

Assessment of Liver Damage in Male Albino Rats after Repetitive Heat Stress of Moderate Level

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ABSTRACT

Background: There are multiple studies showing the effect of severe heat stress on systemic functions. Severe heat stress has been known to affect almost every organ in the body. It is well proved that liver is the organ which is severely affected by heat stress and the biochemical, morphological, and morphometric changes of liver following severe heat stress are well documented. But, the effect of repetitive heat stress of moderate level on the liver has not been studied extensively.

Aims & Objective: In the present study, the effect of repetitive heat stress of moderate level was assessed on biochemical [Serum Glutamic Pyruvate Transaminase (SGPT); Serum Glutamic Oxaloacetic Transaminase (SGOT); and Alkaline phosphatase (ALKP)], morphological, and morphometric changes of liver of adult albino male rats (wistar strain).

Materials and Methods: The experimental animals were subjected to repetitive heat stress for 4 hours daily, at $37 \pm 0.5^\circ\text{C}$ in a Biological Oxygen Demand (BOD) incubator (relative humidity 65 - 82%) for 2, 5, and 10 consecutive days. Biochemical assessment (SGPT, SGOT, and ALKP) was done on blood collected from left ventricle of beating heart of rats. Morphometric and morphological studies were conducted under light microscope on paraffin sections (H&E) of liver from control and experimental animals. The morphometric analysis was done by intersection - point counting method, using simple square lattice test system.

Results: The serum levels of liver enzymes were elevated in all heat exposed animals (statistically significant in five and ten days exposed animals) in comparison to controls. Morphological changes of anisocytosis at some sites, disruption of cell plates in lobules, mild Kupffer cell hyperplasia were present in rats exposed to heat for two consecutive days. After five days heat exposure ballooning degeneration, single cell necrosis along with small foci of necrosis disrupting cell plates in lobules, and sinusoidal compression were noticed. Kupffer cell hyperplasia was observed. At various sites, hepatocytes showed regenerative changes as binucleate cells and anisocytosis. After 10 days exposure, changes became more marked. The volume density of hepatocytes (V_{vh}) and numerical density of hepatocytes (N_{vh}) increased with the increase in heat exposure, despite increasing degenerative changes, confirming regenerative power of liver by hepatocyte proliferation. The increasing numerical density of Kupffer cells on area (N_{ak}) indicated progressive liver damage.

Conclusion: All the observations confirmed that exposure to repetitive heat stress, even of moderate level, leads to liver damage.

KEY WORDS: Anisocytosis; Heat Exposure; Liver; Rat; Regenerative Changes; Sinusoidal Compression

INTRODUCTION

There are a number of studies showing the effect of severe heat stress on systemic functions. Severe heat stress has been reported to affect almost every organ in the body.^[1,2] The first organ affected by heat stress is the skin and consequently, the first response is increased blood flow to it. The second response is reflex vasodilatation in the cutaneous vascular bed and vasoconstriction in the hepato-splanchnic vascular zone due to shifting of blood from this zone to cutaneous vascular zone to overcome the effect of heat stress.^[2]

In heat stressed conditions, the hepato-splanchnic vasoconstriction causes a fall in the hepatic venous oxygen content and a significant increase in the release of hepatic glucose.^[3] These factors probably lead to hepato-splanchnic hypoxia, a condition that is known to cause hepatocellular damage and rise in the levels of Serum Glutamic Pyruvate Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline phosphatase (ALKP), lactic acid dehydrogenase (LDH) and creatine phosphokinase (CPK), as shown in various human^[4-7] and animal^[8] studies.

Although, the biochemical, morphological, and morphometric changes following severe heat stress are well documented, the effect of repetitive moderate heat stress on the liver is not studied extensively.

In the present study, unacclimatized adult albino male rats were exposed to repeated heat stress of moderate level and observed for biochemical (SGPT, SGOT, and ALKP), morphological, and morphometric changes.

MATERIALS AND METHODS

Animals

Adult, male albino rats of Wistar strain, weighing 125–175 grams (5–8 weeks of age) were used, following approval of Animal Ethical Committee of HIMs, Dehradun. The animals were housed in plastic cages (43cm X 29cm X 15cm) under

standard laboratory conditions with ad libitum access to water and freshly cooked food (a mixture gm/kg of porridge – 630, ground nut cake – 100, milk powder – 100, black gram – 100, salt – 10, and fish meal – 60). Each cage contained one to two animals. The room temperature was kept within thermo-neutral zone for albino rats at $25 \pm 3^\circ\text{C}$ (relative humidity 65 - 85%), with the availability of normal day light. The experiment was conducted from September to December.

Experimental Protocol

The animals were divided into two groups, control having 6 animals and experimental with 18 animals.

The experimental animals were subdivided into 3 subgroups of 6 animals each for heat exposure of 2, 5, and 10 consecutive days. All the experimental animals were exposed to moderately high environmental temperatures ($37 \pm 0.5^\circ\text{C}$) in a BOD incubator (relative humidity 65-82%) for 4 hours daily (11:00 am to 03:00 pm). Animals were kept fasting for 3-5 hours prior to heat exposure. During the heat exposure period, only one animal was housed in each cage with liberty of movement and with ad libitum supply of water but no food. Following heat exposure, the animals were restored to room temperature with ad libitum supply of food & water.

Simultaneously, the control animals were kept at normal room temperature ($25 \pm 3^\circ\text{C}$). The controls were also fasted and provided with ad libitum supply of water but no food, just like the experimental animals.

Serum and liver samples for biochemical, morphological, and morphometric evaluation were collected after anesthetizing rats by intraperitoneal injection of a mixture of Ketamine (50 mg/Kg body weight) and Xylazine (6.8 mg/kg body weight)^[9], within one hour of last heat exposure in experimental animals and in control animals simultaneously. The animals were then sacrificed by exsanguination.

Biochemical Observations

Blood samples were collected from the left ventricle of the beating heart. Estimation of serum SGPT, SGOT, & ALKP were done on a semi-automatic RA-50 analyzer using the diagnostic reagent kit by DiaSys international.

Morphological Observations

The abdomens of anaesthetized animals were opened by midline incision and the liver was removed in a petri-dish containing cold formalinized saline (10%). The weight of the liver was measured. Thin slices (less than 3 mm) were cut from the liver lobe. The liver slices were fixed in cold formalinized saline for 24-48 hrs. After fixation, the tissue pieces were dehydrated in graded ethanol and embedded in paraffin. Three to five micrometer thick paraffin sections were cut, and stained with hematoxylin-eosin and special stain reticulin for examination under the light microscope.^[10]

Morphometric Observations

The morphometric observations on the liver sections were done by the intersection-point counting method, using simple square lattice test system A 100, having the quadratic test lines of spacing 5 mm (equivalent to a distance of 0.125 mm in the actual specimen, considering X 40 final magnification). The following morphometric variables were assessed according to the principles described by Loud, Weibel and Weibel et al.^[11-14]

Numerical density of hepatocytes (Nvh) – It tells about the number of hepatocytes in unit volume of liver and was estimated from the numerical density of hepatocytes nuclei (Nvn), since it is easier to count the nuclei in place of cells. The Nvn was calculated from the relationship $Nvn = 1/D \cdot Na/Pt \cdot d^2 \cdot k_2$. Na is the number of profiles (hepatocytes nuclei) per unit area. D is the mean tangent diameter of hepatocytes nuclei. Pt is the test point number. d is the test line distance in the square lattice. k_2 is a constant.

Volume density of hepatocytes (Vvh) – It tells about the volume fraction of liver tissue occupied

by the hepatocytes. It is calculated by the formula $Vvh = Pa/Pt$. Pa is the number of test points found enclosed within profile of the hepatocytes, and Pt is the test point number.

Numerical density of Kupffer cells on the area (Nak) – It expresses the number of Kupffer cells per unit area (per cm^2).

A preliminary study was also performed in six animals to find out the normal body temperature and to observe the changes in core body temperature (rectal temperature), during the heat exposure and to find out the core body temperature of animals after heat exposure.

Along with these observations, to assess the heat stress, WBGT (Wet bulb globe temperature) index was also calculated for animal room and for BOD incubator for five consecutive days of heat exposure.^[15-17]

Data Analysis

The data was analyzed by using both quantitative and qualitative techniques. Analysis of quantitative data was done by using the Student's paired, unpaired (independent) t-test, and ANOVA. All the data are expressed as mean \pm SEM. The program "GraphPad InStat 3.06" was used for this analysis. Qualitative analysis was used for the morphological changes of the liver. The qualitative information was used to support the quantitative findings.

RESULTS

In the preliminary study, the core body temperature of animals was 34.75 ± 0.182 in the morning before first heat exposure and it rose to statistically significant value of 38.53 ± 0.265 °C (just after heat exposure) (P value is < 0.0001).

The calculated WBGT indexes for animal room and BOD incubator respectively were 24.4 ± 0.30 and 36.5 ± 0.03 °C. This change in WBGT index was statistically significant (P value is < 0.0001).

General activity of the animals was sluggish upto about 2 hrs. after the heat exposure, though animals appeared healthy.

Biochemical Observations

The serum levels of liver enzymes in experimental animals were elevated in comparison to controls. The elevations in 5 and 10 days exposed animals were upto statistically significant levels. While in 2 days exposed animals, this elevation was statistically non-significant. The details of biochemical observations are summarized in Table 1.

Morphological Observations

The morphological findings in the control group are depicted in Figure 1 while findings in the experimental group are shown in Figures 2 and 3. In rats exposed to heat for two times, morphological changes of increased variation in size and staining of hepatocytes nuclei (anisocytosis) at some sites with disrupting liver cell plates in lobules, mild Kupffer cell hyperplasia were present. After five times heat exposure ballooning degeneration, single cell necrosis along with small foci of necrosis disrupting cell plates in lobules, and sinusoidal compression were noticed. These changes were more pronounced in peri-portal areas and zone 2 of hepatic lobules. Kupffer cell hyperplasia was observed in all these experimental animals and it was more in comparison to two times exposed animals. At various sites, hepatocytes showed regenerative changes as binucleate cells and anisocytosis. After 10 times exposure, changes became more marked.

Table-1: Effect of Repetitive Heat Exposure (4 hours/day at 37 ± 0.5°C) on Serum Levels of Liver Enzymes

Liver Enzymes	Control (n=6)	Experimental			p value (ANOVA)
		2 Days (n=6)	5 Days (n=6)	10 Days (n=6)	
SGPT(IU/L)	43.7 ± 4.85	52.2 ± 3.24 ^{ns}	81.8 ± 2.55 ^{***}	88.0 ± 0.98 ^{***}	< 0.0001
SGOT(IU/L)	111.8 ± 11.37	124.7 ± 8.73 ^{ns}	188.0 ± 14.14 ^{***}	192.7 ± 4.74 ^{***}	< 0.0001
ALKP(IU/L)	203.2 ± 6.03	232.7 ± 6.83 ^{ns}	441.4 ± 25.95 ^{***}	505.9 ± 5.27 ^{***}	< 0.0001

Values are expressed as Mean ± SEM. *p^{ns}>0.05*, *p*[<]0.05*, *p**[<]0.01*, and *p***[<]0.001*, as compared with control values. Abbreviations: SGPT (Serum Glutamic Pyruvate Transaminase), SGOT (Serum Glutamic Oxaloacetic Transaminase), and ALKP (Alkaline phosphatase)

Table-2: Effect of Repetitive Heat Exposure (4 hours/day for at 37 ± 0.5°C) on Morphometric Parameters

Morphometric Parameters	Controls (n = 6)	Experimental			p value (ANOVA)
		2 Days (n = 6)	5 Days (n = 6)	10 Days (n = 6)	
Vvh	0.796 ± 0.004	0.803 ± 0.002 ^{ns}	0.926 ± 0.004 ^{***}	0.919 ± 0.007 ^{***}	< 0.0001
Nvh (millions/mL)	14.94 ± 0.126	15.08 ± 0.047 ^{ns}	19.01 ± 0.064 ^{**}	19.14 ± 0.053 ^{**}	< 0.0001
Nak (per cm ²)	4905.7 ± 9.472	5045.7 ± 40.829 [*]	7048.7 ± 15.720 ^{***}	7300.0 ± 4.529 ^{***}	< 0.0001

Values are expressed as Mean ± SEM. *p^{ns}>0.05*, *p*[<]0.05*, *p**[<]0.01*, and *p***[<]0.001*, as compared with control values. Abbreviations: Vvh (Volume density of hepatocytes), Nvh (Numerical density of hepatocytes), and Nak (Numerical density of Kupffer cells on the area).

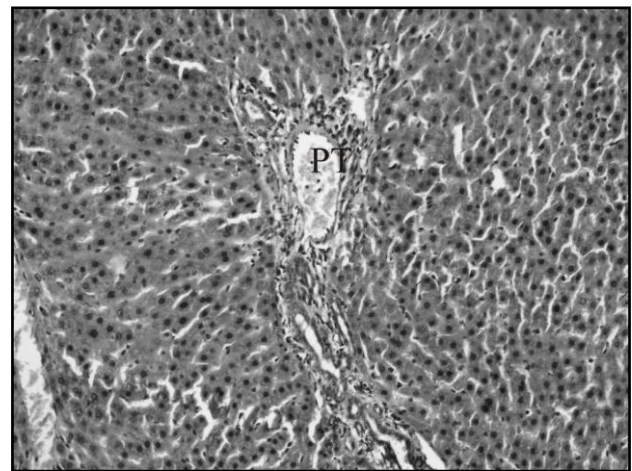


Figure-1: Photomicrograph of liver of control animal showing single cell thick liver cell plate, normal sinusoids, and portal triad (PT). Mild to moderate mononuclear infiltration in portal area. (HE. 400X)

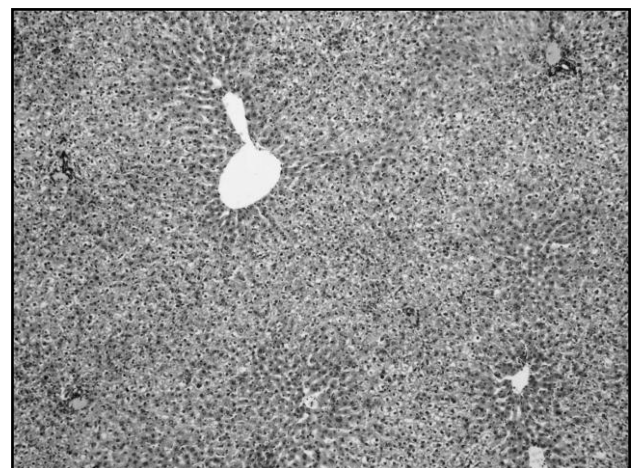


Figure-2: Photomicrograph of liver of experimental animal (05 Days Exposure) showing foci of necrosis, mainly in zone 2, disrupting liver cell plate. Hepatocytes show ballooning degeneration. (HE. 100X)

Morphometric Observations

The morphometric parameters (Vvh, Nvh, and Nak) were much more in all the experimental animals in comparison to controls. All the three parameters were increased in 5 and 10 days

exposed animals, upto a statistically significant level. In 2 days exposed animals, the elevations of Vvh and Nvh were statistically non-significant while the increment in Nak was statistically significant. The details of morphometric observations are summarized in Table 2.

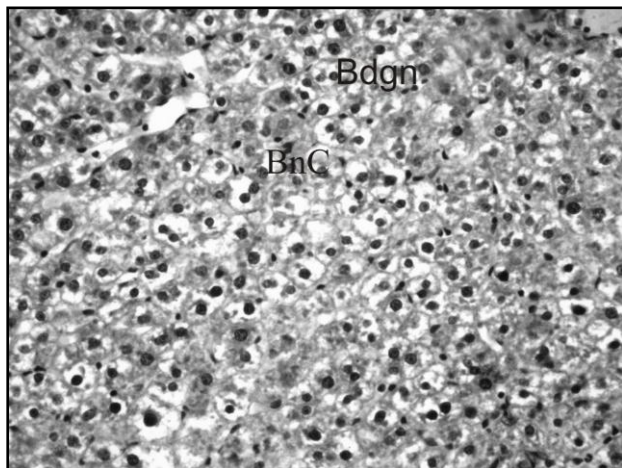


Figure-3: Photomicrograph of liver of experimental animal (05 Days exposure) showing foci of necrosis, mainly in zone 2, disrupting liver cell plate. Ballooning degeneration (Bdgn), Binucleate cells (BnC), and Kupffer cell hyperplasia are also seen. (HE. 400X)

DISCUSSION

Preliminary study of core body temperature indicated that there was substantial increase in core body temperature after the heat exposure.

WBGT index clearly showed that there was significant heat stress to animals when they were exposed even to moderately high environmental temperatures of $37 \pm 0.5^{\circ}\text{C}$ from the ambient temperatures of $25 \pm 3^{\circ}\text{C}$.

Biochemical Observations

SGPT is a liver specific enzyme. High levels of SGPT are indicative of liver injury; SGPT rises dramatically in acute liver damage.^[18,19] High levels of SGOT and ALKP along with high SGPT levels are also indicative of liver injury.^[18,19] Serum SGPT, SGOT, and ALKP are the most sensitive markers of liver damage because they are cytoplasmic in location and are released into the circulation after hepatocellular damage.^[18,20]

As shown in Table 1, the serum levels of liver enzymes in experimental animals (exposed 02 Days) were elevated but not at statistically

significant levels. The serum levels of liver enzymes in experimental animals (05 Days exposed) were elevated to statistically significant levels, in comparison to controls, indicating liver damage. In 10 days exposed animals, the serum levels of enzymes were much more increased (even more than 05 days exposed animals) up to statistical significant levels in comparison to controls. These findings are almost similar to the findings of various Severe Heat Stress studies.^[4-7]

Morphological Observations

In two days heat exposed animals, observed disruption of liver cell plates and anisocytosis indicated increased hepatocytic proliferation though no substantial morphological changes suggestive of liver cell necrosis were present. As evident in Figures 2 and 3, there were degenerative changes such as ballooning degeneration, Kupffer cell hyperplasia, single cell necrosis/drop out as well as foci of necrosis, sinusoidal compression, and disruption of liver cell plates in the liver of 5 days exposed animals. After 10 days exposure these changes were more marked. The occurrence of degenerative changes in the liver parenchyma following heat exposure indicates that even moderate repetitive heat stress may cause significant damage to the liver. These findings are very similar to findings observed by Sharma.^[8]

In some of the specimens, regenerative changes such as anisocytosis and presence of binucleate cells were also noted in the experimental animals. These changes advocated increased proliferation of hepatocytes, also indicative of liver damage due to heat stress.

Morphometric Observations

There was a successive increase in Vvh and Nvh as the number of heat exposures increases (Table 2). It reveals that there is a continued proliferation of hepatocytes. These findings, in presence of increasing degenerative and necrotic changes in liver parenchyma, confirm the regenerative power of the liver. It reiterates the fact that liver has the capacity to regenerate itself after degenerative injury. These changes are very

similar to changes observed by Sharma in his study of morphological and morphometric changes after moderate heat stress.^[8]

Kupffer cell hyperplasia, an established sign of liver injury was observed by Kew and Kent^[5] and by Gupta and coworkers^[7] in their studies of severe heat stress. There is increased hyperplasia of Kupffer cells as revealed by Nak confirming the increased liver injury with increasing repetition of heat exposure.

While doing this study, few limitations were noticed but some of them were inadvertent such as small sample size which was the only size permitted by Animal Ethical Committee. Another limitation was to not to assay any stress related hormone but because of limited funds for the study, biochemical observations were done very selectively, judiciously, and in a justified way.

CONCLUSION

Biochemical, morphological, and morphometric observations prove that exposure to repetitive heat stress, even of moderate level, leads to liver damage of a significant level in adult male albino rats (Wistar strain).

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REFERENCES

- Hoppe J, Sinert R, Kunihiro A, Foster J. Heat Exhaustion and Heatstroke. eMedicine 2008. Available from: URL: <http://www.emedicine.com/emerg/topic236.htm>
- Helman RS, Habal R. Heatstroke. eMedicine 2007. Available from: URL: <http://www.emedicine.com/med/topic956.htm>
- Rowell LB, Brengelmann GL, Blackmon JR, Twiss RD, Kusumi F. Splanchnic blood flow and metabolism in heat-stressed man. J Appl Physiol 1968; 24:475-84.
- Kew M, Bersohn I, Seftel H. The diagnostic and prognostic significance of the serum enzyme changes in heatstroke. Trans Royal Soc Tropical Med Hyg 1971;65(3):325-330.
- Kew M, Kent G. Liver damage in heatstroke. Am J Med 1970;49:192-202.
- Alzeer AH, El-Hazmi MAF, Warsy AS, Ansari ZA, Yrkendi MS. Serum enzymes in heat stroke: prognostic Implication. Clinical Chemistry 1997;43(7):1182-1187.
- Gupta NN, Mehrotra RML, Agarawal NL. Evaluation of functional and morphological status of liver during pyrexia. J Ass Physi India 1963;11:747-754.
- Sharma RK. Morphological and morphometric studies on Liver in rats subjected to repetitive heat stress. Indian J Med Research 1997;106: 20-26.
- Harwood PD. Therapeutic dosages in small and large mammals. Science 1963; 139: 684-685.
- Drury RAB, Wallington EA. Fixation, Preparation of tissues for microtomy, Microtomy, General outline of the theory and practice of staining and general staining procedures. In: Drury RAB, Wallington EA (edi). Carleton's Histological Technique. 4th ed. New York: Oxford university press. 1967. p. 33-137.
- Loud AV. A quantitative stereological description of the ultrastructure of normal rat liver parenchymal cells. JCB 1968;37:27-46.
- Weibel ER. Stereological methods, Practical methods for biological morphometry, Vol. I. London: Academic press; 1979. p. 109.
- Weibel ER, Staubli W, Gnagi HR, Hess FA. Correlated morphometric and biochemical studies on the liver cell, I Morphometric model, stereologic methods, and normal morphometric data for rat liver. JCB 1969; 42: 68-91.
- Reid IM. Morphometric Methods in Veterinary Pathology: A Review. Vet Pathol 1980;17:522-543.
- Occupation safety and health administration. Measurement of Wet Bulb Globe Temperature Index. In: OSHA technical manual. United States: department of labor. Available from: URL: http://www.osha.gov/dts/osta/otm/otm_iii/otm_iii_4.html
- Wet Bulb Globe Temperature. Wikipedia: the free encyclopedia. Available from: URL: http://en.wikipedia.org/wiki/Wet_Bulb_Globe_Temperature
- Health and safety executive. Wet Bulb Globe Temperature Index. Available from: URL: <http://www.hse.gov.uk/temperature/heatstress/measuring/wetbulb.htm>
- Reyers F. Liver Enzyme Profiling: What Works and What Does Not. Proceedings of 28th World Congress of the WSAVA. Bangkok.2003.
- Liver function tests. Wikipedia: the free encyclopedia. Available from: URL: http://en.wikipedia.org/wiki/Liver_function_tests
- Pari L, Amali DR. Protective role of tetrahydrocurcumin (THC) an active principle of turmeric on chloroquine induced hepatotoxicity in rats. J Pharm Pharmacol Sci 2005; 8(1):115-123.

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