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GRADED HYPERTHERMIA AND KIDNEY FUNCTION: A COMPREHENSIVE EVALUATION

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ABSTRACT

Heatstroke is a form of hyperthermia, characterized by systemic inflammatory response resulting in multi-organ dysfunction with delusion, convulsion and coma and other central nervous system disorders. Suitable diagnostic and therapeutic markers for heat stroke need to be established to alleviate associated morbidity and mortality. Acute renal failure has been reported in 25% exertional heatstroke cases. Heat stress induced rhabdomyolysis results in myoglobinuria leading to acute kidney failure; the incidence being as high as 50%. Therefore, kidney serves as an organ of choice to investigate biochemical biomarkers for heat stroke owing to the high incidence of association of kidney damage with heat stroke. We have investigated the effect of graded hyperthermia on Kidney damage via biochemical evaluation.

Key words: Acute heat stress, Heat stroke, Acute renal failure, Biochemical biomarkers.

INTRODUCTION

Rhabdomyolysis is a syndrome characterized by the leakage of muscle-cell contents into the circulation. Heat stress is a known cause of rhabdomyolysis, wherein muscle fibers are damaged, releasing their contents into the bloodstream. Heat stress induced rhabdomyolysis results in myoglobinuria, leading to acute kidney failure; the incidence being as high as 50% (Trujillo *et al*., 2011; Melli *et al*., 2005; Ward, 1988; Bagley *et al*., 2007). Therefore, kidney serves as an organ of choice to investigate biochemical biomarkers for heat stroke owing to the high incidence of association of renal damage with heat stroke.

Heat stroke is a life threatening condition characterized by an elevation in core body temperature beyond 40°C. It is the most severe of the heat related illnesses, which include heat exhaustion, cramps, syncope and stroke. Heat stroke is a particularly pressing problem for inhabitants of hot areas. Heat stroke is an underestimated villain, as the incidence remains under-reported. The pathogenesis of heat stress is only poorly understood, rendering the identification of biomarkers for heat stress, for both diagnostic and therapeutic implications, challenging (Hsieh *et al*., 2011).

Owing to the observed kidney damage upon heat stroke, we have attempted to study the impact of graded hyperthermia on kidney damage biochemical biomarkers. Sprague dawley rats were subjected to varying degree of hyperthermia in a simulation chamber (45°C and 30% relative humidity) and biochemical parameters assayed. A rise in core body temperature was found to be associated with an increase in the level of kidney damage indicators, with the degree of hyperthermia and kidney damage increasing proportionately (Wang *et al*., 1995).

Once a set of biochemical biomarkers is identified, the diagnosis of heat related illnesses would be facilitated, reducing the associated morbidity and mortality. Integrating these findings with genetic level studies will provide deeper insights into development of detection and treatment options for heat stroke.

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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 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 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 into BD vacutainer SST – II Advance (5 ml) for haematological and in BD microtainer brand tube with EDTA (1 ml), for biochemical analysis.

Biochemical Analysis

An automated haematology analyzer (KX-2IN, Sysmex Corporation, Japan) was used for the haematological analysis and Selectra Junior version 04 autoanalyzer (Vital Scientific Bv, Nether- lands) for the biochemical assays. Renal function test (urea, creatinine, potassium, sodium, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzyme assays).

Statistical analysis

All variables were analysed using one way ANOVA. At least a 95% confidence level ($p<0.05$) is used as a thresh hold for statistical significance.

RESULTS

Renal Function test

Significant increase in concentration was observed in BUN and Creatinine in CBT 40 and CBT 41 groups.

Blood electrolyte analysis

Sodium and potassium concentrations were measured in control and heat stressed groups. The concentration of sodium was found to decrease significantly with increase in severity of heat stress which is measured as 140.67±0.23, 141.62±0.13, 141.42±0.08 and 141.35±0.10 g/L ($p<0.001$) in control, CBT 39, CBT 40 and CBT 41 groups respectively. Similarly, the concentration of potassium significantly decreased with increase in heat stress as 3.63±0.01, 3.52±0.06, 3.33±0.01 and 3.21±0.01 ($p<0.01$) in control, CBT 39, CBT 40 and CBT 41 groups respectively.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzyme were measured and following values are observed as shown in figure 2.

Figure 1. Blood electrolytes

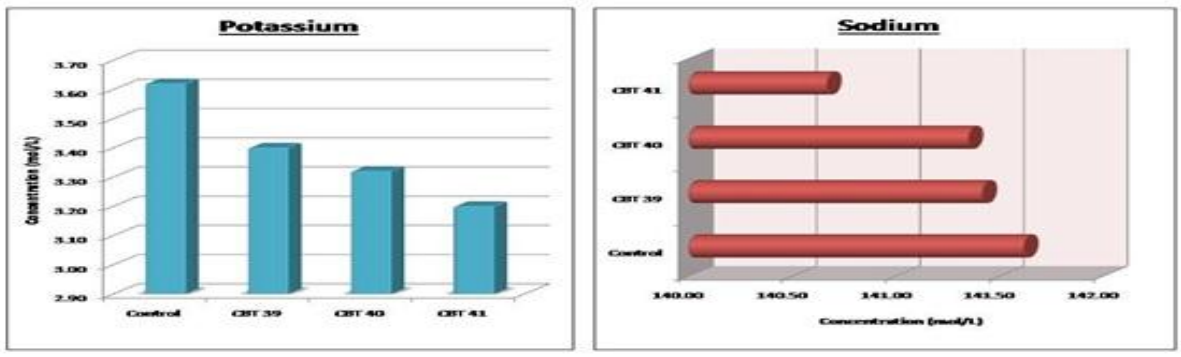

Figure 2. Enzyme Levels

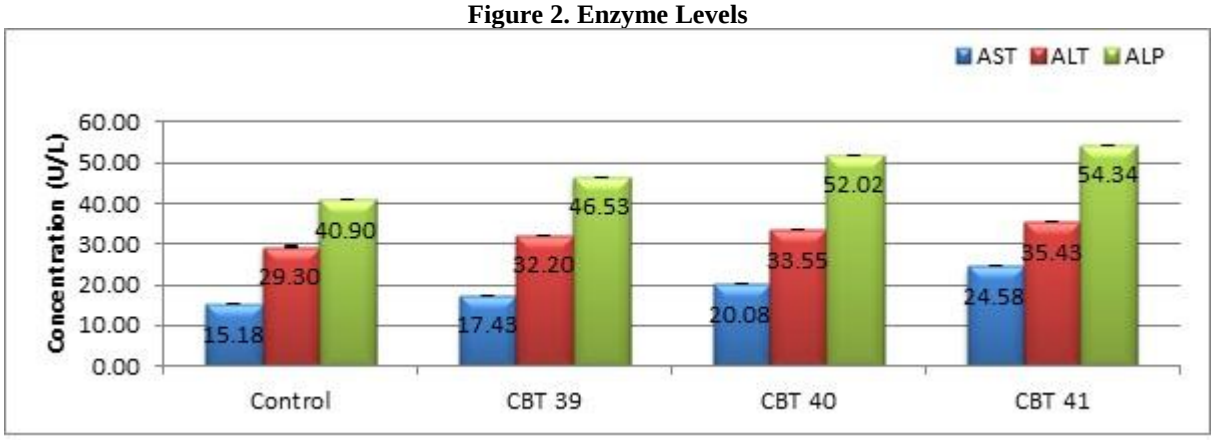

Table 1. Change in BUN and Creatinine concentrations with graded hyperthermia

Group	Blood Urea Nitrogen(mg/dL)	Creatinine (mg/dL)
Control	16.23±1	0.52±0.02
CBT39	17.65±2	0.64±0.01
CBT40	22.79±1*	1.20±0.02*
CBT41	26.67±3*	1.64±0.01*

DISCUSSION

Heatstroke is a form of hyperthermia, characterized by systemic inflammatory response resulting in multi-organ dysfunction with delusion, convulsion and coma and other central nervous system disorders. Suitable diagnostic and therapeutic markers for heat stroke need to be established to alleviate associated morbidity and mortality (1) Acute renal failure has been reported in 25% exertional heatstroke cases (2). Heat stress induced rhabdomyolysis results in myoglobinuria leading to acute kidney failure; the incidence being as high as 50% (Trujillo *et al*., 2011; Melli *et al*., 2005; Ward, 1988; Bagley *et al*., 2007). Therefore, kidney serves as an organ of choice to investigate biochemical biomarkers for heat stroke owing to the high incidence of association of kidney damage with heat stroke. We have investigated the effect of graded hyperthermia on Kidney damage via biochemical evaluation.

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. Rat group not subjected to heat stress served as control. The biochemical findings were compared in all the groups with the objective of facilitating the understanding of biochemical changes in kidney function markers with severity of hyperthermia and its correlation with alteration in functionality.

Sodium and potassium are the key electrolytes regulating the acid-base balance of the body fluids. In this study, the plasma concentration of sodium and potassium decreased with an increase in the magnitude of heat stress, the most likely cause being the fecal and urinary excretion. This is in accordance with the study by Allahverdi et al. wherein heat stress resulted in reduced sodium and potassium plasma levels in poultry (Allahverdi *et al*., 2013).

As the core body temperature increased, Bun and urea concentration in blood were elevated indicating deterioration in kidney function with hyperthermia. The blood levels of creatinine and BUN are the standard kidney function tests reflecting the integrity of kidney function. The BUN test measures the amount of nitrogen coming from urea in blood. Urea is a protein breakdown product excreted in the urine via kidneys. If kidney function is impaired, the BUN level rises.

Creatinine is a product of muscle metabolism, produced from creatine. Creatinine is excreted via kidney after being transported via blood stream. The kidneys, under normal conditions are able to completely eliminate creatinine. Hence, an elevated creatinine level indicated impairment in renal function (Zuo *et al*., 2014).

ALT, ALP and AST increased with an increase in the magnitude of heat stress. AST is found in high concentrations in heart, skeletal muscle, Liver and Kidney, in both cytoplasm and mitochondria. Upon mild tissue injury, the predominant from of AST that is released in circulation is cytoplasmic while severe tissue damage results in increased mitochondrial enzyme release in circulation. A possible cause for increased serum ALP activity could be an injury to the brush border epithelium of the cells of the renal tubule combined with renal function impairment. ALP is a reliable indicator of kidney function (Leibovitch *et al*., 1991).

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 kidney 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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