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GENOTOXIC EFFECTS OF HEAT STRESS IN HOUSE FLY *MUSCA DOMESTICA* L. (DIPTERA: MUSCIDAE)

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ABSTRACT

Any deviation in ambient temperature causes stress and disturbs the cellular homeostasis in the organisms. In the present study, genotoxicity induced by exposure of high temperature (40º, 45º and 50ºC) has been analyzed in the third instar larvae of common housefly, *Musca domestica*, by using chromosome aberration assay, micronucleus assay and mitotic index as cytological end points. The adverse effects of heat stress on development pattern of housefly were also observed. A significant ($p<0.05$, $p<0.01$ and $p<0.001$) increase in chromosome aberrations and micronucleus frequency was observed in all the exposed groups as compared to control. Cell proliferation at 50ºC is inhibited as represented by a decrease in mitotic index. The development pattern in exposed larvae shows increase in larval mortality and delay in adult emergence. The results suggest that heat stress induces structural chromosome aberrations, micronuclei and affects development pattern in housefly *Musca domestica*.

KEYWORDS: Chromosome aberrations, Development pattern, Micronucleus assay, Mitotic index, *Musca domestica.*

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INTRODUCTION

All organisms survive at an optimum environmental condition. Any adverse alteration in their environment such as change in temperature, xenobiotics intrusion and radiations etc. causes stress and results into changes at cellular and biochemical levels in the organisms[1]. To subdue these changes and maintain their homeostasis, cells of all the organisms respond in almost a conserved fashion and synthesize heat shock proteins or stress proteins[2]. Despite this fact, it is well known that the exposure of elevated temperature, one of the environmental stress factors, to the organisms results into severe consequences. Heat stress affects the integrity of structural components of cells like nucleolus and centrosome and almost every cellular process including the cell division and its control[3,4]. The flies belonging to the order Diptera are exposed to extreme variations of temperature in their natural habitats. These flies are, therefore, sensitive to temperature induced injuries [5-9] which some times may regulate their population size. Developmental defects[10], structural chromosome aberrations[11] and defects in centriole organisation and its ability to nucleate microtubules due to heat stress [12,13] have been reported only in *Drosophila*. Therefore, it is imperative that the effects of heat stress be analysed in other dipterans to unravel the mechanism of heat stress in terms of alteration in cellular processes. In the present study, genotoxicity of heat stress in terms of cell division errors has been analyzed in *Musca domestica* by scoring induced chromosome aberrations, micronuclei and other nuclear anomalies. Mitotic index which is an indicator of rate of cell proliferation and cell death and the effects on the development patterns were also observed. Common house fly *M. domestica*, a synanthropic fly, has been widely used in cytogenetic research. The fly has a diploid chromosome complement of 2n=12, with five pairs of meta/submetacentric autosomes and a pair of sex chromosomes XX female/XY male (14-16). The simple karyotype of *M. domestica* renders it to be a suitable model for short term genotoxicity assays.

MATERIALS AND METHODS

Laboratory colonies of *Musca domestica* L. (Diptera: Muscidae) were established from wild flies collected with the help of sweep net following the protocol of Agoze et al[14]. For heat stress, late third instar larvae were kept in petridishes soaked with moist filter paper and placed in a BOD incubator at different temperatures (40º, 45º and 50ºC) for various time intervals. Pattern of emergence was studied to look at the effect of temperature stress on development of house fly. A Group of 50 late third instar larvae were given temperature stress at 40ºC, 45ºC and 50ºC for one hour and subsequently placed in normal culture medium and allowed to continue development at 27 ±1ºC. The larval and pupal mortality, day of emergence of adult flies and the number of flies which emerged in control and treated groups were recorded. Five replicates of each treatment group and control were performed. For genotoxicity assay a group of five larvae (in two batches) were exposed to different temperatures for 20, 40 and 60 minutes. Chromosome preparations were made from neural ganglia of control (27 ±1ºC) and heat stressed late third instar larvae following the standard air drying method of Agoze et al[14]. Slides were stained with 2% lacto-aceto orcein and cells were scored and micro photographed by Nikon Eclipse 80*i* microscope (software, ACT-1). Total dividing cells were observed for analysis of chromosomal abnormalities and nuclear anomalies. Relative frequency of each type of abnormality along with chromosomal aberration index (A.I.) was calculated by using the formula A.I. (%) = (total chromosomal aberrations/ total dividing cells analysed) x 100 [17,18]. Micronucleus frequency was evaluated in 3000 cells per slide. Micronuclei, which were clearly separable from the main nucleus, not exceeding 1/3 in diameter of the main nucleus, and with

distinct borders and of the same colour as the nucleus were scored [19,20]. Number of cells in mitotic division was counted in 2000 cells/ slide in both control and exposed group and mitotic indices (%) were calculated following the formula: mitotic index = (number of cells in division/total number of cells) x 100 [21]. Normality assumptions and homogeneity of variance concerning the data were tested before analysing statistical significance. ANOVA and post-hoc tests (Games-Howell and Tukey-HSD test) were used for pair-wise comparisons and to compare data of treated groups with control with the help of Statistical Package for Social Sciences Software (SPSS, version 10.0). Level of significance was assessed at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$.

RESULTS AND DISCUSSIONS

The present study shows that development pattern of house flies was affected after heat stress, as evinced by increase in larval death, pupal death and delay in emergence of adult flies (Figs. 1a and b). At 45ºC and 50ºC maximum number of flies emerged on 12th day as compared to control and larvae exposed to 40ºC and show a delay of one day in emergence. Tiwari et al[8,9], have also reported that a brief exposure to heat stress results in the reduction in emergence of adult flies in case of *Lucilia cuprina* and *M. domestica*. The delay in emergence of flies may be attributed to the severe genotoxicity as observed in case of cadmium and mercury exposed larvae of *Drosophila* [22] and *M. domestica* [17], respectively.

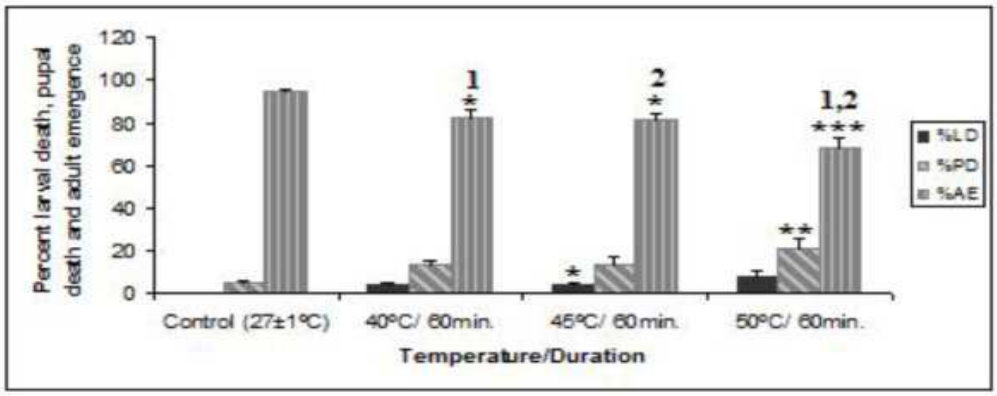

Figure 1

(a). ***The effect of heat stress on survival of larvae and pupae and on emergence of adult houseflies. Values are mean±S.E.; *p<0.05; **p<0.01; ***p<0.001 versus control, similar numerals reveal significant difference between groups at 5% level (Games Howell test for LD% and Tukey HSD test for PD% and AE%; Homogeneity of Variance p<0.05 (LD%); p>0.05 (PD and AE%); ANOVA p<0.05).***

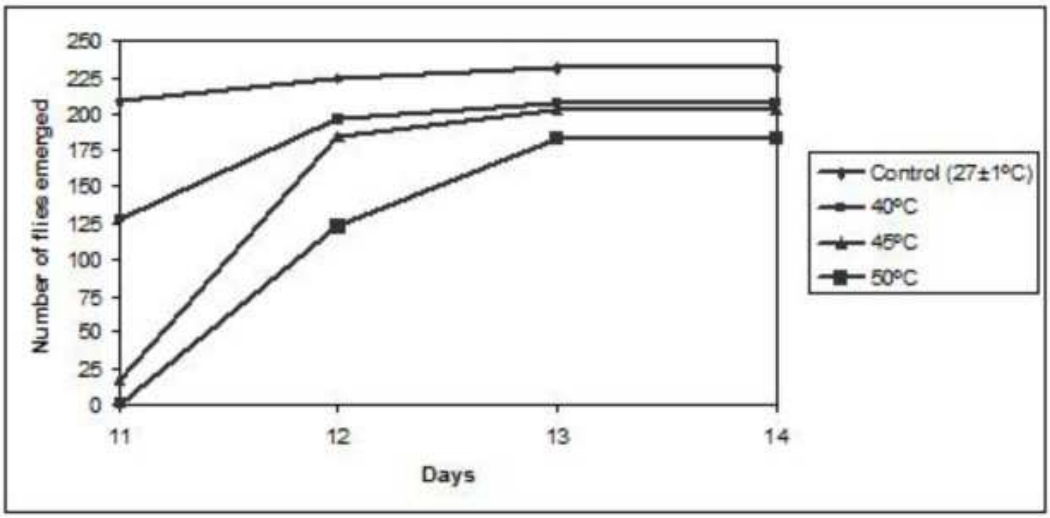

Figure 1
(b). ***Emergence pattern of adult house flies after heat stress at different temperatures.***

The exposure of elevated temperatures leads to genotoxic effects in *M. domestica* by inducing chromosome aberrations, micronuclei and other nuclear anomalies and alterations in mitotic index. Similar results were also observed in *Drosophila melanogaster*[11] and gold fish, *Carassius auratus* [23]. The most commonly observed chromosome aberrations in heat stressed larval neuroganglial cells at prophase (P), metaphase (M), anaphase(A) and telophase (T) were disordered prophase, fragmentation, stickiness (St), constrictions, breaks and gaps (C,B&G), asymmetric bipolar metaphase (Bp), anaphase multipolarity, bridges lagging and sticky chromosomes (OAA) and telophase disorientation and bridges (Figs. 3, 4 and 5). Fig. 2 represents the normal mitotic phases in control groups.

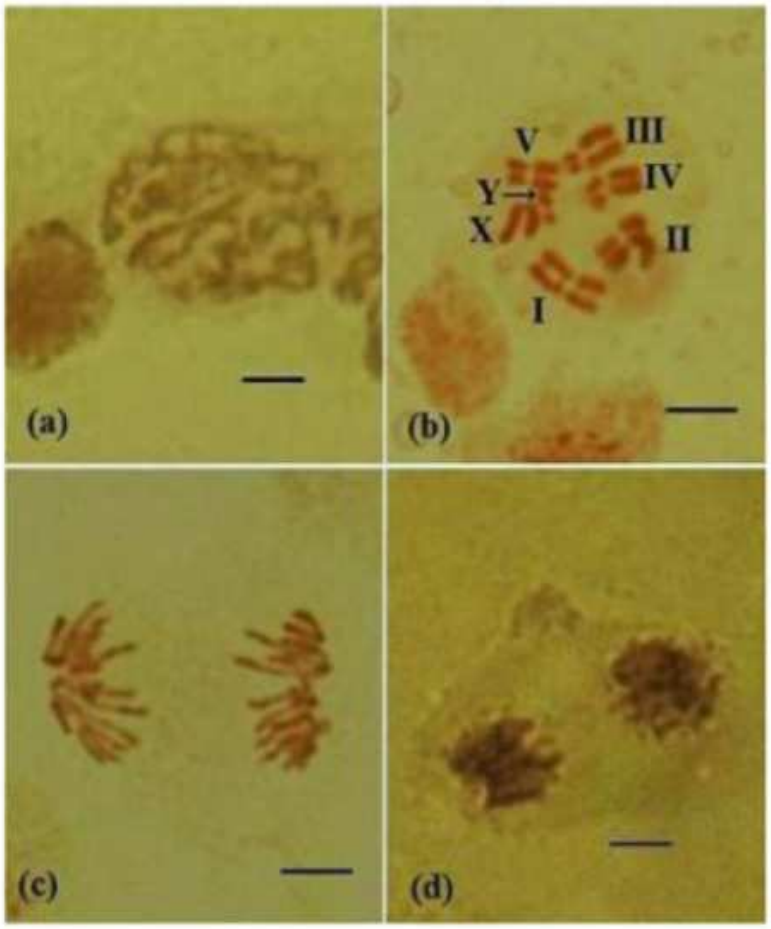

Figure 2

Representative photomicrographs of neuroganglial chromosomes of M. domestica larvae at different stages (a) Normal prophase (b) Normal metaphase (I-V autosomes and X and Y→ sex chromosomes) (c) Normal Anaphase (d) Normal telophase. The bars in all figures represent 10 µ.

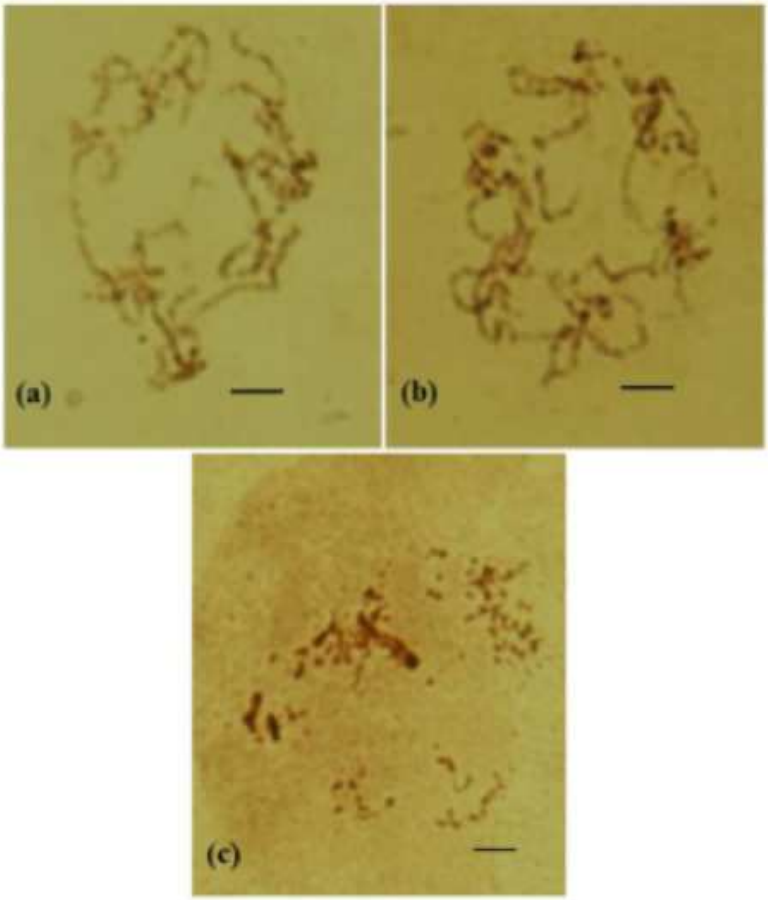

Figure 3
Representative photomicrographs of neuroganglial chromosomes of M. domestica larvae showing different type of aberrations at prophase after heat stress: (a & b) Disorderly-arranged prophase (c) Prophasic chromosome fragmentation. The bars in all figures represent 10 µ.

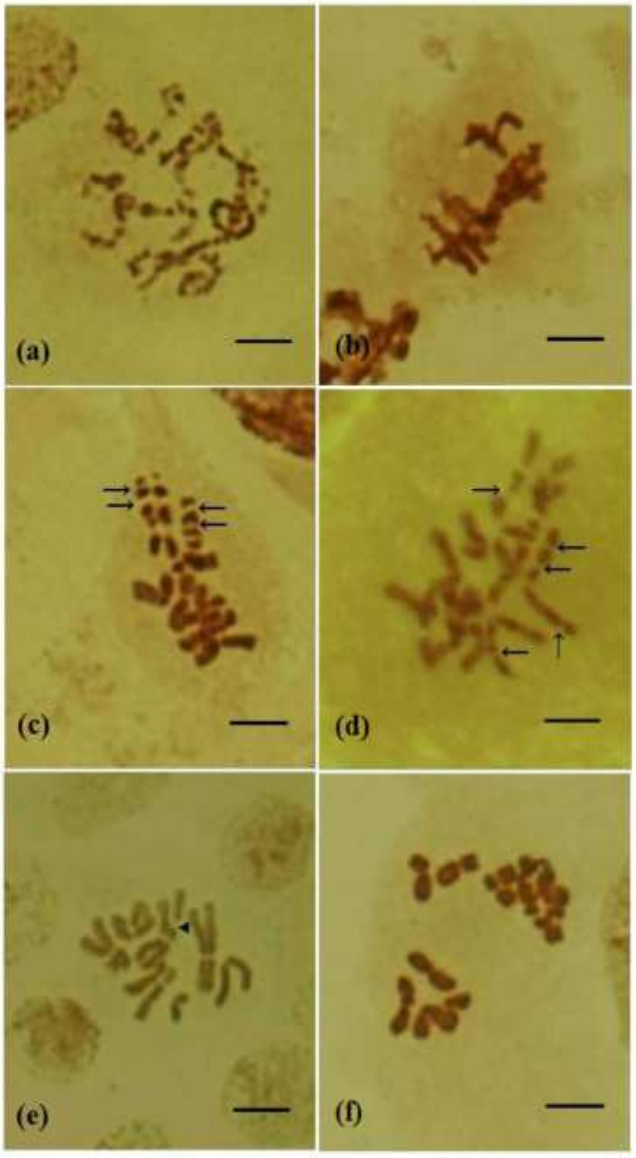

Figure 4

Representative photomicrographs of neuroganglial chromosomes of M. domestica larvae showing different type of aberrations at metaphase after heat stress: (a) Chromosome fragmentation (b) Stickiness of chromosomes (c & d) Chromosome gaps (→) (e) Chromosome break (►) (f) Asymmetrical bipolar metaphase. The bars in all figures represent 10 µ

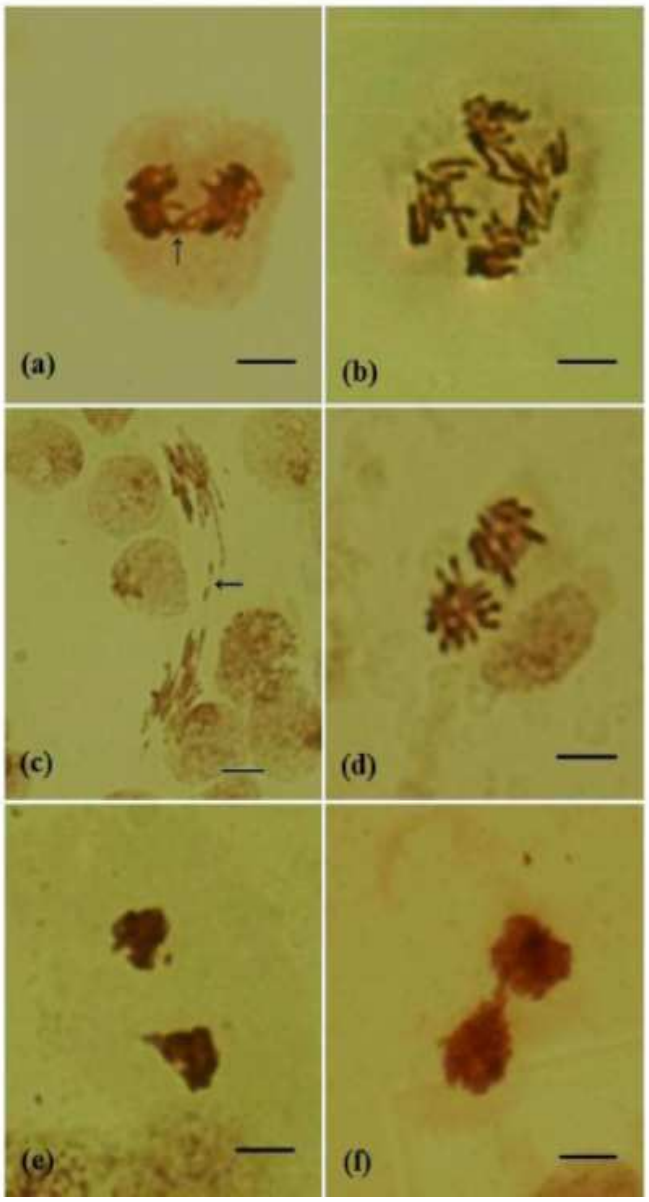

Figure 5
Representative photomicrographs of neuroganglial chromosomes of M. domestica larvae showing different type of aberrations at anaphase and telophase after heat stress: (a) Anaphase bridge (→) with chromosome stickiness (b) Multipolar anaphase with chromosome bridge (c) Lagging chromosome (→) (d) Disoriented anaphase (e) Disoriented telophase (f) Telophase bridge. The bars in all figures represent 10 µ

The relative frequencies of chromosome aberrations at different phases of mitosis, aberration indices (AI) in control and treated groups are represented in Table 1.

Table 1
Mean relative frequencies of chromosomal aberrations and nuclear anomalies after heat stress for different durations.

Temp.	Time	P (*p<0.05)	M			A		T (*p<0.05)	AI (*p<0.05)	NA (*p<0.05)
			St (*p<0.05)	C,B&G (*p<0.05)	Bp (*p<0.05)	PCS (*p>0.05)	OAA (*p>0.05)			
Control	--	0.00 (0.00)	8.86 (1.47)	0.00 (0.00)	0.00 (0.00)	0.54 (0.21)	0.30 (0.12)	0.00 (0.00)	9.70 (1.31)	0.51 (0.19)
40ºC	20 min.	0.47 (0.12)	18.92[a] (2.07)	1.31[c] (0.16)	0.35 (0.17)	0.76 (0.24)	1.40 (0.33)	0.63[a] (0.13)	23.84[a] (2.16)	1.38 (0.20)
	40 min.	0.85[b] (0.14)	29.39[c] (3.03)	0.64[c] (0.07)	0.47[a] (0.10)	0.65 (0.17)	1.17 (0.20)	0.59[c] (0.09)	33.76[c] (3.02)	2.09[b] (0.28)
	60 min.	1.04[a] (0.12)	29.67[c] (2.31)	3.23 (1.17)	0.38 (0.16)	0.49 (0.20)	2.76[c] (0.57)	0.58[c] (0.08)	37.73[c] (2.34)	3.28 (0.71)
45ºC	20 min.	1.36[b] (0.21)	34.26[c] (3.33)	0.96[b] (0.16)	0.13 (0.05)	1.15 (0.22)	2.45[b] (0.40)	1.06[a] (0.20)	41.37[c] (3.48)	4.88[c] (0.41)
	40 min.	1.44[b] (0.21)	38.70[c] (3.01)	1.67[c] (0.19)	0.07 (0.03)	0.48 (0.19)	1.88[a] (0.45)	1.08[b] (0.16)	45.32[c] (3.02)	5.38[c] (0.64)
	60 min.	1.85[c] (0.16)	37.06[c] (2.13)	1.84[b] (0.35)	2.20[a] (0.48)	0.52 (0.20)	1.57 (0.42)	1.39[b] (0.25)	46.49[c] (1.82)	7.27[b] (1.02)
50ºC	20 min.	2.13 (0.55)	39.67[c] (3.54)	1.14[b] (0.19)	0.12 (0.04)	0.53 (0.14)	1.58 (0.39)	1.83 (0.52)	47.00[c] (4.60)	7.74[a] (1.47)
	40 min.	2.00[c] (0.28)	44.64[c] (1.55)	2.27[b] (0.43)	0.25[b] (0.04)	0.20 (0.05)	1.41 (0.27)	1.86[b] (0.38)	52.63[c] (0.99)	6.40[c] (0.46)
	60 min.	1.66[b] (0.29)	45.22[c] (1.37)	1.61 (0.49)	1.38[b] (0.23)	0.31 (0.11)	1.46 (0.29)	1.26[b] (0.24)	52.38[c] (1.44)	6.75[b] (1.07)

P= Prophase; M= Metaphase; A= Anaphase; T= Telophase; St = Stickiness; C, B&G = Constrictions, Breaks and Gaps; Bp = Asymmetrical Bipolar metaphase; PCS = Precocious Chromatid Separation; OAA = Other Anaphase Aberrations; AI = Aberration Index; NA = Nuclear Anomalies; Values are mean ± S.E (values in parentheses). *Homogeneity of variance; ANOVA ($p < 0.05$); [a]$p \leq 0.05$; [b]$p \leq 0.01$; [c]$p \leq 0.001$ versus control (Games Howell and Tukey HSD Tests)

A significant increase in relative frequencies of chromosome aberrations is observed in treated groups as compared to control. Frequency of precocious chromosome separations (PCS) is found to be almost constant in the exposed groups and does not show any significant difference from the control group. The chromosomal aberration indices show a time and dose dependent increase with the increase in duration of exposure and temperature, however, ultimately attain a threshold. Occurrence of asymmetrical bipolar metaphase and multipolar anaphase with the increase in temperature indicates that temperature stress induces the disruption of mitotic spindle and/or destruction of centrosome during G_1 phase of the cell cycle [3,12,13,24,25]. Cytogenetic anomalies such as pulverization and chromosome stickiness in cells of neural ganglia of *Drosophila* larvae have been attributed to heat shock proteins binding to the chromosomes following heat shock, which take part in chromosome condensations and recovery [11]. Nuclear anomalies (NA) observed are nuclear bud, endoreduplication, chromatin bridge, nuclear fragmentation/multinucleated cells, micronuclei and binucleation (Fig. 6).

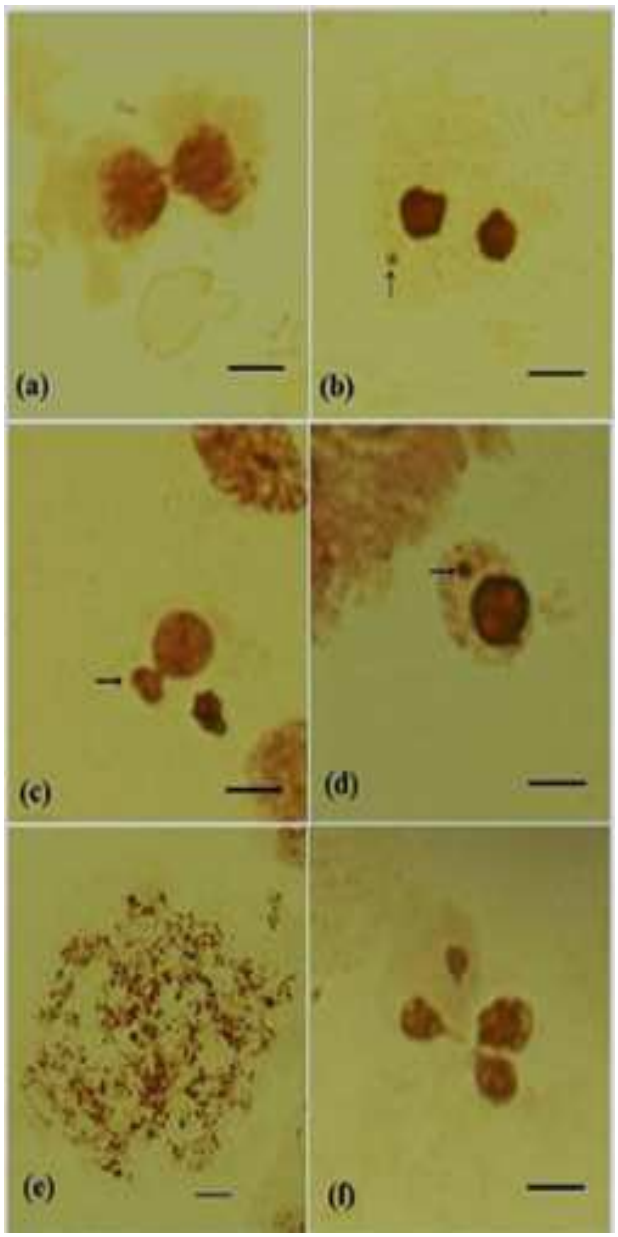

Figure 6

Representative photomicrographs of nuclear anomalies induced in neuroganglial cells of M. domestica larvae after heat stress: (a) Chromatin bridge (b) Binucleated cell with micronucleus (→) (c) Nuclear bud (→) (d) Micronucleus (→) (e) Endoreduplication (f) Nuclear fragmentation with chromatin bridges. The bars in all figures represent 10 µ

Relative frequency of nuclear anomalies increases significantly as compared to control in all treatment groups (Table 1). It is highest in group exposed at 50ºC for 20 min. followed by a decrease. The nuclear anomalies like chromatin bridges, binucleated cells, and nuclear blebs may be due to anaphase bridging [28]. The induction of multinucleated cells may be due to the formation of multipolar anaphase that is unable to undergo cytokinesis [29]. The mean micronucleus frequency of different treated groups and control is presented in Fig. 7. A significant increase in frequency of micronuclei in all

treatment groups was observed as compared to control. The increase seemed to be dose dependent; however, statistically it is not dose dependent. The incidence of micronucleus in *M. domestica* may be ascribed to the DNA strand breakage leading to acentric chromosome fragments or due to chromosome lagging on anaphase [23,25-27].

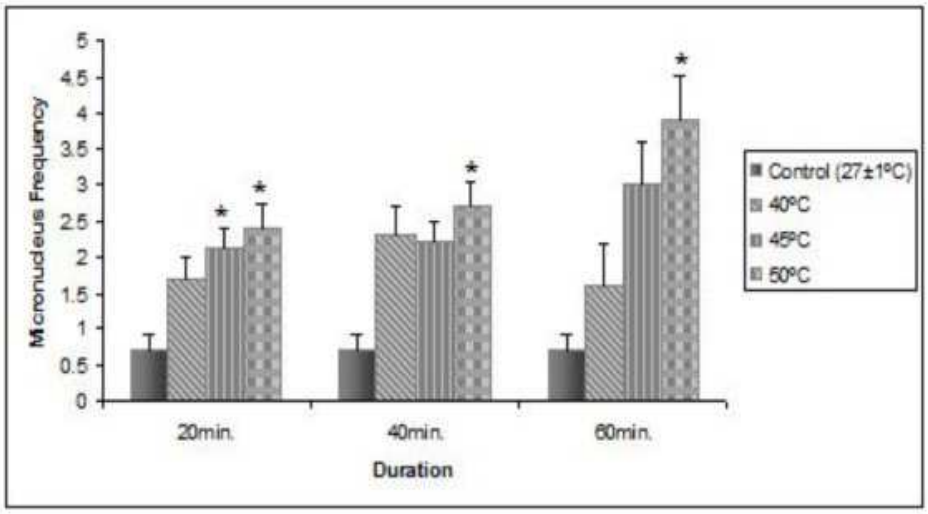

Figure 7

Mean micronucleus frequency induced in M. domestica larvae after heat stress at different temperatures for various durations. Values are mean±S.E.; *p<0.05 versus control (Games-Howell test; Homogeneity of Variance p<0.05; ANOVA p<0.001)

A significant increase in MI is observed with the increase in duration of exposure at 40ºC followed by a decrease with the increase of temperature and its duration as compared to control (Fig. 8). MI is lowest at 50ºC for 60 min. exposure. The increase in mitotic index at 40ºC with the duration of exposure indicates delay in cell cycle due to M phase arrest of the cell cycle which is further reduced due to cellular damage at elevated temperatures as indicated by decreasing mitotic index [4,23,24,31-34].

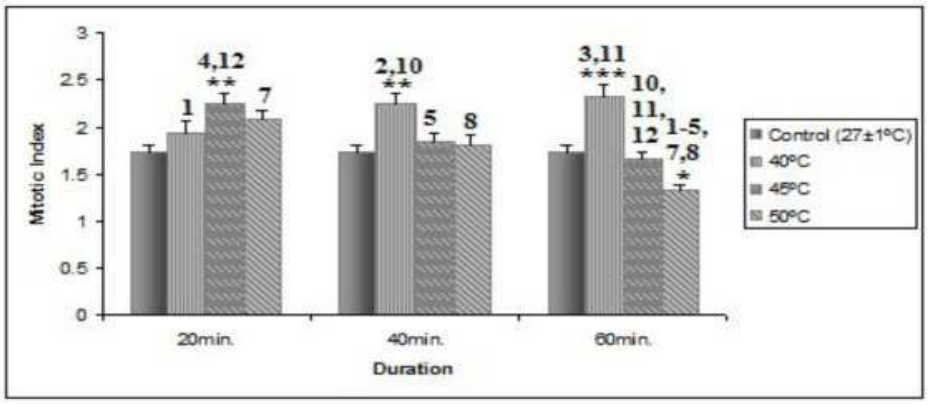

Figure 8

Histogram representing mitotic index (MI) in heat stressed larvae of M. domestica at different temperatures for various durations. Values are mean±S.E.; *p<0.05; **p<0.01; ***p<0.001 versus control, similar numerals reveal significant difference between groups at 5% level (Tukey-HSD test; Homogeneity of Variance p>0.05; ANOVA p<0.001)

CONCLUSION

The present study reveals that heat stress affects the development pattern and induces severe genotoxicity in the house fly *M. domestica*. It seems that the common house fly *M. domestica* may be used to evaluate genotoxic potential of other environmental stress factors.

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