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ORIGINAL RESEARCH ARTICLE

Experimental Study of Effects of Heat Stress on Rat's Liver

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ABSTRACT

In animals and humans, some physiological and biochemical adaptations could occur to protect essential cell functions against the heat stress and to permit a rapid recovery from moderate hyperthermic damage. However, each tissue and organ has a different sensitivity for sustaining thermal injury. Hence, the present study was undertaken to study histological and pathological changes due to chronic heat stress on rat's liver. Thirty albino wistar rats weighing about 150-200gms in experimental group were kept in increased room temperature (i.e 38±2°C) for 15 days. Halogen heater maintained room temperature. Similarly, thirty albino wistar rats weighing about 150-200gms in control group were kept in normal room temperature (i.e 26±5°C) for 15 days. Room temperature was maintained by air conditioner. After 15 days, rats were sacrificed by cervical dislocation, liver tissues were removed and histological slides were prepared. This study showed various changes occur in length and breadth of rat's hepatocyte cell whereas it didn't have any effect on weight of rats. This study provides evidences in support of necrosis, inflammatory changes, irregular cell membrane, enlarged nucleus, binucleated cell, dilated hepatic venules and dilated sinusoids of experimental group of rat's liver in compare to control group.

Key words: Hyperthermic damage, albino wistar rats, dilated hepatic venules and sinusoids.

INTRODUCTION

Exposure to excessive heat adversely affects on health and known as heat stress. This Colloquialism is a source of confusion because in the scientific literature "heat stress" is synonymous with "heat load," which carries the connotation that adverse health effects will occur only if the heat stress exceeds the person's heat tolerance capacity [1]. At lower levels of heat stress there is no risk of health damage, to be even though a person may feel discomfort. ldeally, it would be desirable eliminate heat stress completely by keeping the work place at a comfortable temperature because the state of discomfort has many adverse behavioral effects, such as reduced work rate, increased irritability, carelessness, and a feeling of fatigue [2]. These effects may render a worker more prone to accidental injuries. Unfortunately, the cost of keeping all job sites at comfortable temperature is not possible.

Typical hyperthermia sometimes occurs during severe heat waves in summer and because of hard exercise throughout the world [3,4]. In some temperate large cities, extreme heat stress is associated with an enhanced heat island effect. The incidence of heat-related morbidity, such as heat stroke in aged persons, has been increased because of exposure to extremely hot temperatures in summer. Heat stroke is caused by severe hyperthermia, and rectal temperatures of typical patients are higher than 40°C. Many organs such as liver, kidney and central nervous system (CNS) are damaged by severe hyperthermia [5, 6]. Thrombus, infarct, and death from heat stroke may be caused by injury of these organs. In animals and humans, some physiological and biochemical adaptations could occur to protect essential cell functions against the heat stress and to permit a rapid recovery from moderate hyperthermic damage [7,8,9,10]. However, each tissue and organ has a different sensitivity for sustaining thermal injury. Hence the present study was undertaken to study histological and pathological changes due to chronic heat stress on rat's liver.

MATERIALS AND METHODS

Animal handling

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A total 60 rats were included in this study. Out of which control group consisted of thirty rats (Group-A) and experimental group consisted of another thirty rats (Group-B). Control and Experimental group were included adult albino wistar male and female rats (n = 60) weighing 150-250gms. Animals were produced in the laboratory- breeding house of the Department of Human Anatomy. The control group of rats were maintained under controlled room temperature (26 ± 5°C) and light and dark (24:24 hr) conditions and were given mixed diet to be fed *ad libitum with equal amount of water and light supply*. The experimental groups of rats were maintained under increased room temperature (38±2°C) and light conditions and were given mixed diet to be fed ad libitium with equal amount of water. On day 15, experimental and control group of rats were sacrificed by cervical dislocation. Liver was removed and preserved in the formalin. Further, it was processed for section cutting and staining by Haematoxyllin and Eosin method.

Tissue Preparation

The rat's liver was dissected by cutting on the ventral side. 2 – 3 mm. of the liver tissue was kept in neutral buffered formalin (10% formaldehyde in Phosphate buffered saline) over 24 hours. After fixation, tissues was placed in 70% ethyl alcohol for 1 hour and then in each ascending strength (80%, 90%, 100% ethyl alcohol) for 1 hour each. The amount of alcohol used was 15 times greater than the size of the tissue. Then, the tissue was kept in acetone for a period of 1 – 2 h with periodical shaking. After removing the acetone, xylene was added to check for the milky appearance. Then, the dehydrated tissue was kept in paraffin wax (m.p. = 50°C) for a period of 1h at 58° – 60°C.Then, the tissue was poured in the molten paraffin fixed in L-block and allowed it to become hard. Then, the tissue was sectioned into very thin (2–8 or 5 – 10 micrometer) sections using a microtome. Then, the tissue was mounted on the slides with Mayer's albumin solution (a mixture of equal parts of egg white and glycerin, beaten and filtered with the addition of 1% sodium salicylate) and keep in warm oven for 2 h at 60°C.Then, placed slides containing paraffin sections on a slide holder.

Haematoxyllin and Eosin Staining

Tissues from above step were deparaffinized with Xylene was done for 20 – 30 minutes and blotted the excess xylene.Then, the tissue was rehydrated successively with 100%, 90%, 80% ethyl alcohol for 2–3 min. each & kept it into water for 3 min.Then, Blotted the excess water and the tissue was kept into Haematoxyllin stain for 3 – 5 minute. Then, tissue was removed it from Haematoxyllin stain and then again it was kept into tap water for 3 – 5 minute. Then, the tissue was immersed in Eosin stain for 3-5minute. Then, the tissue was dehydrated successively with 80%, 90%, 100% ethyl alcohol and finally it was kept into Xylene for 5minute. Then, mounting of slides was done by placing cover slip on the slides by using one drop of dibutyl phthalate xylene (DPX).

Data Analysis

Collected data were entered in Microsoft- excel-2007, numbering, coding was done according to different variables after that master chart was prepared. SPSS version 15.0 was used to calculation of frequency, percentage, mean and standard deviation (SD) as well as test of significance, P-value.

RESULTS

Effects of heat stress on experimental group of rat's liver

Thirty albino wistar rats weighing about 150-200gms in experimental group were kept in increased room temperature (i.e 38±2°C) for 15 days. Halogen heater maintained room temperature. After 15days, rats were sacrificed by cervical dislocation, Liver tissue was removed and it was kept in 10% formalin for 24 hours. Histological slides were prepared. All the experimental group of rat's liver slide is shown in (**Fig 1 & 3**). Large sized hepatocytes cells were seen and swollen. Sinusoids were not densely packed. Hepatic venules were dilated. Nuclei of hepatocytes cells were enlarged. Length and breadth of hepatocytes cells were larger than the control group of rat's liver. There was whitish-grey color of masses is seen in rat's liver. Some pathological changes was also seen in slide due to heat stress such as necrosis, inflammatory cell, some eosinophils cell, some Binucleated cell, polymorphism, some cells does not have cytoplasm, irregular cell membrane, single to multiple prominent nucleoli, collagen fiber, congested blood vessels and irregular clumping of chromatin.

Control group of rat's liver

Thirty albino wistar rats weighing about 150-200gms in control group were kept in normal room temperature (i.e 26±5°C) for15days. Room temperature was maintained by air conditioner. After 15 days rats were sacrificed by cervical dislocation. Liver tissue was removed and kept in 10% formalin for 24 hours and histological slides were prepared. All the control groups of rat's liver slide are shown in (**Fig 2 & 4**). Small sized

hepatocytes cells were seen and it was not swollen. Sinusoids were densely packed. Nuclei of the hepatocytes cells were small. Hepatic venule was not dilated. Length and breadth of hepatocytes cells were smaller than experimental group of rats. No mass was seen in control group of rat's liver. There were no pathological changes seen in slide such as necrosis, inflammatory cell, binucleated cells, polymorphism, congested blood vessel, irregular cell membrane, collagen fiber.

Fig 1: Experimental group of rat's liver slide. H&E Stain (Rat: LM×S40/0.65)

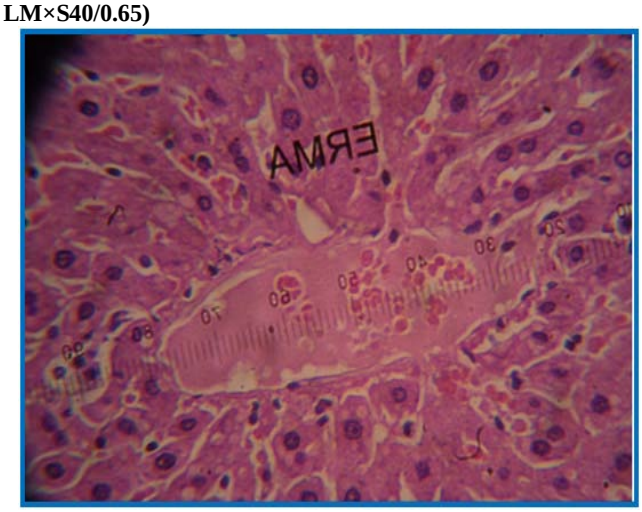


Fig 2: Control group of rat's liver slide. H&E Stain (Rat: LM×S40/0.65)

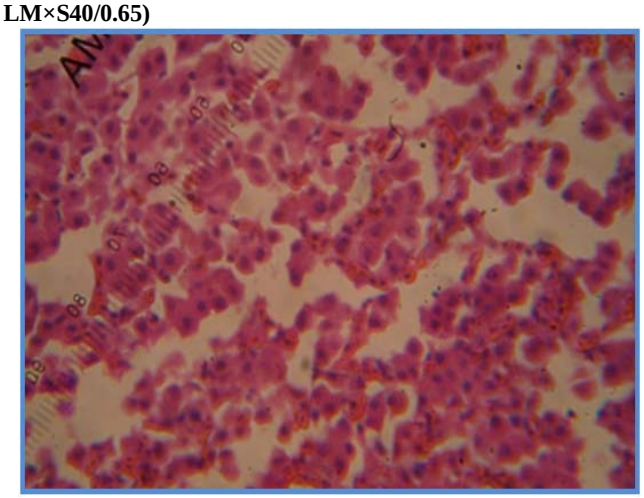


Fig 3: Experimental group of rat's liver slide. H&E Stain (Rat: LM×S40/0.65)

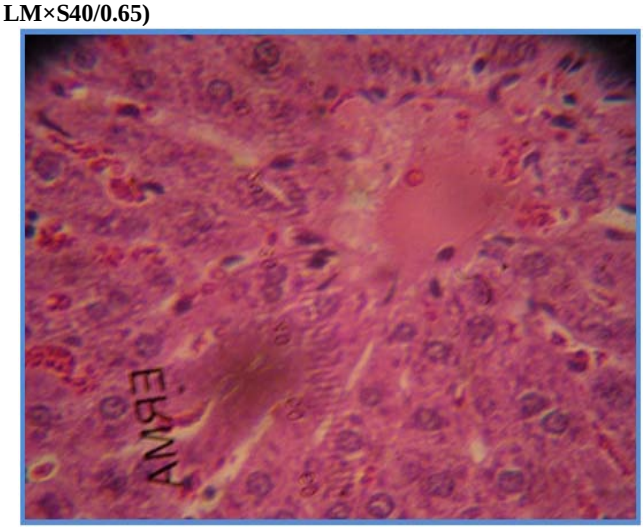
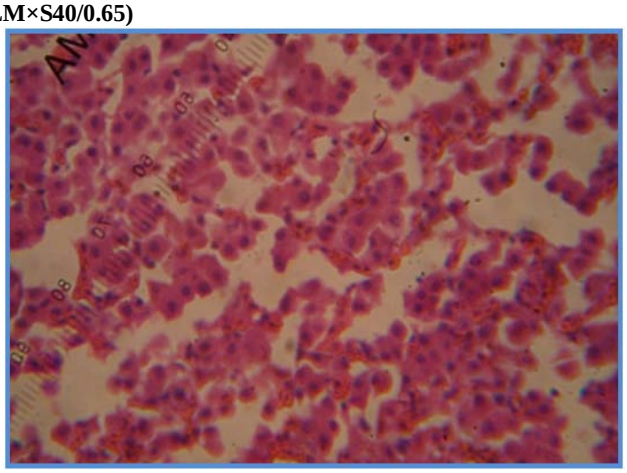


Fig 4: Control group of rat's liver slide. H&E Stain (Rat: LM×S40/0.65)



DISCUSSION

The present study demonstrates the effects of heat stress on rat's liver by using various experimental models. Sixty albino wistar rats weighing about 150-200gms evaluated in this study. The outcome of the present study demonstrates that heat stress produced several changes in liver morphology.

This is probably the first report on this aspect on heat stress. However, there are previous reports of changes in the hepatocytes of rat's liver. For example, Heat stress produced only a small degree of histopathologic damage in young animals, which peaked at 12 hours and recovered at 24 hours points after heating. The overall damage was characterized by cellular vacuolization and sinusoidal congestion. Old animals had widespread liver injury that became more severe over the 24 hours post heating period. At 24 hours there was severe hepatic damage including sinusoidal congestion, monocyte infiltration, hepatocellular vacuolization and widespread necrosis. Heat stress produced only a small degree of histopathologic damage in young animals, which peaked at 12 hours and recovered at 24 hours points after heating. The damage was characterized by cellular vacuolization and sinusoidal congestion. Old animals had widespread liver injury that became more severe over the 24 hours post heating period. At 24 hours there was severe hepatic damage including sinusoidal congestion, monocyte infiltration, hepatocellular vacuolization, and widespread necrosis [11, 12, 13&16]. But in present study it was found that there was densely packed sinusoids, length and breadth of hepatocyte size was highly significant statistically, necrosis of liver was seen.

Heat Shock Proteins (HSPS) are present in both prokaryotic and eukaryotic cells and their high level of conservation suggests that they play an important role in function cell processes [14, 15, 18&20]. HSPS were initially discovered in

drosophilamelanogaster larvae that were exposed to "heat stroke" and subsequent studies [23, 25]. Moreover, this condition stimulated parabrachial nucleus causing increase respiration, Packed cell volume (PCV) and Hemoglobin (Hb) concentration decreased under chronic heat stress might cause hypoxia [20,21,22,&30]. Data from subsequent studies demonstrated that the induction of HSPS was associated with development of tolerance to variety of stresses, including hypoxia, ischemia, acidosis, energy depletion, cytokines such as tumor necrosis factor and ultraviolet radiation [24, 25&27]. In present study shown, there was polymorphism, irregular nuclear membrane, irregular clumping of chromatin, some cell showed cytoplasm vacuolization, single to multiple prominent nucleoli, Binucleated cells and irregular cell membrane. So, increasing core body temperature during heat stress caused endothelial cell damage. Fatty degeneration is the accumulation of neutral lipids in the cytoplasm. This is diagnostic clue for liver injury. Excess lipids in hepatocytes indicate that sub lethal injury has occurred. However, the swollen, yellow, greasy, appearance of fatty degeneration is characteristic of liver and less common in kidney and heart. On microscopic examination, lipid accumulation causes cells to be enlarged, pale and lacy, especially in centrolobular zones-areas of liver where hepatocytes are most susceptible to oxygen [28, 29 &31]. So many cells of these organs were largely damaged and developed cells with fat globules or fatty degeneration [32]. In present study, the inflammatory cells, eosinophils, irregular nuclear membrane, and congested blood vessels were observed. Generally, oxygen deficiency is one of the most common causes of tissue injury when combined with red body temperature and hypoxia becomes a potent cause of death. Besides, necrosis from oxygen deficient develops in centrolobular areas of due to hypoxic liver. In mammal, ischemic hepatic necrosis occurs in cardiac failure, severe anemia and shock with prolonged low circulatory rates [33, 34]. Centritubular necrosis, dissociation of hepatocytes and congestion are often found in the liver [35, 36]. When body temperature rise above normal range, the parenchyma of many cells usually begins to be damaged [38]. Because previous studies specifically focused on the effects of heat stress on age-related mitochondrial responses, the impact of these mitochondrial alterations on cellular death is outside the scope of the present study. However, it would be enlightening in future studies to investigate the impact of heat stress on cellular apoptotic processes in old vs. young animals. While hyperthermic challenge has been shown to induce apoptosis in young mice and rats [39, 40]. In present study, whitish grey color of masses was seen in rat's liver. There was increased length and breadth of hepatocyte size of liver.

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REFERENCES

1. Hawkins-Bell L, Rankin JT. Heat-related deaths - Philadelphia and United States 1993-1994. *Morb Mortal Wkly Rep* 1994; 43:453-5.
2. Donoghue ER, Kalelkar MB, Boehmer MA, Wilhelm J, Whitman S, Good G et al. Heat-related mortality--Chicago. *Morb Mortal Wkly Rep* 1995; 44:577-9.
3. Tamura K, Ono M, Murakami M, Ando M. Relationship between atmospheric temperature and heat stroke by the emergency transportation record. *Jpn J Biometeor1995;* 32:111-4.
4. Burdon RH. Heat shock and the heat shock proteins. *Biochem J* 1986; 240:313-24.
5. Ostermann J, Horwich AL, Neupert W, Ulrich HF. Protein folding in mitochondria requires complex formation with HSP 60 and ATP hydrolysis. Nature 1989; 341:125-30.
6. Hales JRS, Hubbard RW, Gaffin SL. Limitation of heat tolerance. In: Handbook of Physiology (Fregly MJ, Blatteis CM, eds). New York: Oxford University Press, 1996; 279-355.
7. Freeman ML, Malcom AW, Meredith JW. Role of glutathione, thermal sensitivity and thermotolerance in Chinese hamster fibroblasts and their heat resistant varia nts. *Cancer Res* 1985; 46:1984-7.
8. Keatinge WR, Coleshaw SRK, Easton JC, Cotter F, Mattock MB, Chelliah R. Increased platelet and red cell counts, blood viscosity, and plasma cholesterol levels during heat stress, and mortality

from coronary and cerebral thrombosis. *Am J Med* 1986; 81:795-9.
9. Hales JRS, Richards DAB. Heat Stress: Physical Exertion and Environment. Amsterdam: Elsevier Science Publishers, 1987; 14:499-512.
10. Ando M, Katagiri K, Yamamoto S, Asanuma S, Usuda M, et al. Effect of hyperthermia on glutathione peroxidase and lipid peroxidative damage in liver. *J Therm Biol*1994; 19:177-85.
11. Imai M, Shimada H, Watanabe Y, Matsushima-Hibiya Y, Makino R et al. Uncoupling of the cytochrome P-450cam monooxygenase reaction by a single mutation, threonine-252 to alanine or valine: a possible role of the hydroxy amino acid in oxygen activation. *Proc Natl Acad Sci USA 1989;* 86:7823-7.
12. Tarr M, Samson F. Oxygen Free Radicals in Tissue Damage. Boston: Birkhauser, 1993; 12-53.
13. Privalle CT, Fridovich I. Induction of superoxide dismutase in *Escherichia coli* by heat shock. *Proc Natl Acad Sci USA* 84:2723-6.
14. Hruszkewycz AM, Glende EAJr, Recknagel RO. Destruction of microsomal cytochrome P-450 and glucose-6-phosphatase by lipid extracted from peroxidized microsomes. *Toxicol Appl Pharmacol* 1978; 46:695-702.
15. Ando M, Tappel AL. Methyl ethyl ketone peroxide damage to cytochrome P-450 peroxidase activities. *Toxicol Appl Pharmacol* 1985; 81:517-24.
16. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-dependent rat liver. *Biochem Biophys Res Commun*1976; 71:952-58.
17. Burk RF, Lawrence RA. Non-selenium dependent glutathione peroxidase. In Functions of Glutathione in Liver and Kidney (Sies H, Wendel A, eds.) New York: Springer Verlag, 1978; 114-9.
18. Lawrence RA, Burk RF. Species, tissue and subcellular distribution of non Se-dependent glutathione peroxidase activity. *J Nutr* 1978; 108:211-5.
19. Chopp, M. Transient hyperthermia protects against subsequent forebrain ischemic cell damage in the rat *Neurology* 1989; 39:1396-8.
20. Barbe MF, Tytell M, Gower DJ, Welch WJ. Hyperthermia protects against light damage in the rat retina *Science* 1988; 241:1817-20
21. Amin V, Cumming DVE, Latchman DS. Over-expression of heat shock protein 70 protects neuronal cells against both thermal and ischaemic stress but with different efficiencies *Neurosci Lett 1996;* 206:45-8.
22. West JW. Effects of heat-stress on production in dairy cattle. *J Dairy Sci* 2003; 86:2131-44.
23. Pereira AM, Baccari F, Titto EA and Almeida JA. Effect of thermal stress on physiological parameters, feed intake and plasma thyroid hormones concentration in Alentejana, Mertolenga, Frisian and Limousine cattle breeds. Int *J Biometeorol* 2007; 83:47-55.
24. Dale HE, Goberdhan C K, Brody S. A comparison of the effects of starvation and thermal stress on the acid-base balance of dairy cattle. *Am J Vet Res.*1954; 15:197-201.
25. Salo DC, Donovan CM, Davies KJ. HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise. *Free Radic Biol Med* 1991; 11:239-46.
26. Gordon CG, Lagnier J, Lee J, Buhler M, Kieffer S, Perrot M et al. The H2O2 stimulation in Saccharomyces cerevisiae. *J Biol Chem*1998; 273:22480-89.
27. Davidson JF, Whyte B, Bissinger PH, Schiestl RH. Oxidative stress is involved in heat-induced cell death in Saccharomyces cerevisiae. *Proc Natl Sci*1996; 93: 5116-21.
28. Niu KC, Lin MT, Chang C P. Hyperbaric oxygen improves survival in heatstroke rats by reducing multiorgan dysfunction and brain oxidative stress. *Eur J Pharmacol* 2007; 569:94-102.
29. Zhang Y, Mian MA, Chekhovskiy K, So S, Kupfer D, Lai H et al. Differential gene expression in Festuca under heat stress conditions. *J Exp Bot*2005; 56:897-907.
30. Bernabucci U, Ronchi B, Lacetera N, Nardone A. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *J Dairy Sci* 202; 85: 2173-79.
31. Harmon DL, Master DMc, Shields DC, Whiteheat AS, and Rea IM. MTHFR

thermolabile genotype frequencies and longevity in Northern Ireland. Atherosclerosis 131: 137-38.

32. Klatt P, Lamas S. 2000. Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. *Eur J Biochem* 2000; 267:4928-44.
33. Arechiga CF, Vazquez-Flores S, Ortiz O, Hemandez-Ceron J, Porras A et al. Effect of injection of beta-carotene or vitaminE and selenium on fertility of lactating dairy cows. Theriogenology 1998; 50:65-76.
34. Wu C. Heat shock transcription factors: structure and regulation. Annul Rev Cell Dev Boil 1995; 11: 441-69.
35. Malago JJ, Koninkx JFJG, Dijk JEV. The heat shock response and cytoprotection of the intestinal epithelium. Cell Stress Chap 2002; 7:191-9.
36. Santoro MG. Heat shock factors and the control of the stress response. Biochem. Pharmacol 2000; 59:55-63.
37. Horowitz M. Do cellular heat acclimation responses modulate central thermoregulatory activity? News Physiol *Sci* 1998; 13:218-25.
38. Yamashita NS, Hoshida N, Taniguchi T, Kuzuya MHori. Whole-body hyperthermia provides biphasic cardioprotection against ischemia/reperfusion injury in the rat *Circ* 1998; 98:1414-21.
39. Li P, L YM, Chao SH, Chan JY Chan. Potentiation of baroreceptor reflex response by heat shock protein 70 in nucleus tractus solitari confers cardiovascular protection during heatstroke. *Circ* 2001; 24: 2114-9.
40. Kregel K. Heat shock proteins: Modifying factors in physiological stress responses and acquired thermotolarance. *J App Physiol* 2002; 92:2177-86.