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BIOCHEMICAL EVALUATION OF HEPATIC DAMAGE IN MAMMALIAN ACUTE HEAT STRESS AND HEATSTROKE MODELS

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ABSTRACT

Heatstroke is a very common clinical emergency in hot areas. In absence of timely and effective intervention, it may progress to death. The pathophysiological mechanisms underlying heatstroke in dry-heat environment of deserts has not been thoroughly investigated (ou Zhou *et al*., 2014). A strong association has been observed between heat stroke and acute liver failure (Garcin *et al*., 2008) suggesting that hepatic function indicators can serve as potential heat stress biomarkers. We have established rat models of acute heat stress and heatstroke simulating dry-heat environment of desert, to assess the impact of graded heat stress on Liver function indicators. With acute heat stress progressing to heat stroke, the liver damage changed from mild to serious as concluded by assessing blood enzymology parameters such as alanine aminotransferase and aspartate aminotransferase etc. This suggests the applicability of hepatic damage blood enzymology as a potential heat stroke diagnostic tool.

Key Words:- Heat Stroke, Acute heat stress, Liver function test, Biochemical analysis.

INTRODUCTION

Heat stroke is the most severe of all heat related illnesses. It has the potential of being fatal under lack of effective and timely treatment. It is marked by central nervous system dysfunction resulting in delirium, convulsions and coma.

In the times to come, the threat posed by heat stress will only increase owing to a rise in global temperatures. Heat stroke remains under-diagnosed, serving as a research area with tremendous potential to reduce the associated indisposition and death. Currently, heat stroke treatment involves rapidly reducing body temperature and replenishing body fluids coupled with intensive care support. However, only a little is known about the prognosis of heat related illnesses including heat stroke, impeding design of effective management and treatment strategies (Singh *et al*., 2013).

In most cases of heat stroke, acute liver failure has been observed, suggesting that hepatic function indicators can serve as potential heat stress biomarkers. With an aim to test the applicability of hepatic damage blood enzymology as a potential heat stroke diagnostic tool, we investigated the effect of graded heat stress on liver damage induced biochemical changes.

Sprague-dawley Rats served as the ideal model organism for these experiments to allow climatic, physiological, and biochemical manipulations and

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observations for investigating pathophysiology of heatstroke with the same normal core body temperature as humans and similar threshold for heat stroke induction (Hubbard *et al*., 1976)

We investigated the effect of increasing magnitude of heat stress culminating in heat stroke on liver function indicators. Sprague-dawley Rats were divided into 4 groups and subjected to heat stress to attain a core body temperature of 39°C, 40°C, and 41°C along with control group. Rat group not subjected to heat stress served as control and the other group was subjected to heat stress till death.

Biochemical parameters were compared for in all 4 groups with an objective of facilitating the understanding biochemical responses 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 an increase in liver damage biomarkers in a graded fashion. This study, combined with histological and genome-wide studies on heat stress and heat stoke will provide insights into the mechanisms underlying the pathophysiology of liver damage upon progression from mild hyperthermia to heat stroke with increasing core body temperature (in a graded fashion), providing cues for development of appropriate diagnostic and therapeutic measures for heat related illnesses including the potentially fatal heat stroke.

METHODOLOGY

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 4 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 (Control group). 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.

Sample collection

The animals (both control and experimental groups) were anesthetized with an intraperitoneal dose of 80mg/kg ketamine and 5mg/kg xylazine. Dissection was performed to draw blood into BD vacutainer SST – II Advance (5 ml) and BD microtainer brand tube with EDTA (1 ml), for haematological and biochemical analysis respectively.

Biochemical Analysis

For haematological analysis, an automated haematology analyzer (KX-2IN, Sysmex Corporation, Japan) was used. For biochemical analysis, Selectra Junior version 04 autoanalyzer (Vital Scientific Bv, Netherlands) was employed.

RESULTS

Liver Function Test

Total protein, Albumin and Globulin concentrations were measured in control and heat stressed groups. The concentration of total protein was found to decrease significantly with increase in severity of heat stress which is measured as 65.44±0.01, 63.85±0.02, 63.48±0.17 and 63.08±0.02 g/L ($p<0.001$) in control, CBT 39, CBT 40 and CBT 41 groups respectively. Similar results were found for Albumin as 34.96±0.01, 33.88±0.02, 33.86±0.03 and 34.68±0.03 ($p<0.01$) and globulin as 30.50±0.09, 29.16±0.02, 28.80±0.14 and 16.48±0.01 in control, CBT 39, CBT 40 and CBT 41 groups respectively. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzyme along with direct, indirect and total bilirubin, were measured and following values are observed (Table 1).

Lipid Profiling

Total cholesterol, triglycerides, high density lipoproteins (HDL) and low density lipoprotein (LDL) was measured.

Table 1. Biochemical parameters

Biochemical parameters	Control	CBT 39	CBT 40	CBT 41
D. Bilirubin (μmolL^{-1})	1.66±0.01	1.66±0.01	1.84±0.03	2.62±0.01
Ind. Bilirubin (μmolL^{-1}	0.52±0.02	0.66±0.01	1.20±0.14	2.02±0.01
T. Bilirubin (μmolL^{-1})	2.18±0.01	2.50±0.14	3.14±0.01	3.93±0.01
ALT (UL^{-1})	30.30±0.14	33.20±0.14	33.55±0.01	34.43±0.01
AST (IUL^{-1})	11.18±0.01	12.43±0.01	18.08±0.01	22.58±0.01
ALP (UL^{-1})	38.90±0.14	46.53±0.01	51.02±0.01	51.34±0.01

Figure 1. Liver function tests

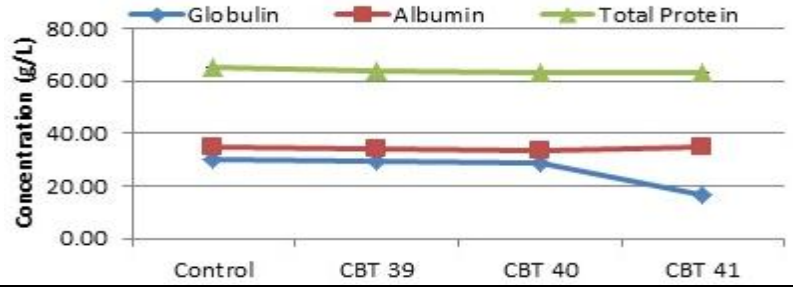


Figure 2. Liver Function Test

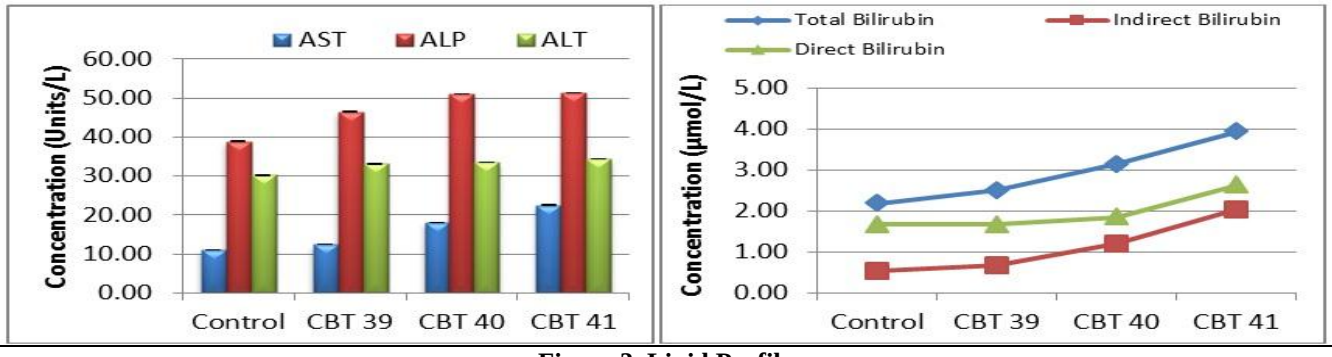
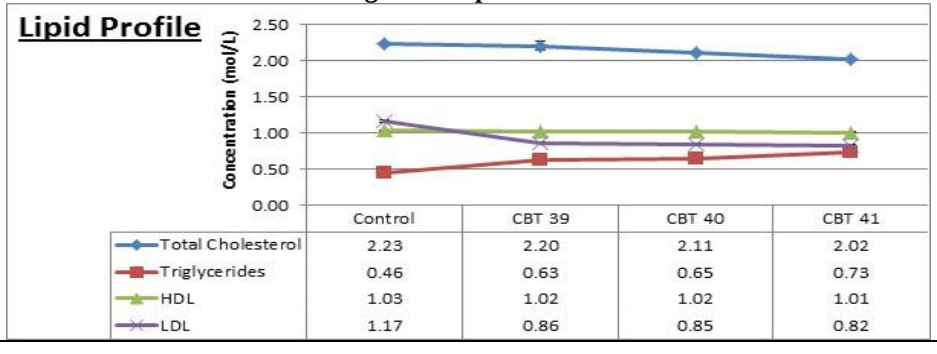


Figure 3. Lipid Profile



DISCUSSION

Heat stress is characterized by an inability of the body's avenues of controlling internal temperature to function. Heat stress either independently or in combination with other stressors such as physical work, loss of fluids, fatigue and medical conditions, culminates in heat illnesses. Heat stress is marked by elevated core temperature, heart rate and sweating (Sieck, 2002 and Keim *et al*., 2002). These conditions can result in heat stroke in absence of timely and effective intervention. Heat stroke remains under-diagnosed, serving as a

research area with tremendous potential to reduce the associated indisposition and death. Currently, heat stroke treatment involves rapidly reducing body temperature and replenishing body fluids coupled with intensive care support. However, only a little is known about the prognosis of heat related illnesses including heat stroke, impeding design of effective management and treatment strategies (Singh *et al*., 2013).

In most cases of heat stroke, acute liver failure has been observed, suggesting that hepatic function indicators can serve as potential heat stress biomarkers. With an aim to test the applicability of hepatic damage blood enzymology as a potential heat stroke diagnostic tool, we investigated the effect of graded heat stress on liver damage induced biochemical changes.

We investigated the effect of increasing magnitude of heat stress culminating in heat stroke on liver function indicators. Sprague-dawley Rats were divided into 4 groups and subjected to heat stress to attain a core body temperature of 39°C, 40°C, and 41°C along with control group. Rat group not subjected to heat stress served as control and the other group was subjected to heat stress till death. Biochemical parameters were compared for in all 4 groups with an objective of facilitating the understanding biochemical responses 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 an increase in liver damage biomarkers in a graded fashion.

In this study, the level of total protein, albumin and bilirubin was found to decrease with an increase in the intensity of heat stress. Albumin and Globulin are the most abundant proteins in blood, synthesized by liver. Evaluating the blood albumin and globulin levels help infer the state of liver functional integrity, with low levels indicating damage. As the severity of heat stress increased, there was heightened liver dysfunction marked by a decreased synthesis of albumin and globulin and hence a decreased total protein concentration (Landry and Bazari, 2011)

AST and ALT are enzymes, which are predominantly found in Liver along with some other body tissues. When liver cells undergo damage, the hepatic enzymes leak out into the blood circulation, where they can be assayed. Hence, an elevated ALT and AST level is indicative of liver damage. Hence, increased heat stress culminated in a concomitant decrease in liver function (Landry and Bazari, 2011).

The level of total, unconjugated and conjugated bilirubin increased with core body temperature. Bilirubin attached to sugar is called as conjugated bilirubin; without sugar is unconjugated bilirubin and the sum total of the two comprises of total bilirubin. High levels of both types of bilirubin indicate liver damage (Limdi and Hyde, 2003).

This study, combined with histological and genome-wide studies on heat stress and heat stoke will provide insights into the mechanisms underlying the pathophysiology of liver damage upon progression from mild hyperthermia to heat stroke with increasing core body temperature (in a graded fashion), providing cues for development of appropriate diagnostic and therapeutic measures for heat related illnesses including the potentially fatal heat stroke.

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