr	-2.40	-14.88	-40.67	-15.64	25.65	29.12	-33.40	25.71	15.74	16.67

Table (3): Mean values of chromosomal aberrations after heat exposure in female rat bone marrow cells at different ages.

Age	Time	Abnormal metaphases	Types and no. of chromosomal aberrations						Total chromosomal aberrations	Total aberrations excluding gaps
			gap	break	Del & frag	CA	End	Numerical aberrations		
2m	control	11±0.41 ^{hi}	5	7	6	13	5	8	11.0±0.41 ^j	9.75±0.63 ^{ij}
	1h	16.25±0.85 ^f	6	3	16	27	0	24	19.0±0.41 ^{gh}	17.5±0.50 ^g
	6h	26.50±1.04 ^b	16	3	19	32	11	37	29.5±0.29 ^{abcd}	25.5±0.29 ^c
	24h	23.75±1.93 ^c	21	5	21	24	5	34	27.5±0.29 ^{bcd}	22.25±0.63 ^d
	72h	19.50±0.65 ^{de}	16	0	0	15	18	29	19.75±0.48 ^{fg}	15.75±0.25 ^g
6m	control	9.50±0.87 ⁱ	5	5	7	12	4	6	9.75±0.63 ^j	8.5±0.87 ^j
	1h	18.0±0.41 ^{ef}	6	0	6	27	23	12	18.5±0.50 ^{ghi}	17.0±0.41 ^g
	6h	27.50±1.26 ^b	9	5	20	40	11	35	30.0±0.0 ^{abc}	27.75±0.25 ^{bc}
	24h	21.0±0.58 ^d	3	0	8	45	11	20	21.75±0.25 ^{efg}	21.0±0.41 ^d
	72h	10.75±0.25 ^{hi}	4	0	7	17	7	8	10.75±0.25 ^j	9.75±1.63 ^{ij}
12m	control	13.50±0.29 ^e	8	4	2	12	5	19	13.50±0.29 ^{ij}	11.50±0.29 ^{hi}
	1h	24.0±0.41 ^c	21	4	15	28	11	19	24.50±0.50 ^{ef}	19.25±0.48 ^{ef}
	6h	32.50±0.29 ^a	8	8	22	59	13	26	34.0±0.41 ^a	32.0±0.41 ^a
	24h	28.0±0.71 ^b	11	3	24	53	8	12	28.75±0.48 ^{abcd}	26.0±0.41 ^{bc}
	72h	12.0±0.41 ^{gh}	11	0	0	19	5	13	12.0±0.41 ^j	9.25±0.63 ^{ij}
24m	control	11.75±0.48 ^{ghi}	3	3	3	15	13	11	12.0±0.41 ^j	11.25±0.48 ^{hi}
	1h	28.50±0.65 ^b	13	5	29	42	16	19	31.0±0.41 ^{ab}	27.75±0.75 ^{bc}
	6h	31.0±0.41 ^a	16	0	24	58	11	21	32.50±0.29 ^a	31.0±2.68 ^a
	24h	28.50±0.29 ^b	9	4	32	41	8	18	30.50±0.29 ^{ab}	28.25±0.25 ^b
	72h	14.0±0.41 ^g	7	0	7	14	14	12	14.50±0.50 ^{hij}	12.75±0.48 ^h

Data were expressed as mean ± S.E

Means with different superscript letters are significantly different (P<0.05)

Del: deletion, Frag: fragment, CA: centromeric attenuation, End: endomitosis

Table (4): Mean values of micronucleated polychromatic erythrocytes after heat exposure in female rat bone marrow cells at different ages

times ages	Mean values of MNPCEs based on 1000 PCEs assessed per animal				
	Control	1h	6h	24h	72h
2 m	2.0±0.41 ^f	1.75±0.48 ^f	2.25±0.48 ^{ef}	6.75±0.48 ^c	4.50±0.65 ^d
6m	2.0±0.41 ^f	2.0±0.41 ^f	2.0±0.41 ^f	4.0±0.41 ^{de}	2.0±0.41 ^f
12m	2.0±0.41 ^f	2.25±0.75 ^{ef}	2.0±0.41 ^f	9.0±0.41 ^b	2.25±0.25 ^{ef}
24m	2.0±0.41 ^f	2.25±0.75 ^{ef}	2.25±0.63 ^{ef}	12.0±1.23 ^a	2.75±0.48 ^{ef}

Data were expressed as mean ± S.E

Means with different superscript letters are significantly different (P<0.05).

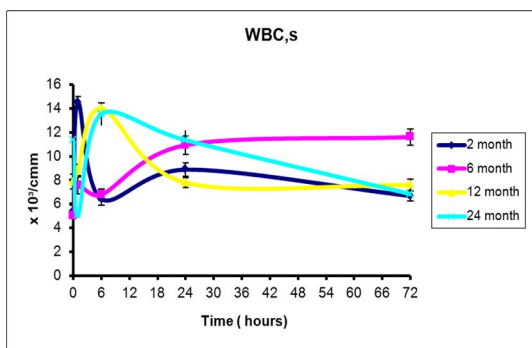


Fig.(1): The effect of WBH on WBC's in rats(2,6,12 and 24 months).

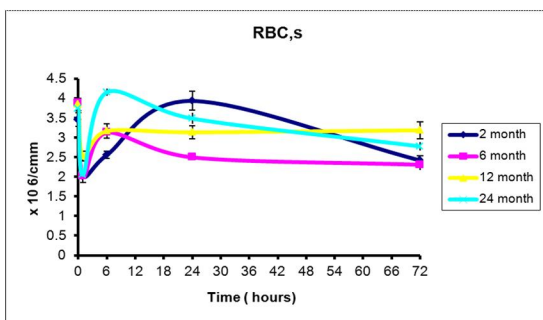


Fig.(2): The effect of WBH on RBC's in rats(2,6,12 and 24 months).

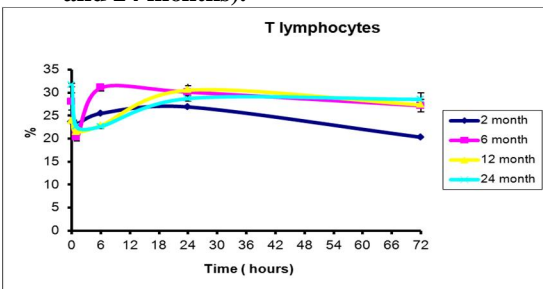


Fig.(3): The effect of WBH on T lymphocytes in rats(2,6,12 and 24 months).

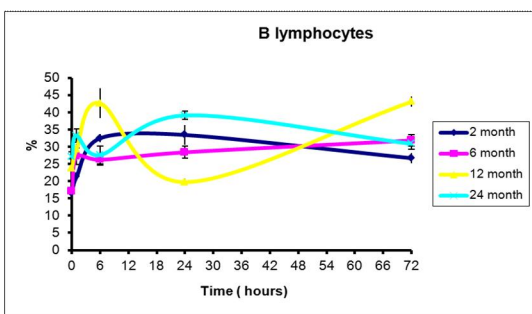


Fig.(4): The effect of WBH on B lymphocytes in rats(2,6,12 and 24 months).

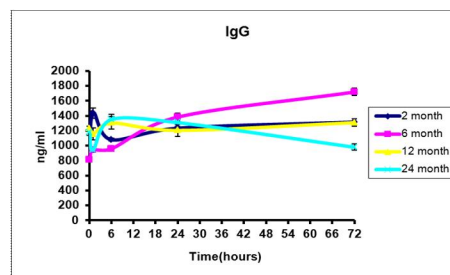


Fig.(5): The effect of WBH on IgG in rats(2,6,12 and 24 months).

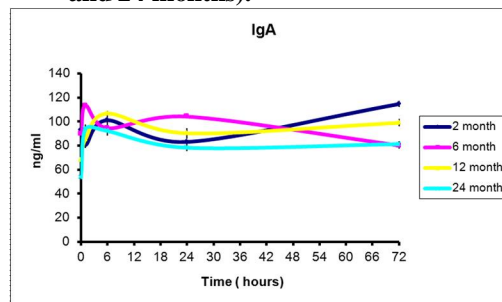


Fig.(6): The effect of WBH on IgA in rats(2,6,12 and 24 months).

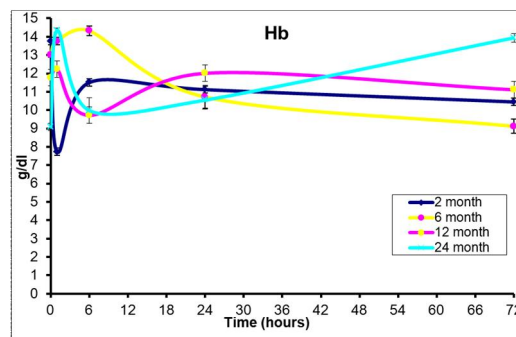


Fig.(7): The effect of WBH on Hb in rats(2,6,12 and 24 months).

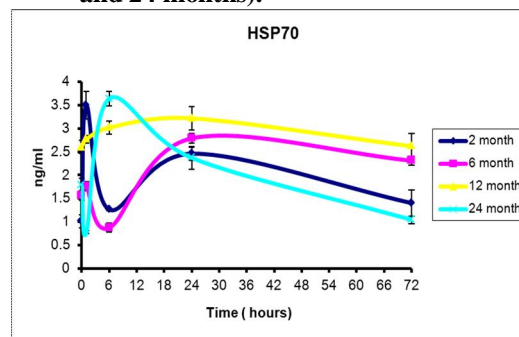


Fig.(8): The effect of WBH on HSP70 in rats(2,6,12 and 24 months).

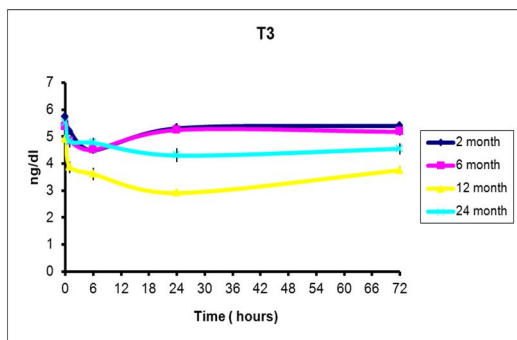


Fig.(9): The effect of WBH on T_3 in rats(2,6,12 and 24 months).

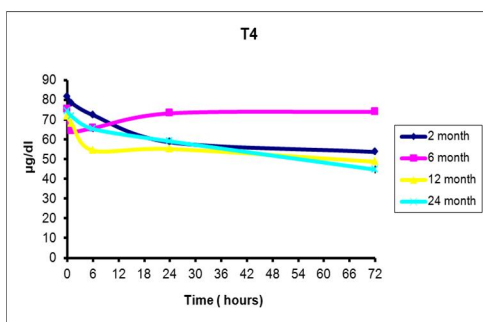


Fig.(10): The effect of WBH on T_4 in rats(2,6,12 and 24 months).

4. Discussions

In animals and humans, some physiological and biochemical adaptations could occur to protect essential cell functions against heat stress and to permit a rapid recovery from moderate hyperthermic damage (Burdon, 1986 and Hales et al. 1996). However, each tissue and organ has a different sensitivity for sustaining thermal injury (Freeman et al. 1985 and Ando et al. 1994). The biochemical impacts of heat stress on different ages to know the ability of tissues to repair them need to be evaluated. In the present study, the whole body hyperthermia induced increase of WBC's in all different ages after WBH at 1hr till 72 hr except in the aged group (24 month) WBC decreased significantly at 1hr post WBH then increased at 6 hr and approached to control value at 24 hr post heat stress. This decrease may be due to aging as a physiological state affect to decrease of WBCs count in aged rats. Also in most mesenchymal tissues a subcompartment of multipotent progenitor cells is responsible for the maintenance and repair of the tissue following stress with increasing age, the ability of tissues to repair themselves is diminished, which may be due to reduced functional capacity of the progenitor cells Stolzing and Scutt (2006). While WBH caused significantly decreased of RBC in all groups after 1 hr post the treatment. The significant decrease in the numbers of RBC count is in agreement with the results

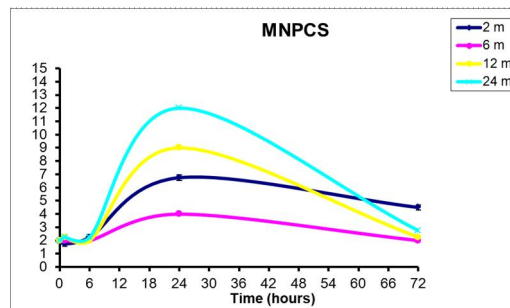


Fig.(11): The effect of WBH on MNPCs in rats(2,6,12 and 24 months).

of Wondergem et al. (1995); Terajima et al.(2000) and Mostafa et al. (2005). The decreased numbers of RBC's may be due to accumulation of blood in different organs as a result of internal hemorrhage as was confirmed by the histopathological alterations observed in liver, kidney and heart in previous studies (Hassan 2006; Abd El- Samea et al. 2007 and Mostafa et al. 2009). These effects might have been also attributed to thrombocytopenia or to decreased blood platelets combined with direct leading to extravasation and escape of red blood cells into the tissue spaces (Terajima et al. 2000 and Mostafa et al. 2005). Another possible reason for the observed decrease of RBCs count could have been the direct hemolytic effect of hyperthermia on the cell membrane resulting in increased membrane fluidity and consequently cell fragility. However, Olayemi and Nottidge (2007) reported the values of RBC, Hb and WBC were similar in the young and adult New Zealand rabbit. The data in the present study revealed a significant increase of Hb level in all groups after 1 hr post WBH except in 2 month's group Hb content decrease in this group till the end of the experiment 72 hr. The present study showed a significant decrease of T lymphocytes percentage in all groups of the experiment after 1hr post WBH which may be due to the stimulating effect of heat on lymphoid organs (Menger ,1983 and Mostafa, 2005).This decreased continued till the end of

experiment in added group 24 months .O Ahlers et al.(1998) The number of *lymphocytes* decreased significantly from 37°C to 42°C . This effect was mainly caused by a significant decrease of the absolute T4-Cell count and a slight decrease of the T8-Cell count with a resulting significant decrease of T-Cells. In addition, IL2-Receptor expression on T-Cells, as a marker for activation, decreased significantly .It seems remarkable, that these effects were reversible in a very short time-period after decrease of temperature. On the other hand, WBH induced a higher significant increase of B lymphocytes percentage in all groups of the experiment.

Induction of Hsp expression appears to correlate with a cytoprotective effect in cultured cells and with improved healing of damaged tissues in animal models and in humans. As well as, the current study was to investigate the impact of both hyperthermia and aging on *in vivo* Hsp70 regulation in response to heat stress. The current results demonstrate that both young animals (2 months) are capable of inducing Hsp70 protein in the early phases of recovery 1 hr after a heat challenge. However, at 1 hr and later time points of recovery, old animals (24 months) failed to maintain the high Hsp70 protein levels that were noted in their young counterparts. These results are in accordance with those of Singh et al. (2006) and Soti and Csermely (2006). Our results indicate that both 6 & 12 month groups are capable of up-regulating Hsp70 level at 24 hr & 72 hr following heat stress, while levels of HSP70 in old rats (24 month) decreased significantly at 1 & 72 hours after heat stress. The decrease in HSP70 levels could potentially result from either lowered transcriptional abilities or accelerated mRNA degradation in old rats Zhang et al. (2006). Nevertheless, Singh et al. (2006) speculated that age dependent decline in the ability of peripheral blood mononuclear cells to respond to heat stress in terms of HSP70 induction. Hall et al. (2000) reported that the inducible HSP70 response in the cytoplasm and nucleus was markedly reduced with age at several time points over a 48-h recovery period, although senescent rats were able to strongly express HSP70 early in recovery. Older animals had extensive zone-specific liver injury, which corresponded to the diminished HSP70 response observed in these regions, and a significant reduction in thermotolerance compared with their young counterparts. The process of aging has been associated with increased oxidative damage to macromolecules such as lipids, proteins and DNA in a wide array of tissue types in many eukaryotic species (Dobson, 2003). Therefore, an alteration in the ability of cells to express heat shock proteins could be physiologically important in aging because all living organisms show a reduced ability to respond to stress with increasing age (Bonelli et

al.1999; Jin et al. 2004; Kregel et al. 1995 and Vasilaki et al. 2002)). We have shown that the induction of HSP70 expression by heat shock is reduced approximately 188.89% at 6 hr. of recovery in an aged group (24 month old rats) as compared to that in 2 month old group. Other investigators have also shown that the induction of HSP70 expression by heat shock as well as other stresses declines significantly with age in a variety of tissues from rats as well as mononuclear cells from human subjects. Therefore, it appears that a reduced ability to express HSP70 in response to stress may be a common phenomenon underlying the aging process (Heydari et al. 2000). Induction of heat shock proteins including HSP70 that gives a cytoprotective effect against further stress. However, HSP70 induction is attenuated in aged cells. The lower HSP70-levels may contribute to the impaired stress response seen in the aged, and to the higher rates of chronic wounds in aged, which arise from repeated ischemia-reperfusion injury (Andrea et al. 2006).

The prevalence of anabolic activity can trigger the increased basal production of HSP70 in young animals (Maiello et al. 1998). Aging is accompanied by a decay of self-defensive mechanisms and by an accumulation of damages at the molecular, cellular, and organic level as a result of a constant exposure to adverse environmental stresses (28, 30). The impaired response to heat stress observed in old rats could be caused by a failure to induce HSP70 (Fargnoli et al. 1990; Nitta et al. 1994; Heydari et al. 2000; Zhang et al. 2003; Tandara et al. 2006). Because HSP70 is believed to promote the correct refolding of denatured or unfolded proteins damaged by stress and can also act as a chaperone in the ubiquitin–proteasome degradation pathway (Riezman, 2004), the failure of senescent organisms to properly induce the expression of HSP70 and other HSPs would lead to the accumulation of damaged proteins that may be toxic to the cell (Kregel, 2002; Riezman, 2004).

The data in the present study revealed a significant increase of IgG in both groups 2 & 6 months after 1 hr till 72 hr the end of the experiment. On the contrary IgG level in remainder two groups 12 & 24 months showed a fluctuated decrease and increase. On the other hand, WBH induced a significant increase of IgA in 6, 12 and 24 months groups after 1hr post heat exposure. This increase still even 72 hr post WBH in both groups 12 & 24 months. On the contrary, Hietala et al. (2006) reported that hyperthermia has no changes occurred in immunoglobulins or cell-mediated immunity. As well as, Koga et al. (2006) reported that total-body hyperthermia (TBHT) therapy may lead to a reduction in the immune response of cancer patients because of the immediate effect of heat on lymphocytes, the authors studied the immunity of

advanced cancer patients receiving combined total body hyperthermia (TBHT) and anticancer chemotherapy. A decrease was found in their lymphocyte blastogenesis and lymphocyte rosette formation, IgG. These parameters returned to their pretreatment levels at 1 week after completion of TBHT therapy. This result indicates that there is no necessity for giving special consideration to a reduction of cell-mediated immunity in TBHT therapy. Further, Salauze et al. (2004) indicated that both IgM and IgG levels increase gradually with age. By contrast Watt et al. (1986) were observed infants under 6 months failed to respond by the production of IgG antibodies, although increases in IgA and IgM levels.

The endocrine system reacts to the change in the environmental temperature, resulting in change in delivery of thyroid hormones and glucocorticoids (Hardy, 1974). Thyroid hormones are known to play an important role in adaptive thermogenesis (Arieli and Chint 1986). In the present study there was a dramatic reciprocal change in T₃ and T₄ during the heat stress of WBH in all groups of the experiment. Although T₃ approached to the control value at 72 hr post heat stress in 2 months group and at 24hr in 6 months group. The decrease of T₃ and T₄ hormones is in agreement with other studies (Boiti et al. 1992; Habeeb et al. 1993; Ashour et al. 1995; Mostafa et al. 2005). The reduction of T₃ and T₄ may be due to the direct effect of high temperature on the thermal receptors, which stimulate the hypothalamus to depress its secretion of thyroid stimulating hormone (TSH) releasing hormone (TRH) and consequently the pituitary TSH and thyroid T₃ and T₄ secretion (Kamal, 1975). This decrease conversion of T₄ to T₃ and faster utilization of T₃ by the respiratory muscles to speed up ventilation in order to enhance energy production. The influence of recovery for 72 in 12 & 24 months groups. So, there is fairly good agreement among authors that the thyroid gland activity in different species increases at moderate cold environment and decreases at high temperature.

There are few reports on the relationship between chromosome aberrations and high body temperature, although there are many *in vitro* reports of the induction of chromosome aberration by heat treatment (Coss et al. 1982 and Yamada et al. 1989). Here, we demonstrated the effect of hyperthermia on the induction of chromosomal aberrations and micronuclei of bone marrow cells in female albino rats. The results accomplished in this work demonstrated that exposure to hyperthermic treatment (42°C) for 1 hr caused chromosomal aberrations and micronucleus of bone marrow cells at all times but in different frequencies. Our results in agreement with Yamada et al. (1989) and Waissenbourn and Obe, (1992) who reported that structural chromosome aberrations

(breaks, stickiness, fragmentation) were observed at 41.5°C and 43 °C respectively. On the other hand, exposure to heat stress in Muscovy ducks leads to negative effects on some physiological, immunological responses and chromosomal aberrations. (El-Badry et al. 2009). Similar findings were obtained in broiler chickens during high temperature exposure (Zhou et al, 1998). These aberrations may because hyperthermia produces little DNA damage (Waters and Axtell, 1992; Jorritsma and Konings, 1993). Heat may induce DNA base damage indirectly via protein damage (Takahashi, 2004) and changes in enzyme complexes for DNA synthesis and repair (Streffer, 1995). Our results demonstrated that at 24 hr, young age (2 month) have more frequencies of chromosomal aberrations than adult (6 months) which have less frequencies than old age (12 and 24 month). 72 hr group show that there were no significant differences between all ages except 2 month which increased statistically than other groups. Mean that young age more affected and need more time than others to recover. This may due to the potential thermotolerance cannot be incorporated into the developing mechanisms of thermoregulation (El-Badry et al. 2009). Laszlo, 1988 suggested that there is a relationship between thermotolerance and heat shock proteins (hsp) synthesis rate. Furthermore, it has been explained that heat shock proteins bind to chromosomes following heat stress and they take part in chromosome condensation and on recovery induce damages like chromatid stickiness. Mamon and Kutsikova, 1993 added that high temperature has a role in inducing damages of mitotic chromosomes in *Drosophila melanogaster*. From the present results, it was observed that adults were more resistance to heat stress than young and old ages due to potential thermotolerance can incorporate into the developing mechanisms of thermoregulation (Rotwell, 1992). On the other hand, some reports indicate no clear correlation between the frequency of chromosome aberrations and age in the occupationally exposed individuals (Jha and Sharma 1991, Monfared et al. 2003).

Our results also finding that heat shock led to the formation of micronucleus at 24 hour after heat treatment in all ages but more frequencies at 24 month of age. These mean values not significant than control in 1 and 6 hour and recovered at 72 hour after heat at all ages except at 2 month of age. Our findings are in good agreement with other investigations conducted on mammals such as Asanami and Shimono, 1997 and Asanami et al. 2001 they observed positive responses of micronucleus at 31, 33 and 40°C for 24h and 42°C for 2hr. Results suggested that in Chinese hamster cells line, hyperthermic conditions can induce both chromosome aberration and micronuclei. The micronucleus is based on observations that

chromosome fragments and/or entire chromosomes separated (lagging) from the main group at anaphase of mitosis tend to be excluded from a daughter cell nucleus at the telophase stage of mitosis. These chromosomes or fragments are often transformed into micronuclei in the cytoplasm of the daughter cell. As this micronucleus formation may occur spontaneously (not under mutagen influence) at a low rate of incidence, a specific cell type known to have just undergone mitosis under mutagen influence is needed. The mammalian erythrocyte offers this opportunity and has the added advantage of not having a main nucleus so micronuclei can be easily observed. After the last mitotic cycle in the bone marrow, mammalian erythroblasts expel their nuclei but retain any micronuclei and take on a different staining ability. These erythroblasts are polychromatic and stain blue for a period of about 24 hours, then become normochromatic or red staining (Chrisman and Baumgartner 1980 and Schmid, 1975). This may give interpretation for our results that micronucleus significantly increased only at 24 hours after heat stress.

In conclusion, aging and hyperthermia have a synergistic effect on hematological indices as well as, HSP70 that may be essential for surviving and recovering from thermal injury in aged animals. Also, exposure to hyperthermic treatment of 42°C for 1 hr induced chromosomal aberrations and micronucleus formation in female rat bone marrow cells, and recovered at 72 hr. after heat treatment in adults and old ages but in young, it take more time to recover.

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