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HISTOLOGICAL CHANGES IN MAMMALIAN LIVER AND HEART IN RESPONSE TO GRADED HYPERTHERMIA

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ABSTRACT

Heat stroke is the most severe and potentially fatal heat related illness. Liver and heart are particularly important organs for heat stroke studies as abnormal liver enzymes' levels and myocardial damage biomarkers are associated with heat stroke. Objective: We aim to investigate the histological changes associated with heat stress as it increases in intensity and duration. Methods: Rats were exposed to 45°C temperature and 30% relative humidity to induce varying degrees of acute heat stress and heat stroke. Results: Histological analysis revealed the association of heat stroke with symptoms of cardiac failure wherein the sinusoidal dilatation and congestion in liver was observed which increased with the degree of hyperthermia. When the venous return through hepatic central vein is reduced, the blood accumulates in the sinusoidal region giving it a dilated appearance. The accumulation of blood in sinusoidal spaces was evident from the engorging of RBCs. The overall damage was characterized by hepatic damage including sinusoidal dilatation and congestion, monocyte infiltration, hepatocellular vacuolization, and widespread necrosis. The tissues from heat stressed rats showed focal areas of necrotic fiber revealing fragmentation of microfilaments, leading to appearance of empty spaces (cardiomyocytes dropout). Also, congestion and extravasations of blood was observed in heat stressed groups resulting from an increase in cardiac output and vasodilatation to cope with heat stress. Conclusion: The histological changes became more severe with the degree of heat stress. Further biochemical and genetic level studies are required to understand the pathogenesis of heat stroke for design of appropriate therapeutic and diagnostic measures.

Keywords: Sinusoidal dilation, Hepatocellular vacuolization, Histological analysis, Heart, Myocardium, Fibres.

INTRODUCTION

Heat stress is the condition wherin the body's avenues of controlling internal temperature start failing. Heat stress, when combined with the stressors such as physical work, loss of fluids, fatigue and medical conditions, culminates in heat related illnesses, which range from mild heat exhaustion to potentially fatal heat stroke. Heat stress is marked by increased core temperature, heart rate and sweating [1,2]. When exaggerated, these conditions can result in heat stroke, a condition that involves a multitude of host – defense responses involving activation of pro-inflammatory and inflammatory cytokines. The data on the incidence of heat stroke undermine the gravity of this illness as it is under diagnosed and lacks a proper definition. An epidemiologic study in urban areas in the United States during heat waves indicated the incidence of heat stroke to vary from 17.6 to 26.5 cases per 100,000 populations. It is observed that the victims of classic heat stroke are very young or elderly, poor, and socially isolated. They generally do not have the access to air conditioning. In Saudi Arabia, the incidence of heat stroke varies seasonally ranging from 22 to 250 cases per 100,000 populations. Heat Stroke showed a very high incidence of death (15,000 individuals) during the 2003 heat wave in France [3].

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The liver is the key player in mediating the onset of systemic inflammatory response syndrome being the major site of endotoxin clearance [4-6]. An association has been observed between liver damage and circulating endotoxin levels in heat stroke patients and animal models. Massive liver cell necrosis occurs in response to thermal shock, circulatory disruption, endotoxinemia (heat sepsis), high blood concentration of cytokines and acute-phase proteins [7,8].

Cell lines and animal model studies have established heat as inducing direct tissue injury with the degree and duration of hyperthermia determining severity of tissue injury and cell death [9-12]. Clinical studies have shown that in most cases, heatstroke induced death occurs soon after the onset of hyperthermia and the associated cardiovascular failure [13].

We investigated the effect of increasing magnitude of heat stress culminating in heat stroke on histological aspects of Liver and heart. Sprague-dawley Rats were divided into 5 groups and subjected to heat stress to attain a core body temperature of 39°C, 40°C, and 41°C. Rat group not subjected to heat stress served as control and the other group was subjected to heat stress till death. The Liver and heart histology was compared for in all 5 groups with an objective of facilitating the understanding of structural changes in these organs with severity of hyperthermia and its correlation with alteration in functionality. Heat stress measured in terms of elevated core body temperature was found to induce organ damage with increase in severity of damage with elevation in core body temperature.

MATERIALS AND METHODS

Experimental Animals

Adult Sprague- dawley rats (weight, 300±50 g) were obtained from the Animal Resource Center of Defence Institute of Physiology and Allied Sciences (DIPAS), Defence Research and Development Organization (DRDO). The animals were housed at an ambient temperature of 25±1°C, with a 12-hour light/dark cycle. Pelleted rat chow and tap water were made available *ad libitum*. All the protocols were approved by the Animal Ethical Committee of the Institute.

Heat stress protocol and experimental groups

All rats were handled daily and familiarized with the rectal temperature probe (rectal Probe for Rats, RET-2, AD instruments) during the week preceding the heat stress protocols for each group of rats. On the day of the heat exposure, each rat was fitted with the rectal temperature probe inserted 6–7 cm into the rectum and then placed in a plastic cage, conscious and unrestrained. Rectal temperature was continuously monitored on a digital display using Lab chart 7, AD instruments. The experiments were terminated when the targeted level of core body temperature was attained.

Animals were randomly assigned to 1 of the following 5 groups with 6 rats in each group (n=6):Group 1 was exposed to an ambient temperature of 25°C and Relative Humidity (RH) of 30% in a temperature-controlled chamber for 30 minutes to reach thermal equilibrium. This group served as the control. Group 2 was exposed to an ambient temperature of 45°C and 30% RH till the rectal temperature reached 39°C. Similarly, animals in group 3 and group 4 were exposed to 45°C and 30% RH till the rectal temperature reached 40 °C and 41°C respectively. For group 5, the exposure to 45°C and 30% was continued till heat stress induced death of Rats.

Surgery and sample collection

The heat exposed and control animals were anesthetized with an intraperitoneal dose of 80mg/kg ketamine and 5mg/kg xylazine and subsequently dissected to draw blood from heart for biochemical evaluation. Blood Urea Nitrogen (BUN) and creatinine was measured using Chem8+ cartridge with *i-STAT* SYSTEM, Abbott. Rat Liver and heart were surgically removed for histological analysis.

Fixing and Sectioning Paraffin-embedded Tissues

The dissected tissue was immersed in 10% formalin solution for 10 hours at room temperature with 50 times the volume of formalin for the given tissue volume.

The tissue was immersed in 70% ethanol three times for 30 minutes each at room temperature followed by immersion in 90% ethanol two times for 30 minutes each. The tissue was then immersed in 100% ethanol three times for 30 minutes each at room temperature. Finally, the tissue was immersed in xylene (mixed isomers) three times for 20 minutes each at room temperature. Now, the tissue was embedded in paraffin at 58 °C. 5 - 15 µm thick tissue sections were obtained using a rotary microtome. The sections were floated in a 56 °C water bath. The sections were mounted onto gelatin-coated histological slides. The slides were dried overnight at room temperature and stored at room temperature till HE staining was performed.

To perform HE staining, the samples were deparfinized by 3 washes of 100% xylene, 3 minutes each. This was followed by 3 washes, 3 minutes each in decreasing concentrations of ethanol (95 and 90% respectively) and a final distilled water wash. The sample was then stained in hematoxylin for 6 minutes and rinsed in running tap water for 20 minutes followed by a 3 second wash in acid alcohol. The sample was rinsed well in tap water for 5 minutes followed by immersion in lithium carbonate for 3 Seconds. The sample was then rinsed in tap water for 5 minutes, followed by counterstaining in eosin for 15 seconds. The sample was dehydrated by incubation in increasing concentration of ethanol (2 washes in 95% ethanol followed by 2 washed in 100% ethanol). The sample was cleared in Xylene and mounted [14].

RESULTS AND DISCUSSION

Figure 1. The histological changes associated with heat stress were evaluated by exposing Rats to 45°C temperature and 30% relative humidity till the core body temperature reached 39, 40°, 41° respectively (group II, III and IV). Heat exposure continued till death for group V and control Rat was not subjected to heat stress and exhibited a core body temperature of 37°C. The liver tissue was surgically removed from each group, fixed in formaldehyde, sectioned, H&E stained and visualized under light microscope.

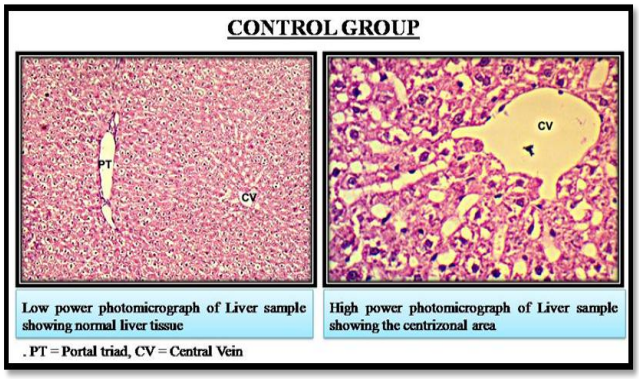


Figure 2. The liver sections from the control group showed a number of lobules with a thin walled central vein from which cords of hepatic cells radiated towards the lobule periphery. The hepatic cords alternated with narrow irregular blood spaces i.e., the hepatic sinusoids. Around the periphery of each lobule, branches of hepatic artery, hepatic portal vein and bile duct were present, constituting the portal triad. Liver sections from Group II, III and IV respectively exhibited an increasing sinusoidal dilation.

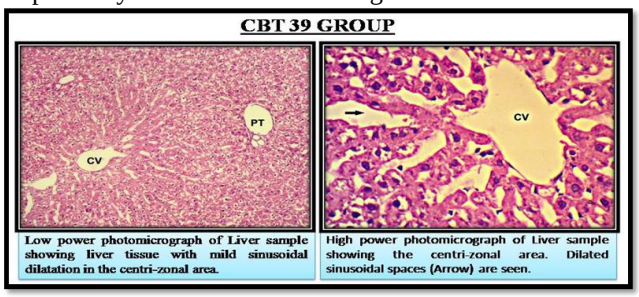


Figure 3. Control Group

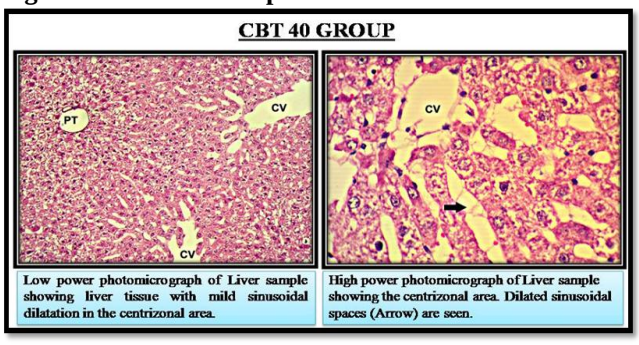


Figure 4. Group V showed marked sinusoidal dilation especially in the centrizonal area with severe vascular congestion throughout the liver parenchyma. Sinusoidal spaces and the central vein were engorged with RBCs.

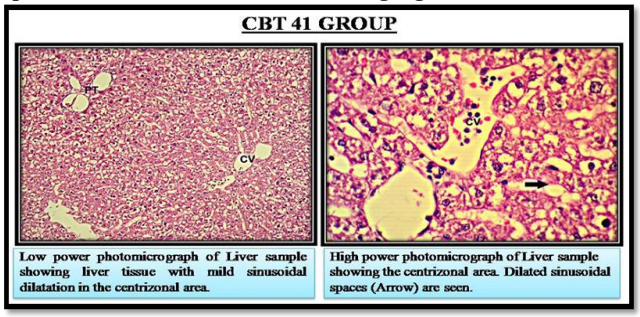


Figure 5. Histological analysis revealed the association of heat stroke with symptoms of cardiac failure wherein the sinusoidal dilation in liver was observed. When the venous return through hepatic central vein is reduced, the blood accumulates in the sinusoidal region giving it a dilated appearance [18]. The accumulation of blood in sinusoidal spaces is evident from the engorging of RBCs.

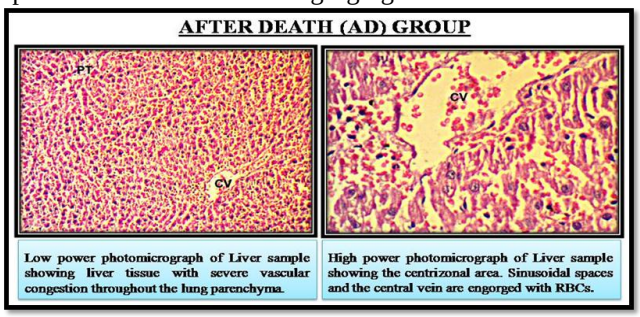


Figure 6. The increasing magnitude of heat stress was associated with increased sinusoidal dilatation indicating a progressive decrease in venous return with magnitude of hyperthermia. The cardiomyocytes appeared as branching, anastmosing cylinders with uniform diameters with acidophilic sarcoplasm and central elongated vesicular nuclei. Group II showed cardiac muscle fibers separated from each other by edematous spaces. Group III showed cardiac muscle fibers with focal RBC extravasation.

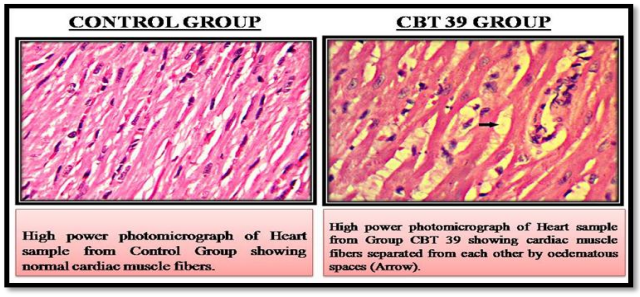
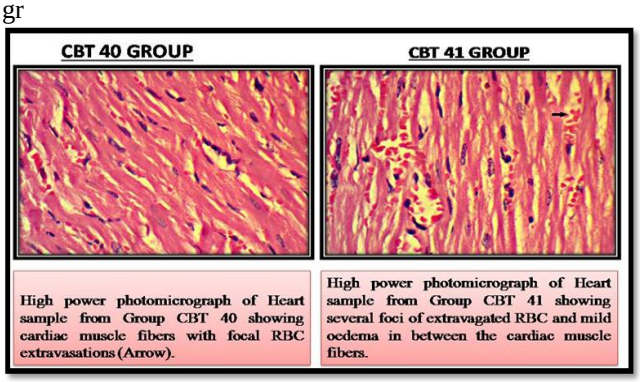


Figure 7. Group IV showed several foci of extravasated RBCs and mild edema in between the cardiac muscle fibers. High power photomicrograph of Heart sample from

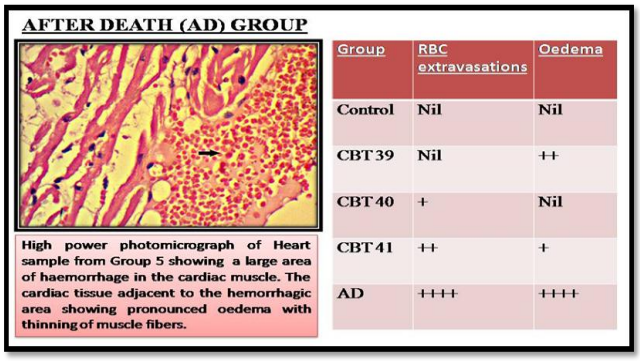
Group V showed a large area of hemorrhage in the cardiac muscle. The cardiac tissue adjacent to the hemorrhagic area showed pronounced edema with thinning of muscle fibers. Sections of hearts of heat stressed rats of group (II, III, IV) showing histological changes in the form of focal areas of necrotic fiber revealed loss of cellular pattern and fragmentation of microfilaments with nuclear changes leading to appearance of empty spaces (cardiomyocytes dropout).

Figure 8. The congestion and extravasation of blood observed in heat stressed gr



oups can be explained on the basis of the observation that cardiovascular system increases its capacity to perform greater amount of work to adapt for heat stress by increase cardiac output, vasodilation and increase blood flow. The magnitude of the histological changes increased with the degree of heat stress [19, 20].

Further molecular and biochemical analysis is required to elucidate the mechanism of decline in organ function to device therapeutic tools to alleviate the morbidity and mortality associated with heat related illnesses.



CONCLUSION

Heat stroke is the most severe of the heat related illnesses, which include heat exhaustion, heat cramps and heat syncope [15]. We investigated the effect of graded hyperthermia on Liver histology in Sprague- dawley Rat. Liver is a particularly important organ for heat stroke studies as abnormal liver enzymes' levels are associated with most of the cases of heat stroke [16]. Liver failure is potentially life threatening as this gland plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification [17].

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