TROPONIN-I LEVEL IN HEAT-EXPOSED FEMALE SPRAGUE-DAWLEY RATS

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHYSIOLOGY IN PARTUAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR OF SCIENCE (BSc) DEGREE IN PHYSIOLOGY

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CERTIFICATION

This is to certify that this project work on **TROPONIN-I LEVEL IN HEAT EXPOSED SPRAGUE-DAWLEY FEMALE RATS** was carried out by **TAPRE ESTHER OYINDEINYEFA** with the matriculation number **BMS2101677** in partial fulfilment of the award of bachelor sciences degree (B.SC) in the department of physiology, school of Basic medical sciences, college of medical sciences, university of Benin, Benin city, Edo state.

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EXTERNAL EXAMINER

DEDICATION

I dedicate this project work to God Almighty, my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding.

I also dedicate this work to my mom and my loving siblings who encouraged me all the way and whose encouragement made sure that I give it all it takes to finish that which I have started. May the blessings of God be with them now and always, Amen. My love for you all can never be quantified.

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Foremost I sincerely appreciate the Almighty God for his grace, strength, sustenance and above all, his faithfulness and love from the beginning of my academic journey up to this level.

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My unalloyed appreciation goes to my amiable, ever supportive and humble supervisor MR. W.J. SILAS for his invaluable contributions and instructions throughout my project.

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ABSTRACT

Troponin I (TnI) is a crucial component of the troponin complex in striated muscle, playing a central role in regulating contraction and relaxation through calcium interaction. Heat stress refers to the overall response of the human body to the combined effects of environmental factors and temperature. It indicates how much heat the body is exposed to within its thermal surroundings. The justification for studying troponin I levels in heat-exposed Sprague-Dawley female rats is grounded in the need to understand cardiac responses to hyperthermia. Elevated troponin I levels can indicate myocardial injury, which is critical during heat stress conditions. The study aimed to explore the connection between heat exposure and myocardial damage by examining variations in cardiac troponin I (cTnI) levels in heat-exposed Sprague-Dawley female rats. The rats were randomly divided into four groups (1, 2, 3 and 4) for a period of eight weeks. The rats were exposed to heat for 1 to 2 hours daily at a temperature of 38°C to 40°C. Group 1 served as the control group which were not exposed to heat. Group 2 was exposed to heat for 14 days and group 3 and 4 were exposed to heat for 28 days and 42 days respectively. At the end of the experiment, each animal was first anaesthetized using chloroform vapour, followed by a dissection procedure to harvest the heart tissue. After harvesting the heart tissue, it was minced into small pieces and homogenised using phosphate buffer solution, thereafter spun and the supernatant was collected and sent for biochemical analysis. Statistical analysis was done using graph pad prism version 10.4. Results were presented as mean ± standard error of mean. One Way Analysis of Variance (ANOVA) was used to compare the means of tests and control value while post hoc test was done using Dunnett's multiple comparison test and a P-value of less than 0.05 was considered statistically significant. The result gotten from this research shows that mild heat exposure may not induce noticeable cardiac stress. In conclusion, long periods of high temperature exposure may indicate possible myocardial alteration, so heat exposure should be kept within reasonable limits.

CHAPTERR ONE

1.0 INTRODUCTION

1.1 BACKGROUND STUDY

Troponin I is a family of proteins present in both cardiac and skeletal muscles and is a component of the troponin protein complex. It binds to actin in thin myofilaments and maintains the actin-tropomyosin complex in place. Troponin is a preferred biomarker for identifying cardiac injuries. For proper utilization, it's important to understand the sensitivity of the specific assay used, acknowledge that elevated troponin levels indicate cardiac injury, and consider key aspects of the protein's basic science and its measurement (Jaffe and Babuin, 2005). After elective direct current cardioversion, cardiac troponin I (cTnI) levels are typically normal or only slightly elevated, indicating that minor myocardial injury may occur due to the direct current transthoracic shocks. In contrast, significant increases in cTnI levels following cardioversion imply that there may be myocardial injury from factors not related to the shocks used during the procedure (Allan *et al*., 1997). Troponin testing is commonly indicated in emergency situations involving symptoms such as chest pain or discomfort, heart palpitations, shortness of breath, fatigue, nausea or vomiting, sweating, dizziness or lightheadedness, and pain radiating to the arm, back, or abdomen (Batra *et al*., 2024). Cardiac troponins T and I (cTnT and cTnI) have been shown to offer high sensitivity and specificity, making them more reliable than creatine kinase-MB (CK-MB) in detecting myocardial injury (Maynard *et al*., 2000). Recently, the development of high-sensitivity troponin assays has allowed for the detection of subclinical organ damage. These advanced tests are being evaluated as potential biomarkers to assess the increased risk of myocardial infarction, coronary heart disease-related death, and heart failure hospitalizations (Wilkins and Lloyd-Jones, 2012). Heat stress is the feeling of discomfort and the strain on the

body caused by exposure to a hot environment, particularly when doing physical work. Body temperature in this situation can be normal or somewhat elevated, registering above 37°C but below 40°C (Wills, 2016). Heat strain, which is often mentioned alongside heat stress is the body's overall physiological reaction to the burden of heat it's experiencing (Huang *et al*., 2021). Heat stress can result from hot weather, industrial processes, physical exertion (which generates heat), or wearing protective clothing. When these factors combine, they can overload the body's ability to regulate temperature, leading to excessive heat strain (Ramsey *et al*., 2000). Climatic factors and environmental conditions are factors that affecting human life, comfort and health, which are studying today in the form of a branch of science called bioclimatology (Ghalhari *et al*., 2019). Heat stress is one of the issues related to climate and environmental conditions that can play a significant role in causing problems and complications affecting human health (Heidari *et al*., 2020).

1.2 STATEMENT OF PROBLEM

Exposure to elevated temperatures can lead to increased serum levels of cardiac troponin I, indicating myocardial injury. Studies show that heat stress induces hyperthermia and hypotension, which correlate with elevated troponin I levels, suggesting potential cardiac dysfunction. Understanding these changes is crucial for developing therapeutic interventions to mitigate heat-induced cardiac damage.

1.3 JUSTIFICATION OF STUDY

The justification for studying troponin I levels in heat-exposed Sprague-Dawley female rats is grounded in the need to understand cardiac responses to hyperthermia. Elevated troponin I levels can indicate myocardial injury, which is critical during heat stress conditions. Troponin I

responses can provide insights into the pathophysiology of heat-related cardiac events, enhancing the applicability of findings to human health outcomes.

1.4 AIM OF STUDY

The study aimed to explore the connection between increased temperatures and myocardial damage by examining variations in cardiac troponin I (cTnI) levels in heat-exposed Sprague-Dawley female rats.

1.5 RESEARCH QUESTION

How does heat exposure affect cardiac Troponin I (cTnI) levels in female Sprague-Dawley rats?

1.6 SPECIFIC OBJECTIVE

To evaluate the level of cardiac troponin-I (cTnI) in heat exposed Sprague-dawley female rats.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 TROPONIN I

Troponin I is a well-established biomarker for cardiac injury. The troponin complex, which is crucial for regulating both skeletal and cardiac muscle contractions, consists of three proteins: troponin T, troponin I, and troponin C (Burklund *et al*., 2020). Troponins I and T are specific to cardiac muscle and are utilized to identify cardiac muscle damage. Following cardiac injury, the concentrations of troponin I and T rise within a few hours and can remain elevated for 5 to 7 days (Tietze, 2012). Troponin C binds calcium, troponin I inhibits actin-activated myosin Mg^{2+}–ATPase, and troponin T serves as the binding protein for tropomyosin. Cardiac troponin C shares complete amino acid homology with skeletal muscle troponin C, which is why it is not used as a cardiac biomarker (Wu, 2017). Troponins are intracellular polypeptides found in both cardiac and skeletal muscles that play a key role in excitation-contraction coupling (The Horse, 2012). As calcium-regulatory proteins, they are integral to the contractile function in these muscles. Troponins are uniformly distributed along the length of thin filaments and form a structured complex with tropomyosin and actin. When intracellular Ca^{2+} levels are low, troponin and tropomyosin inhibit the interaction between myosin and actin; however, an increase in Ca^{2+} concentration triggers the release of this inhibition through Ca^{2+} binding to troponin (Ohtsuki *et al*., 2021). The cardiac-specific isoform of the myofibrillar contractile protein, troponin I, is exclusively found in mammalian hearts (Cummins *et al*., 1987). Cardiac troponin levels are measured on a continuous scale that may correlate positively with increased risk. In clinical practice, results are often interpreted as either positive or negative based on the 99th percentile upper limit of normal (ULN) (Park *et al*., 2021).

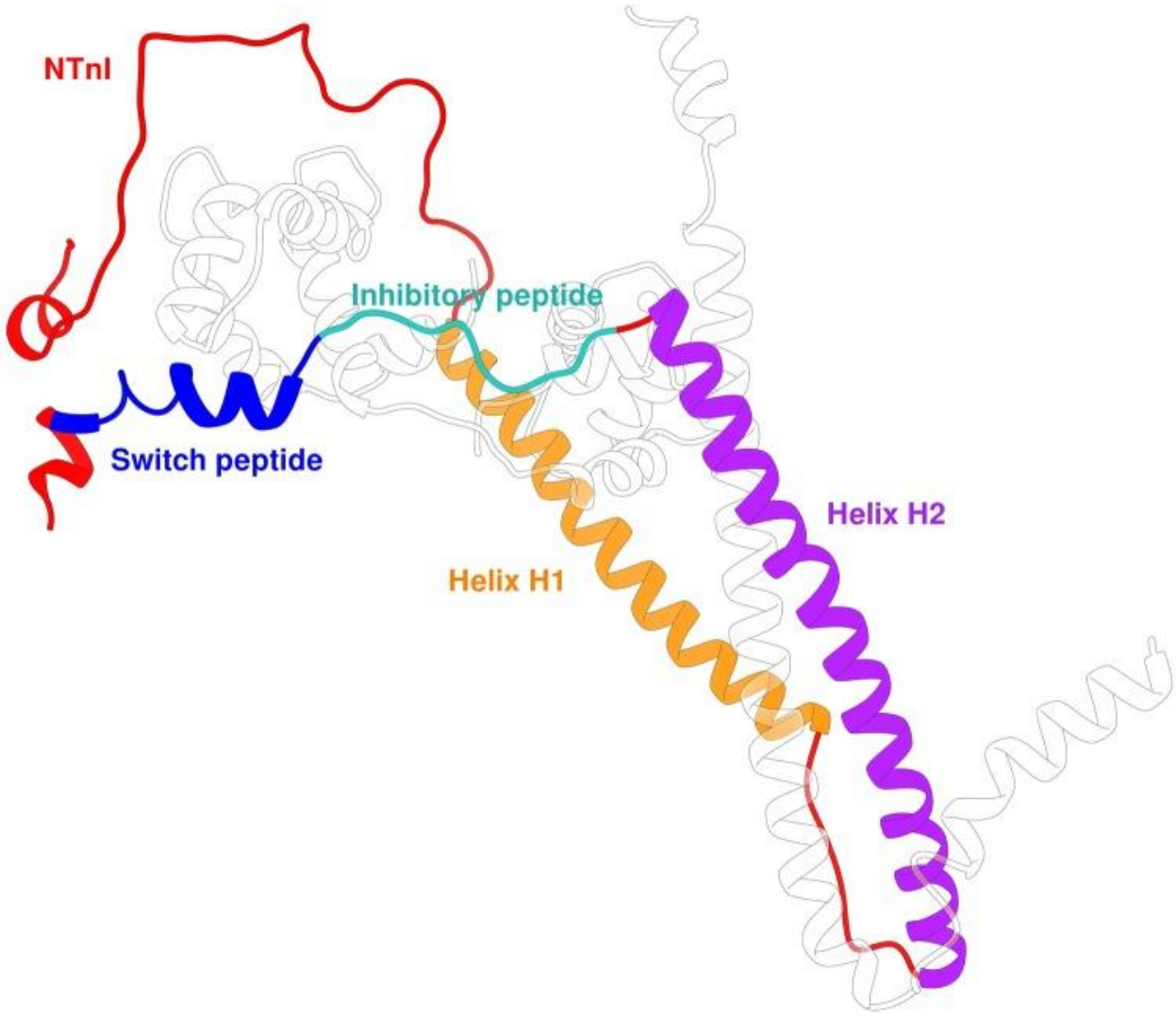

Figure 1: The cTnI molecule with its relevant structural regions highlighted (Marston *et al*., 2020).

2.1.1 TROPONIN TEST

The measurement of cardiac troponin concentrations in the blood is a key element in the evaluation of patients with suspected acute coronary syndromes, according to current guidelines, and contributes importantly to the ruling in or ruling out of acute myocardial infarction (Bingisser *et al*., 2012). Three (3) critical elements are necessary for optimal use of troponin testing in clinical care, as follows:

1) The analytical performance of the assay;

2) The clinical sensitivity and specificity of the test result; and

3) The clinical reasoning for ordering and the proper clinical context for interpreting the test result (Brush *et al*., 2016). The recent advancement of highly sensitive cardiac troponin assays allows for the detection of very low levels of troponin in the bloodstream. Utilizing these sensitive assays enhances diagnostic accuracy for patients suspected of having acute coronary syndromes and provides significant prognostic information for those with stable coronary artery disease and chronic heart failure (Omland, 2010). In real-world practice, troponin test is frequently performed in patients visiting the emergency department not only for chest pain but also for non-cardiac presentations (Park *et al*., 2021).

2.1.2 HIGH SENSITIVITY TROPONIN

High-sensitivity cardiac troponin (hs-cTn) assays have become standard practice in laboratories worldwide for the initial assessment of patients presenting with typical chest pain. Their ability to detect early increases in troponin levels can significantly reduce the time required to identify myocardial infarctions (MI), which is crucial for patients arriving at emergency departments with chest pain (Lazar *et al*., 2022). High-sensitivity troponin assays measure the same proteins targeted by conventional sensitivity assays but are capable of detecting them at much lower concentrations (Sherwood and Newby, 2014). High-sensitivity cardiac troponin (hs-cTn) assays can detect very small troponin concentrations and subtle changes in them. This enables quick identification or exclusion of acute myocardial injury in patients with chest pain, allowing for faster evaluation of acute myocardial infarction. Additionally, these assays offer possibilities for

using troponin release from heart muscle cells to gain prognostic information in other clinical scenarios (Raber *et al*., 2021).

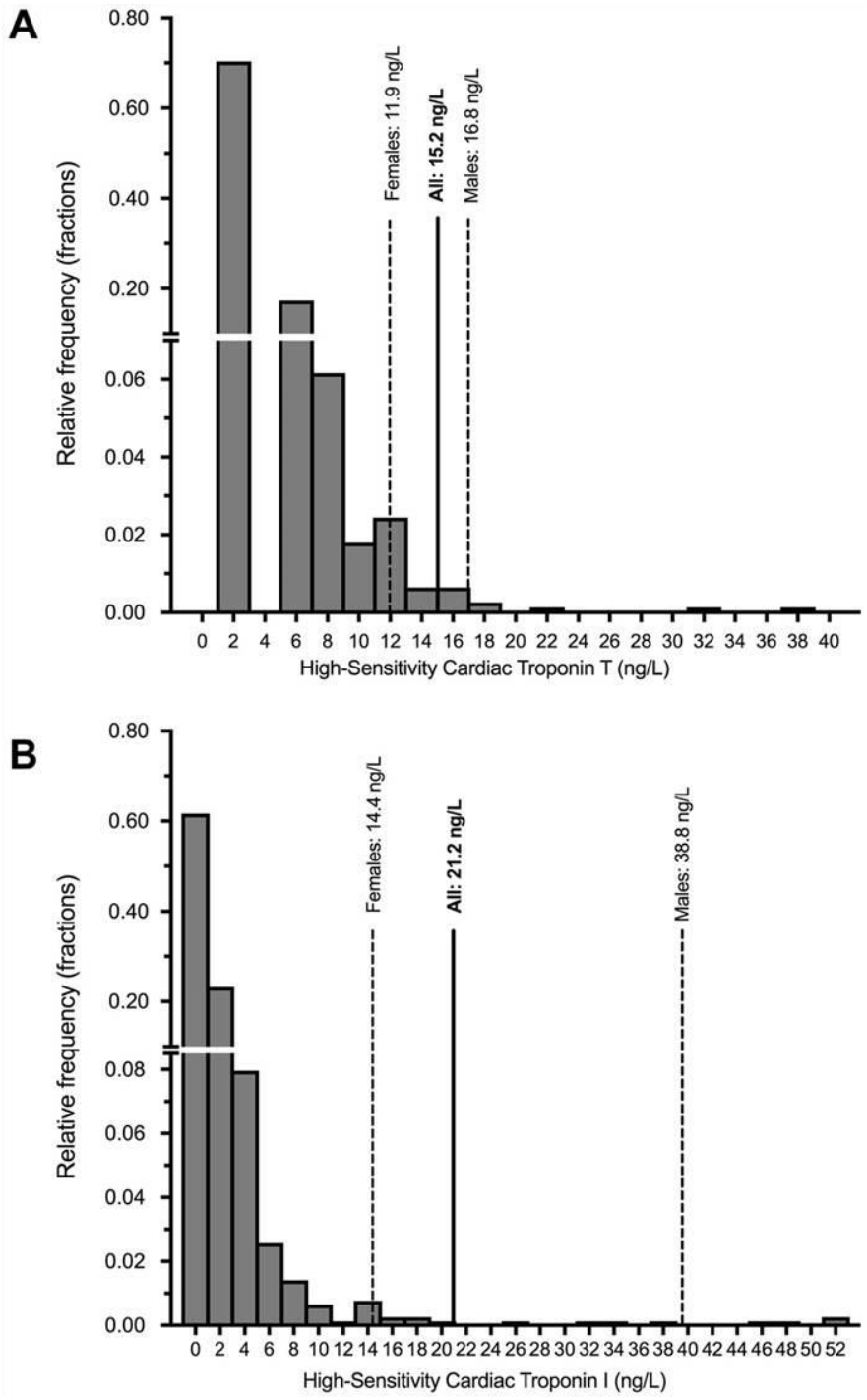

Figure 2: Distribution of High sensitivity cardiac troponin T (Panel A) and I (Panel B) (Aw *et al*., 2019).

2.2 HEAT STRESS

Heat stress happens when the body's heat load surpasses the ability of the thermoregulatory system to adequately dissipate heat (Hodgson, 2014). Initially, while the term "heat stress" is commonly utilized, it actually includes various concepts such as heat shock, heat waves, and warming experiments, each differing in the duration and intensity of temperature increases experienced (Jagadish *et al*., 2021). Elevated heat stress can diminish physical work capacity and impair motor-cognitive functions, leading to decreased productivity and a heightened risk of occupational health issues (Kristie *et al*., 2021). The health impacts linked to exposure to extreme and extended heat seem to be connected to environmental temperatures that exceed what the population is used to (McGeehin and Mirabelli, 2001). Some of the types of heat stress are shown below:

- Heat stroke
- Heat exhaustion
- Dehydration
- Heat syncope (fainting)
- Heat cramps
- Heat rash (prickly heat) (Sutton, 2015).

Heat stress refers to the overall response of the human body to the combined effects of environmental factors and temperature. It indicates how much heat the body is exposed to within its thermal surroundings. This phenomenon is typically associated with the heat transfer dynamics between the environment activity and the human body, as well as the intensity of physical, which generates heat through human exertion (Huang *et al*., 2021). Adjustments in heat production within the body are typically followed by slow, exponential shifts in heat loss, which

in turn result in changes to the amount of heat stored within the body. This dynamic process plays a crucial role in maintaining thermal balance. Throughout the day and night, the body achieves heat balance at different levels of heat production, depending on factors such as activity levels, environmental conditions, and metabolic processes. At each of these distinct levels of heat production, there is a corresponding rectal temperature that reflects the body's internal thermal state. This relationship highlights the body's ability to regulate its temperature and maintain equilibrium despite fluctuations in heat generation and loss (Paul, 1995). When the body is exposed to heat, it can lead to various physiological and pathological effects. To maintain a normal body temperature, the body employs both behavioral strategies, like seeking cooler environments, and autonomic responses, such as skin vasodilation and sweating, to defend against the heat. If these mechanisms are successful, the body develops a tolerance to warmth. With repeated and prolonged exposure to heat, this tolerance increases, resulting in heat acclimation (Székely *et al*., 2015). Climate change is causing environmental heat levels to rise, posing a significant threat to the health, well-being, and long-term viability of communities in already hot regions. This challenge is multifaceted, encompassing both the direct clinical health impacts of daily heat exposure and indirect consequences such as reduced air quality, limited access to safe drinking water and nutritious food, and insufficient protection from disease-carrying organisms and harmful environmental chemicals (Kjellstrom *et al*., 2018). Elevated heat exposure significantly contributes to cardiovascular disease, a risk often underestimated. As temperatures rise, especially within an aging population, public health faces considerable challenges. Understanding how ambient heat affects different cardiovascular diseases and vulnerabilities is crucial for tackling the growing burden of heart-related issues in a changing climate. Evidence-based prevention is needed to lower cardiovascular events during hot periods,

reducing worldwide deaths and illness related to heart issues (Liu *et al*., 2022). The development and application of heat stress indices and models to assess and measure heat stress and strain have significantly decreased morbidity and mortality rates in industrial, military, sports, and recreational activities (Havenith and Fiala, 2011). The most studied aspect of the heat shock response is the activation of heat shock proteins, which act as molecular chaperones and regulate the cell cycle while preventing apoptosis. While research often highlights the harmful effects of intense heat stress, such as cell cycle arrest and apoptosis, the cellular response to mild, fever-like heat stress is not as well understood. However, the body's reaction to mild heat stress is likely more physiologically relevant, as body temperature in warm-blooded animals only increases by 1–2°C during feverish illnesses (Park *et al*., 2005).

2.3 CARDIAC BIOMARKERS IN HEAT STRESS

Cardiac troponin I (cTnI) is recognized as a sensitive and specific biomarker for heart damage (Dervisêvì *et al*., 2023). Biomarkers, which are biological samples collected from patients experiencing heatstroke, are essential for early detection of organ injury and predicting outcomes to develop innovative organ preservation therapies (Schlader *et al*., 2022). Cardiac biomarkers are proteins released into the bloodstream when myocardial injury occurs (Singh *et al*., 2010). Indicators of stress can include thermal stress markers like heat shock proteins (HSPs), innate immune markers such as Acute Phase Proteins (APPs), oxidative stress markers, and chemical compounds found in saliva and urine. Moreover, these stress biomarkers are vital for predicting the progression of stress-related illnesses and guiding treatment strategies (Dhama *et al*., 2019). As the current "gold standard" for diagnosing myocardial infarction, cardiac troponin demonstrates high sensitivity and specificity, and treatment strategies for patients with elevated troponin levels have been shown to positively affect outcomes. While other markers indicating

myocardial necrosis, inflammation, and neurohormonal activity have diagnostic or prognostic value, none surpass the utility of troponin (Aldous, 2013). Climate change has resulted in rising ambient temperatures, leading to extreme heat events globally. July 2023 was noted by the World Meteorological Organization (WMO) as a record-breaking month, with the Earth reaching a critical temperature threshold of 1.5 °C as established by the Paris Agreement. This alarming trend prompted a warning from the United Nations about entering what they term "an era of global boiling." The rise in global temperatures can lead to increased heat stress, contributing to various physiological and biochemical changes in the human body. Given that cardiovascular diseases (CVDs) are a major cause of morbidity and mortality worldwide, heat events exacerbate this public health challenge (Mol *et al*., 2024). Heat shock proteins are produced by cells under stressful conditions, including extreme heat. These proteins function as chaperones to prevent misfolding or aggregation of proteins (Kultz, 2005). Extracellular heat shock proteins (eHSPs) do not perform chaperone functions but instead act as cytokines that engage pattern-recognition receptors like toll-like receptors to trigger an inflammatory response (Chen and Nuñez, 2010), eHSP72 can be released for up to 72 hours in non-human primate models of severe heatstroke and is linked to multiorgan damage and mortality, indicating its potential role as a prognostic biomarker (Dehbi *et al*., 2010). Preliminary evidence of myocardial injury during heatstroke was first reported in a 2006 case study involving a dog with suspected exertional heat stroke and elevated cardiac troponin I levels (Mellor *et al*., 2006), which rise when myocardial cells are damaged. This finding was later supported by a human case report (Whiticar *et al*., 2008), including another report detailing cardiomyopathy following heatstroke (Chen *et al*., 2012). Recent studies have shown that exertional heat stroke can induce metabolic changes in the

myocardium indicative of injury that may last for up to two weeks after the event (Laitano *et al*., 2020).

2.4 COMPARATIVE ANALYSIS OF TROPONIN I LEVEL

Cardiac troponin T (cTnT) and troponin I (cTnI) are structural proteins that play a crucial role in regulating muscle contraction. They are released into the bloodstream from damaged muscle cells during cardiac ischemia, with no overlap with skeletal muscle troponins under normal circumstances. Numerous studies have shown that both cTnI and cTnT serve as significant prognostic indicators for patients experiencing chest pain, even when creatine kinase (CK) MB fraction levels are not elevated. Consequently, troponins are increasingly recognized as valuable tools for stratifying patients with chest pain (Ropollo *et al*., 1999). The lactate dehydrogenase (LD) isoenzyme 1 to isoenzyme 2 ratio (LD1/LD2) is the established marker for diagnosing myocardial infarction at a later stage. A study comparing the sensitivity of cardiac troponin I (cTnI) and LD1/LD2 as late markers of myocardial injury over five days in 36 patients diagnosed with myocardial infarction found that cTnI had significantly greater sensitivity than LD1/LD2 ($P < .05$) (Martins *et al*., 1996). The introduction of high-sensitivity troponin (hsTn) assays has enhanced the identification of chest pain patients who can be safely discharged with a low risk of adverse cardiac events compared to conventional troponin assays. Multiple studies have consistently indicated that patients with hs-troponin levels below the 99th percentile upper reference limit (URL) who rule out for myocardial infarction (MI) have a less than 1% rate of adverse events within 30 days (Mahmoud *et al*., 2020).

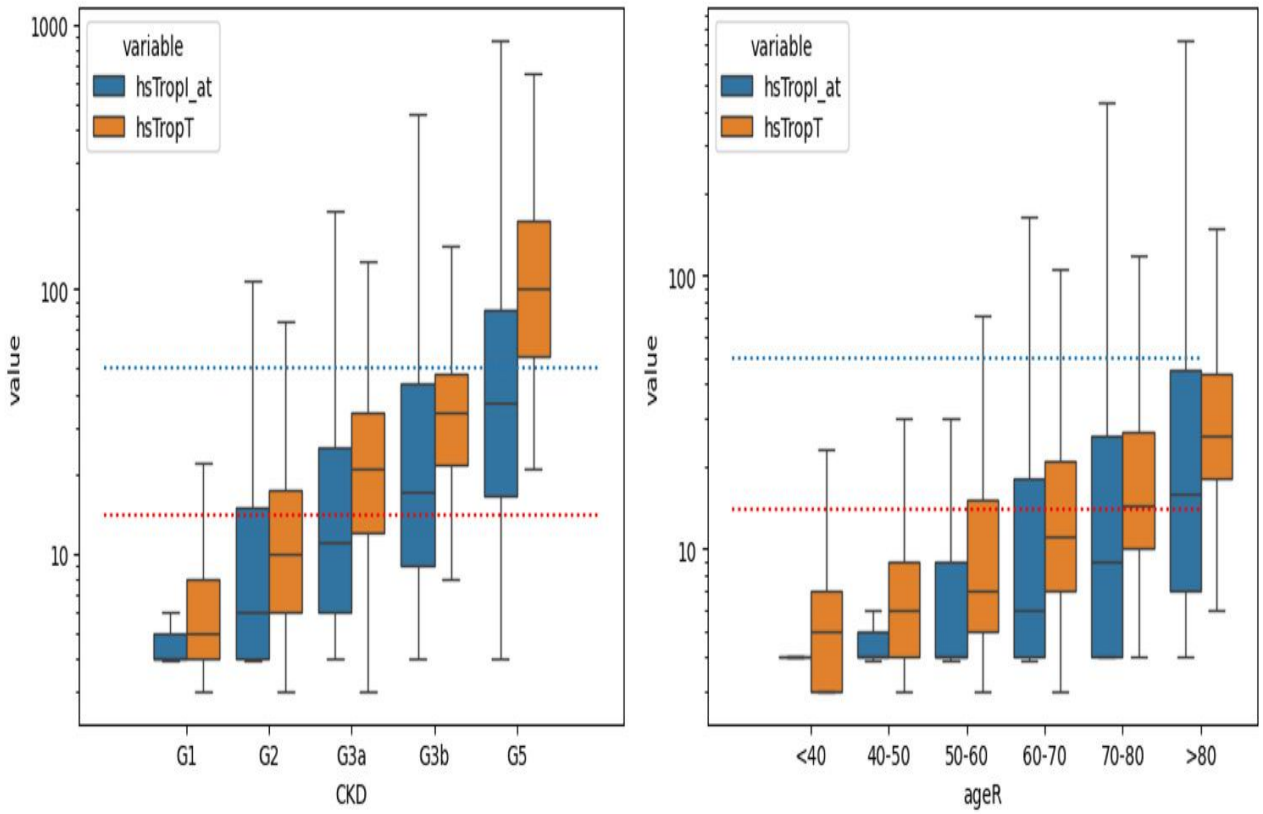

Figure 3: Comparative Analysis of Cardiac Troponin T and Troponin I Assay Positivity: Implications for Myocardial Infarction Diagnosis (Zaheen *et al*., 2024).

2.5 IMPACT OF THERAPEUTIC HYPOTHERMIA

The effects of temperature on human biology have been extensively studied, with numerous experimental findings demonstrating that reducing body temperature can protect tissues from damage (Yamada *et al*., 2021). Therapeutic hypothermia (TH) is a proven treatment for comatose patients, enhancing cardiac and neurological recovery after the restoration of spontaneous circulation following cardiac arrest (Ko *et al*., 2020). A severe lack of oxygen or blood flow around the time of birth, known as perinatal asphyxia, can result in hypoxic-ischemic encephalopathy (HIE), a condition linked to high mortality rates and long-term complications. HIE is identified by clinical and laboratory evidence of brain injury caused by hypoxia and

acidosis (Liu *et al*., 2011). Perinatal asphyxia remains a significant cause of morbidity and mortality, with therapeutic hypothermia being the only effective intervention for neonates with moderate to severe HIE following asphyxia (Leys *et al*., 2023). Furthermore, TH has been shown to significantly reduce myocardial damage in full-term neonates affected by asphyxia (Rakesh *et al.*, 2018). Notably, TH for myocardial infarction is distinct in that it can be initiated before reperfusion, unlike its use for brain injury in cardiac arrest patients who have been resuscitated (Yamada *et al*., 2021). Both hypoxic-ischemic brain injury and traumatic brain injury trigger biochemical and molecular processes that worsen brain damage. The application of therapeutic hypothermia appears to mitigate these molecular cascades, which ultimately lead to neuronal injury. Hypothermia reduces the harmful effects of the initial injury, such as the production of reactive oxygen species, neurotransmitters, inflammatory mediators, and apoptosis (Ma *et al*., 2012).

2.6 PHYSIOLOGICAL RESPONSE TO HEAT STRESS

When environmental or physiological conditions lead to heat gain exceeding heat loss, the internal (core) body temperature rises. An increase of approximately 3°C above the normal range (for example, from 37°C to 40°C) can place significant stress on physiological systems and may even result in death (Crandall and Wilson, 2016). Exposure to high temperatures raises the likelihood of developing heat-related illnesses, which encompass a range of conditions from mild alterations in cardiovascular function (such as heat syncope and heat exhaustion) to potentially fatal heatstroke (Armstrong *et al*., 2007). Heatstroke is characterized by a rapid increase in core temperature (typically exceeding 40.1°C), dysfunction of the central nervous system, systemic inflammation, and injury to organ systems that can lead to death (Bouchama *et al*., 2022a). There are two primary forms of heatstroke: classic heatstroke (CHS) and exertional heatstroke (EHS)

(Bouchama *et al*., 2022). CHS usually occurs during periods of rest in hot and humid environments that limit the body's ability to dissipate heat, affecting primarily the very young, older adults, and individuals with pre-existing health conditions (Bouchama *et al*., 2022). In contrast, EHS primarily affects younger, physically active individuals such as military personnel and athletes, resulting from an inability to effectively dissipate the heat generated by physical exertion (Bouchama *et al*., 2022). Consequently, EHS can arise under a broader range of environmental conditions (Rae *et al*., 2008; Stacey *et al*., 2022). The risk of heatstroke poses a significant threat to the health and well-being of millions globally, a concern that is expected to grow in the coming decades (IPCC, 2021). Elevated body temperatures have significant effects on the cardiovascular system, facilitating increased skin blood flow necessary for heat loss (Crandall and Wilson, 2014). The heart responds by boosting cardiac output, primarily through increased heart rate while maintaining stroke volume due to enhanced cardiac contractility (Crandall and Wilson, 2014). Given these cardiovascular responses—especially when combined with extensive hematological and vascular changes during and after heatstroke—it is unsurprising that a single episode of heatstroke raises the risk of all-cause mortality by 40% (Wallace *et al*., 2007). Specifically, individuals experiencing EHS show a higher incidence of cardiovascular diseases, including myocardial infarction, ischemic heart disease, and acute ischemic stroke as early as 14 years after the initial heatstroke event (Wallace *et al*., 2007; Wang *et al*., 2019). Heatstroke triggers a complex innate immune response marked by neutrophil activation, complement protein activation, and the production of pro- and anti-inflammatory cytokines, chemokines, and acute phase proteins (Bouchama *et al*., 2022b). This immune response activation has been linked to endotoxins leaking from the gastrointestinal tract damaged by heat (Hall *et al*., 1999) and danger signal molecules or alarmins released into circulation from

injured or dying cells (Gallucci and Matzinger, 2001). Alarmins—also referred to as damage-associated molecular patterns—are crucial for clearing necrotic tissue debris and facilitating repair processes (Chen and Nuñez, 2010). However, they have also been implicated in excessive and prolonged inflammatory responses that lead to the production of reactive oxygen species and proteolytic enzymes, which further damage tissues and contribute to multiorgan failure (Chen and Nuñez, 2010.

CHAPTER 3

3.0 MATERIALS AND METHOD

3.1 MATERIALS

20 Sprague Dawley female rats, chloroform, syringes (5ml and 10ml), test tubes, plain tubes, latex gloves, plastic animal cages, wooden cages, saw dust, animal feed, centrifuge, dissection instrument, mortar and pestle, light bulbs, extension.

3.2 STUDY AREA

This study was carried out in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City.

3.3 EXPERIMENTAL ANIMALS

Twenty (20) Sprague Dawley rats obtained from Ibadan were used for this study. The rats were allowed to acclimatize in the new environment (Pharmacology animal house) for a period of two (2) weeks with free access to feed and water before the commencement of the study. Thereafter, the rats were placed in wooden cages that were connected to light bulbs to expose them to heat.

3.4. EXPERIMENTAL DESIGN

After acclimatization, the rats were randomly divided into four groups (1,2,3 and 4) with each group containing 5 rats. The rats were exposed to heat for 1 to 2 hours daily at a temperature of 38°C to 40°C. Group 1 served as the control group which received only feed and water. Group 2 was exposed to heat for 14 days and group 3 and 4 were exposed to heat for 28 days and 42 days respectively.

3.5. STUDY DURATION

The experiment lasted for a period of 8 weeks after which the animals were sacrificed and samples were collected.

3.6 ETHICAL CONSIDERATION

Animal management and experimental protocols were carried out in accordance with the recommendation of the 1996 guide for the care and use of the laboratory animals (Clark *et al*.,1997).

3.7 SAMPLE COLLECTION

At the end of the experiment, the rats were sacrificed. The rats were placed in an enclosed container with chloroform to induce anesthesia. After a few minutes, the rats were removed from the enclosed container and a dissection procedure was done to harvest the heart tissue. After harvesting the heart tissue, it was minced into small pieces and homogenized using phosphate buffer solution, thereafter spun and the supernatant was collected and sent for biochemical analysis at Ibadan.

3.8 SAMPLE ANALYSIS

3.8.1 MEASUREMENT OF TROPONIN I

Serum Troponin I levels were measured using an enzyme-linked immunosorbent assay(ELISA) following the manufacturer's principle.

TEST PRINCIPLE

The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat TNNI3/cTn-I. Samples (or Standards) are added to the micro ELISA plate wells and combined

with the specific antibody. Then a biotinylated detection antibody specific for Rat TNNI3/cTn-I and AvidinHorseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat TNNI3/cTn-I, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat TNNI3/cTn-I. The concentration of Rat TNNI3/cTn-I in the samples can be calculated by comparing the OD of the samples to the standard curve.

TISSUE HOMOGENATES

The tissues were minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces were weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. The homogenates were then centrifuged for 5-10 min at 5000×g at 2-8℃ to get the supernatant.

3.9 ASSAY PROCEDURE

1. 100µL standard or sample was added to the wells and incubated for 90minutes at 37°C.

2. Liquid was discarded and 100µL Biotinylated Detection Ab working solution was added immediately to each well and incubated for 60 minutes at 37°C.

3. Solution was aspirated and plate was washed 3 times.

4. 100µL HRP conjugate working solution was added and incubated for 30 minutes at 37°C. Solution was aspirated and plate was washed 5 times.

5. 90µL substrate reagent was added and incubated for 15minutes at 37°C.

6. 50µL stop solution was added.

7. Plate was read at 450nm immediately.

3.10 STATISTICAL ANALYSIS

Statistical analysis was done using Graph pad prism version 10.4. Results were presented as mean ± standard error of mean. One Way Analysis of Variance was used to compare the means of tests and control value while post hoc test was done using Dunnette's multiple comparison test and a p-value of 0.05 was considered statistically significant**.**

CHAPTER 4

4.0 RESULTS

4.1 RESULTS OF STATISTICAL ANALYSIS

Table 4.1: Mean ± SEM (standard error of mean) of Troponin I concentrations in Heat-Exposed Sprague Dawley Female Rats

Parameter	Group A	Group B	Group C	Group D	p-value
Troponin-I (pg/ml)	32.26 ± 1.817	28.74±1.436	52.52 ± 2.775	46.07± 1.650	<0.001

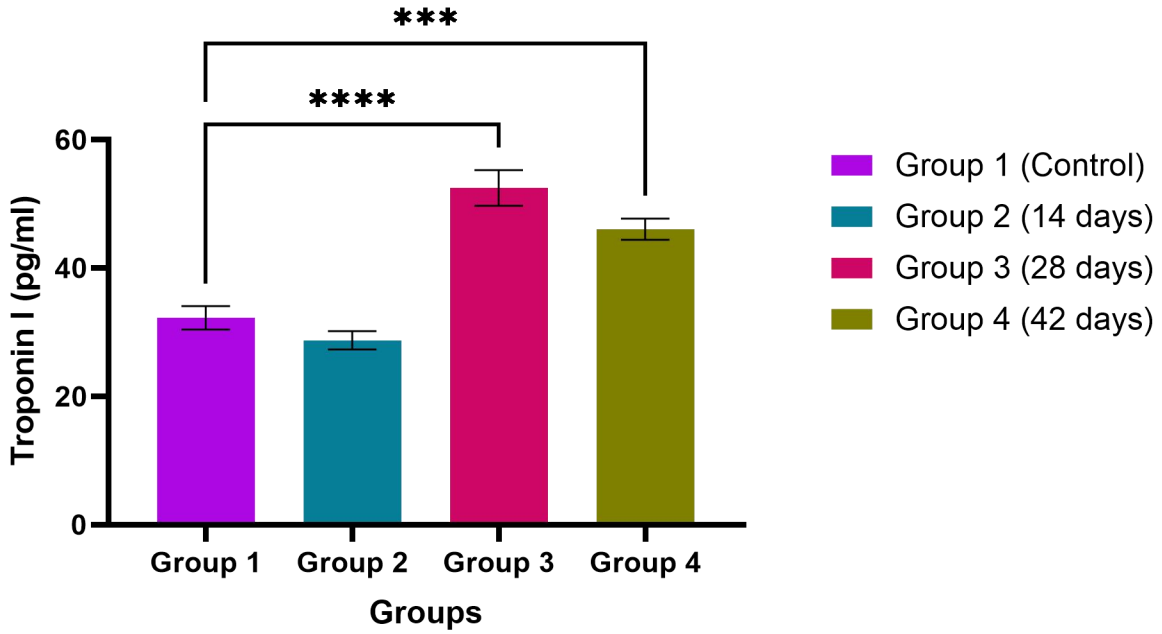

Figure 1: Chart showing varying troponin concentration (pg/ml) of Sprague-Dawley female rats following heat exposure for 6 weeks.

The result above shows no statistically significant difference in the troponin level concentrations between group 2 and the control group ($p > 0.05$). However, it shows a statistically significant increase in the troponin concentrations between the control group and group 3 ($p < 0.05$) and also between the control group and group 4 ($p < 0.05$).

CHAPTER 5

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Findings in this study indicate that prolonged heat exposure significantly affects troponin I levels in female Sprague-Dawley rats. Groups 3 and 4, which experienced prolonged heat exposure, showed significantly increased troponin I concentrations compared to the control group ($p < 0.05$). However, group 2 did not show a statistically significant difference from the control group, suggesting that mild heat exposure may not induce noticeable cardiac stress.

Studies have shown that chronic heat exposure increases cardiac biomarkers, including troponin, due to thermal-induced myocardial injury. Research on rodents exposed to high ambient temperatures revealed increased oxidative stress and inflammatory markers, which contribute to myocardial damage.

Also Human studies have reported elevated troponin levels in individuals experiencing heatstroke, indicating cardiac injury. A study by Leon and Helwig (2010) found that heat stress leads to increased cardiac strain, particularly in vulnerable populations.

Additionally a research by Lin *et al*. (2017) found that prolonged heat exposure in rats induced oxidative damage, increasing cardiac enzyme levels. This aligns with the observed elevated troponin levels in groups 3 and 4 in this study.

5.2 CONCLUSION

With the data gotten from this research, it was concluded that mild heat exposure with a short duration may not induce noticeable cardiac stress. However long periods of high temperatures may induce possible myocardial alteration, so heat exposure should be kept within a minimal possible.

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